

## BIOCHEMICAL AND HISTOPATHOLOGICAL CHANGES IN ALBINO RATS AFTER LONG TERM CONSUMPTION OF SOME HEATED OILS

BY

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### ABSTRACT

This study was carried out on 70 adult male albino rats with average weight  $102.57 \pm 14.39$  gram. A pilot survey study among 1200 females in Mansoura Faculty of Medicine was done to choose the oils to be tested. These oils were used once for frying potatoes chips for different periods (1/4, 1/2, and 1 hour), repeatedly used 2, 3 and 4 times; each 1/4 hour and repeatedly used 2 times; each 1/2 hour. The effect of heating on oils was determined by assessment of Thio Barbituric Acid Reactive Substances (TBARS) in the fresh and heated studied oils. Results revealed that the deteriorative effect of heat increases by increasing the duration of oil use and the repetition of heating was much more destructive than the continuous use of the oil. Oils heated repeatedly for 4 times; each 1/4 hour was found to have the maximum TBARS content and so they were chosen for the preparation of diet used during the study on experimental animals. The animals were randomly divided into seven groups; 10 rats each and fed for three months the prepared experimental diet. First group of animals was fed oil free diet (negative control group). Animals was fed diet containing fresh (non- heated) sunflower (FS) oil in the second group (positive control group), repeatedly heated sunflower (HS) oil in the third group, fresh (non- heated) cottonseed (FC) oil in the fourth group (positive control group, repeatedly heated cottonseed (HC) oil in the fifth group, fresh (non-heated) mixed (sunflower and cottonseed) (FM) oil in the sixth group (positive control group) and repeatedly heated mixed (sunflower and cottonseed) (HM) oil in the seventh group. Rats were sacrificed and dissected at the end of third month of the study. Organs (liver, kidney, colon, heart, and testis) were immediately excised and prepared for pathological studies and blood samples were collected for biochemical studies including estimation of "TBARS" and glutathione peroxidase in serum, separation of  $\alpha$ -,  $\delta$ - tocopherols and tocopherol acetate and estimation of serum ALT, AST, albumin, creatinine and urea. In experimental animals it was found that chronic consumption of any of the repeatedly heated studied oils increases the serum level of the peroxidative products (TBARS), decreases the antioxidant defense of the body (serum glutathione peroxidase and tocopherols) and compromising the liver and kidney functions presented as increase in the serum hepatic enzymes, decrease in serum albumin and increase in both

*serum creatinine and urea. Pathological changes were found in liver, kidney, colon, and testes of animals fed repeatedly heated oils. These effects were more pronounced in animals fed repeatedly heated cottonseed oil followed by the repeatedly heated mixed oil then the repeatedly heated sunflower oil.*

## INTRODUCTION

The role of dietary fat and oils in human nutrition is one of the important areas of concern and investigation in the field of nutritional science. The findings of these investigations have wide ranging implications for consumers, health care providers and nutrition educators, as well as food producers, processors and distributors. New evidence concerning the benefits and risks associated with particular aspects of dietary fat is constantly emerging in both the scientific literatures and the popular media. At times, controversies about these findings evolve (F.A.O., 1994). Fats and oils are heated at high temperatures during baking, grilling and frying; however, deep fat frying is the most common method of high temperature treatment. Deep fat frying is a popular food preparation method produces the desirable fried food flavour, golden brown colour and crisp texture. Because of the great consumption of frying oils and fats, the effects of high temperature on oils and fats is of major concern both for product quality and nutrition (Warner, 1999).

Deep fat frying is an important, ubiquitous and highly versatile process, which has been used since antiquity to cook a

wide spectrum of products. Its unique contribution to sensory characteristics, together with the relatively low cost of large-scale frying, has made fried foods the staples of the ever growing late 20th century fast food industry (Saguy and Dana, 2003).

The use of oil remains one of the most popular methods for the preparation of foods. This is particularly true in Egypt and the majority of the Arab world where fallafel (a deep fried vegetable patty) forms the staple food item in the diet and is widely available at street level from vendors (Tewfik et al., 1998).

The purpose of the present work is to study the effect of heating on oils and the effect of chronic consumption of repeatedly heated oils on experimental animals.

## MATERIAL AND METHODS

The most commonly used oils (sunflower, cottonseed, and mixed oils) determined by a pilot study were chosen for the study. These oils were purchased from supermarket and were used one time in frying potatoes chips in aluminum wok for different periods (1/4, 1/2, and 1 hour), repeatedly used 2, 3 and 4 times; each 1/4



hour and repeatedly used 2 times; each 1/2 hour. Repeated heating of oils was done without any topping up (without replenishing the oil in the fryer with fresh oil) under conditions corresponding to the normal household frying practices (domestic frying). The effect of heating on oils was predicted by estimation of the content of the TBARS in fresh and heated studied oils according to the method described by Draper et al. (1993).

The study was carried out on 70 adult male albino rats with average weight  $102.57 \pm 14.39$  gram obtained from animal house of Mansoura Faculty of Medicine. The rats were housed in metallic cages at room temperature with day and night light rhythm. After a week of acclimatization to the housing conditions, rats were randomly divided into seven groups; 10 rats each and fed the prepared experimental diet.

Diets were prepared as a 15 % (W/W) mixture of the test oil (sunflower, cottonseed or mixed) fresh or heated (repeatedly heated for 4 times; each 1/4 hour have the maximum TBARS content) and the rodent chow.

This study used the oil concentration as 15 % in the diet because the fat content of the potatoes when fried as chips increases to 7-15 gram fat per 100 gram potatoes (Fox and Cameron, 1995).

#### Classification of animals:

Rats were classified into 7 groups. First group of animals was fed oil free diet (negative control group). Animals were fed diet containing fresh (non-heated) sunflower (FS) oil in the second group (positive control group), repeatedly heated (4 times; 1/4 hour each) sunflower (HS) oil in the third group, fresh (non-heated) cottonseed (FC) oil in the fourth group (positive control group, repeatedly heated (4 times; 1/4 hour each) cottonseed (HC) oil in the fifth group, fresh (non-heated) mixed (sunflower and cottonseed) (FM) oil in the sixth group (positive control group) and repeatedly heated (4 times; 1/4 hour each) mixed (sunflower and cottonseed) (HM) oil in the seventh group.

Rats were kept on diet for three months. Rats were sacrificed and dissected at the end of third month of the study. Organs (liver, kidney, colon, heart, and testis) were immediately excised and prepared for pathological studies according to the method of Stevens (1982).

#### Biochemical studies :

Blood samples were collected for biochemical studies as heparinized blood samples prepared by using 0.3 ml heparin on 2 ml blood (heparin "5000 IU" of Nile Co. Batch No. 90071) and Serum (blood samples were centrifuged at 3500 Round Per Minute (RPM) for 5 minutes on Sigma 2-15 centrifuge). Samples were kept in re-

frigerator until the time of biochemical studies.

Effect on lipid peroxidation was determined by estimation of "TBARS" in serum according to method described by Draper et al. (1993).

