Hepato-nephroprotective Role of *Lepidium sativum* against Oxidative Stress Induced by Dexamethasone in Rats

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Abstract

The current research was prepared to explore the hepato-nephroprotective effect of *Lepidium sativum* seed aquatic extract against oxidative stress stimulated by dexamethasone in rats. Animals were classified as follows; group one was used as control while groups 2, 3 and 4 were treated orally with *Lepidium sativum* seed extract (LSSE; 20 mg/kg, daily), interperitoneally with dexamethasone (DEX; 1 mg/kg BW) and LSSE plus DEX for 14 days, respectively. Administration of DEX elevated thiobarbituric acid reactive substances (TBARS), hydrogen peroxide (H_2O_2), and kidney and liver function biomarkers level, and lactate dehydrogenase activity. While enzymatic (SOD, CAT, GPx, GR, GST) and non-enzymatic (GSH) antioxidants, protein content, and alkaline phosphatase activity were significantly decreased. Otherwise, rats supplemented with LSSE singly declined lipid peroxidation and improved most of the studied parameters. Moreover, rats pretreated with LSSE then received DEX showed significant alleviation in lipid peroxidation, antioxidant status and biochemical indices with respect to DEX treated group. Conclusively, LSSE has beneficial protective effects and has the capability to counteract the harmful influence of DEX. So, *Lepidium sativum* might represent a novel approach in the therapy of dexamethasone because of its antioxidant properties.

Keywords: *Dexamethasone; Lepidium sativum; oxidative stress; antioxidant enzymes; liver and kidney dysfunction.*

Introduction

Glucocorticoids (GCs), the primary stress hormones secreted by adrenal glands, are essential for life. Several synthetic glucocorticoids such as dexamethasone, prednisone, and betamethasone are more potent than natural cortisol. They are involved in the treatment of disturbances induced by an overactive immune system as allergies, asthma, autoimmune diseases and sepsis [1]. Additionally, GCs are involved in several diseases including obesity, metabolic syndrome, hypertension, and depression [2]. GCs have several side effects including the activation of the mineralocorticoid receptors leading to the growth of renal injury. However, dexamethasone that given in vivo can also induce albuminuria and promote also renal injury [3]. Furthermore, high GCs concentrations result in overproduction of reactive oxygen species (ROS) leading to alteration in mitochondrial permeability, cellular energy and calcium levels followed by apoptosis [4]. The over production of ROS in dexamethasone therapy is predominantly due to increased NADPH oxidase activity (Zhang et al., 2004) [5]. Previous study showed that dexamethasone treatment prompted gluconeogenesis, increased insulin resistance in addition to lipid disturbances [6]. Reactive oxygen species are ordinarily created in a regulated manner in human and animals. Nevertheless, xenobiotics can provoke oxidative stress and consequently hurt the cellular macromolecules . Essentially, the cellular defense mechanism versus ROS toxicity involved non-enzymatic (glutathione (GSH), vitamin E, ascorbic acid, β-carotene, and uric acid) and enzymatic antioxidants (superoxide dismutase, catalase, selenium-dependent glutathione peroxidase, selenium-independent glutathione peroxidase, alkyl hydroperoxide reductase, glutathione S-transferase and glutathione reductase) [7].

Great interest is directed to many plants because of their antioxidant and anti-inflammatory potential, among them *Lepidium sativum* Linn which belongs to

Cruciferae family. *Lepidium sativum* is known as "El-Rshad" is an edible herb with aroma flavor. In traditional medicine, different parts of this plant have been adopted for several diseases therapy (jaundice, liver problems, spleen diseases, gastrointestinal disorders, menstrual problems, arthritis, asthma, headache, nasal polyps, breast cancer, and other inflammatory conditions) [8,9]. *Lepidium sativum* seeds essential oils contains fatty oils, protein, carbohydrate, vitamins, fatty acids, flavonoids, and isothiocynates glycoside [10]. Interestingly, experimental evidences showed that *Lepidium* sativum has multiple useful properties as antihypertensive, diuretic [38], anti-asthmatic [37], hypoglycaemic, antioxidant [10] and anti-inflammatory [39]. Therefore, the existent study was prepared to evaluate the hepato-renal protective effect of *Lepidium sativum* seed aqueous extract against oxidative stress and biochemical perturbations induced by dexamethasone in rats.

