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Protective effect of grape seeds oil (Vitis Vinifera) on methomyl induced liver damage in albino rats

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SUMMARY

Liver damage was induced in adult male albino rats, weighting about 130-140 g, by oral administration of methomyl with single dose at two concentrations (1/10 or 1/20 LD_{50}/day for short term (4 weeks) and long term (8 weeks). The markers of liver damage were investigated by evaluating biochemical parameters in serum and liver tissues such as activities of aspartate aminotransferase (AST), alanine aminotrasferase (ALT)), alkaline phosphatase (ALP) , total bilirubin (TB), total protein (TP), total lipids (TL), total cholesterol (TC), triacylglycerols (TAG) and malondialdhyde (MDA) levels. In addition serum urea, creatinine and uric acid were assessed after 4 and 8 weeks. The effect of oral administration of grape seed oil (GSO) 4 ml/kg. Body weight/day on the above parameters for 4 and 8 weeks were investigated as a protective and antihepatotoxic effect.

Oral administration of methomyl with single dose of concentration (1/10 or 1/20 LD_{50}/day for 4 and 8 weeks, showed significant increase ($p < 0.05$) in serum liver enzymes activity (ALT, AST, ALP), TB, and MDA levels, while total protein showed a highly significant reduction as compared to negative control and control group received GSO, and the more effect was observed at the high dose of methomyl 1/10 LD_{50}. ALT was not affected at the methomyl dose 1/20 LD_{50}/day after 4 weeks. Serum lipid profile (TL, TC and TAG), creatinine and uric acid were also elevated in methomyl intoxicated rats with slight changes in TC and TL as compared to normal group at dose 1/20 LD_{50}/day after 4 and 8 weeks. A significant decrease ($p < 0.05$) and a high decrease ($p < 0.01$) in urea values were observed after short and long term in methomyl intoxicated rats. In liver tissues, elevation was found in ALP, TB, MDA, TC and TL levels after long term, while ALT, AST and total

protein were significantly decreased, and intoxicated group treated with methomyl 1/10 LD_{50}/day for 4 and 8 weeks was more acute effect as compared to control groups.

Oral treatment with grape seed oil GSO (4 ml/kg. body weight/day improved all the above parameters being almost similar to control values with treated low dose of methomyl.

Key words: Liver damage – methomyl - biochemical parameters - oral administration grape seed oil (GSO).

INTRODUCTION

Carbamates are widely used in industry, agriculture and public health purposes. They were detected in well water and certain plants (Abdel-Rahman et al., 1985). Insecticides, even in very low concentration, have been reported to interfere with basal metabolism (Write et al., 1977).

Methomyl is a carbamate pesticide, markted as an aqueous solution and in solid form with brand names like Lannate, Nudrin, Metomex and Terlate (Tsatsakis et al., 1996). The active ingredient in Lannate is methomyl, (S – methyl – N - (methyl carbomyl) - thioacetimidate), a compound of the oxime carbamate group. The methomyl might have affected cell metabolism, and cell membrane permeability and detoxification system in liver (Manawadi and Kaliwal 2010).

Methomyl inhibits acetylcholinesterase activity that results in increased acetylcholine levels. The accumulation of acetylcholine disrupts the normal nerve synaptic function which affects mainly the peripheral nervous system (Ekins and Geller 1994). In mice and rats, levels and activities of antioxidant defense system enzymes (e.g. glutathione-S-transferase and superoxide dismutase) and lipid peroxidation were altered due to methomyl intoxication which indicates oxidative stress. Pesticidies like methomyl have shown to cause overproduction of reactive oxyg molecute. When the reactive forms of oxygen are produced faster than they can be safely neutralized by antioxidant mechanisms, oxidative stress and potential tissue damage are likely to occur (Garg et al., 2009 and Maansour et al., 2009).

El-khawaga 92005) found that the distribuon of methomyl in different organs of mice injected with methomyl in a dose of 7 mg/kg body weight (µg/g tissue) was for: liver 37. 69±3.4, spleen 295±1ti9.0, kidney 225.7±34.0, stomach 72.97±5.6, intestine 142.2±17.0 and lung 803.3±54.0.

Free radicals occur naturally in the body, but different environmental toxins (ultraviolet light, radiation, smoke, certain prescriptive and

non- prescriptive as well as drugs, and air pollution) can also increase the number of these damaging particles

Grape seed oil (GSO) is a vegetable oil pressed from the seeds of various varieties of vitis vinifera grapes. Grape seed oil is obtained from grape seeds after the wine pressing . The GSO contains 75% linoleic acid, 15% oleic acid, 6% palmitic acid, 3% stearic acid, and 1% linolenic acid, (Natella,et al., 2002).

Grape seeds contain antioxidants as polyphenols, including proanthocyanidis (Joshi et al., 2001). Moreover, grape seed oil not only contains nutritionally useful essential fatty acids but also tocopherols (El-Mallah and Murui 1993).

Some studies suggested the use of grape seed oil as a chemoprotictive and cytoprotective agent (Rasmussen et al.,(2005), also it, acts as a potent antioxidant that prevented genotoxicity of bone marrow cells and sperm abnormalities as well as DNA fragmentation (Abd El-Rahim and Hafiz 2009).