Effect on antioxidants was determined by estimation of serum glutathione peroxidase (Paglia and Valentine, 1967).

Furthermore, Separation of  $\alpha$  - ,  $\delta$  - tocopherols using tocopherol acetate as an internal standard was done by high performance liquid chromatography (HPLC) using Philips PU 4100 liquid chromatography system with 20  $\mu$ l sample injector loop. The detector used was a Unicam PU 4225 UV with variable wave length detection and adjusted at UV 292 nm. The mobile phase was 100% methanol at a flow rate equilibrated at 0.7 ml / min. The column used for the chromatographic separation was reversed phase Spherisorb ODS1 column. Serum was mixed with tocopherol acetate and extracted with n-hexan using an electric shaker for 5 minutes then the mixture was centrifuged to separate the hexan layer. The extract was completely dried at 40°C and reconstituted with 300  $\mu$ l of methanol (Liu et al., 2000).

Effect on liver functions was determined by estimation of serum albumin (Domas et al., 1971); Serum aspartate

aminotrasferase (AST) and Serum alanine aminotrasferase (ALT) according to the method described by Reitman and Frankel (1957).

Effect on kidney functions was determined by estimation of both serum urea and creatinine concentration according to the methods described by Sampson et al. (1980) and Heinegard and Tiderstrom (1973), respectively.

#### Statistical analysis :

Statistical analysis was done by using the Statistical Package for Social Science (SPSS) program version 10, 1999. The following statistical parameters were utilized arithmetic mean ( $\bar{x}$ ), standard deviation (SD), Student t-test and correlation coefficient. Significance was considered when p value less than 0.05.

### RESULTS

#### I. Effects of heating on oils :

Results of this study showed that there was an increase in the TBARS content of the studied heated oils which was more rising on increasing the duration of heating. It was found that the TBARS content of sunflower, cottonseed and mixed oils increased significantly in different durations of heating in comparison to the fresh oil (Table 1).

Moreover, the repetition of heating was



much more destructive than the continuous use of the oil (e.g., TBARS content of oils used for one hour frying session was less TBARS content of oils used for two sessions; each 1/2 hour and was much less than TBARS content of oils used for four sessions; each 1/4 hour). TBARS content was found to be increased in heated cottonseed oil more than heated mixed oil and heated sunflower oil (Figure 1).

II. Effects of chronic consumption of repeatedly heated {four times; each 1/4 hour (H4/4)} oils [sunflower, cottonseed, mixed] on experimental animals:

Serum TBARS was found to be significantly increased ( $p < 0.001$ ) in animals fed any of the studied heated oils in comparison to both the negative and the positive control groups while, there was insignificant increase in its value in the positive control groups (animals fed fresh oils) in comparison to the negative control group (animals fed oil free diet) (Table 2).

In comparison to animals in negative control group, serum glutathione peroxidase was insignificantly decreased in animals fed fresh oils while significantly decreased in those fed repeatedly heated sunflower oil ( $p \leq 0.01$ ) and in animals fed repeatedly heated cottonseed or mixed oil ( $p \leq 0.001$ ). In comparison to animals of positive and negative control group, serum glutathione was insignificantly de-

creased in animals fed repeatedly heated sunflower or mixed oil while, significantly decreased in those fed repeatedly heated cottonseed oil ( $p \leq 0.001$ ) (Table 3).

Both  $\alpha$  - and  $\delta$  - tocopherols showed insignificant difference between positive and negative control groups ( $p > 0.05$ ) and significant decrease in animals fed repeatedly heated oils in comparison to negative and positive control groups.  $\delta$  - tocopherol decreased in animals fed repeatedly heated cottonseed or mixed oils ( $p < 0.001$ ) more than in animals fed repeatedly heated sunflower oil ( $p < 0.01$ ) (Figures 2, 3, 4 and 5) and (Table 4).

Results of the present study showed positive correlation between the TBARS content of the oil, either fresh or heated, and the serum levels of TBARS (Figure 10). In addition a negative correlation was showed between the serum concentration of TBARS and that of glutathione peroxidase (Figure 11),  $\alpha$  - tocopherol (Figure 12) and  $\delta$  - tocopherol (Figure 13). Whereas glutathione peroxidase was positively correlated with  $\alpha$  - tocopherol (Figure 14), and  $\delta$  - tocopherol (Figure 15).

The present results showed that animals fed heated studied oils have a significantly higher levels ( $p < 0.001$ ) of serum ALT and AST in comparison to both negative and positive control groups. Serum albumin concentration insignificantly in-

creased in animals fed any of the fresh studied oils but it decreased significantly in animals fed heated studied oils specially heated cottonseed oil (Table 5).

Serum creatinine and urea concentration showed insignificant difference between positive and negative control groups ( $p > 0.05$ ) while they were significantly increased in animals fed heated studied oils when compared to both negative and positive control groups (Table 6).

Pathological changes were found in liver, kidney, colon, and testes of animals fed heated oils whereas, the heart did not show any pathologic effects. Maximum pathological effects were found in animals fed repeatedly heated cottonseed oil then animals fed repeatedly heated mixed oil while those fed repeatedly heated sunflower oil had the least pathological changes. These results were correlated with the biochemical changes associated with the chronic use of the heated studied oils.

Lesions in the liver were vacuolation of hepatocytes with areas of lymphocytic infiltration and necrosis (Figure 6).

The renal pathology appeared in the form of glomerular and interstitial lymphocytic infiltrate with cloudy swelling which occurred nearly in all animals fed heated oils. In addition, tubular casts ap-

peared in those fed repeatedly heated cottonseed or mixed oil and renal tubular necrosis which occurred in one animal only of those fed repeatedly heated cottonseed oil (Figure 7).

Erosion of the colonic mucosa occurred in animals fed repeatedly heated cottonseed oil while those fed repeatedly heated mixed oil had mucosal hyperplasia. Degenerative mucosal changes appeared in both groups. Chronic colitis was found in all animals but was maximally and presented as follicular colitis in animals fed repeatedly heated cottonseed oil with haemosidrin inside the macrophages indicating recurrent bleeding (Figure 8).

Testicular pathology presented as hypospertogenesis with degenerated spermatogonia and accumulation of spermatid debris in animals fed repeatedly heated cottonseed or mixed oil (Figure 9).

## DISCUSSION

Because of concern about the types of changes that take place in fats during oxidative and thermal deterioration, many chemical and biological studies have been carried out (Alexander, 1981). The use of oil remains one of the most popular methods for the preparation of foods. This is particularly true in Egypt and the majority of the Arab world where fallafel (a deep fried vegetable patty) forms the staple



food item in the diet and is widely available at street level from vendors (Tewfik et al., 1998).

The increased TBARS content of the studied heated oils in this study which was more rising on increasing the duration of heating coincide with observations in other studies on heated sunflower oil (Eder and Stangl, 2000) and heated cottonseed oil (El - Mehallawi et al., 2000).