Materials and Methods

Materials

Lepidium sativum seeds (LSS) were collected from local market of Baghdad, Iraq and stored in air tight containers. Dexamethasone phosphate (DEX ≥ 98%, was bought from Sigma Chemical Company. All other chemicals were of analytical grade.

Lepidium sativum seed extract preparation

Lepidium sativum seed aqueous extract was prepared by boiling 1 g of dry seeds powder in 100 ml of distilled water for 10 min and left for 15 min to soak then chilled and filtered. The obtained filtrate was lyophilized and the required dose was prepared and reconstituted in 10 ml of distilled water per kilogram bodyweight prior oral administration directly.

Experimental design

Twenty-eight male Wister rats (150–170 g) were bought from the Faculty of Medicine, Alexandria University, Alexandria, Egypt. The protocol was approved by the local University Committee in conformity with the ethics and guidelines of the National Institutes of Health. Rats were distributed in cages seven per each and kept on commercial diet and water *ad libitum* and acclimated (temperature, 21°C; photoperiod, 7 a.m. to 7 p.m.) for two weeks. Animals were classified into four groups: group 1 used as the control, while group 2 was treated orally with *Lepidium sativum* seed aqueous extract (LSSE; 20 mg/kg), group 3 was injected interperitoneally with dexamethasone (DEX; 1 mg/Kg) dissolved in saline and the fourth group received LSSE one hour before DEX treatment, respectively. *Lipidium sativum* and dexamethasone were administered daily for 14 days according to [19]. At the experiment termination, rats were anesthetized using isoflurane, and then killed via cervical dislocation and livers and kidneys were immediately removed.

Blood and tissue samples

Blood samples were assembled for serum preparation and permitted to stand for 30 min for blood clotting at 25°C then centrifuged at 3000 ×g for 15 min. Serum of each sample was taken and stored at −20°C till utilized in the determination of biochemical parameters. Livers and kidneys were taken away and homogenized in ice-cold 0.01 mol/l sodium-potassium phosphate with 1.15% KCl buffer (pH 7.4) and centrifuged at 10,000 g (4°C) for 20 min then the supernatants were taken and utilized for the determination of different assays.

Determination of TBARS, H_2O_2 and glutathione content

Thiobarbituric acid-reactive substances (TBARS), hydrogen peroxide (H_2O_2), and reduced glutathione (GSH) content were measured in liver and kidney homogenates using the methods of Ohkawa et al. (1979), Velikova et al. (2000), and Ellman (1959), respectively.

Determination of antioxidant enzyme activities

The activities of superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6) and glutathione S-transferase (GST; EC 2.5.1.18) were assessed by the methods of Misra and Fridovich (1972), Aebi (1984) and Habig et al. (1974), respectively. While the activities of glutathione peroxidase (GPx; EC 1.11.1.9) and glutathione reductase (GR; EC 1.6.4.2) were evaluated according to Hafeman et al. (1974).

Determination of biochemical parameters

Alanine aminotransferase (ALT; EC 2.6.1.2) and aspartate aminotransferase (AST; EC 2.6.1.1) activities were assayed using kits from SENTINEL CH. (via

principle Eugenio 5-20155 MILAN-ITALY). Lactate dehydrogenase (LDH; EC 1.1.1.27) and alkaline phosphatase (ALP; EC 3.1.3.1) activities, protein content, and total bilirubin were estimated by the methods of Cabaud and Wroblewski (1958), Principato et al. (1985), Bradford (1976) and Walters and Gerade (1970), respectively. Urea and creatinine concentrations were measured according to Patton and Crouch (1977) and Henry et al., (1974), respectively.

