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Clinicopathological Effect of Thermally Processed Oil on Albino Rats with Albumin as Modulating Effect

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Abstract: Repeated heated oils (RHO) at high temperatures are resulting in thermal oxidation of the oils which eventually can promote formation of compounds with adverse nutritional implications and potential hazards to human health. The present study was designed to evaluate deleterious effect of RHO on albino rats and the effect of albumin supplementation on the impact of RHO. Twenty four rats were established and divided into 4 groups (n= 6) for the experiment. Group 1 was fed 7ml fresh oil on 93gm ration and served as control. groups 2, 3& 4 were treated with 7 wt% RHO, 7 ml repeated heated oils plus 7% of (2.4 gm of albumin dissolved in 100 ml of distilled water shaked for 20 minute), distilled water plus 2.4% albumin, daily for one month. Blood and serum samples were collected for hematological and biochemical examinations. No significant changes were observed in hematological parameters in all groups in comparison with control. Alkaline phosphatase, Gamma glutamyl transferase (gamma-GT) and Malondialdehyde (MDA) were significantly increased in group 2 in comparison with control, while (gamma-GT) and MDA were significantly decreased in groups 3& 4 when compared to group 2. Urea and creatinine were significantly increased in groups 2& 3 in comparison with control. Superoxide dismutase was significantly decreased in group 2 when compared with control and significantly increased in groups 3& 4 in comparison with group 2. It could be concluded that RHO disrupted the hepatic and renal parameters with increases in the oxidative stress, which was repaired by albumin protein treatment.

Key words: Albino rats · Albumin · Repeated heated oils

INTRODUCTION

Deep frying oil is the most common and one of the oldest methods of food preparation worldwide. However, using frying oils repeatedly can produce constituents that not only compromise food quality but also can promote the formation of compounds with adverse nutritional implications and potential hazards to human health [1]. Cooking oil is sometimes reused due to its stability at high temperatures [2]. During frying process, various chemical reactions occur, such as thermal oxidation, hydrolysis and polymerization, due to the exposure of the oil to high temperatures in the presence of air and moisture. As a result, cooking oil decomposes and forms volatile compounds and various monomers and polymers [3]. At higher temperatures, thermal oxidation of the oil causes configuration of the fatty acid to change from cisisomer to transisomer. This configuration change causes the polyunsaturated fatty acids to acquire undesirable properties associated with saturated fatty acids, such as their correlation with increased serum total cholesterol levels and low-density lipoprotein [4]. Other studies reported that reactive oxygen species (ROS), also present in these oils, as inhibitors of mitochondrial function which may also affect health negatively [5].

Nowadays, most of Egyptian people fed on frying foods, used several times for long period, such as French fries, food (Timia) from popular restaurants. In Japan it

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was reported that administration of diet containing 7% practically used frying oil for 12 weeks induced severely damaged liver and kidneys in Wister rats [6].

Many antioxidative substances have been isolated from natural materials including food [7] especially from both hen egg yolk and egg white protein hydrolysates [8-10]. It has been reported that egg-yolk hydrolysates exhibit antioxidant capacity in a linoleic acid oxidation system [10]. Therefore, peptides with these properties could be considered as alternative antioxidative to the synthetic preservatives.

The aim of this work was to evaluate deleterious effect of RHO (Repeated heated oils) on albino rats and the effect of albumin and casein supplementation on the impact of RHO.

MATERIALS AND METHODS

Materials

Experimental Animals: Twenty four adult male albino rats weighing 100±20 gm and obtained from animal house, Laboratory Animal House, Qena, Egypt were used in this study. Animals were housed in stainless-steel cages in a clean ventilated room at 40-60% relative humidity and temperature of 25±2°C. They were fed on rodent diet and water was provided *ad libitum* throughout the experiment period. They were left for two weeks to adapt the place of the experiment. Housing and management of the animals and the experimental protocol were conducted as stipulated in the Guide for Care and Use of Laboratory Animals Guidelines of the National Institutes of Health (NIH) and approved by research ethical committee of Faculty of Veterinary Medicine, South Valley University, Qena, Egypt.