Studies by Nash and Nash, (1993) the beneficial HDL-cholesterol effect of GSO and the research showed that pearsons indviduals subjects were advised to use up 45 ml of GSO in their daily diet as a substitute for their oil and within 2 weeks there was 13-14% increase in HDL level. Bagchi et al., (2002) found that GSO has a very high level of antioxidant vitamin E (60-120 mg/100g), which makes the oil very stable. The antioxidant property of GSO is claimed to have the hepatoprotective mechanism activity.

Another study by Bagchi et al., (2002) found that GSO reduces the platelet aggregation, prevents hypertension caused by excess sodium intake and normalizes the lesions caused by obesity and diabetes. Jung Kim et al., (2010) revealed that GSO fed rats had a significant reduction in total cholesterol, low density lipoprotein cholesterol, and the ratio of high density lipoprotein cholesterol (HDL-C) to total cholesterol (0.5) was significantly higher than those of groups fed soybean oil or lard.

The antioxidants effect found in grape seeds can neutralize free radicals and may reduce or even help to prevent some of the damage (P. Antioxidants found in grape seeds can neutralize free radicals, and may reduce or even help prevent some of the damage (Pâunescu et al., 2009).

Objective: The present investigation aimed to prove the clinical changes in biochemical parameters due to acute toxicity and liver damage caused by the oral administration of methomyl pesticides and revealed the possible role of grape seed oil (GSO) to protect against methomyl toxicity in rats.

MATERIALS AND METHODS

Material and Chemicals:

Grape seed oil (Vitis Vinifera) was obtained from the Unit of Squeeze and extraction of National Oils in National Research Center, Dokki, Cairo - Egypt. Methomyl was obtained from Agriculture Pesticides Laboratory, Agriculture, Research Center, Giza, Egypt and used in the present studies (methomyl LD_{50} = 48mg/kg. body weight orally) according to Thomson (1992).

Experimental Animals:

The total number of experimental animals was 72 adult male albino rats, (Sparague Dawely), weighting about 130 to 140 g. and were obtained from the National Research Center, Dokki, Cairo, Egypt. Animal were kept to be acclimatized for two weeks prior to experimentation. Rats were kept in wire cages at room temperature 25C° and provided with food and water *ad-libitum*. All rats were fed on standard diet prepared according to National Research Council (1995).

Experimental design:

The duration of the present study was divided into two experimental periods, four weeks and eight weeks. In each experimental duration a total number of 36 adult male albino rats were divided into 6 groups, each of 6 rats. The first group was kept untreated and served as negative control, these received a daily oral administration of saline by gastric tube. The second group oraly administrated grape seed oil GSO (4 ml/kg. body weight) according to Uma Maheswar and Rao (2005). The third and fourth groups were given daily oral dose of 1/10 and 1/20 of LD_{50}/day of methomyl, respectively. Fifth and sixth groups were received daily oral dose of 1/10 and 1/20 of LD_{50}/day of methomyl and treated with 4 ml/kg. Body weight/day of grape seed oil respectively. At the end of each treatment period (4 or 8 weeks), 6 rats from each group were anaesthetized by ether and blood samples were collected from portal vein. Serum samples were prepared by centrifuging the blood samples for 20 minutes at 3000 rpm.to separate the sera which were kept at -20 C° till biochemical analysis.

Preparation of liver homogenate:

Livers of the sacrificed rats were removed for preparing liver extract (25% w/v), 1 g. of liver tissue was dissolved in 4 ml of 0.1M potassium phosphate buffer solution (pH 7.5), then homogenize in ice –cold phosphate buffer. Liver homogenate was centrifuged at 7000 rap for 20 min. then separated the supernatant, which proceed in the manner of blood serum and stored at -20 C° for subsequent biochemical analysis. (Koriem et al., 2009). Another liver extraction was prepared to determed total lipid and total cholesterol (Bligh and Dyer, 1959).

Serum and liver extract were used to assess the activity of hepatic liver enzymes AST and ALT according to the methods described by Young, (2001), and ALP activity by Tietz, (1976). Total lipids, total cholesterol and TAG were measured on the basis of the method of Frings and Dunn (1970) , Richmond (1973) and Fossati and prencipel (1982) , respectively. Total bilirubin, and total protein were measured using colorimetric method by Walter and Gerade (1970), and Doumas et al., (1975) respectively.

Serum urea, uric acid and creatinine were determined according methods described by Patton and Crouch (1977), Fossati et al., (1980) and Bartles et al., (1972). Thiobarbituric acid reactive substance (TBARS) as MDA was determined as an index of lipid peroxidation according to the method described by Draper and Hadley (1990). GSO was analized by GCMS Spectrum in Pharmacay College El-Azhar University.

Data analysis: One – way ANOVA was used to detect differences. Differences were considered significant at $p < 0.05$ and high significantly at $p < 0.01$ (Snedecor, and Cochran.1967).

RESULTS

General behaviour of rats treated with methomyl received sublethal doses 1/10 or 1/20 revealed many symptoms of toxicity. The severity of the symptoms was increased at high dosing. Signs of toxicity started with reduced activity, loss of appetite, salivation and diarrhea. Also, deep and violent respiratory movement were observed. The beneficial effects and the active compounds the essentially unsaturated fatty acids in grape seed oil are believed to have antioxidant properties. Antioxidants are those substances that destroy free radicals- damaging compounds in the body that alter cell membranes and even cause cell death.

The present study indicated that GSO contains high percentage of essential unsaturated fatty acid (Linolenic acid, 72% Liolenic acid 1%, olic acid 16%, and saturated fatty as stearic acid 4%, and palmitic acid 7%, theses results were similar with finding by Natella et al., (2002).