The increased level of TBARS in oils following repetition of heating more than continuous heating in the present work is in agreement with Andrikopoulos et al. (2002). This may be explained by that at a high temperature (during continuous heating) the water is lost more quickly producing a steam blanket over the frying kettle and protect the fat against atmospheric oxygen (Alexander, 1981). FAO (1994) stated that the continuous use provides a protective water vapour blanket that protects against oxidation.

Results of the present work revealed that the cottonseed oil was exposed to the deteriorative effect of heating more than the mixed oil and the sunflower oil, as in the study of Abdel -Khalek and Abdel -Rahman (1996). The lower TBARS contents found in sunflower oil than in cottonseed oil is in contrast with the fact that sunflower oil contains more polyunsaturated fatty acids (Gutebrie et al., 1995).

This agree with the concept that fat high in monoenes may be considerably more toxic than more highly unsaturated fats when heated under the same conditions (Alexander, 1981) and support the opinion that fatty acid unsaturation of dietary oils is not the only determining factor of the antioxidant capacity. Therefore, the optimal balance between the content of unsaturated fatty acids and natural antioxidants in dietary oils appear to be of major importance (Scaccini et al., 1992). Moreover, recent work suggest that the benefits occurred by lowering polyunsaturated fatty acids could be lost if the levels of antioxidants such as tocopherols are reduced (Botha et al., 2003).

The significantly increased serum TBARS in animals fed heated studied oils, is in harmony with Liu and Huang (1995). Increased serum TBARS may be due to absorption of TBARS from the heated oil in the gastrointestinal tract or due to peroxidation of plasma lipids (Sheehy et al., 1994). The results of present study showed positive correlation between the TBARS content of the oil, either fresh or heated, and the serum levels of TBARS in accordance with the findings of Nwanguma et al. (1999).

In our study, the significant decrease in serum glutathione peroxidase in animals fed heated studied oils coincide with Narasimhamurthy and Raina (1999). In fact,



glutathione peroxidase enzyme activity is influenced by several factors like heating and frying conditions, nature of fat, extent of peroxidation, presence of antioxidants, duration of feeding and the degree of unsaturation of the diet (Maraschiello et al., 1999).

In addition, the study showed significant decrease in both  $\alpha$ - and  $\delta$ -tocopherols by feeding heated studied oils in comparison to negative and positive control groups in agreement with findings of Liu and Huang (1995). The reduced levels of  $\alpha$ -tocopherol could be due to the destruction of  $\alpha$ -tocopherol in the gastrointestinal tract by free radicals present in the heated oil. Another possibility could be that  $\alpha$ -tocopherol in plasma is oxidized by peroxy radicals or lipid hydroperoxides absorbed from the diet. Diet supplementation with  $\alpha$ -tocopherol acetate reduced tissue susceptibility to lipid peroxidation with varying degrees depending on the tissue (Sheehy et al., 1994).

The significant negative correlation between the serum TBARS and that of glutathione peroxidase,  $\alpha$ -tocopherol and  $\delta$ -tocopherol, and the positive correlation of glutathione peroxidase with  $\alpha$ -tocopherol, and  $\delta$ -tocopherol are in harmony with Eder and Stangl (2000), and Liu et al. (2000). These results are expected as the lipid peroxidation is a suitable index to evaluate vitamin E requirements. On the

other hand, Maraschiello et al. (1999) observed a negative relationship between glutathione peroxidase activity and tissue levels of  $\alpha$ -tocopherol and a positive relationship was evidenced between TBARS and the antioxidant enzyme activity. The biochemical mechanism of interaction between glutathione peroxidase and vitamin E has remained a fascinating question. Although selenium could prevent some, but not all, of the symptoms of vitamin E deficiency, vitamin E do not replace glutathione peroxidase in protecting from acute peroxidative stress (Cheng et al., 1999).

The higher levels of serum ALT, AST, creatinine and urea as well as lower serum albumin concentration in the present study is in contrast to the study of Eder and Stangl (2000) which revealed no effect of thermoxidized sunflower oil, on plasma biochemical variables (ALT, AST, albumin, creatinine, and urea) but their experiment period was shorter than that of the present work. The lower serum albumin in this study can be explained by the fact that; almost all amino acids react with primary and secondary products of oxidized lipids, thereby decreasing the digestive utilization of protein, amino acids (Nielsen et al., 1985).

Histopathological examination of albino rat organs in this study revealed that animals fed heated studied oils had pathological changes in liver, kidney, colon, and



testes whereas, the heart did not show any pathologic effects. Maximum pathological effects were found in animals fed repeatedly heated cottonseed oil then animals fed repeatedly heated mixed oil while those fed repeatedly heated sunflower oil had the least pathological changes. These results were correlated with the biochemical changes associated with the chronic use of the heated studied oils.

The pathological lesions in the liver and kidney coincide with those found in the study of Alexander (1987). Burk et al. (1995) concluded that depletion of glutathione in the liver and kidney leads to necrosis in these organs associated with evidence of lipid peroxidation.

The enhancement of cell proliferation induced by heated polyunsaturated oil may be caused by non specific tissue injury and the irritating effects of free fatty acids and their oxidation products (Bruce, 1987). This may explain mucosal hyperplasia occurred in animals fed repeatedly heated mixed oil in the present study.

The pathological changes in testes may be attributed to the rapid and continuous cell turnover in testicles, which render them sensitive to stimuli such as chemical agents arising from thermally oxidized fats. Dysfunctional Sertoli cells may be causative factor of decreased primary and secondary spermatogonia, because these

cells function to organize the germinal epithelium and regulate the cyclic production of sperm in the tubules. Injury of stem cells may also be an etiological factor as these cells are responsible for somniferous tubules renewal (Alexander, 1987). Hypospermatogenic effect of long term consumption of repeatedly heated oils may be explained by a change in spermatozoa membrane fluidity, which is a major consequence of lipid peroxidation. Change in fatty acid composition of phospholipids of sperm membrane by reactive oxygen species may be another cause of hypospermatogenesis (Zalata et al., 1998).

#### **CONCLUSION & RECOMMENDATIONS**

In conclusion deteriorative effect of heat on dietary oils increases by increasing the duration of oil use and the repetition of heating is much more destructive than the continuous use of the oil. In addition chronic consumption of any of the repeatedly heated studied oils increases the serum level of the peroxidative products (TBARS), decreases the antioxidant defense of the body and compromising the liver and kidney functions in experimental animals. These effects were more pronounced in animals fed repeatedly heated cottonseed oil followed by the repeatedly heated mixed oil then the repeatedly heated sunflower oil. According to the results of this study it is recommended that fry-

ing should be avoided as much as possible and be replaced by other methods of cooking and frying oil must not be overheated and must be changed frequently. In addition it is better to use sunflower oil in fry-

ing as it showed better tolerance to effect of heating than mixed and cottonseed oil. Human risks can be modulated by reduction of fried food consumption or food fortification with synthetic antioxidants.



**Table ( 1 ):** Comparison of TBARS content ( nmol / ml ) in the fresh and repeatedly heated sunflower , cotton seed and mixed oils.

