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Biochemical studies on the effect of fixed oil extracted from *Rosmarinus officinalis* on blood lipid level in male albino mice

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Abstract: This work has been carried out to investigate the Hyperlipidemia mirrors the onset of abnormalities in lipid metabolism secondary to the manifestation and progression of the atherosclerotic disease in the patient. In addition to diet, use of medicinal plant as a pharmacologic modality in preventing alteration in lipid metabolism has received wide attention from several works. In the present study, we have therefore investigate the effect of 100 µg/ml doses of fixed oil extract from *Rosmarinus officinalis* on the levels of lipid parameters by using the protective effect of the test herbs against hyperlipidemia for two weeks. The results of experiment elicited the protective effect of 100 µg/ml doses of fixed oil extract from *Rosmarinus officinalis* were marked improvements in the level of all the test parameters were indicated.

Keywords: Hyperlipidemia**,** *Rosmarinus officinalis*, Lipid profile, Liver enzyme, Histopathological studies.

I. INTRODUCTION

Many of the chronic diseases that affect human have an uneven geographic distribution. Although the general perception that several diseases, specially the various types of cancer, kidney and liver diseases as well as coronary heart disease (CHD) often result from an exposure to pollutants and toxic environmental such as chemicals, pesticides, fungicides and food additives. The high incidence of CHD is often correlated with high fat, high cholesterol and low fiber diets and also the consumption of fried foods [Betul and Nesrin , 2012]. Cholesterol presents a great health hazard when its consumption is unduly increased. Hypercholesterolemia arteriopathies whether coronary or cerebral take the biggest tool of middle aged and elderly deaths. Intake of fatty diets, lack of exercise, smoking habits and mental stress participate in hypercholesterolemia and the resultant arteriopathies. Atherosclerosis, a chronic inflammatory disease which Is characterized by the accumulation of plasma lipoproteins that carry cholesterol and triglycerides in the arteries, Atherosclerosis results in coronary heart disease (CHD), one of the major cause of morbidity and

mortality worldwide [Stocker and Keaney, 2014]. Although the focus of research so far has been mainly on the vascular effects of hyperlipidemia, i.e. arteriosclerosis, it is now quite evident that hyperlipidemia exerts direct effects on the myocardium in addition to the development of atherosclerosis. Hyperlipidemia is often linked to oxidative/nitrosative stress in the vasculature and in the myocardium. We have previously shown an increased formation of peroxynitrite, a toxic reaction product of superoxide and nitric oxide, in the rat myocardium in cholesterol-enriched diet - induced hyperlipidemia [Tamas *et al*., 2013]. Strong evidences have been put forward by various investigators for the involvement of free radicals production and lipid peroxidation in the onset of atherosclerosis [Bansal and Sapna , 2011]. One of the initial events in the development of atherosclerosis is the accumulation of cells contained excess lipids within the arterial wall. Hypercholesterolemia, especially elevated level of serum cholesterol and low-density lipoprotein (LDL),Furthermore, oxidative stress is also suggested as a mechanism underlying hypercholesterolemia, which is an important factor in atherosclerosis. According to the oxidative modification hypothesis, oxidation of LDL is crucial to the cellular uptake of LDL in the first stages of atherosclerotic development. [Lifeng *et al*., 2012]. Large acute doses of chronic exposure to toxic agents can overpower to the antioxidant defense system which cause hepatic cell damage. Natural or synthetic compounds having antioxidant properties can scavenge the free radicals which damage lipid, protein, DNA molecules and cell membrane. Their removals prevent the development of certain diseases [Cintra *et al*., 2006]. Antioxidants had important role in decreasing serum lipids and retarding atherosclerosis. The observational epidemiological studies have suggested that individuals with high dietary antioxidant intake have lower risks of CHD which remains the leading cause of death in most countries [Sun *et al*., 2010]. Diet rich in fruits and vegetables are associated with decreased risk of CHD. Biochemical functions of naturally occurring antioxidants in biological systems such as flavonoids, polyphenols, vitamin C and E have been reported to protect the body system against reactive oxygen species. Antioxidant compounds, various efforts are now concentrated on many herbal plant extracts because of their potential to induce antioxidant effects [Olorunnisola *et al*., 2012]. Plant products are widely used in testing because of their low toxicity and great medicinal value. Much of research has concentrated on different plant extracts' abilities to induce antioxidant effects [Jeon *et al*., 2015]. Rosemary essential oil is used as brain and nerve tonic, and as a remedy for mental fatigue [Laybourne *et al.*, 2013] as well as antiseptic, diuretic and anti-depressant, it is also used to treat cold, influenza and rheumatic pain [Erenmemisoglu, 2014] and has the ability to enhance the performance of memory [Moss *et al.*, 2003]. Diversity in the chemical contents of rosemary as polyphenolic compounds have been ascribed to many factors, involving the environment [Tigrine-Kordjani *et al.*,

