Suez Canal University

Faculty of Science

Department of Chemistry

Hepatoprotective Effect of *Moringa oleifera* Extract on Lead Induced DNA Damage in Rats

A Thesis Submitted by

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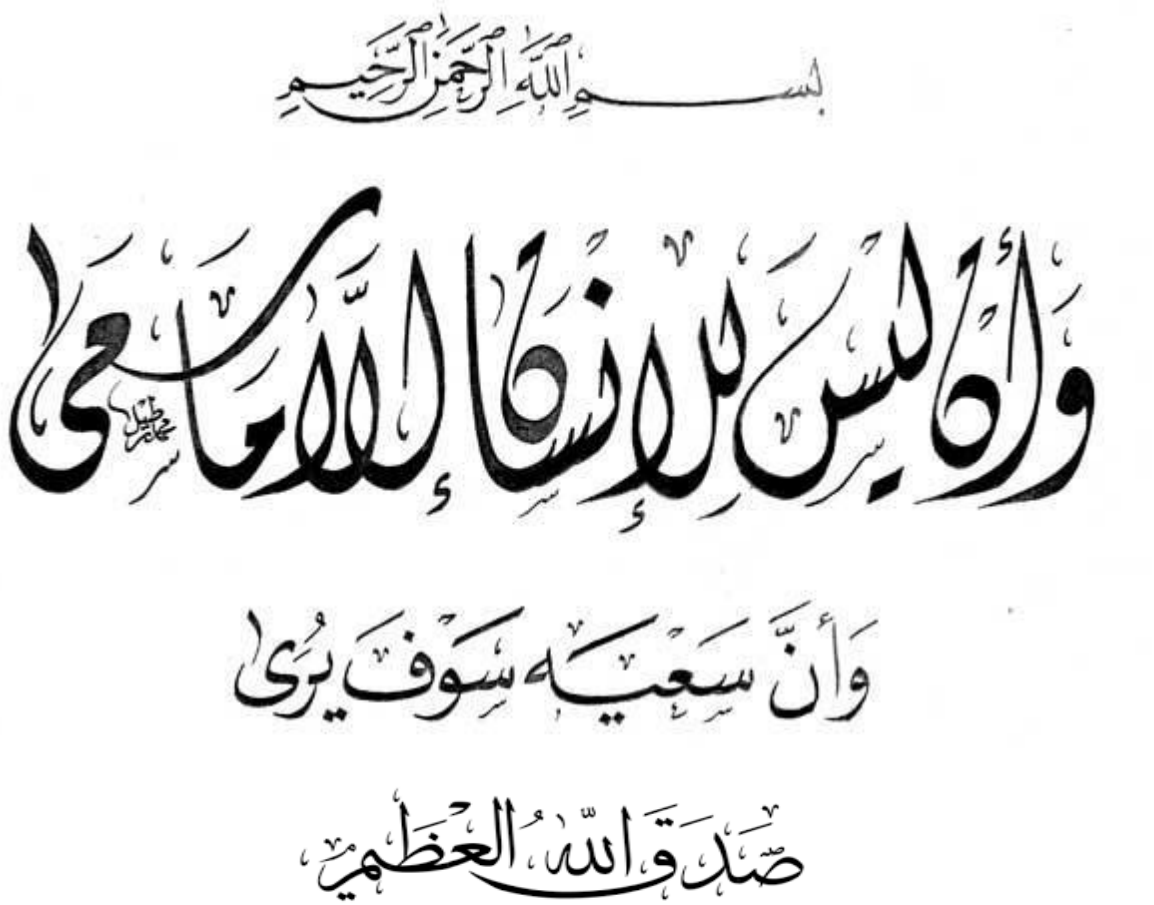
بسم الله الرحمن الرحيم
وأن ليس للإنسان إلا ما سعى
وأن سعيه سوف يرى
صدق الله العظيم

سورة النجم

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List of Abbreviations

Abbreviation	Full Name
%	Percent
A/G	Albumin/Globulin
ALP	Alkaline phosphatase
ALT	Alanine amino transaminase
APAP	Acetaminophen
AST	Aspartate amino transaminase
BUN	Blood urea nitrogen
b.wt	Body weight
CAT	Catalase
CCl4	Carbon tetrachloride
DEN	Diethyl nitrosamine
dL	Decilitre (s)
DNA	Deoxy ribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
G	Gram
GAE	Gallic acid
GLDH	Glutamate dehydrogenase
GM	Gentamicin
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GST	Glutathione-S-transferase
H & E	Hematoxylin and Eosin
HCD	High cholesterol diet
HFD	High fat diet
i.p	Intraperitoneal
IU	International unit (s)
HDL-C	High density lipoprotein
HPLC	High performance liquid chromatography
kg	Kilogram (s)
L.A	Lead acetate
LDH	Lactate dehydrogenase
LPO	Lipid peroxidation
LDL-C	Low-density lipoprotein
LD50	Lethal dose

Abbreviation	Full Name
µg	Microgram (s)
µL	Miciolitter
MDA	Malondialdehyde
Mg	Milligram (s)
mg/dL	Milligram/decilitter
Min	Minutes
mL	Milliliter
Mmoles	Millimoles
MO	*Moringa oleifera*
MOL	*Moringa oleifera* leaves
MOLE	*Moringa oleifera* leaves extract
MOLM	*Moringa oleifera* leaves meal
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NBT	Nitro blue tetrazolium
Pb	Lead
QE	Quercetin
r.p.m	Round per minute
RE	Rutin
ROS	Reactive oxygen species
S.E.M.	Standard error of mean
Sec.	Second(s)
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamate pyruvate transaminase
SH	Sulfhydryl group
SLD	Standard laboratory diet
SOD	Super oxide dismutase
Spp	Species
TAG	Triacylglycerol
TC	Total cholesterol
TFC	Total flavonoid content
TG	Triglycerides
TL	Total lipids
TPC	Total phenolic content
U/L	Unit/littre
Vit C	Vitamin C
Vit E	Vitamin E
Viz.	Namely
WK	Week


Introduction

1. Introduction

The environmental pollution by heavy metals is a growing worldwide problem, especially in developing countries. Many studies indicate that the heavy metals act as catalysts in the oxidative reactions of biological macromolecules. Therefore, the toxicities with these metals might be due to oxidative tissue damage **(Flora *et al.*, 2009)**. Among those heavy metals, lead which is one of the well-known widespread metal poisons that occur naturally in the environment. The levels of lead in the environment are constantly increasing due to industrial activities, such as leaded gasoline, lead paints, leaded plumbing components, car repair, refining, smelting, battery manufacturing, fuel combustion, building construction, solder and ceramics manufacture for many years **(Karrari *et al.*, 2012)**. Many investigations have indicated even small quantities of lead are harmful to humans and other organisms because lead exposure could induce a wide range of biochemical and physiological dysfunctions in humans and animals include neurotoxicity, hepatotoxicity and nephropathy **(Soliman *et al.*, 2015)**.

Absorbed lead is conjugated in the liver and passed to the kidney, where a small quantity is excreted in urine and the rest accumulates in various body organs. Autopsy studies of lead-exposed humans indicated that the liver tissue is the largest repository (33%) of lead among soft tissues followed by kidney (**Mudipalli, 2007)**. Accumulated Pb not only enhances production of Reactive Oxygen Species (ROS) such as hydroxyl radical (HO), superoxide radical (O_2^-) or hydrogen peroxide (H_2O_2), but also inhibits the antioxidant defense system by inhibiting the activities of antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), glutathione reductase (GR) and glutathione S-transferase (GST) and depletion of the non-antioxidant defense system, such as reduced glutathione (GSH) **(Adikwu *et al.*, 2013)**.

Enhanced generation of ROS as well as depletion of intrinsic antioxidant defenses increase the susceptibility of tissues to free radical and result in a condition known as ‘‘oxidative stress’’ **(Ahamed and Siddiqui, 2007)**. Cells under oxidative stress display various dysfunctions due to lesions caused by ROS to lipids, proteins and DNA **(Ercal *et al.*, 2001)**.

Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to human and animals against infections and degenerative diseases. The administration of antioxidants reduced the uptake and accumulation of lead in tissues and diminished the lead-induced biochemical alterations in blood, kidney and liver in rats **(Flora *et al.*, 1984)**.

Current research is now directed towards natural antioxidants originated from plants due to safe therapeutics without side effects **(Sreelatha and Padma, 2009)**. Among myriad of natural plants, *Moringa oleifera* Lam (MO), which is one of the best known and most distributed species of Moringacae family and is an edible plant with wide variety of nutritional and medicinal benefits have been attributed to its roots, bark, leaves, flowers, fruits, and seeds **(Kumar *et al.*, 2010)**.

Phytochemical studies have shown that *Moringa oleifera* leaves (MOL) are particularly rich in potassium, calcium, phosphorous, iron, vitamins A and D, essential amino acids, as well as such known antioxidants such as β -carotene, vitamin C, and flavonoids **(Aslam *et al.*, 2005; Manguro and Lemmen, 2007)**. Therefore leaves act as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities **(Anwar *et al.*, 2007)**.

Aim of the work:

The present study aimed to investigate the possible hepatoprotective effect and antioxidant activity of aqueous extract of *Moringa olrifera* leaves (MOLE) against lead induced toxicity in male albino rats through determination of:

1. Growth performance parameters.
2. Serum liver enzymes (ALT, AST, ALP).
3. Serum total protein, albumin, globulin and A/G ratio.
4. Serum kidneys functions (BUN and creatinine).
5. Serum lipid profile (TC, LDL-C, HDL-C and TG).
6. Hepatic antioxidant enzymes (GSH level and SOD activity).
7. Hepatic DNA fragmentation test.
8. Histopathological examination on both liver and kidney.

Review of Literature

2. Review of Literature

2.1. Environmental pollution by heavy metal

The excessive amount of pollutants such as heavy metals in animal feed and feedstuffs is often due to human actions and their results from either agricultural, industrial production, through accidental or deliberate misuse **(Amal *et al.*, 2009; Aboul-Enein *et al.*, 2010; El-Beltagi *et al.*, 2010; Afify and El-Beltagi, 2011).** Heavy metals are defined in physiochemical terms as metals with a density at least 5 times as great as water **(John Neustadt and Pieczenik, 2011)**. By definition, this would account for 60 metals, several of which are biologically essential like iron, zinc, calcium, magnesium, vanadium and trivalent chromium that have bio-importance to man and their daily medicinal and dietary allowances had been recommended. Many others have bio-toxic effect such as lead, cadmium, arsenic, mercury, hexavalent chromium and essential metals at high dose as vanadium leading to production of reactive oxygen species, oxidative stress and tissue damage **(Ahamed and Siddiqui, 2007)**.

Pollution of the environment by heavy metals has received considerable attention in recent years because of anthropogenic activities in soils such as metal mining, smelting, irregular agricultural and horticultural practices (i.e., excess application of fertilizers, pesticides, over-irrigation), and waste disposal **(Fahr *et al.*, 2013)**.

2.2. Lead

Khan *et al.* (2008) reported that lead is a soft, grey blue heavy metal found ubiquitously is a common cause of poisoning of domestic animals throughout the world.

2.2.1. Occurrence, sources and distribution of lead

El-Sokkary *et al.* (2005) mentioned that lead has been widely used in anthropogenic activities such as leaded gasoline, lead paints, leaded plumbing components, car repair, refining, smelting, battery manufacturing, automobile manufacturing, fuel combustion, building construction, solder and ceramics for many years because of its higher density, softness, ductility, malleability and poorer electrical conductivity compared to other metals.

El-Beltagi and Mohamed (2010) stated that traces of lead occur in many rocks, and find their way into soil and water and hence into food, animals. Also they added that lead toxicity depends on its chemical form administrated to the animal, the route of administration, the frequency and duration of administration to animals.

(Rodriguez *et al.*, 2011) told that lead is considered one of the most toxic heavy metals involved in many fertilizers, which can be absorbed and accumulated in plants. From the soil, it easily enters the food chain and poses a threat to life. The downward movement of elemental and inorganic lead compounds from soil to the groundwater is very slow because of its slow mobility, and therefore, it is easily absorbed by plants and other organisms.

Romero-Freire *et al.* (2015) told that lead's half-life is quite long compared with other elements in nature (150– 5000 years) and can be retained in the environment for ever. Therefore, the effect of lead contamination in agricultural areas can be quite devastating and last for many years. Without remedial studies, high soil or water lead levels will never return to normal level.

2.2.2. Toxicity of lead

ATSDR (2007) reported that the toxicity of lead is closely related to age, sex, route of exposure, level of intake, solubility, metal oxidation state, retention percentage, duration of exposure, frequency of intake, absorption rate and mechanisms, and efficiency of excretion. Furthermore, as a result of the long persistence of lead in the environment, it is ranked second of all hazardous substances.

Ibrahim *et al.* (2013) mentioned that accumulation of lead produces damaging effects in the hematopoietic, hepatic, renal, and gastrointestinal systems. Furthermore, lead has been associated with various forms of cancer, nephrotoxicity, central nervous system effects, and cardiovascular diseases in human.

2.2.2.1. Mechanism of lead toxicity

Godwin (2001) reported that one of the suggested molecular mechanisms of lead toxicity is binding to sites in a variety of proteins, thereby competing with endogenous cations, principally calcium and zinc. Biophysical studies have revealed that lead tightly binds zinc and calcium sites and alters the structural conformation and biological activity of the corresponding proteins. This ability for lead to substitute divalent ions could alter many cellular functions. Furthermore, lead is similar in chemical structure to calcium and competes with it for absorption in the gastrointestinal tract and deposition in bone.

Hsu and Guo (2002) mentioned that the mechanism of lead-induced oxidative stress involved an imbalance between generation and removal of ROS

in tissues and cellular components causing damage to membranes, DNA and proteins. The presence of double bonds in the fatty acid on cell membrane weakens the C–H bonds on the carbon atom adjacent to the double bonds and makes H removal easier. Therefore, fatty acids containing zero to two double bonds are more resistant to oxidative stress than polyunsaturated fatty acids with more than two double bond.

Kosnett (2007) reported that absorbed lead following oral ingestion is absorbed by the gastrointestinal tract is directly bound with erythrocytes and distributed primarily to soft tissues such as liver.

Abdel-Moneim *et al.* (2011b) found that L.A induced an elevation in free radicals with a decrease in the endogenous antioxidant enzymes in liver which leads to disturbance of prooxidant and antioxidant balance.

Kim *et al.* (2016) stated that absorption of lead through the gastrointestinal tract is the major exposure pathway in children, only one hour after intestinal absorption, lead accumulates in all the organs of the body with maximum concentration per gram weight of tissue being recorded in liver and kidneys, which could be because these two organs are primarily responsible for its excretion.

2.2.3. Effect of lead on body weight, food intake and food conversion

Kang *et al.* (2004) found that administration of L.A to rats at three doses low dose (0.1% L.A), medium-dose (0.3% L.A) and high-dose (1.0% L.A) in drinking water for 8 weeks significantly reduced the body weights of the animals in the high-dose group when compared to control, low dose and medium dose groups. While no statistically significant differences were found between the body weights of the low-dose, medium-dose and control groups.

El-Nekeety *et al.* (2009) stated that that animals treated with L.A at a dose (20 ppm) alone for 30 days showed a significant decrease in feed intake and consequent reduction in the body weight gain.

Ponce-Canchihuamán *et al.* (2010) found that rats treated with (25 mg L.A /week, intraperitoneal (i.p) injection) for one month revealed a significant decrease in body weight and weight gain when compared to control group.

Seddik *et al.* (2010) mentioned that administration of (500 mg/L) L.A for 90 days reduced the body weight and weight gain of in L.A intoxicated rats significantly when compared to control rats.

Xia *et al.* (2010) stated that the weight gain and food intake of L.A exposed rats at a dose (20 mg/kg b.wt) for 21 days showed moderate decrease compared with the control group ($0.05 < P < 0.10$).

Moneim *et al.* (2011) said that rats received i.p injection of (20 mg/kg b.wt) L.A for 5 days revealed a significant reduction in the body weight when compared to control rats.

Ibrahim *et al.* (2012) reported that the final body weights of lead-intoxicated rats with L.A at three doses 1/20, 1/40, and 1/60 of the oral LD_{50}, each two days for 14 weeks were significantly lower than those of healthy controls. This harmful effect of lead on the body weight gain was elevated parallel with the increases of L.A doses. Furthermore, the food intakes of lead groups showed significant elevation in relation to control group, this indicates that neither the food intake nor the feed efficiency affected the rates of growth. Also, feed efficiency was decreased under the effect of L.A relative to the healthy controls which was concurred with the gain in body weight but not with food intake.

Liu *et al.* (2012b) reported that L.A exposed rats at (500 mg /L) as the only drinking fluid for 75 days caused retardation in weight gain by 10% and decrease in food and water intakes of rats.

Ramah *et al.* (2015) told that administration of (1.5 g/L) L.A daily for 8 weeks showed a significant reduction in body weight when compared to control group. These results may be due to impaired intestinal absorption of some essential trace elements.

El-Tantawy (2016) stated that in the lead treated rats with (100 mg/kg b.wt) daily for 4 weeks by gavage tube resulted in a significant reduction in the body weight (140± 2.9) as compared to their corresponding control animals after 4 weeks (175± 5), P< 0.05.

Badary (2017) mentioned that administration of (10 mg/kg b.wt) L.A through i.p injection for five consecutive days/week for 4 weeks revealed a significant decrease final body weight and a significant increase in relative liver weight in L.A intoxicated rats when compared to control rats. While, insignificant difference in absolute liver weight between L.A intoxicated rats and control rats was observed.

2.2.4. Effect of lead on liver functions

2.2.4.1. Effect of lead on liver enzymes

Shalan *et al.* (2005) reported that rats received (500 mg/kg diet) L.A daily in diet for 2, 4 and 6 weeks revealed a significant increase in serum ALT, AST and ALP levels.

Rahman and Sultana (2006) told that administration of (100 mg/kg b.wt, i.p injection) L.A for 5 days caused 237%, 273% and 147% elevation in the levels

of SGOT, SGPT and LDH, respectively, when compared with saline administered group.

(Abdou *et al.*, 2007) stated that 1/20 LD_{50} of L.A highly increased the activity of AST and ALT when compared to control group. These results proved that lead has a hepatotoxic effect.

El-Nekeety *et al.* (2009) reported that rats administered (20 ppm) L.A alone orally for 30 days induced a significant elevation in ALT and AST enzymes when compared to control rats.

Geraldine and Venkatesh (2009) stated that administration of (500 ppm) L.A in the drinking water for 10 months resulted in a significant increment in ALT and AST activities noted from 10 days and 20 days of lead exposure till the end of the exposure period.

Liu *et al.* (2010b) found that rats administered with aqueous solution of L.A at a concentration of (500 mg/L) as the only drinking fluid for 10 weeks, significantly ($P< 0.001$) increased the activities of ALT and AST by 285% and 117% as compared with vehicle controls, respectively.

Ponce-Canchihuamán *et al.* (2010) stated that rats treated with L.A (25 mg/week through i.p injection) for one month revealed a significant increase in ALT level. Further they reported that liver is considered one of the target organs affected by lead toxicity owing to its storage, biotransformation and detoxification in the liver after lead exposure.

Sharma *et al.* (2010) reported that administration of (40 mg/kg b.wt per day) lead nitrate for 40 days showed significant increase in serum ALT, AST and ALP activities in lead intoxicated rats when compared to control rats. They added

that lead alter the level of ALT activity in the blood by disrupting hepatocytes membrane. Consequently, there will be a discharge of the cell content into the blood stream.

Xia *et al.* (2010) told that rats treated with (20 mg/kg b.wt) L.A for 21 days reduced ALT and ALP activities significantly when compared to control group.

Hanan *et al.* (2012) found that rats treatment with (0.5 g L.A/100 ml distilled water) via drinking water for 2 months expressed significant increase in the plasma activity of liver enzymes ALT, AST and ALP and significant decrease in total protein indicating a damaged structural and functional hepatic integrity when compared to control rats.

Liu *et al.* (2012a) said that the activities of ALT and AST in lead treated rats with (500 mg /L in the drinking water) for 75 successive days, significantly increased by 281% and 120% as compared with vehicle controls, respectively.

Mehana *et al.* (2012) mentioned that rats received (0.4% L.A) orally for 8 weeks showed a significant increase liver enzymes, ALT, AST and ALP activities in comparison with control group.

El-Tantawy (2016) stated that administration of (100 mg/kg b.wt) L.A daily for 4 weeks by stomach tube resulted in a significant increase of ALT and AST activities when compared to the control group. These results showed that the exposure to lead affects hepatic tissue.

Badary (2017) reported that rats treated with (10 mg/kg b.wt) L.A intraperitoneally (i.p) for five consecutive days/week for 4 weeks revealed a significant increase in serum levels of hepatic enzymes AST and ALT with

disruption of liver structure in lead-exposed group when compared to control group.

2.2.4.2. Effect of lead on liver proteins

Shalan *et al.* (2005) demonstrated that rats received L.A in diet as (500 mg/kg diet) daily, for 2, 4 and 6 weeks revealed a significant decrease in hepatic total protein content in response to lead intoxication when compared to control rats. These results attributed to a decreased utilization of free amino acids for protein synthesis.

Murray *et al.* (2006) mentioned that ingestion of L.A induced change Protein profile of plasma. The results reported significant reduction in total soluble protein and albumin, while plasma globulin value was insignificantly changed. The reduction in plasma total soluble protein and albumin levels may be due to inhibition of protein biosynthesis through the specific enzymes in cell processes and low significant excretion of hormones (such as triiodothyronine T3 and T4) which regulated protein biosynthesis.

El-Nekeety *et al.* (2009) found that rats administered L.A at a dose (20 ppm) orally for 30 days induced a significant reduction in total proteins and albumin levels when compared to control rats.

Geraldine and Venkatesh (2009) stated that administration of (500 ppm) L.A in the drinking water for 10 months resulted in a significant reduction in total protein and albumin levels in lead intoxicated rats when compared to normal rats seen from 6 months ($p< 0.01$) till the end of the exposure period.

Sharma *et al.* (2010) stated that administration of (40 mg/kg b.wt) lead nitrate daily for 40 days to rats showed a significant decrease in total protein in lead intoxicated rats when compared to control rats.

Mehana *et al.* (2012) mentioned that rats received (0.4% L.A) orally for 8 weeks induced a significant decrease in plasma levels of total protein and albumin in comparison with control rats.

El-Tantawy (2016) stated that administration of (100 mg/kg b.wt) L.A daily for 4 weeks by stomach tube resulted in a decrease in total serum protein in lead treated group as compared to the control group.

2.2.4.3. Mechanism of hepatotoxicity induced by lead

El-Sokkary *et al.* (2005) said that the competition of lead with calcium, a mineral that is necessary for cell membrane integrity and stability, may also increase the hepatocyte membrane permeability and thus resulting in release of hepatocyte enzymes into the blood stream.

Ashry *et al.* (2010) reported that lead produced excess of free radicals which induced lipid peroxidation (LPO) of hepatocyte membrane. Cell membrane LPO leads to various membrane damage (fluidity loss, potential change, permeability increase and hepatocellular damage), which all cause leakage of liver enzymes into blood circulation and elevation of these enzymes.

2.2.5. Effect of lead on kidney functions

Garçon *et al.* (2007) reported that lead is a multitargeted toxicant that causes alterations in different organs of the body, including the kidney. The absorbed lead is conjugated in the liver and passed to the kidney, where a small

quantity is excreted in urine and the rest accumulates in various body organs and interferes with their function, especially the kidney.

El-Nekeety *et al.* (2009) mentioned that L.A administration at a dose at a dose (20 ppm) alone orally for 30 days showed a significant increase in serum blood urea nitrogen (BUN) levels in lead intoxicated rats when compared to control rats.

Liu *et al.* (2010a) mentioned that the serum levels of BUN, uric acid and creatinine that considered serum biochemical markers of renal damage were significantly increased in lead-intoxicated rats with (500 mg Pb/L in the drinking water) for 75 successive days by 55%, 103% and 111% when compared with vehicle controls, respectively ($P< 0.01$).

Abdel-Moneim *et al.* (2011a) observed that rats treated with (20 mg/kg b.wt) L.A for 5 days via i.p injection revealed a significant increase ($p< 0.05$) in the levels of uric acid, BUN and creatinine. These results indicate that lead had a nephrotoxicity effect.

Moneim *et al.* (2011) said that rats received i.p injection of (20 mg/kg b.wt) L.A for 5 days revealed a significant elevation in the levels of uric acid, BUN and creatinine by approximately 35.7%, 55.6% and 32.0%, respectively when compared to control group.

Liu *et al.* (2012b) mentioned that in lead-treated rats with 500 mg Pb/L as the only drinking fluid for 75 days, the levels of serum urea, creatinine and uric acid significantly increased by 40%, 63% and 69% as compared with those of the controls, respectively.

2.2.5.1. Mechanism of nephrotoxicity induced by lead

Liu *et al.* (2010a) stated that one possible molecular mechanism involved in lead nephrotoxicity is that lead induced over generation of ROS which resulted in the disruption of delicate oxidant/antioxidant balance, which can lead to kidney injury via oxidative damage.

2.2.6. Effect of lead on lipid profile

Shalan *et al.* (2005) concluded that treatment with (500 mg/kg diet) of L.A for 2, 4 and 6 weeks showed no significant changes in serum total lipid (TL), total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL-C) levels, however, serum low density lipoprotein (LDL-C) was decreased significantly.

El-Nekeety *et al.* (2009) found that rats received (20 ppm) L.A orally for 30 days expressed a significant decrease in serum levels of TG when compared to control rats.

Newairy and Abdou (2009) reported that TL, TC, TG and LDL-C were significantly increased after treatment with (200 mg/L) L.A daily for 3 weeks, while HDL-C levels were decreased in the sera and liver extracts when compared to control group.

Ponce-Canchihuamán *et al.* (2010) mentioned that in both plasma and liver, TC, triacylglycerols (TAG) and TL levels were higher in the group treated with (25 mg L.A each/weekly, through i.p injection) for one month compared to the control group ($p< 0.05$). The increased levels of lipids with increased levels of ALT may indicate liver dysfunction.

Sharma *et al.* (2010) told that rats treated with (40 mg/kg b.wt/ day) lead nitrate for 40 days showed significant increase in TC when compared to control rats.

2.2.6.1. Mechanism of the effect of lead on lipid profile

Ademuyiwa *et al.* (2009) stated that lead indeed perturbs the metabolism of lipids in different compartments of the organism. These perturbations were reflected as up-/down regulation of the concentrations of the major lipids (TC, TG, phospholipids and free fatty acids). Indirectly, the results also suggest that the activities of some of the enzymes in the lipid metabolism spectrum are either up- or down-regulated as a result of lead exposure.

Newairy and Abdou (2009) reported that the association between lead exposure and high serum lipid levels is biologically plausible and could be due to either increased synthesis or decreased removal of lipoproteins. Decreased removal may occur as a result of the alteration of cell surface receptors for lipoproteins or as a result of the inhibition of hepatic lipoprotein lipase activity. Furthermore, lead has been shown to depress the activity of cytochrome P- 450. This can limit the biosynthesis of bile acids, which is the only significant route for elimination of cholesterol from the body. Increased denovo cholesterol synthesis may be due to a lead induced increase in hepatic enzymes at important control points or it may be due to impaired feedback inhibition.

2.2.7. Effect of lead on antioxidant enzymes

Sivaprasad *et al.* (2004) stated that the activities of hepatic antioxidant enzymes in rats, including SOD, CAT, GPx and GSH were dramatically

decreased by treatment with (25 mg L.A/kg b.wt / day i.p) for 5 weeks. These results indicated that lead exposure induced the oxidative stress by inhibiting the activities of antioxidant enzymes. Also, GSH level reduction is owning to the high ability of lead to bind with the sulfhydryl (SH) group of GSH thereby interfering with the antioxidant activity.

Rahman and Sultana (2006) mentioned that administration of (100 mg L.A/kg b.wt, i.p) for 5 days caused 56% decrease in hepatic GSH , 62% decrease in GST , 67% decrease in GPx and 30% reduction in CAT in lead intoxicated rats when compared to control ones.

El-Nekeety *et al.* (2009) reported that rats treated with L.A at a dose (20 ppm) orally for 30 days resulted in a significant increase in hepatic malondialdehyde (MDA) in liver tissue accompanied with a significant decrease in GSH level.

Newairy and Abdou (2009) observed that treatment with L.A with (200 mg/L) in drinking water, daily for three weeks decreased the GSH concentration in the liver which may be one of the mechanisms of peroxidative action of lead in this organ. In addition, treatment with L.A also significantly ($p< 0.05$) decreased the activities of GPx, GST, and SOD in the liver extract, compared to the control group.

Seddik *et al.* (2010) said that the administration of (500 mg/L) L.A for 90 days in rats significantly reduced GSH level when compared to their corresponding control.

Xia *et al.* (2010) found that rats exposed to (20 mg/kg b.wt) L.A for 21 days significantly decreased GSH level in relation to control ones.

Abdel-Moneim *et al.* (2011b) told that i.p injection of (20 mg/kg b.wt) L.A for 5 days resulted in significant increase in serum and hepatic MDA as well as a significant decrease in GSH level, catalase and SOD, GST, and GPx activities when compared to control rats.

Liu *et al.* (2012a) found that in lead-treated rats with (500 mg/L in the drinking water) for 75 successive days, hepatic Cu/Zn-SOD, CAT and GPx activities were significantly($P< 0.01$) decreased by 31%, 46% and 42% when compared to vehicle controls, respectively.

Mehana *et al.* (2012) revealed that rats treated with (0.4%) L.A in for 8 weeks induce a significant decrease in the SOD and GST activities in tissue homogenates of liver and kidney when compared to control rats.

Ramah *et al.* (2015) told that rats received (1.5 g/L) L.A daily for 8 weeks showed a significant decrease in level of GSH and GST beside significant increase in level of MDA when compared to control rats.

El-Tantawy (2016) demonstrated that rats treated with (100 mg/kg b.wt) for 4 weeks by gavage tube revealed a significant increase in MDA level and a significant decrease in SOD activity and GSH levels when compared to control rats. The possible explanation could be related to the proposed role of GSH in the active excretion of lead through bile by binding to the thiol group of GSH and then being excreted. A decrease in GSH levels could induce oxidative stress and a consequent increase in MDA and decrease in SOD activity.

2.2.7.1. Mechanism of oxidative stress induced by lead

Ercal *et al.* (2001) reported that lead toxicity induced oxidative damage by two separate, although related, pathways: (1) the generation of ROS, including

hydroperoxides, singlet oxygen, and hydrogen peroxides, evaluated by MDA levels as the final products of lipid peroxidation, and (2) the direct depletion of antioxidant reserves.

Hsu and Guo (2002) told that lead can alter antioxidant activities by inhibiting functional SH groups in several enzymes such as SOD, CAT and GPx, because of its high affinity for sulfhydryl (SH) groups in these enzymes. GPx, CAT and SOD depend on various essential trace elements for proper molecular structure and activity, so these antioxidant enzymes are potential targets for lead toxicity. Moreover, if the balance between ROS production and antioxidant defenses is broken, the enzyme may be exhausted and its concentration depleted then oxidative stress occurred.

2.2.8. Effect of lead on DNA damage

Pagliara *et al.* (2003) isolated DNA from hepatocytes of control rats at 1, 3 and 5 days after (10 µg/110 g b. wt) lead nitrate injection and observed that the ladder-like pattern of apoptosis is detectable only in hepatocytes isolated at 5 days after lead nitrate injection.

