

## **BIOLOGICAL EVALUATION FOR FRYING BLEND OIL OF JOJOBA AND SUNFLOWER ON EXPERIMENTAL RATS**

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### **ABSTRACT**

The current study was undertaken to assess the effect of utilization of non-conventional oil, jojoba oil (more heat stable oil) blend with less heat stable sunflower oil. Main objective of this study was concern on evaluate potential adverse affects fresh and frying of this blend on rats, following deep frying potato fingers at 180 °C for internment frying up to consecutive 3 days. The blending fresh and frying processed proceed a remarkable changes in their fatty acid profiles by increase monounsaturated while reduce polyunsaturated fatty acids. Long term feeding as subchronic protocol of using fresh blend and its frying blend oils, four groups of male albino rats were fed with diets supplemented with 10 % of each corn oil (group 1) , fresh blend (group 2), and oils obtained from 20<sup>th</sup> and 30<sup>th</sup> frying (group 3 and group 4 , respectively) , for along 95 days. The studies were focused on effect of feeding rats on fatty acid profile of adipose tissues in liver, kidney and heart, hematology; organs weigh indices (hepatosomatic; kidney and cardiosomatic indices), serum analysis, liver functions and histological studies among all of four groups. The results shows that an alteration effects of hemoglobin; hematocrite; platelets; mean corpuscular volume and mean corpuscular hemoglobin concentrate occurrence and extent a increase in red blood cells in most of group of 20<sup>th</sup> and 30<sup>th</sup> dietary groups. No adverse effects on serum analysis of total cholesterol ranged from 86.23± 18.13 to 92.08 ± 10.69 mg/dl among all groups with normal range less than 100 mg/dl. While, the triglyceride tended to decrease in groups 2; 3 and 4. Based on the results of liver function; it shows a significant difference between the studied dietary groups.  $\gamma$ -GT shows a significant increase among groups of feeding frying oils. Whereas, it's feeding to group 1 had a lowering level of  $\gamma$ -GT (41.66 U/L). Generally, increase of  $\gamma$ -GT can be taken as one of phase carcinogenesis of liver preneoplastic foci. Organs weight indices were assessed, most of increase in this indices concerning among group 3 and 4 comparing to group1. An increase in arachidonic acid among lipid profile of frying oils dietary groups, whereas, linoleic acid was decline in the organs adipose fatty acids. This contributed to role of body and liver to maintain the oxidized stress on the cells and organs. A dramatically impaired in level of  $\alpha$ -tocopherol and followed by liver retinol could be obvious among group 4. Adverse effect in histological examination of various liver and kidney show an alteration in liver and kidney tissues, especially it positively among group 3 and4. Generally, there was an adverse effect of using jojoba oil frying after 20<sup>th</sup> frying. However, it can use this blend (jojoba and Sunflower oils) in fresh blend and / or in frying process up to 20<sup>th</sup> frying.

**Keywords:-** Biological, fatty acids profile, jojoba oil , hematology , histology, liver retinol, liver  $\alpha$ -tochopherol ,  $\gamma$ -GT, liver function , organs weight indices, oil blend, sunflower oil , serum analysis

### **INTRODUCTION**

Deep-fat frying is one of method to prepare foods. Despite potato being the leading products to be fried, a large number of other foods are prepared by frying technology. Both frying oils and lipids in foods undergo many

reactions taken place in oil as a result of thermal degradation. It is including decomposition oxidation, hydrolysis and polymerization (Sánchez-Muniz and Bastida 2006). The rate of decomposition depends on the composition of the oil and typed of fried foods. Similar this progressive deterioration occurred in frying oil and such fried food products. The complex series of reactions were producing a great number of volatile and nonvolatile products. The nonvolatile decomposition products are produced primarily by thermal oxidation and polymerization of unsaturated fatty acid (Warner 1999). These compounds are concern because they accumulate in frying oil medium and promote further degradation and observed by fried foods, entire in the diet and effect on public health and recognized as potentially serious health hazards. Some deteriorated compounds from frying fat can present certain toxicity (Varela and Ruiz-Roso 1998 and Gertz 2002).

The potential toxic products produced in frying oils, during biological tests conducted by Billek (1979), rats fed used frying oil displayed haematological changes indicating liver malfunction. Consequently there is a need to characterize the compounds produced during frying and the nutritional consequences of their ingestion. Although the effects of feeding heated and used frying oils on body weight and liver are already well documented (Cuesta *et al.*, 1988)

In the liver , there is a toxic effect confirmed by the increase in thiobarbituric acid reactive substance (TBARs) which concentrated after feeding on thermal oxidized sunflower oil (Ammouche *et al.*, 2002).

Several researches are studied effect of repeatedly used of frying oils on rats. Hageman *et al.*, (1991) studied that repeatedly used frying oils such as saturated fatty acid rich coconut oil (CO) and polyunsaturated (PUFA) which heated palm oil and heated corn oil showed tendency to be increase in plasma alkaline phosphotase activity, cell proliferation of esophageal tissues and excretion of TBARS in plasma were significantly higher than rats fed on heated palm oil (PO). The oxidation products of linoleic acid produced indications of cellular damage to liver and kidney and increase urine mutagenicity for Ames test, as well as enhanced cell proliferation in esophagus.

Heating or deep-frying may also generate harmful mutagenic or carcinogenic by-products, Kasamatsu *et al.*, (2005) investigated the potential genotoxic effects of heated oils series of standard *in vitro* and *in vivo* genotoxicity tests.

Moreover, the long term feeding on heated and fried oils, the plasma and liver fatty acid were affected after feeding on frying oils with no deleterious reaction of continuously effect on other tissue lipids profile (Narasimhamurthy *et al.*, 1998). Considering that also, organ weight would appear to be highly dependent on body size and / or body weight. The hepatosomatic index (liver weight/body weight  $\times 100$ ) and cardiosomatic index was higher after fed rats for along time on used frying oil (Dolphin 1981). Frying oil is convenes to increase glutathione reductase, that have a role in the detoxifying of accumulated peroxides (Saka *et al.*, 2004). Furthermore, frying oil does not only toxic for rats and human being but also increase the atherogenic probability. Excessive decomposition of culinary oil polyunsaturates, such

extreme thermal stressing episodes can also give rise to substantial losses of Alfa tocopherol (Vit E) therein. Although the dietary polyunsaturated fatty acid are protective against the development of atherosclerosis, the frequent utilization of culinary oils contain high levels of these agents after cooking or frying and consequent ingestion of pro-atherogenic aldehydes peroxidation products clearly pose health hazard worthy of increase clinical and public concern (Grootveld *et al.*, 1998 and Oya *et al.*, 2002).

The traditional approach has been taken to improve the oxidation stability as well as thermal stability of the frying oil through hydrogenation, addition of antioxidants or blending different type of oil stabilized to minimize there detritions reaction (Gupta 2000).

The thermal stability of used frying oils can be maintained by blending more stable oil with less stable oil such as sunflower with a new oil is named jojoba oil. The seeds of jojoba (*Simmondsia Chinensis*) are attributed to decrease food intake and weight loss when fed on oil if included in the diet of rats (Cokelaere *et al.*, 1992a;1992 b). No clinical chemistry, hematology and necropsy resulted along fed experimental rats for long time on jojoba oil (Nutrition Research Newsletter 1989). The fatty acid composition of jojoba oil is mainly monounsaturated, which can be reduced level of LDL in the diet (Reaven *et al.*, 1994). Parallel to intake jojoba oil led to significant reductions in blood cholesterol among experimental rats animal which fed on jojoba oil up to 3-6 weeks (Anantharaman 1994).

Now a day, jojoba oil, it was an alternative to conventional fat source. Such alternative are either fat substitutes or fats that may be used as partial fat replacers because of their low digestibility *in vivo* and highly resistance to oxidation and posses desirable physical and culinary properties. The jojoba oil has oxidation stability index approximately 60 hours, which means that it is more shelf stable than oils of safflower, canola and almond oils, not only stable but also can enter normally in food stuff (Bernasconi *et al.*, 2006). Jojoba oils does not oxidize or become rancid, it is added to other oils to extend their shelf life (Baldwin 1989). The research efforts on jojoba oils are a means to reduce fat energy intake, not by exclusion of fat from the diet but also by preventing its absorption. Jojoba oil is not hydrolyzed *in vitro* by pancreatic lipase. Jojoba also can be used as coating of food stuffs (e.g. chocolate, dried fruits) which greatly improved stability and impassive moisture loss even in comparison with hydrogenated oil coating (Clarke and Yermanos 1980). In addition to jojoba wax can be used as food additive and replacing as edible fat and oil in margarine and mayonnaise as well as deep-fat frying which stand up to high temperature (Tada *et al.*, 2005).The utilization of jojoba oil to replace frying fat in large or small good frying operation still not feasible nevertheless its application could be used for extended industrial frying period (Anika 1987).