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2007], abiotic stress [Tounekti *et al.*, 2011], genetic heritance [Yosr *et al.*, 2013 and Jordan *et al.*, 2013] and the phenological stages of the plants [Singh *et al* ., 2013]. The aim of this study was to extract the fixed oil of *Rosmarinus officinalis* grow in Al-Jabal Al Akhdar, also to investigate the protective effect of the test herbs against hyperlipidemia in experiment animals.

II. MATERIALS AND METHODS

2.1Materials:

2.1.1 Plant material: The leaves of *Rosmarinus officinalis* L, were collected from Al-Jabal Al Akhdar area in Libya 2017.

2.1.2 Chemicals: 1,1-Diphenylpicrylhydrazyl ($DPPH^{\cdot}$) was supplied from Sigma and Merck company. Ascorbic acid, Folin-Ciocalteu reagent, ferric chloride, potassium ferricyanide, monobasic dihydrogen phosphate, dibasic monohydrogen phosphate, trichloro acetic acid, sodium carbonate, petroleum ether, anhydrous sodium sulfate and pyrogallol were obtained from the biochemistry laboratory of chemistry department-Benghazi University.

2.1.3 Experimental animals: Male albino mice weighing between 25 and 35gm used in this study were housed in biochemistry laboratory at Benghazi University under standard conditions of temperature (22 ±3C◦) with a dark light cycle. The animals were fed with standard diet and water ad libitum.

2.2 Methods:

2.2.1 Sample preparation:

2.2.1.1 Extraction of fixed oil: The fixed oil from the dry powdered leaves of *Rosmarinus officinalis* (100g) was extracted with light petroleum ether (40-60C°) in a soxhlet apparatus for about 4h and the solvent was removed by rotary vacuum evaporator. The oil sample were stored at 7°C in dark air-tight container.

2.2.1.2 Determination of LD_{50}: A total Number of 80 male albino mice used in this study to determine the lethal dose of fixed oil extracted from *Rosmarinus officinalis*. In each experiment were used 40 mice, divided into five groups were given different amounts of the oil "10, 50, 100, 150, 200µg/ml" oral. The animals were monitored for 24hrs for mortality. The number of animals survived a specific dose (S) and the number of those died at that dose (D) was determined [Al-Ali *et al.*, 2008].The percent mortality was calculated for each dose group as in the following:

% mortality= D/(S+D) X 100

Calculated lethal dose for half the number depending on the equation of [Al-Ammar, 2001].

LD_{50}=Biggest dose - $\sum a \times b / n$

a :The difference between the doses in two consecutive terms.

b :the average number of dead animals for two consecutive terms.

n : number of animals in each group.

2.2.2 Experimental design: The following experiments were conducted as follows:

2.2.2.1 The prophylactic effect of the fixed oil against hyperlipidemia: To study the protective effect of the oil against hyperlipidemia, a total of 45 mice were used and the experiment lasted for 2

weeks. Animals were divided randomly into three groups (15 mice each) as follows: Group 1: mice were fed on the standard synthetic diet (S.d) and served as negative control group (-ve).Group 2: mice were daily attained to the hyperlipidemic diet (H.L.D) and served as positive control group (+ve).Group 3: mice were daily administered fixed oil at a dose of 100 µg/ml oral (added to the H.L.D).