Shalan *et al.* (2005) observed that administration of (500 mg L.A/ kg diet) daily for 2, 4 and 6 weeks decreased the basal DNA in liver of lead intoxicated rats and increased the intensity of apoptotic bands at 180, 360 and 540 base pair in liver of L.A group than control rats.

Xu *et al.* (2008) stated that treatment with (50 or 100 mg/kg b.wt) L.A for 4 weeks, caused DNA breakage in mouse lymphocytes , as well as induced elevated generation of ROS and MDA, which strongly suggested that oxidative stress might play an essential role in lead-induced DNA damage and the corresponding toxicity. The possible pathway could be due to lead induces

excessive production of ROS which attack the polyunsaturated fatty acid and cause lipid peroxidation. Subsequently, ROS and MDA attack DNA together or individually, leading to irreversible DNA damage.

Abdel-Moneim *et al.* (2011b) reported that i.p. injection of (20 mg/kg b.wt) L.A for 5 days markedly caused DNA fragmentation in the hepatic tissue of lead intoxicated rats with a longer tails appeared on the electrophoresis gel with 8% change in optical density compared to the control.

2.2.8.1. Mechanism of lead induced DNA damage

Xu *et al.* (2008) told that DNA damage can occur directly from ROS. Among the ROS, the highly reactive hydroxyl radical (OH) reacts with DNA by addition to double bonds of DNA bases and by abstraction of an H atom from the methyl group of thymine and each of the C–H bonds of 2-deoxyribose, leading to single- or double- strand breaks, purine, pyrimidine, or deoxyribose modifications and DNA cross- links.

García-Lestón *et al.* (2012) supposed that lead could change the secondary structure of DNA by covalently binding with the oxygen atom of nucleic acid or nitrogen atom of base pairs of DNA.

Kumar *et al.* (2013) suggested that lead can interfere with the process of base excision repair (BER) and nucleotide excision repair (NER) to inhibit DNA repair machinery.

Zhang *et al.* (2014) reported that the indirect mechanisms of DNA damage caused by lead have been studied through several aspects, lead can induce the production of ROS and then result in DNA strand breaks.

Dkhil *et al.* (2016) mentioned that lead itself is genotoxic or enhances the effectiveness of other DNA-damaging agents. Lead exposure, may stimulate the formation of ROS affecting free radical scavenging enzymes and glutathione.

2.2.9. Effect of lead on histopathological changes

2.2.9.1. Effect of lead on histopathological changes in liver

El-Nekeety *et al.* (2009) demonstrated that the histological results revealed that the liver section of the control group showed normal hepatocytes and central vein. While liver sections of rats treated with (20 ppm) L.A orally for one month showed dilated portal tract with massive aggregation of mononuclear inflammatory cells which scattered in different spaces, hepatocytes focal necrosis, fatty degeneration and internuclear inclusions and fragmented as well as pyknotic nuclei.

Xia *et al.* (2010) mentioned that rats treated with (20 mg/kg b.wt) L.A for 21 days showed aggregation of mononuclear cells which was scattered in different spaces. Hepatocytes focal necrosis, internuclear inclusions and fragmented nuclei were also observed.

Abdel-Moneim *et al.* (2011b) stated that hepatic tissue sections reveals that injection of rats with (20 mg L.A/kg b.wt) for 5 days caused a severe inflammatory response of the liver, as indicated by inflammatory cellular infiltration as well as cytoplasmic vacuolation and degeneration of hepatocytes. In addition, the hepatic sinusoids were dilated and apparently contained more Kupffer cells as compared to the control rat's livers.

Liu *et al.* (2012a) observed treatment with (500 mg Pb/L as the only drinking fluid) for 75 successive days caused visible histological changes

including structure damage hepatocellular necrosis, leukocyte infiltration in rat liver. Beside sinusoidal enlargement in lead treated rats. These changes were absent in livers of control rats.

Mehana *et al.* (2012) observed that intoxication with (0.4%) L.A alone for 8 weeks in male albino rats, showed cloudy swelling, vacuolar, hydropic and fatty changes (steatosis) as well as congestion, edema and hyperactivation of Kupffer cells. Some other specimens showed apoptotic necrotic changes, multifocal to diffuse, type of coagulative necrosis that finally replaced by lymphocytic cells infiltration, finally the portal tirade showed congestion, perivascular edema, mild fibrosis, biliary hyperplasia and apoptotic necrotic cells.

Narayana and Al-Bader (2011) reported that rats treated orally with lead nitrate at different dose (0.5% and 1%) for 60 days showed hepatic structure damage, hepatocellular necrosis, leukocyte infiltration and massive hemorrhage.

Badary (2017) found that hepatic tissue sections from lead-exposed rats with (10 mg/kg b.wt L.A i.p) for five consecutive days/week for 4 weeks showed chronic portal inflammation in the portal triads, periportal zones and hepatocytes (lobular inflammation); the cell infiltrates were mainly lymphocytes and plasma cells. The sinusoidal Kupffer cells became prominent and increased in number due to lead intoxication.

2.2.9.2. Effect of lead on histopathological changes in kidney

Liu *et al.* (2010a) observed that treatment with (500 mg /L in the drinking water) for 75 caused several visible histopathological changes. Renal sections in lead treated rats showed extensive tubular damage by presence of necrotic epithelial cells. Tubular degeneration, necrosis, cell swelling, mononuclear cell

infiltration, and degenerated organelles were also observed in the kidney following L.A treatment.

Xia *et al.* (2010) reported that rats treated with (20 mg/kg b.wt) L.A for 21 days showed tubular dilatation, vacuolar, hemorrhage, cellular debris and glomerulal hyper cellularity.

Abdel-Moneim *et al.* (2011a) found that treatment of rats with (500 mg /L in the drinking water) L.A induced marked alterations in renal tissues when compared to the non-treated control group. These changes were in the form of tubular dilatation, vacuolar and cloudy epithelial cells lining, interstitial inflammatory cells and appearance of some cellular debris. The renal tubules contained some apoptotic nuclei.

Moneim *et al.* (2011) stated that i.p. injection of (20 mg/kg b.wt) L.A for 5 days caused progressive glomerular and tubular alterations ,tubular vacuolization, necrosis and dilation as compared to kidneys of the control rats.

Liu *et al.* (2012b) told that treatment with (500 mg/L) as the only drinking fluid for 75 days caused several visible histopathological changes, including inflammatory cellular infiltrations, cytoplasmic vacuolation and dilatation of some kidney tubules, renal tubules with protein casts, swelling, vacuolization and proximal tubule necrosis. Moreover, the number of tubules with cellular necrosis from the renal cortices and outer medulla also increased in the lead treated rats as compared with vehicle controls.

2.3. *Moringa oleifera*

Figure (1): *Moringa oleifera* plant

2.3.1. Taxonomic classification of *Moringa oleifera* (Olson, 1999)

Kingdom: Plantae – Plants

Subkingdom: Tracheobionta – Vascular plants

Superdivisio: Spermatophyta – Seed plants

Division: Magnoliophyta – Flowering plants

Class: Magnoliopsida – Dicotyledons

Subclass: Dilleniidae

Order: Capparales

Family: Moringaceae – Horse-radish tree family

Genus: *Moringa* Adans. – Moringa

Species *Moringa oleifera* lam. – horseradish tree

2.3.2. Historical background of *Moringa oleifera*

Anwar *et al.* (2007) mentioned that MO is a medium- sized tree, growing in Asia, Africa and tropical areas of the world as a valuable food source. It provides a rich combination of nutrients, amino acids, antioxidants, anti-aging and anti-inflammatory compounds and is employed as medication for a variety of ailments particularly in South Asia and India. Since 1998, the World Health Organization has promoted MO as an alternative to imported food supplies to treat malnutrition. Additionally MO is used as a leafy vegetable with leaves that can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and without loss of nutritional value.

Khalafalla *et al.* (2010) reported that MO is one of the most widely distributed and it is highly valued plant in tropic and subtropic countries where it is mostly cultivated.

Farooq *et al.* (2012) said that MO, belongs to family Moringaceae, which bears 14 species among which MO is most commonly found. The plant is native to northern India. MO is a highly nutrient-rich plant with exceptional medicinal properties widely used to treat various health care problems

Berkovich *et al.* (2013) told that MO is a tree that grows widely in the tropics and subtropics of Asia and Africa. Its leaves have been traditionally consumed by Asian village people, but it is a relatively novel food material in the western world.

Leone *et al.* (2015) stated that MO, the native of the sub-Himalayan mountains of northern India; had been cultivated for a variety of purposes in the whole tropical and subtropical regions of the world.

2.3.3. Names and synonymes

Verma *et al.* (1976) stated that MO commonly called as 'Sahanjana' was one of the best known and most widely distributed species of Moringaceae family.

Johnson (2005) reported that MO which is native to South Asia and with high potential medicinal value and is sometimes described as "Mother's Best Friend", "Miracle Tree", "Tree of Life" and "God's Gift to Man".

Yassa and Tohamy (2014) declared that MO is cultivated in many tropical and subtropical countries of Asia and Africa. It is commonly known there as the drumstick tree or horseradish tree, while in the Nile valley, its name is 'Shagara al Rauwaq', which means "tree for purifying".

2.3.4. Chemical composition of *Moringa oleifera*

Verma *et al.* (1976) demonstrated that MO plant is native to northern India, that pods and leaves contained 2.5 and 6.7 g protein/100 g, respectively.

Ogbe and Affiku (2011) reported that MOL contain high crude protein (17.01%± 0.1) and carbohydrate (63.11%± 0.09). The leaves also contained appreciable amounts of crude fibre (7.09%± 0.11), ash (7.93%± 0.12), crude fat (2.11%± 0.11) and fatty acid (1.69%± 0.09).

El-Gammal *et al.* (2017) stated that dried MOL contained (24.47 ± 0.11) crude fibre, (22.86± 0.05) crude protein, (33.69± 0.12) carbohydrate, (8.54± 0.21) moisture and (7.72± 0.13) ash.

2.3.5. Phytochemistry of *Moringa oleifera*

Dahiru *et al.* (2006) reported that phytochemical screening of the aqueous *Moringa oleifera* leaf extract (MOLE) revealed the presence of alkaloids, glycosides, phenols, saponins, tannins, volatile oils and tannins.

Dasgupta and De (2007) told that aqueous MOLE had antioxidant activity which may be attributed to the presence of antioxidant compounds like vitamins C, E and β-carotene in concentrated form. The aqueous extract also showed good phenol content (184 µg/mg gallic acid equivalent) and flavonoids content (15.9 µg/mg catechin equivalent).

Lako *et al.* (2007) mentioned that MOL have been reported to have sufficient amount of major groups of phytochemicals contributing to the total antioxidant capacity, these phytochemicals include. Polyphenols, carotenoids and antioxidant vitamins such as vitamins C and E. MOL also used as green leafy vegetables of high total antioxidant capacity (260 mg/100 g).Leaves have also been found to be rich in polyphenols (250 mg/100 g), and β-carotene (34 mg/100 g) and flavonols such as quercetin (100 mg/100 g) kaempferol (34 mg/100 g)

Pari *et al.* (2007) stated that MOL contain various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids therefore, act as a good source of natural antioxidant.

Singh *et al.* (2009) reported that analysis of the aqueous extract of leaves, seeds and fruits of MO demonstrated that the aqueous extract of leaves had

comparatively higher total phenolics content (105.04 mg/g gallic acid equivalents (GAE)/g), total flavonoids content (31.28 mg quercetin equivalents (QE)/g), and ascorbic acid content (106.95 mg/100g) and showed better antioxidant activity (85.77%), anti-radical power (74.3) and reducing power (1.1 ascorbic acid equivalents (ASE)/ml) than did the fruit and seed extracts. Additionally leaves extract, seed extracts and fruit extracts contain gallic acid, chlorogenic acid, ellagic acid, ferulic acid, kaempferol, quercetin and vanillin.

Verma *et al.* (2009) found that antioxidant polyphenolics investigation for ethyl acetate/polyphenolic fraction of MO leaves done by HPL-C indicated the presence of phenolic acids (gallic, chlorogenic, ellagic and ferulic acid) and flavonoids (kaempferol, quercetin and rutin).

Amaglo *et al.* (2010) stated that the therapeutic effects of MOL have been attributed to the combined actions of various bioactive components found in the plant that include trace metal ions, especially potassium, calcium, phosphorous, zinc, manganese and iron, vitamins A, D, E and C, alkaloids such as moringinine, carotenoids such as β-Carotene and essential amino acids.

Atawodi *et al.* (2010) observed that the methanolic MOLE was found to contain chlorogenic acid, rutin, quercetin, glucoside, kaempferol and rhamnoglucoside.

Ramakrishnan and Venkataraman (2011) stated that MOL contain very high amount of total polyphenolics, quercetin and kaempferol along with β-carotene and lutein.

Mbikay (2012) reported that MO contains three structural classes of phytochemicals which have several medicinal benefits. They are glucosinolates such as glucomoringin, flavonoids such as quercetin and kaempferol and phenolic

acids such as chlorogenic acid. These phytochemicals have been reported to possess antioxidant, hypoglycemic, hypotensive, antidyslipidemic, anticancer, and anti- inflammatory properties.

Coppin *et al.* (2013) found that in the leaves of MOL, the amount of quercetin and kaempferol was found to be in the range of 0.07–1.26 and 0.05–0.67%, respectively.

Berkovich *et al.* (2013) stated that MOL contains several phytochemicals like flavonoid pigments, such as kaempferol, rhamnetin, isoquercitrin and kaempferitrin. In addition, these leaves are rich in a group of the glycoside compounds, glucosinolates and isothiocyanates as well as Beta- sitosterol.

Atsukwei *et al.* (2014) stated that phytochemical screening of the ethanol MOLE revealed the presence of alkaloids, tannins, carbohydrates and cardiac glycosides while saponins, steroids and anthraquinones were not detected.

Kamble (2014) reported that the phytochemical analysis of aqueous extract of MO seeds revealed the presence of sterols, alkaloids, proteins, reducing sugar, saponin, tannins, glycosides and flavonoids.

Yassa and Tohamy (2014) told that MOL have been reported to contain the major groups of phytochemicals that contribute to the total antioxidant capacity of plant foods. They include polyphenols, flavonols, carotenoids and antioxidant vitamins such as vitamins C and E.

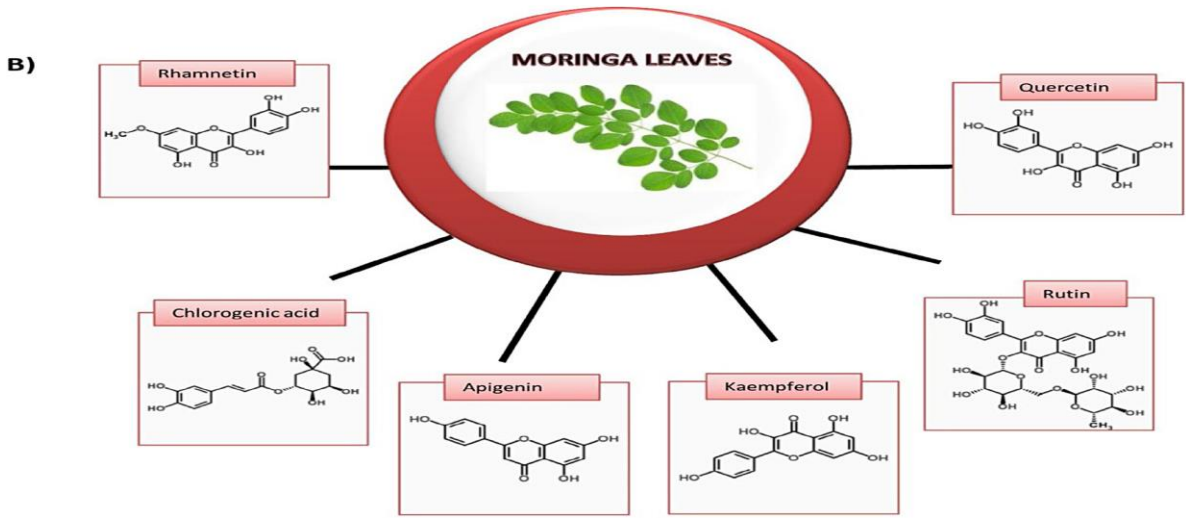
Karthivashan *et al.* (2015) reported that MOL have been enduringly used as a traditional medicinal source and employed for treatment of many diseases because it is enriched with many bioactive compounds such as kaempferol, rhamnetin, quercitin, chlorogenic acid, rutin and apigenin.

Figure (2): Major bioactive constituents of *Moringa oleifera* leaves, holding high therapeutic properties **(Karthivashan *et al.*, 2015)**.

2.3.6. Biological and therapeutic role of *Moringa oleifera*

Hartwell (1967) reported that the plant parts are used in folk remedies for tumors, abdominal discomfort, boils, cold, conjunctivitis, high blood pressure, hysteria, relapsing fever, skin diseases.

Mazumder *et al.* (1999) told that the leaves, as well as the flowers, roots, gums, fruits and seeds are extensively used for treating hematological, hepatic



and renal function, inflammation, cardiovascular action, and liver disease.

Ghasi *et al.* (2000) reported that MOL are traditionally known for or reported to have various biological activities, including hypocholesterolemic

agent, regulation of thyroid hormone status, anti-diabetic agent, gastric ulcers, anti-tumor agent and hypotensive agent.

Pari and Kumar (2002) declared that MOL had hepatoprotective effect against antitubercular drug such as isoniazid and rifampicin.

Ndong *et al.* (2007) stated that MO is beneficial for the prevention of hyperlipidemia and hepatocytic disorders caused by iron deficiency, and its seed extract has been shown to protect liver from necrotic injury and fibrosis in rat model.

Sulaiman *et al.* (2008) told that the MO also bears some bioactivities, like, anti-inflammatory, anti-asthmatic, antioxidant, hepatoprotective, antimicrobial, antiurolithiatic and antitumor effect.

Singh *et al.* (2009) mentioned that MO could protect DNA from oxidative damage in vivo and in vitro experimental models.

Patel *et al.* (2010) said that bark, sap, root, leaves, seed, oil and flower of MO are used as folk remedy for stomach complaints, catarrh, cancer, gastric ulcers, skin diseases, lowering blood sugar, moreover they increased bone density, nervous conditions, diabetes, fatigue, increased lactation. As well as they could treat hay fever, impotence, edema, cramps, hemorrhoids, headaches, sore gums. They are also used to improve sight, cause better brain development, enhance better functioning of the liver, the gall, digestive respiratory and immune system, and as blood cleanser and blood builder.

Biswas *et al.* (2012) mentioned MOL possessed antifungal, antimicrobial, antiatherosclerotic, antifertility, relieving pain, central nervous system

depressant, anti- inflammatory and diuretic activities, as well as regulating effect to hyperthyroidism.

Karthivashan *et al.* (2013) studied effect of MOL in animal models and found that they had various therapeutic properties such as antimicrobial, anti-inflammatory, anticancer and antidiabetic effects.

2.3.7. Effect of *Moringa oleifera* on body weight, food intake and food conversion

Awodele *et al.* (2012) observed that the food intake of rats treated with 3 doses (250, 500 & 1500 mg/kg b.wt) for 60 days was reduced compared with the control rats without subsequent reduction in body weight of animals. These results augmented that MO may serve as food supplements.

Atsukwei *et al.* (2014) demonstrated that normal rats treated with (300 and 600 mg/kg b.wt) ethanolic MOLE for 21 days did not significantly affect the body weight when compared with the control animals, so it "may" also be recommended in maintaining body weight.

Allam *et al.* (2016) reported that treatment of infected *E. coli* and *Salmonella spp* broiler chickens with (200 mg/kg b.wt) aqueous and ethanolic MOLE daily for 30 successive days in drinking water respectively induced significant increase in body weight gain and improved the feed conversion rate compared to infected broiler chickens.

Elabd *et al.* (2018) found that treatment of HFD mice with (200 mg/kg b.wt) aqueous MOLE for three months revealed a significant reduction in body weights and weight gain when compared to HFD mice.

2.3.8. Effect of *Moringa oleifera* on liver functions

2.3.8.1. Effect of *Moringa oleifera* on liver enzymes

Ghasi *et al.* (2000) observed that administration of crude MOLE (1 mg/g b.wt) to hypercholesterolemic male wister rats decreased hepatic enzymes when compared to hypercholesterolemic rats.

Pari and Kumar (2002) showed that methanolic MOLE at doses (150, 200 and 250 mg/kg b.wt) for 45 days has significantly restored the elevation of ALT and AST as well as ALP level in rats treated with antitubercular drugs such as isoniazid (INH), rifampicin (RMP), and pyrazinamide (PZA) to the normal level.

Nadro *et al.* (2006) demonstrated that (100 and 200 mg/kg b.wt) aqueous MOLE has prevented the release of hepatic enzymes from hepatocytes into the bloodstream when administered with high level of alcohol in rats. Therefore, the level of these enzymes in groups treated orally with (100 and 200 mg/kg b.wt) of aqueous MOLE significantly decreased when compared to rats received alcohol only.

Fakurazi *et al.* (2008) reported that treatment of male Sprague–Dawley rats with (200 and 800 mg/kg b.wt) aqueous MOLE for 14 days prior acetaminophen APAP administration has provided a significant protection to the liver, preventing the elevation of ALT, AST and ALP enzymes induced by APAP. These results indicated that MO possess hepatoprotective effect which may attributed to the fact that MO may preserve the structural integrity of hepatocytes when challenge with hepatotoxicants and subsequently preventing enzyme leakage into plasma.

Hamza (2010) said that oral co-administration of (1 g/kg b.wt) hydro-ehanolic extract of MO seeds to CCL4 intoxicated rats, twice weekly and for 8

weeks reduced liver damage as well as symptoms of liver fibrosis by reducing the serum activities of AST and ALT which were elevated by CCl4.

Liu *et al.* (2010b) found that oral administration of (10 mg/kg/day b.wt) quercetin to rats exposed to 500 mg L.A /L as the only drinking fluid for 10weeks decreased ($P< 0.001$) the elevated levels of ALT and AST activities significantly by 52% and 30% , respectively when compared to the lead-intoxicated rat.

Chattopadhyay *et al.* (2011) told that treatment of arsenic intoxicated rats with (500 mg/100 g b.wt/ day) aqueous MO seed extracts for 24 days significantly reduced the elevated ALT, AST, ALP enzymes when compared to arsenic intoxicated rats.

Awodele *et al.* (2012) declared that the oral administration of aqueous MOLE at three doses (250, 500 & 1500 mg/kg b.wt) for 60 days showed no significant differences in ALT, AST, ALP activities when compared with the control animals ,however, there were slight increase in the level of these enzymes without any significance at the higher doses.

Das *et al.* (2012) found that co-administration of (150 mg/kg b.wt) ethanolic MOLE to HFD rats significantly ($p\geq 0.05$) decreased the elevated ALT, AST, ALP levels when compared to HFD rats. These results indicated that MO had a hepatoprotective effects.

Ewuola *et al.* (2012) mentioned that normal rabbits fed with (5, 10 &15%) *Moringa oleifera* leaf meal (MOLM) based diets did not show any significant difference with control rabbits concerning ALT, AST & ALP activities.

Fakurazi *et al.* (2012) observed that administration (200 mg/kg or 400 mg/kg b.wt, via i.p injection) of hydroethanolic extracts of MO flowers and leaves

extract an hour after APAP administration significantly reduced the serum ALT level and extent of liver damage when compared to APAP intoxicated rats.

Sharifudin *et al.* (2013) revealed that administration of hydroethanolic extract of *Moringa oleifera* leaves and flowers at a dose (200 & 400 mg/kg b.wt by i.p injection) to APAP intoxicated rats reduced the elevated levels of ALT and AST induced by APAP significantly.

Singh *et al.* (2014) said that treatment with ethanolic MOLE at the three (dose levels; 100, 200 and 400 mg/kg b.wt/day), orally for 60 days simultaneously with CCl4 attenuated the increased ALT, AST and ALP enzyme activities produced by CCl4 with a subsequent recovery towards normalization of these enzymes.

Karthivashan *et al.* (2015) stated that treatment of mice with (100 and 200 mg/kg of b.wt) ethanolic MOLE extract simultaneously an hour after treatment with APAP significantly ($P< 0.05$) reduced the level of serum AST, and ALT compared to APAP group untreated with MO.

Toppo *et al.* (2015) mentioned that oral administration of (200 and 500 mg/kg b.wt) hydro- ethanolic MOLE to cadmium intoxicated rats daily for 28 days significantly ($P< 0.05$) reduced the serum biomarker levels of ALT, AST, and ALP which elevated by cadmium, but still significantly higher than control rats.

Mallya *et al.* (2017) reported that co-administration of MOLE to cadmium intoxicated rats showed a decrease in the levels of AST and ALT activities that were elevated in cadmium intoxicated rats.

Sadek *et al.* (2017) reported that co-administration of (500 mg/kg b.wt) ethanolic MOLE orally to diethyl nitrosamine (DEN) intoxicated rats for 16 weeks significantly decreased the elevation in ALT, AST and ALP activities induced by DEN treatment. However, there were no significant difference between ethanolic MOLE group only and control group in ALT, AST and ALP activities.

2.3.8.2. Effect of *Moringa oleifera* on liver preoteins

Ghasi *et al.* (2000) observed that administration of crude MOLE (1mg/g b.wt) to hypercholesterolemic male wister rats increased serum albumin and didn't alter serum total protein when compared with hypercholesterolemic rats.

Hamza (2010) said that oral administration of (1 g/kg b.wt) hydro-ehanolic extract of MO seed CCl4 intoxicated rats for 8 weeks and increased serum total protein, albumin and globulin which were decreased by CCl4.

Ewuola *et al.* (2012) mentioned that growing rabbits fed with (5, 10 & 15%) MOLM based diets did not show any significant difference with control rabbits in levels of serum total proteins, albumin & globulin.

Gupta *et al.* (2012) stated that treatment of normal rats with (150 or 300 mg⁄ kg b.wt) MO methanolic extracts of pods had no adverse effects and did not show any significant difference in total protein and albumin levels when compared to control rats. Though there were a significant increase in the level of total protein and albumin in diabetic rats treated with MO in comparison with diabetic rats only.

Allam *et al.* (2016) observed that treatment of infected *E. coli* and *Salmonella spp* broiler chickens with (200 mg/kg b.wt) of aqueous and ethanolic

MOLE daily for 30 successive days in drinking water respectively induced significant increase in total proteins, albumin, globulins.

Omodanisi *et al.* (2017) mentioned administration of (250 mg/kg b.wt) methanolic MOLE for 6 weeks to diabetic rats increased ($p< 0.05$) total protein and albumin significantly when compared to diabetic control .on other hand normal rats received methanolic MOLE only increased total protein and albumin concentrations in relation to control group and diabetic control.

Sadek *et al.* (2017) reported that co-administration of (500 mg/kg b.wt) ethanolic MOLE orally to rats intoxicated with DEN for 16 weeks relieved and deflected the decrease in the total protein and albumin induced by DEN.

2.3.9. Effect of *Moringa oleifera* on kidney functions

Karadi *et al.* (2006) said that administration of (200 mg/kg b.wt) aqueous and alcoholic MO root-wood extract for 28 days significantly lowered the elevated serum levels of creatinine, uric acid and BUN induced by ethylene glycol feeding.

Liu *et al.* (2010b) observed that the treatment of lead intoxicated rats with (10 mg/kg b.wt) quercetin (QE) daily for 10 weeks significantly inhibited the elevation of serum BUN, creatinine and uric acid. These results were may be due to QE, one of the flavonoids, is also a potential metal chelator.

Awodele *et al.* (2012) observed that the oral administration of aqueous MOLE to normal rats at three doses (250, 500 & 1500 mg/kg b.wt) for 60 days showed no significant differences in serum BUN and creatinine levels when compared with the control animals.

Liu *et al.* (2012b) mentioned that treatment of lead intoxicated rats with 25 and 50 mg/kg b.wt) QE for 75 days decreased the elevated levels of serum BUN, creatinine and uric acid significantly when compared to lead group. While normal rats administered QE alone showed no differences in levels of serum BUN, creatinine and uric acid when compared with control rats.

Sharifudin *et al.* (2013) revealed that administration of hydroethanolic extract of MO leaves and flowers at a dose (200 & 400 mg/kg b.wt by i.p injection) to rats treated with APAP did not extent any significant variations in serum creatinine and BUN levels.

Ouédraogo *et al.* (2013) stated that oral administration of aqueous-ethanolic MOLE at doses (150 and 300 mg/kg b.wt.) to gentamicin (GM) intoxicated rabbits for10 days significantly ($p< 0.01$) attenuated the increase of serum BUN and creatinine levels that was elevated by GM when compared to GM treated group. These results proved that MOLE restored the renal function after daily administration; suggesting that the contents of MO not only protected the integrity of kidney but, at the same time increased its regenerative and reparative capacity.

Maduka *et al.* (2014) found that oral treatment of APAP intoxicated male albino rats with aqueous MOLE at different doses (200 mg/kg b.wt and 400 mg/kg b.wt) significantly reduced BUN and creatinine levels that elevated by APAP toxicity.

Karthivashan *et al.* (2016) said that treatment of mice with varying doses of aqueous- ethanolic MOLE (100 mg/kg b.wt) and (200 mg/kg b.wt) an hour after treatment with APAP significantly reduced the elevated levels of serum creatinine and BUN induced by APAP.

Mallya *et al.* (2017) demonstrated that pretreatment cadmium exposed rats with MOLE in, presented a significant decrease in the levels of serum BUN and creatinine levels indicating anti- nephrotoxic potential. The reason of this decrease may be due to the leaves of this plant are a good source of phenolic compounds, β-carotene etc.

Omodanisi *et al.* (2017) mentioned administration of (250 mg/kg b.wt) methanolic MOLE for 6 weeks to diabetic rats showed insignificant difference in creatinine level when compared to diabetic control. Also normal rats treated with methanolic MOLE showed insignificant difference in creatinine level when compared with control group.

2.3.10. Effect of *Moringa oleifera* on lipid profile

Ghasi *et al.* (2000) found that observed that co- administration of aqueous crude MOLE (1 mg/g b.wt) to hypercholesterolemic male wister rats for 30 days reduced serum TC significantly.

Mehta *et al.* (2003) observed that administration of (200 mg/kg b.wt) MO fruits to rabbits fed with standard laboratory diet (SLD) or high cholesterol diet (HCD) for 120 days decreased the serum TC, TG, LDL-C, VLDL-C and TL when compared to the corresponding control groups. Furthermore, treatment of normal rabbits with MO fruits decreased the HDL-C levels as compared to the normal control group. However, treatment of HCD fed rabbits with (200 mg/kg b.wt/day) MO for 120 days showed a significant increase in HDL-C levels when compared to HCD rabbits.

Ara *et al.* (2008) found that co-administration of (200 mg/kg b.wt) ethanolic MOLE to rats administered (30 mg/155 g b.wt) adrenaline via i.p injection everyday morning for one week significantly decreased serum TG and

TC when compared to adrenaline group. These results revealed that MOLE has an ameliorate effect on lipid profile and hypolipidemic activity.

Chumark *et al.* (2008) reported that addition of (0.1 g/kg b.wt /day) water MOLE to HCD fed rabbits, for 12 weeks significantly ($P< 0.05$) lowered the elevated TC, TG, HDL-C and LDL-C levels when compared to compared to the HCD group.