The present study was designed to envisage the effect of utilization jojoba oil and sunflower blend after frying process within the following aims: (1) to look for a possible use of this jojoba oil in regular frying duty. (2) To manifest effect of using jojoba oil in blend at nutrition, biochemical chemistry, structure and function of internal organs in rats fed on both fresh and used blend oils in frying. Subsequent study will evaluate lipid stores in rat's organ tissues

attributes of this blend. (3) To study the effect of used blend jojoba oil on the level of retinol and Vit. E as normal antioxidants in liver of fed rats on these used oil. (4) To examine the effect of fried jojoba oil in blend on the histopathology of fed animal rats. Generally, it can be detected the effect of jojoba oil uses as general on the human food as alternative oil in the food stuff.

## **MATERIALS AND METHODS**

### **Frying performance:**

Refined sunflower oil (RBD and free antioxidants), cold press jojoba oil and potatoes were purchased from a local market in Egypt. The oils were stored below than 5 °C in the dark and used as purchased the oils were blended (2:1) w/w sunflower and jojoba oil then kept up to used in refrigerators.

Uniform size of potatoes were washed, peeled by stainless steel knife, and then cut as finger shaped about 0.5 cm diameter and length 5-8 cm, frying after stripping in 3 % sodium chloride solution. Domestic deep-fat fryer with large jar 5 L aluminum vessel was used for frying. The fried potatoes in frying oil with repeating frying was kept at 200g / 5 L without replenish the oil. The consecutive 10 frying cycle were carried out every day up to 3 days with intermittent time up to 24 hr; total carried time was 2 hr per day. The fryer was filled with 5 liters blend oils (Sunflower + jojoba oil 2:1) and heated at  $180 \pm 5$  °C. For the analytical determinations of oils and diets of experimental rats animals were preserved after each frying cycle (zero, 20<sup>th</sup> and 30<sup>th</sup> fried oils) and samples from unused blend oil (fresh) was also retained. All the oil samples were stored in freezer at -20 °C before analysis.

### **Biological study:**

Subchronic study was performed as protocol based on FDA Red book guidelines (with regard to numbers of animals and parameters assessed) and OECD Guidelines for Testing Chemicals, Health Effects Test Guidelines, Section 408, July 1995, and in compliance with the United States Food and Drug Administrative for the subchronic study. forty weaning male Albino rats weighing approximately 70 - 75 gm were obtained from Agriculture experimental house of rats, MOA, Egypt and assigned randomly to one of the four dietary groups, each group 10 rats. The animals were housed in couple on stainless cage as *add libitum* fed. They housed in the room with control light cycle about 12 h light/ dark, 23-25 °C /60-70% humidity. *Add libitum* diets were provided to rats for 95 days and animal were weighted weekly to access growth. Diets were prepared freshly every 7 days and thus injected with nitrogen gas, high purity to preserve the diet from rancidity. The diets were prepared from formula using enriched by fresh and used oil from different number of frying as well as control diet oil (corn) at level 10 % of each appropriate diet according to modified method described by Eggum (1967) guide lines. Blood samples were collected from rats every 21 days after fasting period not exceeding 12 hr. Blood was drawn by cannulation of the carotid artery into heparinized plastic vials. Serum was obtained by

centrifugation for 10 min at 1000 xg and stored at -20 ° C until analysis. By the end of treatment on day 95, animals were sacrificed by decapitation after overnight fast and after obtain blood sampling. The organs (liver, kidney and heart) were deposited from diet groups of rats, washed by buffer solution and then preserve in formol 10 % up to histopathology study. Another of selected organs were excised and washed with saline, weighted and stored in frozen state at -20 ° C to extraction lipid from these organs.

Fatty acid composition of liver, kidney and heart tissues were determined, approximately 1 g of liver or other organs was homogenized in 12 ml chloroform-methanol (1: 1v/v), after which the extraction was continued according to the method described by Folch *et al.*, (1957). The fatty acids (FA) profiles of different fresh blend, frying blend oils and corn oil as well as extracted organs lipid were received to analyze by methylation using BF<sub>3</sub> / methanol described by AOAC, (2005) and identified by GC Shimadzu 2010 auto system model of gas chromatography fitted with an auto sampler, and DB-wax column 30m x 0.25 mm (I.D) x 0.25 µ m film of fused silica, and fitted with FID detector. The temperature of injection port and FID was maintained 250 ° C and the oven was 200 ° C and the carrier gas was helium 30 ml / min. The sample size was 1 µl. Peaks area was automatically integrated. The relationship between the fatty acid composition of the liver, kidney, and heart tissues according to their composition of ingested oils by assessing FA composition index according to diet oil of groups.

#### **Hematological parameters:**

Blood parameters were determined freshly with heparinized plastic tube and CBC counter blood cells (model ABC, Vet, user manual RAB 015 a Ind A. Animal blood counter, France). Blood picture such as determined WBC: White blood cells, RBC: Red blood cells, HGB: Hemoglobin, HCT: Hematocrite, PLT: Platelets, MCV: Mean corpuscular volume and MCH: Mean Corpuscular Hemoglobin and MCHC: Mean Corpuscular Hemoglobin Concentration was performed for all decapitated blood samples.

#### **Serum analysis:**

Total cholesterol (TC) and Triglyceride (TG) were determined by enzymatic colorimetric method using Human Kit (Germany and Biomerieux France).

#### **Liver functions:**

Asparate aminotransferase (GOT) and Alanine aminotransferase (GPT) were determined by using method according to Cambeau and Hornby (1975) at blood serum to avoid hemolysis.

**γ-GT (Gamma Glutamyl Transferase)** it was determined using method Persijn G ., (1976) in serum and colorimetric kits . The enzymatic activity produce color was measured at length 405 nm. Calculation of γ-GT activity by using factor  $\Delta A/\text{min}$  (Hy 405 nm x 1158).

**Organs weight ratio:** was calculated finally by divided organs weight at final weight multiply by 100. Hepatosomatic index (liver weight/ body weight × 100); Kidney index (Kidney weight / body weight × 100) and Cardio index (Heart weight / body weight × 100).

**Determination vitamin A and E:**

- **Vitamin A (retinol):** was determined in the extracted liver lipid using method described by Leth and Jacobsen (1993). Using HPLC equipped by kieselgel-column and spectrophotometric detection at 325 nm for all Vit.A acetate and Trans retinol. From area of all identified peaks. The amount of Vit A was calculated.
- **Vitamin E ( $\alpha$  – tocopherol):** was determined by using HPLC method described by Leth and Sonderyaro (1983) , the extracted lipid from liver of sacrificed rats by using method according to Folch *et al.*, (1957) . The HPLC equipped with a kiesel gel L-column.

**Histopathological examination:**

It was carried out by using method according to Banchroft *et al.*, (1996) as follows: The specimens organs were taken from the various examined organs (kidney, heart and liver) after scarification and during the post mortem examination of experimental rat animals. Them fixed in 10% formol saline for 24 hour trimming was done on the fixation tissue specimens and washed in tap water for 12 hrs. Series alcohol (methyl, ethyl and absolute) were used for dehydration of the tissue samples. Tissue specimens were cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned at micron thickness by slide microtome. The obtained tissues sections were collected on the glass slide and stained by hematoxylin and eosin stain for histopathological examination by the light microscope.

**Statistical analysis:**

Two ways ANOVA were used to analyze difference tests. Data were analyzed using the SAS, (1999). Least significant differences were considered at  $p < 0.05$ .

## **RESULTS AND DISCUSSION**

**Fatty acid profile of oils:**

Fatty acid profile of sunflower, jojoba, corn, fresh blend of sunflower and jojoba oils (2:1) and frying blend oil are shown in Table (1). It is clear from the data that linoleic acid is the most predominant fatty acid (FA) in fresh blend oil (51.6%); followed by oleic acid (21.0%) and Gondoic acid (14.8%).

In addition, linoleic acid was generally lower in fresh blend (51.6%), than found in reference sunflower (63.0%). Moreover, improving heat stability could be extended by increase of total mono unsaturated fatty acids (MUFA) in fresh and blend frying oils as compared by reference alone of either sunflower or jojoba oil. The direction towards lowering linoleic and increase oleic acid as MUFA, seem to appropriate increase frying stability and less oxidation, polymerization and hydrolysis (Warner *et al.*, 1994 and Firestone *et al.*, 1991).

From this previous result obviously that, blending of jojoba oil with sunflower oil would except to improve thermal oxidization and frying stability due to their fatty acids pattern. Also, blending jojoba with sunflower will improve the shelf life for extended time and reducing the deteriorate reduction related to either lipid peroxide or thermal peroxidation and hence

improve the quality of sunflower oil during frying process. Moreover, frying sunflower oil is potentially toxic (López-Varela *et al.*, 1995) while jojoba oil is proved as low energy expenditure and protects sunflower oil from thermal deterioration for a long time during frying process.