As shown in table (1), the activities of serum ALT, AST, Alp and TB levels were markedly elevated after 4 weeks, except for (G4) which was not affected and more elevation was found with high dose and after long term (8 weeks) in methomyl treated animals with dose 1/10 LD_{50} and 1/20 LD_{50} as

compared to control groups (negative and group received GSO). The present study indicated that, the level of serum protein was found to be significantly reduced ($p<0.05$) and highly significant reduced ($p<0.01$) in intoxicated groups (G3 and G4) as shown in table (1). The extent of reduction depends upon the dosage level. Administration of grape seed oil (GSO) induced decrease in serum enzymes, ALP and TB return towards the normal values in some intoxication groups (G5 and G6) which might be due to the antihepatotoxic effect of GSO.

A significant improvement in protein values after short term was found as compared to intoxicated groups (G3 and G4) and almost similar to the negative control. But, there was less improvement of protein values after 8 weeks in (G5 and G6) as compared to intoxicated groups (G3 and G4). (Serum proteins are influenced by a variety of factors such as infection, stress, injury, toxicity, etc.).

Regarding to table (2): Serum lipids profiles: TC, TAG, TL and lipid peroxidation (MDA) were elevated after 4 weeks specially, in intoxicated group (G3) received high dose of methomyl (1/10 $LD_{50,}$) and more elevation in TC, TAG were found after 8 weeks in the same group (G3). Total lipids were reduced after long term in intoxicated groups (G3 and G4). MDA were elevated but values were not affected with short or long term of methomyl toxicity.

Intoxication groups (G5 and G6) who administered GSO, induced significant decrease ($p<0.05$), in the above lipids profile and MDA levels as compared to control groups (G3 and G4), showed less values or similar to control (G1 and G2).

Table (3) illustrated the levels of serum creatinine, urea and uric acid which treated and untreated intoxicated groups. Creatinine and uric acid levels were elevated in (G3) and (G4) in indicating the stress on kidney due to methomyl toxicity. Intoxicated groups (G5 and G6) which had administered the GSO showed a decrease in serum creatinine and uric acid levels similar to the control groups. These effects might be attributed of GSO. Significant decrease were found in serum urea levels in groups 2, 3, 4, 5, and 6 as compared to negative controls especially after long term(8 weeks). Uric acid did not change after 8 weeks and being less than the negative control.

Biochemical parameters in liver tissues: Table 4, indicates that there are highly significant differences in liver enzymes level in intoxicated groups after 4 and 8 weeks. Whereas liver enzymes activities, ALT, AST and TP were reduced after short and long term of intoxication, ALP was elevated after the end of long term intoxication (G3) and (G4) and TB was elevated after 4 weeks and return to reduced level after 8 weeks.

Oral treatment with GSO in groups (G5 and G6) resulted a significant improvement as compared to intoxicated groups (G3 and G4) and being similar in some parameters. Compared to both controls (G1 and G2).

Table (5) revealed the values of total cholesterol, total lipids and MDA in liver tissues in treated and untreated groups after 4 and 8 weeks. It was found a slightly increase in TC, TAG and MDA in intoxicated groups received 1/10 and 1/20 LD_{50} doses of methomyl with improvement in groups received oral GSO as compared to intoxicated groups.

DISCUSSION

The great hazard caused by pesticides on lives tocks are due to their accidental exposure to these pesticides either by ingestion or inhalation (Hernandez et al., 2006 and Gokhan et al., 2008). The purpose of this study was to evaluate the hepatoprotective effect of grape seed oil against methomyl induced hepatoxocity and liver damage in rats.

Garg et al., (2008) revealed the role of vitamin E in mitigation methomyl induced acute toxicity in blood of male Wister rats. They studied the effect of either a single oral dose of 9 mg/kg body weight of methomyl, vitamin E alone injected intraperitoneally on alternate days (4 injections) at 50 mg/kg body weight for one week prior to methomyl treatment, or methomyl plus vitamin E given in a similar manner. They found that vitamin E given pretreatment improved the morphology of red blood cells and affords protection in methomyl-induced toxicity in the rats. Vitamin E enhances oxidative stress and reduced the level of lipid peroxidation in serum and hepatic tissue but not to be the control value, (El-Sayed 2000 and Ozden et al.,2009). Theses results are in agreement with the present study whereas, GSO contains high amount of vitamin E.

Measurement of the activities of serum marker enzymes as AST, ALT and ALP, can make assessment of liver function (Ulican et al., 2003; Porchezhian and Ansari, 2005).

The present investigation are in accordance with those reported by Patil et al.,(2008) who found that methomyl dose levels of 2 or 4 mg/kg body weight treatment of old male and adult female rats showed significantly elevation of in ALP, SGPT and SGOT activities as markers of liver toxicity, methomyl inhibits cholinesterase activity and causes toxicity.

Fayez and Bahig, (1991) who revealed that rats received oral dose of methomyl at 4.8 and 8 mg/ kg body weight /day, for 33 days showed significantly elevation in serum liver enzymes ALT, AST and ALP activities in rats , theses elevation due to toxic hepatitis.

However, the enzyme activities returned to normal values by the end of intoxication period which, suggested repair of the damaged liver cells. Serum creatine level was significantly altered after 25 days with no dose relashinship. The elevation pattern was almost restored to normal thereafter, which may be attributed to the inhibition of the circulating enzyme by methomyl and or its metabolites.