Gunjal *et al.* (2010) stated that oral administration of (250 and 500 mg/kg b.wt) aqueous extract of MO barks to rats administered isoproterenol revealed improvement in lipid profile which proved by the significant decrease in serum levels of TC, TG, LDL-C and VLDL-C along with the significant increase in serum HDL-C levels in relation to isoproterenol group.

Jain *et al.* (2010) reported that treatment HFD fed animals with methanolic MOLE, at three different doses (150, 300 and 600 mg/kg b.wt) significantly lowered the serum TC, TG, VLDL-C, LDL-C, and increase the HDL-C when compared to the corresponding HFD fed group.

Chattopadhyay *et al.* (2011) observed that the co-administration of aqueous seed extract of MO at dose (500 mg/100 g b.wt/day) for 24 days to the arsenic intoxicated rats caused a significant decrease ($P< 0.001$) of elevated TC, TG and LDL-C ($P< 0.001$), along with increase of HDL-C levels.

Rajanandh *et al.* (2012) found that treatment of hyperlipidemic rats with 200 mg/kg hydroalcoholic extract of MO for 4 weeks significantly decreased TC, TG and LDL- C ($P< 0.001$), as well as increased HDL-C levels significantly when compared to untreated hyperlipidemic rats.

Chatterjee *et al.* (2013) said that pretreatment with MOLE in cadmium exposed rats presented a decrease in the TC level, TG, LDL-C and VLDL-C levels along with an increase in the HDL-C levels thereby having beneficial effect on lipid profile in cadmium exposed rats.

Oyedepo *et al.* (2013) found a significant reduction in TG, LDL- C and TC levels along with a significant increment in HDL-C level in the diabetic rats treated with (400 mg/kg b.wt) aqueous MOLE for 28 days when compared with the diabetic control rats. This hypolipidemic effect of MO could be related to its chemical composition, which showed the presence of alkaloids, flavonoids, saponin and cardiac glycosides. All these components are known to reduce serum lipid level in animals.

Atsukwei *et al.* (2014) found that administration of ethanolic MOLE at two doses (300 mg/kg b.wt and 600 mg/kg b.wt) to both female and male HFD rats for 14 days significantly ($p< 0.05$) decreased the serum TC levels in both male and female rats that received low (300 mg/kg body weight) and high (600 mg/kg b.wt) doses of the extract. While HDL-C level significantly ($p< 0.05$) increased in both male and female rats that were administered high dose (600 mg/kg b.wt) of the extract only. Furthermore, LDL-C reduced significantly ($p< 0.05$) in both male and female rats that were administered high dose (600 mg/kg b.wt) of the extract, but was not significantly altered in those that received low doses (300 mg/kg b.wt) of the extract.

Mansour *et al.* (2014), reported that, administration of (300 mg/kg b.wt) aqueous MOLE orally for 15 days 1h prior to gamma-irradiation resulted in significant recovery in TC, TG and LDL-C levels and LDH activity and increased HDL-C levels compared to irradiated groups. While administration of MO only

induced significant increase in serum LDL-C level and no significant change in other serum lipid profiles compared to the control group.

2.3.11. Effect of *Moringa oleifera* on antioxidant enzymes

Gupta *et al.* (2005) said that administration of (500 mg/kg b.wt) powdered seeds of MO once daily post arsenic exposure for 4 months improved the decrease in the GSH level and SOD activity induced by arsenic treatment.

Fakurazi *et al.* (2008) told that pretreatment of male Sprague–Dawley rats with (200 and 800 mg/kg b.wt) aqueous MOLE for 14 days prior to APAP significantly increased the level of GSH in the liver compared to group receiving APAP only. These results showed that MO has been found to restore the activity of GSH which was diminished following APAP.

Verma *et al.* (2009) observed that the co-treatment of CCl4 intoxicated Male Sprague–Dawley rats with (50 and 100 mg/kg b.wt/day, respectively) of ethyl acetate/polyphenolic fraction of MO leaves for 14 days effectively enhanced the decline of SOD, CAT activities and GSH level in liver and kidney. This is due to enhanced antioxidant status in the liver as well as in the kidney.

Liu *et al.* (2010b) found that treatment of lead intoxicated rats with 10 mg/kg b.wt QE daily for 10 weeks significantly increased GSH level and SOD activity when compared to lead intoxicated rats. The results showed that quercetin could renew the activities of these antioxidant enzymes in the liver of lead-treated rats.

Chattopadhyay *et al.* (2011) observed higher values of SOD activity close to control level after co-administration of (500 mg/100 g b.wt) of aqueous extract

of MO seeds for 24 days in MO+ arsenic treated group when compared to arsenic group only.

Awodele *et al.* (2012) reported that treatment of normal rats with aqueous MOLE at three doses (250, 500 & 1500 mg/kg b.wt) for 60 day orally showed non-significant difference in levels of SOD and GSH at low dose when compared to control rats.

Fakurazi *et al.* (2012) told that administration (200 mg/kg or 400 mg/kg b.wt via i.p injection) of MO flowers or MO leaves extracts an hour after APAP administration, respectively enhanced the reduction in the level of GSH and SOD activity of rats treated with APAP.

Mansour *et al.* (2014) said that administration of (300 mg/kg b.wt, orally) for 15 days aqueous MOLE 1h prior to gamma irradiated group resulted in resulted in a significant 61.4%, 165.5%, 99.6% and 306.5% increase in the activities of SOD, CAT and GSHPx and GSH content, respectively, compared to the irradiated group, and significant 21.7% decrease ($P< 0.01$) in MDA levels compared to the irradiated group ($P< 0.001$), However, treatment of normal healthy rats with aqueous (300 mg/kg b.wt, orally) MOLE alone for 15 days showed no change in all measured parameters compared to the control group.

Yassa and Tohamy (2014) reported that administration of (200 mg/kg b.wt) aqueous MOLE to diabetic rats increased GSH level significantly when compared to diabetic group. However, they found that there was no difference in GSH level between control rats and rats received aqueous MOLE only.

Toppo *et al.* (2015) mentioned that oral administration of 200 mg/kg hydro- ethanolic MOLE to rats along with oral administration of (200 ppm/kg b.wt) cadmium chloride daily for 28 days significantly increased SOD activity in

liver which reduced by cadmium chloride. However, it was still significantly lower than control rats.

Karthivashan *et al.* (2015) found that Administration of (200 mg/kg of b.wt) hydro ethanolic MOLE to APAP intoxicated rats decreased the elevated MDA levels and increased significantly the SOD, CAT and GPX activities induced by APAP significantly. These results due to the existence of flavonoids such as kaempferol, apigenin, quercetin, and multiflorin in the MO leaf extract, which are likely responsible for advancing antioxidant potential.

Allam *et al.* (2016) observed that treatment of infected *E. coli* and *Salmonella spp* broiler chickens with (200 mg/ kg b.wt) of Aqueous and ethanolic MOLE daily for 30 successive days in drinking water respectively resulted in significant increase in SOD beside significant decrease in MDA compared to infected ones.

Sadek *et al.* (2017) found that GSH level and SOD activity were increase significantly after co- administration of (500 mg/kg b.wt) ethanolic MOLE for 16 weeks to rats DEN intoxicated rats in comparison to DEN treated group only. In addition a significant increase in GSH level and SOD activity in the group received ethanolic MOLE only when compared with control group.

2.3.12. Effect of *Moringa Oleifera* on DNA damage

Singh *et al.* (2009) mentioned that there was some evidence from animal model research that MO protects cells from oxidative DNA damage because MO

had high phenol content, chelates iron and prevented the formation of catalysts to cause lipid peroxidation as well as oxidative DNA damage.

Chattopadhyay *et al.* (2011) reported that co-administration of an aqueous extract of MO seeds at a dose (500 mg/100 g b.wt/day) for 24 days to arsenic intoxicated rats showed that DNA was partially and significantly protected from fragmentation when compared to untreated arsenic intoxicated rats .

Sinha *et al.* (2011) reported that, administration of aqueous- ethanolic MOLE to mice at a dose (300 mg/kg of b.wt) for 15 consecutive days before exposing them to a single dose of γ-irradiation showed protection of oxidative DNA damage induced by irradiation. These results suggested that the inhibitory effect of MOLE on oxidative DNA damage could be attributed to their free radical scavenging activities.

Mansour *et al.* (2014) observed that control samples and aqueous MOLE sample of heart and lung tissues did not show any band indicating no DNA fragmentation, while treatment of irradiated group with aqueous MOLE (300 mg/kg, oral gavage) for 15 days showed a small release of DNA in heart and lung tissues compared to irradiated group only.

2.3.13. Effect of *Moringa oleifera* on histopathological changes

2.3.13.1. Effect of *Moringa oleifera* on histopathological changes in liver

Pari and Kumar (2002) observed that addition of methanolic MOLE at doses (150, 200 and 250 mg/kg b.wt) for 45 days also decreased dilation of hepatic sinusoids, portal inflammation, and fatty acid-induced microvesicular and macrovesicular changes and preserved the structural integrity of the hepatocellular membrane induced by antitubercular drugs.

Fakurazi *et al.* (2008) reported that centrilobular necrosis, fatty infiltration, lymphocytic, neutrophil infiltration as well as pathological hallmark such as bridging necrosis were not observed in the liver sections obtained from rats that were pretreated with aqueous extract of MOL (200 and 800 mg/kg b.wt) for 14 days prior to APAP administration.

Hamza (2010) said that administration of MO seed extract (1 g/kg b.wr) twice weekly and for 8 weeks has significantly improved histopathological scores of inflammation and necrosis in comparison with CCl4 livers. These results indicated that Moringa possessed hepatoprotective, anti-inflammatory and anti-fibrotic properties against CCl4 induced liver damage and fibrosis.

Liu *et al.* (2010b) found that co-administration of (10 mg/kg day b.wt) QE to lead intoxicated rats for 10 weeks significantly alleviated and improved the liver damage in lead-treated rats. Furthermore, there were no visible changes in the liver of the QE + L.A treated rats when compared to control rats. These results of histopathological evaluation showed that QE exhibited hepatoprotective effect against lead-induced liver injury.

Singh *et al.* (2014) observed that the degenerative changes observed in liver sections of ccl4 were shown to be minimal or absent after oral treatment with ethanolic MOLE at three different doses (100, 200 and 400 mg/kg b.wt/day) for 60 days. This might be due to lower fat accumulation and re-establishment of the antioxidant defense system in the liver tissue through the antioxidant and hepatoprotective nature of MOL.

Karthivashan *et al.* (2015) stated that the histological photographs of ethanolic MOLE extract treated mice liver at a dose of 100 mg/kg of b.wt showed reduction of necrotic area and declined infraction induced by APAP. However, at a higher dose of ethanolic MOLE extract (200 mg/kg of b.wt), MO preserves the

hepatocyte centrilobular architecture as same as the control group. These results indicatively represent the hepatoprotective role of MO leaves.

Sadek *et al.* (2017) mentioned that the morphology of the livers in the control group was typical hepatic architecture, and there were no noticeable, visible knobs. Shockingly, huge diminishments in liver broadening, knob events and normal knob numbers per knob-bearing liver were recognized in DEN intoxicated rats treated with (500 mg/kg b.wt) for 16 weeks compared with DEN treated rats. These histopathological appearances were extraordinarily enhanced in the livers of the rats that were treated with MOLEE.

2.3.13.2. Effect of *Moringa oleifera* on histopathological changes in kidney

Karadi *et al.* (2006) said that administration of (200 mg/kg b.wt) of aqueous and alcoholic root-wood extract for 28 days to ethylene glycol fed rats decreased renal damage and decreased glomerular filtration rate. The findings suggest that MO improves kidney function and decreases the incidence of kidney damage.

Liu *et al.* (2010b) reported that treatment of lead intoxicated rats with (10 mg/kg day b.wt) QE for 10 weeks significantly alleviated the tubulointerstitial and glomerular lesions damage induced by lead in rat kidney.

Ouédraogo *et al.* (2013) reported that kidney of GM intoxicated rabbits groups which treated with ethanolic MOLE at doses (150 and 300 mg/kg b wt) showed reparative tendencies than those of GM treated ones. These changes can be considered as an expression of the functional improvement of renal tubules, which might be caused by an accelerated regeneration of tubular cells.

Karthivashan *et al.* (2016) found that the histopathological analysis of kidney sections of APAPA mice treated with MO (100 mg/kg b.wt) showed some sparsely disorganized glomerulus, tubular dilation with moderate tubular casting and inflammation. However, treatment with aqueous-ethanolic MOLE at a dose (200 mg/kg b.wt) the glomerular and tubular architecture were well preserved. They showed negligible amount of granular casting in the renal tubules, similar to that observed in the control group.

Materials and Methods

3. Materials and Methods

3.1. Materials:

3.1.1. *Moringa oleifea* leaves

Fresh leaves of *Moringa oleifera* were collected, from the "farm of Faculty of Agricultural, Suez Canal University".

3.1.2. Chemicals

- Lead acetate trihydrate (Lead (II) acetate) (CH_3COO) 2Pb. $3H_2O$ Oxford lab Co., Mumbai 400002 (India) Container 500 g
- Chloroform: EL-Naser Pharmaceutical Chemicals Co., Cairo
- Concentrated sulphuric acid: EL-Naser Pharmaceutical Chemicals Co., Cairo
- Molish reagent: El-gomhoria, Cairo
- Ninhydrin solution : El-gomhoria, Cairo
- Concentrated nitric acid : EL-Naser Pharmaceutical Chemicals Co., Cairo
- Fehling reagent: El-gomhoria, Cairo
- Sodium hydroxide solution: El-gomhoria, Cairo
- Ferric chloride solution: El-gomhoria, Cairo
- Sodium bicarbonate: EL-Naser Pharmaceutical Chemicals Co., Cairo
- Acetic anhydride: EL-Naser Pharmaceutical Chemicals Co., Cairo
- Picric acid: El-gomhoria, Cairo
- Folin ciocalteu reagent: Oxoford Laboratory Co., India
- Gallic acid: Oxoford Laboratory Co., India
- Rutin: Rajasthaa Co., India
- EDTA: Bioshop Co., Canada
- Ethedium bromide: Bioshop Co., Canada
- Agarose: Bioshop Co., Canada

- Proteinase K: Roth Co., Germany
- Canada balsam: El-Nasr Co., Cairo
- Ethyl Alcohol: El-Nasr Co., Cairo
- Neutral formalin 10%: El-gomhoria, Cairo
- Hematoxylin and Eosin stain: El-gomhoria, Cairo
- Paraffine wax: El-Nasr Co., Cairo
- Xylene: El-Nasr Co., Cairo
- Citrate buffer pH 4.5.

3.1.3. Animals

Twenty four male albino rats weighting 150-190 g were purchased from animal house of Faculty of Veterinary Medicine (Suez Canal University). Throughout the period of experiment they were fed on standard rations supplying the essential vitamins, trace elements and water supply was given *ad-libitum*.

3.1.4. Basal diet composition

As outlined in the result section, the rations was analyzed for their dietary values composition by determining the various parameters. Rations was free of aflatoxin.

3.2 Methods:

3.2.1. Analytical measurement for dietary constituent:

3.2.1.1. Determination of crude protein content:

Crude protein in basal diet was determined as nitrogen content using micro-Kjldahl method according to **William (1980)**.

3.2.1.2. Determination of fat content:

The fat content in dried MOL was determined by ether extraction as described by **William (1980)**. Two grams of sample were extracted with anhydrous ether, using thimble apparatus, at a rate of 2.3 drops/sec for 16 hours. The extract was then dried for 30 minutes, at 100 °C and the weight of dried extract determined.

Fat content percentages were calculated by the formula:

$$= \frac{\text{Weight of dried ether extract}}{\text{Weigh of samples prior to extraction}} \times 100$$

Calculation of acid numbers in fat was according to **Koch (1953)**.

3.2.1.3. Determination of aflatoxin content:

The content of aflatoxin in samples was determined by ultraviolet absorption chromatography as described by **Nabney and Nesbitt (1965)**. Aliquots of finely ground homogenized samples were extracted in light petroleum for 6 hours in a soxhlet, allowed to dry and re-extracted with methanol for 6 hours. Chloroform solutions were concentrated under reduced pressure and made to 10 ml with chloroform in calibrated flask. A 0.2 ml portions of this solution were then applied to chromatoplates**,** and developed with diethyl ether until a solvent path. Length of 12 cm (from the base-line has been obtained. The plates were allowed to dry and aflatoxins bands identified under ultraviolet illumination.

3.2.1.4. Determination of crude fiber percentage:

The percentage of crude fiber in basal ration determined according to the method described by **Less (1975)**. Two grams of sample were treated with 200 ml

sulphuric acid and the mixture allowed for boiling for 30 minutes under constant stirring. Loss in volume was compensated by distilled water and after the indicated time, the mixture was filtered through a Whatman filter paper. The resulting residue was then treated with aliquots of 100 ml of hot distilled water and 2.5 N NaOH for 30 minutes at 105 °C. Dried residue was weighed and crude fiber contents was calculated as percentage.

3.2.1.5. Determination of ash and moisture contents:

Moisture (%) and ash were determined according to **William (1980)**.

3.2.1.6. Determination of carbohydrate content:

Carbohydrate determination by the difference we had as follow:

Total carbohydrate (%) =100-(% moisture + % protein + % ash + % fiber).

3.2.2. Analytical measurement for dried *Moringa oleifera* leaves constituent:

3.2.2.1. Determination of crude protein content:

Total nitrogen content was determined according to the method described by **Subba Rao (1990)** using micro- Kjldahl method. Crude protein content was calculated by multiplying total nitrogen percentage by 6.25.

3.2.2.2. Determination of fat content:

The fat content in ration was determined by extractor using soxhlet apparatus according to **William (1980)**. Calculation of acid numbers in fat was according to **Koch (1953)**.

3.2.2.3. Determination of ash contents

Ash content was determined according to the method described by **Subba Rao (1990)** using muffle furnace at 550 °C until constant weight.

3.2.2.4. Determination of carbohydrate content:

Carbohydrate determination by the difference we had as follow:

Total carbohydrate (%) =100-(% moisture + % protein + % ash + % fiber).

3.2.3. Preparation of *Moringa oleifera* leaves extract

Fresh MOL were washed under running water, air dried and then ground to powder and kept dry in an air-tight container prior to the extraction. The plant derived aqueous extract tested in this study was prepared in the laboratory by mixing 1 g dried and powdered leaves with 10 ml boiling water for 15 min. The mixture was then filtered twice through a 2 µm pore sterile filter paper into a sterile tube and left to cool. The aqueous extract stock solution (100 mg/ml) was kept for further study **(Berkovich *et al.*, 2013)**.

3.2.4. Qualitative phytochemical analysis of active compounds in aqueous MOLE

The phytochemical study was undertaken for determination of various active constituents of aqueous MOLE by the various tests described by **Rosenthaler and Ghosh (1930).**

3.2.4.1. Detection of sterols by Libermann Buchard reaction:

Ten milligrams residues of extract were taken in 2 ml of chloroform and sulphuric acid was added by the side of the test tube. The test tube was shaken for a few minutes, red colour development in the chloroform layer and greenish yellow fluorescence in the lower layer indicated the presence of sterols in the extract.

3.2.4.2. Detection of carbohydrates by Molish test:

2-3 drops of molish reagent added to 2 ml of plant extract in test tube. After mixing, a small amount of concentrated sulfuric acid is slowly added down the sides of sloping test-tube, without mixing. Development violet ring indicates the presence of carbohydrates.

3.2.4.3. Detection of amino acids by ninhydrin test:

One ml of 0.1 % solution of ninhydrin in alcohol, where added to the extract, development of violate or purple colour indicates the presence of amino acids.

3.2.4.4. Detection of proteins by xanthoprotein test:

Ten milligrams residue of extract was taken in 2 ml of water and to it 0.5 ml of concentrated nitric acid was added. The appearance of (white or yellow) precipitate indicated the presence of proteins.

3.2.4.5. Detection of reducing sugars by Fehling's reagent:

Two ml of extract was added to 0.5 ml of Fehling's reagent (Fehling's solution A and B mixed immediately before use) plus 2 ml of sodium hydroxide solution. The mixture was then heated on a water bath for 10 minutes. The appearance of reddish brown precipitate indicated the presence of reducing sugars.

3.2.4.6. Detection of tannins by ferric chloride test:

Five milligrams residue of the extract were taken into a beaker and 10 ml distilled water were added. This mixture boiled for 5 minutes then few drops of ferric chloride solution $FeCl_3$ were added to the mixture. Development of green colouration indicated the presence of tannins.

3.2.4.7. Detection of saponins by foam test:

Ten milligrams of MOLE residue were taken in a test tube with small amount of sodium bicarbonate and distilled water, they were shaken vigorously. Formation of froth indicated the presence of saponins.

3.2.4.8. Detection of triterpenoids:

Five milligrams of MOLE extract residue were dissolved in 2 ml chloroform and 1ml acetic anhydride then 1ml of concentrated sulfuric acid was added to them. Appearance of reddish violet colour indicated presence of triterpenoids.

3.2.4.9. Detection of flavonoids

Two drops of dilute sodium hydroxide was added to one milliliter of the crude stock of MOLE extract. Development of intense yellow color in the plant crude extract, which became colorless after the addition of a few drops of dilute acid indicates the presence of flavonoids.

3.2.4.10 Detection of alkaloids

Two milliliter of picric acid were added to 2 ml of MOLE extract. The formation of orange coloration indicated the presence of alkaloids.

3.2.5. Quantification of phenolic compounds and flavonoids

The total phenolic content of MOLE extracts was determined using Folin–Ciocalteu reagent **(Wong et al., 2006)**. About 0.5 ml of (1 mg/ml) plant extracts were mixed with 2.5 mL of 10% (v/v) Folin–Ciocalteu reagent and 2 ml of 7.5% (w/v) sodium carbonate. The mixture was allowed to stand for 30 min. Then the absorbance of the resulting blue colour was measured at 760 nm by a Shimadzu UV-VIS spectrophotometer. These data were used to estimate the phenolic contents using a standard curve obtained from various concentration 0.01, 0.02, 0.03, 0.04 and 0.05 mg/mL of gallic acid.

The flavonoids content were determined according to **Kumaran and Karunakaran (2007)** using rutin as a reference compound. One milliliter of plant extract in methanol (10 mg/ml) was mixed with 1ml aluminium trichloride in ethanol (20 mg/ml) and a drop of acetic acid, then diluted with ethanol to 25 ml. The absorption of this mixture was read after 40 min at 415 nm. Blank samples were prepared from 1 ml of plant extract and a drop of acetic acid, and then diluted to 25 ml with ethanol. The absorption of standard rutin solution (0.5 mg/ml) in ethanol was measured under the same conditions. The amount of flavonoids in plant extracts in rutin equivalents (RE) was calculated by the following formula:

$X= (A.m_0)/(A_0.m)$,

Where X is the flavonoid content, mg/g plant extract in RE

A is the absorption of plant extract solution.

A_0 is the absorption of standard rutin solution.

m_0 is the weight of rutin in the solution, mg.

m is the weight of plant extract.

3.2.6. HPLC analysis for aqueous extract of *Moringa oleifera* leaves

HPLC analysis was carried out using Agilent Technologies 1100 series liquid chromatograph equipped with an auto sampler and a diode-array detector. The analytical column was an Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 0.8 ml/min for a total run time of 70 min and the gradient programme was as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. The injection volume was 50 µl of MOLE peaks were monitored simultaneously at 280 and 320 nm for the benzoic acid and cinnamic acid derivatives, respectively. All samples were filtered through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI) before injection. Peaks were identified by congruent retention times and UV spectra and compared with those of the standards **(Kim *et al.*, 2006)**.

3.2.7. Animal care and management

Rats kept for 2 weeks for acclimatization at the laboratory animal house of Faculty of Veterinary Medicine, Suez Canal, University before the beginning of the experiment. They were housed in separate polyethylene cages under controlled environmental and nutritional conditions (25ºC and 55-60% relative humidity), where a day light rhythm was allowed. Throughout the period of experiment they were fed on standard rations supplying the essential vitamins, trace elements and other nutrients. Water supply was given *ad-libitum*.

3.2.8. Experimental Design

Rats were divided into 4 main equal groups each consisted of 6 rats, placed in individual cages (3 rat/cage) and classified as follows:

Group 1 (Control –ve group):

Served as control group and gavaged daily with 2 ml distilled water all over the experimental period (4 weeks).

Group 2 (MOLE group):

Administered aqueous MOLE orally at a dose of 200 mg/kg/ day by gavage tube for 4 weeks **(Dahiru *et al.*, 2006; Jaiswal *et al.*, 2009; Jaiswal *et al.*, 2013).**

Group 3 (lead group):

Administered lead acetate orally at a dose of 100 mg/kg/ day for daily 4 weeks **(Xu *et al.*, 2008; El-Tantawy, 2016)**.

Group 4 (MOLE + lead acetate):

Administered a mixture of aqueous MOLE extract at a dose of 200 mg/kg **(Dahiru *et al.*, 2006; Jaiswal *et al.*, 2009; Jaiswal *et al.*, 2013)** plus lead acetate at the dose of 100 mg/kg **(Xu *et al.*, 2008; El-Tantawy, 2016)** daily for 4 weeks orally by gavage tube.

Body weight, weight gain and feed conversion of all groups were recorded weekly. All these groups were sacrificed at the 28th day form the start of experiment.

3.2.9. Growth performance parameters:

3.2.9.1. Mean live body weight:

Each rat was weighed at the beginning of the experiment and at the end of every week of 4 weeks of the experiment.

3.2.9.2. Weight gain (g / week):

The body weight gains for each week were determined by subtracting the previous week's body weight from the current week's body weight. Body weight for each rat was recorded at the onset of the experiment.

Weight Gain = Final weight – Initial weight

3.2.9.3. Feed intake (g):

The amount of feed consumed weekly by each group was calculated by subtracting the remaining feed from the total allowed amount. The feed intake for each rat was obtained by dividing the amount of consumed food per cage by the number of rats per cage.

Feed Intake = Introduced parts of feed – Residual parts of feed

3.2.9.4. Feed efficiency:

The feed efficiency was determined from the average weight gained and average food consumed by the rats in each group.

$$\text{Feed efficiency} = \frac{\text{Average feed intake (g)/rat/week}}{\text{Average body weight gain (g)/rat / week}}$$

3.2.10. Sampling:

3.2.10.1. Serum samples

Blood samples were collected from overnight fasted rats of all groups (6 rats from each group) at the end of experimental period under effect of diethyl ether. Blood samples were collected from retro orbital capillary plexus in the medial canthus of eye using micro-hematocrite tubes. The blood samples were taken into a clean and dry screw capped centrifuge tubes and left to clot at room temperature, then centrifuged at 3000 r.p.m for 15 minutes to separate clear serum samples for determination of different biochemical parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, globulin, blood urea nitrogen (BUN), creatinine, triglycerides (TG),total cholesterol (TC),low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C).

3.2.10.2. Tissue specimens:

Organs were carefully examined by naked eye for detection of any abnormalities.

3.2.10.2.1. Hepatic tissue:

Rats were decapitated under the effect of diethyl ether anesthesia, and then the liver was dissected and excised then washed with normal saline and plotted by filter paper then weighed to determine the absolute liver weight and relative liver weight %.

Relative liver weight calculated by the following formula:

$$\text{Relative liver weight \%} = \frac{\text{Absolute liver weight of rat}}{\text{Final body weight of the same rat}} \times 100$$

The liver of each rat was divided into three parts, first part kept immediately at -80 °C for DNA fragmentation test, the second part kept at -80°C for determination of reduced glutathione level (GSH) and superoxide dismutase activity (SOD). Third part was immersed directly in 10% formalin for histopathological examination.

3.2.10.2.2. Kidney tissue:

The two kidneys were excised and weighed then immersed in10% neutral buffered formalin for histopathological examination.

3.2.11. Serum biochemical analysis:

3.2.11.1. Hepatic enzymes:

A. Determination of aspartate amino transferase (AST) (U/L)

Test principle according to **Lorentz (1998)**:

Aspartate amino transferase in the sample catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH) to form NAD^+. The rate of the NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance.

Calculation

Roche/Hitachi cobas c systems automatically calculate the analyte activity of each sample.

Conversion factor: U/L x 0.0167 = µkat/L

B. Determination of alanine amino transferase (ALT) (U/L)

Test principle was carried according to **Lorentz (1998)**, alanine amino transferase

catalyzes the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD^+. The rate of the NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance.

Calculation

Roche/Hitachi cobas c systems automatically calculate the analyte activity of each sample.

Conversion factor: U/L x 0.0167 = µkat/L

C. Determination of alkaline phosphatase (ALP) (U/L)

Test principle was according to **Tietz *et al.* (1983)**, colorimetric assay in accordance with a standardized method in the presence of magnesium and zinc ions, p-nitrophenyl phosphate is cleaved by phosphatases into phosphate and p-nitrophenol. The p-nitrophenol released is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance.

Calculation

Roche/Hitachi cobas c systems automatically calculate the analyte activity of each sample.

Conversion factor: U/L x 0.0167 = µkat/L

3.2.11.2. Estimation of serum proteins

A. Estimation of serum total proteins (g/dl)

Serum total proteins were estimated colorimetrically using Biodiagnostic kits, Egypt according to **Gornall *et al.* (1949)**.

Principle:

The method was based on a reaction between the alkaline copper sulfate and protein giving violet color. The intensity of color was directly proportional to the amount of protein which could be measured colorimetrically at 550 nm.

Calculation:

$$\text{Serum protien concentration (g/dl)} = \frac{\mathbf{A}_{sample}}{\mathbf{A}_{standard}} \text{ X } 5$$

B. Estimation of serum albumin (g/dl)

Serum albumin was estimated colorimetrically using Biodiagnostic kits, Egypt according to **Doumas *et al.* (1971).**

Principle:

The method was based on a reaction between albumin and bromcresol green at pH 4.2 forming green color. The intensity of color was directly proportional to the amount of albumin which could be measured colorimetrically at 630 nm.

Calculation:

$$\text{Albumin concentration (g/dl)} = \frac{\mathbf{A}_{sample}}{\mathbf{A}_{standard}} \text{ X } 4$$

C. Calculation of serum globulins calculation (g/dl)

Serum globulins were determined by substraction of the obtained albumin level from the level of total proteins as described by **Doumas *et al.* (1972).**

D. Calculation of albumin/globulin ratio (A/G ratio):

$$\text{A/G ratio} = \frac{\text{Albumin (g/dl)}}{\text{Globulins (g/dl)}}$$

3.2.11.3. Estimation of kidney functions

A. Blood urea nitrogen (BUN) (mg/dl)

The BUN was determined according to **Sampson *et al.* (1980).** Kinetic test with urease and glutamate dehydrogenase.

Urea is hydrolyzed by urease to form ammonium and carbonate

$$\text{Urea} + 2\ H_2O \xrightarrow{\text{Urease}} 2\ NH_4^+ + CO_3$$

In the second reaction 2-oxoglutarate reacts with ammonium in the presence of glutamate dehydrogenase (GLDH) and the coenzyme NADH to produce L-glutamate. In this reaction two moles of NADH were oxidized to NAD+ for each mole of urea hydrolyzed.

$$NH_4^+ + \text{2-oxoglutarate} + NADH \xrightarrow{GLDH} \text{L-glutamate} + NAD^+ + H_2O$$

The rate of decrease in the NADH concentration was directly proportional to the urea concentration in the specimen and is measured photometrically.