**Table (1): Fatty acids profile of the fresh blend oil (sunflower and jojoba oils 2:1 w/w) and there different numbers of frying potatoes fingers.**

Fatty acids		Sunflower oil	Jojoba oil	Corn oil	Frying cycle		
					Fresh blend	F20 (20 <sup>th</sup> frying oil)	F30 (30 <sup>th</sup> frying oil)
C14:0	Myristic acid	0.7	--	--	--	--	--
C16:0	Palmitic acid	6.0	1.3	7.5	5.1	5.2	5.4
C18:0	Stearic acid	3.2	--	3.6	3.2	2.6	2.6
C18:1ω9	Oleic acid	24.4	9.2	23.6	21.0	21.1	21.4
C18:1ω7	Vaccenic acid	1.3	0.4	0.2	1.0	1.0	1.2
C18:2 ω6	Linoleic acid	63.0	1.0	62.6	51.6	52.4	51.4
C18:3 ω4	Octadecatetrienic acid	--	--	--	0.6	0.6	0.9
C18:3 ω3	Linolenic acid	0.3	--	--	0.2	--	0.2
C20:0	Arachidic acid	0.2	0.3	0.3	0.2	--	--
C20:1 ω9	Gondoic acid	--	72.8	0.3	14.8	14.4	13.9
C22:0	Arachidic acid	0.3	--	--	--	--	--
C22:2	Docosadienoic acid	--	14.0	--	--	--	--
C22:4 ω6	Arachidonic acid	--	0.2	--	2.3	2.2	1.9
Total saturated acids		10.4	1.6	11.4	8.5	7.8	8.0
Total unsaturated acids		89.3	96.6	86.7	91.5	91.7	90.9
Total ω-6		63.0	1.0	62.6	53.9	54.6	53.3
Total ω-3		0.3	--	--	3.1	--	0.2
Total mono unsaturated acids		26.0	82.4	24.1	36.8	36.5	36.5
Total polyunsaturated acids		63.3	14.2	62.6	54.7	55.2	54.4

For the general public health, it considers both practically and safety limit that total fat = 30% of total energy with oleic acid intake 15-16 % as recommended currently by the American Health Association for the general public health. This range will allow for a ratio of oleic to linoleic acid to vary from 1:1 to 3:1 from most people this range of dietary oils to be health (Grundy 1997). While, the fresh and frying blend oil have fairly difference than appropriate normal ratio as stated before. From Table (1) blend oil have a ratio between oleic and linoleic acid as 1:2.5. According to this ratio frying stability from blend oil is low perception to maintain the cholesterol level.

**Hematological parameters:**

Table (2) shown that the hematological parameters of male albino rats administrated on fresh blend experiment oil containing sunflower oil with jojoba oil 2:1 w/ w and their frying oils obtained from different deep frying process on potato fingers; comparison to control dietary fresh corn oil group (1).

The white blood cells (WBCs) count of the group 2 ( $16.95 \pm 3.69 \times 10^3 / \text{mm}^3$ ) was significantly ( $p < 0.05$ ) higher than that of group 1 ( $9.59 \pm 2.21 \times 10^3 /$

mm<sup>3</sup>) and 20<sup>th</sup> frying oil group (9.26 ± 1.77 10<sup>3</sup> / mm<sup>3</sup>) as well as group 4 dietary of 30<sup>th</sup> frying oil (10.16 ± 2.70 10<sup>3</sup> / mm<sup>3</sup>). There was no significance differences between that the frying blend oil groups 3 and 4 that of control group 1 (Table 2). According to this obtained result, there was a remarkable increase in WBCs could be seen in the group 2 after feeding on fresh blend oil. While another groups (1, 3 and 4) in this study showing a similar WBCs count with little difference between each other. The increase of WBCs among group 2 may behave as with normal physiological response following the perception of an insulting the body. Finlayson *et al.*, (1999) and Mesembe *et al.*, (2004) demonstrated that composition of thermoxidized palm oil diets cause damaging liver. It is likely; therefore that damage to the liver cells may have caused that contributed to observe increase in WBCs count. Similar found by this carried out study, there was indicated that the increase of WBCs among group 2 contributed to, that rats could not able to tolerate jojoba oil and / or different oxidized components in there dietary blend oil

**Table (2): Hematological parameters of albino rats fed on corn oil, fresh blend sunflower with jojoba and their different numbers of frying oils.**

Blood parameters	WBC 10 <sup>3</sup> /mm <sup>3</sup>	RBC 10 <sup>6</sup> /mm <sup>3</sup>	HGB g/dl	HCT %	PLT 10 <sup>3</sup> /mm <sup>3</sup>	MCV µm <sup>3</sup>	MCH pg	MCHC g/dl
<b>Normal range</b>	3.0-15.0	5.0-12.0	11.1-18.0	36.0-52.0	140-600	44-69	12.0-24.5	21.6-42.0
<b>Group 1</b>	9.59 ±2.21 <sup>a</sup>	6.74 ±0.35 <sup>a</sup>	13.39 ±1.40 <sup>a</sup>	43.02 ±1.58 <sup>a</sup>	483.1 ±37.75 <sup>a</sup>	66.5 ±11.45 <sup>a</sup>	19.92 ±5.64 <sup>a</sup>	31.87 ±2.72 <sup>a</sup>
<b>Group 2</b>	16.95 ±3.69 <sup>a</sup>	8.00 ±0.85 <sup>b</sup>	13.97 ±0.32 <sup>a</sup>	45.60 ±2.08 <sup>a</sup>	563.0 ±50.91 <sup>a</sup>	57.25 ±3.90 <sup>b</sup>	17.36 ±1.94 <sup>a</sup>	31.70 ±1.42 <sup>a</sup>
<b>Group 3</b>	9.26 ±1.77 <sup>a</sup>	8.40 ±0.25 <sup>b</sup>	13.17 ±0.39 <sup>a</sup>	44.48 ±0.71 <sup>a</sup>	577.3 ±36.06 <sup>a</sup>	53.25 ±0.35 <sup>b</sup>	15.40 ±21.0 <sup>a</sup>	30.81 ±0.57 <sup>ab</sup>
<b>Group 4</b>	10.16 ±2.70 <sup>b</sup>	8.31 ±0.76 <sup>b</sup>	11.89 ±1.17 <sup>c</sup>	42.53 ±2.89 <sup>b</sup>	621.4 ±58.1 <sup>a</sup>	53.25 ±3.08 <sup>b</sup>	14.27 ±1.75 <sup>b</sup>	29.40 ±1.91 <sup>b</sup>

Group 1: corn oil based diet; Group 2 fresh blend oil based diet; Group 3: 20<sup>th</sup> frying oil based diet and Group 4: 30<sup>th</sup> frying oil based diet

Values are mean ± SD of four diet oils (corn, fresh blend sunflower and jojoba (2:1), used frying F20 and F30 oils at different blood analysis time.

Values in vertical raw bearing same letters are significantly (p<0.05) different

WBC: White blood cells, RBC: Red blood cells, HGB: Hemoglobin , HCT : Hematocrite, PLT : Platelets , MCV: Mean Corpuscular Volume and MCH: Mean Corpuscular Hemoglobin and MCHC: Mean Corpuscular Hemoglobin Concentration .

The red blood cells count (RBCs) of group1 (6.74 ± 0.35 10<sup>3</sup> / mm<sup>3</sup>) was significantly (p<0.05) lower than that of group 2 (8.00 ± 0.85 10<sup>3</sup> / mm<sup>3</sup>); group 3 (8.40 ± 0.25 10<sup>3</sup> / mm<sup>3</sup>) and group 4 (8.31 ± 0.76 10<sup>3</sup> / mm<sup>3</sup>). There was no significant difference between that of all frying blend oils and fresh blend oils group as shown in Table 2.

There was an appreciable increase occurred among all of experiment groups in the hemoglobin concentration as attributed to Table (2). The hemoglobin concentration of deteriorated dietary frying oils (group 4) (11.89 ± 1.17 g / dL) has a significantly (p<0.05) lower than that of control group (13.39 ± 1.4 g / dL); group 2 (13.97 ± 0.32 g / dL) and group 3 (13.17 ± 0.39 g / dL). There was no significant difference between that of corn oil and



group (2). Therefore, the hemoglobin concentration of group 3 was significantly ( $p < 0.05$ ) lower than groups 1 and 2. Both groups 1 and 2 were a quite similar in their hemoglobin concentration in between 13-14 g/dl. From this obtained data obvious, the hemoglobin was significantly ( $p < 0.05$ ) lower among group 4 (fed on 30<sup>th</sup> frying oil) compared to hemoglobin concentration of rats fed on either fresh corn oil or fresh blend (jojoba and sunflower oils) and 20<sup>th</sup> frying oil. This decrease in hemoglobin concentration may be occurred due to reduce iron uptake by the damaged intestinal mucosa and resulting in reduced bioavailability of iron in system. This is borne out by the work of Igiri *et al.*, (1994) which showed that the intestinal mucosa of rats was severely damaged by chronic consumption of the thermoxidized palm oil diet and also attributed to decrease storage iron in the deterioration hepatocellular of liver (Finlayson *et al.*, 1999).

Continuously by Table (2), the hematocrite percentage of dietary frying oil of group 4, shows a significant decrease ( $42.53 \pm 2.89\%$ ) in hematocrite ratio by observed on all experiment groups. The hematocrite level of group 4 ( $42.53 \pm 2.89\%$ ) was significantly ( $p < 0.05$ ) lower than control group 1 ( $43.02 \pm 1.58\%$ ); group 2 ( $45.60 \pm 2.08\%$ ) and group 3 ( $44.48 \pm 0.71\%$ ). Otherwise, the hematocrite level among all of experiment groups was within normal range (36-52 %)(Kiraly 1980). From this previous result, appears that the hematocite concentration is the function of RBCs count, it represents the percentage of RBCs count in blood (Kiraly 1980).

