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Ameliorative effects of silymarin and *Nigella sativa* extract on paracetamol induced hyperlipidemia and oxidative stress in heart tissues in male mice

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ABSTRACT

Paracetamol (APAP-Acetaminophen) has been used extensively as antipyretic drug. The purpose of this study was to evaluate the ameliorative role of silymarin or/and Nigella sativa extract against APAP-induced hyperlipidemia in male mice at the biochemical, levels. The mice were divided into seven groups (10/group). The first group was served as control. While, the second group was treated with dose of APAP. The third and fourth groups were treated with silymarin alone and Nigella sativa extract alone respectively, the fifth and sixth groups were treated with combination of APAP with silymarin and APAP with Nigella sativa extract respectively. The seventh group was treated with combination of both ameliorative compounds (Silymarin and Nigella sativa extract) with APAP and all animals were treated for a period of 30 days. Exposure to APAP at the treated dose to mice led to an alternation of lipid function parameters, increase the TG, total cholesterol, LDL and vLDL levels, decreased HDL level as well as decreased SOD and CAT, while increasing MDA in heart tissues homogenates in APAP treated group. The effects of APAP on the biochemical parameters of mice were dose-dependent. Administration of silymarin or/and Nigella sativa extract to APAP-treated mice alleviate the toxicity of APAP, and this appeared clearly by biochemical improvement of lipid profile picture. But, the alleviation is more pronounced with the both antioxidants. Thus, the pronounce effect of silymarin and Nigella sativa extract is most effective in reducing the toxicity induced by APAP and improving the lipid profile of male mice.

Key words: Paracetamol, Silymarin, *Nigella sativa* extract, Liver function.
Abbreviations: APAP; Paracetamol, N. sativa; Nigella sativa, TG; Triglycerides, TC; Total cholesterol.

INTRODUCTION

Acetaminophen (paracetamol - APAP) is a derivative of para-aminophenol used as an analgesic and antipyretic drug belonging to the Para-aminophenol class of the non-steroidal anti-inflammatory drugs (NSAIDs) [1].

APAP is quickly absorbed from the gastric intestinal tract and reaches peak serum levels in 1- 4 hours. Although it is safe at therapeutic doses [2].

APAP distributes throughout most tissues and fluids, reaching a tissue: plasma concentration ratio of about unity in all tissues except fat and cerebrospinal fluid. With normal therapeutic dose, APAP is slightly bound to plasma proteins; only 20 to 50% may be bound at the concentrations encountered during acute intoxication [3].

The seeds of *N. sativa* have been subjected to a range of pharmacological, phytochemical and nutritional investigations in recent years .It has been shown to contain more than 30 %(*w/w*) of a fixed oil with 85 %of total unsaturated fatty acid [4].

Nigella sativa seeds decrease the serum total lipids and body weight in rats [5], decrease the fasting plasma glucose in rabbit, increase serum total protein, and shows diuretic and hypotensive effects in spontaneously hypertensive rat [6]**.**

There is some scientific evidence for the hypolipidemic effect of black seed as its oil [6], extract or some pure compounds such as TQ [7]. Moreover, the effect of *N. sativa* on serum or tissue lipid peroxidation and antioxidant status has been studied previously with inconsistent results [8] .However, to date; little attention has been paid to the effects of whole or crushed black seed on serum lipid profile and other biochemical parameters.

Silybum marianum (Milk thistle) has been used to treat liver diseases since the 16th century .Its major constituents are the flavonoids silibinin, silidianin, silichristin, and isosilibinin of which silibinin is the biologically most active compound and used for standardisation of pharmaceutical products [9].

In addition, as antioxidant, silymarin regulates the intracellular contents of the reduced glutathione (GSH) and Chelates metal ions (iron and copper. Recently silymarin received attention due to its alternative beneficial activities as hypocholestrolimic and cardiprotective agent [10].