2.2.2.2 Blood sampling: Blood samples were collected before treatment and then after 2 weeks. Blood samples were collected from retro-orbital veins according to the procedure described by [Janet Hoff, 2000] by microhematocrit blood tube into the corner of the eye socket underneath the eyeball. Serum and plasma samples were carefully separated and stored frozen until using in the different biochemical analysis.

2.2.2.3 Biochemical analysis: The following parameters were assayed; serum total cholesterol [Alan *et al.*, 2006] , HDL-cholesterol [Allain *et al.*, 1974], Triglycerides [Fossati and Precipe, 1996], Blood urea [Tabacco *et al.*,1979]Transaminases enzymes (ALT& AST) [Bergmeyer *et al.*, 1986] ,Alkaline phosphatase (ALP) [Bretaudiero and vassault, 1977], Total proteins (TP) [Doumas and Biggs, 1981], Gamma-Glutamyltransferase (GGT) [Shaw *et al.*, 1983], Superoxide dismutase (SOD)[Nishikimi *et al.*, 1972], Malondialdehyde (MDA) [Ohkawa *et al.*, 1979], Glutathione Reductase (GR) [Goldberg and Spooner, 1983], Glutathione Peroxidase (GPx) [Paglia and Valentine, 1967] and Catalase (CAT) Paglia and Valentine, 1967. All biochemical examination is carried out in Benghazi medical center. The obtained results were statistically analyzed [Chase ,1967].

III. RESULTS

Effects of fixed oil in prophylactic experiment after 2 weeks of treatment: All the plasma lipids parameters were significantly increased in induced hyperlipidemia mice (positive group), when compared to normal control values. The concentration of TC, T- chol/HDL-chol, LDL, VLDL and TG were significantly increased by (121.6%, 270.5%, 573%, 28.98%, 28.98%) respectively, but the level of HDL-cholesterol is decreased by (40.5%) . Fixed oil had significant decrease in serum lipid profile levels as compared with positive group. TC, T-chol/ HDL-chol, LDL, VLDL and TG were significantly decreased by (16.9%, 2.9%, 21.7%, 36.7% and 36.7%) respectively at dose of 100µg/ml of fixed oil for two weeks, while the concentration of HDL-chol is increased by (18%) at dose of 100µg/ml of fixed oil respectively. Liver function tests in hyperlipidemia mice displayed a mild to moderate increase in serum alanine aminotransferase (37.2%), aspartate aminotransferase (23.5%), G-glutamyl transferase (136.5%), alkaline phosphatase (66%) in comparison with normal control group and significantly decrease in the serum TP by (5.75%). The sequential changes in serum ALT, AST, G-GT, ALP and TP are summarized in Figures (15,16,17,18 and 19) respectively, however in G3 which that ingested 100µg/ml of fixed oil with hyperlipidemia diet for two weeks, serum ALT, AST, G-GT and ALP was

lowered by (16.1%, 8.11%, 22.4% and 21.6%,) respectively , while the serum TP showed no significant difference as compared with the positive group. The mean values of blood urea in the positive control were non-significantly compared with the positive control. The average value of SOD ,GR, GPX and Catalase were significantly decreased in hyperlipidemia group by (26.9% ,41.6%,31.6% and 31.3%) respectively. The mice fed with hyperlipidemia diet showed significant increase in the plasma levels of MDA (147.7%) when compared with normal group, while the administration of fixed oil decrease the MDA level by (27.5%).