Ali Saeed et al., (1995) revealed that two sublethal doses of profenofos (7.5 and 15 mg/kg body weight) during 7-16 days in pregnant female Swiss mice. There were a marked and highly significant reduction in ALT and AST in liver tissues with no remarkable change with low dose of profenofos while, the dose revealed highly significant reduction in the ALp enzyme activity, besides produced a highly significantly elevation in creatinine and urea levels at two doses. Al-Shinnawy et al., (2008) investigated some diagnostic parameters in the serum of rats treated orally with 1/10 LD50 dose of thiodicarb incecticide (carbamate group) daily for 7 days(short term) and 30 days (long term). They showed that a highly significant elevation in serum AST, ALT, urea, creatinine accompanied by highly significant decrease in serum cholesterol. While ALP activity and total protein were not affected.

Heibatollah et al., (2008) and Mohamed et al., (2009) found that, the level of serum markers such as AST, ALT, ALP and TB were significantly increased in CCL_4 –induced liver injury in treated rats. Also there was a significant decrease in the concentration of TP and Alb in CCL_4 treated rats compared to the control group. Studies by Lohitnavy and Sinhason (1998) and Vijayaraghavan and Nagarajan (1994) reported that the elevation in serum ALT and AST was due to degeneration and necrosis of liver cells which was accompanied by damage of cell-walls and cytolysis, thereby pouring considerable amount of theses mitochondrial enzymes in the blood stream. Damage induced by toxin are similar to those of acute viral hepatitis.

Koriem et al., (2009) revealed the toxic effect of the alcoholic extract of faba beans on albino rats injected with 1/3 orally daily for 15 days. They showed that highly significantly decrease in serum and liver enzymes AST, ALT, total protein, albumin and increase MDA levels, while treatment with the protective dose of vitamin E (100 mg/kg body weight or β–carotene 70 mg/kg body weight, both antioxidants shift all the above parameters to be near the control value. The decrease in the activities of AST and ALT may be correlated with the decrease in the level of serum and hepatic total protein, where biosynthesis of protein in favism group was decreased. Anther studies revealed that this decrease in protein

and albumin levels explained by liver injury is associated with decreased albumin level secondary to decreased protein synthesis and increased globulin level due to deteriorated hepatic activity, while the decline in serum albumin attributed to enhanced degradation and loss of albumin through the gastrointestinal tract (Marquardt et al., 1997 and Rosengren et al., 1995).

Biochemical studies in patients affected with oral consumption of organophophorous (OP) insecticides recorded elevation in AST, Alt, ALP activities and slightly elevated in creatinine and urea levels indicating the stress on kidney due to OP toxicity whereas, total protein and albumin were reduced than that of the control subjects, (Hariprasad et al.,2009). The present study are similar of these finding. In contrast, serum urea was reduced in intoxicated rats due to liver damage, and this damage affect urea formation in liver.

Al–Attar (2004), showed that the exposure of frogs (*Rana ridibunda)* to7,12 dimethylbenza-anthracene (DMBA) alone or DMBA plus grape seed oil caused significant physiological alterations reflected in decreasing of GPT, GOT and ALP activities of liver tissues and frogs treated with grape seed oil were not different from theses of controls.

Excessive exposure to pesticide caused cytotoxic changes in the hepatic and renal biochemical markers which were positively correlated with pesticide residu (Khan et al., 2006). The results of the present study are in harmony with finding by theses authers. A highly significant increase in these enzymes activites was observed at long term period of treated group, indicating liver damage .The decrease in the activities of these enzymes may be correlated with the decrease in the levels of serum and hepatic total protein, where biosynthesis of protein in intoxicated groups were deceased. This decrease in protein levels explained by liver injury.

Hypoalbuminemia and decline in TP levels can be seemed as a useful index of severity of hepatocellular damage. The lowered levels of TP and Alb recorded in the serum as well as in the liver of CCL_4 treated rats revealed the severity of hepatopathy (Aniya et al., 2005). Oral administration of GSO (3.7g./kg., body weight orally) for 7 days resulted in a significant reduced in serum AST, ALT and ALP activites and liver MDA and hydroperoxides and improved, TP, when compared with CCL_4 damage rats. The antioxidant effect of GSO at 3.7g./kg.for 7 days was found t o be comparable with vitamin E (100 mg/kg, orally) in treated rats (Uma Maheswari and Rao 2005).

The present investigation recorded decreases in concentration of cholesterol and

triacylgylcerols which may be explained by the fact that the toxic effect of methomyl is diminished by daily oral administration of GSO having hepatoprotective effects.

Administration of CCL_4 caused significant elevation in serum lipids (TL, TAG and TC compared to negative control group (Mohamed et al., 2009). Rats treated with CCL_4 alone developed significant hepato - cellular damage as was evident from a significant ($p < 0.01$) increase in the serum levels of ALT, AST, ALP and total billirubin concentration, when compared with the controls. Peroxidation products inhibit protein synthesis and cause leakage of serum enzymes and an elevation in the thiobarituric acid reactive substances (Valarmathi et al., 2010).

Manswadi and Kaliwal (2010) revealed that mice treated with 4mg/kg/day methomyl for 10 and 20 days caused significant increase in the levels of MDA as compared to control. The present study revealed the reason for increased MDA level in serum or liver tissues under the influence of methomyl treatment in high dose in rats might be caused due to the conjugation of methomyl or its metabolites to the polyunsaturated fatty acids or by production of reactive oxygen species (ROS) reacts with polyunsaturated fatty acids or accumulation of liphophilic components of pesticides conjugated with the fatty acids.