EXPERIMENTAL SECTION

2.1. Chemicals

APAP (APAP, N-acetyl-p-aminophenol) was purchased from the Egyptian International Pharmaceutical Industries Company (EIPICO); Silymarin was obtained from "Sedeco Pharmaceutical Co-6-october city. Egypt. The *Nigella Sativa* seeds were purchased from a local herb store with a fair degree of quality assurance. Seeds were washed to remove sand and other debris and air-dried and finely powdered with an electric microniser according to traditional mode of preparation [11]. Crude extract was obtained by the maceration of 800gm of these seeds by boiling in distilled water (1200ml) for 24h and filtered through muslin [12]. After 24h, the aqueous extract was filtered, concentrated at room temperature [13] then the dried extract was stored at 4^{o}C until use).,other chemicals and reagents were of the highest analytical grade and were bought from standard commercial suppliers and were purchased from Roche (Germany).

2.2. Animals

SWR albino male mice weighing approximately 30-35g were obtained from Animal breeding house, King Fahd Center for medical research, king Abdul-Aziz University. The animals were maintained in solid bottom shoe boxtype polycarbonate cages with stainless steel wire-bar lids, using a wooden dust-free litter as a bedding material. Animals were located in air-conditioned room and were allowed free access to pellet diet and tap water for a week before starting the experiment. The European Community Directive (86/609/EEC) and National rules on animal care have been followed. After 2 weeks of acclimation, animals were randomly divided into seven groups with 10 animals in each one as following.Groups1was served as untreated control (1ml/kg of physiological saline), Group 2 was treated with paracetamol (2 g/Kg; Chen et al. [14], Group 3 was treated with silymarin (50 mg/Kg; *Hale et al*.,[15] Group 4 was treated with *Nigella sativa* extract (0.25gm/100g; *Schleicher and Saleh*[11]. Group 5 was treated with paracetamol and silymarin respectively, Group 6 was treated with paracetamol and *Nigella sativa* and the final 7^{th}group was treated with paracetamol followed by silymarin and *Nigella sativa* respectively. All the groups were treated orally for 30 successive days.

2.3. Collection of blood samples

At the end of experimental period, blood samples of the fasted mice were collected from the medial retro-orbital venous plexus immediately with capillary tubes (Micro Hematocrit Capillaries, Mucaps) under ether anesthesia**[16]**. Then, the blood was centrifuged at 3000 rpm for 15 min and serum was collected for different biochemical analyses.

2.4. Lipid profile determination

The serum total cholesterol (TC) and triglycerides (TG) were determined by the method of *Carr et al.* [17]. High density lipoprotein-cholesterol (HDL-c) was determined according to the methods of *Warnick et al*.[18]. Serum

LDL-cholesterol (LDL-c) level was calculated according to *Friedewald* [19] formula: Very low density lipoprotein cholesterol (VLDL-c) levels were calculated by using the following formula of *Prakasam et al*. [20]: VLDL-c = triglyceride/5.LDL-c = Total cholesterol − (HDL-c + Triglyceride)/5.

2.5. Preparation of tissues for measurement of oxidative/ antioxidant parameters

The tissues of heart were used for the analysis of oxidative stress and antioxidant parameters. Prior to dissection, tissues were perfused with a 50 mM (sodium phosphate buffer saline (100 mM Na_2HPO_4/NaH_2PO_4) (pH 7.4) and 0.1 m ethylenediaminetetraacetate (EDTA) to remove any red blood cells and clots. Then tissues were homogenized in 5 mL cold buffer per gram tissue by a Potter - Elvehjem type homogenizer. The homogenate was centrifuged at 10,000 × g for 20 min at 4 ° C, and the resultant supernatant transferred into Eppendorf tubes and preserved in a deep freezer until used. The supernatant was used for the determination of some biochemical parameters of liver tissues.

2.6. Lipid peroxidation assay

The extent of LPO was estimated as the concentration of thiobarbituricacid reactive product MDA by using the method of *Ohkawa et al.*, [21]. MDA concentrations were determined using 1, 1, 3,3-tetraethoxypropane as standard and expressed as micromoles per gram of tissue.

2.7. Antioxidant enzymes

SOD activity was measured according to the method described by *Marklund and Marklund*, [22] in which pyrogallol underwent autoxidation at 440 nm for 3 min. One unit of SOD activity was calculated as the amount of protein that caused 50% pyrogallol autoxidation inhibition. The SOD activity was expressed as units per gram tissue. Before determination of the CAT activity, samples were diluted 1:9 with 1% Triton X-100 (v/v). CAT activity was measured according to the method described by *Aebi*, [23]. The hydrolysis of $H_2O_2$and the resulting decrease in absorbance at 240 nm over a 3-min period at 25 ° C was measured. CAT activity was expressed as units per gram tissue.