		Fresh	H 1/4 (once)	H 2/4 (twice)	H 3/4 (three times)	H 4/4 (four times)	H 1/2 (once)	H 2/2 (twice)	H 1 (once)
Sunflower oil	Mean ± SD	1.15 ± 0.029	1.54 ± 0.15	5.72 ± 0.088	7.15 ± 0.038	9.06 ± 0.059	2.92 ± 0.075	6.92 ± 0.106	4.07 ± 0.007
	T		4.78	8.12	10.1	20.1	5.1	9.5	7.1
	P		0.017*	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001**
Cotton seed oil	Mean ± SD	5.53 ± 0.023	6.31 ± 0.14	9.45 ± 0.92	12.72 ± 0.15	16.66 ± 1.69	9.57 ± 0.18	12.08 ± 0.068	10.47 ± 0.004
	T		5.22	8.95	10.23	12.19	8.1	10.1	9.1
	P		0.014*	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001**
Mixed oil	Mean ± SD	1.5 ± 0.13	5.62 ± 0.23	7.54 ± 0.16	11.45 ± 0.51	11.72 ± 0.86	5.98 ± 0.026	7.55 ± 0.27	6.95 ± 0.003
	T		9.1	11.1	13.1	13.5	9.3	11.17	10.1
	P		< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001**

Insigificant when p > 0.05. ( ) Significant when p ≤ 0.05.  
 ( \*\* ) Highly significant when p ≤ 0.01. ( \*\*\* ) Very highly significant when p ≤ 0.001

**Table ( 2 )** The statistical comparison of the serum TBARS concentration (nmol/ml) in animals after chronic consumption of the fresh or repeatedly heated studied oils.

	Control	FS	HS	FC	HC	FM	HM
Mean ± SD	3.45 ± 0.85	3.73 ± 0.78	6.39 ± 0.89	4.49 ± 1.03	10.33 ± 2.22	4.33 ± 0.67	9.02 ± 0.97
T <sub>1</sub>		0.76	6.82	1.93	10.1	1.94	13.51
P <sub>1</sub>		0.45	< 0.001***	0.054	< 0.001***	0.053	< 0.001***
T <sub>2</sub>		7.11		7.53		12.54	
P <sub>2</sub>		< 0.001***		< 0.001***		< 0.001***	

Insigificant when p > 0.05. ( ) Significant when p ≤ 0.05.  
 ( \*\* ) Highly significant when p ≤ 0.01. ( \*\*\* ) Very highly significant when p ≤ 0.001

**Table ( 3 ):** The statistical comparison of the serum glutathione peroxidase concentration (U/L) in animals after chronic consumption of the fresh or repeatedly heated studied oils.

	Control	FS	HS	FC	HC	FM	HM
Mean ± SD	1418.7 ± 208.42	1281.6 ± 218.46	1132.7 ± 213.05	1147.8 ± 218.67	575.4 ± 136.88	1191 ± 191.96	1041.7 ± 202.22
T <sub>1</sub>		1.43	3.58	1.79	10.1	1.71	4.9
P <sub>1</sub>		0.16	0.002**	0.09	< 0.001***	0.1	< 0.001***
T <sub>2</sub>		1.55		5.91		1.71	
P <sub>2</sub>		0.13		< 0.001***		0.105	

Insigificant when p > 0.05. ( ) Significant when p ≤ 0.05.  
 ( \*\* ) Highly significant when p ≤ 0.01. ( \*\*\* ) Very highly significant when p ≤ 0.001

**Table (4) :** The statistical comparison of both serum  $\alpha$ -tocopherol and serum  $\delta$ -tocopherol concentration (Ug/ml) in animals after chronic consumption of the fresh or repeatedly heated studied oils.

		Control	FS	HS	FC	HC	FM	HM
serum $\alpha$ -tocopherol	Mean	21.43	20.74	15.27	18.95	9.01	19.3	10.96
	+SD	+ 2.7	+ 2.45	+ 1.93	+ 2.42	+ 2.2	+ 2.4	+ 2.13
	T <sub>1</sub>		0.68	5.7	2.03	9.24	1.99	12.06
	P <sub>1</sub>		0.51	< 0.001***	0.07	< 0.001***	0.08	< 0.001***
	T <sub>2</sub>		10.22		10.02		6.86	
P <sub>2</sub>		< 0.001***		< 0.001***		< 0.001***		
serum $\delta$ -tocopherol	Mean	5.79	5.65	3.87	4.39	1.46	4.96	2.42
	+SD	+ 1.38	+ 1.56	+ 0.99	+ 0.77	+ 0.67	+ 0.99	+ 0.68
	T <sub>1</sub>		0.21	3.58	1.63	8.12	1.95	0.93
	P <sub>1</sub>		0.83	0.002**	0.117	< 0.001***	0.052	< 0.001***
	T <sub>2</sub>		3.97		6.91		5.04	
P <sub>2</sub>		< 0.001***		< 0.001***		< 0.001***		

Insignificant when  $p > 0.05$ . ( ) Significant when  $p \leq 0.05$ .  
 ( \*\* ) Highly significant when  $p \leq 0.01$ . ( \*\*\* ) Very highly significant when  $p \leq 0.001$ .

**Table (5) :** The statistical comparison of serum ALT (U/L), serum AST (U/L) and serum albumin (gm/dl) in animals after chronic consumption of the fresh or repeatedly heated studied oils.

		Control	FS	HS	FC	HC	FM	HM
serum ALT (U/L)	Mean	11.76	12.57	21.95	14.18	42.72	13.95	30.88
	+SD	+ 2.23	+ 2.51	+ 4.8	+ 2.63	+ 10.87	+ 2.71	+ 7.97
	T <sub>1</sub>		0.76	6.09	1.93	8.82	1.9	7.3
	P <sub>1</sub>		0.45	< 0.001***	0.054	< 0.001***	0.059	< 0.001***
	T <sub>2</sub>		5.94		8.52		6.5	
P <sub>2</sub>		< 0.001***		< 0.001***		< 0.001***		
serum AST (U/L)	Mean	10.75	11.43	22.23	13.74	39.95	12.78	32.03
	+SD	+ 2.17	+ 2.41	+ 4.3	+ 2.98	+ 9.3	+ 2.89	+ 7.07
	T <sub>1</sub>		0.66	7.53	1.88	9.66	1.78	9.09
	P <sub>1</sub>		0.51	< 0.001***	0.72	< 0.001***	0.92	< 0.001***
	T <sub>2</sub>		7.28		9.17		8.12	
P <sub>2</sub>		< 0.001***		< 0.001***		< 0.001***		
serum albumin (gm/dl)	Mean	5.32	5.75	4.41	5.42	4.18	5.4	4.24
	+SD	+ 0.87	+ 1.26	+ 0.84	+ 0.74	+ 0.82	+ 0.8	+ 0.93
	T <sub>1</sub>		0.88	2.38	0.28	3.005	0.218	2.68
	P <sub>1</sub>		0.38	0.029*	0.78	0.008**	0.83	0.015*
	T <sub>2</sub>		2.8		3.54		2.99	
P <sub>2</sub>		0.012*		< 0.001***		0.008**		

Non significant when  $p > 0.05$ . ( ) Significant when  $p \leq 0.05$ .  
 ( \*\* ) Highly significant when  $p \leq 0.01$ . ( \*\*\* ) Very highly significant when  $p \leq 0.001$ .