IV. DISCUSSION

4.1 Evaluation of fixed oil against hyperlipidemia: There is a wealth of scientific data coming from in vitro studies or from different animal models, supporting the validity of the oxidative hypothesis of atherosclerosis which states that the oxidative modifications of lipoproteins is a pivotal event in the evolution of atherosclerotic plaques. A corollary of this hypothesis is that antioxidant enzymes should therefore prevent LDL oxidation and protect against the development of atherosclerosis [Kallol and Biswadev, 2009]. Several epidemiological studies concluded that a high intake of food rich in natural antioxidants increases the antioxidant capacity of the body and reduces the risk of several diseases. Herbal remedies or food supplements have increasingly become attractive alternatives to prevent or treat hyperlipidemia, especially for those with cholesterol at the borderline levels [Nyangono *et al.*, 2012]. Antioxidant phytochemicals play an important role in human health by scavenging reactive oxygen and nitrogen species and modulating several defense systems [Dhan and Gupta, 2009].[Khatun *et al.*, 2006] found that clove had the highest radical-scavenging activity followed by all spice and cinnamon. The phenolic and the flavonoids compounds are groups of secondary metabolites with broad range of biological properties such as: antioxidant, anti-atherosclerosis, cardiovascular protection and improvement of the endothelial function, it has been reported that antioxidant activity of the phenolic compounds is mainly due to their redox properties which allow them to act as reducing agents, hydrogen donors play an important role by adsorbing and neutralizing reactive free radicals, and chelating ferric ions which catalysis lipid peroxidation, and regarded as promising therapeutic agent for free radical-linked pathologies [Nyangono *et al.*, 2012]. The result obtained from the GC-MS technology found that the most important components are 1,8-cineole , α-pinene , camphor , camphene , borneol . The high concentration of 1,8-cineole in fixed oil make it potentially useful in the medicines because they exhibit antibacterial, antifungal, anti-inflammatory activity and antioxidant properties according to [Greiner *et al*., 2014].

The hypolipidemic effect of oil extracted fro*m* *Rosmarinus officinalis* may be due to its ability to combat oxidative stress by quenching free radicals generated in the body as a result of HFD. A significant increase in HDL-chol levels with a

simultaneous lowering in serum cholesterol level definitely indicates the beneficial role of the antioxidant compounds such as 1,8-cineole, α-pinene, camphor camphene, Borneol which that found in the oil. The increased levels of HDL-cholesterol concentrations also suggest that fixed oil may protect against cardiovascular diseases that result from hyperlipidemia. [Nyangono, 2012] suggests that the increase in HDL cholesterol may be due to mobilizes cholesterol from extra-hepatic tissues where it is catabolized and stimulate cholesterol 7-α-hydroxylase which converts cholesterol to bile acids, an important pathway of degradation of cholesterol, the earlier reports show that some of the major constituents of the rosemary oil were eucalyptol, α- pinene, myrcene, p-cymene, pinene, camphor, camphene, limonene, borneol, verbenone, bornylacetate, etc. It has been found that location and seasonal change affect the variation in composition and ultimately the biological activity of the essential oil of R. officinalis L. [Zaouali *et al*., 2010].

This result suggests that other phenolic compounds, beyond those found in the fixed oil, may be responsible for these responses. This effect on cholesterol reduction may be attributed to a decrease in the micellar solubilization of cholesterol in the digestive tract, to an increase in bile flow, bile cholesterol and bile acid concentration and to a subsequent increase in the fecal excretion of steroids, as previously described [Gorinstein *et al*., 2005]. This protection could be attributed to rosmarinic acid, which has been reported to inhibit the expression of inducible nitric oxide synthase (iNOS) and suppress the production of superoxide and 3-nitrotyrosine in Raw 264.7 macrophages [Qiao *et al*., 2005].

We suggest the oil rich in phenolic constituent has the highest antioxidant activity against LDL oxidation.[Omer *et al* ., 2013] reported that the 1,8-cineole and α- pinene have an antioxidant property and can change the affinity of the LDL particles for the LDL receptor. Also,[Ramesh *et al.,* 2008] showed that dietary intake of phenolic compounds could inhibit oxidation of LDL and thereby reduce risk factors for cardiovascular disease. Flavonoids are a class of polyphenolic compounds that seem to have antioxidant properties by suppressing reactive oxygen and nitrogen species formation and protecting the antioxidant defense system, in addition flavonoids may act by making liver cells more efficient to remove LDL. from blood. To do this, flavonoids increase LDL receptor densities in liver and by binding to apolipoprotein B [Pourghassem-Gargari *et al.,* 2009].