2.8. Measurement of enzymes involved in glutathione metabolism

The GPx activity was measured by the method of *Hafeman et al*,[24].The reaction mixture contained 0.5 mL of 0.4 M sodium phosphate buffer (pH 7.0) and 0.4 mM EDTA, supplemented with 0.25 mL of sodium azide (1 mM), 0.5 mL of GSH (2 mM) and 0.25 mL of distal water. About 0.5 mL of homogenate was added and allowed to equilibrate for 5 min at 37 ° C. The reaction was initiated by adding 0.5 mL of H_2O_2 (1.25 mM). Absorbance at 340 nm was recorded at 1, 3, and 6 min. The activity of GPx was expressed in terms of nanomoles GSH consumed per minute per gram of tissue (U/g).

The GRx activity was measured by the method of *Hafeman et al.*, [24].The reaction mixture contained 1.2 mL of 67 mM sodium phosphate buffer (pH 7.0), 0.2 mL of sodium azide (1 mM) and 0.1 mL of oxidized glutathione (7.5 mM). Homogenate (0.5 mL) was added and allowed to equilibrate for 5 min at 37 ° C. Reaction was initiated by adding 0.25 mL of reduced nicotinamide adenine dinucleotide phosphate (NADPH) (2 mM). Absorbance at 340 nm was recorded at 1, 2 and 3 min. The activity of GRx was expressed in terms of micromoles-mol GSH produced per minute per gram of tissue (U/g).

2.9. Statistical analysis

Statistical analysis was performed using SPSS for Windows version 17.0. Data was given in the form of arithmetical mean values ± standard error (S.E). Differences between groups were evaluated by one-way ANOVA according to $p < 0.05$ and post-hoc Duncan test. For each histological parameter, a score has to be performed analyzing a number of different histological sections in each organ for each animal and then the median value has to be calculated for each group; finally a comparison could be done by a semi-quantitative test using SPSS.

RESULTS

3.1. Lipid profile assessments

The triglycerides increased significantly in paracetamol treated group, but significantly decreased by 9.54 and 2.72 fold in the groups treated with the silymarin and *Nigella sativa* extract respectively when compared with control group (Table 1).Meanwhile combination between (paracetamol ,silymarin and *Nigella sativa* extract have ameliorated triglycerides level as compared with control group (Fig.1). Serum total cholesterol level of *Nigella*

sativa extract groups was non-significantly increased by2.49 % when compared with control group. In the paracetamol-treated group, the total cholesterol was increased by 61.11foldincrease when compared to control (Fig. 1). Treatment the mice with 1000 mg/kg paracetamol with the combination of silymarin and *Nigella sativa* extract decreased the activity of TC more than each compound separately. The same observation has been noticed in the LDL and vLDL activities that increased by giving the dose of paracetamol and decreased by the treatment of each antioxidant separately (Fig. 2) and this adversely to that happened in HDL level in paracetamol treated group and in combined groups with both antioxidants.

Table 1: Changes of serum lipid functions in male mice treated with silymarin, *Nigella sativa* extract and paracetamol separately or in combination

Groups	Triglycerides (mg/dl)	Total Cholesterol (mg/dl)	HDL(g/dl)	LDL(g/dl)	VLDL(g/dl)
Control group	73.33±4.52^{de}	66.50±4.29^{f}	37.16±1.24^{b}	18.60±3.65^{de}	14.66±0.92^{d}
Acetaminophen (Paracetamol)	115.00±0.42^{a}	171.00±3.16^{a}	29.33±0.98^{e}	65.21±2.03^{a}	23.00±0.85^{a}
Silymarin	66.33±3.11^{f}	71.08±2.93^{e}	37.16±1.24^{b}	16.02±1.025^{e}	13.26±0.65^{e}
Nigella sativa extract	71.33±3.44^{e}	68.16±2.43^{f}	38.66±0.80^{ab}	18.02±1.52^{de}	14.26±1.02^{d}
Acetaminophen + Silymarin	90.33±4.37^{b}	97.16±2.26^{bc}	30.16±0.90^{d}	43.08±3.26^{b}	18.06±0.36^{b}
Acetaminophen + *Nigella sativa* extract	85.66±2.75^{c}	93.33±5.05^{c}	31.16±0.90^{d}	40.25±1.25^{b}	17.13±1.02^{c}
Acetaminophen + Silymarin+ *Nigella sativa* extract	84.60±0.42^{c}	82.50±2.41^{d}	33.16±0.60^{c}	31.25±3.25^{c}	16.92±1.65^{c}