Chemicals:

- Fresh oils composed of edible oil refined sunflower and soybean oil, were obtained from Holding companies for food industries (Cairo, Egypt).
- Repeated heated oils that was used for frying several times through one day, obtained from restaurant at Sohag governorate, Egypt.
- Albumin was obtained as pure white powder in a package of 200 gm capacity, was purchased from Biodiagnostic Co. (Giza, Egypt).
- Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) estimated using the technique delineated by Reitman and Frankel [11]. Alkaline phosphatase (ALP) was assessed via method of Belfield and Goldberg [12]. Gamma glutamyl transferase (GGT) was performed [13]. Serum urea, creatinine and uric acid kits were obtained from Spectrum Diagnostic Laboratories (Cairo, Egypt),respectively catalog Nos.(291 000), (393 000),(246 002),(318 001), (235001).
- Serum urea, creatinine and uric acid were estimated by methods of Fawcett and Scott [14], Bartels *et al*. [15] and Barham and Trinder [16] respectively. Kits were obtained from by Spectrum Diagnostic Laboratories (Cairo, Egypt).
- Lactate dehydrogenase (LDH) was estimated by using the method of kits obtained from Spectrum Diagnostic Laboratories (Cairo, Egypt) (catalog No.278 002).
- Serum total cholesterol and triglyceride were assessed by the techniques of respectively. Serum high density lipid (HDL) was calculated according to Lopez-Virella *et al*. [17]. Kits were obtained from by Spectrum Diagnostic Laboratories (Cairo, Egypt). Respectively catalog No. (230 002), (314 002), (266 001)
- Superoxide dismutase (SOD) was evaluated by the technique of Nishikimi *et al*. [18]. Malondialdehyde (MDA) concentration was measured according to Ohkawa *et al*. [19]. kits were obtained from Biodiagnostic company (Giza, Egypt).Respectively catalog No.(SD 25 21), (MD 25 29).

Methods:

Experimental Design: The rats were weighed and divided into 4 groups (n=6).

Group 1: The rats given fresh oil orally at dose 7 ml oil on 93 gm ration (7% of weight of ration) daily for one month.

Group 2: The rats were administered repeated heated oils orally at dose 7 ml. oil on 93 gm ration (7% of weight of ration) daily for one month.

Group 3: The rats were given 7 ml repeated heated oils, plus 7% of (2.4 gm of albumin dissolved in 100 ml of distilled water shaked for 20 minute) (0.16 gm albumin/ 6 rats added on 93 gm ration), daily for one month.

Group 4: All rats were given 7% of (2.4 gm of albumin dissolved in 100 ml of distilled water shaked for 20 minute) (0.16 gm albumin/ 6 rats added on 93 gm ration) daily for one month.

Clinical signs and mortality rate of animals were daily recorded along experiment period.

Blood samples were collected under general anesthesia through injection by use of [xylazine (10mg/kg) and ketamine (100mg/kg)] for hematological and biochemical examinations. All rats were sacrificed after 30 days.

Specimens from livers were collected and washed by physiological saline immediately after scarification. Small part from each tissue (0.5 g) was homogenized using electrical homogenizer in phosphate buffer (5 ml), pH 7.4 on ice. Tissue homogenates were centrifuged at 4000 r.p.m. for 15 min at 4°C. Resultant supernatants were kept at -80°C till used for assessment of oxidative stress biomarkers in hepatic and renal tissues.

Statistical Analysis: Statistical analysis was done using one-way analysis of variance (ANOVA). It was done to compare between control and other treated groups, followed by post-hoc analysis (Dunnett's test) using SPSS (Statistical Package for Social Sciences) version 17 [20]. The data were presented in form of Mean ± Standard Deviation. The difference was considered statistically significant when P< (0.05).

RESULTS

Hematological Findings: Table 1 shows non-significant changes in the RBCs counts, Hb. conc., PCV%, MCV, MCH and MCHC% values of groups 2, 3& 4 in comparison with group 1.

Table 2 shows non-significant changes in WBCs counts and differential leucocytic counts of groups 2, 3& 4 in comparison with group 1.

