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Silymarin and *Nigella sativa* extract ameliorate paracetamol induced oxidative stress and renal dysfunction in male mice

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ABSTRACT

Objective: To evaluate the ameliorative role of silymarin or/and *Nigella sativa* (*N. sativa*) water extract against N-acetyl-p-aminophenol (APAP)-induced renal function deterioration in male mice at the biochemical levels.
Methods: The mice were divided into seven groups (10/group). The first group was served as control. The second group was treated with dose of APAP. The third and fourth groups were treated with silymarin alone and *N. sativa* water extract alone, respectively. The fifth and sixth groups were treated with combination of APAP with silymarin and APAP with *N. sativa* water extract, respectively. The seventh group was treated with a combination of both ameliorative compounds (silymarin and *N. sativa* water extract) with APAP and all animals were treated for a period of 30 days.
Results: Exposure to APAP at the treated dose for mice led to an alteration of kidney function parameters, increase in the level of serum urea and creatinine. Also, paracetamol administration induced oxidative stress in kidney homogenates by increasing malondialdhyde level and decreasing superoxide dismutase and catalase activities and this stress was ameliorated by administration of either silymarin or *N. sativa* water extract.
Conclusions: Administration of silymarin or/and *N. sativa* water extract to APAP-treated mice alleviate the toxicity of APAP, and this appeared clearly by biochemical improvement of kidney function parameters and antioxidant parameters. But, the alleviation is more pronounced with the both antioxidants. Thus, the pronounce effect of silymarin and *N. sativa* water extract is most effective in reducing the toxicity induced by APAP and improving the kidney function parameters and antioxidant status of kidney of male mice.

1. Introduction

Paracetamol is an extensively used analgesic and antipyretic drug and, thought safe when used at therapeutic doses, is associated with significant hepatotoxicity when taken in overdose[1]. Under normal conditions, paracetamol is primarily metabolized in the liver by glucuronidation and sulfation.

Paracetamol, widely applied as an analgesic and antipyretic, produces acute liver damage at higher dose. The hepatotoxicity of paracetamol has been ascribed to the formation of n-acetyl parabenzoquineimine (NAPQI) which causes oxidative stress and glutathione depletion[2]. It is a well-known antipyretic agent which produces hepatic necrosis at higher doses[3].

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Nigella sativa L. (*N. sativa*) is a member of family Ranunculaceae. It is an annual herbaceous plant growing in the Mediterranean countries and is likewise cultivated in the north of Morocco[4].

N. sativa (Ranunculaceae), commonly known as black cumin, is an erect herbaceous annual plant. *N. Sativa* seeds have been used in Middle Eastern folk medicine as a natural remedy for various diseases as well as a spice for over 2000 years. The seeds of *N. sativa* have been subjected to a range of pharmacological, phytochemical and nutritional investigations in recent years[5]. It has been shown to contain more than 30% (w/w) of a fixed oil with 85% of total unsaturated fatty acid[6].

Thymoquinone is a phytochemical compound found in the plant *N. sativa*. It has antioxidant effects and can protect heart, liver and kidney damage in animal subjects as well as has possible anticancer effects[7].

Silybum marianum (milk thistle) is a plant of the Asteraceae family. This fairly typical thistle has red to purple flowers and shiny pale green leaves with white veins. Originally a native of Southern Europe through to Asia, it is now found throughout the world. The

medicinal parts of the plant are the ripe seeds, as other flavonoids has been shown to inhibit p-glycoprotein-mediated cellular efflux[8].

Silymarin contains a bit of active constituents called flavolignans which is also used to assist in protecting the liver from poisoning[9]. Silymarin treatment in type II diabetic patients has a beneficial effect on improving the glycemic profile[10].

Therefore, the primary aim of the study is to assess the possible ameliorative role of either silymarin or *N. sativa* extract in alleviating the oxidative stress and renal function impairment induced by paracetamol drug.