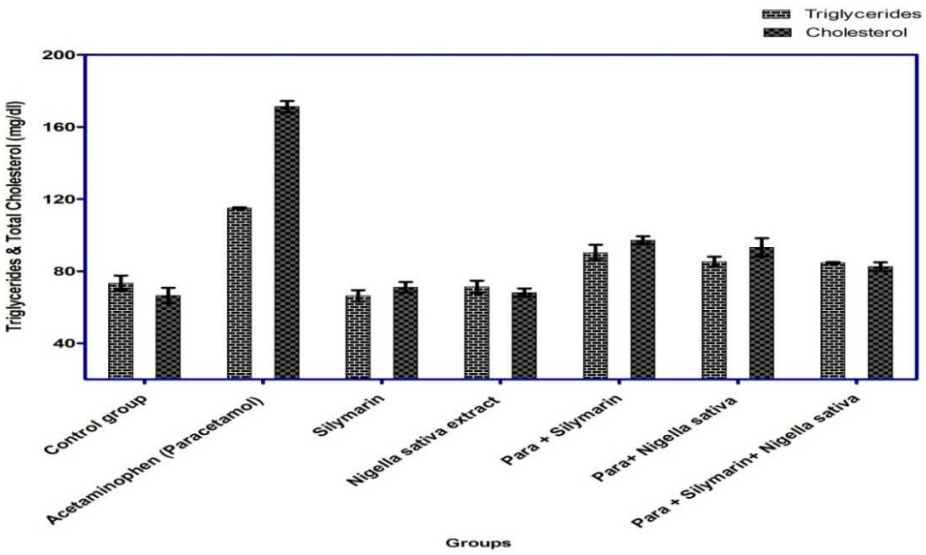

Fig (1): Changes of Triglycerides and Total cholesterol levels in male mice treated with silymarin or *Nigella sativa* extract or both of them with paracetamol. Values are expressed as means ± SE; n = 10 for each treatment group

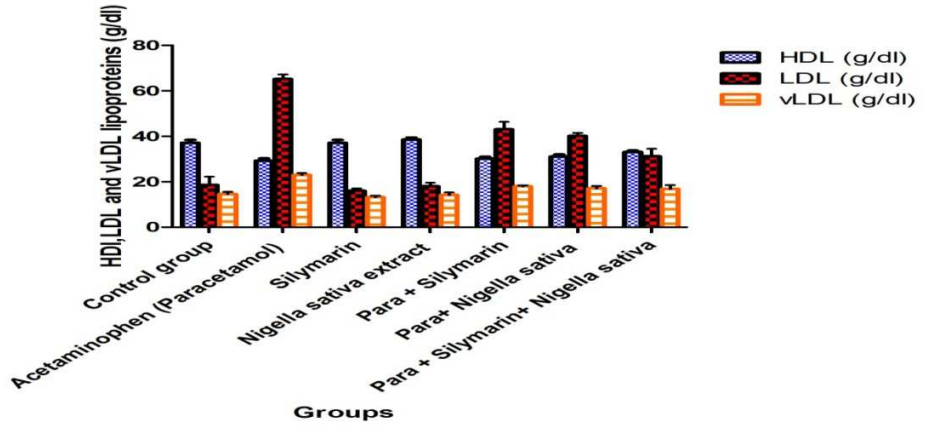

Fig (2): Changes of serum (HDL, LDL and vLDL) levels in male mice treated with silymarin or *Nigella sativa* extract or both of them with paracetamol. Values are expressed as means ± SE; n = 10 for each treatment group

3.2. Oxidative and antioxidant responses

As shown in Table 2 and Fig.3, the data showed that treatment with paracetamol caused a significant decrease in the activity of SOD in Heart tissue homogenates. Administration of silymarin caused non-significant increase in SOD activity in heart tissues as compared with those of control mice. Meanwhile, significant increase was observed in *Nigella sativa* treated group as compared to control group. In addition, a significant recovery relating to SOD was observed in response to the presence of either silymarin or *Nigella sativa* extract or both with paracetamol in the tested tissues. However, co-administration of silymarin or *Nigella sativa* extract with paracetamol increased the SOD activity in tested heart tissues but still better than administration of paracetamol alone.