Statistical analysis

Data from different groups were represented as means ± standard errors (SEM) then analyzed utilizing SPSS software (version 22, IBM Co., Armonk, NY). Comparison between groups was done through one-way ANOVA followed by Tukey's post-hoc test. *P* value ≤ 0.05 was approved to be significant.

Results

General health

None of the dexamethasone treated rats showed signs of morbidity or mortality during the study.

Lipid peroxidation and reduced glutathione

The present results revealed significant ($P< 0.05$) increase in TBARS and H_2O_2 levels, the indicators of lipid peroxidation, in liver and kidney homogenate after DEX treatment versus control while rats pretreated with LSSE then treated with DEX presented a significant reduction in TBARS and H_2O_2 levels as compared to DEX- treated rats. On the other hand, GSH content was significantly decreased in DEX -treated rats. While rats treated with both LSSE and DEX, induction in GSH content was observed as compared with DEX-treated rats. Supplementation with LSSE alone reduced the concentrations of TBARS and H_2O_2 and induced GSH content in liver (Table 1) and kidney (Figure 1) homogenates.

Antioxidant enzymes

A significant reduction ($P< 0.05$) in SOD, CAT, GPx, GR, and GST activities was observed in liver (Table 2) and kidney (Figure 2) homogenates of DEX- treated rats. Furthermore, rats taken LSSE + DEX showed significant alleviation in the activities of antioxidant enzymes as compared to DEX - treated ones ($P<0.05$). Moreover, LSSE supplementation alone improved antioxidant enzyme activities significantly versus the control group.

Liver and kidney function biomarkers

Data showed that AST, ALT, and ALP activities and protein content decreased while LDH activity as well as serum urea, creatinine and total bilirubin increased significantly ($P < 0.05$) in rats received DEX as compared to control. Moreover, a significant modulation in enzyme activities and protein content in rats received LSSE then treated with DEX versus DEX group was observed. LSSE supplementation alone had insignificant alteration on the enzymes and protein contents in (Table 3 and 4).

Table 1. Effect of *Lipidium sativum seed* extract (LSSE), dexamethasone (DEX) and their combination on the level of thiobarbituric acid reactive substances, hydrogen peroxide and reduced glutathione content in rat liver

Parameters	Groups			
	Cont.	LSSE	DEX	LSSE + DEX
TBARS (nmol/g tissue)	36.74±0.951c	29.60±0.814d	54.23±1.42a	44.43±1.71b
H2O2 (µmol/g tissue)	64.56±2.05c	51.80±1.86d	90.48±4.03a	76.40±2.71b
GSH (mmol/mg protein)	2.19±0.071b	2.61±0.083a	1.17±0.038d	1.65±0.043c

Values are expressed as means ± SE; n=7 for each treatment group. [abcd]Mean values within a row not sharing common superscript letters were significantly different, $p< 0.05$. Statistically significant variations are compared as follows: LSSE and DEX groups are compared to control group while LSSE + DEX group is compared to DEX group.

Table 2. Effect of *Lipidium sativum seed* extract (LSSE), dexamethasone (DEX) and their combination on the activities of antioxidant enzymes in rat liver