Ibrahim and El-Gamal (2003) suggested that diazinon insecticide may interfere with lipid metabolism in mammals. Separated daily oral administration of diazinon at ½ LD50, 1/8 and 1/32 LD_{50} doses. Whereas, a significant decrease in the levels of TC, HDL as well as LDL-C was observed with the 1/8 LD_{50} dose. resulted in a significant decrease in HDL-C and phospholipids and elevated LDL-C and TAG with no change in TC levels. A decrese of serum TC could be a result of the organophosphate-induced stimulation of the LDL receptors which increase the clearance of cholesterol from circulation (Brown et al., 1981).

GSO has significantly reduced the levels of liver enzymes, namely AST, ALP and ALP. Further, GSO has increased the level of TP, which indicated hepatoprotective activity. Stimulation of protein synthesis accelerates the regeneration process and the production of liver cells. The increase in MDA and hydroperoxide levels in liver in intoxicated animals, suggest enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanisms. Treatment with GSO significantly prevents these changes. Hence, the mechanism of hepatoprotection of GSO may be due to antioxidant effect (Uma Maheswari and Rao, 2005).

It has been established that the elevated serum or plasm HDL –C levels are antiatherogenic, whereas the reduced levels are associated with an increased risk for coronary artery disease (Stein 1987). Previous studies demonstrated an increase of the serum TAG concentration in the animals that were treated with different insecticides, including the organophsphate and carbamate (Gupta et al., 1986). This elevation of TAG has been attributed to an inhibition of the lipase enzyme activity of both the hepatic TAG and plasma lipoproteins (Musliner et al., 1979 and Goldberg et al., 1982). While, the decrease of serum TAG that was recorded in rats were treated with the organophosphate insecticide acephate may be a reflection of the insecticide-induced reduction of this lipid fraction in all lipoproteins classes, particulary LDL-C. Methomyl have been reported to produce a rise in insecticide in serum TC (Antal et al., 1979). This result was in harmony with the present investigation.

.Jung Kim et at., (2010) revealed that, grape seed oil supplementation (4.2 g. /day) has significant health benefits through favorable alterations in plasma lipid profile and may provide health benefits in hyperlipidemia and related complications, whereas, GSO fed rats had a significant reduction in total cholesterol (TC, 60.6 mg/dl), low density lipoprotein-cholesterol (LDL-C.16.8 mg/dl), and atherogenic index (AL, 0.9). A study by Pâunescu et al., (2009) showed that the animals were intoxicated with CCL4 in a dose of 30 µl/100 g body weight for 12 days caused decreased triacylglycerols and cholesterol by 27% as compared to control value and group was treated with grape seeds oil (1ml/kg B.W. for 12 days) had protective effect and cholesterol value in these group being similar to control value. The decreases of triacylglycerols and cholesterol may be explained by the fact that the toxic effect of CCL_4 is diminished by daily administration of grape seeds oil being similar to control value.

From the nutritional and therapeutic point of views, GSO have a high linoleic acid content (58-78%), high tocopherol content (534-450% mg./kg), and high polyphenolics (10-34%mg/kg) and therefore it is recognized that its intake may be beneficial to prevent heart and circulatory problems (Kim et al., 2008 and Pardo et al., 2009). In another study, GSO feeding resulted in more favorable plasma lipid response. It is thought that higher tocotrienols and polyphenols in GSO could have play roles in reducing plasma cholesterol in animal model. The hypolipidemic effects of tocotrienls in animal model have been reported. Epidemiological studies suggest that tocopherol may protect against cardiovascular disease.

Tocotrienols which are rich in GSO also have antioxidant activity and, unlike the tocopherols, lower plasma concentration of atherogenic LDL-C in various animal species, (Parker et al., 1993). This is probably due to the postrasciptional suppression of the key enzyme in cholesterol synthesis, 3 –hydroxy – 3-mehlglutary – CoA reducatse (HMG –CoA reductase), and a concomitant upregulation of the LDL receptor (Theriault et al., 1999). Experiments in vitro and vivo indicate that–tocotrienol , which is rich in GSO may be more potent inhibitors of HMG –COA reducatase than other tocopherols and yocotrienols (Parker et al.,1993).

In addition , virgin GSO contains a large amount of polyphenols , i .e., the oligomeric proanthocyanosides, at levels of 1,000 fold higher than in other seed oils and this makes GSO more resistant to lipid peroxidation (Rao, 1994). Polyphenols in vegetable oils have been reported to exert a variety of biological actions such as free radical scavenging and modulation of antioxidant enzyme activities. These compounds are also capble of decreasing the total and LDL-cholesterol in serum and tissues as well as increasing the antioxidant statues (Khor et al., 1998).

Conclusion:

The present investigation suggested that the organophsphate and carbamate as methomyl may interfere with lipid metabolism in mammalian animals. A significant elevation in serum or liver tissues especially at higher dose and long term in investigated biochemical parameters induced liver damage. Methomyl inhibits cholinesterase activity and causes toxicity. It can be concluded that feeding GSO resulted in a more favorable serum and liver lipid improvement. The grape seed oil when used properly is considered one of the best medicine food to lower and maintain the normal rate of cholesterol and has a protective effect against many intoxication cases due to its higher antioxidant contents

Table (1): Effect of grape seed oil (GSO) on serum enzymes activities (ALT, AST, ALP), total, bilirubin (TB) and total Protein (TP) in methomyl intoxicated rats after 4 and 8 weeks (Mean+ SD).