Calculation

Roche/Hitachi cobas c systems automatically calculate the analyte concentration of each sample.

B. Determination of serum creatinine (mg/dl)

Test principle was done according to **Fabiny and Ertingshausen (1971)**

This kinetic colorimetric assay is based on the Jaffé method. In alkaline solution, creatinine forms a yellow-orange complex with picrate. The rate of dye formation is proportional to the creatinine concentration in the specimen. The assay uses "rate-blanking" to minimize interference by bilirubin. To correct for non-specific reaction caused by serum/plasma pseudo-creatinine chromogens, including proteins and ketones, the results for serum or plasma were corrected by -26 µmol/L (-0.3 mg/dL).

$$\text{Creatinine} + \text{picric acid} \xrightarrow{\text{Alkalin pH}} \text{yellow- orange complex}$$

Calculation

Roche/Hitachi cobas c systems automatically calculate the analyte concentration of each sample.

Conversion factors: µmol/L x 0.0113 = mg/dL

3.2.11.4. Determination of lipid profile:

A. Assay for total cholesterol (TC) (mg/dl)

Serum level of total cholesterol was determined by enzymatic colorimetric

method, using (ELITech Diagnostic, France) kits (cat. NO .303113050) according to **(Richmond, 1973; Allain *et al.*, 1974).**

Principle:

The method was based on hydrolysis of cholesterol esters to cholesterol and free fatty acids by cholesterol esterase. H_2O_2 was formed in the subsequent enzymatic oxidation of cholesterol by oxidase, then it was reacted with phenol and aminoantipyrine giving red color (quinoneimine dye). The intensity of color was directly proportional to the amount of T.C which could be measured colorimetrically at 500 nm.

Calculation

Serum total cholesterol (mg/dl) = $\frac{\text{O.D.of sample}}{\text{O.D.of standard}}$ X conc. of standard (200 mg/dl)

B. Assay for serum triglycerides (TG) (mg/dl)

Serum triglycerides were determined colorimetrically using (ELITech Diagnostic, France) kits (cat. NO .303113050) according to **Fossati and Prencipe (1982).**

Principle:

- Triglycerides $\xrightarrow{\text{Lipase}}$ Glycerol + fatty acids
- Glycerol + ATP $\xrightarrow{\text{Glycerokinase}}$ Glycerol-3-phosphate + ADP
- Glycerol-3-phosphate $\xrightarrow{\text{Glycerol-3-phosphateoxidase}}$ Dihydroxyacetone phosphate + H_2O_2
- 2 H_2O_2+4-Chlorophenol+4-Aminoantipyrin $\xrightarrow{\text{Peroxidase}}$ Quinoeimine + H_2O_2 + HCl

Calculation

Serum triglycerides (mg/dl) = $\frac{\mathbf{A}_{\text{sample}}}{\mathbf{A}_{\text{standar}}}$ X 200

C. High density lipoprotein-choleasterol (HDL-C)

HDL-cholesterol was determined colorimetrically using commercial kits (Cat.NO 0599, Stanbio Laboratory,USA) according **(Burstein *et al.*, 1970; Lopes-Virella *et al.*, 1977)**

Principle:

The method was based on the precipitation of LDL-C and VLDL by phosphotungastic and magnesium ions. After centrifugation the HDL-C fraction remained in the supernatant and then measured by the same method of total cholesterol.

Calculation:

Serum HDL-C (mg /dl) = $\frac{\text{O.D.of sample}}{\text{O.D.of standard}}$ X conc. of standard (55 mg /dl)

D. Calculation of serum low density lipoprotein-choleasterol (LDLC)

LDL–C was calculated by formula of **Friedewald *et al.* (1972).**

Serum LDL–C (mg /dl) = T.C – HDL.C - $\frac{TG}{5}$

3.2.12. Determination of hepatic enzymatic and non-enzymatic antioxidants

The liver kept at -80 °C was subjected to homogenization in phosphate buffer saline solution in a rate 1:5 (w/v). Then centrifugation of the homogenate was performed and supernatants were collected for estimation of enzymatic and non-enzymatic antioxidants

3.2.12.1. Determination of hepatic enzymatic superoxide dismutase activity (SOD):

SOD was determined by superoxide dismutase (SOD) Colorimetric Activity Kit provided by Kamiya biomedical company (Cat. No. KT-745) according to **Peskin and Winterbourn (2000)**.

Principle:

The K-ASSAY Ò superoxide dismutase (SOD) Activity Kit is designed to quantitatively measure SOD activity in a variety of samples. The assay measures

all types of SOD activity, including Cu/Zn, Mn, and FeSOD types. A bovine erythrocyte SOD calibrator is provided to generate a calibrator curve for the assay and all samples should be read off of the calibrator curve. Samples were diluted in our specially colored sample diluent and added to the wells. The Substrate is added followed by xanthine oxidase reagent and incubated at room temperature for 20 minutes. The xanthine oxidase generates superoxide in the presence of oxygen, converted a colorless substrate in the detection reagent into a yellow colored product. The colored product was read at 450 nm.

Increasing levels of SOD in the samples caused a decrease in superoxide concentration and a reduction in yellow product. The activity of the SOD in the sample was calculated after making a suitable correction for any dilution, using software available with most plate readers. The results were expressed in terms of units of SOD activity per mL.

3.2.12.2. Determination of hepatic non-enzymatic reduced glutathione level (GSH):

GSH was determined by GSSG/GSH Quantification Kit provided by Kamiya Biomedical Company Cat. No. KT-768 according to **Beutler *et al.* (1963).**

Principle

The method based on the reduction of 5, 5` dithiobis-2-nitrobenzoic acid (DTNB) with glutathione (GSH) to produce a yellow compound. The reduced chromogen directly proportional to GSH concentration and its absorbance could be measured at 405 nm.

3.2.13. DNA fragmentation test

The test performed according to **Bortner *et al.* (1995)**

The liver samples were crushed with extraction buffer. Then 50 µl of proteinase-K solution (10 mg/ml) were added with invention of the tube for mixing.

- The tubes incubated at 55 ˚C for 12 hour to 3 days with occasional vigorous mixing. Then samples incubated with RNase (2 µl /ml) for 10 minut at room

temperature. Then tubes removed from incubator and 0.7-0.8 ml of phenol: chloroform: isoamyl alcohol 25:24:1 were added.

- Samples centrifuged at 12,000 rpm for 3-5 min, then 400-500 µl aqueous layer for each sample were carefully removed into new tube and 40-50 µl sodium acetate pH 5.3 were added to each tube followed by addition of 100% ethanol with invention to mix the tube. Then the precipitate DNA let to set at -20˚C overnight.
- Centrifugation was done at 12,000 rpm for 20 min and the supernatant was removed then 50 µl of tries EDTA buffer was added for overnight for complete dissolving.
- Samples were run on electrophoresis using 1.2% agarose gel stained with ethidium bromide at 50 volt. Finally samples were analyzed using image analyses software

3.2.14. Histopathological Examination

Formalin fixed liver and kidneys were dehydrated in ethyl alcohol gradients, cleared in xylol then embedded and casted in paraffin. Thin paraffin sections of 5 micron thickness were stained with hematoxylin and eosin stain according to **Bancroft *et al.* (1990)**.

3.2.15. Statistical analysis

The present studies were analyzed using Statistical Package for Social Sciences (SPSS) version 23 (SPSS Inc., Chicago). Results were expressed as Mean ± S.E (n = 5). One way ANOVA followed by Duncan test were used for analysis. The level of statistical significance was taken as $P< 0.05$.


Results

4. Results

The present study was carried out to investigate the effect of aqueous MOLE in normal and lead acetate intoxicated male albino rats. During this study, the following results were obtained:

4.1. Analytical measurement for dietary constituent

Analysis showed that diet contained 14.6% crude protein, 3.6% crude fiber, 2.18% moisture, 10.7% ash, 7.2% acid number of fat and 61.09% carbohydrates, whereas the diet was free of aflatoxins as showed in **Table (1)**.

Table (1): percentage composition of ration in experiment (g/100g)

Constituents	**%**
Crude proteins	14.6
Crude fiber	3.6
Moisture	2.18
Ash	10.7
Aflatoxins	None
Acid number of fat	7.2
Carbohydrate	61.09

4.2. Chemical composition of dried *Moringa oleifera* leaves

Analysis showed that dried MOL contained 6.72 g crude fiber, 23 g crude protein, 1.98 g fat, 6.7 g moisture, 16 g ash and 45.6 g carbohydrate as recorded in **Table (2)**.

Table (2): Chemical composition of *Moringa oleifera* leaves (g/100 g)

Constituents	**g/100 g**
Crude proteins	23
Crude fiber	6.72
Moisture	6.7
Ash	16
Fat	7.2
Carbohydrate	45.6

4.3. Qualitative phytochemical screening of active compounds in aqueous MOLE

Our results showed that the qualitative phytochemical analysis of aqueous MOLE revealed the presence of sterols, alkaloids, amino acids, proteins, flavonoids, reducing sugars, glycosides, saponins, tannins and carbohydrates **Table (3)**.

Table (3): Qualitative phytochemical screening of aqueous MOLE

No.	Active principle	Test Applied	Observation	Result
1	Sterols	Libermann Buchard Reaction	Development of red greenish yellow fluorescence in lower layer	Present
2	Carbohydrates	Molish test	Development violet ring	Present
3	Amino acids	Ninhydrin test	Development of violet colour.	Present
4	Proteins	Xanthoprotein test	yellow precipitate was formed	Present
5	Reducing Sugars	Fehling's reagent	Appearance of reddish brown precipitate	Present
6	Saponins	Foam test	Formation of froath	Present
7	Tannins	Ferric chloride test	Occurrence of green colour	Present
8	Triterpenoids	Test for triterpenoids	Formation of reddish violet colour	Present
9	Flavonoids	Test for flavonoids	Development of red or pink colour	Present
10	Alkaloids	Hager's reagent	Development of orange colour	Present

4.4. The amount of phenolic and flavonoids compounds in aqueous MOLE

Total phenolics in the aqueous MOLE was determined using the Folin–Ciocalteu assay, calculated from regression equation of calibration curve ($y = 5.57x + 0.1929$; $r^2 = 0.6341$) and is expressed as gallic acid equivalents (GAE) as shown below in **Figure (1)**. Finally the amount of phenolics in plant extracts in gallic equivalents (GAE) was calculated by the following formula:

C (GAE) = (c×v)/m.

Where C is the concentration determined from calibration curve mg/mL, v is the volume of plant extract during the assay (mL) and (m) is the mass of plant extract during the assay (g).

Current results showed that the total phenolic content in aqueous MOLE was 128.64 mg/g plant extract (in GAE).

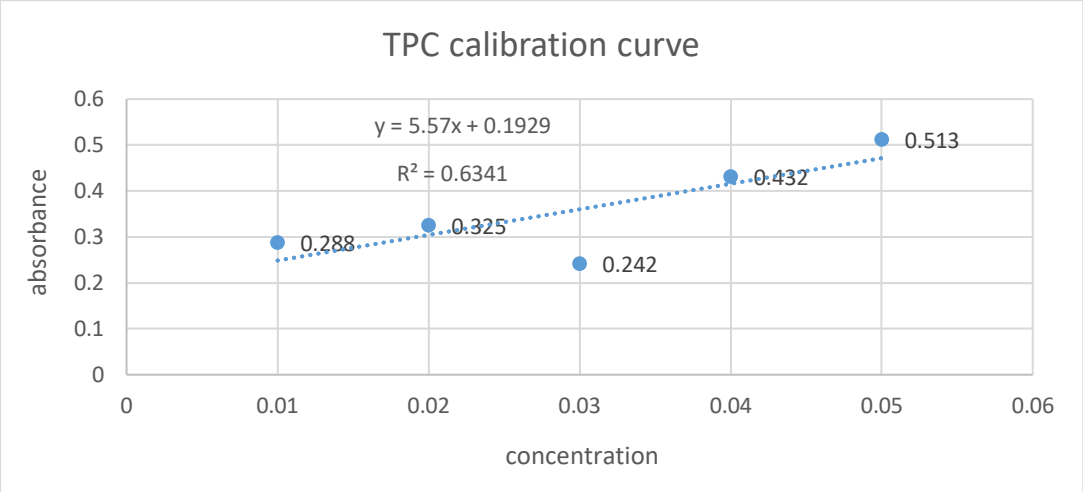


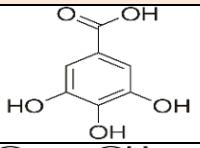
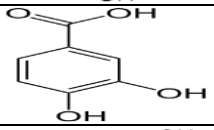
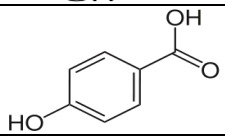
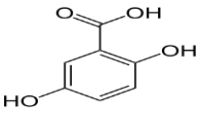
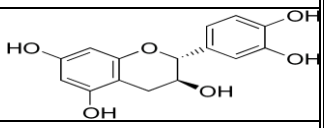
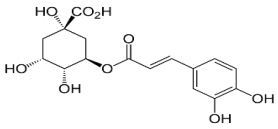
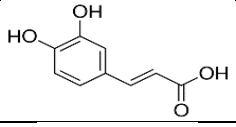
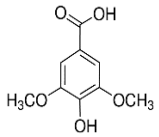
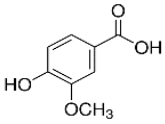
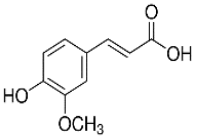
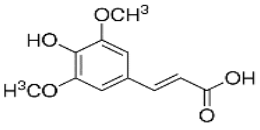
Figure (1): Calibration curve of different concentration of gallic

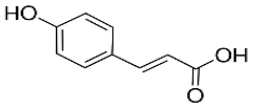
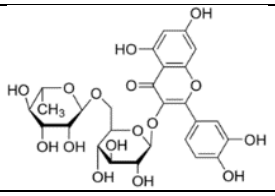
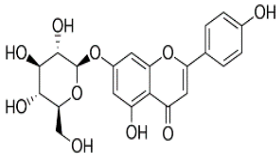
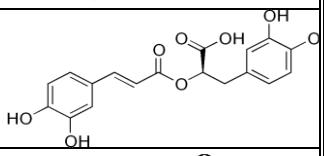
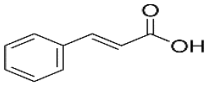
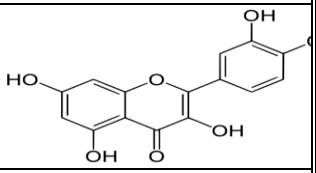
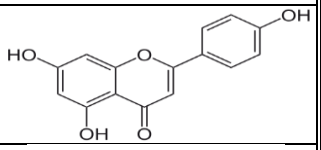
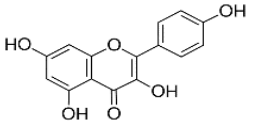
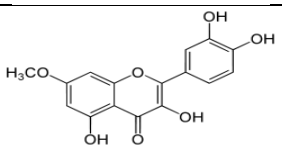
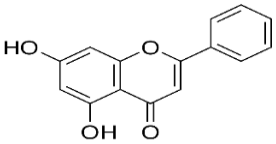
Our results showed that the total flavonoid content in aqueous MOLE was 28.39 mg/g plant extract (in RE).

4.5. HPLC analysis of aqueous extract of *Moringa oleifera* leaves

HPLC analysis showed that aqueous MOLE contained gallic acid, protocatechuic, p-hydroxy benzoic acid, gentisic acid, cateachin, chlorogenic acid, caffeic acid, syringic acid, vanillic acid, ferulic acid, sinapic acid, *p*-coumaric acid, rutin, apigenin-7-glucoside, rosmarinic acid, cinnamic acid, qurecetin, apigenin, kaempferol, rhamnetin and chrysin. The concentration and the retention time of these components were recorded in **Table (4)**.

Table (4): HPLC analysis of aqueous extract of *Moringa oleifera* leaves

No.	Compound	Ret. Time	Concentration (µg/g)	Structure
1	Gallic acid	3.9	10.481	
2	Protocatechuic acid	7.7	0.516	
3	p-hydroxy benzoic acid	12.1	0.313	
4	Gentisic acid	12	0.920	
5	Cateachin	15.2	2.317	
6	Chlorogenic acid	16.5	4.260	
7	Caffeic acid	17.2	20.172	
8	Syringic acid	19.3	1.743	
9	Vanillic acid	21.2	2.458	
10	Ferulic acid	28.9	1.461	
11	Sinapic acid	30.7	1.880	

No.	Compound	Ret. Time	Concentration (µg/g)	Structure
12	*p*-coumaric acid	35	24.533	
13	Rutin	34.1	39.293	
14	Apigenin-7-glucoside	38	26.474	
15	Rosmarinic acid	38.9	5.948	
16	Cinnamic acid	46.2	0.234	
17	Qurecetin	48.6	39.921	
18	Apigenin	48.156	0.995	
19	Kaempferol	54.9	6.968	
20	Rhamnetin	55.6	14.221	
21	Chrysin	59	0.229	

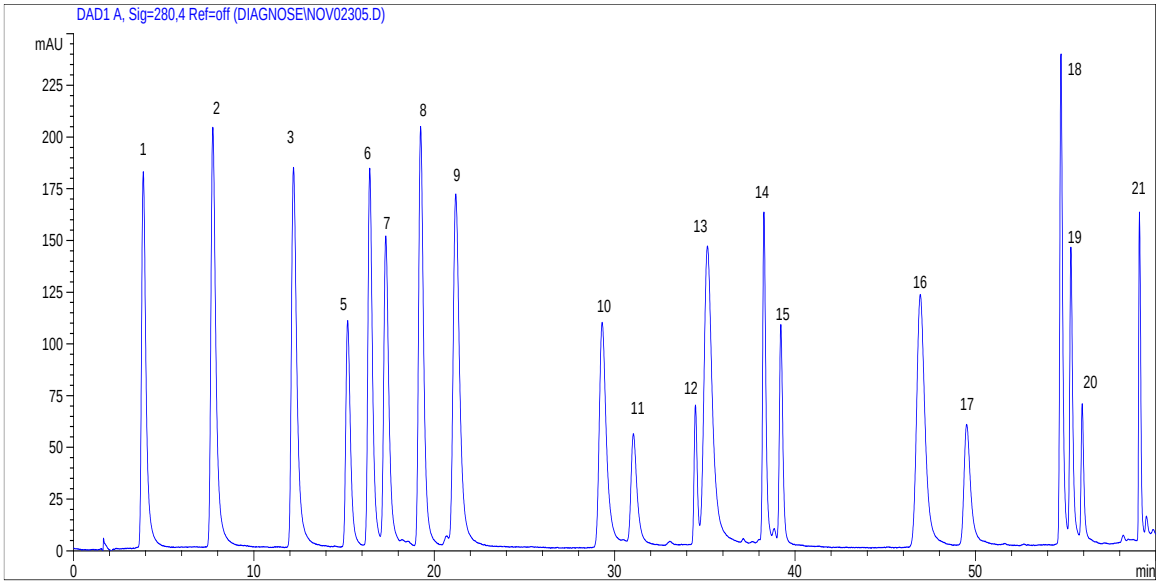


Figure (2): showing the HPLC chromatogram of standards

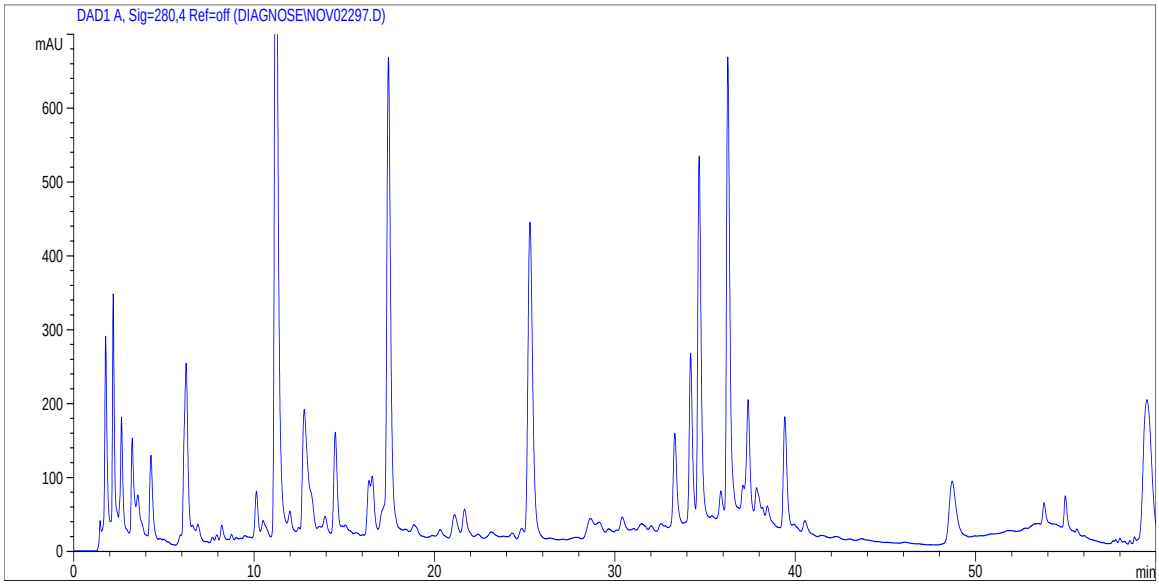


Figure (3): showing the HPLC chromatogram of *Moringa oleifera* leaves extract

4.6. Effect of MOLE on body weight of L.A intoxicated rats

Our results showed that initial body weights of all experimental groups weren't significantly altered at the beginning of the experiment. At the 1st week and 2nd week of the experimental period the body weights of all experiment groups weren't differ statistically.

At the 3rd and 4th week L.A group revealed a significant decrease in body weight in relation to control group. While treatment of lead intoxicated rats with aqueous MOLE increased body weight significantly (P< 0.05) than lead intoxicated groups to a level comparable to control. However, normal rats administered aqueous MOLE didn't express any significant change when compared with control rats **Table (5) and Figure (4)**.

Table (5): Effect of MOLE on body weight of normal and L.A intoxicated rats

Weeks / Groups	initial bwt	1st week bwt	2nd week bwt	3rd week bwt	4th week bwt
Control	152.67± 8.51 [a]	180.67± 8.63 [a]	208.67± 9.47 [a]	236.17± 9.01 [a]	250.67± 8.87 [a]
MOLE	156.67± 7.49 [a]	182.67± 7.64 [a]	206.17± 7.91 [a]	231.00± 6.39 [a]	246.50± 7.33 [a]
Lead acetate	150.66± 5.54 [a]	173.17± 6.59 [a]	187.67± 7.51 [a]	198.00± 5.83 [b]	217.83± 6.0 [b]
MOLE+ L.A	152.50± 10.42 [a]	177.67± 11.18 [a]	197.17± 12.05 [a]	225.00± 9.18 [a]	242.00± 9.34 [a]

Values are means ± SE, means carrying different superscripts (a, b) within the same column are considered significant at (P< 0.05).

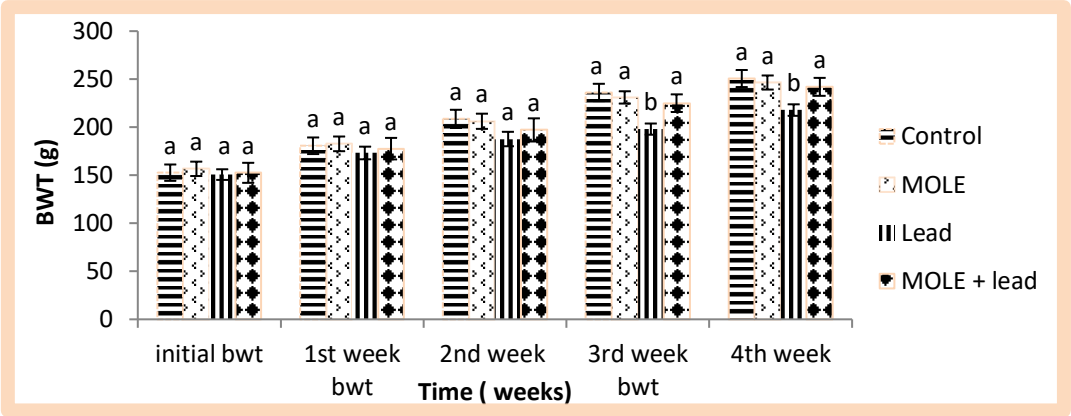


Figure (4): Effect of MOLE on body weight of normal & L.A intoxicated rats

4.7. Effect of MOLE on body weight gain of L.A intoxicated rats

At end of the 1st and 2nd weeks there were no significant changes in weight gain in all treated groups. At end of the 3rd and 4th weeks, the L.A intoxicated rats revealed a significant ($P< 0.05$) decrease in weight gain when compared to the control group. Treatment of lead intoxicated rats with aqueous MOLE increased weight gain significantly ($P< 0.05$) when compared to the L.A group to a level comparable to control group. MOLE treated rats didn't express any significant changes when compared with the control group **Table (6) and Figure (5)**.

Table (6): Effect of MOLE on body weight gain of normal and L.A intoxicated rats

Weeks / Groups	1st week bwt gain	2nd week bwt gain	3rd week bwt gain	4th week bwt gain
Control	28.00±0.68 [a]	28.00± 1.36 [a]	27.83±1.47 [a]	21.83 ± 1.87 [a]
MOLE	26.00±0.57 [a]	26.50±1.18 [a]	22.33±1.76 [a]	19.50 ± 0.764 [a]
Lead acetate	25.67±1.56 [a]	25.67±.66 [a]	15.83±1.35 [b]	14.33 ±1.67 [b]
MOLE+ L.A	25.67±.843 [a]	24.67±1.28 [a]	23.00±2.35 [a]	20.00 ± 2.25 [a]

Values are means ± SE, means carrying different superscripts (a, b) within the same column are considered significant at ($P< 0.05$).

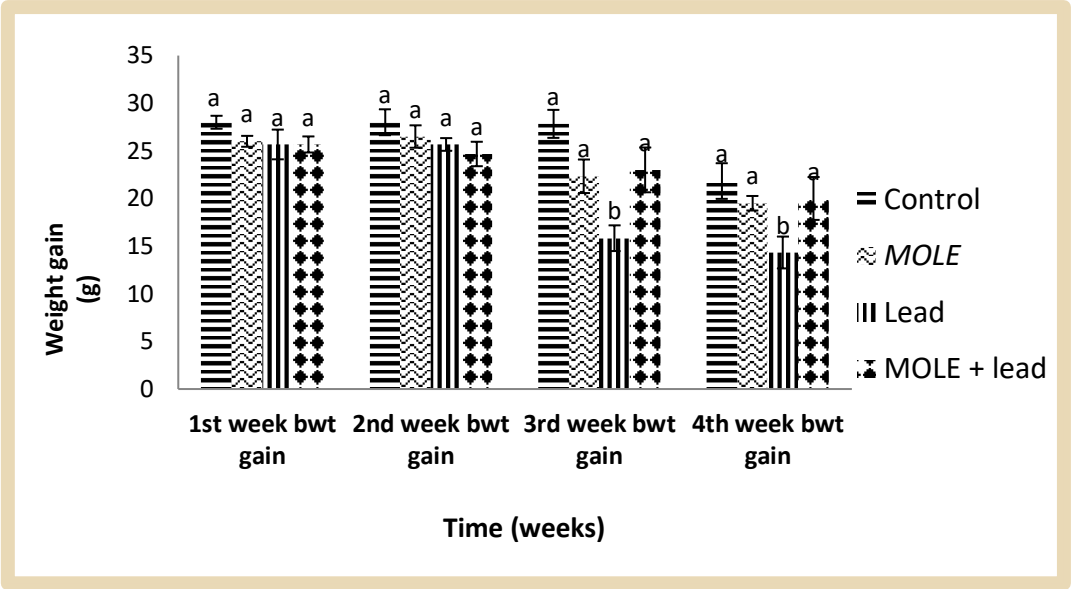


Figure (5): Effect of MOLE on body weight gain of normal and L.A intoxicated rats

4.8. Effect of MOLE on feed intake and feed efficiency of L.A intoxicated rats

Current results showed that L.A group had a significant (P< 0.05) increase in feed intake compared to control group. Treatment of L.A intoxicated rats with MOLE significantly (P< 0.05) decreased feed intake than L.A, however it didn't reach the control group. Rats treated with MOLE only expressed a significant (P< 0.05) decrement in feed intake in comparison to control rats **Table (7) and Figure (6)**.

Regarding feed efficiency, our results revealed that L.A group significantly (P< 0.05) decreased feed efficiency in comparison to control group. Whereas treatment of lead intoxicated rats with MOLE produced a significant (P< 0.05) elevation in feed efficiency when compared to L.A group, although it was significantly (P< 0.05) lower than control group. MOLE treated group showed no significant difference with control group **Table (7) and Figure (7)**.

Table (7): Effect of MOLE on feed intake and feed efficiency of normal and L.A intoxicated rats

Parameters / Groups	Feed intake	Feed efficiency (%)
Control	881.7± 0.09^{b}	12.11± 0.19^{a}
MOLE	849.2± 0.12^{d}	11.11± 0.25^{a,b}
Lead acetate	950± 0.08^{a}	7.70± 0.44^{c}
MOLE+L.A	861± 0.09^{c}	10.83± 0.45^{b}

Values are means ± SE, means carrying different superscripts (a, b, c, d) within the same column are considered significant at (P< 0.05).

