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Modulating impacts of coustus Sassura lappa extract against oxidative stress and genotoxicity induced by deltamethrin toxicity in rat kidneys

***Najla O. Ayaz*[1*]**

[1]*Biochemistry Department, Faculty of Science-Al Faisaliah, King Abdulaziz University, Jeddah, Saudi Arabia.*
[*]*Corresponding authors: Najla O. Ayaz*
Biochemistry Department, Faculty of Science-Al Faisaliah, King Abdulaziz University, Jeddah, Saudi Arabia.
E-mail, nayaz@kau.edu.sa

ABSTRACT

The objective of the current investigation was to explore the reno- protective impact of 70% methanolic extract of Saussura lappa (coustus) against oxidative stress and oxidative DNA fragmentation induced renal damage in rat models in response to chronic exposure to the pesticide, deltamethrin. Deltamethrin was administered orally to rats at two doses (1/15 LD50 and 1/30 LD50) daily for 28 days. The results illustrated that oral intake of S lappa extract (300mg/Kg b.w) for 28 consecutive days to animals ingested by either of the two doses, significantly modulated the elevation in plasma malondialdehyde (MDA) and protein carbonyl as well as the decreases in plasma antioxidants markers, including protein thiol, glutathione S transferase (GST), catalase (CAT) and, superoxide dismutase (SOD) compared with control animal group. The plant extract could also reverse the inhibitory effect of deltamethrin on plasma ATPase in animals intoxicated by either of the two doses. In addition, the plant extract successfully modulated the oxidative DNA damage in kidneys of deltamethrin intoxicated rat groups. The beneficial renoprotective impact of the S lappa extract against deltamethrin toxicity was reflected by improvement in plasma renal function markers (urea and creatinine) and histomorphological pictures of renal tissues.
***Conclusion**: The current investigation may suggest that early administration of S lappa extract may protect renal tissue from oxidative damage promoted by the toxic effects of deltamethrin pesticide.*
***Key words:** Saussura lappa, deltamethrin, renal tissues, DNA damage*

INTRODUCTION

Natural extracts of plant origin have been investigated recently worldwide because of their multi-functions properties. Many of these plants have therapeutic effects against different diseases and have a protective effect against the hazards of chemical pollutants (1). *Saussurea lappa* (*S lappa*), commonly known as costus, family Asteraceae, is one of the medicinal plants which contains many active constitutes with therapeutic activities. The active principles of this plant include terpenes (sesquiterpenes, costunoloids and dehydrocostunoloids), anthraquinones, alkaloids and flavonoids (2). The plant also contains mokko lactone, betulinic acid and betulinic acid methyl ester (3). These compounds have antitumor, anti-inflammatory, antifungal, antidiabetic, antiulcer, antimicrobial and antihepatotoxic effects (4-5).

Insecticides constitute the real wellspring of potential environmental risks for people (6). Exposure to these products for an extended period causes numerous disorders in the body and reduces the life expectancy of organisms (7-8). It was reported that three million cases of pesticide toxicities happen every year and more than 250,000 deaths all over the world had been found (9).

Pyrethroids are highly active insecticides with a low toxicity and an easy biodegradability with respect to organochlorine and organophosphorus pesticides (10). In the last decade, the use of such pesticides has increased several-fold as compared to organochlorine insecticides, due to their low mammalian toxicity and limited remaining in the soil (11). Deltamethrin [((s)-a-cyano-3-phenoxybenzyl(R1-R2)-3-(2,2 dibromvinyl)-2,2dimethylcyclo-propancarboxylate] is a synthetic pyrethroid which is widely used as insecticide and acaricides (10). Propagation of pyrethroids applications induced many adverse health problems on human and environment (12). The toxicity of pyrethroids on different biological organs and glands has been documented (13). Overgeneration of reactive free species, stimulation of lipid peroxidation, and changes in antioxidant ability of the vital organs, including liver and kidney, are mechanisms of pyrethroids toxicity (14). Induction of oxidative stress and propagation of free radical production can cause serious effects on different cell components, including carbohydrates, lipids, proteins, and nucleic acid (15).

To the best of our knowledge, there are no in vivo experimental studies on the antioxidant impact of *S. lappa* extracts, consequently, measuring some oxidative stress and antioxidant markers as well as oxidative DNA damage can be used as interesting markers for therapeutic ability of S *lappa* extract against deltamethrin toxicity in rats.

The aim of the current work was to explore the potential impact of 70% methanolic extract of *S lappa* (coustus) against oxidative stress and oxidative DNA damage induced renal damage in rats under the effect of deltamethrin exposure.

MATERIALS AND METHODS

Insecticide

Deltamethrin insecticide, (formulated as agrodelta 25 EC/ULV contain 2.5%., deltamethrin) was purchased from the local market, Jeddah, KSA.

Preparation of *S lappa* extract

The *S lappa* roots were obtained from the herbal market in Jeddah, KSA. 100 g of the plant roots were crushed and then extracted using 70% methanolic solution. The supernatant of the root extract was separated by filtration and then evaporated using a rotary evaporator at 45°C. The semi solid paste of the plant extract was dissolved in a distilled water before administration.