The CAT activity decreased after paracetamol treatment in the examined tissue (Heart) (Figure.3). The administration of either silymarin or *Nigella sativa* extract non-significantly increased the CAT activity in heart tissues as compared with the control group. The treatment of the mice with silymarin and *Nigella sativa* extract in combination with paracetamol elevated the CAT activity in heart tissues as compared with paracetamol + silymarin or paracetamol + *Nigella sativa* extract.

The activity of GPx was significantly decreased in heart tissues of mice treated with paracetamol by 66.92 % as compared with the control group (Table 2).Treatment of the animals with silymarin caused slight significant decrease in the activity of this enzyme as compared with control group. Meanwhile, *Nigella sativa* extract treated group induced non-significant decrease in GPX activities as compared with normal control group. While the presence of silymarin or *Nigella sativa* extract in combination with paracetamol minimized the observed alterations in the examined enzyme activity induced by paracetamol intoxication in the examined tissues. Thus there was elevation in GPX activity after administration of both silymarin and *Nigella sativa* extract, respectively.

As shown in Table (2), treatment of the mice with paracetamol caused a significant decrease ($p < 0.05$) in the activity of GRX in heart tissues as compared with control animals. Administration of silymarin or *Nigella sativa* extract alone caused non-significant decrease in GRX activity respectively as compared with control mice. In addition, a significant recovery relating to GRX was observed in response to the presence of silymarin and *Nigella sativa* extract in combination with paracetamol which showed amelioration in the enzyme activity as compared with paracetamol treated group only.

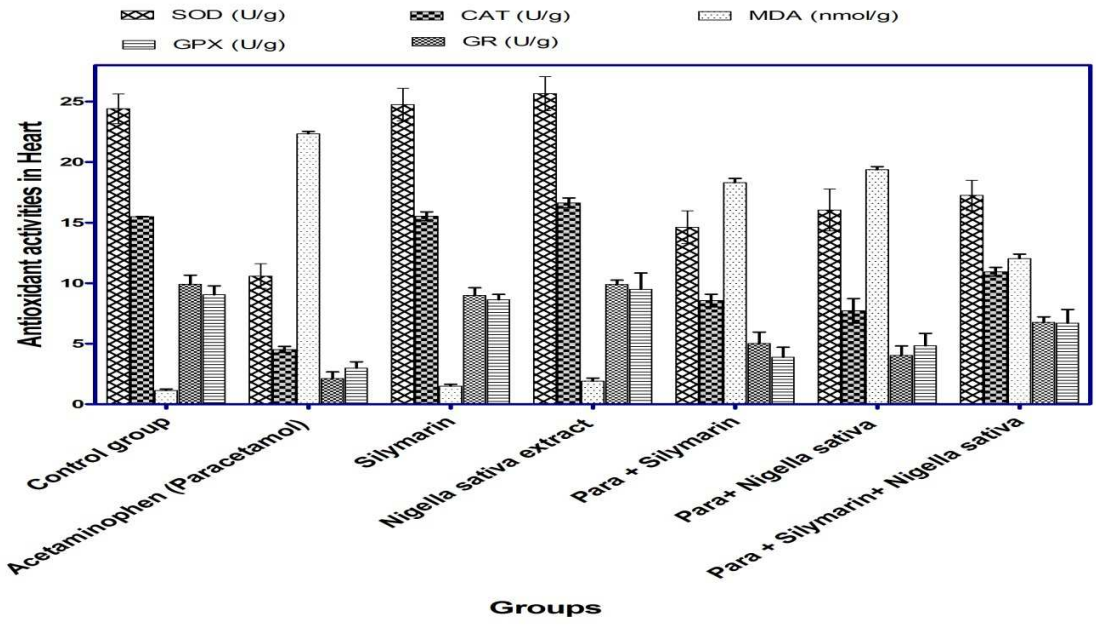

Fig (3): Changes of antioxidant parameters in liver homogenates in male mice treated with Silymarin or *Nigella sativa* extract or both of them with paracetamol. Values are expressed as means ± SE; n = 10 for each treatment group