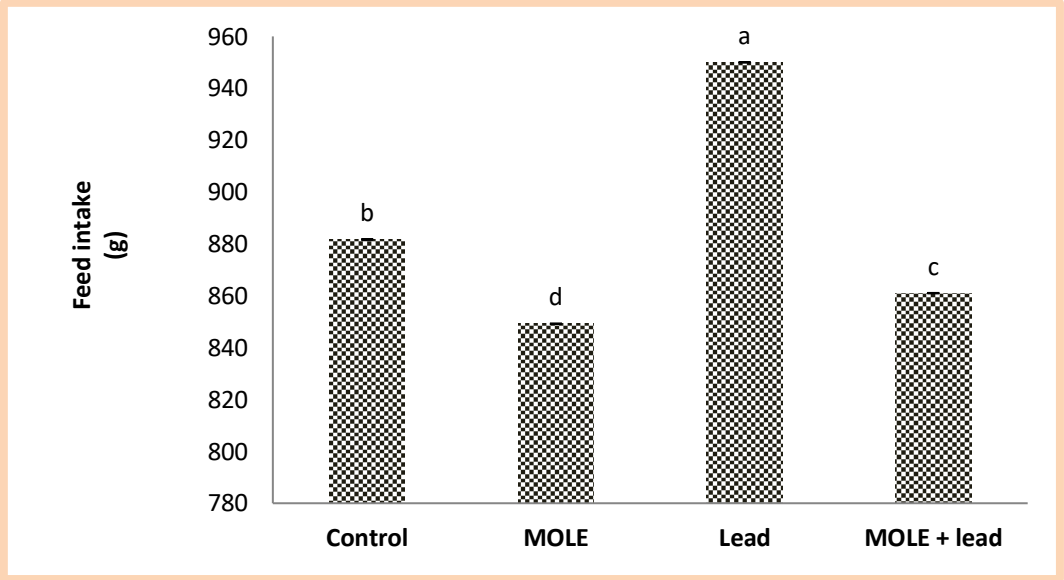


Figure (6): Effect of MOLE on feed intake of normal and L.A intoxicated rats

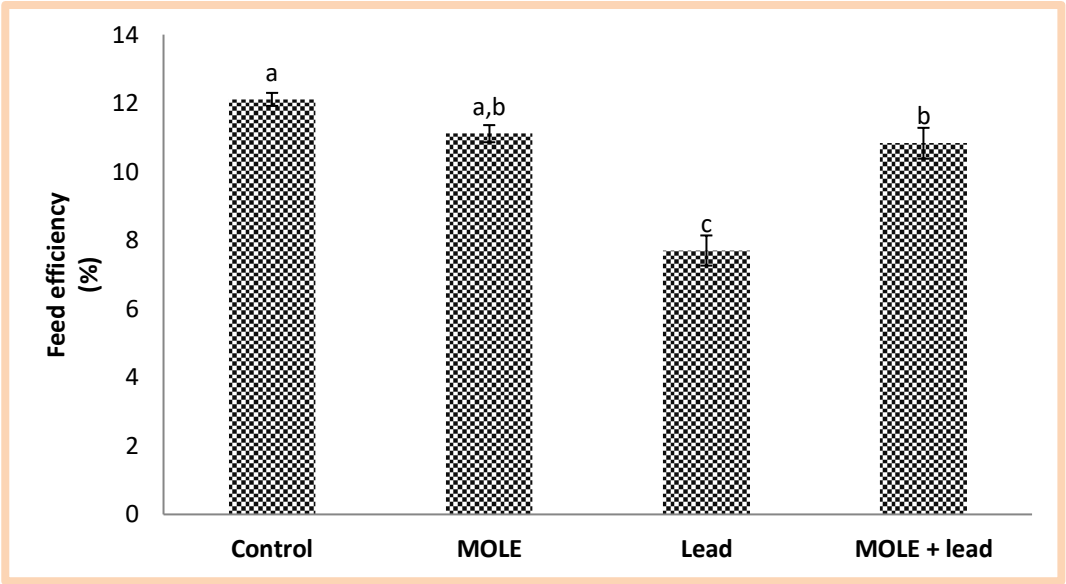


Figure (7): Effect of MOLE on feed efficiency of normal and L.A intoxicated rat

4.9. Effect of MOLE on absolute livers weight and relative liver weight of normal and L.A intoxicated rats

Current results showed that there were no significant difference in absolute liver weight between the four groups **Table (8) and Figure (8)**.

Regarding to relative liver weights, our results showed that there was a significant ($P< 0.05$) increase in the relative liver weight of L.A treated group when compared to control group. However, treatment of L.A group with MOLE showed slight numerical decrease in relative liver weight when compared to L.A and control groups. MOLE treated rats revealed non-significant alternation in relative liver weight when compared with control rats as shown in **Table (8) and Figure (8)**.

Table (8): Effect of MOLE on liver weights and relative liver weights of normal and L.A intoxicated rats

Parameters / Groups	Absolute liver weight	Relative liver weight (%)
Control	7.86± 0.44[a]	3.06± 0.09[b]
MOLE	7.49± 0.27[a]	2.99± 0.12[b]
Lead acetate	7.92± 0.37[a]	3.49± 0.08[a]
MOLE+ L.A	7.96± 0.17[a]	3.25± 0.09[a,b]

Values are means ± SE, means carrying different superscripts (a, b, c, d) within the same column are considered significant at (P< 0.05)

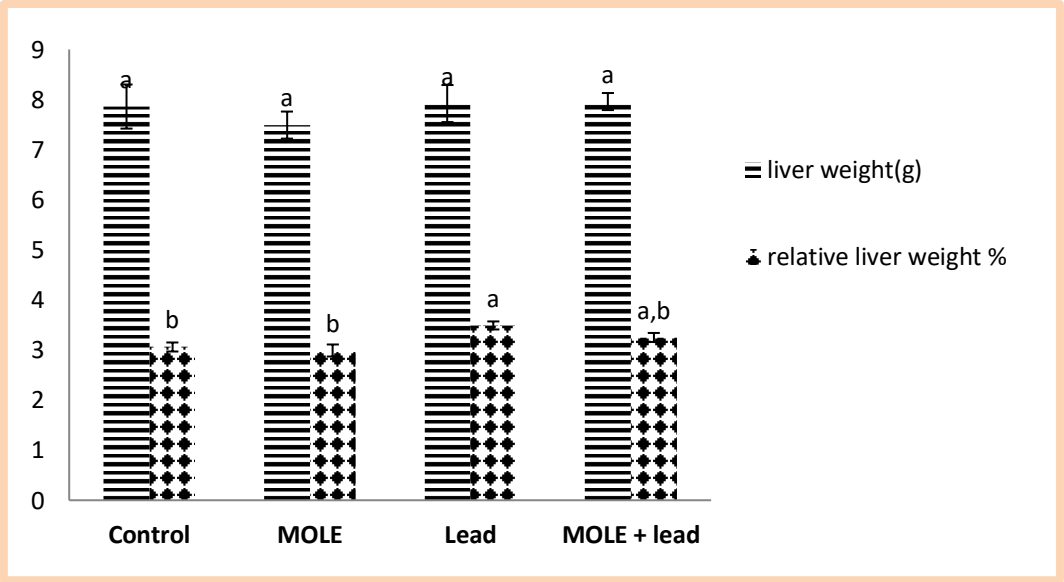


Figure (8): Effect of MOLE on liver weights and relative liver weights % of normal and L.A intoxicated rats

4.10. Biochemical parameters

4.10.1. Effect of MOLE on serum liver enzymes activity in normal and L.A intoxicated rats

4.10.1.1. AST (U/L)

Current study showed that L.A treated group significantly (P< 0.05) increased AST values when compared to control group, Treatment of lead intoxicated rats with MOLE expressed a significant (P< 0.05) decrease in AST values in relation to the L.A group but showed significant (P< 0.05) elevated values when compared to control group. Normal rats treated with MOLE showed insignificant changes in AST values in compare with the control **Table (9) Figure (9)**.

4.10.1.2. ALT (U/L)

Current study showed that L.A treated group significantly (P< 0.05) increased ALT value when compared to control group. Treatment of lead intoxicated rats with MOLE expressed a significant (P< 0.05) decrease in ALT in comparison to the L.A group, however it was significantly (P< 0.05) elevated in comparison to the control group. Rats treated with MOLE showed insignificant changes in ALT values in comparison with the control group **Table (9) Figure (9)**.

4.10.1.3. ALP (U/L)

The L.A treated group revealed a significant (P< 0.05) increase in ALP values when compared to control group. Treatment of lead intoxicated rats with MOLE expressed a significant (P< 0.05) decrease in ALP values in comparison to L.A group to a level comparable to control group. Rats treated with MOLE showed insignificant changes in ALP values when compared with the control as showed in **Table (9) Figure (9)**.

Table (9): Effect of MOLE on serum liver enzymes activity in normal and L.A intoxicated rats

parameters / Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
Control	49.00± 1.22 ^{c}	149.80± 5.41^{c}	94.00± 3.36 ^{b}
MOLE	55.60± 1.50^{c}	147.6± 12.50^{c}	94.60± 5.42 ^{b}
Lead acetate	82.40± 3.69^{a}	238.4± 4.30 ^{a}	181.40± 5.48 ^{a}
MOLE+ L.A	66.20± 2.08^{b}	192.2± 2.52 ^{b}	105.60± 4.20 ^{b}

Values are means ± SE, means carrying different superscripts (a, b, c) within the same column are considered significant at (P< 0.05).

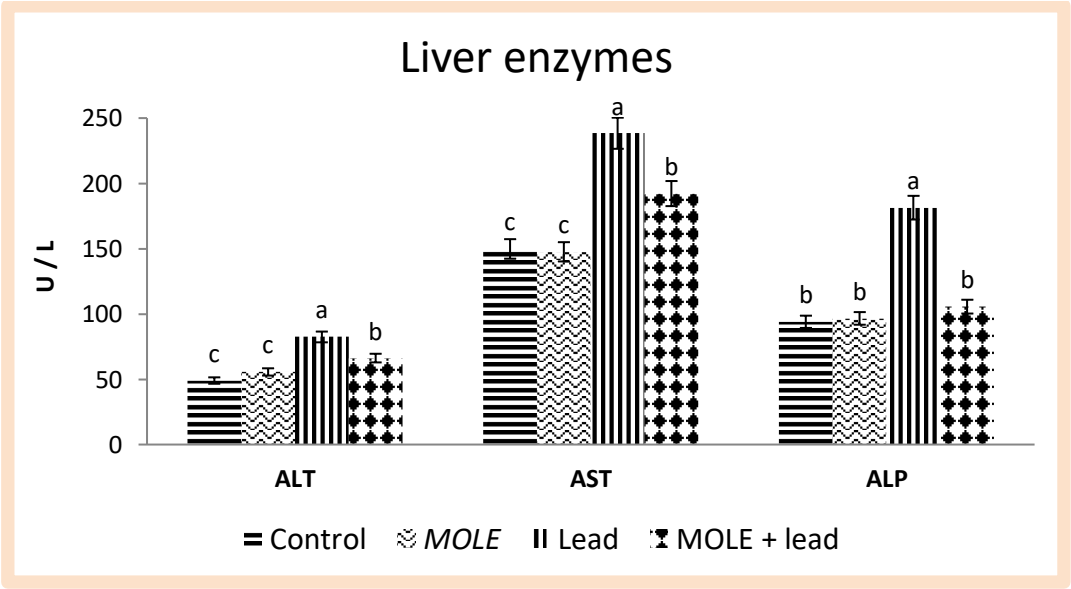


Figure (9): Effect of MOLE on serum liver enzymes activity in normal and L.A intoxicated rats

4.10.2. Effect of MOLE on serum proteins in normal and L.A intoxicated rats

4.10.2.1. Serum total protein (mg/dL)

Our results revealed that the L.A treated group expressed a significant (P< 0.05) decreases in total protein values in comparison to the control group. Treatment of lead intoxicated rats with MOLE expressed a significant (P< 0.05) increment in total protein values in comparison to L.A group, whereas MOLE treated rats weren't differ statistically with control rats in total protein **Table (10) Figure (10)**.

4.10.2.2. Serum albumin (mg/dL)

Our revealed a significant (P< 0.05) decreases in albumin values in L.A group when compared to the control group. Co-administration of MOLE to lead intoxicated rats revealed a significant (P< 0.05) elevation in albumin values when compared to the L.A to a comparable level of MOLE group. Rats treated with MOLE only didn't express any significant difference with control rats **Table (10) Figure (10)**.

4.10.2.3. Serum globulin (mg/dL)

There was a significant (P< 0.05) decrease in globulin values in L.A group compared to the control groups. Co-administration of MOLE to L.A intoxicated rats showed insignificant difference in comparison with L.A group. MOLE group expressed insignificant changes in relation with control group **Table (10) Figure (10)**.

4.10.2.4. Albumin / globulin ratio (A/G ratio)

Lead acetate intoxicated rats expressed a significant (P< 0.05) decrease in A/G ratio in comparison to the control group. Co-administration of MOLE to L.A intoxicated group expressed a significant (P< 0.05) elevation in A/G ratio when compared to L.A group which is comparable to control group. Rats administer

with MOLE only didn't express any significant difference with control group **Table (10) Figure (10)**.

Table (10): Effect of MOLE on serum proteins of normal and L.A intoxicated rats

parameters / Groups	T. protein (mg/dL)	Albumin (mg/dL)	Globulin (mg/dL)	A/G ratio
Control	7.36± 0.88 [a]	5.04± 0.12 [a]	2.32± 0.99 [a]	2.172± 0.12 [a]
MOLE	7.05± 0.10 [a]	4.8± 0.15 [a,b]	2.26± 0.11 [a]	2.123± 0.15 [a]
Lead acetate	5.34± 0.27 [c]	3.300± 0.25 [c]	2.04± 0.10 [b]	1.62± 0.15 [b]
MOLE+ L.A	6.52± 0.07 [b]	4.45± 0.15 [b]	2.07± 0.89 [b]	2.14± 1.70 [a]

Values are means ± SE, means carrying different superscripts (a, b, c) within the same column are considered significant at ($P < 0.05$).

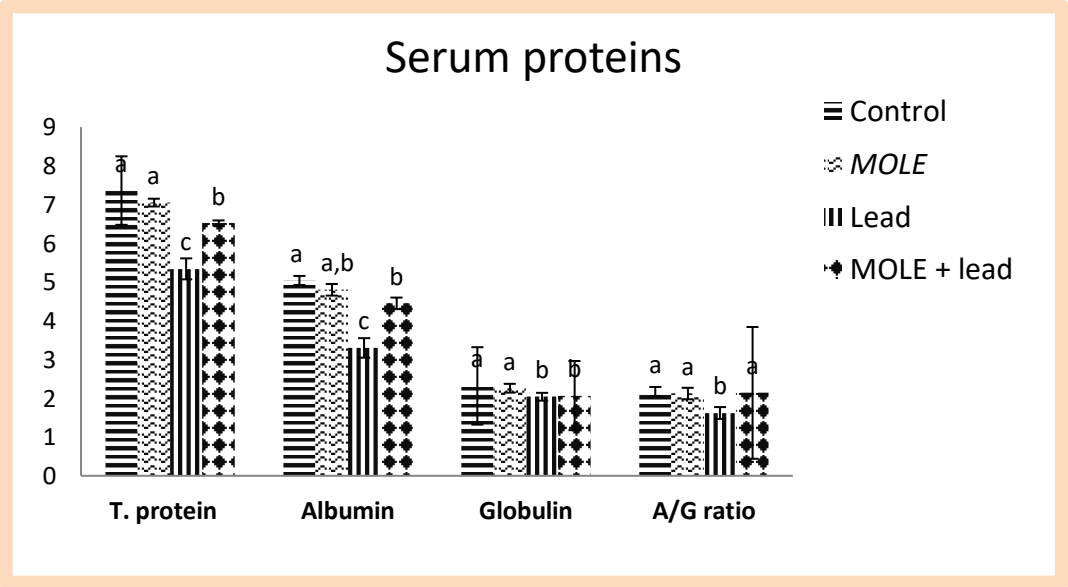


Figure (10): Effect of MOLE on serum proteins in normal and L.A intoxicated rats

4.10.3. Effect of MOLE on kidney functions in normal and L.A intoxicated rats

4.10.3.1. BUN (mg/dL)

Administration of L.A revealed a significant (P< 0.05) increase in BUN level in comparison to the control group. Co-administration of MOLE to L.A intoxicated group gave a significant (P< 0.05) decrease in BUN in relation to the L.A group to level comparable to MOLE group but were significantly (P< 0.05) higher than control group. Rats treated with MOLE showed non-significant difference in comparison to control group in BUN level as showed in **Table (11) Figure (11).**

4.10.3.2. Creatinine (mg/dL)

Current study showed that the L.A group significantly (P< 0.05) increased creatinine in comparison to the control group. Co-administration of MOLE to lead intoxicated group revealed a significant (P< 0.05) decrease in comparison to the L.A group. MOLE group showed insignificant difference in relation with the control group **Table (11) Figure (12)**.

Table (11): Effect of MOLE on renal biomarkers (urea and creatinine) in L.A intoxicated rats

parameters / Groups	Urea (mg/dL)	Creatinine (mg/dL)
Control	26.40±1.28 ^{c}	0.40±0.05 ^{c}
MOLE	30.80±2.67^{b,c}	0.47±0.02 ^{c}
Lead acetate	41.80±1.82^{a}	1.06±0.07^{a}
MOLE+ L.A	34.60±1.03 ^{b}	0.73±0.04 ^{b}

Values are means ± SE, means carrying different superscripts (a, b, c) within the same column are considered significant at (P< 0.05).

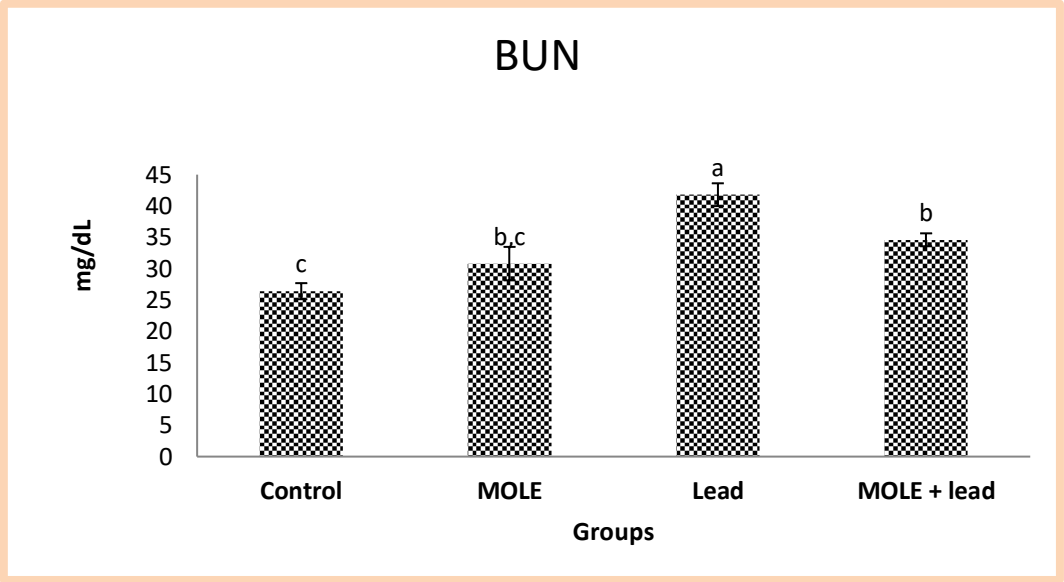


Figure (11): Effect of MOLE on BUN in normal and L.A intoxicated rats

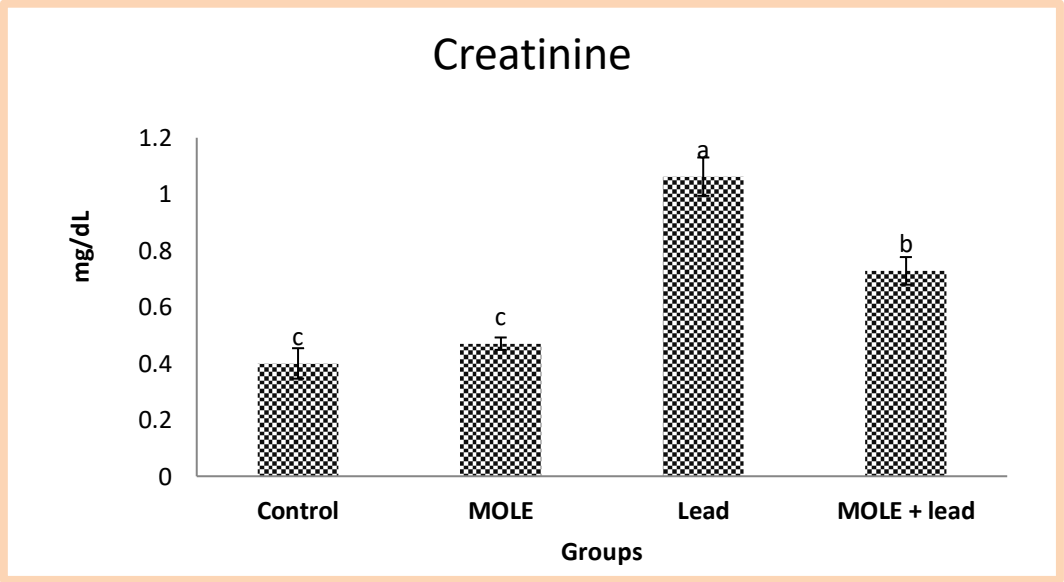


Figure (12): Effect of MOLE on creatinine level of normal and L.A intoxicated rats

4.10.4. Effect of MOLE on serum lipid profile in normal and L.A intoxicated rats

4.10.4.1. Serum cholesterol (TC) (mg/dL)

Our results showed that the L.A group expressed a significant (P< 0.05) increase in TC values in comparison to the control group. Co- administration of MOLE to lead intoxicated rats revealed a significant (P< 0.05) reduction in TC values in relation to L.A group but was of significant (P< 0.05) higher values when compared to control group. MOLE treated rats weren't differ statistically with normal rats in TC values **Table (12) Figure (13)**.

4.10.4.2. Serum triglycerides (TG) (mg/dL)

Rats administered L.A only expressed a significant (P< 0.05) increment in TG values in comparison to the control group. Co- administration of MOLE to L.A intoxicated rats revealed a significant (P< 0.05) reduction in TG values in relation to L.A group, however showed higher values when compared to control group. Normal rats administered with MOLE only weren't differ statistically with normal rats in TG level as shown in **Table (12) Figure (13)**.

4.10.4.3. Serum high density lipoprotein cholesterol (HDL-C) (mg/dL)

The lead acetate group expressed a significant (P< 0.05) decrease in HDL-C Values in comparison to the control group. Co-administration of MOLE to L.A intoxicated rats revealed a significant elevation in HDL-C Values when compared to L.A group. MOLE group showed a significant elevation in HDL-C Values when compared to the control group as revealed in **Table (12) Figure (13)**.

4.10.4.4. Serum low density lipoprotein cholesterol (LDL-C) (mg/dL)

Rats treated with L.A group expressed a significant (P < 0.05) increase in LDL-C values when compared with the Control group. Co-administration of MOLE to L.A intoxicated group revealed a significant reduction in LDL-C values when compared to the L.A group. Moreover, MOLE group revealed slight increase in

LDL-C values without any significance when compared with control group as revealed in **Table (12) Figure (13)**.

Table (12): Effect of MOLE on serum lipid profile in normal and L.A intoxicated rats

Parameters / Groups	TC (mg/dL)	TG (mg/dL)	HDL(mg/dL)	LDL (mg/dL)
Control	56.80± 1.93 ^{c}	66.60± 1.21 ^{c}	29.00± 0.71 ^{b}	14.60± 1.67^{c}
MOLE	64.60± 1.9 ^{c}	73.60± 1.72 ^{c}	32.80± 1.39 ^{a}	18.00± 1.43 ^{c}
Lead acetate	91.61± 1.96 ^{a}	104.80± 2.28 ^{a}	17.20± 1.92 ^{d}	53.41± 1.25 ^{a}
MOLE+ L.A	78.22± 2.92 ^{b}	81.40± 3.14^{b}	22.00± 1.30 ^{c}	39.90± 1.37 ^{b}

Values are means ± SE, means carrying different superscripts (a, b, c) within the same column are considered significant at (P< 0.05).

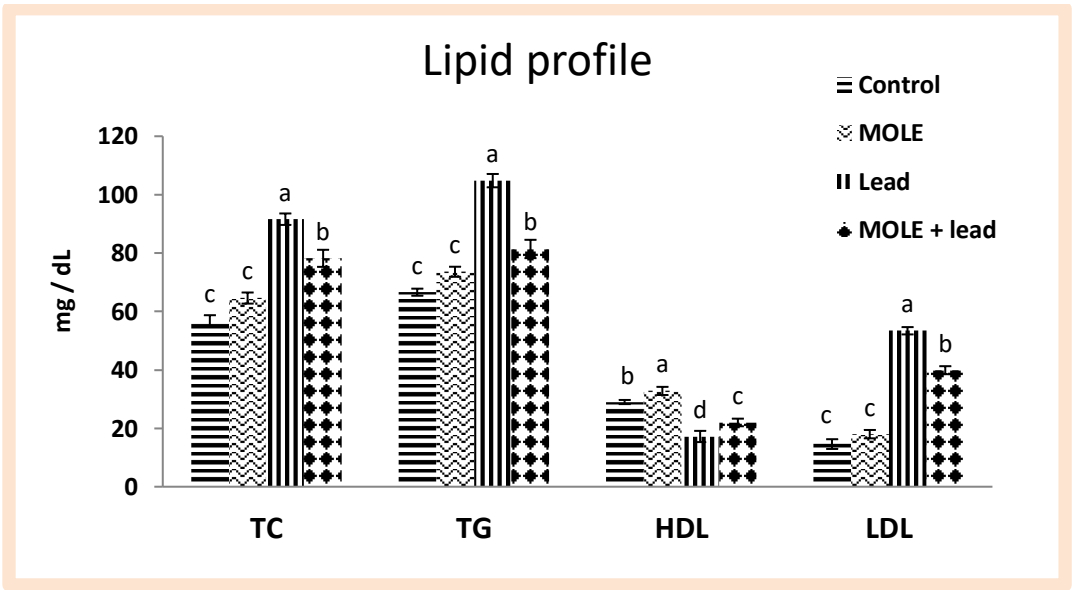


Figure (13): Effect of MOLE on serum lipid profile in normal and L.A intoxicated rats

4.11. Effect of MOLE on hepatic enzymatic and non-enzymatic antioxidants in normal and lead intoxicated rats

4.11.1. Effect of MOLE on reduced glutathione level (GSH) µmol/L in normal and L.A intoxicated rats

Our results showed that the L.A intoxicated group had a significant (P< 0.05) decrease in GSH level in comparison to the control group.co-administration of MOLE to L.A intoxicated group expressed a significant (P< 0.05) increase in GSH level when compared to the L.A group. Rats treated with MOLE only didn't show any significant difference with control group in GSH level as showed in **Table (13) Figure (14)**.

4.11.2. Effect of MOLE on superoxide dismutase activity (SOD) U/mL in rats in normal and L.A intoxicated rats

There was a significant (P< 0.05) decrease in L.A intoxicated in SOD activity in comparison to the control group. Co-administration of MOLE to L.A intoxicated group expressed a significant increase in SOD activity in relation to the L.A group. Rats treated with MOLE only didn't show any significant difference with control group in concerning SOD activity **Table (13) Figure (15).**

Table (13): Effect of MOLE GSH level GSH (µmol/L) and SOD (u/mL) in lead intoxicated rats

parameters / Groups	GSH (µmol/L)	SOD (U/mL)
Control	20.69 ± 0.10 $^{\alpha}$	7.73 ± 0.589 $^{\alpha}$
MOLE	20.20 ± 0.11 ^{a}	7.47 ± 0.468 ^{a}
Lead acetate	12.52 ± 0.22 ^{c}	4.50 ± 0.123 ^{c}
MOLE+ L.A	16.80 ± 0.52 ^{b}	6.51 ± 0.171 ^{b}

Values are means ± SE, means carrying different superscripts (a, b, c) within the same column are considered significant at (P< 0.05).

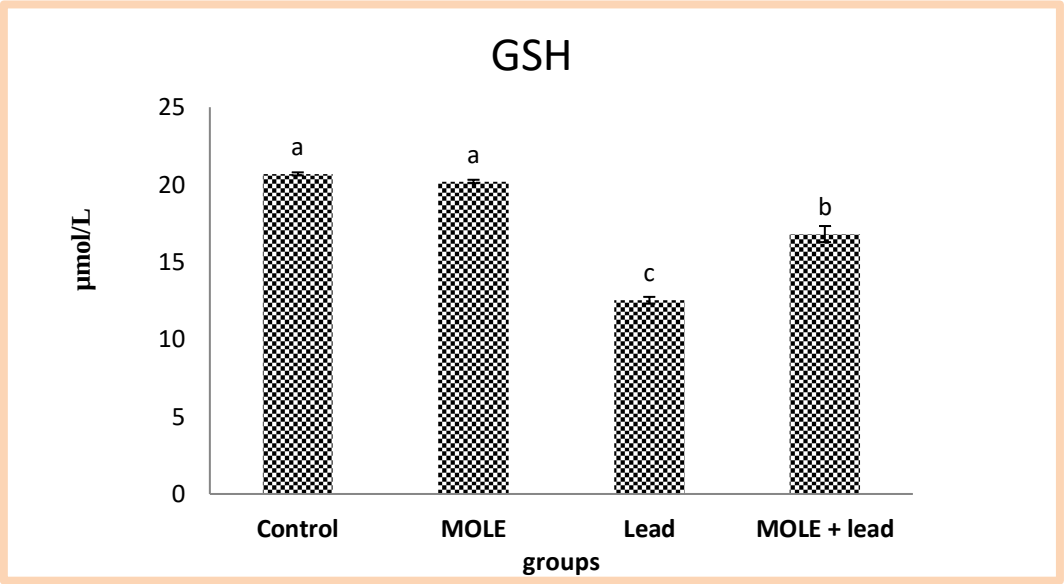


Figure (14): Effect of MOLE on reduced glutathione level (GSH) µmol/L in normal and L.A intoxicated rats

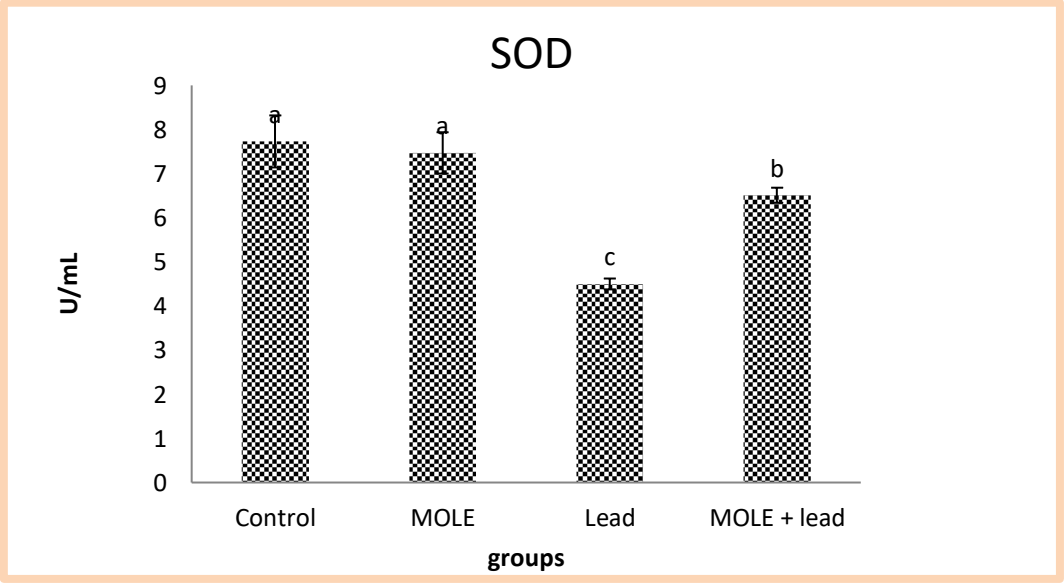


Figure (15): Effect of MOLE on Superoxide dismutase activity (SOD) u/ml in rats in normal and L.A intoxicated rats

4.12. Effect of MOLE on hepatic DNA fragmentation of lead L.A intoxicated rats on agarose gel

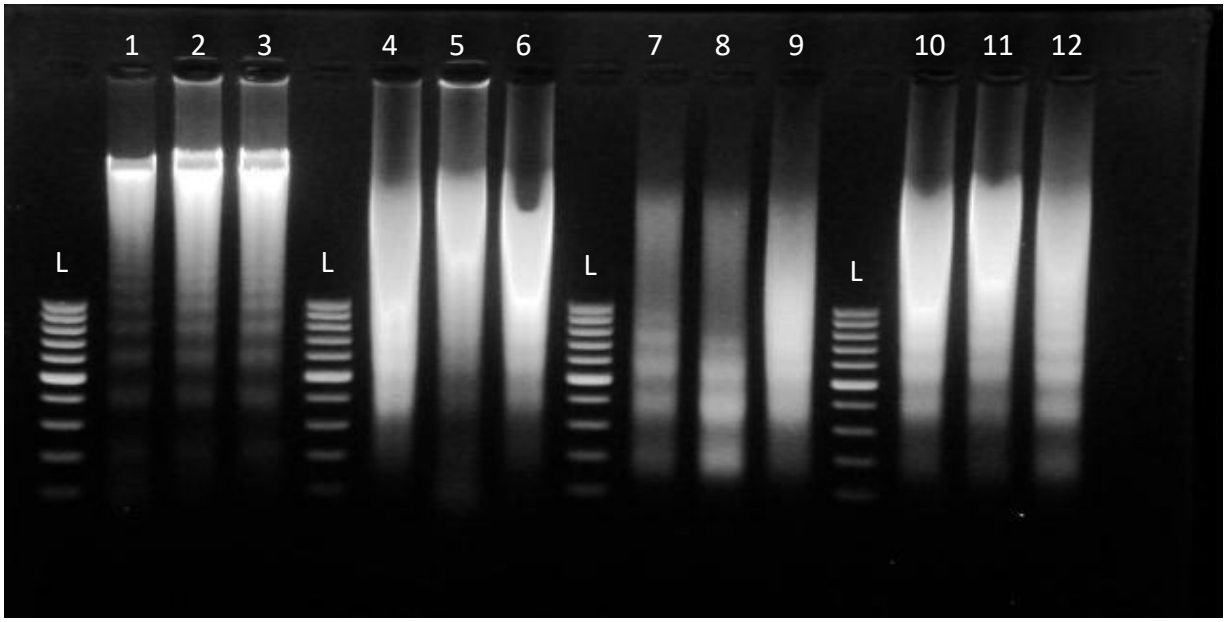


Photo (3) DNA fragmentation of rat's liver of different treated groups on agarose gels/ ethidium bromide gel. DNA isolated from experimental liver tissues was loaded into 1 %(w/v) agarose gel, 100 pb ladder. Lane (1, 2, 3) represents DNA isolated from control rats; lane (4, 5, 6): represents DNA isolated from rats received aqueous MOLE alone; lane (7, 8, 9): represents DNA isolated from rats received L.A alone; lane (10, 11, 12): represents DNA isolated from rats received aqueous MOLE and L.A. (L) means ladder.