**Table (6) :** The statistical comparison of both serum creatinine concentration and serum urea concentration (mg/dl) in animals after chronic consumption of the fresh or repeatedly heated studied oils.

		Control	FS	HS	FC	HC	FM	HM
serum creatinine	Mean	0.49	0.53	0.66	0.67	1.22	0.57	0.98
	+SD	0.07	+ 0.13	+ 0.1	+ 0.15	+ 0.29	+ 0.09	+ 0.21
	T <sub>1</sub>		0.94	4.6	1.74	7.74	1.63	7.05
	P <sub>1</sub>		0.35	< 0.001***	0.098	< 0.001***	0.117	< 0.001***
	T <sub>2</sub>		2.22		5.34		6.7	
P <sub>2</sub>		0.039*		< 0.001***		< 0.001***		
serum urea	Mean	12.24	13.36	17.28	14.67	30.52	13.49	24.56
	+SD	+ 1.69	+ 3.31	+ 3.4	+ 3.8	+ 7.26	+ 2.2	+ 5.25
	T <sub>1</sub>		0.95	3.03	1.71	9.75	1.42	7.06
	P <sub>1</sub>		0.35	0.007**	0.099	< 0.001***	0.17	< 0.001***
	T <sub>2</sub>		2.95		4.34		5.7	
P <sub>2</sub>		0.008**		< 0.001***		< 0.001***		

Insignificant when  $p > 0.05$ . ( ) Significant when  $p \leq 0.05$ .  
 ( \*\* ) Highly significant when  $p \leq 0.01$ . ( \*\*\* ) Very highly significant when  $p \leq 0.001$ .



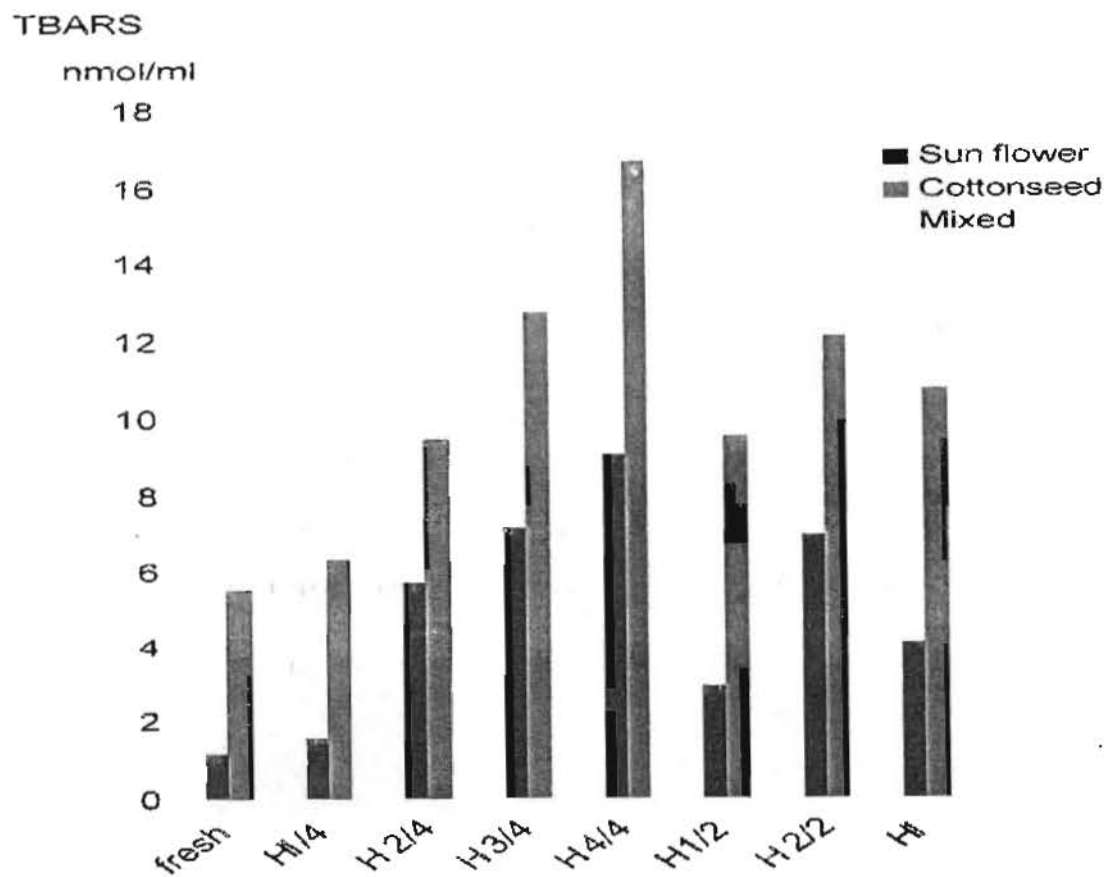


Fig. (1): Comparison of TBARS content in fresh and repeatedly heated sunflower, cottonseed, and mixed oils.

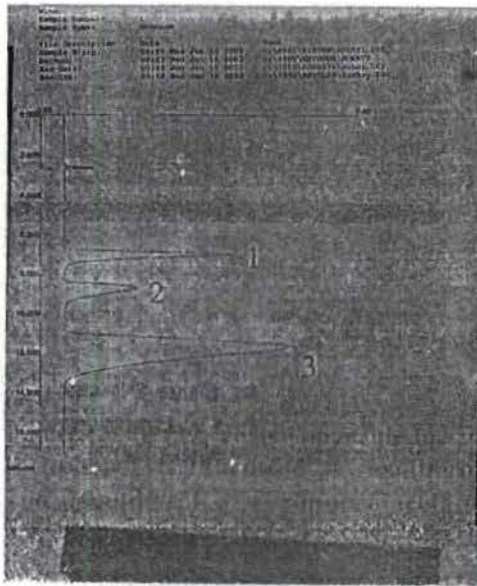


Fig. (2) : HPLC chromatograph for tocopherol standards. (1.  $\delta$  - tocopherol, 2.  $\alpha$  - tocopherol, 3. Tocopherolacetate).

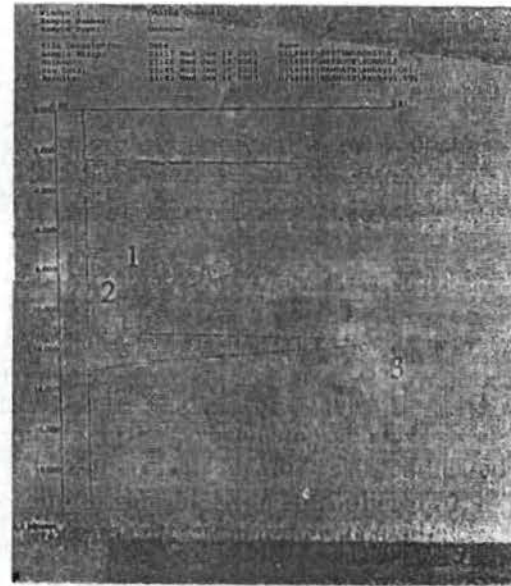


Fig. (3) : HPLC chromatograph for tocopherols in serum of rat fed heated sunflower oil. ( 1.  $\delta$  - tocopherol , 2.  $\alpha$  - tocopherol, 3. Tocopherolacetate).