2. Material and methods

2.1. Chemicals

APAP (N-acetyl-p-aminophenol) was purchased from the Egyptian International Pharmaceutical Industries Company. Silymarin was obtained from Sedeco Pharmaceutical Co-6-October city, Egypt. The *N. 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 grinded. Whole *N. sativa* seeds were dissolved in distilled water for 24 h and filtered through muslin[12]. After 24 h, the aqueous extract was filtered, concentrated at room temperature then the dried extract was stored at 4 ˚C until use[13]. Other chemicals and reagents were of the highest analytical grade and were bought from standard commercial suppliers in Roche, Germany.

2.2. Animals

ICR male mice, weighing approximately 35-40 g, were provided by Faculty of Veterinary Medicine, Zagazig University. The animals were maintained in the solid bottom shoe box, type 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 admittance to a pellet diet and tap water for a week before using in 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 follows. Groups 1 was served as untreated control (1 mL/kg of physiological saline). Group 2 was treated with paracetamol (2 g/kg[14]. Group 3 was treated with silymarin 50 mg/kg[15]. Group 4 was treated with *N. sativa* extract 300 mg/kg[11]. Group 5 was treated with paracetamol and silymarin respectively. Group 6 was treated with paracetamol and *N. sativa* and the final group was treated with paracetamol followed by silymarin and *N. sativa* respectively. All the groups were treated orally for 30 consecutive days.

2.3. Collection and handling of blood samples

At the final stage of the 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 3 000 r/min for 15 min and serum was collected for different biochemical analyses.

2.4. Renal function determination

The levels of uric acid and creatinine in serum were estimated using commercial diagnostic kits according to the manufacturer's instructions. The data were expressed as mg/dL.

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

The tissues of kidney were used for the analysis of oxidative stress and antioxidant parameters. Prior to dissection, tissues were perfused with 50 mmol/L [sodium phosphate buffer saline (100 mmol/L Na_2HPO_4/NaH_2 PO_4] (pH 7.4) and 0.1 m ethylene diamine tetra acetic acid 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 kept up in a deep freezer until used. The supernatant was applied for the purpose of some biochemical parameters of kidney tissues.

2.6. Lipid peroxidation assay

The extent of lipid peroxidation (LPO) was estimated as the concentration of thiobarbituric acid reactive product malondialdhyde (MDA) by using the method of Ohkawa *et al.*[17]. MDA concentrations were determined using 1, 1, 3,3-tetraethoxypropane as standard and expressed as micromoles per gram of tissue.

2.7. Antioxidant enzymes

Superoxide dismutase (SOD) activity was measured according to the method described by Marklund and Marklund, in which pyrogallol underwent autoxidation at 440 nm for 3 min[18]. Single unit of SOD activity was estimated as the quantity of protein that caused 50% pyrogallol autoxidation inhibition. The SOD activity was expressed as units per g tissue. Before determination of the catalase (CAT) activity, samples were diluted 1:9 to 1% Triton X-100 (v/v). CAT activity was evaluated according to the method described by Aebi[19]. The hydrolysis of H_2O_2 and the ensuing decrease in absorbance at 240 nm over a 3 min period at 25 ˚C was measured. CAT activity was expressed as units per g tissue.

2.8. Statistical analysis

Statistical analysis was done using SPSS for Windows version 17.0. Data were given in the form of arithmetical mean ± SE. Differences between groups were evaluated by One-way ANOVA according to $P < 0.05$ and *post-hoc* Duncan test.

3. Results

3.1. Renal functions assessments

The urea level increased significantly in paracetamol treated group,

but significantly decreased by 16.57 and 17.36 fold in the groups treated with a combination of the silymarin and acetaminophen and also combination of *N. sativa* extract with acetaminophen when compared with control group (Table 1). Meanwhile combination between (paracetamol, silymarin and *N. sativa* extract) have ameliorated urea level as compared with control group and recorded the best ameliorative result. Serum creatinine level of *N. sativa* extract groups was non-significantly decreased when compared with the control group. In the paracetamol-treated group, the creatinine level increased by 56.32 fold increase when compared to control. Treatment of the mice with 1 000 mg/kg paracetamol with the combination of silymarin and *N. sativa* extract in combination with paracetamol decreased the activity of creatinine more than each compound separately.

Table 1

Changes of kidney function parameters in male mice.