It was observed that the enzymes such as AST, ALT, G-GT and ALP were decreased by the effect of oil when compared with positive group. [Olorunnisola *et al.,* 2012] reported that the co-administration of Phytochemical compounds such as flavonoids prevented liver damage resulting in the improvement of the status of liver function test. [Saleh *et al.*,2013] reported that the decrease in the serum levels of transaminases enzymes might be due to the presence of various phenolic, flavonoid compounds and sesquiterpenes that may be responsible for the protective effect of liver damage. In humans the over-production of ROS

can result in tissue injury and has been implicated in disease progression and oxidative damage of nucleic acids and proteins [Middleton *et al.*, 2008]. When there is a lack of antioxidants to quench the excess reactive free radicals, cardiovascular, cancer, hyperlipidemia, diabetes, Alzheimer's and inflammatory diseases may develop in the body [Ashwell *et al.*, 2010]. Pharmaceutical products are normally supplemented with synthetic antioxidants such as butylated hydroxytoluene (BHT) and tetrabutylhydroxyquinone (TBHQ). However, natural antioxidants from plant products may be more effective in reducing ROS levels compared to synthetic antioxidants. In addition, the dietary intake of synthetic antioxidants could be cause genotoxicity and carcinogenicity at high concentrations [Ashwell *et al.*, 2010]. A possible mechanism for protection by *Rosmarinus officinalis* against liver damage could involve *Rosmarinus officinalis* components acting as free radical scavengers intercepting those free radicals [Nassar *et al.*, 2007]. The significant decreasing in the plasma MDA and increasing of antioxidant enzymes levels of hyperlipidemic mice and their tendency to return to near normal levels after administration of oil revealed their powerful antioxidant effect. Antioxidants function as rupturing the antioxidant chain that prevents the propagation of free radical reactions; this protects cells from oxidative stress. Vitamin E is particularly important, protecting cells against lipoperoxidation, during which free radical attack the fatty acids, causing structural damage to the membrane, resulting in the formation of secondary cytotoxic products such as MDA [Jervis *et al.*, 2004]. Antioxidants are believed to neutralize the free radicals in lipid chains by contributing a hydrogen atom usually from a phenolic hydroxyl group, which in turn converts phenolic groups into stable free radicals that do not initiate or propagate further oxidation of lipids [Hamid *et al.*, 2010]. [Potapovich and Kostyuk , 2003] reported that, of a variety of flavonoids (rutin, dihydroquercetin, quercetin, epigallocatechin gallate, and epicatechin gallate), the catechins were most effective in inhibiting microsomal lipid peroxidation. All were able to chelate $Fe^{2+,}$ Fe^{3+}, and Cu^{2+} and effective $O_2^{\cdot}$ scavengers at varying degrees.

Our study suggested that the health promoting capabilities effects of this oil in animal models of hyperlipidemia could be attributed to the high polyphenolic content along with the enhancement of the total antioxidant defense system of body. As preventive antioxidants, the oil can directly intercept free radical retard or slow the oxidative processes leading to decrease MDA level.[Bhosale *et al.*, 2012] reported that the lower levels of MDA and higher levels of SOD activities could be attributed to polyphenols and flavonoids. SOD catalyzes dismutation of superoxide anions into hydrogen peroxide, which was converted to water by both CAT and GPx. Nutrient antioxidants, included in the dietary antioxidants, are chain breaking antioxidants, which work with enzyme antioxidants, to regular the ROS within physiological limits [Singh *et al.*, 2010]. Natural plant antioxidants include phenolic compounds may produce beneficial effects by scavenging free

radicals [Chun *et al.*, 2003]. Thus, phenolic compounds may help protect cells against the oxidative damage caused by free radicals,[Korakot *et al.*, 2006]. reported that the extract plant rich in phenolic compounds leads to decrease MDA and increase the SOD activity. In agreement with our study[Singh *et al.*, 2010].indicated that the administration of *Rosmarinus officinalis* in hyperlipidemic rat decreased plasma lipid profile and enhanced the activity of the antioxidant enzymes.