Groups / parameters	**Group (1)** Negative control	**Group (2)** **Control** GSO	**Group (3)** Methomyl 1/10 LD_{50}	**Group (4)** Methomyl 1/20 LD_{50}	**Group (5)** Methomyl 1/10 LD_{50} +GSO	**Group (6)** Methomyl 1/20 LD_{50} +GSO
ALT (U/L) after 4 weeks	29.26± 1.78	22.92± 5.16^{a}	44.56± 3.6^{ab}	26.04± 4.94^{c}	28.73± 2.80^{bc}	22.86± 2.86^{ace}
ALT (U/L) after 8 weeks	28.92± 3.46	25.23± 2.86	43.52± 5.89^{ab}	35.48± 1.87^{abc}	28.43± 3.41^{cd}	27.45± 2.78^{cd}
AST (U/l) after 4 weeks	41.58± 6.89	41.52± 1.77	85.92± 14.61^{ab}	66.26± 7.87^{abc}	65.26± 2.65^{abc}	48.35± 1.99^{cde}
AST (U/L) after 8 weeks	58.64± 5.12	48.52± 3.08^{a}	120.04± 7.91^{ab}	76.28± 5.56^{abc}	82.00± 3.99^{abc}	54.55± 5.38^{cde}
ALP (U/L) after 4 weeks	96.63± 12.92	84.51± 6.19	190.24± 20.17^{ab}	118.14± 2.57^{c}	97.44± 12.55^{cd}	87.35± 15.22^{cd}
ALP (U/L) after 8 weeks	86.55± 6.01	78.09± 5.73	201.18± 18.30^{ab}	120.62± 18.42^{abc}	117.81± 11.86^{abc}	93.40± 17.55^{cde}
TB (U/L) after 4 weeks	0.54± 0.93	0.41± 0.30	1.58± 0.30^{ab}	1.18± 0.08^{abc}	1.14± 0.28^{abc}	0.78± 0.64^{bcde}
TB (U/L) after 8 weeks	0.85± 0.18	0.55± 0.79^{a}	1.90± 0.15^{ab}	0.79± 0.14^{bc}	1.19± 0.25^{abcd}	0.57± 0.43^{ace}
TP (mg/dl) after 4 weeks	5.06± 0.63	5.27± 1.11	1.91± 0.11^{ab}	2.78± 0.21^{abe}	4.43± 0.25^{bcde}	5.29± 0.35^{cd}
TP (mg/dl) after 8 weeks	5.65± 1.15	5.69± 0.47	1.73± 0.28^{ab}	2.95± 0.13^{ab}	3.05± 0.37^{ab}	3.99± 0.90^{abcd}

Significane at P<0.05

a- Significant difference compared to group (1)
b- Significant difference compared to group (2)
c- Significant difference compared to group (3)
d- Significant difference compared to group (4)
e- Significant difference compared to group (4)

Table (2): Effect of grape seed oil (GSO) on serum total cholesterol (TC), triacylgelycrol (TAG) total lipids (TL) and malondialdehyde (MDA) in methomyl intoxicated rats after 4 and 8 weeks (Mean± SD).

Groups / parameters	**Group (1)** Negative control	**Group (2) control** GSO	**Group (3)** Methomyl 1/10 LD_{50}	**Group (4)** Methomyl 1/20 LD_{50}	**Group (5)** Methomyl 1/10 LD_{50} +GSO	**Group (6)** Methomyl 1/20 LD_{50} +GSO
TC (mg/dl) after 4 weeks	110.56± 4.26	74.21±3.11[a]	135.56±3.11[b]	121.48± 3.46[b]	99.77± 10.73[acd]	77.94±24.49[cd]
TC (mg/dl) after 8 weeks	137.38± 5.89	101.50± 8.23[a]	151.33± 6.12[ab]	129.48± 7.52[bc]	119.89± 8.66[abc]	107.67± 9.78[acde]
TAG (mg/dl) after 4 weeks	74.35±4.55	56.95±2.16[a]	177.53±17.29[ab]	99.05±8.47[abc]	105.63±7.30a[bc]	68.66±2.41[cde]
TAG (mg/dl) after 8 weeks	80.50± 3.11	67.11± 9.59	19 5.93± 26.18[ab]	133.62± 16.44[abc]	132.96± 24.10[abc]	83.66±10.61[cde]
TL (mg/dl) after 4 weeks	308.14±19.66	280.82±23.17[a]	403.84±17.61[ab]	319.01±15.26[bc]	300.54±6.88[ce]	276.26±13.37[acd]
TL (mg/dl) after 8 weeks	266.04± 43.94	208.84± 28.19[a]	361.93± 24.80[ab]	255.19± 34.98[bc]	254.58± 11.31[bc]	212.71± 13.33[acd]
MDA (nm/l) after 4 weeks	2.39± 0.44	2.37± 0.25	4.78± 0.33[ab]	3.82± 0.24[abc]	3.55± 0.49[abc]	2.64± 0.53[cde]
MDA (nm/l) after 8 weeks	2.68± 0.38	2.47± 0.33	4.76± 0.29[ab]	4.19± 0.27[abc]	3.78± 0.40[abc]	3.83± 0.56[abc]

Significane at $P<0.05$

a- Significant difference compared to group (1)
b- Significant difference compared to group (2)
c- Significant difference compared to group (3)
d- Significant difference compared to group (4)
e- Significant difference compared to group (4)

Table (3): Effect of grape seed oil (GSO) on serum creatinine, urea and uric acid in methomyl i intoxicated rats after 4 and 8 weeks (Mean± SD).