Groups	Urea (mg/dL)	Creatinine (mg/dL)
Control group	20.31 ± 1.50^{e}	0.38 ± 0.01^{fg}
Acetaminophen (paracetamol)	41.40 ± 3.08^{a}	0.87 ± 0.02^{a}
Silymarin	21.20 ± 1.88^{de}	0.39 ± 0.02^{ef}
N. sativa extract	20.10 ± 1.51^{e}	0.37 ± 0.02^{g}
Acetaminophen + silymarin	34.54 ± 0.70^{b}	0.71 ± 0.01^{bc}
Acetaminophen + *N. sativa* extract	34.10 ± 2.77^{b}	0.65 ± 0.01^{c}
Acetaminophen + silymarin + *N. sativa* extract	28.08 ± 2.10^{c}	0.55 ± 0.01^{d}

Means within the same column in each category carrying different litters are significant ($P \leqslant 0.05$) using Duncan's multiple range test, where the highest mean value is symbol (a).

3.2. Oxidative and antioxidant responses

As indicated in Table 2 and Figure 1, the data indicated that treatment with paracetamol caused a substantial reduction in the activity of SOD in kidney tissue homogenates. Administration of silymarin caused non-significant increase in SOD activity in kidney tissues as compared with those of control mice. Meanwhile, a significant reduction was observed in *N. 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 *N. sativa* extract or both with paracetamol in the tested tissues. However, co-administration of silymarin or *N. sativa* extract with paracetamol increased the SOD activity in tested kidney tissues but still better than administration of paracetamol alone.

Table 2

Changes in SOD, CAT and MDA activities of kidney homogenates in male mice.

Groups	SOD (IU/g)	CAT (IU/g)	MDA (nmol/g)
Control group	23.32 ± 0.87^{ab}	5.49 ± 0.05^{ab}	8.67 ± 0.02^{f}
Acetaminophen (paracetamol)	9.61 ± 1.52^{g}	1.31 ± 0.04^{e}	38.68 ± 0.42^{a}
Silymarin	22.14 ± 2.98^{b}	5.28 ± 0.07^{b}	8.60 ± 0.18^{f}
N. sativa extract	18.82 ± 0.91^{d}	5.11 ± 0.01^{b}	9.42 ± 0.01^{e}
Acetaminophen + silymarin	14.94 ± 1.66^{e}	4.51 ± 0.07^{d}	14.79 ± 0.03^{c}
Acetaminophen + *N. sativa* extract	12.92 ± 1.59^{f}	4.47 ± 0.07^{d}	18.27 ± 0.15^{b}
Acetaminophe + silymarin + *N. sativa* extract	19.91 ± 0.88^{cd}	4.94 ± 0.08^{c}	10.59 ± 0.05^{d}

Means within the same column in each category carrying different litters are significant at ($P \leqslant 0.05$) using Duncan's multiple range test, where the highest mean value is symbol (a).