Also table (2) shown that the platelets concentration in blood of experiment dietary oil groups, there was a wide fluctuation in concentration of platelets among all of different dietary oil groups. There were a significant increases in platelets in group 4 ( $621.4 \pm 58.1 \times 10^3 / \text{mm}^3$ ), while group 1 has a lower concentration ( $483.1 \pm 37.75 \times 10^3 / \text{mm}^3$ ) in their platelets comparing to other different type of dietary oil groups. Incontrast, the platelets of group 2 ( $563.0 \pm 50.91 \times 10^3 / \text{mm}^3$ ) and group 3 ( $577.3 \pm 36.06 \times 10^3 / \text{mm}^3$ ) were some what similar with slightly significance difference in platelets concentration in group 2. While, the platelets concentration of group 2 has some what different than group 3.

In addition, MCV of RBCs was significantly lower ( $p < 0.05$ ) in all groups which fed on fresh blend oil and frying blend oil than group 1. The increase of RBC volume (MCV) is also reflected in release in the MCH or amount of hemoglobin per cell.

MCV ( $\mu\text{m}^3$ ) of control group 1 ( $66.5 \pm 11.45 \mu\text{m}^3$ ) was significantly ( $p < 0.05$ ) higher than that of group 2 ( $57.25 \pm 3.90 \mu\text{m}^3$ ), group 3 ( $53.25 \pm 0.35 \mu\text{m}^3$ ) and group 4 ( $53.25 \pm 3.08 \mu\text{m}^3$ ). There was no significant difference between that of the fresh and used in dietary frying oil groups. This result indicated that increasing of MCV of group 1 and RBCs volume were markedly increase in the groups 2, 3 and 4. In contrast, MCV was significantly ( $p < 0.05$ ) lower than control group 1 whereas their RBC count was significantly ( $p < 0.05$ ) higher in group 2, 3 and 4.

Both group 3 ( $15.40 \pm 21.0 \text{ pg}$ ) and group 4 ( $14.27 \pm 1.75 \text{ pg}$ ) had significantly ( $p < 0.05$ ) lower than group 1 ( $19.92 \pm 5.64 \text{ pg}$ ) and group 2 ( $17.36 \pm 1.94 \text{ pg}$ ) of those mean corpuscular hemoglobin (MCH). This obtained result, it could be appeared that the MCH and MCV concentration

lower among group 3 and 4. Science, the dietary on deteriorated dietary frying oil of group 3 and 4, both hematological parameters of hemoglobin (Hb) concentration and RBCs volume (MCV) were lower than other experiment dietary oil, whereas, their RBC count was higher among this groups (3 and 4) of frying dietary.

However, the RBCs count was increase in the groups 3 and 4, RBC cells being in smaller size with containing abnormal of hemoglobin concentration (Cokelaere *et al.*, 2000).

Most of blood parameters indicated that frying oil and fresh blend oil may be caused a microcytic normochromic anemia, as decrease could be seen in the MCV or RBCs volume.

Mean corpuscular hemoglobin concentration was determined among group 3 ( $30.81 \pm 0.57$  g/dl) and group 4 ( $29.41 \pm 1.91$  g/dl). This result elucidated that; the bone marrow snears showed very active blood cells formation. Moreover, from data of (Table 2), there was no significant difference being in group 1 ( $31.87 \pm 2.72$  g/dl), group 2 ( $31.70 \pm 1.42$  g/dl) and group 3 ( $30.81 \pm 0.57$  g/dl) in the level of MCHC, thus, the MCHC was remained within the normal range in all groups.

Generally, this study of subchronic consumption of frying blend oil with jojoba oil based in blend diets at animal rats has been observed an alteration of hemoglobin concentration, hematocrite, MCH, MCV and MCHC occurred, with level of platelets and count of WBC and RBCs were higher concentration but within normal range. This extent increase in RBCs of groups fed on fresh and blend frying oil which was containing jojoba oil, it could be recognized that the jojoba oil induced the liver to release blood hemosiderin and ferritin, and then suppressed the bone marrow to synthesis RBC cells (Finlayson *et al.*, 1999).

In conclusion, frying edible blend oil containing jojoba oil, may be induced degenerative hepatocellular of liver nodes and follows by hematological adverse after consumption this blend in frying process. While, there were no appreciable adverse from feeding rats on the fresh blend containing jojoba oil have been obtained on the hematological parameters.

#### **Serum analysis:**

Effect of fresh blend oil and its frying on plasma total cholesterol (TC), triglyceride (TG) and liver function parameters were presented in Table (3). All groups resulted in nonsignificant ( $p < 0.05$ ) difference in TC level after long time experiment. TC ranged from  $86.23 \pm 18.13$  to  $92.08 \pm 10.69$  mg/dl among all of experiment groups. Meanwhile, there were significant increases in TC during the experimental period and its interaction with oil type.

From the results in Table (3), serum cholesterol in normal cholestromic rats, generally of sure around 100 mg/dl according to Ranhotra *et al.*, (1990).

Feeding on different types of blend oil in diet administrated into experimental rats for a long time of subchronic protocol, decrease significantly plasma triglycerides after experimental periods.

From previous obtained result, it could estimated that jojoba oil in blend with sunflower oil in formation either fresh or frying process and then feeding into rats behave a tendency to decline triglyceride level in plasma in comparison to control group. Jojoba oil may be acting as HMG-COA (3

hydroxy -3methylglutryl-COA reductase) inhibitors, do as lower in both plasma cholesterol and triglyceride (Bocan *et al.*, 1994). On the other hand, the mean of triglyceride level of group 4 (fed on exhausted frying blend oil of 30<sup>th</sup> frying) was lower than group 2 and 3, the thermoxidized alteration which consequence after reputedly frying process increase the level of oxidized triacylglycerols and dimmers as well as polymers, these compounds act as retardation of hydrolytic process that contributed to use jojoba oil in blend, however, jojoba oil has generally not hydrolyzed by lipase and pancreatic lipase (Bizzi *et al.*, 1985) reported that jojoba was impaired the oil absorption of hydrolyzed oil form, Moreover, Gonzalez-Muñoz *et al.*, (2003) found that , the tendency to lower plasma triglycerol level in animal receiving on heated oil was due to slow fat absorption and delay chylomicron synthesis.

Table (3): Mean serum analysis of rats fed on corn oil, fresh and versus blend frying of sunflower and jojoba oils (2:1).

Group diet oil	Plasma total cholesterol TC (mg/dl)	Plasma triglyceride TG (mg/dl)	Liver function		
			GOT (U/L)	GPT (U/L)	γ-GT (U/L)
Group 1	85.38 ± 10.81 <sup>a</sup>	104.14 ± 41.79 <sup>a</sup>	26.42 ± 10.83 <sup>a</sup>	103.83 ± 36.50 <sup>a</sup>	41.66 ± 12.92 <sup>b</sup>
Group 2	86.23 ± 18.13 <sup>a</sup>	90.11 ± 21.47 <sup>b</sup>	21.42 ± 15.21 <sup>b</sup>	98.66 ± 19.88 <sup>a,b</sup>	53.75 ± 7.77 <sup>a</sup>
Group 3	88.66 ± 8.48 <sup>a</sup>	89.09 ± 23.34 <sup>b</sup>	25.54 ± 4.96 <sup>a</sup>	92.17 ± 15.55 <sup>b</sup>	57.25 ± 10.46 <sup>a</sup>
Group 4	92.08 ± 10.69 <sup>a</sup>	79.08 ± 11.75 <sup>b</sup>	24.17 ± 9.11 <sup>ab</sup>	85.00 ± 16.66 <sup>b</sup>	57.58 ± 9.67 <sup>a</sup>
LSD	10.094	11.186	2.939	15.774	7.829
Two-way ANOVA					
Oil	NS	SN	SN	SN	SN
Experimental duration	SN	SN	SN	SN	SN
Oil*Experimental duration	SN	SN	SN	SN	SN

Values are mean ± SD of ten animals

Values in vertical raw bearing same letters are significantly (p<0.05) different

- \*Aspartate aminotransferase (GOT) \*\* Alanine aminotransferase (GPT)
- \*\*\* γ-GT Gamma Glutamyl Transferase

It noticed from the result in Table (3) that, group 4 have a lesser tolerate in spite of increase the TC (92.08) than other groups 1, 2 and 3 meanwhile, vise reverse TG was tended to decrease of its concentration in same group. This may be credible by Ammouche *et al.*, (2002) who reported that, the decrease in serum triglyceride and liver hypertrophy reflected on prevent TC synthesis resulting in lipid metabolism that is the consequence of ingested oxidized ester toxicity.