Biochemical Parameters: Table 3 shows significant elevation (P<0.05) in ALT and (gamma-GT) of group 2 when compared to group 1. In contrast, (gamma-GT) significantly decreased (P<0.05) in groups 3& 4 when compared with group 2. ALP was not significantly changed in groups 3& 4 when compared to group 1. AST& ALT were not significantly changed in all treated groups 2, 3& 4 in comparison with group 1.

Table 4 shows significant elevation (p<0.05) in urea and creatinine in groups 2& 3 when compared with group 1. Moreover**,** uric acid level in group (2) was significantly increased (p<0.05) in comparison with group 1. There were non significant changes in levels of urea, creatinine and uric acid in group 4 in comparison with group 1. The levels of urea and creatinine in groups 3& 4 were significantly decreased (p<0.05) when compared to group (2). Moreover, level of uric acid was significantly decreased in group 4 (p<0.05) when compared to group (2)

Table 5 shows the level of lactate dehydrogenase (LDH) activity in groups, 2, 3& 4 was significantly increased (p<0.05) when compared to group (1). However, LDH activity was significantly decreased (p<0.05) in groups 3& 4 when compared to group 2.

Table 6 shows significant increases in cholesterol level of groups 2 & 4 was detected when compared with control group (P<0.05). In case of triglyceride, there was significant elevation detected in group 2 when compared with control. Level of HDL was significantly increased in groups 2& 3 in comparison with control one. While group 4 recorded significant decrease in HDL

Table 1: Effect of RHO on hematological finding

	Blood parameters					
Groups	RBC's(x 10^6/mm^3)	Hb.(gm/dl)	PCV(%)	MCV(fl)	MCH(pg)	MCHC(%)
Group (1)	5.7±0.7	10.0±2.2	27.7±5.1	44.4 ±5.3	17.3±1.4	39.1±2.1
Group (2)	6.1±0.6	10.8±1.4	32.4±3.1	53.2±1.6	17.7±1.3	33.5±3.3
Group (3)	6.7±0.6	12.6±1.7	35.2±4.7	52.3±2.3	18.7±0.8	35.8±0.7
Group (4)	6.3±0.2	11.2±1.3	32.0±3.1	50.2±3.7	17.5±1.2	34.9±1.1

Table 2: Effect of RHO on differential leucocytic count.

		Differential leukocytic counts		
GroupsGroups	WBCs(x 10^6/mm^3)	LymphocytesCount	MonocytesCount	GranulocytesCount
Group (1)	7.9±0.8	5.9±0.4	0.5±0.1	1.5±0.4
Group (2)	5.7±1.2	4.3±1.2	0.5±0.1	0.9±0.2
Group (3)	7.6±2.4	1.6±5.4	0.1±0.7	0.7±1.5
Group (4)	7.2±1.3	0.8±5.5	0.1±0.4	0.5±1.2

Table 3: Effect of RHO on liver function tests

	Liver function tests			
Groups	(gamma-GT) (IU/I)	ALP(IU/l)	ALT (IU/l)	AST (IU/l)
Group (1)	8.2±0.3 b	374.0±3.6	5.6±2.1	8.3±1.5
Group (2)	9.7±0.5a	385.0±5.0a	8.3±1.5	10.6±1.1
Group (3)	8.0±0.5 b	372.3±2.5	5.0±1.0	8.3±1.5
Group (4)	8.1±0.3 b	377.0±2.0	5.0±1.0	7.0±1.0

a→ significant difference when compared with normal group (G.1) when P<0.05.
b→significant difference when compared with group (G. 2) when P<0.05.

Table 4: Effect of RHO on kidneys function tests

	Kidney function tests		
ParametersGroups	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Group (1)	20.0±4.5	0.6±0.1	4.5±0.4
Group (2)	41.7±1.2 a	1.4±0.1 a	5.4±0.4a
Group (3)	17.3±3.2 b	0.5±0.1	3.7±0.1b
Group (4)	16.6±3.1b	0.6±0.1	3.7±0.2b

a→ significant difference when compared with normal group (G.1) when P<0.05.
b→ significant difference when compared with group (G. 2) when P<0.05.