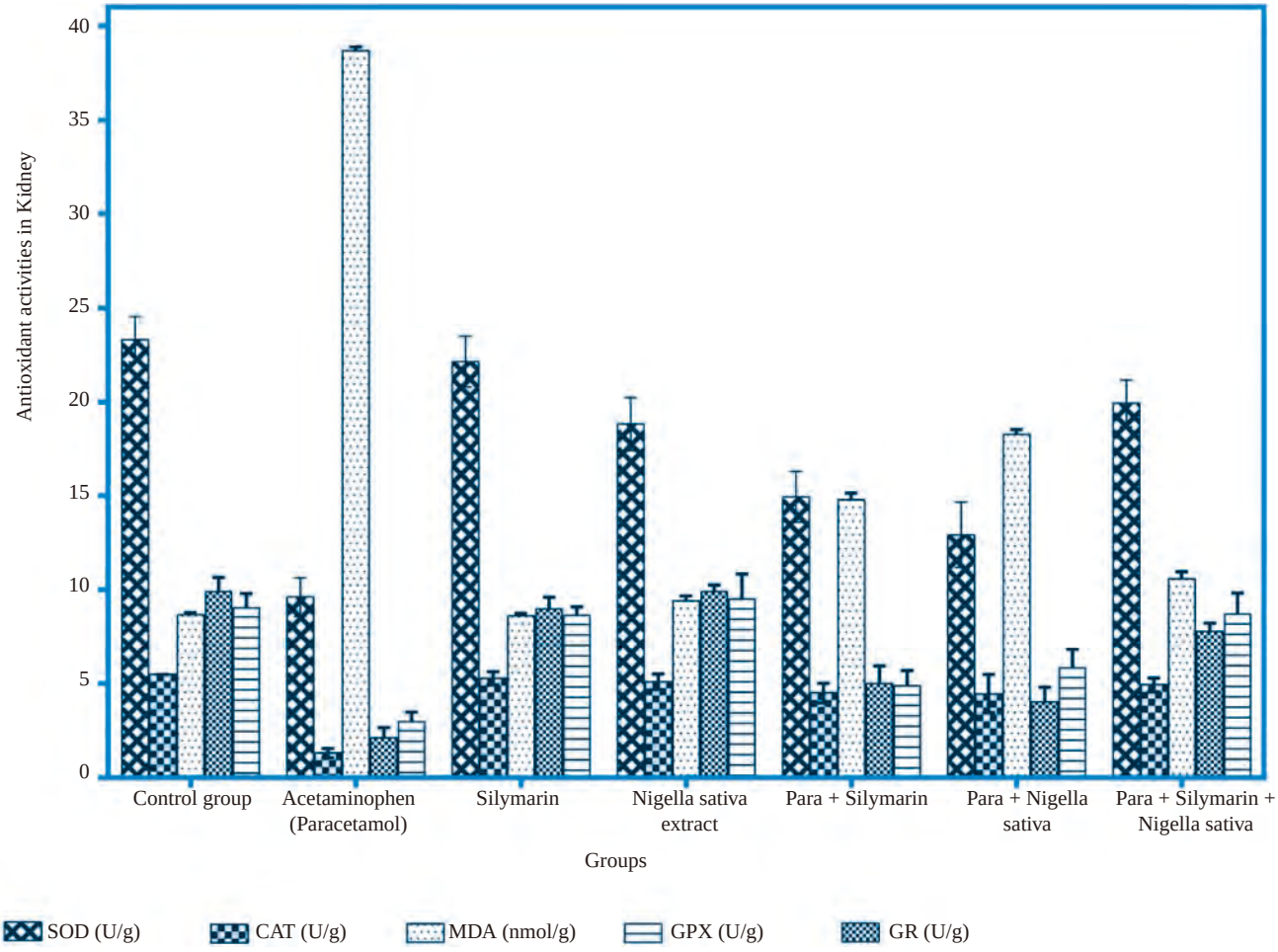

Figure 1. Changes of antioxidant parameters in kidney homogenates in male mice.
GPX: Glutathione peroxidase; GR: Glutathione reductase.

The CAT activity decreased after paracetamol treatment in the examined tissue (kidney) (Figure 1). The administration of either silymarin or *N. sativa* extract non-significantly decreased the CAT activity in kidney tissues as compared with the control group. The treatment of the mice with silymarin and *N. sativa* extract in combination with paracetamol elevated the CAT activity in kidney tissues as compared with paracetamol + silymarin or paracetamol + *N. sativa* extract.

MDA level was significantly increased in the paracetamol treated group as compared with the normal control group. On the other hand, silymarin treated group elicited non-significant decrease in MDA as compared with the normal control group, while *N. sativa* extract treated group afforded slight significant increase in MDA activity as compared with the normal control group. At the meantime, administration of *N. sativa* extract in combination with silymarin has greatly ameliorated MDA level as compared with paracetamol treated group only.

4. Discussion

The major objective of this study was to assess the possible benefit of silyamrin and *N. sativa* extract administration on APAP tissue injury, compare to silymarin or *N. sativa* extract treatment alone. To our knowledge, no survey has been borne along the co-effect of silymain and *N. sativa* extract on APAP toxicity at the biochemical level related to kidney function parameters as well as antioxidant parameters in kidney homogenates. This study investigated the ability of silyamrin and *N. sativa* extract to alleviate the APAP-induced renal function impairments and oxidative stress in mice. Exposure to APAP at their recommended dose to mice led to an alteration of renal parameters and antioxidant capacities decrease SOD and CAT levels, increased MDA, urea levels as well as increasing the creatinine.