The biological activity of such frying oil and its oxidized has a role in increase the level of LDL-cholesterol in the serum of fed experimental animals (Andrikopoulos *et al.*, 2002). This LDL-cholesterol act as precursor to produce VLDL-cholesterol cholesterol by liver lipogenesis and produced a fatty liver (Dominique 1994).The decrease of TG synthesis has been proposed as contributing to the hypolipidemic effect of used frying oil, this may be attributed to the containing of jojoba oil an a precious amount of long chain fatty acids of MUFA (about 14%) table (1), that able to impaired the level of TG in the plasma of treated dietary groups as mentioned before.

Control group corn oil dietary have a significantly ( $p < 0.05$ ) higher in plasma TG than group 2, 3 and 4 (Table 3). This result is agreement with Sánchez-Muniz *et al.*, (1998) who found that PUFA primarily linoleic acid are known to be hypocholesterolemic but the linoleic acid has a reverse role on increase the TG in serum rats fed on corn oil. Such corn oil has linoleic acids about 63% that increase the TG of received rats on corn oil and decrease cholesterol level on the other side.

**Liver function parameters:**

Effect of using blend oil and its frying at the liver function GPT; GOT and  $\gamma$ -GT were shown in Table (3). There were a significant ( $p < 0.05$ ) difference between all of experiment dietary oil groups, experimental duration and the interaction between oil & duration. Higher mean GOT in group 1, 3 and 4 were 26.42, 25.54 and 24.17(U/L), respectively when compared to group 2 fed on fresh blend oil. While all of the experiment groups shows a significant ( $p < 0.05$ ) decrease in GPT of groups 2, 3 and 4 compared to control group 1. Meanwhile, there were pronounced increases of  $\gamma$ -GT obvious among treatment groups that fed on fresh and frying blend oil. These differences were attributed to significant toxicological effect on frying jojoba and sunflower blend oils occurrence after used this blend in the frying. However, corn oil group had a lowest level of  $\gamma$ -GT ( $41.66 \pm 12.92$  U/L).

Generally, increase and development of  $\gamma$ -GT level was expressed as liver presumptive preneoplastic foci, thus also attributed to type of dietary fat and modifying the postinitiation on phase of carcinogenesis with mammary tumors (Hopkins and West 1967). In addition to, the suppressive effect of frying dietary oil on liver  $\gamma$ -GT positive foci development compared to corn oil diet. Increase of  $\gamma$ -GT may occurred through regulation on liver detoxifying role in phase I and II detoxifying enzyme activity as well as role of liver to biotransformation system of rats (Chong-Kuei *et al.*, 2000).

**Organs weight indices:**

This subchronic study aimed to investigate adverse effect of administrated blend of jojoba oil with less stable sunflower oil and its usage probability in frying process as well as examined both histological and biological effect on experimental rat's. The current study was undertaken to assess the effect of ingested 10% fresh and frying blend jojoba and sunflower oil (1:2 w/w) used repeatedly for different frying cycles up to 30 frying for potato fingers.

As a consequence of ingestion frying oils, a various changes appear in liver, kidney and heart weight among groups as shown in Table (4). Rats which consumed the freshly and frying oil has a tendency to increase their livers kidneys and hearts weight indices than those fed on freshly corn oil (Table 4). Generally, this present study shows that fresh and frying oils among groups 2, 3 and 4, have a markedly increase in their different weight index than group 1 (corn oil based diet). In agreement by Sánchez-Muniz *et al.*, 1998) whom indicated that frying oils used at high temperature or for a long time produce a liver weight increase. In addition Ammouche *et al.*, (2002) founded that the following ingestion of thermally oxidized oil, there is a concomitant evaluation of very cytotoxic and destructive by products, which are injurious to cell tissues and organs.

Hepatosomatic index (liver / body weight × 100) among rats group 2, 3 and 4 which were fed on fresh and frying blend oils, increase in hepatosomatic index of group 1. Also, kidney / body weight index was significantly higher among groups 2 than other groups. These results indicated that, both group 3 and 4 were reasonably well tolerated by the rats than group 2 which fed on fresh blend (Sunflower: jojoba oils 2:1). The consumption of frying blend jojoba and sunflower, altered or damaged the nephron with respect to decrease kidney weigh ratio, similar to kidney weight of group 3 and 4 comparing to both group 1 and 2 , this agree with comments by Aruoma (1994). This result may be indicated by Davidson *et al.*, (1999) whom found that demonstrated that thermoxidized palm oil diet cause tissue damage to the kidney especially portal tubular a trophy and decrease erythropoietin production caused decrease RBC count.

From Table (4) the cardiosomatic index (heart / body weight × 100) among group 2 , 3 and 4 there were a relative increases by 33.3 % , 50% and 30%, respectively, than group 1.

**Table (4): Influence of dietary fresh and frying blend oils (Sunflower: Jojoba oils 2:1 w/w) on different organs weight % of albino rats group.**

Organ /body weight %	Experimental diet groups			
	Group 1	Group 2	Group 3	Group 4
Hepatosomatic index (weight liver /body %)	2.73	3.80	2.90	3.32
Kidney /body %	Right /Left	Right /Left	Right /Left	Right /Left
	0.31/0.29	0.43/0.4	0.36/0.42	0.37/0.35
Cardiosomatic index (weight heart / body %)	0.30	0.40	0.45	0.39

Data are presented as average among each group 5 rats.

This result indicated that inverse tendency of rats whose diets contained alter used frying to excrete more oxidized components, by different detoxification enzymes in liver and kidney which may be responsible to increase organs weight (Aruoma 1994 and Lopez-Varela *et al.*, 1995). Agree with this reports the carried out on experiment shows an reasonable increase in the liver of feeding groups on frying blend oil meanwhile their was a shrinkage in the glomeruli of kidney in this groups fed on frying oil accompanied with lower weight of rats kidney.

**Fatty acids profile of extracted lipids from experimental albino rats organs (Liver, Kidney and Heart):**

The fatty acids profile of liver , kidney and heart extracted from rats fed on both fresh and frying blend oils as well as fresh corn oil diet groups are presented in Table (5).

The fatty acid profiles of liver tissues were fairly similar pattern in all experiment groups. There was a little partial destruction of the fatty acids by different frying oils obtained from frying process on blend sunflower and jojoba oils. From data in Table (5) it noticed that, the fatty acid of corn oil and

fresh blend oil groups (1,2) contains appreciable amount of C18:2 $\omega$ 6 (15.2-16.4 %) in the dietary oil, but found in lower concentration in the liver tissues among different lipid oil groups (3,4) flocculated trends increases in between 14.5-21.0 %. These resulted in agreement with Ammouche *et al.*, (2002) and Viejo (1992) whom reported that low ratio of linoleic acid in liver tissue among all groups may be attributed mainly to its initial amount in the dietary oils and also due to alteration of fatty acids biosynthesis of dietary oils and their fatty acids.

On the other hand, the fatty acid deposition in adipose tissue bears some resemblance to the fatty acid composition of diet (Thomas *et al.*, 1981).

Moreover, extent of oxidative stress after fed on dietary oxidized fat or frying oil, assumed to play a crucial role in the development of several chronic disease including coronary heart disease and cancer (Halliwell and Gutteridge 1999). In addition, dietary fatty acids can influence the susceptibility of cells to oxidative stress, perhaps due to changes in cell membrane fatty acid composition (Battio *et al.*, 1999).

Also, it is clear from the resulted in Table (5) that there were increase of arachidonic acid (C20:4  $\omega$ 6) those results agree with Ammouche *et al.*, (2002) and Alexander *et al.*, (1983) who reported that increases of arachidonic acid content in liver fed on deteriorated frying oil accompanied by reduction of linoleic acid this may be the consequence of cellular protection with a view to maintaining a normal physiological state. Moreover, high level of arachidonic acid C20:4  $\omega$ 6 in oxidized lipids of heated corn oil dietary for animal attributed to increase activity of enzyme system functioning to elongated and desaturate C18:2  $\omega$ 6 to form C20:4.

These results were indicated by (Harris 1990) whom found that, arachidonic acid can block the production of several products related to the inflammatory process such as cytokines and interferon released by macrophages and lymphocytes and soluble phase inflammatory mediators released by neutrophils at the site injury. Furthermore, the increase of arachidonic acid (C20:4  $\omega$ 6) is duty to maintain the oxidation stress on the cell of different organs after ingested dietary thermal oxidized frying oils. Another suggestion Lin *et al.*, (2000), by increasing dietary oxidized oil and frying oil administrative to mice could be result in increase of prostaglandin production and subsequent higher Microsomal Enzyme Cytochrome P450 content was noted, the microsomal enzyme is more important to prostaglandin metabolism, certain enzymes such as prostaglandin hydrogenase synthase implicated in the metabolism of arachidonic acid metabolism which may also increase in mice fed oxidized and frying oils. In addition, degree of lipid oxidation can be affect lymphoid organs and influence immune responses of normal mice and aggravated of such immune disease and allergic and tumors as well as tumor necrosis (Lin *et al.*, 1996).

Finally, after all above statements the higher level of arachidonic in different rat's organs would be needed for regeneration to palliate liver injury (López-Varela *et al.*, 1995).