Table 2: Changes in superoxide dismutase (SOD), Catalase (CAT), Malondialdhyde (MDA) , Glutathione reductase (GR) and Glutathione peroxidase (GPX) activities of (Heart homogenates) in male mice treated with Silymarin, *Nigella sativa* extract and paracetamol separately or in combination

Groups	SOD (U/g)	CAT(U/g)	MDA (nmol/g)	GR (U/g)	GPX (U/g)
Control group	24.41±1.79^{b}	15.49±0.01^{b}	1.14±0.10^{f}	9.91±0.75^{ab}	9.04±0.74^{b}
Acetaminophen (Paracetamol)	10.59±1.22^{f}	4.52±0.03^{f}	22.35±0.26^{a}	2.12±0.48^{f}	2.99±0.51^{g}
Silymarin	24.76±1.51^{b}	15.53±0.03^{b}	1.50±0.66^{g}	8.99±0.58^{b}	8.64±0.44^{c}
Nigella sativa extract	25.66±1.18^{a}	16.63±0.04^{ab}	1.91±0.12^{e}	9.90±0.94^{ab}	9.50±1.34^{ab}
Acetaminophen + Silymarin	14.62±1.22^{e}	8.57±0.04^{d}	18.30±0.25^{c}	5.01±0.82^{d}	3.89±0.82^{f}
Acetaminophen + *Nigella sativa* extract	16.05±1.24^{d}	7.72±0.05^{e}	19.38±0.38^{bc}	4.03±1.56^{e}	4.83±1.00^{e}
Acetaminophen + Silymarin+ *Nigella sativa* extract	17.25±1.03^{cd}	10.95±0.02^{c}	12.04±0.26^{d}	6.77±1.19^{cd}	6.70±1.13^{d}

DISCUSSION

The major aim of this work was to evaluate the potential benefit of silyamrin and *Nigella sativa* extract administration on APAP tissue injury, compare to silymarin or *Nigella sativa* extract treatment alone. To our knowledge, no study has been conducted on the co-effect of silymarin and *Nigella sativa* extract on APAP toxicity at the biochemical level related to lipid profile picture as well as antioxidant parameters in heart homogenates of the mice. This study investigated the ability of silyamrin and *Nigella sativa* extract to alleviate the APAP-induced hyperlipidemia and oxidative stress in mice. Exposure to APAP at the recommended dose to mice led to an alternation of lipogram and antioxidant capacities decrease LDH-c level, increased Triglycerides, Total cholesterol and LDL-c and vLDL-c levels as well as increasing the MDA while decreasing SOD, CAT, GPX and GR.

Effect on lipid profile:

The obtained results revealed that administration of Acetaminophen (paracetamol) in its recommended dose for 30 successive days afforded significant increases in serum triglycerides when compared with control group. Meanwhile, *Nigella sativa* treated group elicited non-significant decrease in triglycerides level when compared with normal control group. The administration of Acetaminophen (paracetamol) afforded significant increase in serum total cholesterol when compared with control group. However, the group treated with combinations of silymarin and *Nigella sativa* afforded significant decrease in total cholesterol level when compared with acetaminophen with either *Nigella sativa* or silymarin and thus recorded the best amelioration value in total cholesterol level. The results revealed that the administration of Acetaminophen (paracetamol) elicited a significant decrease in serum HDL-c when compared with control group. However combination of acetaminophen (paracetamol) with silymarin and *Nigella sativa* extract exhibited a slight decrease when compared with control group. This effect was much better than that produced with acetaminophen (paracetamol) alone.

Acetaminophen (paracetamol) treated group showed a significant increase in serum LDL-c when compared with control group. The combination of acetaminophen (paracetamol) and silymarin and *Nigella sativa* extract as it showed a slight significant increase in LDL-C value as compared to normal control group. It was apparent that treatments of mice with Acetaminophen (paracetamol) elicited a significant increase in serum vLDL-c when compared with control group. The group treated with combination of acetaminophen, silymarin and *Nigella sativa* extract which showed a significant increase compared with control group.