Bearing on the effects of the test plant drugs on the kidney function parameters, our results revealed that serum urea and creatinine were significantly elevated in serum of acetaminophen treated mice compared to the control group. Our results coincide with Jones *et al*.[20].

Our results were reinforced by the results of Yousef *et al*.[21] who also revealed a significant renal impairment in animals treated with paracetamol demonstrated by the increase in plasma urea and creatinine. In renal diseases, urea accumulates because the rate of serum urea production exceeds the rate of clearance, while the elevation in plasma creatinine is observed only with marked damage to functioning nephrons.

At doses within the therapeutic range, paracetamol has been found to affect renal function by lowering renal blood flow, glomerular filtration rate, sodium excretion and prostaglandin E2 excretion in both human and rat[22].

This is defined as ingestion of an analgesic which leads to renal papillary necrosis and chronic interstitial nephritis with progressive renal failure[23].

Yousef *et al*. reported that paracetamol treatment did not produce gross renal histological changes[21]. These results coincided with those obtained by Ahmed *et al*.[24]. who observed that the ingestion of paracetamol (500 mg/kg/day) N-acetyl-p-benzoquinone imine for 30 days did not produce papillary necrosis nor interstitial nephritis. However, it reduces the urinary concentrating ability of the animals[24].

Creatinine, a more reliable renal function marker, is a breakdown product of creatine phosphate in muscle metabolism at a constant rate. Increased creatinine concentration reflects the reduced glomerular filtration rate. An indication of renal dysfunction which is greatly reinforced our results as the paracetamol treated group showed an elevation in creatinine level and this also reflects the ameliorative role of *N. sativa* and silymarin in reducing this dysfunction by decreasing creatinine level in their treated groups[25].

On the same basis, urea, a major nitrogenous end product of protein/amino acid catabolism, also acts as a renal function marker as its elevation indicates impaired glomerular filtration and this also coincides with our results.

In correspondence with our results, Peng *et al*. showed that paracetamol-induced defects on glomerulus, pronephric tube and pronephric duct could be easily and dynamically observed[26].

Similarly, Sonnenbichler *et al*. showed that isosilibin and silidianin and components of silymarin could stimulate proliferation as well as protein and DNA synthesis in kidney cells[27]. Silymarin increases the renal activity of antioxidant enzymes, and also restores renal morphology, suggesting that silymarin may have therapeutic potential for the treatment of nephropathy.

Our results go hand in hand with Dollah *et al*. as they showed that there was a significant reduction in serum creatinine in the rats treated with high dose *N. sativa*[28].

Our results coincide with Dollah *et al*. and they indicated that the supplementation of *N. sativa* to the diets of rats for five weeks did not change the biochemical parameters of kidney function as well as histopathological investigations which illustrated normal architecture of the kidney[28]. It's proved by the presence of no significant change of serum urea of all treatment groups and creatinine level in low and normal doses groups compared with the control group.

In conformity with our findings, it was previously demonstrated that oral administration of aqueous extract of *N. sativa* seeds showed no significant changes in kidney function Ali and Blundes[29]. Another study also failed to show any toxicity for *N. sativa* fixed oil in mice.

Our study showed that oral administration of *N. sativa* has no toxicity. These results are in agreement with previous data reporting that *N. sativa* has a wide margin of safety. With the evidence of normal urea and creatinine level in blood and normal kidney tissue in histology examination for all handling groups. It is proposed that there is no toxic effect on kidney function of *N. sativa* at different doses for five-week period.

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

Our results are in harmony with Hamza *et al*. as they revealed that the mean level of LPO was increased significantly associated with a reduction in antioxidant defense systems that protect tissues against oxidative damage through a decrease in GSH,

SOD and CAT levels in $CdCl_2$ treated group[30]. In spite of that, there was a significant decrease of LPO values in Cd + TQ (active component of *N. sativa*) treated group when compared with $CdCl_2$ treated group.