The fatty acid profile of liver of rats provided corn oil, fresh blend oil and different frying oil showed substantial increases of C16:0 and C18:0 content. Similar by Alexander *et al.*, (1983) who found that major distribution of

unaltered fatty acids present after dietary treatment. However, the heated palm oil increased the C16:0 and C18:0 of neutral liver lipids and the C16:0 of oxidized oil with reference to levels of C20:4 in all groups. Different dietary fats showed some effect on all activity of elongation desaturation enzymes involved in metabolism of C18:2.

**Table (5): Fatty acids composition of extracted lipids from experimental albino rats organs (Liver, Kidney and Heart) , those fed on diet containing 10% fresh corn oil, unused blend oil and used frying blend oils**

Fatty acids	Group 1			Group 2			Group 3			Group 4		
	Liver	Kidney	Heart	Liver	Kidney	Heart	Liver	Kidney	Heart	Liver	Kidney	Heart
C14:0	1.1	1.9	1.3	0.0	0.0	1.7	0.4	1.4	-	1.2	1.3	-
C16:0	30.8	35.4	28.0	21.0	24.7	27.3	21.2	27.9	22.2	25.6	26.8	25.0
C16:1 $\omega$ 7	4.8	9.9	2.6	1.4	3.0	2.6	1.8	3.2	-	3.9	4.1	-
C18:0	8.4	4.2	13.0	12.3	10.3	11.9	14.4	14.3	32.5	9.4	9.2	34.8
C18:1 $\omega$ 9	28.0	26.8	35.0	24.0	26.6	32.8	18.8	26.1	20.4	24.6	26.5	26.1
C18:1 $\omega$ 7	3.2	1.1	-	2.5	2.1	1.9	1.0	1.7	3.4	1.8	2.0	4.0
C18:2 $\omega$ 6	15.2	18.9	18.0	16.4	17.7	15.0	21.0	14.9	16.5	14.5	15.9	8.8
C18:3 $\omega$ 6	1.3	-	-	-	-	-	0.6	-	-	1.0	-	-
C18:3 $\omega$ 3	-	-	-	-	5.5	-	-	-	-	-	1.2	-
C20:1 $\omega$ 9	-	-	6.8	9.6	-	6.6	3.0	5.3	4.9	4.2	4.6	6.3
C20:4 $\omega$ 6	7.2	0.8	-	11.7	10.0	-	16.2	5.0	-	11.1	8.1	-
$\Sigma$ Saturated	40.3	42.4	42.3	33.3	35.1	41.0	36.0	43.6	54.7	35.2	37.4	59.8
$\Sigma$ unsat	59.7	57.5	62.4	65.6	64.9	44.0	62.0	56.3	45.2	61.2	62.6	40.1
$\Sigma$ $\omega$ -6	23.7	19.7	18.0	28.1	27.8	15.0	17.0	19.9	16.5	20.9	24.1	8.8
$\Sigma$ $\omega$ -3	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	1.0	1.2	0.0
$\Sigma$ monounsaturated	36.0	37.8	44.4	37.5	37.2	44.0	24.6	36.4	28.7	39.2	37.3	35.8
$\Sigma$ polyunsaturated	23.7	19.7	18.0	28.1	27.8	15.0	37.8	19.9	16.5	22.0	25.3	8.8

From obvious results , it appears that frying blend oil as general really has a toxic effect , which is confirmed by increase the liver malonaldehyde and also increase trans fatty acids in lipid tissue for disturbance and trouble enzymatic activity which implying as a detoxification on mechanism with changes in metabolic regulation . System of fatty acids synthesis, also Trans fatty acids were trouble membrane fluidity with enzymatic and reaction disorders in cells (Ammouche *et al.*, 2002). By this suggestion, it can be assumed that jojoba oil as dietary oil has a similar implication of normal or conventional usage. Moreover, jojoba was acting as frying stability with no more alters in nutrition similar to conventional types of oil. Revealed to the fatty acid pattern of kidney there were fairly similar in C16:0 among kidneys lipid of groups 2, 3 and 4. Otherwise, it is a higher ratio in C16:0 in group 1.

The most obvious relative increase in C18:0 ranged 119 up 240 % among groups 2, 3 and 4 compared to kidney lipid of group 1 (fed on corn oil).

The higher content of C18:0 and C16:0 especially in kidneys of group 2, 3 and 4 indicated that both jojoba and sunflower and their blend may be cause tissue damage to kidney especially partial tubular atrophy. With

ranged to fresh and frying oils, a marked raise was observed in C18:0 and C20: 4  $\omega$ 6. Meanwhile, the fatty acids of kidney group 1 have a decline in C20:4 and C18:0 with greater level of C16:0 (35.4%) vice reverse among groups 2, 3 and 4.

Furthermore, the fatty acids profile of kidney among all groups is quite similar in C18:1  $\omega$ 9 and C18:2  $\omega$ 6. Also it is pronounced that kidney of group 1 has a low level of C20:4 (0.8%). This result indicated that, there is no alteration found among group 1 which received on corn oil.

Table (5) shows of heart lipids, their were an increase in level of C18:0 in group 3 and 4 (32.5 and 34.8%), whereas group 1 and 2 have a lower ratio in C18:0 (13.0 and 11.9%, respectively). This indicated that, may be an alteration occurred in biosynthesis pathways in ingestion of frying oils appears on hearts of such group 3 and 4 comparing to group 1 and 2. Also, these results proved that jojoba oil in blend with sunflower oil have no adverse in heart of rats and / or human dietary oil.

**Adverse effect of used frying blend oils on liver retinol and liver  $\alpha$ -tocopherols:**

The effect of frying oils on the alteration procedure to the experimental administrative fresh and frying blend of sunflower and jojoba oils for animal rats in the present study, shown in Table (6).

Liver retinol (mg/100gm) levels were varied according to dietary type of oils among all groups reference to its level in corn oil dietary group. Levels of retinol in the livers of groups diet was drastically decreased from group 1 up to group 4. as shown in Table 6. The livers of total retinol concentration in both of group 3 and 4 were decrease by quarter of those of group 1. Otherwise, liver retinol levels of rats fed on a corn oil was higher than rats fed on the fresh and frying blend oil. Vitamin A is stored particularly in the liver and body is reverse in a normally nourished rats are only depleted after about one year (Chong-Kuei *et al.*, 2000). Weber *et al.*, (1983) found that, feeding three treatment groups on 10% jojoba oil fed group, liver stores of vitamin A were high, indicating absence of vitamin A deficiency.

From these obtained data, liver retinol level in the two typical altered groups 3 and 4 was decreased; it may be to use retinol of liver to regenerate of alters membrane. This is in agreement with that reported by Nancy Misslbeck *et al.*, (1984) they appreciated that the increase of  $\gamma$ -GT activity of liver Foci was a reason to lack of liver vitamin A level. Also the deficiency intake of vitamin A was enhanced the liver tumorigenic. Parallel to this report, jojoba oil is used in blend oil diet interpret that the latter observation is evidence for the presence of antivitamin A hydrolysis factors in jojoba oil, although a more convincing argument would be the lack of stable fat esters hydrolyase internal mammals as mention by Weber *et al.*, (1983). Moreover, the group 3 was affected similarly to the group 4, the effect on level of liver retinol resulting from investigation of dietary oxidized frying oils. This is agreed by Chong-Kuei *et al.*, (2000). Before inclusion into the retinol of liver influence of repeated frying on the digestive utilization of various dietary oils, a notice decrease was occurred with repeated frying. The extent of vitamin destruction during different frying process on used oil and dissolved in the frying oil medium. Emilia *et al.*, (2006) studied the effect of processing on



carotene content of Thai vegetables and showed that the average losses of vitamin A were 14 % for boiling and 24 % for frying.

Some carotenes were lost in the cooking water losses during frying were greatest due to leaching into frying oil. In agreement by Pokorny (1999) investigated that,  $\beta$ - carotene and total carotenes were depleted after first frying but the second and further consecutive frying resulted in a sharper fall in carotenes . From this previous observation, it can be assumed that the diets containing different status of frying oils or fresh blend oil had a wide variation of carotenes or  $\alpha$ - tocopherol concentration.

**Table (6): Liver retinol and  $\alpha$ -tocopherols fed rats on diet containing fresh and frying oil blend sunflower and jojoba oils (2:1)**

Groups of dietary oil base	Liver retinol mg/100gm	Liver $\alpha$ -tocopherol mg/100gm
Group 1	1.830	31.80
Group 2	1.317	30.34
Group 3	1.189	30.52
Group 4	1.134	5.5

Chong-Kuei *et al.*, (2000) found that administration fresh corn oil for experimental animal increase the liver and plasma vitamin E as well as vitamin A, whereas the heated corn oil is impair these concentration of both liver vitamin A and vitamin E.

Generally, an straightforward relationship apparent between level of vitamin A or carotenes in dietary lipids and their level in liver retinol . Also indicated by Dutra-de-Oliveira *et al.*, (1998) reported that deep heating of soya bean oil at 170 °C/ 20min had a decreased of liver vitamin A storage in rats after fed on this heated Soya oil.