Table 5: Effect of RHO on LDH

Groups Groups	Lactate dehydrogenase (LDH) (IU/l)
Group (1)	1334.0±150.4
Group (2)	2242.0±102.8 a
Group (3)	1593.0±61.2 ab
Group (4)	1591.0±58.0 ab

a→ significant difference when compared with normal group (G.1) when P<0.05.
b→ significant difference when compared with group (G. 2) when P<0.05.

Table 6: Effect of RHO on lipid profiles

	Parameters			
	Lipogram			
Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL(mg/dl)	LDL(mg/dl)
Group (1)	48.3±2.8	70.0±8.6	61.6±1.5	27.3±2.1
Group (2)	60.6±4.0a	107.3±11.2a	66.6±1.5a	27.46±2.1
Group (3)	56.0±5.2	80.0±20.0	63.2±0.7a	23.2±1.7
Group (4)	56.6±1.5a	72.0±9.6	60.6±1.5b	18.4±0.6ab

a → significant difference when compared with normal group (G.1) when P<0.05.
b → significant difference when compared with group (G. 2) when P<0.05.

Table 7: Effect of RHO on oxidant and antioxidant status

	ParametersGroups		
	Oxidative and antioxidants enzymes		
Groups	MDA(µmol/l)	SOD(IU/l)	GSH(IU/l)
Group (1)	6.3±0.9	584.0±6.0	0.001±0.0003
Group (2)	8.8±0.9 a	95.0±3.0 a	0.001±0.0006
Group (3)	5.9±1.1 b	660.7±24.9 b	0.002±0.0006
Group (4)	5.7±0.25 b	661.0±13.5 b	0.001±0.0006

a → significant difference when compared with normal group (G.1) when P<0.05.
b → significant difference when compared with group (G. 2) when P<0.05.

in comparison with group 2 (P<0.05%). There was a significant decrease in LDL value in group 4 in comparison with groups 1& 2. Other groups don't exhibit any changes with P<0.0.

Table 7 shows significant increase (p<0.05%) in the activity of MDA of group 2 in comparison with group 1, while it was decreased (p<0.05%) in group 3&4 when compared with group 2. Also, SOD level was significantly

decreased in group 2 when compared with control & significantly increased in groups 3& 4 in comparison with group 2.

DISCUSSION

Our work revealed non-significant changes of RBCs counts, Hb. conc., PCV%, WBCs counts, MCV, MCH and MCHC% values of all exposed and treated groups in comparison with control. Moreover, differential leucocytic counts showed non significant difference between groups when compared with control one. These results are opposite to views of Mesembe *et al*. [21] who mentioned that there was significant decrease in RBCs count, hemoglobin conc. and PCV% on blood of albino rats fed on thermoxidized palm oil diet. Also, El-bialy *et al*. [22] recorded significant increase in total leukocyte count, neutrophil and basophil percentages while lymphocyte percentage showed a significant decrease when albino rats were fed basal diets mixed with 15% g/g of fresh oil.

Biochemical analysis exhibited that ALP and GGT enzymes recorded significant elevation ($P<0.05\%$) in group (2) when compared with control, while, groups treated with albumin recorded significant improvement in comparison with group 2. Level of urea in groups 2& 3 showed significant increases when compared with control. Moreover, creatinine level of groups 3 was significantly increased when compared with control. On contrast, uric acid level showed significant increase in group 2 in comparison with control. This due to generating reactive oxygen species (ROS) present in repeatedly heated vegetable oils which are potential in causing deleterious effects on normal function of cells [23]. Oxidative damage may accumulate over time, thereby contributing to cell injury and pathologies [24]. Over production of reactive oxygen species and lipid peroxidation result in wide range of disorders in variety of the organs such as liver and kidney [25, 26]. Moreover, oxidative damage to lipid architectures can ultimately lead to disorganization and dysfunction as well as damage to membranes, enzymes and proteins [27].