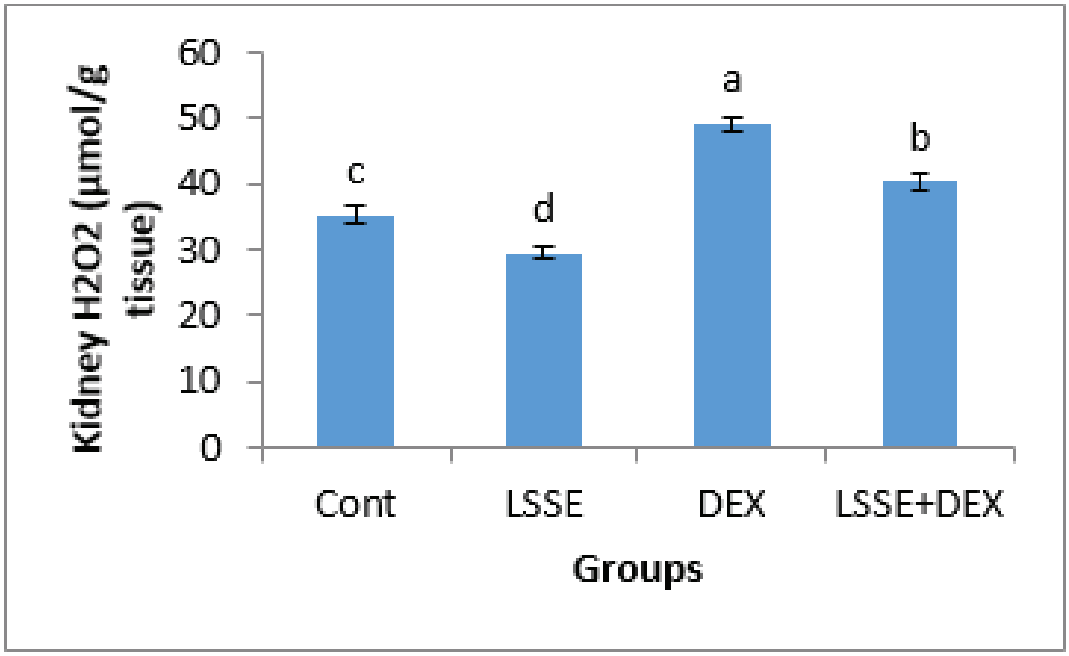
Parameters	Groups			
	Cont.	LSSE	DEX	LSSE + DEX
SOD (U/mg protein)	70.59±1.89b	84.48±2.46a	36.99±1.19d	54.72±1.45c
CAT (µmol/hr/mg protein)	41.57±1.38b	49.29±1.15a	22.73±0.966d	32.71±1.08c
GPx (U/mg protein)	1.19±0.040b	1.42±0.052a	0.688±0.026d	0.954±0.026c
GR (U/mg protein)	1.31±0.041b	1.56±0.059a	0.717±0.023d	1.038±0.027c
GST (µmol/hr/mg protein)	0.984±0.033b	1.17±0.041a	0.565±0.021d	0.802±0.020c

Values are expressed as means ± SE; n=7 for each treatment group. [abcd]Mean values within a row not sharing common superscript letters were significantly different, $p< 0.05$. Statistically significant variations are compared as follows: LSSE and DEX groups are compared to control group while LSSE + DEX group is compared to DEX group.

Table 3. Effect of *Lipidium sativum seed* extract (LSSE), dexamethasone (DEX) and their combination on the enzyme activities and protein content in rat liver

Parameters	Groups			
	Cont.	LSSE	DEX	LSSE + DEX
Liver AST (U/mg protein)	88.94±2.45a	92.28±2.04a	52.30±1.68c	73.20±1.89b
ALT (U/mg protein)	119±4.09a	115±4.12a	71.49±2.59c	95.22±1.32b
LDH (U/mg protein)	659±20.62c	630±20.30c	919±17.79a	793±20.35b
ALP (U/mg protein)	360±9.07a	381±10.17a	216±7.00c	288±5.94b
protein (mg/g tissue)	171±4.81a	176±3.28a	103±5.07c	146±5.94b
Serum Total bilirubin (mg/dl)	0.727±0.015c	0.703±0.021c	0.966±0.036a	0.817±0.82b

Values are expressed as means ± SE; n=7 for each treatment group. [abcd]Mean values within a row not sharing common superscript letters were significantly different, $p< 0.05$. Statistically significant variations are compared as follows: LSSE and DEX groups are compared to control group while LSSE + DEX group is compared to DEX group.