The rat's livers of the control group and MOLE group showed slight DNA fragmentation on agarose gel electrophoresis as shown in **Photo (3)** lane **(1, 2, 3)** and lane **(4, 5, 6)** respectively. On the other hand the L.A group showed more DNA fragmentation on agarose gel electrophoresis than control and aqueous MOLE groups as shown in **Photo (3)** lane **(7, 8, 9)**. Co-administration of aqueous MOLE to L.A intoxicated group dramatically lowered degradation than L.A group as showed in **Photo (3)** lane **(10, 11, 12)**.

4.13. Effect of MOLE on histopathology of rat's livers and kidneys of normal and L.A intoxicated rats

4.13.1. Effect of MOLE on histopathology of rat's livers of normal and L.A intoxicated rats

Livers of both control and MOLE groups showed normal hepatic lobules with centrally located central veins, radiating hepatic cords with normal polyhedral hepatic cells and normal hepatic areas **(Photo 4 & 5)**. The L.A group revealed focal lymphocytic infiltration of hepatic parenchyma **(Photo 6**). In addition to hyperplasia of hepatic triad with hyperplastic bile ducts and early fibrous connective tissue proliferation **(Photo 7)**. Diffuse moderate vacuolation of hepatocytes, congestion of central veins, mild to moderated centro-central fibrosis were also observed **(Photo 8)**. The L.A+ MOLE treated group revealed pronounced improvement of hepatic lesions with mild to moderate vacuolar degeneration of hepatic cells and mild congestion of central veins **(Photo 9).**

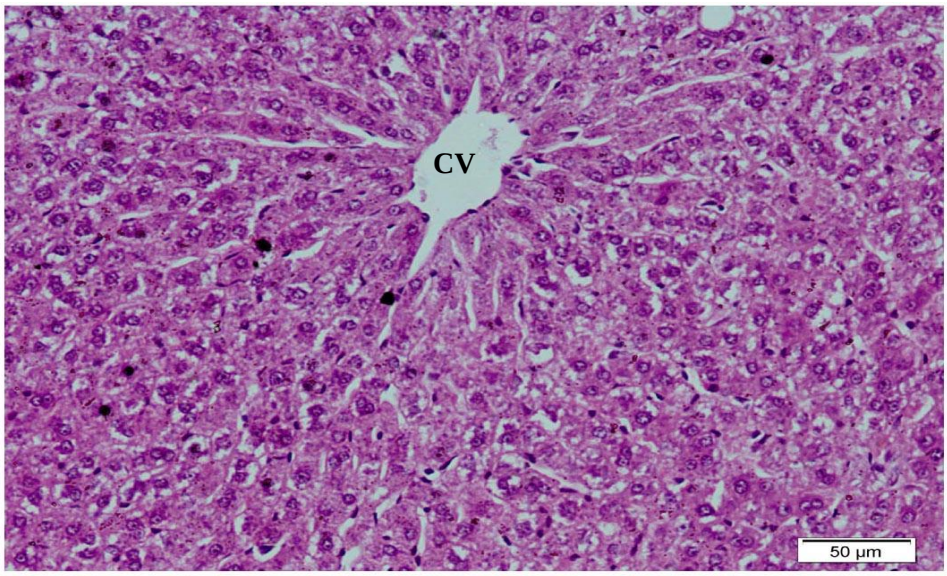


Photo (4): Liver of control group showed normal central vein (c) and normally arranged polyhedral hepatic cells. H & E. X 200.

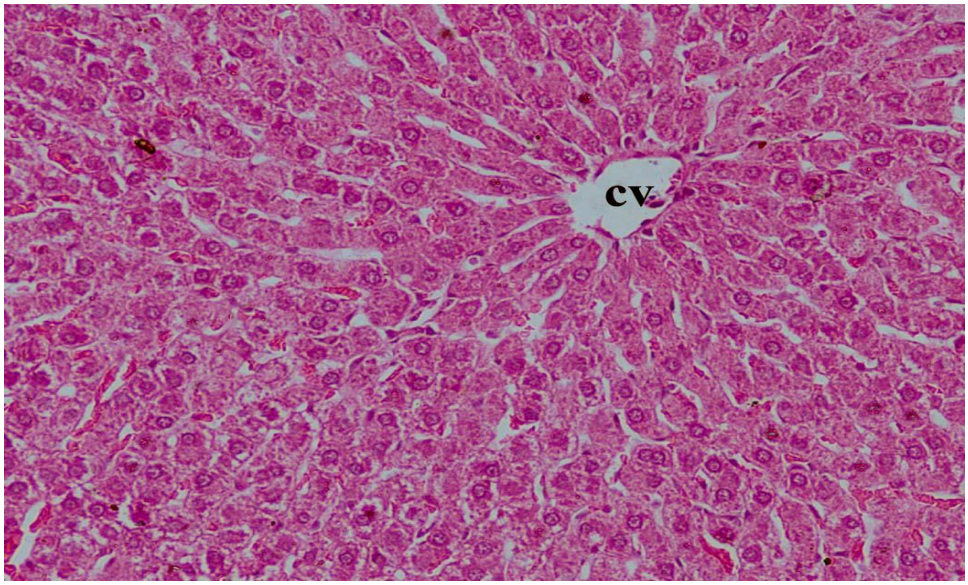


Photo (5): Liver of MOLE treated group showed normal central vein (c) and normally arranged polyhedral hepatic cells. H & E. X 200.

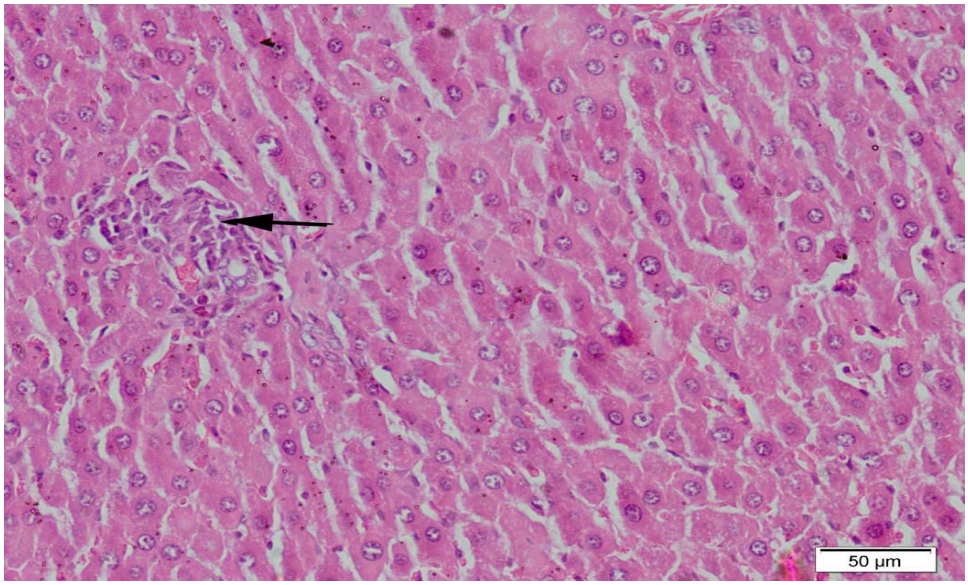


Photo (6): liver of L.A intoxicated group showed focal lymphocytic infiltrations (arrow). H & E. X 200.

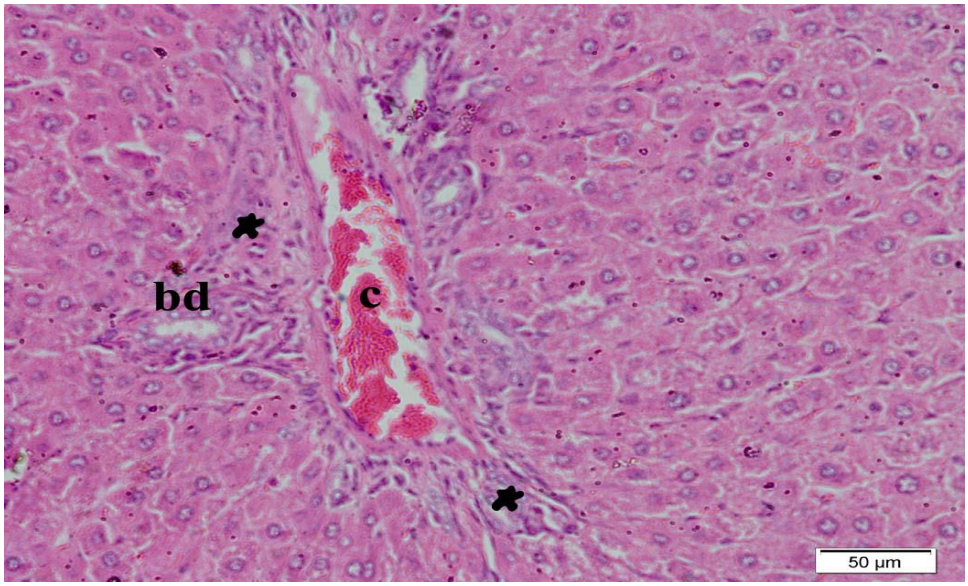


Photo (7): Liver of L.A intoxicated group showed hyperplasia of bile duct (bd) fibrosis (stars tick). H & E. X 200.

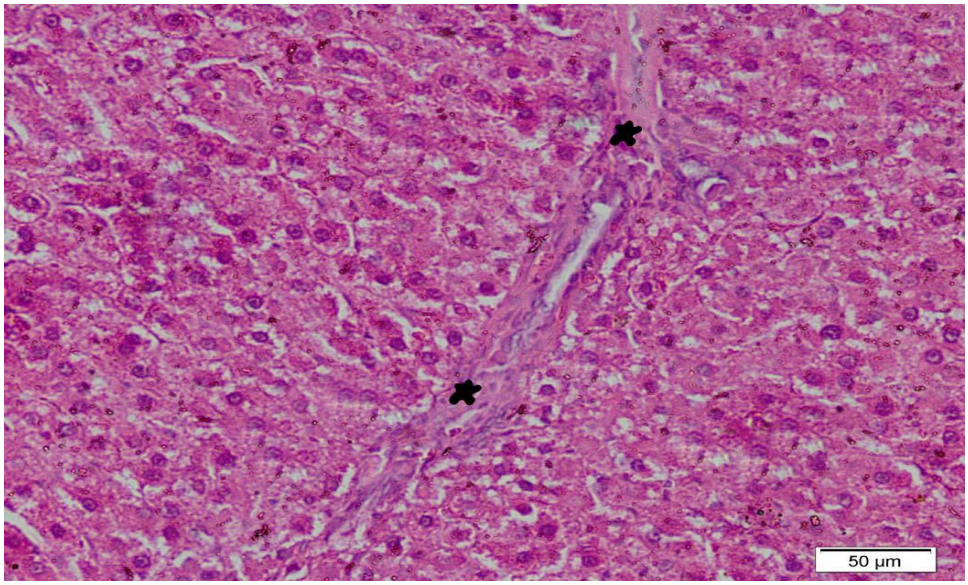


Photo (8): liver of L.A intoxicated group showed diffuse vacuolar degeneration along with moderate centro-central fibrosis (stars stick). H & E. X 200.

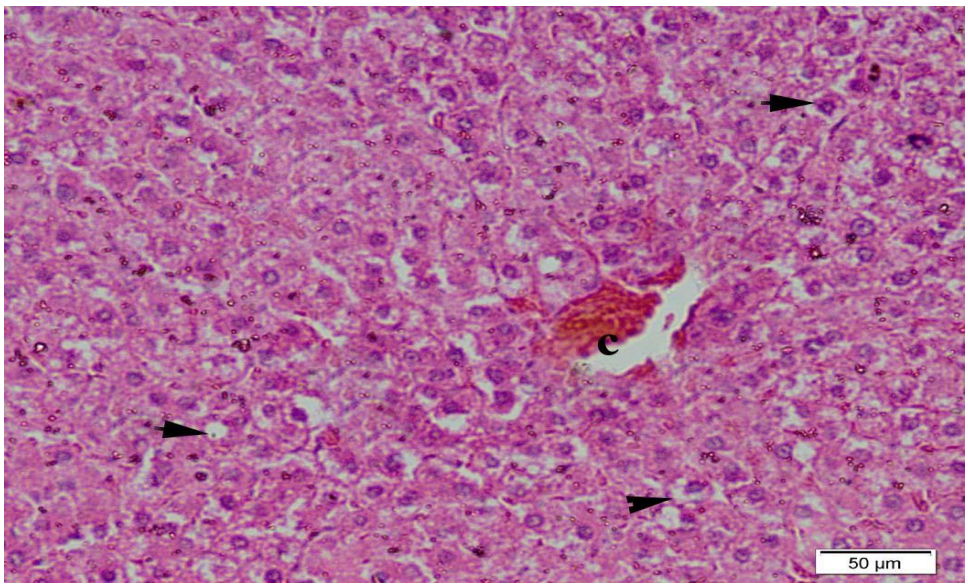


Photo (9): Liver of lead-Moringa treated group showed mild congestion of central vein (c) and mild to moderate vacuolation of hepatic cells (arrow heads). H & E. X 200.

4.13.2. Effect of MOLE on histopathology of rat's kidneys of normal and L.A intoxicated rats

Kidneys of control group revealed normal histological structure of both cortex and medulla. In cortex numerous normal nephrons were observed. Each nephron formed from glomerulus, proximal and distal convoluted tubules, loops of Henle and the collecting ducts. All glomeruli had a normal capillary tufts and clear Bowman's space and normal Bowman's capsule **(Photo 10)**. Proximal and distal convoluted tubules showed normal intact tubular epithelium and normal non-engorged intertubular capillaries. In medulla, most of the collecting ducts were normal with intact epithelium (**Photo 11**). MOLE group revealed a normal cortical and medullary tissues **(Photo 12 & 13)**. The L.A group showed severe multifocal congestion of both cortex and medulla with focal hemorrhages in medullas **(Photo 14 & 15)**. Marked perivascular edema with mild proliferation of collagen and few lymphocytes, degeneration and focal necrosis of some tubular epithelium were also observed **(Photo 16).** Focal proliferative glomerulonephritis characterized by proliferation of capillary tufts, adhesion of parietal and visceral layers of Bowman's capsule, proliferation and mesangial cells and absence of urinary space were seen, in addition to degeneration and focal necrosis of some tubules **(Photo 17 & 18)**. Periglomerular lymphocytic infiltrations and mild proliferation of fibrous tissue was also observed **(Photo 19)**. Moderate to severe lymphocytic infiltrations along with early intertubular fibrosis were also observed in medullary tissue **(Photo 20 & 21)**. Pronounced improvement and protection were observed in L.A+MOLE treated group as it showed mild renal tubular nephrosis, mild congestion few to null inflammatory reactions (**Photo 22 & 23)**.

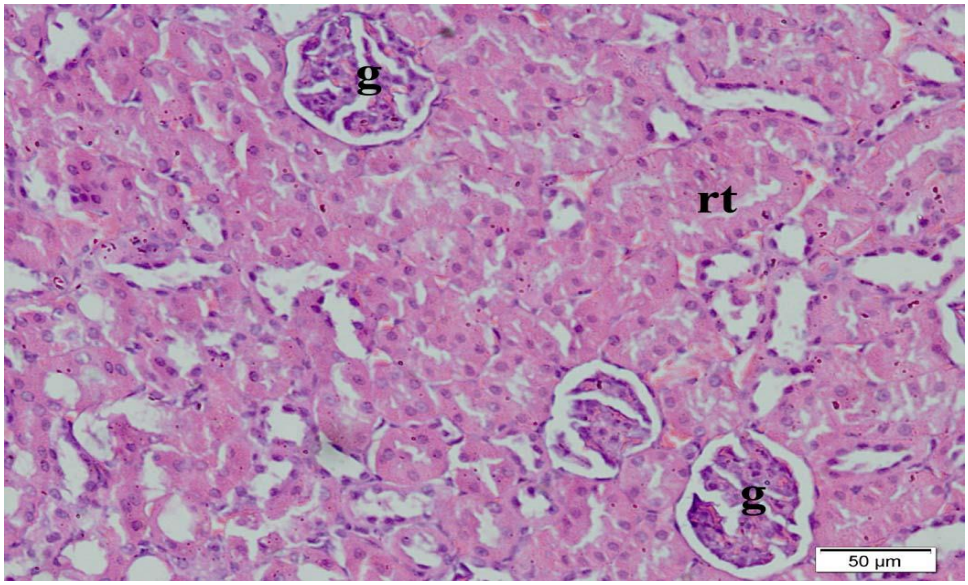


Photo (10): kidney of normal control group showed normal glomeruli (g) and normal renal tubules (rt). H & E. X 200.

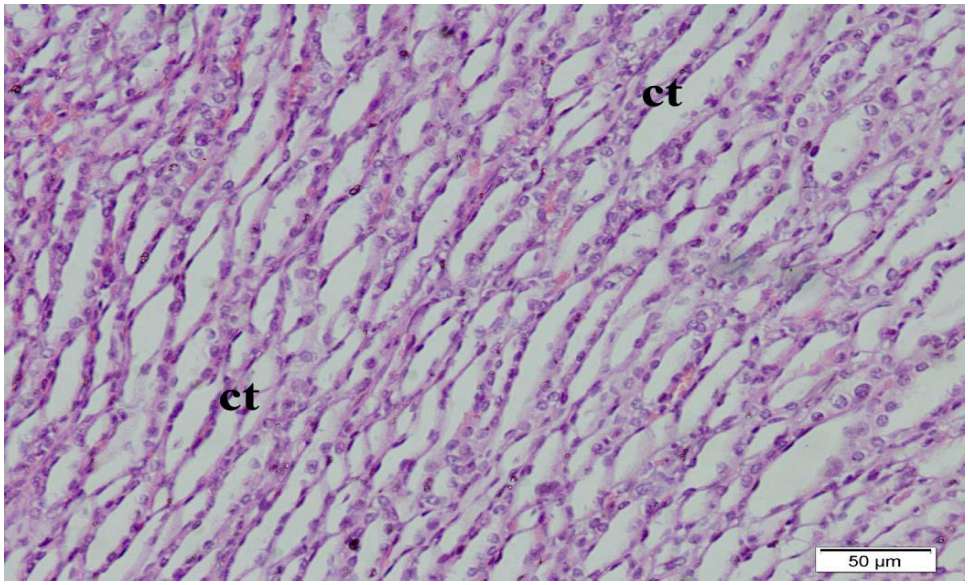


Photo (11): kidney of normal control group showed medulla with normal collecting ducts (ct). H & E. X 200.

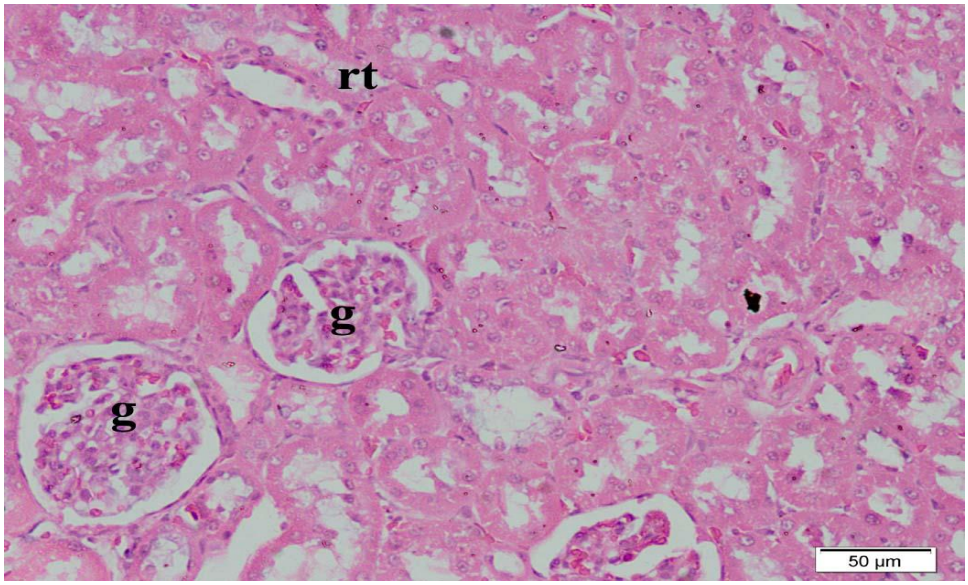


Photo (12): kidney of MOLE treated group showed normal glomeruli (g) and normal renal tubules (rt). H & E. X 200.

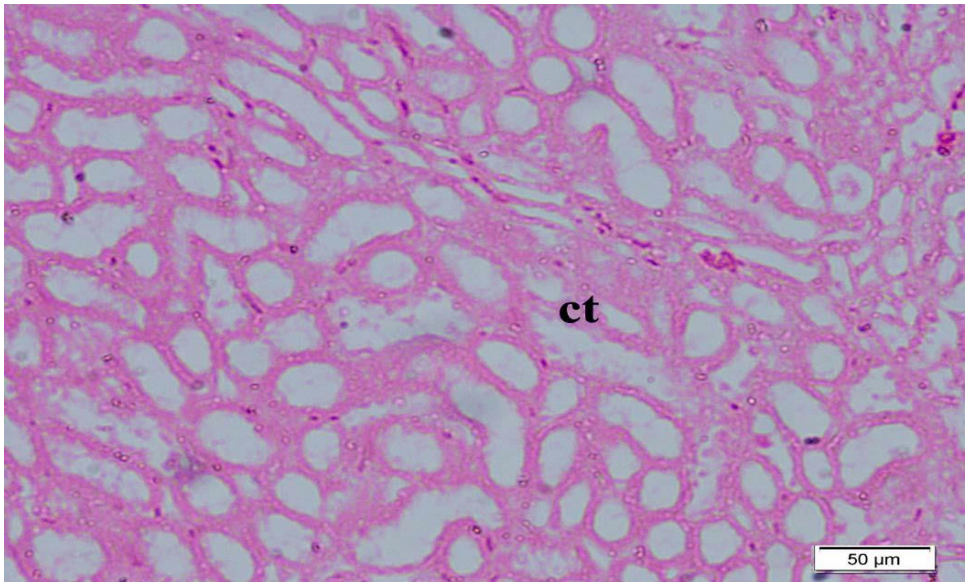


Photo (13): kidney of MOLE treated group showed medulla with normal collecting ducts (ct). H & E. X 200.

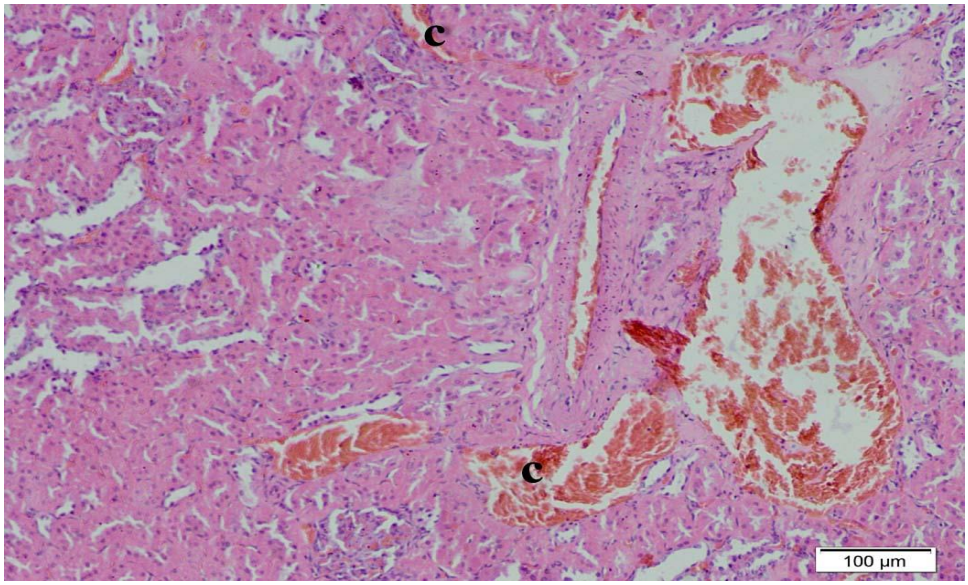


Photo (14): kidney of L.A intoxicated group showed severe multifocal congestion of cortical blood vessels (c). H & E. X 100

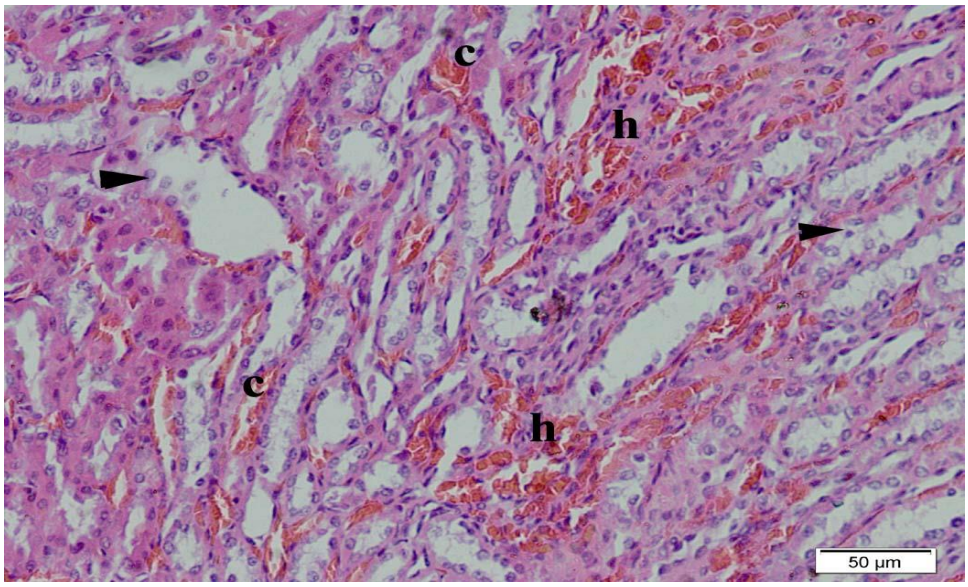


Photo (15): kidney of L.A intoxicated group showed severe multifocal congestion of medullary blood vessels (c), focal hemorrhages (h) and necrotic changes of some tubules (arrow heads). H & E. X 200.

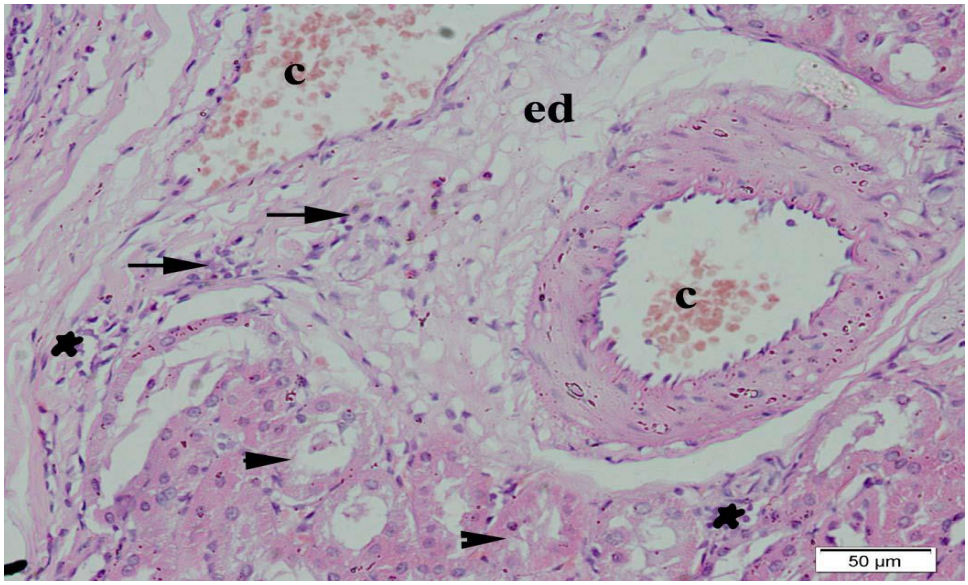


Photo (16): kidney of L.A intoxicated group showed congestion of blood vessels (c), leukocytic infiltrations (arrows), early mild fibrosis (starstick) and focal tubular necrosis (arrow heads). H & E. X 200.

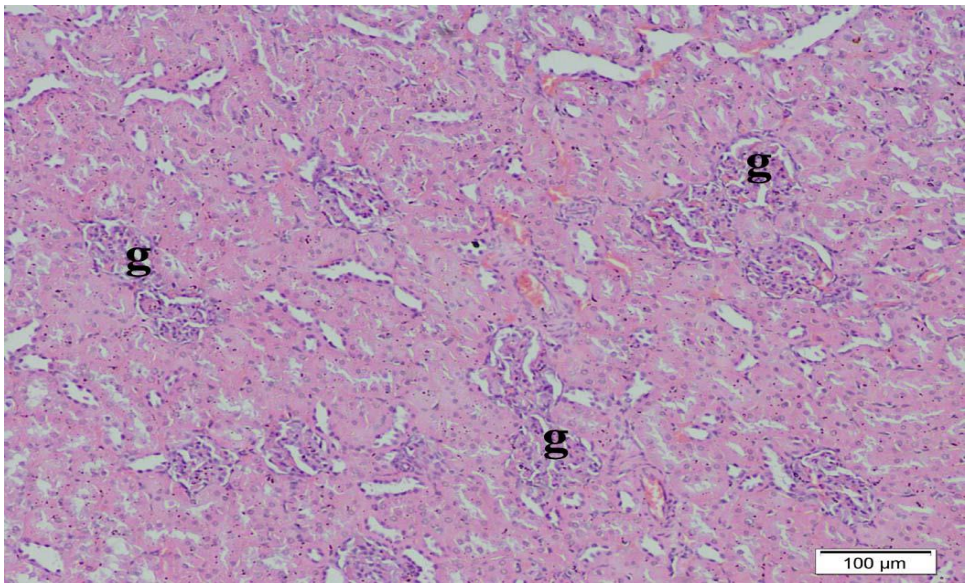


Photo (17): kidney of L.A group showed multiple proliferative glomerulonephritis (g) and multifocal congestion of cortical blood vessels. H & E. X 100.