In agreement with the obtained results, *Kanchana and Sadiq*.[25]reported that paracetamol seems to cause impairment in lipoprotein metabolism and also alterations in cholesterol metabolism. The levels of cholesterol and triglyceride were significantly increased in paracetamol treated rats, when compared to control, silymarin and Plumbagozeylanica treated rats.

Similar findings were obtained by *Kanchana and Sadiq*.[25]as they showed elevation of triglycerides level during paracetamol intoxication and this could be due to increased availability of free fatty acids, decreased hepatic release of lipoprotein and increased esterification of free fatty acids.

The decreased level of triglycerides total cholesterol LDL-vLDL-c obtained in the study as a result of treatments with silymarin goes hand in hand with the results of *Metwally et al*.[26]. They reported that administration of silymarin to high cholesterol fed rats afforded a significant decrease in serum levels of total lipids, triglycerides, total cholesterol, LDL-c and VLDL-c as well as HDL-c.

Silymarin is a flavonoid found in the herb milk thistle *Silybum marianum* (L.). *Anderson et al.* [27] reported that flavonoids led to an inhibition of cholesterol synthesis, cellular cholesterol esterification, triglycerides and phospholipids.

A similar result was recorded by *Labhal et al.* [28], they showed that *Nigella sativa* petroleum ether extract significantly lowered fasting plasma levels of insulin and triglycerides and normalized HDL–cholesterol in normal and diabetic animal models. Our results were in accordance with the previous authors, they reported that *Nigella sativa* seeds decrease the serum total lipids and body weight in obsess rat.

Furthermore, our results were reinforced with *Helal,* [29] who reported that *Nigella sativa* seed have a significant lowering effect on total cholesterol level and LDL cholesterol level.

Le et al. [30] reported that since *Nigella sativa* reduced the total cholesterol level, there is a probable decrease in intracellular cholesterol level which causes an up-regulation of LDL– receptor. Their results suggest that *Nigella sativa* has a protective role in atherosclerosis due to its hypolipidemic activity. The authors added that treatment of rats with *Nigella sativa* petroleum extract for 4 weeks afforded lowered triglycerides and increased HDL-cholesterol. Nearly similar results were previously reported by *Helal,* [29]. They studied thymoquinone (Active ingredient of *N. sativa* seeds) on Doxorubicin-induced hyperlipidemic nephrophathy in rats. They found that thymoquinone afforded a significant lowering of triglycerides and total cholesterol.

The obtained results were in full agreement with *El-Dakhakhny et al.* [31] they reported that when *N. sativa* was administered in a dose of 800mg/kg of rats for 4 weeks elicited a significant decrease in serum total cholesterol, LDL-c, triglycerides and a significant elevation in serum HDL-c level.

The obtained findings were compatible also with *Skottova et al.* [32] they reported that silymarin induced a decrease of plasma cholesterol, LDL-c, VLDL-c and increase in HDL-c. These changes are considered to be of benefit in pharmacological treatment of hypercholesterolemia and the removal of LDL by the liver represents one from the most important mechanisms regulating the level of plasma LDL *Goldstein and Brown,* [33].

The LDL activity of normal mice treated with Acetaminophen (paracetamol) for 30 successive days showed a significant elevation when compared with normal control group along the entire period of the experiment. The increased triglycerides, total cholesterol were strongly supported in the previous mentioned studies. They found marked increase of serum triglycerides, cholesterol and LDL-cholesterol of abnormal lipid profile known as dyslipidemia in Acetaminophen (paracetamol) and this may be the main cause of increase risk of cardiovascular disease as evidenced by atherosclerosis and increased body weight which is characterized by low HDL-c, raised triglycerides and a predominance of small, dense LDL-c particles and increase in free fatty acids FFA. The hepatic over production of triglycerides is probably a consequence of increased flux of glucose and free fatty acids.

The increased serum levels of cholesterol in Acetaminophen treated mice observed in the present study is compatible with the observations of *Fujimura et al*. [34]. They attributed this increase in serum cholesterol level due to the increase in the hepatic concentration of acetyl-Co-A used for cholesterol synthesis, which comes from increased oxidation of long chain fatty acids and increased oxidation of ketogenic amino-acids.

Effect on antioxidant capacities:

The obtained results revealed that administration of paracetamol induced significant decrease in SOD, CAT, GR and GPX activities and elicited a significant increase in MDA level and there is ameliorative role of either *Nigella sativa* extract or silymarin in improving the antioxidant capacities of male mice.