Similar results obtained by Helal *et al*. who found that the administration of TQ led to decreased MDA levels in different toxicity studies[31]. They also reported that increase in LPO levels and histological damage caused by *Escherichia coli* which improved markedly with TQ treatment through its restored antioxidant defense against the generation of reactive oxygen species (ROS) in different tissues.

Fouda *et al*. who reported that orally administered TQ (active component of *N. sativa*) increased the activities of CAT and GSH-Px enzymes in rats[32]. So in agreement with our results, Sayed *et al*. showed that the rats treated with TQ and l-cysteine revealed improvement of oxidants/antioxidant mechanism[30].

Helal *et al*. attributed these effects of TQ to its anti-inflammatory, antioxidant and antiapoptotic properties[31]. Previous study Fouda *et al*. showed that TQ and l-cysteine are potent antioxidative compounds, and because of this property, it strongly protects tissues against the oxidative stress and this coincides with our obtained results[32].

The obtained findings are reinforced by Yousef *et al*.[21]. They reported that paracetamol treatment caused a significant elevation in thiobarbituric acid reactive substance levels by 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 and kidney Newton *et al*.[33]. The basic mechanism of paracetamol toxicity in the liver is the covalent binding of N-acetyl-p-benzo-quinone imine, the reactive metabolite of paracetamol, to sulfhydryl groups of GSH and various proteins and their subsequent oxidation Lee *et al*.[34].

On the other hand, several mechanisms were suggested as probable pathways for paracetamol-mediated nephrotoxicity. These included the oxidative metabolism to N-acetyl-p-benzo-quinone imine 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[35].

The elevation in thiobarbituric acid reactive substance level is an indicator of lipid peroxidation, which has been suggested to be closely related to paracetamol-induced tissue damage El-Megharbel *et al*. and this finding go hand in hand with our obtained results[36]. It has been proven that hydrogen peroxide and superoxide anion are produced during metabolic activation of paracetamol in the CYP450 system and from mitochondria during paracetamol intoxication. 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[37]. In addition, both paracetamol and N-acetyl-p-benzo-quinone imine 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 and this explanation is greatly confirmed by our finding[38].

The fall in catalase activity which proven in our results indicated a reduction in the antioxidative capacity as easily. It has been proven that the diminished activity of SODs may be ascribed to the use of these enzymes in ROS detoxification and also its increasing is due to increased lipid peroxidation. It is likewise known that antioxidant enzymes can be inactivated by lipid peroxides and ROS[39]. Superoxide dismutase is inhibited by hydrogen peroxide, while GPx and catalase are inhibited by an excess of superoxide radical on the same ground as indicated in our study[40].

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[41]. 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 *N. sativa* extract.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Scialli AR, Ang R, Breitmeyer J, Royal MA. A review of the literature on the effects of acetaminophen on pregnancy outcome. *Reprod Toxicol* 2010; **30**(4): 495-507.

[2] Shah VN, Deval K. Hepatoprotective activity of leaves of *Parkinsonia aculeata* Linn against paracetamol induced hepatotoxicity in rats. *Int J Pharm* 2011; **1**(2): 59-66.

[3] Hurkadale PJ, Shelar PA, Palled SG, Mandavkar YD, Khedkar AS. Hepatoprotective activity of Amorphophallus paeoniifolius tubers against paracetamol-induced liver damage in rats. *Asian Pac J Trop Biomed* 2012; **2**: S238-42.

[4] Saad SI. *Classification of flowering plants*. 2nd ed. Alexandriak: The general Egyptian Book Co.; 1995, p. 412-3.

[5] Al-Nazawi MH, El-Bahr SM. Hypolipidemic and hypocholestrolemic effect of medicinal plant combination in the diet of rats: black cumin seed (*Nigella sativa*) and *Turmeric* (curcumin). *J Anim Vet Adv* 2012; **11**: 2013-9.

[6] Houghton PJ, Zarka R, de las Heras B, Hoult JR. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Planta Med* 1995; **61**: 33-6.