Groups / parameters	**Group (1)** Negative control	**Group(2)** GSO	**Group(3)** Methomyl 1/10 LD_{50}	**Group (4)** Methomyle 1/20 LD_{50}	**Group (5)** Methomyl 1/10 LD_{50} +GSO	**Group (6)** Methomyl 1/20 LD_{50} +GSO
Creatinine (mg/dl) after 4 weeks	0.44± 0.47	0.42± 0.33	0.79±0. 44 ^{ab}	0.57 ± 0.54 ^{abc}	0.56± 0.07 ^{abc}	0.47± 0.57 ^{cde}
Creatinine (mg/dl) after 8 weeks	0.39± 0.52	0.42±0.57	0.20±0.83 ^{ab}	0.22±0 ^{ab}.33	0.51± ^{acd} 0.73	0.46±0.62 ^{cd}
Urea (mg/dl) after 4 weeks	29.08± 1.43	26.50± 3.81 ^{cd}	16.87± 1.06 ^{abdf}	20.23± 1.83	21.4± 1.65 ^{abc}	23.29±0.56 ^{abcd}
Urea (mg/dl) after 8 weeks	29.66± 2.97	23.98± 1.75 ^{acde}	17.55±2 ^{ab}.3	19.87± 2.28 ^{ab}	17.04± 1 ^{ab}.88	20.68± 3.53 ^{abce}
Uric acid (mg/dl) after 4 weeks	2.50± 0.30	1.91± 0.18 ^{ac}	3.59± 0.32 ^{abef}	2.87± 0.10 ^{abcf}	2.39± 0.14 ^{abcdf}	2.01± 0.14 ^{acd}
Uric acid (mg/dl) after 8 (weeks	1.82± 0.09	1.76± 0.20	2.71± 0.15 ^{abd}	1.88± 0.1 °8	1.55± 0.10 ^{ac}	1.79 ±0.24 ^{c}

Significane at P<0.05

a- Significant difference compared to group (1)
b- Significant difference compared to group (2)
c- Significant difference compared to group (3)
d- Significant difference compared to group (4)
e- Significant difference compared to group (4)

Table (4): Effect of grape seed oil (GSO) on enzymes liver tissue (ALT,AST,ALP) , total bilurobuin and total Protein, in methomyl intoxicated rats after 4 and 8 weeks (Mean SD).

Groups / parameters	**Group (1)** Negative control	**Group (2)** GSO	**Group (3)** Methomyle 1/10 LD_{50}	**Group (4)** Methomyl 1/20 LD_{50}	**Group (5)** Methomyl 1/10 LD_{50} +GSO	**Group (6)** Methomyl 1/20 LD_{50} +GSO
ALT (U/L) after 4 weeks	20.06± 4.32	20.56± 1.19	13.70± 1.28^{abde}	17.27± 1.46^{cd}	20.30± 2.61^{cd}	19.05± 1.73^{cd}
ALT (U/L) after 8 weeks	22.79± 4.64	17.61± 1.20^{a}	9.88± 1.08^{ab}	24.23±1.10^{bc}	20.51± 1.98^{cd}	24.58± 2.86^{bce}
AST (U/L) after 4 weeks	27.72± 3.14	26.99± 2.56	14.33± 1.49^{ab}	21.04± 1.00^{abc}	21.47± 1.67^{abc}	24.5± 2.47^{cd}
AST (U/L) after 8 weeks	28.69± 2.99	26.41± 3.37	11.91± 2.50^{ab}	17.48± 2.75^{abc}	18.85± 2.60^{abc}	24.05± 2.68^{acde}
ALP (U/L) after 4 weeks	211.00± 9.14	165.71± 6.50^{a}	137.31± 4.84^{ab}	153.24± 14.64^{a}	142.49± 19.21^{ab}	117.94 ± 7.51^{abcde}
ALP (U/L) after 8 weeks	214.75± 9.01	105.61± 13.77^{a}	237.25± 22.05^{ab}	240.0± 7.74^{ab}	130.20± 10.55^{abcd}	143.86± 6.82^{abcd}
TB (U/L) after 4 weeks	0.44± 0.09	0.33± 0.09	2.44± 0.28^{ab}	1.14± 0.03^{abc}	0.78± 0.06^{abcd}	0.37± 0.08^{cde}
TB (U/L) after 8 weeks	0.25± 0.05	0.22± 0.08	0.85± 0.18^{ab}	0.60± 0.13^{abc}	0.49± 0.07^{abc}	0.49± 0.15^{abc}
TP (g/dl) after 4 weeks	3.85± 0.44	3.21± 0.66^{a}	1.88± 0.29^{ab}	2.52± 0.3^{abc}	3.07± 0.39^{ac}	3.65± 0.21^{cd}
TP (g/dl) after 8 weeks	1.17± 0.26	1.28±0.14	1.54± 0.28	1.37± 0.16	2.37± 0.39^{abcd}	2.64± 0.42^{abcd}

Significane at P<0.05

a- Significant difference compared to group (1)
b- Significant difference compared to group (2)
c- Significant difference compared to group (3)
d- Significant difference compared to group (4)
e- Significant difference compared to group (4)

Table 5: Effect of grape sees oil (GSO) on liver total cholesterol (TC) and total lipids (TL) and malondialdehyde (MDA) in methomyl intoxicated rats after 4 and 8 weeks (Mean ±SD).