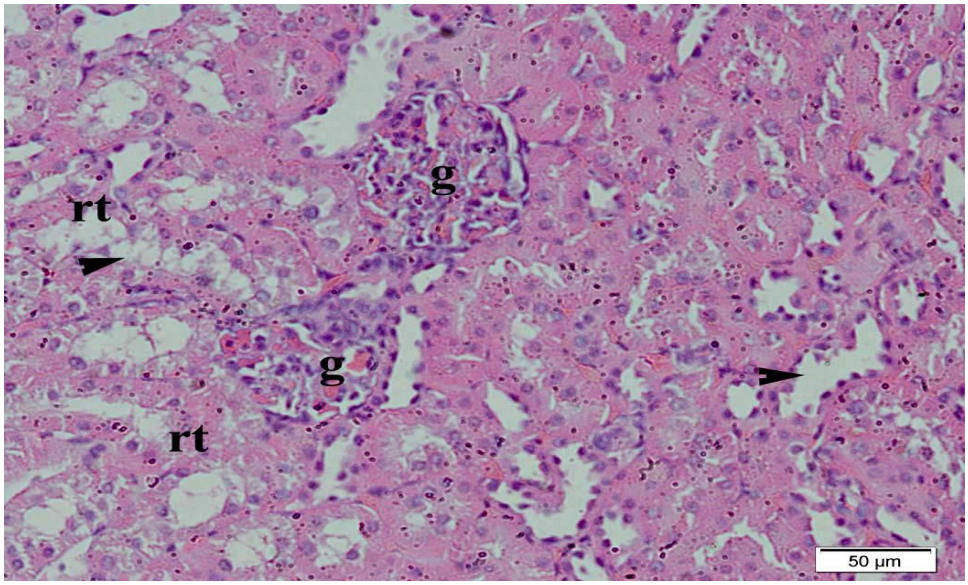


Photo (18): kidney of L.A intoxicated group, showed proliferative glomerulonephritis (g) and multifocal degeneration, necrosis and cystic dilatation (arrow heads) of renal tubules (rt). H&E. X 200**.**

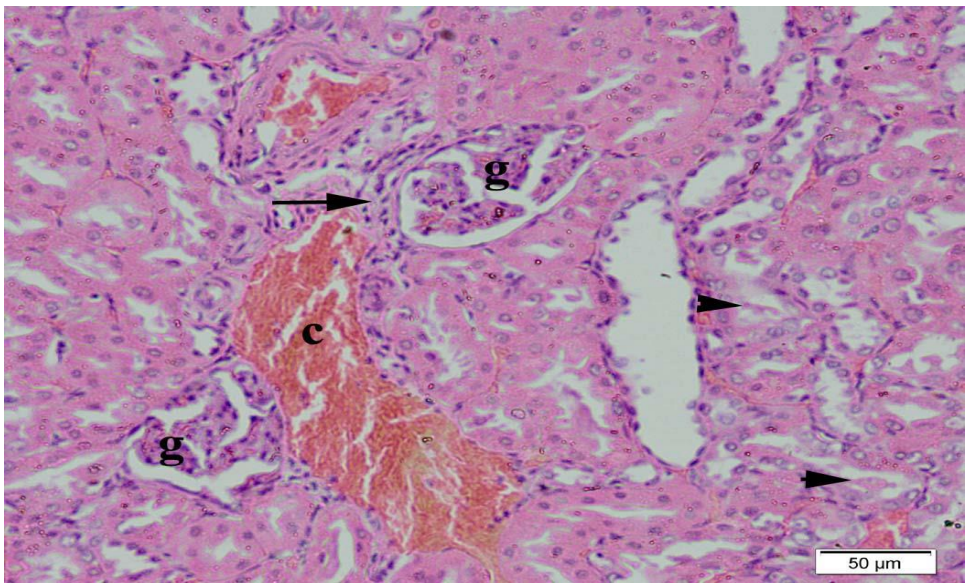


Photo (19): kidney of L.A intoxicated, showed periglomerular leukocytic infiltrations (arrow), tubular nephrosis (arrow heads) and congestion of cortical blood vessels (c). H & E. X 200.

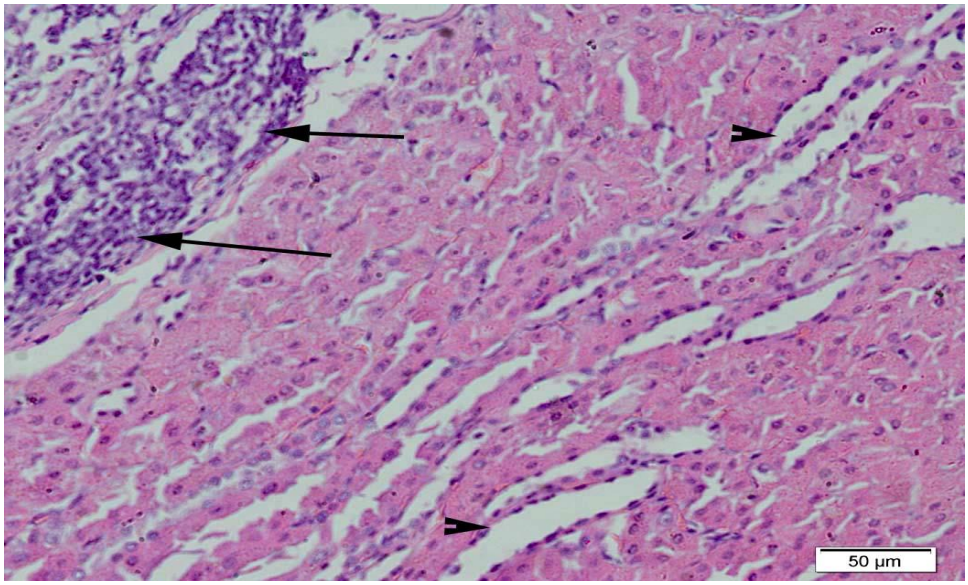


Photo (20): kidney of L.A group showed cystic dilations of some tubules (arrow heads) and lymphocytic infiltrations (arrows). H & E. X 200.

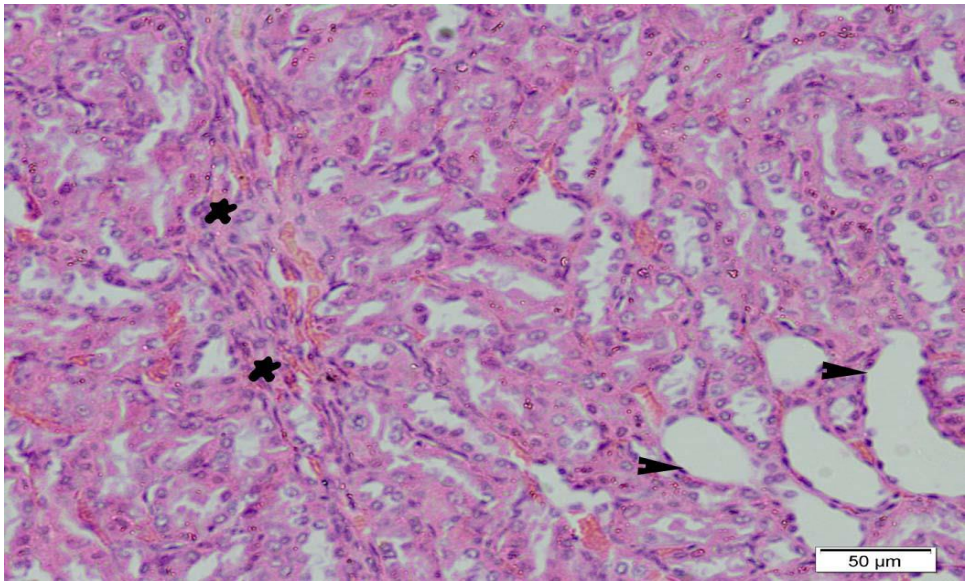


Photo (21): kidney of L.A group showed congestion of blood capillaries, cystic dilations of some tubules (arrow heads) and mild intertubular fibrosis (starstick). H & E. X 200**.**

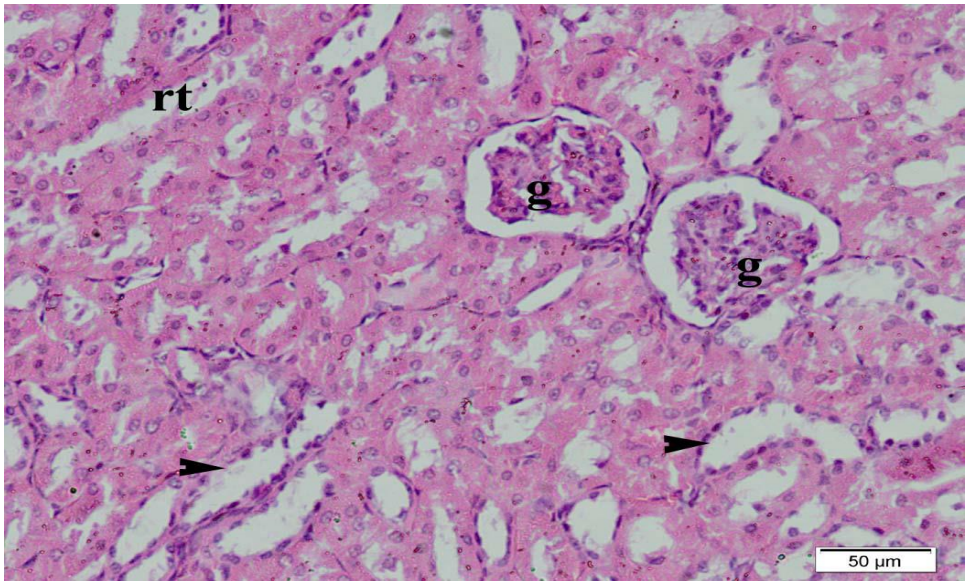


Photo (22): kidney of L.A+MOLE treated group, showed pronounced improvement of cortical tissue, focal degeneration and cystic dilatations of some tubules (arrow heads). H & E. X 200.

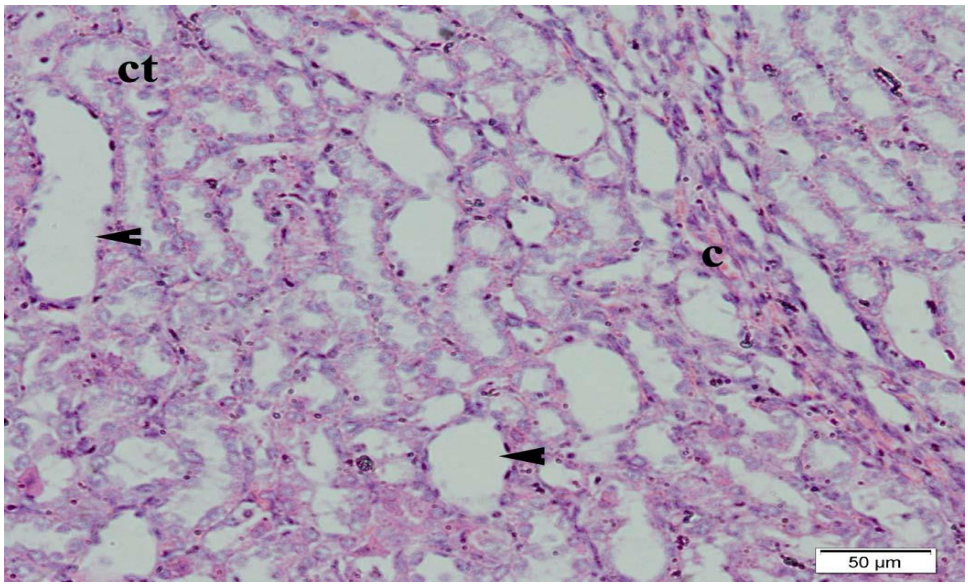


Photo (23): kidney of L.A+MOLE treated group, showed pronounced improvement of renal medulla, only focal degeneration and cystic dilatations of some tubules (arrow heads) along with mild congestion (c). H &.E. X 200.

Discussion

5. Discussion

Lead constitutes a major health problem as it is widely used in different industries such as paint, ceramic, plastics, etc. It induces deleterious effects on various body organs including the liver and its toxicity has also been associated with health hazards **(Flora *et al.*, 2012; Badary, 2017)**. Liver is considered one of the target organs affected by lead toxicity owing to its storage, biotransformation and detoxification there **(Ohkawa *et al.*, 1979)**.

Moringa oleifera, known as a "miracle tree" and possess antitumor, anti-inflammatory, antiulcer, diuretic, antihypertensive, cholesterol lowering, antidiabetic, hepatoprotective, antibacterial and antifungal activities as well as antioxidant activity against free radicals which prevent oxidative damage to major biomolecules and afford significant protection against DNA damage **(Singh *et al.*, 2009)**.The effect of lead acetate on liver and kidney was investigated in the current study with a trial to counteract the toxic effect of lead by using aqueous MOLE.

In the present study chemical composition of 100 g dried MOL revealed the presence of 6.72 g crude fiber, 23 g crude protein, 1.98 g fat, 6.7 g moisture, 16 g ash and 45.6 g carbohydrate. These results were parallel to finding of **El-Gammal *et al.* (2017)** who demonstrated that dried MO leaves contained (24.47± 0.11) crude fibre, (22.86± 0.05) crude protein, (33.69± 0.12) carbohydrate, (8.54± 0.21) moisture and (7.72± 0.13) ash. The variations in the ratio of chemical constituents may be attributed to agroclimatic locations and seasons **(Iqbal and Bhanger, 2006)**.

In the present study the qualitative phytochemical analysis of MOLE revealed the presence of sterols, alkaloids, amino acids, proteins, flavonoids, reducing sugars,

glycosides, saponins, tannins and carbohydrates. These results were in agreement with previous studies of **Fahey (2005), Iqbal and Bhanger (2006), Anwar and Rashid (2007)** and **Arora *et al.* (2013)** who reviewed the presences of phytoconstituents of MO as sterols, glycosides, carbohydrates, alkaloids, flavonoids, saponins, resins, tannins and amino acids.

Our results showed that the total phenolic content (TPC) in aqueous MOLE is 128.64 mg/g gallic acid equivalent. These results were higher than those obtained by **Sreelatha and Padma (2009)** and **Singh *et al.* (2009)** who found that TPC of aqueous MOLE was 45.81 mg/g gallic acid equivalents and 105.04 mg/g gallic acid equivalents, respectively. However, our results showed lower TPC than obtained by **El-Gammal *et al.* (2017)** who found that aqueous MOLE contain 340 (mg/g) gallic acid equivalent.

Present study showed that the total flavonoid content (TFC) in aqueous MOLE was 28.39 mg/g rutin equivalent which was very close to the results obtained by **Sreelatha and Padma (2009)** who observed that TFC in aqueous MOLE contained 27 mg/g quercetin equivalents. However, our results showed lower TFC than obtained by **Singh *et al.* (2009)** who mentioned that aqueous MOLE contained 31.28 mg/ g quercetin equivalents. The variations in the amounts of TFC and TPC were explained by **Iqbal and Bhanger (2006)** who reported that agroclimatic locations and seasons have profound effects on the antioxidant activity of MOL. antioxidant activity of samples from cold areas was relatively higher than those from temperate regions.

In the current study, HPLC analysis showed that the aqueous MOLE contained rutin, qurecetin, rhamnetin, kaempferol, gallic acid, chlorogenic acid, apigenin-7-glucoside, cateachin, apigenin, vanillic acid, ferulic acid, caffeic acid, sinapic acid, rosmarinic acid, cinnamic acid, chrysin, protocatechuic acid, p

hydroxybenzoic acid, syringic acid and gentisic acid. These results matched to those obtained by **Ashok Kumar and Pari (2003) and Verma *et al.* (2009)** who reported that MOLE contained various bioactive compounds such as chlorogenic acid, rutin, quercetin glucoside, and kaempferol which are responsible for the antioxidant activity of MO leaves. Moreover, **Singh *et al.* (2009)** found that the aqueous extracts of MO leaves, seeds and fruit contained gallic acid, chlorogenic acid, rutin, ellagic acid, ferulic acid, kaempferol, quercetin and vanillin.

Regarding to body weights and weight gain, the present results showed that the administration of L.A at a dose 100 mg/kg for four weeks significantly ($P< 0.05$) decreased body weight and weight gain at the 3rd and 4th weeks when compared to the control group. These results were in agreement with **Ibrahim *et al.* (2013)** who found that lead caused decreases in rats' growth rate. Also **El-Tantawy (2016)** found that rats treated with (100 mg/kg) for four weeks revealed a significant reduction in the body weight as compared to their corresponding control animals. The reduction in body weight could be due to enhanced gluconeogenesis as a result of lead-induced impairment in pancreatic function and insulin secretion. Additionally, weight reduction could be a part of impaired metabolism as a result of impaired zinc-dependent enzymes. In contrast to our results **Pandya *et al.* (2010)** and **Mabrouk *et al.* (2016)** found that there were no significant change in lead intoxicated rats with 0.025 mg L.A/kg b.wt and 2000 ppm of L.A through i.p injection, respectively when compared to control rats.

Our results showed that normal rats treated with 200 mg MOLE /kg b.wt only for four weeks didn't express any significant changes with the control group in body weights and weight gain. These results were in agreement with **Awodele *et al.* (2012)** and **Atsukwei *et al.* (2014)** who found that normal rats treated with aqueous MOLE and ethanolic MOLE, respectively showed non-significant difference in body weights and weight gain when compared to control rats. These

results indicate that the extract didn't alter the metabolic processes of the treated animals which may subsequently affect the hormones and body weight so it is recommended in maintaining body weight.

The treatment of lead intoxicated rats with 200 mg/kg b.wt aqueous MOLE significantly (P< 0.05) promoted the body weight and weight gain at the 3rd and 4th weeks to a level that is comparable to control rats when compared to L.A group. These results may be attributed to the nutritive and antioxidant power of MO. In contrast to our results **Elabd *et al.* (2018)** observed that treatment with 200 mg/ kg aqueous MOLE was effective in reducing weight gain, body weights and the consequent metabolic disturbance in obese group mice following high fat diet (H.F.D) feeding.

Regarding to feed intake and feed efficiency our results showed that L.A intoxicated rats resulted in a significant (P< 0.05) increase in feed intake and a significant (P< 0.05) reduction in feed efficiency in relation to control group. These results matched with those obtained by **Ibrahim *et al.* (2013)** who found that there was a significant decrease in feed efficiency along with a significant increase in feed intake in lead intoxicated rats when compared to control group. These results indicated that feed efficiency was concurred with the gain in body weight but not with feed intake. In contrast to our results **El-Nekeety *et al.* (2009)** and **Liu *et al.* (2012b)** who observed that rats administered L.A showed a significant decrease in feed intake with subsequent reduction in body weights and weight gain when compared to control rats.

Current results demonstrated that rats treated with 200 mg/kg b.wt aqueous MOLE only showed a significant (P< 0.05) decrease in feed intake without affecting feed efficiency when compared to control group. These results were in harmony with those of **Awodele *et al.* (2012)** who found that rats treated with

different doses of aqueous MOLE revealed a significant reduction in feed intake without subsequent reduction in body weight and feed efficiency when compared to control rats. These results indicated that, MO may serve as food supplements owning to its nutritional value that attributed to presence of important minerals, proteins, vitamins, β-carotene, amino acids and various phenolics.

Current results demonstrated that treatment of lead intoxicated rats with 200 mg/kg b.wt aqueous MOLE revealed a significant ($P< 0.05$) decrease in feed intake along with a significant increase in feed efficiency when compared to L.A group. In contrast to our results, **Chattopadhyay *et al.* (2011)** found that treatment of arsenic intoxicated rats with aqueous extract of MO seeds didn't show any alternation in feed intake.

Regarding to liver weights and relative liver weights our results revealed that there were no significant changes in absolute liver weight between L.A intoxicated rats and control rats. These results came in agreement with **Pandya *et al.* (2010)** who observed that rats treated with L.A didn't show change in absolute liver weight when compared to control rats. However, L.A treated animals expressed a significant elevation in relative liver weight when compared to corresponding control. These results were in agreement with **Ibrahim *et al.* (2012)** and **Badary (2017)** who found that there was a significant ($P< 0.05$) elevation in relative liver weight in L.A intoxicated rats when compared to control rats. They attributed the increment in relative liver weight to liver necrosis, accumulation of lipid in it and cholesterogenesis. Opposite to our results **Mabrouk *et al.* (2016)** noticed no significant difference in relative liver weight in L.A intoxicated rats when compared with control rats, they indicated the absence of overt general toxicity.

Current results revealed that MOLE+ L.A treated group showed non-significant difference in absolute liver weight and relative liver weight when compared to L.A group. These results matched to those obtained by **Chattopadhyay *et al.* (2011)** who observed that co-administration of aqueous extract of MO seeds to arsenic intoxicated rats showed insignificant change in relative liver weight when compared to arsenic group.in contrast to our result **Sadek *et al.* (2017)** found that administration of ethanolic extract of MO leaves to DEN treated rats diminished the relative liver weight compared to the DEN group. Furthermore, our results showed that MOLE treated group didn't express any significant difference in both absolute liver weight and relative liver weight when compared to control group. These results came in agreement with **Sadek *et al.* (2017)** who observed that there was no significant difference in the relative liver weights and liver weights between control rats and normal rats treated with ethanolic MOLE.

Hepatic enzymes (AST, ALT and ALP) are widely used as the most specific indicators of liver injury. They help in diagnosis of any abnormalities in liver. Leakage of these enzymes into plasma indicates the sign of hepatic tissue damage **(Badary, 2017)**. ALT is the most sensitive indicator of acute liver damage and elevation of this enzyme in non-hepatic disease is unusual because the largest pool of ALT is found in cytosol of hepatic parenchymal cells. Whereas, AST is found in cytosol and mitochondria of hepatocytes and also found in cardiac muscle, skeletal muscle, pancreas and kidney **(Shyamal *et al.*, 2006)**.

Our results revealed that ALT, AST and ALP levels in L.A intoxicated rats increased significantly ($P< 0.05$) when compared to control group. The same results obtained by **El-Tantawy (2016)** who observed that rats administered 100 mg/kg L.A for four weeks significantly ($P< 0.05$) increased ALT and AST activities when compared to the control group. The elevation in these enzymes because lead disrupt the tissues membrane. Consequently, there will be a

discharge of the cell content into the blood stream and led to these elevations. Also our results were in agreement with those obtained by **Abdel-Moneim *et al.* (2011)** and **Badary (2017)** who found that there were a significant elevation in ALT, AST and ALP activities in L.A intoxicated rats when compared to control rats. Our results contradict with earlier studies of **Stoimenov-Jevtović *et al.* (2003)** who demonstrated non-significant alternations in the activity of ALT and AST enzymes in lead treated rats when compared with control rats. Moreover, **Sivaprasad *et al.* (2004)** noticed that L.A administration caused a significant decrease in the activities of ALT, AST and ALP when compared to control rats.

Current data also demonstrated that treatment of L.A intoxicated rats with aqueous MOLE induced a significant reduction in ALT, AST and ALP enzymes in relation to rats treated with lead only. These results indicated that aqueous MOLE have hepatoprotective effect. Our results were parallel to findings of **Nadro *et al.* (2006)** who demonstrated that oral co-administration of 200 mg/kg aqueous MOLE to alcohol treated rats significantly decreased elevated levels of ALT, AST and ALP. These results were attributed to the ability of MO to stabilize cell membrane and prevent the release of hepatic enzymes from hepatocytes into the blood stream, hence decreased these enzymes. Moreover, our results came in agreement with previous studies of **Toppo *et al.* (2015)** and **Mallya *et al.* (2017)** who observed that co-administration of hydro-ethanolic extract of MO leaves to cadmium intoxicated rats reduced the elevated levels of ALT, AST and ALP significantly when compared to cadmium treated rats. These results proved the non-toxic nature as well as the hepatoprotective effect of MO against various toxic metabolites that attributed to presence of quercetin and kaempferol.

In the present study our results showed that MOLE treated group showed no significance difference in ALT, AST and ALP in relation to control group. The same result obtained by **Awodele *et al.* (2012)** who found that normal rats treated

with different doses of aqueous MOLE revealed non-significant alternations hepatic enzymes in MOLE treated rats when compared to control rats.

Regarding to serum proteins our results showed that L.A group had a significant ($P< 0.05$) reduction in total protein, albumin, globulin and A/G ratio in relation to control group. These results were in harmony with those of **El-Nekeety *et al.* (2009)**; **Mehana *et al.* (2012)** and **El-Tantawy (2016)** who found that rats treated with L.A showed a significant ($P< 0.05$) decrement in the levels of total protein, albumin and globulin when compared to control rats. These results may be attributed to that lead binds to plasma proteins, where it causes alterations in a high number of enzymes. It can also perturb protein synthesis in hepatocytes or may be attributed to a decreased utilization of free amino acids for protein synthesis. Also, this decrease in protein values may be a result of damage of liver responsible for protein biosynthesis as well as renal tissue damage. On contrast to our results **Mahaffey *et al.* (1981)** found that lead exposed rats showed normal serum total proteins and albumin when compared with normal rats.

Present results revealed that administration of 200 mg/kg aqueous MOLE to normal rats didn't produce any significant difference in total protein, albumin, globulin and A/G ratio in relation to control group. These results were similar to those obtained by **Gupta *et al.* (2012)** who found that treatment of normal rats with methanolic MOLE had no effects total protein and albumin levels when compared to control rats. Also, **Sadek *et al.* (2017)** found that treatment of normal rats with ethanolic MOLE didn't produce any significant change in the total proteins when compared to normal control group.

Current study also demonstrated that treatment of lead intoxicated rats with 200 mg/kg aqueous MOLE expressed that there were a significant elevation in total protein, albumin, globulin and A/G ratio in relation to L.A group. These results

were matched with results obtained by **Hamza (2010)** who found that treatment of CCl4 with hydro-ehanolic extract of MO seeds was markedly abolished the decrease in serum albumin level induced by CCl4 and increased serum globulin level . Also, **Sadek *et al.* (2017)** observed that administration of ethanolic MOLE to DEN group relieved and deflected the decrease in the total protein and albumin significantly when compared to DEN group. These results indicated that MO extracts stabilized the serum total protein and albumin levels. The stabilization of proteins might be considered as an indication of enhanced protein synthesis in the hepatic cells due to inhibition of lipid peroxidation and scavenging of the free radicals.

Regarding to renal function our results showed that administration of L.A produced a significant increase in serum creatinine and urea levels in compare with the control group. These results came in agreement with previous studies of **Liu *et al.* (2012b)** and **Missoun *et al.* (2010)** who observed that there were a significant increase ($P< 0.05$) in serum urea and creatinine in lead treated rats when compared to control rats. These enzymes are considered as indicators for renal function in mammals. These results may be due to lead induce disruption of delicate oxidant/ antioxidant balance, which can lead to kidney injury via oxidative damage or because kidney is vulnerable to damage due to larger perfusion and the increased concentrations of excreted compounds which occur in renal tubular environment.

Current results showed that treatment of normal rats with aqueous MOLE produced non-significant changes in serum urea and creatinine in comparison with the control group. The decrease in in serum urea and creatinine indicating the anti-nephrotoxic effect of MO. These results matched with those obtained by **Awodele *et al.* (2012)** who found that normal rats treated with different doses of

aqueous MOLE didn't alter serum urea and creatinine levels when compared with control rats.

Also our results showed that administration of aqueous MOLE to lead intoxicated rats resulted in a significant ($P< 0.05$) reduction in creatinine and urea levels in relation to the L.A group. These results were in agreement with previous studies of **Maduka *et al.* (2014)** who found that treatment with aqueous MOLE to APAP intoxicated rats significantly ($P< 0.05$) reduced the elevated serum creatinine and urea levels when compared to APAP treated group and **Karthivashan *et al.* (2016)** who observed that co-administration of varying doses of MOLE to APAP intoxicated mice significantly ($P< 0.05$) decreased the serum creatinine and urea levels when compared to APAP treated group. They demonstrated the capability of MO leaves to modulate and restore serum creatinine and urea levels induced by several nephrotoxic agents. They attributed these results to the ability of MO leaves to scavenge free radicals, which is attributed to presence of phenolic compounds, β-carotene.

Regarding to lipid profile our results showed that L.A significantly ($P< 0.05$) increased TC, TG and LDL-C along with a significant decrease in HDL-C levels when compared to control rats. Our results concided with those obtained by **Newairy and Abdou (2009)** who found that in lead treated rats there were a significant increase in TC, TG and LDL-C along with a significant decrease in HDL-C levels and **Ponce-Canchihuamán *et al.* (2010)** who observed the same alternations in lipid profile with lead administration. The association between lead exposure and high serum lipid levels is attributed to either increased synthesis or decreased removal of lipoproteins. Decreased removal of lipoproteins may occur as a result of the alteration of cell surface receptors for lipoproteins or as a result of the inhibition of hepatic lipoprotein lipase activity. Furthermore, lead has been shown to depress the activity of cytochrome P-450.

This can limit the biosynthesis of bile acids, which is the only significant route for elimination of cholesterol from the body. Increased de novo cholesterol synthesis may be due to a lead induced increase in hepatic enzymes**.** The increased levels of lipids with increased levels of ALT may indicate liver dysfunction.

In contrast to our results **Shalan *et al.* (2005)** noticed no significant changes in lead treated rats in serum total lipid, cholesterol, triglycerides and HDL-C levels. However, serum LDL-C decreased significantly when compared to control rats. Also **El-Nekeety *et al.* (2009)** found there was a significant decrease in TG levels of lead intoxicated rats when compared to control rats.

In the present study administration of 200 mg/kg aqueous MOLE to normal rats showed slight increase without any significant difference in TC, TG and LDL-C levels when compared to control rats .However, it increased ($P< 0.05$) HDL-C level significantly when compared to control rats. These results were similar to the results obtained by **Aborhyem *et al.* (2016)** who found that normal rats treated with 400 mg/ kg hydro-ethanolic MOLE for 4 weeks revealed significant increase in HDL-C level when compared to control rats, while there was insignificant change in TG level when compared to control rats. Also **Oyewo *et al.* (2013)** observed that normal rats administered with (250 mg/kg b.wt) aqueous MOLE resulted in a significant increase in HDL-C level as well as insignificant difference in TC and TG levels when compared to control rats. The increase in HDL-C could be attributed to presence of flavonoids in aqueous MOLE which enhance the biosynthesis of HDL-C in the liver. In contrast to our results **Mansour *et al.* (2014)** observed that normal rats treated with aqueous extract of MO leaves induced significant increase in serum LDL-C ,TC and TG levels and significant decrease in HDL-C levels when compared to the control group.