Table (6) shows that the liver  $\alpha$ -tocopherol concentration in the liver of experimental rats fed on different dietary fresh and frying blend oil used in frying of potato fingers compared to normal based fresh corn oil diet. There was a decline in liver  $\alpha$ -tocopherol after use blend oil in frying. Decrease in liver  $\alpha$ -tocopherol appears among group 4 which fed on 30<sup>th</sup> frying oil. Meanwhile, the  $\alpha$ -tocopherol was a quite similar between group 1, 2 and 3 at about 30-31 mg/100mg (Table 6) . The worst result was observed with group 4 of rat showed consecutive decreases in the amount of liver  $\alpha$ -tocopherol into 5.5 mg/100mg. This reduction in vitamin E may be due to increase activity of plasmatransaminase and alkaline phosphotase which act as evident to oxidative stress on liver under experiment, observation that may be viewed as indirect evidence of the lowered plasma and liver vitamin E concentration in group 4. The vitamin E was responsible to present against damage the erythrocytes and decrease the oxidative stress in the body. This vitamin E is acting as antioxidants, which was depletion from the plasma and liver stores (Weber *et al.*, 1997 and Allard *et al.*, 1997).

Furthermore, these previous results could be appropriated that both group 2 and 3 in healthy status depending on liver  $\alpha$ -tocopherol, these groups were well nourished animal group.

Another point of view, the frying process on blend oil have not affect on vitamin E concentration up to 20<sup>th</sup> frying cycle , and also natural vitamin E in used oil is protect oils from deterioration for a prolonged time of frying abuse. Meanwhile, repeated frying up to consecutive 30<sup>th</sup> frying was declined the level of vitamin E in used frying oil, which decline level of liver  $\alpha$ -tocopherol after dietary based on 20<sup>th</sup> frying oil from 30.52 mg/100gm into 5.5 in 30<sup>th</sup> frying oil. This result also indicated by Gordon and Kourimská (1995) they reported that a significant increase in vitamin loss in the frying oil during 4 day of commercial frying. Tang *et al.*, (1998) indicated that administration of 15% oxidized frying oils into male weanling rats was attributed indirectly to the deteriorated vitamin E in liver and serum status resulting from the ingestion of these dietary oxidized oil. In addition to, plasma concentration vitamin E were significantly ( $p < 0.001$ ) reduced in both sexes of rats fed on jojoba oil treatment group. Reduction in Vit E liver stores although significant in the 10% jojoba oil fed group compared to control of corn oil diet. The increase activities of alkaline phosphatase and plasma transaminase was extended in the subchronic 90 day study, may be viewed as indirect evidence of the lowered plasma vitamin E concentrations in the jojoba oil group as convinced by Verbiscar *et al.*, (1980)

**Histopathological changes of liver and kidney tissues following the diet containing fresh and frying blend (sunflower with jojoba oils):**

Effect of dietary fresh and frying blend oil on the liver structure are spontaneous in Figures 1, 2, 3 and 4 comparing to livers of group 1 fed on corn oil. Microscopic examination of the different tissues kidney and liver demonstrated changes. Dietary fresh corn oil on tissues of group 1 was related to a normal histopathological structure of the central vein (CV), portal area and hepatocytes. There is no adverse of fed corn oil on the hepatocellular of experimental animal rats.

Meanwhile, a mild to severe necrosis of the liver cells of animals fed on fresh blend, 20<sup>th</sup> frying oil and 30<sup>th</sup> frying oil were investigated in this carried study. Otherwise, fresh blend (Sunflower with Jojoba 2:1) was showing a dilated and ingested central vein (CV) with diffuse Kuffer cells proliferation in between the hepatocytes. This result is disintegration with normal cellular structure and central vein observed in the control group 1 (fed on fresh corn oil). However, susceptible severe dilation of central vein with degenerative changes in the surrounding adjacent hepatocytes and diffuse kuffer cells in-between were investigated after feeding rats on 20<sup>th</sup> frying oil. These degenerative fibrotic areas in liver were found attributed to concurrent regenerative process. Therefore, revealed to the Table (5) , there was apparent increase in arachidic acids (C20:4  $\omega$ 6) among this group (3), that would suggested an alteration in the biosynthesis of this fatty acids and also to important to repaired the degenerative liver cells this suggestion is established also by López-Varela *et al.*, (1995).

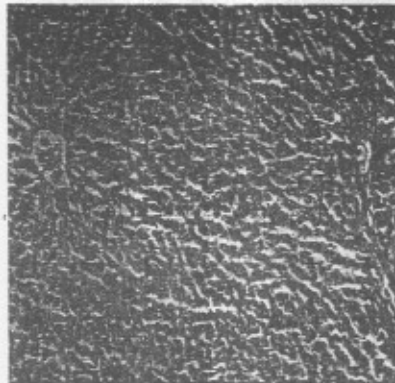


Fig (1):Section of liver of rats fed on fresh corn oil showing the normal histological structure of the central vein (CV), portal area (PA) and hepatocytes (magnification× 40)

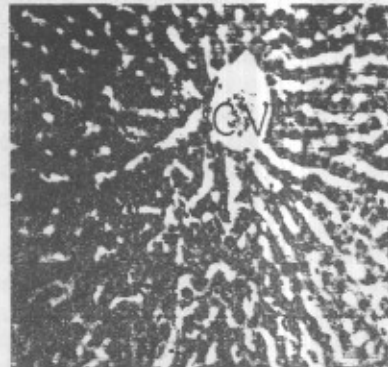


Fig (2):Section of liver of rats fed on fresh blend sunflower and jojoba oils, showing dilated and ingested central vein (CV), with diffuse kuffer cells proliferation in between the hepatocytes (arrow) (magnification× 64)

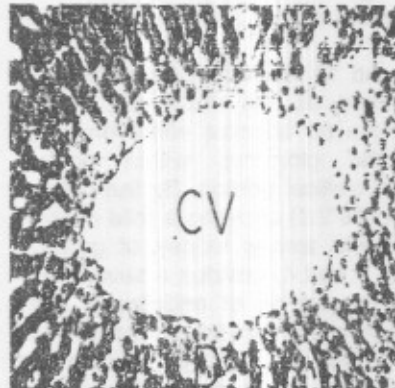


Fig (3):Section of liver of rats fed on 20<sup>th</sup> frying oil, showing sever dilation of central vein (CV) with degenerative changes in the surrounding adjacent hepatocytes and diffuse kuffer cells in between (magnification× 64)

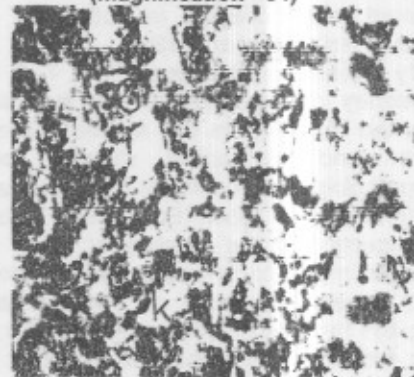


Fig (4):Section of liver of rats fed on 30<sup>th</sup> frying oil showing granular (G) and vacuolar degenerations and fatty change in cytoplasm of the hepatocytes (arrow) with diffuse kuffer cells proliferation in between (K) (magnification× 160)

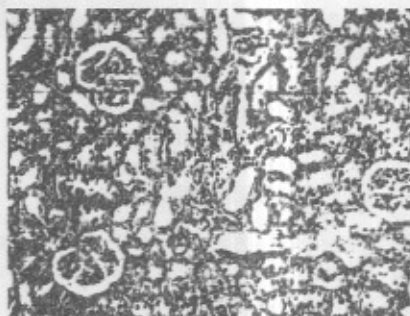
Investigation of Fig (4) shows that, the liver of group 4 after fed on 30<sup>th</sup> frying oil, there was obvious a highly severe of granular and vacuolar degeneration and appear fatty acid change in the cytoplasm of the hepatocytes. In addition to a severe diffuse in kuffer cells proliferation in between were observed. This proliferation of cells and granular degeneration has been reported as consequences of oxidized oil ingestion. Similar in agreement by Battio *et al.*, (1999) whom reported that dietary fatty acid influence in the susceptibilty of cells to oxidation stress, perhaps due to changes in cell membrane fatty acid composition. Since, the ingestion of aldyhedic products from oxidized oil and / or frying oil damaging the human health. In addition to many changes in histopathological investigation of liver and those cells injury apparent only after some critical biochemical system within the cell have been deranged. Disruption of the liver cell as reflected by altered morphological structure is suggested as chiefly reason to raise serum level of liver enzymes as previously reported (Jimoh and Odutuga 2001).

Incorporated with this previous statement on the carried study, development of  $\gamma$ -GT positive was affected by increase the deteriorated cycles of used frying oil up to 30<sup>th</sup> frying as shown in Table (3). The biochemical investigated that, a significant increase ( $p < 0.05$ ) in  $\gamma$ -GT among groups 2, 3 and 4 compared to control group 1. No significant difference could be shown among group 3 and 4 in their  $\gamma$ -GT. Therefore, the degeneration of hepatocellular and changes in fatty acids of cytoplasm composition of liver cells as well as changes in liver lipid profile were indicated that increases in both  $\gamma$ -GT concentration and liver functions (Nancy Misslbeck *et al.*, 1984).