Significant increase was detected in cholesterol and triglyceride level of groups 2 when compared with control. Level of HDL was significantly increased in groups 2 & 3 in comparison with control one. Significant decrease in LDL value in group 4 in comparison with groups 2, other groups didn't exhibit any changes when $P<0.05$. Additionally, significant changes were characterized by decrease in lactate dehydrogenase level of groups 3, 4 in comparison with group 2. Repeated deep fried oil usually changes in chemical composition due to oxidation, polymerization and denatured oil. During biochemical changes like lipid profile, cholesterol, ketone body and NO level increase in plasma in blood [28, 29]. The primary lipid oxidation products such as peroxides and hydro peroxides are unstable; they react rapidly with each other to form secondary lipid oxidation products. It was demonstrated that lipoprotein triacylglycerol and total lipids were more oxidized in diet rich in oxidized sunflower seed [30].

Cells are protected against ROS by a complex antioxidant system present within the cells, which includes superoxide dismutase, catalase and glutathione peroxidase [31]. In the present study, significant decrease in antioxidants enzymes was observed in group 3. This may be due to oxidative damage arising from lipid peroxidation caused by repeated heated oils. As well, there is an inverse relationship between reduced activities of antioxidants as SOD and increased lipid peroxidation products in blood and cardiovascular disease [32].

CONCLUSIONS

It could be concluded that repeated heated oils disrupted the hepatic and renal parameters with increases in the oxidative stress, which can be repaired by albumin protein treatment.

REFERENCES

1. Sanibal, E. and J. Mancini-Filho, 2004. Frying oil and fat quality measured by chemical, physical and test kit analyses. J. Am. Oil Chem. Soc., (81): 847-852.
2. Gupta, M.K., 2005. Edible Oil and Food Products: Products and Applications. New Jersey: John Wiley & Sons Inc, pp: 1-31. Make references like this style.
3. Andrikopoulos, N.K., N. Kalogeropoulos, A. Falirea and M.N. Barbagianni, 2002. Performance of virgin olive oil and vegetable shortening during domestic deep-frying and pan- frying of potatoes. Inter. J. Food Sci. Tech., (37): 177-190.
4. Mensink, R.P. and M.B. Katan, 1990. Effect of dietary trans fatty acids on high-density and low-density lipoprotein cholesterol levels in health subjects. N. Engl. J. Med., 323, pp: 439-45, doi: 10.1056. . Make references like this style.
5. Costantini, P., E. Jacotot, D. Decaudin and G. Kroemer, 2000. Mitochondrion as a novel target of anticancer chemotherapy. J. Nat. Cancer Inst., (92): 1042-1053.