The obtained findings are reinforced by *Yousef et al*.[35]They reported that paracetamol treatment caused a significant elevation in TBARS levels with simultaneous inhibition in the activities of antioxidant enzymes; GST, GPx, SOD and CAT in rat plasma, liver, kidney, brain, lung, heart and testis. Furthermore, it decreased GSH content significantly in rat liver, kidney and lung. These features might be attributed to the metabolic activation of paracetamol, which is considered a major mechanism of its toxicity.

In accordance with the present results, It was found that paracetamol trigger a rapid loss of GSH and lipid peroxidation in both liver *Jaeschke et al*.[36] and kidney *Newton et al*.[37]. The basic mechanism of paracetamol

toxicity in the liver is the covalent binding of N-acetyl p-benzoquinone imine (NAPQI), the reactive metabolite of paracetamol, to sulfhydryl groups of GSH and various proteins and their subsequent oxidation *Lee et al*.[38].

On the other hand, several mechanisms were suggested as probable pathways for paracetamol-mediated nephrotoxicity. These included the oxidative metabolism to NAPQI similar to that in the liver, deacetylation to p-aminophenol and further oxidation to an aminophenoxy radical and benzoquinone imine, and also involved are the hepatically-derived metabolites from paracetamol- GSH conjugates *Trumper*., [39].

The elevation in TBARS level is an indicator of lipid peroxidation, which has been suggested to be closely related to paracetamol- induced tissue damage *Sener et al.[*40]and this finding go hand in hand with our obtained results. It has been proven that hydrogen peroxide and superoxide anion are produced during metabolic activation of paracetamol in the CYP450 system *Dai and Cederbaum.* [41]and from mitochondria during paracetamol intoxication *Knight et al*. [42]. It has been further suggested that the generation of ROS appears as an early event which precedes intracellular GSH depletion and cell damage in paracetamol hepatotoxicity.

The superoxide formation may promote peroxynitrite generation and protein nitration that may further result into oxidative damage to proteins, DNA and lipids *Abdel-Zaher et al*. [43].In addition, both paracetamol and NAPQI can interact with mitochondria, thereby inducing depletion of mitochondrial GSH content, decline in ATP content, and uncoupling of the mitochondrial respiratory chain combined with electron leakage *Donnelly et al.* [44] and this explanation is greatly supported by our finding.

Glutathione is a ubiquitous tripeptide present in all cell types in millimolar concentrations. The major roles of GSH are to maintain the intracellular redox balance and to eliminate xenobiotics and reactive oxygen species (ROS) *Myhrstad et al.* [45]

The obtained results are completely in agreement with *Halliwell,* [46] who showed that they had chosen the liver, kidney and lung to estimate the changes in GSH because they possess a high content of this protein. According to the decline in hepatic, renal and lung GSH content, it was evident that paracetamol-induced toxicity involved a change in cellular redox status toward a state of oxidative stress. A wide variety of oxidizing molecules such as ROS and/or depleting agents can alter glutathione redox state, which is normally maintained by the activity of GSH-depleting (GPx ,GST) and GSH-replenishing (GR) enzymes. Therefore, it can be assumed that the decrease in GSH concentration might cause the effectiveness of GST and GPx activity to be restricted, as evident by the intensification of lipid peroxidation.

The fall in catalase activity which proven in our results indicated a decrease in the antioxidative capacity as well. It has been shown that the decreased activity of SODs may be attributed to the consumption of these enzymes in ROS detoxification and also its increasing is due to increased lipid peroxidation. It is also known that antioxidant enzymes can be inactivated by lipid peroxides and ROS *Halliwell and Gutteridge* [47]. Superoxide dismutase is inhibited by hydrogen peroxide, while GPx and catalase are inhibited by an excess of superoxide radical *Pigeolet et al*.[48] on the same ground as indicated in our study.

Thus in conclusion lipid peroxidation is supposed to cause the destruction and damage to cell membranes, leading to changes in membrane permeability and fluidity and enhancing the protein degradation in mice *El-Megharbel et al.* [49] In the present study, the levels of LPO were increased, indicating an increase in the generation of free radicals in the paracetamol treated group and this level was decreased in other groups treated with silymarin and *Nigella sativa extract*.

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