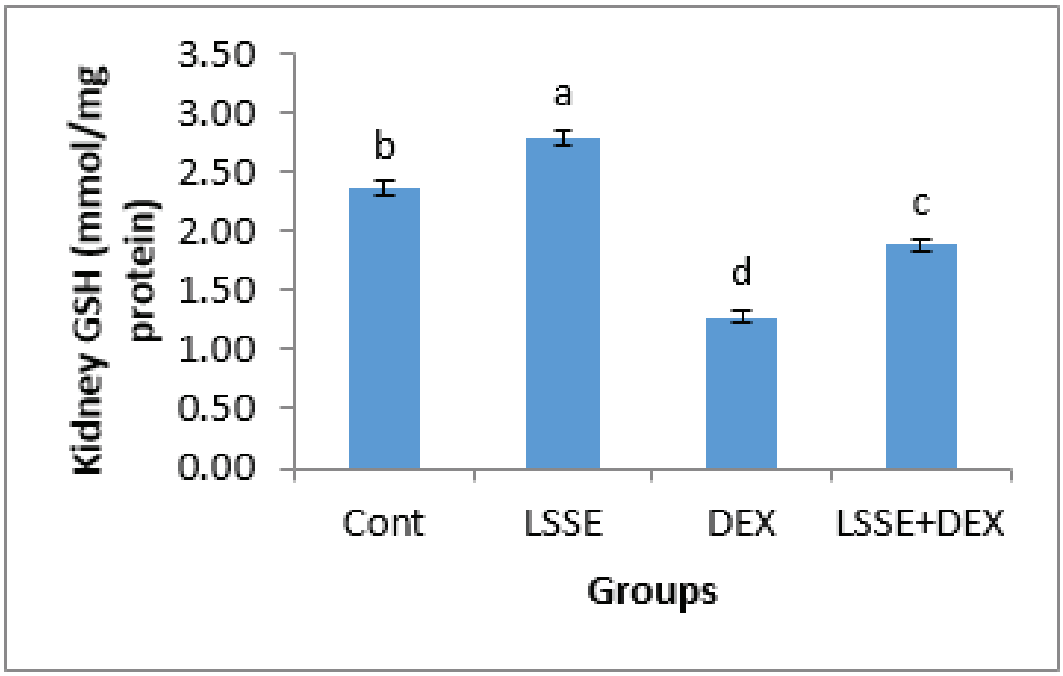
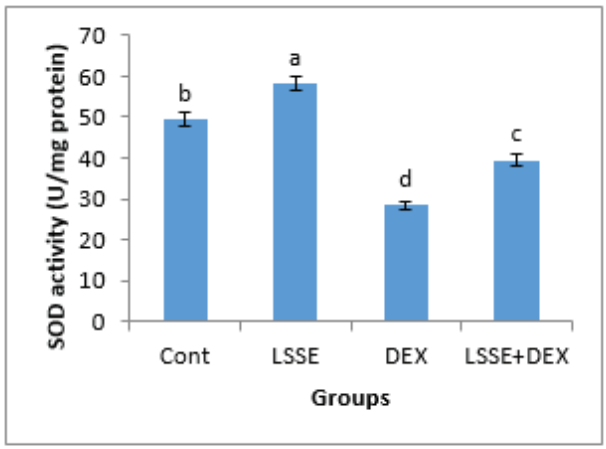
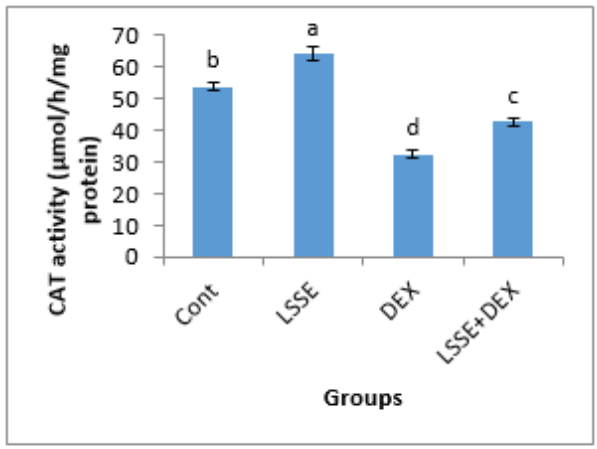
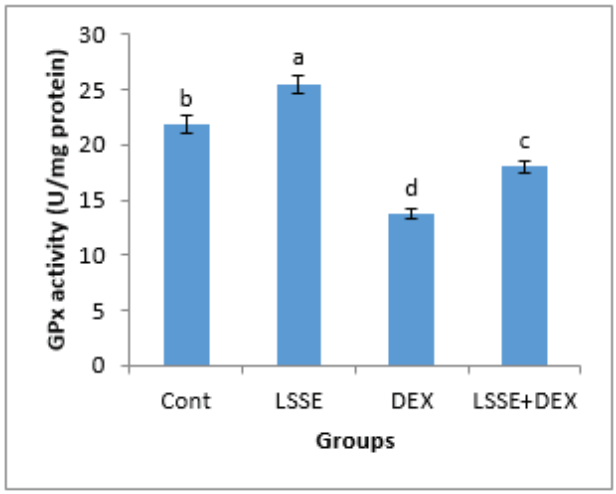
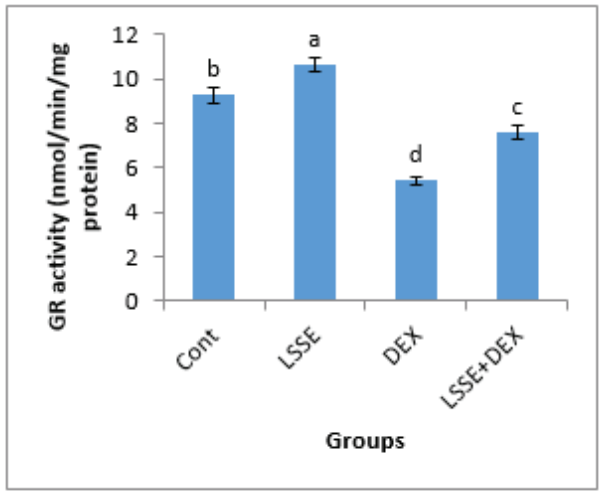