By focusing on the figures 5, 6, 7, 8, 9 and 10, microscopic examination the kidney of experimental rats fed on frying oil and fresh oils, shows remarked changes. In this figures, there were no adverse effect on renal tubular and histological structure of the glomeruli within normal histopathological structure of renal tubular in cortical portion. By the dietary supplement of oil blend (Sunflower + Jojoba oils 2:1) showing a mild dilation and ingestion in the intertubular blood vessels among kidney of group2. However, microscopic investigation for group 3 and 4, obvious a severe and highly severe symptoms as swelling and vacuolation of endothelial cells lining the hyperemic glomerular tuft with degeneration in the epithelial cells lining the surrounding tubules in cortex after fed on 20<sup>th</sup> frying oil on the kidney of group 3 . Similar, a higher hyperemic capillaries inbetween the degenerated tubules at the corticomedullay junction and cystic dilation in renal tubules at the corticomedullay junction when rats were administration on 30<sup>th</sup> blend fried oil.

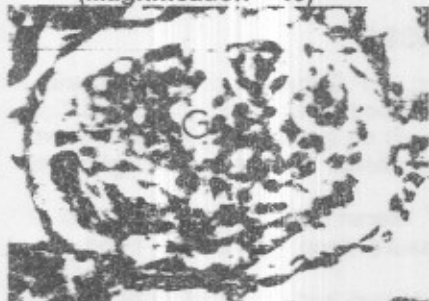
This result is in agreement by Ologan (2002); Jimoh and Odutuga (2001) Agula *et al.*, (2005) whom found that the disintegration of cytoplasmic membrane may likely lead to disruption in the filtration and concentration in urine, it may also affect fluid and electrolyte balance of the rats as well as regulation of total body homeostasis due of ingestion thermal oxidized lipids and found a ruminant glomeruli surrounding by variable extensions of scar tissue interstitial fibrosis . According to these previous reports, on this carried study, the hemoglobin was significantly ( $p < 0.05$ )

decrease among group 4 that other carried experiment on rats as shown in Table (2). There was no significant difference between fresh corn group, fresh blend group and 20<sup>th</sup> frying group (Table 2). This is in agreement with Mesembe *et al.*, (2004) who found that, there were no significant difference between the hemoglobin of fresh palm oil and control group, whereas group which fed on thermoxidized oil showing significantly ( $p < 0.001$ ) lower than control group ( $13.14 \pm 0.4$  g/dl).



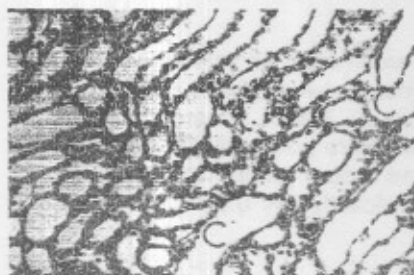
Fig(7): Section of the kidney of rats fed with fresh corn oil showing the normal histological structure of the glomeruli (G) and renal tubular © in cortical portion (Magnification  $\times 40$ )

Fig(8): Section of the kidney of rats fed with fresh blend sunflower with jojoba oils 2:1 showing dilation and ingestion in the intertubular blood vessels (bv) (Magnification  $\times 40$ )



Fig(9): Section of the kidney of rats fed with 20<sup>th</sup> frying blend oil showing swelling and vacuolation of endothelial cells (arrow) lining the hyperemic glomerular tuft (G) with degeneration in the epithelial cells lining the surrounding tubules in cortex (Magnification  $\times 160$ )

Fig(10): Section of the kidney of rats fed with 20<sup>th</sup> frying blend oil showing degeneration and swelling in the epithelial cells lining the tubules (D) (Magnification  $\times 160$ )



Fig(11): Section of the kidney of rats fed with 30<sup>th</sup> frying blend oil showing hyperemic capillaries (arrow) in between the degenerated tubules (D) at the corticomedullary junction (Magnification × 64)



Fig(12): Section of the kidney of rats fed with 30<sup>th</sup> frying blend oil showing cystic dilation in renal tubules at the corticomedullary junction (C) (Magnification × 64)

This result, proved that, feeding rats on exhausted frying oil up to 30<sup>th</sup> frying cycle, cause tissue damage of kidney especially partial tubular and consequence to failure erythropoietin production from kidney. After this failure in production of erythropoietin caused to decrease the RBCs count and decrease hemoglobin concentration (Mesembe *et al.*, 2004), this somewhat as shown in this carried study, as general, the result of different section tissues of rats fed on either fresh corn or blend in fresh status showed that there were no adverse histopathological affect, except a few alteration in the liver and kidney. While, section of different animal tissues appearing that mild and severe deleterious effects on the architecture of the tissue after fed on diet containing 10% 20<sup>th</sup> frying oil and 30<sup>th</sup> frying oil.

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## التقييم البيولوجي لإستخدام مخلوط من زيت الجوجوبا وعباد الشمس في القلي على فئران التجارب .

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المركز الإقليمي للأغذية والأعلاف - مركز البحوث الزراعية - الجيزة - مصر .

استهدفت هذه الدراسة تقييم إستخدام زيت غير تقليدي الإستخدام مثل زيت الجوجوبا الثابت حرارياً بخلاطة مع زيت أقل في الثبات الحراري مثل العباد بنسبة ٢:١ واستخدامه في صورته المخلوطة بدون قلي أو باستخدامها للقلي المتقطع والمستمر لمدة ٣ أيام متتالية على ١٨٠ م<sup>٢</sup> في قلي أصابع من البطاطس .

ووجد أنه بالخلاط حدث ارتفاع مستوى الأحماض أحادية عدم التشبع على حساب الأحماض الدهنية عديدة عدم التشبع. وبالتقييم البيولوجي لهذة الخلاطة متبعاً لجراء التغذية لتحديد التأثيرات تحسنت المزمنة لهذة الزيوت سواء طازجة الخلاط أو المستخدمة في القلي بالاعتماد على أربعة مجموعات من فئران التجارب وبالتغذية على وجبات تحتوي على ١٠% لأي من الزيوت المستخدمة ولتجهت الدراسة بالتغذية المفتوحة على زيت الذرة للمجموعة الأولى (مجموعة كنترول) والزيت الطازج للخلاطة للمجموعة الثانية والمجموعة الثالثة على الزيت الناتج بعد القلي العشرين والمجموعة الرابعة على الزيت الناتج بعد القلي الثلاثين لمدة كلية ٩٥ يوم.

وركزت الدراسة على تقدير تركيب الأحماض الدهنية للدهون المخزنة بالكبد والكلي والقلب لهذة المجموعات مع إجراء تحديد معدلات أوزان تلك الأعضاء بالنسبة لوزن الجسم مع إجراء التحاليل الكيماوية ووظائف الكبد وتركيب الدم والتأثيرات لهستولوجية على تلك الأعضاء. ولتضح من الدراسة لثة حدثت تأثير سلبي على تركيب الدم للمجموعات الثالثة والرابعة المغذلة على زيت القلي العشرين والثلاثين على التوالي من حيث تركيز هيموجلوبين الدم و الهيماتوكريت والصفائح الدموية وتركيز وحجم كرات الدم الحمراء. لا يوجد تأثيرات سلبية على نسبة الكوليسترول الكلي لجميع المجموعات والذي تراوح ما بين ١٨,١٣ ± ٨٦,٢٣ لسي ٩٢,٠٨ ± ١٠,٦٩ مجم / ديسيلتر وعمامة وجدت في النسبة الامنة بينما أتجه التراي جليسيريد نحو الأنخفاض للمجموعات ٢ و ٣ و ٤. وبالاعتماد على تحاليل وظائف الكبد وجد إختلافات معنوية ما بين المجموعات تحت الدراسة. حدثت زيادة معنوية للجاما جلوتاميل ترانسفيراز (γ-GT) للفئران المغذلة على زيوت الناتجة من القليات ٢٠ و ٣٠ بينما وجد تركيزها أقل في المجموعة الأولى المغذلة على زيت الذرة (٤١,٦٦ وحدة/لتر) وعمامة يتخذ هذا الإنزيم واحد من القرائن لحدوث بدايات سرطان الكبد. وبتقييم معدلات وزن الأعضاء بالنسبة للجسم وجد أن المجموعة ٣ و ٤ حدثت زيادة لتلك القياسات بالمقارنة للمجموعة الأولى (الكنترول). حدثت زيادة في حمض الارشيدونيك للمجموعات المغذلة على زيوت القلي مع انخفاض حمض اللينوليك لدهون الكبد والكلي والقلب والذي قد يعزى إلي دور الجسم والكبد لأصلاح تأثير جهد الأوكسدة الواقع على الخلايا وأعضاء الجسم. كما وجد انخفاض حاد في نسبة الريبوتول والفاتوكينول للكبد للمجموعة الرابعة.

ثبتت الدراسات لهستولوجية على الكبد والكلي من حدوث تأثيرات سلبية واضحة على تلك الأعضاء للمجموعات الثالثة والرابعة المغذلة على زيت القلي. واستخلصت للدراسة من أمكانية إستخدام زيت الجوجوبا بخلاطة مع زيت العباد في الإستخدام بصورته الطازجة أو المستخدمة في القلي بحيث لا يزيد مرات القلي عن ٢٠ قلي.