[7] Effenberger-Neidnicht K, Breyer S, Mahal K, Diestel R, Sasse F, Schobert R. Cellular localisation of antitumoral 6-alkyl thymoquinones revealed by an alkyne-azide click reaction and the streptavidin-biotin system. *Chembiochem* 2011; **12**(8): 1237-41.

[8] Zhou SF, Lim LY, Chowaby B. Herbal modulation of P-glycoprotein. *Drug Metab Rev* 2004; **36**(1): 57-104.

[9] John Wiley & Sons, Inc. Herbal medicine silymarin may help sugar-control in people with type II diabetes. New York: John Wiley & Sons, Inc; 2006. [Online] Available from <http://www.sciencedaily.com/releases/2006/10/061030071127.htm> [Accessed on 15th April, 2015]

[10] Diab AEA, Zahra MH, Hendawy AA, Samih IE, Hamza R. Possible ameliorative role of some compounds on the side effects of avandia (drug). *Biosci Biotechnol Res Asia* 2010; **7**: 139-51.

[11] Schleicher P, Saleh M, Peter S, Mohamed S. *Black cumin: the magical Egyptian herb for allergies, asthma and immune disorders*. Rochester: Healing Arts Press; 2000, p. 90.

[12] el Daly ES. Protective effect of cysteine and vitamin E, *Crocus sativus* and *Nigella sativa* extracts on cisplatin-induced toxicity in rats. *J Pharm Belg* 1998; **53**: 87-93.

[13] Benhaddou-Andaloussi A, Martineau LC, Spoor D, Vuong T, Leduc C, Joly E, et al. Antidiabetic activity of *Nigella sativa* seed extract in cultured pancreatic β-cella. Skeletal muscle cells and adipocytes. *Pharm Biol* 2008; **46**: 96-104 .

[14] Chen X, Sun CK, Han GZ, Peng JY, Li Y, Liu YX, et al. Protective effect of tea polyphenols against paracetamol-induced hepatotoxicity in mice is significantly correlated with cytochrome P450 suppression. *World J Gastroenterol* 2009; **15**(15): 1829-35.

[15] Toklu HZ, Tunali-Akbay T, Erkanli G, Yüksel M, Ercan F, Sener G. Silymarin, the antioxidant component of *Silybum marianum*, protects against burn-induced oxidative skin injury. *Burns* 2007; **33**: 908-16 .

[16] Boussarie D. [Hematology of company rodents and lagomorphs]. French. *Bull Acad Vet Fr* 1999; **72**: 209-16.

[17] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; **95**: 351-8.

[18] Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* 1974; **47**: 469-74.

[19] Aebi H. Catalase *in vitro*. *Methods Enzymol* 1984; **105**: 121-6.

[20] Jones R, Mann T, Sherins R. Peroxidative breakdown of phospholipids in human spermatozoa, spermicidal properties of fatty acid peroxides, and protective action of seminal plasma. *Fertil Steril* 1979; **31**: 531-7.

[21] Yousef MI, Omar SA, El-Guendi MI, Abdelmegid LA. Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat. *Food Chem Toxicol* 2010; **48**: 3246-61.

[22] Trumper L, Monasterolo LA, Elias MM. Probenecid protects against *in vivo* acetaminophen-induced nephrotoxicity in male Wistar rats. *J Pharmacol Exp Ther* 1998; **284**: 606-10.

[23] Henrich WL. Analgesic nephropathy. *Trans Am Clin Climatol Assoc* 1998; **109**: 147-58.

[24] Ahmed MH, Balment RJ, Ashton N. Renal action of acute chloroquine and paracetamol administration in the anesthetized, fluid-balanced rat. *J Pharm Exp Ther* 2003; **306**(2): 478-83.

[25] Ismail HAA, Hamza RZ, El-Shenawy NS. Potential protective effects of blackberry and quercetin on sodium fluoride-inducedimpaired hepato-renal biomarkers, sex hormones and hematotoxicity in male rats. *J Appl Life Sci Int* 2014; **1**(1): 1-16.

[26] Peng HC, Wang YH, Wen CC, Wang WH, Cheng CC, Chen YH. Nephrotoxicity assessments of acetaminophen during zebrafish embryogenesis. *Comp Biochem Physiol C Toxicol Pharmacol* 2010; **151**: 480-6.