Figure 1. Effect of *Lipidium sativum seed* extract (LSSE), dexamethasone (DEX) and their combination on the level of thiobarbituric acid reactive substances (TBARS), hydrogen peroxide (H_2O_2) and reduced glutathione (GST) content in rat kidney. Values are expressed as means ± SE; n=7 for each treatment group. Mean values with different letters were significantly different, $p< 0.05$.









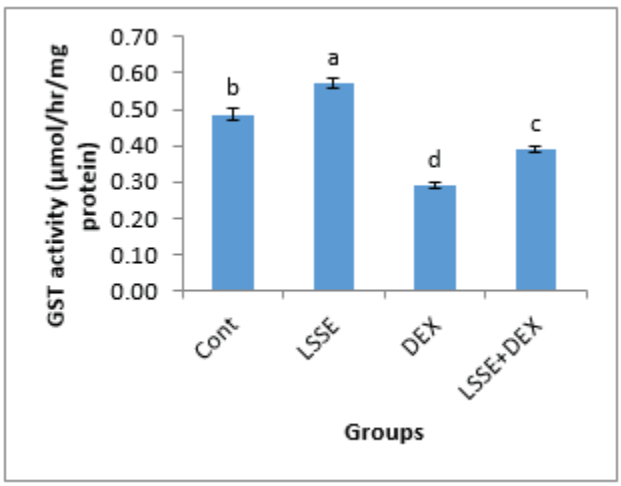


Figure 2. Effect of *Lipidium sativum seed* extract (LSSE), dexamethasone (DEX) and their combination on the antioxidant enzymes activity in rat kidney. Values are expressed as means ± SE; n=7 for each treatment group. Mean values with different letters were significantly different, $p< 0.05$.

Table 4. Effect of *Lipidium sativum seed* extract (LSSE), dexamethasone (DEX) and their combination on the concentration of urea and creatinine, enzyme activities and protein content in rats.