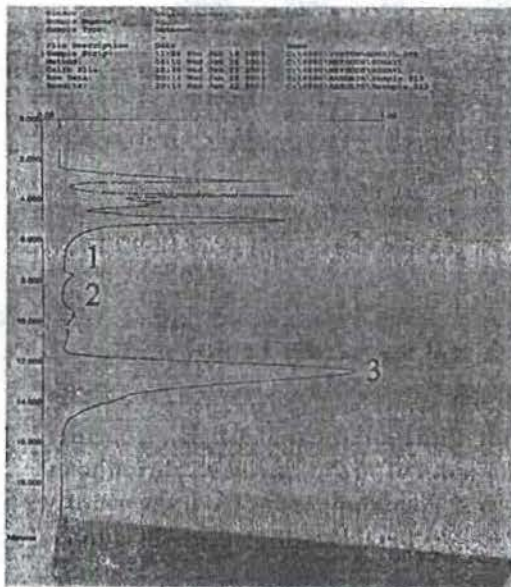


Fig. (4) : HPLC chromatograph for Tocopherols in serum of rat fed heated cottonseed oil. (1.  $\delta$  - tocopherol, 2.  $\alpha$  - tocopherol, 3. Tocopherolacetate).

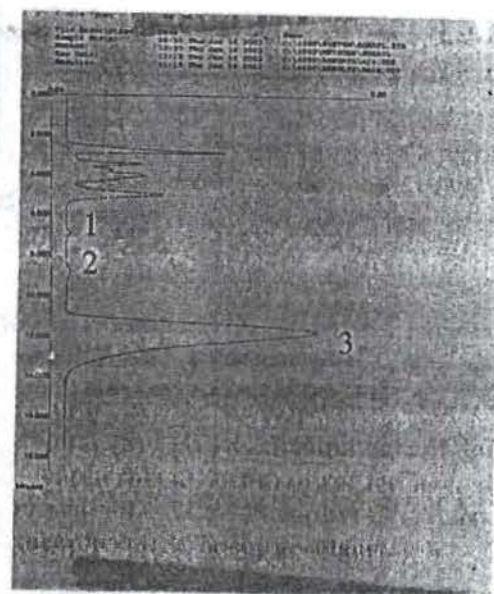
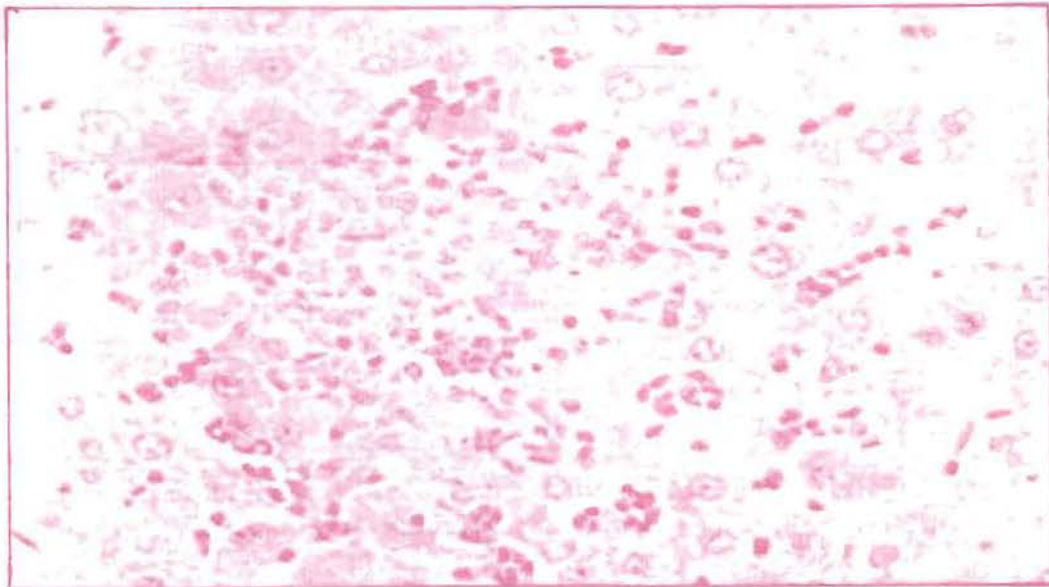
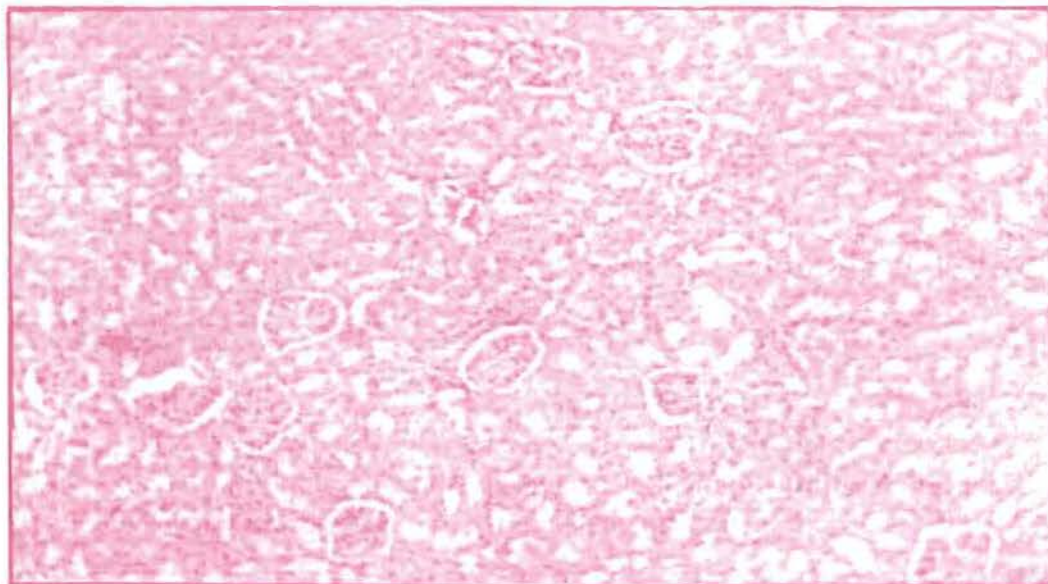


Fig. (5) : HPLC chromatograph for tocopherols in serum of rat fed heated mixed oil. ( 1.  $\delta$  - tocopherol , 2.  $\alpha$  - tocopherol, 3. Tocopherolacetate).





**Fig. (6) :** Section in liver of rats fed repeatedly heated cottonseed oil . It shows mild vacuolation and focal confluent necrosis with apoptotic bodies. (Hx & E x 100).