Groups / parameters	**Group 1** Negative control	**Group 2** GSO	**Group 3** Methomyle 1/10 LD_{50}	**Group 4** Methomyl 1/20 LD_{50}	**Group 5** Methomyl 1/10 LD_{50} +GSO	**Group 6** Methomyl 1/20 LD_{50} +GSO
TC (mg/dl) after 4 weeks	47.69± 1.14	38.88± 5.36^{a}	48.90± 3.12^{b}	40.88± 1.45	30.98± 2.06^{acd}	20.13± 12.01^{abcde}
TC (mg/dl) after 8 weeks	40.65± 2.03	38.75± 1.45	50.98± 1.21^{ab}	45.89± 3.37^{abc}	37.42± 2.88^{cd}	37.75± 4.25^{cd}
TL (mg/dl) after 4 weeks	454.06± 20.18	222.73± 19.24^{a}	367.96± 25.48^{ab}	284.85± 18.21^{abc}	210.91± 16.99^{acd}	170.99± 13.76^{abcde}
TL (mg/dl) after 8 weeks	317.31± 22.25	135.21± 5.08^{a}	480.27± 21.26^{ab}	290.48± 16.91^{abc}	379.19± 16.38^{abcd}	243.13± 17.61^{abcde}
MDA (nm/g) after 4 weeks	3.57± 1.16	3.29± 0.85	3.83± 0.48	3.33± 0.73	2.90± 0.40	2.63± 0.81^{c}
MDA (nm/g) after 8 weeks	2.67± 0.67	3.45± 0.37^{a}	4.18± 0.56^{ab}	2.85± 0.42^{c}	2.35± 0.28^{bc}	2.49± 0.39^{bc}

Significane at $P<0.05$

a- Significant difference compared to group (1)
b- Significant difference compared to group (2)
c- Significant difference compared to group (3)
d- Significant difference compared to group (4)
e- Significant difference compared to group (4)

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التأثيرالوقائى لزيت بذور العنب على المبيد الحشرى ميثوميل المسبب لتلف الكبد فى الجرزان

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أجريت هذة الدراسة على عدد ٧٢ من ذكور الجرزان البيضاء من فصيلة سبراج دولى والتى تزن من – ١٣٠الى 140 جم بهدف إظهار الدور الواقى لزيت بذور العنب المصرى على كبد الجرزان التى تناولت المبيد ميثوميل(من مجموعة الكاربامات). قسمت الجرزان على تجربتين (٣٦ فى كل تجربة تم تقسيمها على ٦ مجموعات فى كل مجموعة ٦ جرزان) مجموعة ١ ضابطة طبيعية ومجموعة ٢ ضابطة تناولت زيت بذور العنب فقط ومجموعة ٣و ٤ اعطيت جرعتين المبيد الحشرى ميثوميل تحت المستوى المميت عن طريق الفم (١٠/١ و ٢٠/١ منLD_{50}) ومجموعة ٥ و٦ تناولت المبيد +زيت العنب وذلك لمدة ٤ أسابيع (فترة قصيرة الامد) و٨ أسابيع (فترة طويلة الامد)على التوالى . وقد أظهرت النتائج أن المبيد أحدث خللا واضحا فى وظائف كل من الكبد والكلية للجرزان مع زيادة جرعة المبيد .ولدراسة التغيرات البيوكيميائية نتيجة التسمم بالمبيد تم تقدير نشاط الانزيمات المختلفة فى السيرم و الكبد مثل الترانس امينيز والفوسفاتيز القلوى والبيلوربين الكلى ، كما تم تقدير الكوليسترول الكلى والجليسريدات الثلاثية والدهون الكلية والبروتين الكلى والمالون د اى الديهيد. كما تم تقدير كل من حامض اليوريك والكرياتينين واليوريا فى سيرم الدم.

وقد أوضحت النتائج مايلى: ظهرت أعراض التسمم بالمبيد بقلة حركة الحيوان ، إفراز غزير للعاب ، فقدان الشهية للغذاء مع عمق شديد لحركات التنفس وتقل هذه الأعراض بعد ساعات من تعاطى الجرعات من المبيد.

التغيرات البيوكيميائية :حدث إرتفاع معنوى له دلالة إحصائية لنشاط إنزيمات الكبد فى المصل (AST، ALP ALT) والبيلوروبين الكلى والكوليسترول الكلى والجليسريدات الثلاثية والدهون الكلية. كما لوحظ أرتفاع كل من الكرياتينين وحمض البوليك فى مصل الدم مع زيادة القيم فى الفترة طويلة الأمد (٨ أسابيع).

- حدث نقص شديد فى البروتين الكلى واليوريا فى سيرم المجموعات التى تناولت المبيد وخصوصا مع الجرعة LD_{50} ١٠/١بالمقارنة بالمجموعات الضابطة. وقد أظهرت النتائج ان هناك تحسن واضح فى هذه التغيرات البيوكيميائية فى كل من سيرم الدم والكبد عند تعاطى الجرزان المسممة بالمبيد زيت بذور العنب بمعدل ٤ مل/كيلو جرام من وزن الجسم يوميا الى درجة مشابه للمجموعات الضابطة وذلك عند المستوى المنخفض من المبيد وهذا التحسن راجع لما يحتويه الزيت من مركبات مضادة للاكسدة .