Parameters	Groups			
	Cont.	LSSE	DEX	LSSE + DEX
Serum Urea (mg/dl)	38.49±0.949c	36.46±1.09c	53.23±1.99a	46.33±1.81b
Creatinine (mg/dl)	0.699±0.025c	0.655±0.024c	0.972±0.036a	0.814±0.023b
Kidney LDH (U/mg protein)	617±22.82c	595±13.57c	827±15.43a	739±20.41b
ALP (U/mg protein)	183±6.27a	190±5.56a	115±2.58c	149±4.29b
Protein (mg/g tissue)	60.04±2.05a	63.54±1.26a	39.14±0.918c	50.44±2.10b

Values are expressed as means ± SE; n=7 for each treatment group. [abcd]Mean values within a row not sharing common superscript letters were significantly different, $p< 0.05$. Statistically significant variations are compared as follows: LSSE and DEX groups are compared to control group while LSSE + DEX group is compared to DEX group.

Discussion

In the current investigation, the protective role of LSSE against DEX - induced oxidative injury and biochemical perturbations was studied. To our knowledge there are no findings have been pointed out the efficiency of LSSE as natural products for overcoming DEX harmful effect. Dexamethasone is known to disrupt oxidants/antioxidant balance in tissues, leading to various biochemical and physiological dysfunctions. Rats treated with DEX exhibited an imbalanced oxidant/antioxidant status as apparent in TBARS and H_2O_2 elevation accompanied by depletion in enzymatic (SOD, CAT, GPx, GR, GST) and non-enzymatic antioxidants (GSH) in the liver and kidney homogenates indicating the failure of antioxidant defense system to overcome the flow of ROS. The current results are in consistent with Kiersztan et al. (2017) [5] and Jatwa and Kar (2010) who demonstrate the generation of hydroxyl free radicals and lipid peroxidation in the kidney of rabbits and mice treated with dexamethasone, respectively. Moreover, several authors showed that dexamethasone induced ROS generation in different tissues and cells via the mitochondrial electron transport chain, vascular endothelial xanthine oxidase and NADPH oxidase (Almeida et al., 2011; Feng and Tang, 2014; Assaf et al., 2012). So, this study showed that administration of DEX impaired antioxidant defense system since it has the capability to induce oxidative stress.

Antioxidant enzymes are so important in the preservation of homeostasis for normal cell function in addition they are used as indicators of oxidative stress (Gutteridge, 1995). Superoxide dismutase, as part of the defense system versus oxidative hurt in aerobic organisms, catalyzes superoxide anion (O_2-) to O_2 and H_2O_2, which then is reduced to H_2O by H_2O_2-scavenging enzyme, catalase. The decrease in both SOD and CAT activities might be related to inhibition of enzyme protein synthesis [5]. Glutathione plays a crucial role in cellular protection versus xenobiotics toxicity because of its thiol group (Halliwell and Gutteridge, 2007). Glutathione acts as a reducing non-enzymatic antioxidant and as a substrate for GPx and GST antioxidant enzymes (Cossu et al., 1997). GPx preserves the lipid-cellular membrane from oxidative injury (Kantola et al., 1988) and spurs hydroperoxide reaction with reduced glutathione to form disulphide glutathione (GSSG) (Kalaiselvi et al.,

2013). While, glutathione S-transferases (GSTs), play a critical role in the detoxification process of xenobiotic to non-toxic products, protecting against electrophiles and oxidative stress (Ghosh et al., 2012). Moreover, inhibition of GST activity in the liver and kidney of DEX -treated rats might repress the elimination of cellular free radicals leading to renal damage displayed by elevation of lipid peroxidation and creatinine (Kiersztan et al., 2017). DEX may affect the synthesis of GSH through the inhibition of glutathione-synthase and glucose 6-phosphate dehydrogenase activities. Additionally, DEX retards the diversion of oxidized glutathione (GSSG) into its reduced form (GSH) via GR inhibition (Yeh et al., 2005). In consistence with the present results, GCs may also indirectly participate in the induction of oxidative stress through inhibiting antioxidant enzymes activity [5]. So, antioxidant enzymes, which prohibit the chain reaction of free radicals, are so important in alleviating DEX hurtful effect in liver and kidney tissues.