6. Totani, N., M. Yatawa, Y. Ojiri and Y. Fujioka, 2007. Effects of trace acrylamide intake in Wister rats. J. Oleo Sci., 56(9): 501-506.
7. Sakanaka, S. and Y. Tachibana, 2006. Active oxygen scavenging activity of egg-yolk protein hydrolysates and their effects on lipid oxidation in beef and tuna homogenates. Food Chem., 95, pp: 243-249.
8. Tsuge, N., Y. Eikawa, Y. Nomura, M. Yamamoto and K. Sugisawa, 1991. An-tioxidative activity of peptides prepared by enzymatic hydrolysis of egg-white albumin. J. Agric. Chem. Soc. Jpn., 65: 1635-1641.
9. Davalos, A., M. Miguel, B. Bartolome and R. Lopez-Fandino, 2004. Antioxidant activity of peptides derived from egg white roteins by enzymatic hydrolysis. J. Food Protect., 67: 1939-1944.
10. Sakanaka, S., Y. Tachibana, N. Ishihara and L.R. Juneja, 2004. Antioxidant activity of egg-yolk protein hydrolysates in linoleic acid oxidation system. Food Chem., 86: 99-103.
11. Reitman, S. and S. Frankel, 1957. Colorimetric method for estimation of GOT/AST and GPT/ALT transaminases. Am. J. Clin. Pathol., 28: 56-63.
12. Belfield, A. and D.M. Goldberg, 1971. Colorimetric determination of alkaline phosphatase activity. Enzyme, 12,: 561-573.
13. Szasz, G., 1969. Colorimetric method for GGT determination. Clin Chem., 22: 124-136.
14. Fawcett, J.K. and J.E. Scott, 1960. Enzymatic determination of urea. J. Clin. Path., 13: 156-159.
15. Bartels, H., M. Bohmer and C. Heirli, 1972. Estimation of creatinine clearance in patients with unstable renal function. Clin. Chim. Acta., pp: 37:173.
16. Barham, D. and P. Trinder, 1972. An improved color reagent for the determination of uric acid. Analyst., 97: 142-145.
17. Lopez-Virella, M.F., P. Stone, S. Ellis and J.A. Colwell, 1977. Colorimetrically method used for HDL measurement Clin. Chem., 23: 882.
18. Nishikimi, M., N. Appaji and K. Yagi, 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun., 46: 849-854.
19. Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides. In: Animal tissue by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.
20. Borenstein, M., H. Rothstein and J. Cohen, 1997. Sample Power Statistics 1.0.SPSS, Inc., Chicago.
21. Mesembe, O. E., I. Ibanga and E. E. Osim, 2004. The effects of fresh and thermoxidized palm oil diets on some haematological indices in the rat. Nigerian Journal of Physiological Sciences, 19(1-2): 86-91.
22. El-bialy, B., El. Nahed, S. Th. and Reham, M. Abou-Elkhair, 2015. Potential hazards of feeding albino rats on diet containing repeatedly boiled cooking oil: Clinicopathological and Toxicological studies. International Journal of Advanced Research (IJAR), 3 (3): 134-147.
23. Leong, X.F., A. Aishah, U. Nor Aini, S. Das and K. Jaarin, 2008. Heated palm oil causes rise in blood pressure and cardiac changes in heart muscle in experimental rats," Archives of Medical Research, 39(6): 567-572.
24. Siegel, G., E. Ermilov, A.R. Pries, K. Winkler, A. Schmidt and L. Ringstad, 2014. The significance of lipid peroxidation in cardiovascular diseases. Colloid Surface A., 442: 173-180.
25. Thirunavukkarasu, C. and D. Sakthisekaran, 2003. Influence of sodium selenite on glycoprotein contents in normal and N-nitrosodiethylamine initiated and phenobarbital promoted rat liver tumors. Pharmacol. Res J., 48: 167-173.
26. Jaarin, K., U. Nor-Aini, M.A. Siti-Aishah and S. Das, 2015. Palm Oil Fat Diet Consumption and its Effects on Serum Liver Enzymes and Microscopic Changes in Experimental Rats. Pakistan Journal of Nutrition, 14 (9), pp: 575-580. ISSN 1680-5194.
27. Halliwell, B., 1997. Antioxidants and human disease: a general introduction. Nutr Rev., 55: S44-49.
28. Medeiros, F.J., C.G. Mothe, M.B. Aguila and C.A. Mandarim-de-Lacerda, 2005. Long term intake of edible oils benefits blood pressure and myocardial structure in spontaneously hypertensive rat (SHR) and streptozotocin diabetic SHR. Prostaglandins Other Lipid Mediat, 78: 231e248.
29. Osim, E.E., D.U. Owu and K.M. Etta, 1996. Arterial pressure and lipid profile in rats following chronic ingestion of palm oil diets. Afr J Med Sci., 25:335e340.
30. Suomela, J.P., M. Ahotupa and H. Kallio, 2005. Triacyl-glycerol oxidation in pig lipoproteins after a diet rich in oxidized sunflower seed oil. Lipids, 40: 437-444.
31. Lakomkin, V., G. Konovalova, E. Kalenikova, I. Zabbarova, A. Kaminnyi, A. Tikhaze, V. Lankin, E. Ruuge and V. Kapelko, 2005. Changes in antioxidant status ofmyocardium during oxidative stress under the influence of coenzyme Q10. Biochemistry (Moscow), 70, pp: 79-84.
32. Kalenikova, E.I., E.A. Gorodetskaya, A.N. Murashev, E.K. Ruuge and O.S. Medvedev, 2004. Role of Reactive Oxygen Species in the Sensitivity of Rat Hypertrophied Myocardium to Ischemia. Biochemistry (Moscow), 69: 311-316.