[27] Sonnenbichler J, Scalera F, Sonnenbichler I, Weyhenmeyer R. Stimulatory effects of silibinin and silicristin from the milk thistle *Silybum marianum* on kidney cells. *J Pharmacol Exp Ther* 1999; **290**: 1375-83.

[28] Dollah MA, Parhizkar S, Izwan M. Effect of *Nigella sativa* on the kidney function in rats. *Avicenna J Phytomed* 2013; **3**(2): 152-8.

[29] Ali BH, Blunden G. Pharmacological and toxicological properties of *Nigella sativa*. *Phytother Res* 2003; **17**: 299-305.

[30] Hamza RZ, Al-Harbi MS. Monosodium glutamate induced testicular toxicity and the possible ameliorative role of vitamin E or selenium in male rats. *Toxicol Rep* 2014; **1**: 1037-45.

[31] Helal GK. Thymoquinone supplementation ameliorates acute endotoxemia-induced liver dysfunction in rats. *Pak J Pharm Sci* 2010; **23**: 131-7.

[32] Fouda AM, Daba MH, Dahab GM, Sharaf El-Din OA. Thymoquinone ameliorates renal oxidative damage and proliferative response induced by mercuric chloride in rats. *Basic Clin Pharmacol Toxicol* 2008; **103**: 109-18.

[33] Newton JF, Hoefle D, Gemborys MW, Mudge GH, Hook JB. Metabolism and excretion of a glutathione conjugate of acetaminophen in the isolated perfused rat kidney. *J Pharmacol Exp Ther* 1986; **237**: 519-24.

[34] Lee DG, Kim HK, Park Y, Park SC, Woo ER, Jeong HG, et al. Gram-positive bacteria specific properties of silybin derived from *Silybum marianum*. *Arch Pharm Res* 2003; **26**(8): 597-600.

[35] Al-Harbi MS, Hamza RZ, Al-Sofiani TA. Ameliorative roles of silymarin and *Nigella sativa* on hematological parameters and immunological capacities of male mice affected by paracetamol. *Biosci Biotechnol Res Asia* 2015; **12**(1): 379-85.

[36] El-Megharbel SM, Hamza RZ, Refat MS. Synthesis, chemical identification, antioxidant capacities and immunological evaluation studies of a novel silver(I) carbocysteine complex. *Chem Biol Interact* 2014; **220**: 169-80.

[37] Al-Harbi MS, Hamza RZ, Dwary AA. Ameliorative effect of selenium and curcumin on sodium fluoride induced hepatotoxicity and oxidative stress in male mice. *J Chem Pharm Res* 2014; **6**: 984-98.

[38] Al-Harbi MS, Hamza RZ, Dwari AA. Sodium fluoride induced antioxidant defense impairment and impaired renal biomarkers and the ameliorative role of selenium and curcumin in male mice. *Asian Pac J Trop Dis* 2014; **4**: S990-7.

[39] Hamza RZ, El-Shenawy NS, Ismail HA. Protective effects of blackberry and quercetin on sodium fluoride-induced oxidative stress and histological changes in the hepatic, renal, testis and brain tissue of male rat. *J Basic Clin Physiol Pharmacol* 2015; **26**(3): 237-51.

[40] El-Megharbel SM, Hamza RZ, Refat MS. Synthesis, spectroscopic and thermal studies of Mg(II), Ca(II), Sr(II) and Ba(II) diclofenac sodium complexes as anti-inflammatory drug and their protective effects on renal functions impairment and oxidative stress. *Spectrochim Acta A Mol Biomol Spectrosc* 2014; **135C**: 915-28.

[41] El-Megharbel SM, Hamza RZ, Refat MS. Preparation, spectroscopic, thermal, antihepatotoxicity, hematological parameters and liver antioxidant capacity characterizations of Cd(II), Hg(II), and Pb(II) mononuclear complexes of paracetamol anti-inflammatory drug. *Spectrochim Acta A Mol Biomol Spectrosc* 2014; **131**: 534-44.