**Fig. (7) :** Section in kidney of rats fed repeatedly heated cottonseed oil. It shows cloudy swelling with interstitial lymphocytic infiltrate. (Hx & E x 100).

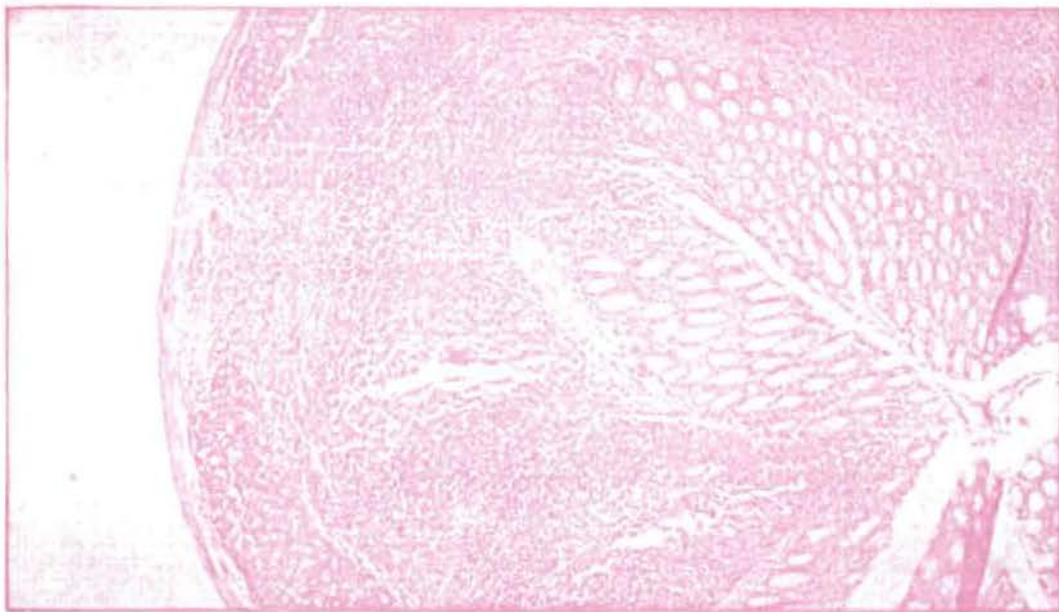


Fig. (8)<sup>6</sup>: Section in colon of rats fed repeatedly heated cottonseed oil. It shows follicular colitis. (Hx & E x 100).



Fig. (9)<sup>6</sup>: Section in testis of rats fed repeatedly heated cottonseed oil. It shows hypospermatogenesis with spermatid debris inside the lumina. (Hx & E x100).



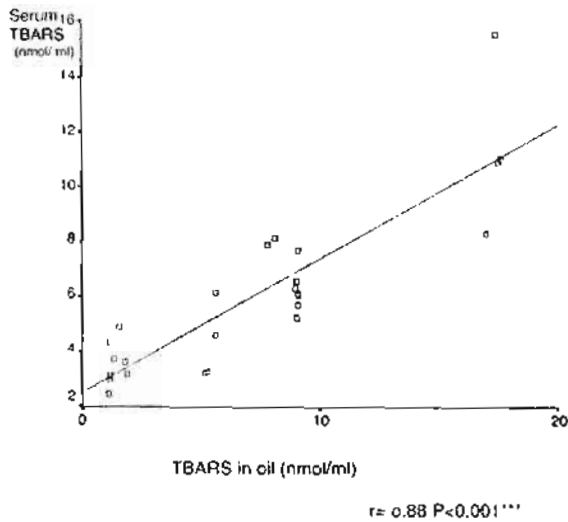


Fig. (10) : The statistical correlation between the oil content of TBARS and serum concentrations of TBARS.

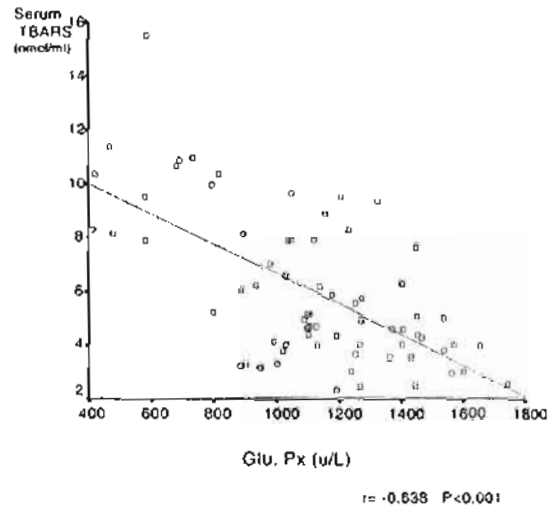


Fig. (11) : The statistical correlation between serum concentrations of TBARS and glutathione peroxidase.

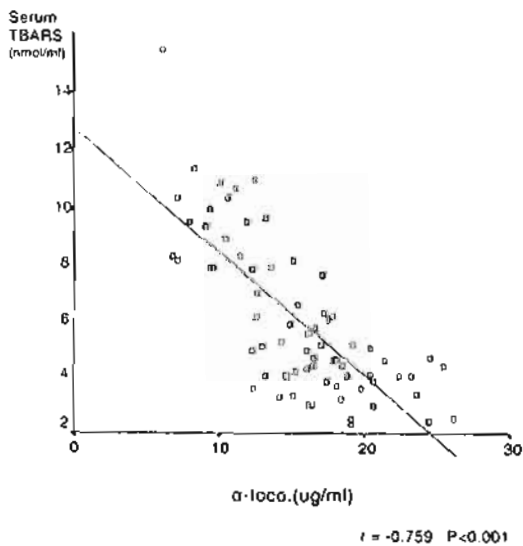


Fig. (12) : The statistical correlation between serum concentrations of TBARS and  $\alpha$  - tocopherol.