Our results also revealed that co-administration of MOLE to L.A intoxicated produced a significant reduction in TC, TG and LDL-C along with a significant increment in HDL-C levels in relation to L.A group. These results were similar to those obtained by **Chatterjee *et al.* (2013)** who reported that treatment with aqueous MOLE to cadmium exposed rats presented a decrease in TC, TG, LDL-C and VLDL-C with an increase in the HDL-C levels when compared to cadmium group. Moreover,**Mansour *et al.* (2014)** who found that oral administration aqueous MOLE prior gamma irradiation resulted in significant recovery in TC, TG and LDL-C levels and increased HDL-C levels compared to gamma irradiated groups.

These results indicated that aqueous MOLE has hypolipidaemic effect which could be related to its chemical composition, which indicated the presence of alkaloids, flavonoids, saponin and cardiac glycosides. All these components are known to reduce serum lipid level in animals. Briefly saponins may lower cholesterol by binding with cholesterol in the intestinal lumen, preventing its absorption, and/or by binding with bile acids, causing a reduction in the enterohepatic circulation of bile acids and increase in its fecal excretion **(Rotimi *et al.*, 2011)**. The increased bile acid excretion is offset by enhanced bile acid synthesis from cholesterol in the liver and consequent lowering of the plasma cholesterol. Also β- sitosterol present are the major cholesterol-reducing components of the MO leaves. β- sitosterol helped in reducing cholesterol levels by limiting the amount of cholesterol that is able to enter the body, by inhibiting cholesterol absorption in the intestines **Bordia and Verma (1998)**. Additionally the MOL extract contains polyphenols, which inhibit lipid peroxidation by acting as a chain-breaking peroxyl radical scavenger, and can protect LDL from oxidation. On the other hand, polyphenolic compounds possess a variety of biological activities, such as reduction of plasma lipids, which might be due to

the up-regulation of LDL receptor expression, inhibition of hepatic lipid synthesis and lipoprotein secretion and increase in cholesterol elimination via bile acids **Pari *et al.* (2007)**.

Regarding to hepatic GSH level and SOD activity, our results showed that in L.A intoxicated rats there were a significant ($P< 0.05$) decrease in hepatic GSH level and SOD activity when compared to control group. These results came in agreement with previos studies of **Abdel-Moneim *et al.* (2011)** and **El-Tantawy (2016)** who found that there was a significant decrease in in hepatic GSH level and SOD activity in lead-intoxicated rats when compared to normal rats. The possible explanation could be related to the proposed role of GSH in the active excretion of lead through bile by binding to the thiol group of GSH and then being excreted. A decrease in GSH levels could lead to oxidative stress and a consequent decrease in SOD activity or may be due to the overproduction of ROS induced by lead which induced depletion of antioxidant defense.

Present study showed that treatment of normal rats with aqueous MOLE didn't alter the hepatic GSH level and SOD activity when compared with their corresponding control rats. These results were similar to results obtained by **Awodele *et al.* (2012)** and **Mansour *et al.* (2014)** who found that treatment of normal rats with aqueous MOLE induced no change in GSH level and SOD activity when compared to control rats.

Also our results showed that co-administration of aqueous MOLE to lead intoxicated rats resulted in a significant ($P< 0.05$) increase in hepatic GSH level and SOD activity when compared to L.A group. These results were matched with those of **Mansour *et al.* (2014)** who reported that administration of aqueous MOLE to gamma irradiated group resulted in a significant increase in the activities of SOD and GSH content compared to the irradiated group. The

increase in the SOD activity after the administration of aqueous MOLE because MO protected the tissues from the effects of superoxide ions by enhancing the activities of superoxide dismutase which attributed to the antioxidant properties of MO which may be mediated through direct trapping of the free radicals and also through metal chelation. Also, the increase in these activities due to the existence of high amounts of phenolic compounds and flavonoids such as kaempferol, apigenin, quercetin, and multiflorin in the MOLE, which are likely responsible for advancing antioxidant potential.

Also, **Yassa and Tohamy (2014)** found that administration of 200 mg/kg bwt aqueous MOLE to diabetic rats elevated GSH level significantly in comparison to diabetic group. The increase in the GSH level because leaves of MO contained the major groups of phytochemicals including polyphenols, flavonols, carotenoids and antioxidant vitamins such as vitamins C. and E that contribute to the total antioxidant capacity of plant foods.

Regarding to DNA fragmentation, in the present study the rat of the control group and MOLE group showed slight hepatic DNA fragmentation on agarose gel electrophoresis. These results were in agreement with **Chattopadhyay *et al.* (2011)** who demonstrated that these results may be a normal feature owing to the nature of function of liver, as it receives all absorbed nutrients and toxins in the process of detoxification, that might impart genotoxicity.

Our results revealed that L.A group showed more hepatic DNA fragmentation in rat on agarose gel electrophoresis than control. These results were in agreement with previous studies of **Abdel-Moneim *et al.* (2011)** who observed that the administration of L.A markedly caused more DNA fragmentation in the hepatic tissue than control rats. These results may be due to lead itself genotoxic or potentiate the efficiency of other DNA-damaging agents. Moreover, lead

exposure, may stimulate the formation of ROS affecting free radical scavenging enzymes and glutathione.

Current study also showed that administration of MOLE to lead intoxicated rats resulted in less fragmentation when compared to L.A. These results supported by the observed improvement in GSH level and SOD activity in L.A group treated with MOLE. Our results were matched with **Chattopadhyay *et al.* (2011)** who observed that administration of aqueous extract of MO seeds to arsenic intoxicated rats dramatically lowered the degradation than arsenic group. These results confirmed that MO protect hepatic tissue from oxidative DNA damage. The most probable reason for the DNA protection might be due to presence of high phenolic content. Polyphenols are potential protecting agents against the lethal effects of oxidative stress and offered protection of DNA by chelating redox-active transition metal ions, or may be due to the free radical scavenging activities of MO.

Regarding to liver's histopathology the present study revealed that livers of both control and MOLE groups showed normal hepatic lobules with centrally located central veins, radiating hepatic cords with normal polyhedral hepatic cells and normal hepatic areas. These results came in agreement with earlier studies of **Das *et al.* (2012)** who reported that normal hepatic tissue was observed in control mice and normal mice treated with ethanolic MOLE.

The hepatic tissue of L.A groups revealed focal lymphocytic infiltration of hepatic parenchyma. In addition to hyperplasia of hepatic triad with hyperplastic bile ducts and early fibrous connective tissue proliferation. Diffuse moderate vacuolation of hepatocytes, congestion of central veins, mild to moderated centro-central fibrosis were also observed. These results were supported by increase in serum liver enzymes and hepatic DNA fragmentation. Our results

were matched with the results obtained by **Abdel-Moneim *et al.* (2011)** who found that the hepatic histopathology of lead intoxicated rats showed a severe inflammation, cellular infiltration as well as cytoplasmic vacuolation and degeneration of hepatocytes. Also **Liu *et al.* (2012a)** found that exposure to L.A resulted in histopathological changes in rat's liver such as structure damage, hepatocellular necrosis, leukocyte infiltration and massive hemorrhage. In contrast to our results **Pandya *et al.* (2010)** found that the liver of lead exposed groups did not show marked alteration compared to control group. These variations in histopathological changes could be attributed to the differences in lead dose and route of administration.

In the present study treatment of L.A intoxicated rats with aqueous MOLE revealed pronounced improvement of hepatic lesions with mild to moderate vacuolar degeneration of hepatic cells and mild congestion of central veins. These findings suggested that MO improves liver functions and prevents hepatic damage. Our results were parallel to those obtained by **Singh *et al.* (2014)** who found that the hepatocellular necrosis, congestion and leukocyte infiltration evoked by CCL4 were shown to be minimal or absent with MOLE treatment. These alternations were due to re-establishment of the antioxidant defense system in the liver tissue through the antioxidant and hepatoprotective nature of MO leaves which are attributed to the ß-carotene in MO that trapped free radicals and increased antioxidant activity. The findings suggested that MO improved liver functions and prevented hepatic damage through decreasing the presence of oxidative free radicals. Also **Oliveira *et al.* (2005)** and **Karthivashan *et al.* (2015)** observed that administration of MO to APAP intoxicated mice decreased the degree of liver fibrosis, congestion, inflammation and cell infiltration compared to APAP group**.**

Regarding to renal histopathology, in the present study L.A group showed severe multifocal congestion of both cortex and medulla with focal hemorrhages in medullas. Marked degeneration and focal necrosis of some tubular epithelium were observed, as well as focal proliferative glomerulonephritis and moderate to severe lymphocytic infiltrations along with early intertubular fibrosis were also observed in medullary tissue. Our results came in agreement with **Liu *et al.* (2010)** and **Liu *et al.* (2012b)** who stated that in lead treated rats the renal sections showed extensive glomerular and tubular damage by presence of necrotic epithelial cells, tubular degeneration, necrosis, cell swelling, mononuclear cell infiltration and degenerated organelles. These tubular alterations might be a result lead intoxication that yields a partial failure in the ion pump transport of kidney tubules cells which in turn produces tubular swelling and causes necrosis and vacuolization of the tubules.

Current study revealed that treatment of lead intoxicated rats with aqueous MOLE ameliorated the renal injury induced by lead. These results were parallel to the findings of **Karthivashan *et al.* (2016)** who observed that after treatment APAP intoxicated mice with MO, the glomerular and tubular architecture were well preserved in relation to APAP group. The restoration of normal renal architecture after MO administration to APAP intoxicated mice may be attributed to its antioxidant potential of MO due to presence of polyphenols, flavonols, carotenoids and antioxidant vitamins such as vitamins C and E.

Taking together all data, it was clear that lead can cause serious perturbation in hepatic and renal tissue that accompanied with increments in hepatic and renal injury biomarkers. This perturbations could be attributed to its ROS producing powers that caused cellular injury liberation of hepatic enzymes as well as increments in urea and creatinine. Also, lipid profile abnormalities beside reduction in total protein, albumin, globulin and A/G ratio which are major liver

products. All this manifested by increased DNA breakage. The administration of aqueous MOLE with its various antioxidant and protective constituents; restored the lead induced perturbations to a significant level better than lead group and sometimes to comparable level of control values.

Conclusion

6. Conclusion

From the overall observation in the present study, it is concluded that:

Lead had toxic effects on several body organs especially liver and kidney. The different adverse effect of lead intoxication could be ameliorated via aqueous MOLE through:

1. Amelioration of lead-induced impairment on body weight, weight gain, feed intake and feed efficiency via MOLE co-administration.
2. Amelioration of lead-induced deteriorations in ALT, AST, ALP, total protein, albumin, globulin, A/G, urea and creatinine through co-administration of aqueous MOLE.
3. Amelioration of lead-induced lipid profile abnormalities via co-administration of aqueous MOLE
4. Amelioration of lead-induced oxidative stress via restoring hepatic GSH level and SOD activity with co-administration of aqueous MOLE.
5. Reducing lead-induced DNA fragmentation in hepatocytes via aqueous MOLE co-administration.
6. Improvement of lead-induced hepatic and renal tissue damages via aqueous MOLE co-administration.

Recommendations

7. Recommendations

1- Animals and humans exposure to Lead should be avoided as much as possible as it induces a wide range of hepatic and renal biochemical and physiological dysfunctions.
2- Aqueous MOLE has an excellent antioxidant effect, hepatic, renal and DNA protection against lead toxicity so, it's recommended for oral use to counteract lead toxicity.
3- MOLE organic solvents extract may contain less-polar active substances those may achieve higher intercellular and intracellular concentrations. Hence, further studies on MO still needed.

Summary

8. Summary

Lead is one of the most widespread heavy metal in the environment that used for both domestic and industrial purposes and possesses serious harms to both humans and animals. Previous studies on lead exposed animals showed that liver is considered the main reservoir (33%) for lead among the soft tissues.

MOLE as phytotherapy has been widely used for its antioxidant activity against the free radicals due to its active ingredients like flavonoids, carotenoids, phenolics and ascorbic acid. MOLE was tested in the current study to counteract lead intoxication.

Current study was carried out using 24 male albino rats weighting 150-190 g. The rats kept two weeks for adaptation period prior to start of the experiment and they housed in cages under optimal environmental conditions. Rats were divided into to 4 equal groups' six rats each.

Group I (control group): fed on the basic diet and gavaged with 2 ml distilled water daily for 4 weeks.

Group II (MOLE group): fed on the basic diet and administered with 200 mg/kg b.wt of aqueous MOLE daily by oral gavage for 4 weeks.

Group III (lead acetate group): fed on the basic diet and administered with 100 mg/kg b.wt of L.A daily by oral gavage for 4 weeks.

Group IV (lead acetate group+ MOLE): fed on the basic diet and administered with 100 mg/kg b.wt of L.A plus 200 mg/kg b.wt of aqueous MOLE daily by oral gavage for 4 weeks.

The body weight, body weight gain were recorded weekly, feed intake and feed efficiency were recorded weekly. At the end of experiment at day 28^{th}, all

rats were sacrificed under the effect of diethyl ether inhalation anesthesia blood samples were drawn into labeled tubes for sera separation. Liver was excised, weighed to determine relative liver weight and sliced into three parts, two parts of them were quickly frozen in dry ice and stored at -80 $^{\circ}$C for GSH and SOD analysis and DNA fragmentation test. Also the remaining part of the liver in addition to the kidney specimens were preserved in 10% formalin for histopathological study. Serum biochemical parameters as liver enzymes, liver proteins, kidney enzymes and lipid profile were determined.

The obtained results can be summarized as follow:

Regarding to growth performance parameters, there were a significant ($P< 0.05$) decrease in body weight, weight gain at the 3rd and 4th weeks and feed efficiency as well as a significant ($P< 0.05$) increase in food intake in L.A group when compared to control group. Administration of aqueous MOLE to lead intoxicated rats revealed a significant ($P< 0.05$) increase in body weight, weight gain at the 3rd and 4th weeks to a level comparable to control group and feed efficiency with a significant decrease in food intake when compared to L.A group. However, there were no significant changes in MOLE group in body weight, weight gain and feed efficiency, while a significant ($P< 0.05$) decrease in food intake was observed when compared to control group.

Regarding to absolute and relative liver weights, current results showed that there were no significant difference in absolute liver weight between the four groups. However, there was a significant ($P< 0.05$) increase in relative liver weight in L.A group when compared to control group. Administration of aqueous MOLE to lead intoxicated rats revealed a significant ($P< 0.05$) decrease in relative liver weight to a level comparable to control group when compared to L.A group. Whereas MOLE group didn't express any significant difference in relative liver weight when compared to control group.

Regarding to liver enzymes activity, L.A group revealed a significant (P< 0.05) increase in ALT, AST and ALP when compared to the control group. Administration of aqueous MOLE to lead intoxicated rats expressed a significant (P< 0.05) decrease in ALT and AST activities in comparison to the L.A group, however they were significantly (P< 0.05) higher than the control group as well as a significant (P< 0.05) decrease in ALP in comparison to the L.A group to a level comparable to control. MOLE didn't differ statistically when compared with control group.

Concerning the serum proteins, there were a significant (P< 0.05) reduction in L.A group in T.protein, albumin, globulin values and A/G ratio when compared to the control group. Administration of aqueous MOLE to lead intoxicated group expressed a significant (P< 0.05) increment in T.protein and albumin values in comparison to L.A group as well as insignificant difference in globulin and a significant (P< 0.05) elevation in A/G ratio when compared to L.A group. Whereas MOLE group showed insignificant difference in T.protein, albumin, globulin values and A/G ratio when compared with control group.

Regarding to renal functions, creatinine and urea levels significantly (P< 0.05) increased in L.A group when compared to control group. Co-administration of aqueous MOLE to lead intoxicated group revealed a significant (P< 0.05) decrease in creatinine and urea levels in comparison to the L.A group. MOLE group showed insignificant difference in creatinine and urea levels in relation with the control group.

Regarding to lipid profile, our results showed that the L.A group expressed a significant (P< 0.05) increase in TC, TG and LDL-C values along with a significant (P< 0.05) decrease in HDL-C value. Co- administration of aqueous MOLE to L.A intoxicated rats revealed a significant (P< 0.05) decrease in TC, TG and LDL-C values along with a significant (P< 0.05) increase in HDL-C value

when compared to control group. However, control rats administered with aqueous MOLE revealed a significant ($P< 0.05$) increase in HDL-C values without any significant difference in TC, TG and LDL-C values when compared to control rats.

Regarding to antioxidant enzymes, there were a significant ($P< 0.05$) decrease in GSH level and SOD activity in L.A group when compared to control group. Co-administration of MOLE to L.A intoxicated group expressed a significant ($P< 0.05$) increase in GSH level and SOD activity when compared to the L.A group. Control rats administered with aqueous MOLE insignificant alternation when compared with control group.

Regarding to DNA fragmentation, the rat's livers of the control group and MOLE group showed slight DNA fragmentation on agarose gel electrophoresis. On the other hand the L.A group showed more DNA fragmentation on agarose gel electrophoresis than control and MOLE groups. Co-administration of MOLE to L.A intoxicated group dramatically lowered degradation than L.A group.

Concerning the histopathological examination, control and MOLE groups showed normal hepatic and renal tissue architecture. On the other hand in L.A group the hepatic tissue showed focal lymphocytic infiltration of hepatic parenchyma, hyperplasia of hepatic triad with hyperplastic bile ducts and early fibrous connective tissue proliferation as well as diffuse moderate vacuolation of hepatocytes, congestion of central veins, mild to moderated centro-central fibrosis were also observed, while renal tissue showed severe multifocal congestion of both cortex and medulla with focal hemorrhages in medullas and multifocal degeneration, necrosis and cystic dilatation of renal tubules. Co-administration of aqueous MOLE to L.A group revealed pronounced improvement and protection in hepatic and renal tissues.

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الملخص العربى

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يعتبر الرصاص أحد أكثر المعادن الثقيلة إنتشارا في البيئة حيث يستخدم في معظم الأغراض المنزلية والصناعية محدثا للكثير من الأضرار الخطيرة لكل من البشر والحيوانات. وقد أظهرت الدراسات السابقة على الحيوانات المعرضة للرصاص أن الكبد هوالعضو الأكثر تضررا بالرصاص من بين الأنسجة، كذلك يؤدى الرصاص إلى زيادة الجهد التأكسدى نتيجة خلل فى التوازن بين إطلاق وإزالة جزيئات الاكسجين الحرة فى الأنسجة مما يؤدى إلى تلفها.

حصل العلاج بالنباتات الطبية فى الأونة الأخيرة على إهتمام الباحثين في جميع انحاء العالم. حيث يتم استخدام النباتات الطبية التي تحتوى علي مضادات الأكسدة للحماية من السموم المختلفه بما في ذلك المعادن الثقيلة. وأوضحت الأبحاث دور مستخلص أوراق نبات المورينجا اوليفيرا فى منع الأضرار التأكسدية التي تتعرض لها الأنسجه حيث أن لها قدرة كبيرة في منع الضرر التأكسدي اعتمادًا على وجود العديد من المركبات المضادة للأكسدة مثل الفلافونويد والكاروتينات و الفينولات وحامض الأسكوربيك.

أجريت هذه الدراسة لأختبار التأثيرالواقى لمستخلص أوراق نبات المورينجا أوليفيرا علي الكبد والكلي وكذلك الحمض النووي بالكبد من التسمم الناجم عن التعرض لخلات الرصاص.

أجريت الدراسة علي عدد 24 من ذكورالجرذان تتراوح أوزانهم من 150 الى 190 جم وتم تقسيمهم الى أربع مجموعات وهي:

- **المجوعه الأولي**: المجموعة الضابطة السالبه والتى تم تغذيتها على العليقة الاساسية مع تجريع 2 مللى/ كجم مياه مقطره يوميا لمدة أربعة أسابيع.
- **الجموعة الثانية:** تغذت على العليقة الاساسية وتم تجريعها بستخلص نبات المورينجا اوليفيرا بجرعة 200 مجم/ كجم من الوزن الحي يوميا لمدة أربعة اسابيع.
- **المجموعة الثالثة:** تغذت على العليقة الاساسية وتم تجريعها بمادة خلات الرصاص بجرعة 100 مجم/ كجم من الوزن الحي يوميا لمدة أربعة اسابيع لأحداث التأثير السمي المطلوب.
- **المجموعة الرابعة:** تغذت على العليقة الاساسية وتم تجريعها بمادة خلات الرصاص بجرعة 100 مجم/ كجم من الوزن الحي ومستخلص نبات المورينجا اوليفيرا بجرعة 200 مجم/ كجم من الوزن لمدة أربعة اسابيع .

تم تسجيل كلا من وزن الجرذان ومعدل زيادة الوزن وكذلك كمية العلف المستهلكة أسبوعيا مع حساب الكفاءة الغذائية.

تم قتل الحيوانات بشكل رحيم فى نهاية التجربة وتجميع الدم لفصل مصل الدم لقياس الدلالات البيوكيميائيه وهى (انزيمات الكبد و بروتينات الدم و وظائف الكلي والدهون) وكذلك تم استخراج الكبد ووزنه وتقسيمه إلى ثلاثة أجزاء بحيث تم تجميد جزئين لقياس مستوي الجلوتاثيون المختزل (GSH) والسوبر أوكسيد ديسميوتيز (SOD) وكذلك معدل تكسير الحمض النووي الديوكسي ريبوز، والجزء المتبقى من الكبد مع جزء من الكلى تم وضعه فى محلول 10 ٪ فورمالين لعمل قطاعات نسيجيه.

وقد أسفرت الدراسة عن النتائج التالية:

1. تسببت خلات الرصاص في انخفاض معنوي فى أوزان الجران ووزن الجسم المكتسب وكذلك الكفاءة الغذائية بالمقارنة بالمجموعة الضابطة بينما مستخلص نبات المورينجا أوليفيرا لم يسبب اي فروق معنويه في كل من وزن الجران ووزن الجسم المكتسب وكذلك الكفاءة الغذائيه بالمقارنه بالمجموعه الضابطة، بينما اظهرت المجموعه التى تناولت مستخلص نبات المورينجا اوليفيرا والرصاص زيادة معنويه فى كل من وزن الجرذان ووزن الجسم المكتسب بالاضافه الي الكفاءة الغذائيه مقارنة بالمجموعة التى تناولت خلات الرصاص فقط.
2. نتيجة لقياس وزن الكبد المطلق وجد انه لايوجد فروق معنويه بين المجموعات الاربعه بينما أظهرت نتائج قياس الوزن النسبى للكبد وجود زياده معنوية فى المجموعه التى تناولت خلات الرصاص بالمقارنة مع المجموعه الضابطه السالبه، كذلك أظهرت النتائج ان استخدام مستخلص نبات المورينجا اوليفيرا مع الرصاص فى المجموعه الرابعه سبب انخفاضا معنويا فى الوزن النسبى للكبد مقارنة بمجموعة خلات الرصاص، بينما لم يظهراستخدام مستخلص نبات المورينجا اوليفيرا فى المجموعه الثانيه أى فروق معنوية فى الوزن النسبى للكبد مقارنة بالمجموعه الضابطه السالبه.
3. وجدت زيادة معنوية فى انزيمات الكبد فى المجموعه التى تناولت خلات الرصاص مقارنة بالمجموعة الضابطة السالبة، بينما سبب إستخدام مستخلص نبات المورينجا اوليفيرا مع الرصاص فى المجموعه الرابعه انخفاضا معنويا في انزيمات الكبد مقارنة بمجموعة خلات الرصاص، وفى المجموعه الثانيه التي تم فيها استخدام مستخلص نبات المورينجا اوليفيرا فقط لم يحدث اى زيادة معنوية في انزيمات الكبد مقارنة بالمجموعة الضابطة السالبة.

4. تحليل وظائف الكلى أوضح ان استخدام خلات الرصاص قد تسبب في احداث زيادة معنوية لكل من يوريا الدم وكذلك الكرياتينين مقارنة بالمجموعات الضابطة، بينما استخدام مستخلص نبات المورينجا اوليفيرا مع الرصاص فى المجموعه الرابعه قد سبب انخفاض معنوي في يوريا الدم وكذلك الكرياتينين مقارنة بمجموعة خلات الرصاص، اما بالنسبة لاستخدام مستخلص نبات المورينجا اوليفيرا فقط فى المجموعه الثانيه لم يحدث اى زيادة معنوية في انزيمات الكبد مقارنة بالمجموعة الضابطة السالبة.
5. أظهر قياس بروتينات الدم أن خلات الرصاص قد تسببت في إحداث إنخفاض معنوي لكل من البروتين الكلي والألبيومين والجلوبيولين وكذلك نسبة الألبيومين/الجلوبيولين مقارنة بالمجموعة الضابطة السلبية بينما إستخدام مستخلص نبات المورينجا اوليفيرا مع الرصاص في المجموعه الرابعه قد تسبب في إرتفاع معنوي لكل من البروتين الكلي، الألبيومين و نسبة الألبيومين/الجلوبيولين لكنه لم يحدث اى زيادة معنوية فى الجلوبيولين مقارنة بمجموعة الرصاص، لم يحدث استخدام مستخلص نبات المورينجا اوليفيرا فقط فى المجموعه الثانيه اى فروق معنوية في البروتين الكلي، الالبيومين، الجلوبيولين وكذلك نسبة الألبيومين/الجلوبيولين مقارنة بالمجموعة الضابطة السلبية.
6. أظهرت نتائج تحليل دهون الدم أن استخدام خلات الرصاص قد تسبب في إحداث زيادة معنوية فى الكوليسترول والدهون الثلاثية وكذلك الكوليسترول منخفض الكثافة بينما سبب إنخفاض معنوي في الكوليسترول عالي الكثافة مقارنة بالمجموعة الضابطة، بينما أدى إستخدام مستخلص نبات المورينجا اوليفيرا مع الرصاص في المجموعه الرابعه إلى إنخفاض معنوي للكوليسترول والدهون الثلاثية وكذلك الكوليسترول منخفض الكثافة مع زيادة معنوية للكوليسترول عالي الكثافة مقارنة بمجموعة خلات الرصاص، كما أسفر استخدام مستخلص نبات المورينجا اوليفيرا فقط فى المجموعه الثانيه عن إحداث زيادة معنويه فى الكوليسترول عالي الكثافة لكنه لم يحدث أى زيادة معنوية في كل من الكوليسترول و الدهون الثلاثية وكذلك الكوليسترول منخفض الكثافة مقارنة بالمجموعة الضابطه السالبه.
7. وجد أن إستخدام خلات الرصاص قد تسبب في احداث انخفاض معنوى لمستوى الجلوتاثيون المختزل والسوبر أوكسيد ديسميوتيز مقارنة بالمجموعة الضابطة السلبية، بينما أدى إستخدام مستخلص نبات المورينجا اوليفيرا مع الرصاص في المجموعات الرابعه الي إرتفاع معنوي في مستوي الجلوتاثيون المختزل وكذلك مستوى السوبر أوكسيد ديسميوتيز مقارنة بمجموعة خلات الرصاص، لم يحدث استخدام مستخلص نبات المورينجا اوليفيرا فقط فى المجموعه

الثانيه اى فروق معنويه فى مستوي الجلوتاثيون المختزل وكذلك مستوى السوبر أوكسيد ديسميوتيز مقارنة بالمجموعة الضابطة.

8. أدى استخدام خلات الرصاص الي احداث تكسير في الحمض النووي الديوكسي ريبوزى الكبدى بينما استخدام مستخلص نبات المورينجا اوليفيرا مع الرصاص في المجموعة الرابعه ادى الى حدوث تحسن ملحوظ في منع تكسير الحمض الديوكسي ريبوزى.
9. أظهرت القطاعات النسيجية أن إستخدام خلات الرصاص أدى إلي إحداث تلف في انسجة الكبد والكلى بينما إستخدام مستخلص نبات المورينجا اوليفيرا أدى إلى تحسين الصورة المرضية الناتجة عن إستخدام خلات الرصاص.

الخلاصه والتوصيات

يظل الرصاص واحدا من أكثر المعادن الثقيله إنتشارا وأكثرها خطرا لما له من أضرار جسيمه على العديد من أجهزة الجسم المختلفه وخصوصا الكبد والكلى. كما أتضح من هذه الدراسه أن هذه الأثار السامة يمكن أن تخفف باستخدام مستخلص نبات المورينجا اوليفيرا من خلال:

- مستخلص المورينجا اوليفيرا له القدرة علي تخفيف الأثار السمية للرصاص ويظهر ذلك من خلال تحسين وزن الجسم ومعدل زيادة وزن الجسم ومعدل تناول الغذاء وكذلك الكفاءه الغذائية.
- قام مستخلص المورينجا اوليفيرا بتحسين الأثر المدمر الذي لحق بالانسجه ويظهر ذلك من خلال التحسن الواضح في وظائف الكبد المتمثلة فى انزيمات الكبد والألبيومين والجلوبيولين والنسبه بين الألبيومين و الجلوبيولين بالاضافه الي تحسن وظائف الكلى التي تمثلت فى مستوى الكرياتينين واليوريا.
- تخفيف الاثر السام للرصاص على دلالات الدهون من خلال تعاطى مستخلص المورينجا اوليفيرا مع الرصاص.
- قدرة مستخلص نبات المورينجا اوليفيرا علي تقليل الجهد التأكسدى الناجم عن التسمم بالرصاص من خلال إعادة مخزون الجلوتاثيون المختزل بالكبد وكذلك انزيم السوبر أوكسيد ديسميوتيز.
- حماية الحمض النووى فى خلايا الكبد من التكسير والتلف الناجم عن السمية بالرصاص وذلك باستخدام مستخلص المورينجا اوليفيرا مع الرصاص.
- تناول مستخلص المورينجا اوليفيرا مع الرصاص أدى الى تخفيف الضرر الناجم عن السمية بالرصاص فى القطاعات النسيجية للكبد والكلى.

وبناءا علي هذه النتائج توصي الرساله بالاتي:

- مراعاة تجنب تعرض الانسان والحيوان للرصاص لما له من أثار سميه عديده خاصة علي الكبد والكلى.
- يوصى بإستخدام مستخلص نبات المورينجا اوليفيرا لاحتوائه على فوائد طبية عظيمة تقي وتعالج الكثير من الأمراض والسموم.

جامعة قناة السويس- كلية العلوم

ادارة الدراسات العليا والبحوث

موافقة مجلس الكلية

وافق مجلس الكلية بتاريخ / /2019م كما وافق السيد أ.د/ نائب رئيس الجامعة بتاريخ / /2019م على منح درجة الماجستير فى العلوم كيمياء تخصص كيمياء حيوية وبيولوجية للطالبة / أريج عبد الرحمن رضا عبد السلام من الخارج:

عنوان الرسالة

التأثير الوقائى الكبدى لمستخلص نبات المورينجا اوليفيرا على تلف الحمض النووى الناجم عن الرصاص فى الجرذان

Hepatoprotective Effect of *Moringa oleifera* Extract on Lead Induced DNA Damage in Rats

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قسم الكيمياء

التأثير الوقائى الكبدى لمستخلص نبات المورينجا اوليفيرا على تلف الحمض النووى الناجم عن الرصاص فى الجرذان

رسالة مقدمة من الطالبة

أريج عبد الرحمن رضا عبد السلام

(بكالوريوس فى العلوم، قسم الكيمياء، جامعة قناة السويس 2012)

كمتطلب للحصول على درجة الماجيستير فى العلوم

فى

الكيمياء الحيوية والبيولوجية

الى

قسم الكيمياء

كلية العلوم

جامعة قناة السويس

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