The induction in kidney function biomarkers (urea and creatinine) in DEX -treated rats reflected the renal dysfunction. This may be attributed to the metabolic impairment in liver function, as urea is the end-product of protein breakdown. While, high creatinine concentration is related to muscle creatine catabolism that leads to kidney damage (El-Demerdash and Nasr, 2014). Also, DEX may contributes to disturbances in kidney functions, resulting in development of albuminuria which is considered as an early indicator of renal damage or inflammation (Xu et al, 2010).

Xenobiotics are transformed in the liver into less harmful products leading to hepatocytes damage. In the current study, rats received DEX showed significant variations in AST, ALT, ALP and LDH activities as well as total bilirubin and protein levels. These parameters are important biomarkers for hepatocellular damage (Chen et al. 2019) and its alterations pointed out hepatocytes damage that altered the transport function and membrane permeability as well as leakage of enzymes from the cells to the bloodstream indicating hepatotoxicity (Gokcimen et al., 2007; Chen et al. 2019; Albasher et al., 2020). Also, lipid peroxidation has a fundamental role in the disruption of hepatocellular membrane integrity, leading to the leakage of cytoplasmic enzymes and this confirmed the possible mechanism of oxidative stress in liver lesion induced by DEX (Bhadauria, 2012). Lactate dehydrogenase is known as a potent marker in the toxicity assessment of many xenobiotics. The observed induction in LDH activity in DEX- treated rats may be attributed to cellular decay that leads to impairment in carbohydrate and protein metabolism in addition to energy depletion (Sivakumari et al., 1997). Alkaline phosphatase is an important membrane-bound enzyme used as a biomarker for xenobiotics toxicity and critical enzyme in the biological processes. It is responsible for detoxification, metabolism, and biosynthesis of macromolecules that are required for many biological functions and its inhibition in organs could be attributed to tissue necrosis that leads to seepage of the enzyme into the bloodstream (Yarbrough et al. 1982). The elevation in total bilirubin may be related to diminished liver uptake, conjugation, or prolonged bilirubin output from hemolysis (El-Demerdash, 2004). Protein is an essential cellular component susceptible to damage by free radicals and its depression might be because of exaggerated leakage via nephrosis (Chatterjea and Shinde 2002). Additionally, the decrease in protein may be related to disturbance in protein anabolic and catabolic processes.

Our results inferred that LSSE pre-supplementation significantly repaired the liver and kidney biomarkers induced by DEX confirming its guarding potential and its important role in preventing oxidative stress related liver damage due to its anti-inflammatory effect. Protective and therapeutic use of ethanolic LSSE in rat's renal failure reduced urea and creatinine concentration significantly indicating excess of glomerular filtration rate. Also, administration of aqueous LSSE for 3 weeks exhibited antihypertensive and diuretic activities and suppress free radical attack in rats [10,43]. So, this study is a successful attempt that introduces *Lipidium sativum* as a wonderful antioxidant for mitigation of DEX- induced oxidative stress in rats due to its antioxidant properties.

Coclusion

In conclusion, the present results pointed out that dexamethasone has the potency to cause hepatorenal dysfunction via oxidative injury, alterations in antioxidant defense system, enzyme activities, and biochemical parameters. Furthermore, *Lepidium sativum* administration in combination with dexamethasone

attenuates its harmful effect by quenching and chelating free radicals. So, *Lepidium sativum* had a powerful antioxidant role in alleviating dexamethasone side effects by potentiating antioxidant defense system status and depressing free radicals' generation.

Competing of Interest: The author reports no conflict of interest.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Misan University and all experiments were carried out in accordance with approved guidelines.

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