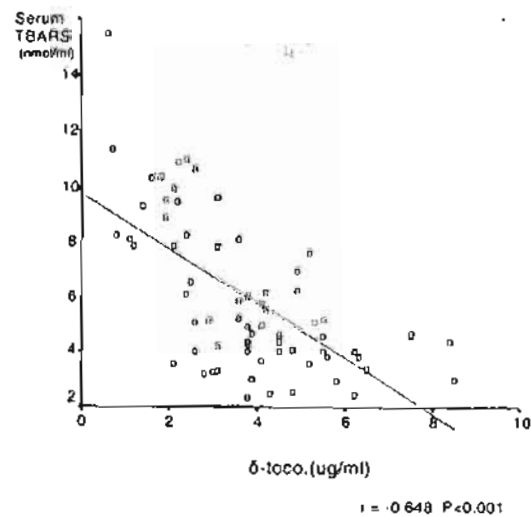
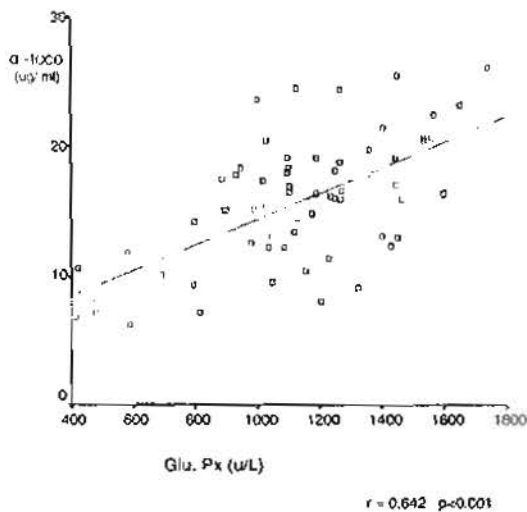
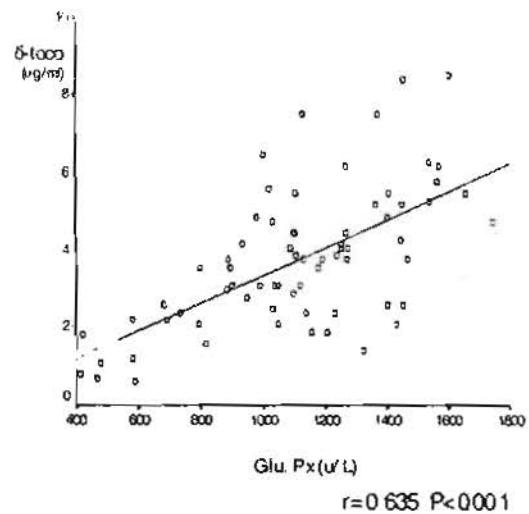


Fig. (13) : The statistical correlation between serum concentrations of TBARS and  $\delta$  - tocopherol.



**Fig. (14) :** The statistical correlation between serum concentrations of glutathione peroxidase and  $\alpha$  - tocopherol.



**Fig. (15) :** The statistical correlation between serum concentrations of glutathione peroxidase and  $\delta$  - tocopherol.



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## التغيرات الكيميائية الحيوية والهستوباثولوجية في الجرذان البيضاء بعد الاستهلاك طويل المدى لبعض الزيوت المسخنة

المشركون في البحث

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تم اختيار الزيوت التي أستخدمت في هذه الدراسة بعد عمل مسح إسترشادي على الأنواع التي تستعملها ١٢٠٠ سيدة بكلية الطب جامعة المنصورة ووجد أن أكثر الزيوت إستخداماً هي زيت بذرة القطن وزيت عباد الشمس والزيت الخليل (زيت عباد شمس مع زيت بذرة القطن). ثم أستخدمت هذه الزيوت في قلى رقائق البطاطس لفترات مختلفة من الوقت (ربع ساعة و نصف ساعة و ساعة كاملة) كما تم تكرار إستخدامها أيضاً (مرتين و ثلاث وأربع مرات كلا منها ربع ساعة و كذلك تكرار إستخدامها مرتين كلا منها نصف ساعة). أختبرت نسبة الشوارد الجامحة النشطة في كل الزيوت المستخدمة في الدراسة (الطازجة - المسخنة) وقد أظهرت النتائج أن تسخين الزيوت أثناء عملية القلى يؤدي الى تكوين الشوارد الجامحة النشطة في هذه الزيوت وأن تكرار التسخين يؤثر على الزيت أكثر من التسخين المستمر لمرة واحدة وتبين أن الزيوت المتكررة التسخين أربع مرات كلا منها ربع ساعة محتوى على أكبر نسبة من الشوارد الجامحة النشطة و لهذا تم اختيارها ليتم تغذية حيوانات التجارب عليها. أجريت الدراسة على ٧٠ من ذكور جرذ التجارب البيضاء، البالغة حيث قسمت الجرذان إلى سبعة مجموعات كلا منها عشرة جرذان. المجموعة الأولى (مجموعة ضابطة سالبة) تم تغذيتها على غذاء خالى من الزيوت. المجموعة الثانية (مجموعة ضابطة موجبة) تم تغذيتها على غذاء مخلوط بزيت عباد شمس طازج. المجموعة الثالثة تم تغذيتها على غذاء مخلوط بزيت عباد شمس متكرر التسخين (أربع مرات كلا منها ربع ساعة). المجموعة الرابعة (مجموعة ضابطة موجبة) تم تغذيتها على غذاء مخلوط بزيت بذرة القطن متكرر التسخين (أربع مرات كلا منها ربع ساعة). المجموعة السادسة (مجموعة ضابطة موجبة) تم تغذيتها على غذاء مخلوط بالزيت الخليل (زيت عباد شمس مع زيت بذرة القطن) طازج. المجموعة السابعة تم تغذيتها على غذاء مخلوط بالزيت الخليل المتكرر التسخين (أربع مرات كلا منها ربع ساعة). إستمرت تغذية الجرذان لمدة ثلاث شهور ثم ذبحت في نهاية الشهر الثالث وتمت دراسة تأثير الاستهلاك طويل المدى للزيوت المسخنة على مختلف أعضاء الجسم و ذلك عن طريق أخذ عينات من الأنسجة الكبد والكلى والقولون و الخصية و القلب) لعمل دراسات باثولوجية و عينات من الدم لعمل دراسات كيميائية والتي اشتملت على قياس



الشوارد الجامعة النشطة في الدم قياس التأثير على مضادات الأكسدة في الدم ( إنزيم بيروكسيد الجلوتاثيون - فيتامين "التوكوفيرول" ) قياس التأثير على وظائف الكبد ( إنزيمات الكبد - الألبومين ) والكلية (الكرياتينين - اليوريا) .. وأظهرت النتائج أن الإستعمال طويل المدى للزيوت المتكررة التسخين في غذاء جرذان التجارب البيضاء أدى إلى زيادة في الشوارد الجامعة النشطة و إنخفاض مضادات الأكسدة في دم هذه الجرذان بصورة ملحوظة أكثر من الجرذان التي أعطيت غذاء خالي من الزيوت أو غذاء مخلوط بالزيوت الطازجة. كما حدث إضطراب في وظائف الكبد في صورة إرتفاع إنزيمات الكبد وإنخفاض الألبومين ووظائف الكلية في صورة إرتفاع الكرياتينين و اليوريا. وبالإضافة إلى ذلك أظهر البحث أيضاً حدوث تغيرات مرضية بأنسجة كل من الكبد والكلية والقولون و الخصية و لم تظهر أى تغيرات مرضية بأنسجة القلب. و كانت هذه التغيرات الكيميائية والمرضية بالأنسجة أكثر وضوحاً في الجرذان المغذاه على الزيوت المتكررة التسخين خصوصاً زيت بذرة القطن و يليه الزيت الخليط ثم زيت عباد الشمس.

ولذلك ينصح الباحثون بتجنب استخدام القلي كطريقة لتحضير الأطعمة كما يجب تغيير زيت القلي باستمرار و عدم تكرار تسخين الزيت ويوصى باستخدام زيت عباد الشمس بدلاً من الزيت الخليط و زيت بذرة القطن لأنه أقل تأثيراً بالإجهاد التأكسدي نتيجة الحرارة.

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