Conclusion:

In conclusion, our results suggest that phenolic compounds from *Rosmarinus officinalis* protect against hyperlipidemia-induced oxidative stress, increasing the activities of antioxidant enzymes and reducing the amount of thiobarbituric acid reactive substances. The fixed oil extract was also able to improve the serum lipid profile, contributing to cardiovascular disease reduction. Future analysis involving the bioavailability of rosemary phenolic compounds and their role on modulating the signaling pathways activated under oxidative stress-related diseases may help to clarify the role of these antioxidant compounds in cholesterol homeostasis and parameters of oxidative stress.

Table.1 Results for the determination of lethal dose LD_{50} of the fixed oil extracted from *Rosmarinus officinalis*. after oral ingestion in male albino mice (n = 5). LD_{50} = 180µg/ml

Dose(µg/ml)	(a)	Number of mice			(b)	(a×b)	% mortality
		Total	Survived	Died			
10	0	5	5	0	0	0	0%
50	40	5	5	0	0	0	0%
100	50	5	5	0	0	0	0%
150	50	5	4	1	1	50	20%
200	50	5	3	2	1	50	40%

a: The difference between the doses in two consecutive terms. b: the average number of dead animals for two consecutive terms

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Table. 2 Prophylactic Effects of fixed oil in prophylactic experiment on serum lipids (mg/dl ± S.D) and % variation from the corresponding control during the induction of hyperlipidemia for2 weeks in male mice.

I. GROUP NUMBER	II. TREATMENT	III. ITEM	Time intervals (Wk's)		
			0	2	% var.
1	Negative control	Trig.	48.7 ± 3.47	49.0 ± 3.70 †	0.62↑
		T.Ch.	55.5 ± 5.78	58.7 ± 4.10 †	5.77↑
		HDL.Ch.	34.6 ± 1.42	34.5 ± 1.30 †	0.28↓
		T./HDL	1.6±0.09	1.7±0.08†	6.25↑
		LDL	± 1.1716 .11	± 1.034 .14	29↑
		VLDL	9.74 ± 2.03	9.8 ± 2.11	0.62↑
2	Positive control	Trig.	52.1 ± 2.11	63.2 ± 2.21*	28.98↑ a
		T.Ch	57.4 ± 4.71	130.1 ± 3.24 ***	121.6↑ a
		HDL.Ch.	32.0 ± 1.93	20.5 ± 1.81***	40.5↓ a
		T./HDL	1.8±0.01	6.3±0.17 ***	270.5↑ a
		LDL	± 0.9298.14	± 1.00 .9696	573↑ a
		VLDL	10.42 ± 1.59	12.64 ± 2.22**	28.98↑ a
3	Fixed oil (100µg/ml)	Trig.	55.3 ± 5.03	40.0 ± 4.20 *	36.7↓ b
		T.Ch.	55.0 ± 4.06	108.1 ± 2.95**	16.9↓ b
		HDL.Ch.	33.9 ± 1.71	24.2 ± 1.73***	18↑ b
		T./HDL	1.62±0.01	4.47± 0.22***	2.9↓ b
		LDL	± 1.8204 .10	75.9 ± 1.90	21.7↓ b
		VLDL	11.06 ± 0.99	8.00±1.73	36.71↓ b

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†P > 0.1. * P < 0.05. ** P < 0.01. *** P < 0.001. T.L.: total lipids. T.C.: total cholesterol. TG.: triglycerides. HDL-C: HDL-cholesterol. T. /HDL-C: total cholesterol / HDL-cholesterol.

Table.3 Prophylactic effect of fixed oil on serum enzymes (U/L ± S.D) and % variation from the corresponding control during the induction of hyperlipidemia for 2 weeks in male mice.

IV. GROUP NUMBER	V. TREATMENT	VI. ITEM	Time intervals (Wk's)		
			0	2	% var.
1	Negative control	ALT	28.8±1.95	30.4±2.23 †	5.55↑
		AST	54.3±0.38	54.9±1.65 †	1.10↑
		ALP	30.3±4.03	30.9±3.17 †	1.98↑
		S. GGT	8.21± 0.43	8.88±2.11 †	8.16↑
2	Positive control	ALT	30.2±1.78	41.7±2.09 *	37.17↑ a
		AST	50.73±2.86	67.8±3.10 *	23.5↑ a
		ALP	28.9±2.77	51.3±2.75 †	66↑ a
		S. GGT	8.92 ± 2.00	21.0 ± 1.12*	136.5↑ a
3	Fixed oil (100µg/ml)	ALT	30.1±1.09	35.0±1.16 †	16.1↓ b
		AST	50.0±1.89	62.3±2.99 *	8.11↓ b
		ALP	31.0±1.90	40.2±3.14**	21.64↓ b
		S. GGT	9.11± 3.01	16.3±2.61 *	22.4↓ b

†P > 0.1. * P < 0.05. ** P < 0.01. *** P < 0.001. ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. ALP: Alkaline phosphatase. S. GGT: Gama-Glutamyl transferase

Table. 4 Prophylactic effect of Fixed oil on serum proteins, blood urea and antioxidant enzyme (± S.D) and % variation from the corresponding control during the induction of hyperlipidemia for 2 weeks in male mice.

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VII. GROUP NUMBER	VIII. TREATMENT	IX. ITEM	Time intervals (Wk's)		
			0	2	% var.
		T.P. (mg/dl)	6.15±0.62	6.44±0.19 †	4.72↑
		UREA (mg/dl)	22.9± 0.34	22.7 ± 0.90†	0.87↓
		SOD (U/mol)	7.11±1.90	7.00±1.21†	1.55↓
		MDA (nmol/ml)	10.3± 0.44	10.7±1.25 †	3.9↑
		P. GR (U/l)	18.5± 0.59	18.5±1.06 †	Zero
		P. GPX (mu/ml)	33.0± 5.01	32.3±3.46 †	2.12↓
		S. CAT (U/l)	48.7±2.90	47.3±2.99†	2.9↓
2	Positive control	T.P. (mg/dl)	6.12±0.61	6.07±0.38†	5.75↓ a
		UREA (mg/dl)	21.5 ± 0.25	24.1 ± 1.11†	6.17↑ a
		SOD (U/mol)	7.52±1.23	5.12±0.90*	26.9↓ a
		MDA (nmol/ml)	10.1 ± 1.02	26.5 ±1.87 **	147.7↑ a
		P. GR (U/l)	18.1 ± 1.72	10.8 ± 1.22*	41.6↓ a
		P. GPX (mu/ml)	33.7 ± 2.11	22.1 ±2.78*	31.6↓ a
		S. CAT (U/l)	49.3 ± 1.88	32.5 ± 2.01*	31.3↓ a
3	Fixed oil (100µg/ml)	T.P. (mg/dl)	6.22±0.20	6.34±0.39 †	4.45↑ b
		UREA (mg/dl)	23.0 ± 1.92	24.6 ± 1.34†	2.07↑ b
		SOD (U/mol)	7.22±1.11	5.79±1.05*	13.1↑b
		MDA (nmol/ml)	10.1± 1.11	19.2±1.00 *	27.5↓b
		P. GR (U/l)	17.9± 1.38	11.9±1.19*	10.2↑ b
		P. GPX (mu/ml)	32.9±2.90	26.8± 2.05*	21.3↑ b
		S. CAT (U/l)	49.2± 5.21	37.6± 2.33*	15.7 ↑b

†P > 0.1. * P < 0.05. ** P < 0.01. *** P < 0.001. T.P.: Total protein. superoxide dismutase (SOD), Malondialdehyde (MDA) , Glutathione Reductase (GR) , Glutathione Peroxidase (GPx) and Catalase (CAT).

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