Animals and experimental design

Thirty male Wistar rats (150-180g) were utilized for this investigation. The rats were gotten from King Fahd research center, King Abdulaziz University, Jeddah, KSA. The animals were kept under standard conditions of lighting, moistness (50-60%) and temperature (23-25°C), Rats were given a pellet chow with standard constituents and tap water *adlibitum*. The animals were stayed in these conditions for a week before beginning the experiment. Rats were then divided into six groups, each of five animals as follows,

Group I (control group), Rats were orally given bi-distilled water (1ml/ rat)

Group II (CT), Animals were orally supplemented *S lappa* extract, (16).

Group III (DH), Rats were ingested a high dose of deltamethrin (1/15 LD50, 4mg/ Kg b.w).

Group IV (CTDH), Rats were orally given *S lappa* extract one hour prior administration of the high dose of deltamethrin (1/15 LD50).

Group V (DL), Rats were ingested a low dose of deltamethrin (1/30 LD50, 2mg/ kg bw).

Group VI (CTDL), Rats were given *S lappa* extract one-hour prior administration of the low dose of deltamethrin.

All animals were intoxicated with an oral deltamethrin repeated dose for 28 consecutive days according to the standard procedures laid down by OECD guidelines 407 (17). *S lappa* extract was dissolved in bi-distilled water and administered (300 mg / Kg.bw) to rats orally for 28 successive days.

Sampling,

After the experimental period, the blood samples were collected on heparinized tubes. The blood samples were centrifuged at 3600 rpm for 15 min for plasma separation. Plasma were maintained at -20 ºC for biochemical assays. After blood collection, animals were sacrificed under anesthesia and the kidneys from all experimental animals were removed for histopathological study. Parts of kidney samples were kept in liquid nitrogen for studying DNA damage by a comet assay

Plasma biochemical assay

Lipid peroxidation (biomarker of oxidative stress) was estimated by determining the concentration of thiobarbituric acid reactive products (malondialdehyde) (18). Concentration of protein carbonyl was also determined (19). Plasma protein thiol was measured using 5,5-dithiobis 2-nitrobenzoic acid (DTNB) (20). Activity of superoxide dismutase (SOD) (21), catalase (22) and glutathione s-transferase (GST) (23) were measured as antioxidant biomarkers. Total adenosine triphosphates (ATPase) activity was determined according to Samson and Quinn (24), the method is based on measuring the inorganic phosphorus that liberated from the substrate. The urea concentration was evaluated by the method of Fawcett and Scott (25), while, the content of plasma creatinine was estimated according to Siest *et al*. (26).

Histopathological microscopic examination

The kidney specimens were fixed in a formalin solution (10%) overnight for 15 hours after dissection into small pieces. The tissue samples were further processed by immersion in a series of graded ethanol and then embedded in a paraffin wax. The sections of kidneys (4-5 µm) were stained using hematoxylin and eosin. The morphological changes were observed by a light microscope.

Comet assay

The comet assay on the level of the individual cells was applied in this investigation for DNA fragmentation analysis (27). Tail length and the percentage rate of DNA in the tail were assessed as markers of DNA fragmentation.

Statistical analysis,

Data analysis were carried out by comparing the results of various experimental groups with the control values. The results are represented as mean ± SD. Significance between groups were performed utilizing one-way analysis of variance (ANOVA-LSD). $p \leq 0.05$ was considered as significant.

RESULTS

Biochemical results

The impacts of S *lappa* extract on the plasma oxidative stress indices in deltamethrin (DH) treated rats are shown in Figure 1. The data revealed that intoxication of rats with the high (1/15LD50) or the low (1/30 LD50) dose of deltamethrin induced significant increases in oxidative stress parameters, malondialdehyde (MDA) and protein carbonyl compared with the control animal group. The alterations in these indices were pronounced in rats ingested the high deltamethrin dose. Supplementation with *S lappa* extract (300 mg/Kg) to deltamethrin treated animals, markedly reduced the oxidative stress parameters with relation to deltamethrin treated counterpart group. The plant extract was more effective in down modulating these markers close to their normal level in rats intoxicated with the low dose of deltamethrin. Significant decreases in antioxidant defense markers, including protein thiol, glutathione S transferase (GST), catalase (CAT) and, superoxide dismutase (SOD) were recorded in rats intoxicated with the high or the low dose of deltamethrin versus control animals (Figure2). Ingestion of *S lappa*extract to deltamethrin

intoxicated rat groups, markedly improved the alteration in these antioxidant markers compared with intoxicated counterpart animal group.

The impact of *S lappa* extract on the activity of adenosine triphosphatase (ATPase) in deltamethrin intoxicated animal groups is shown in Figure 3. The result demonstrated that intoxication of rats with either the high or the low dose of deltamethrin, induced a remarkable reduction in plasma ATPase activity. The reduction in the enzyme activity was more pronounced in rats administered the high dose of deltamethrin. Prophylactic administration of *S lappa* extract to deltamethrin intoxicated animal groups, significantly ameliorated the decrease in plasma ATPase activity. The extract was more successful in modulating the enzyme activity in rats intoxicated with the low dose of deltamethrin..

The markers of kidney function (urea and creatinine) in different experimental groups are depicted in Figure 4. The result showed pronounced elevation in plasma urea in rats treated with the high or the low deltamethrin dose versus control groups, however a marked elevation in plasma creatinine was observed only in rats intoxication with the high deltamethrin dose compared with control rats. Supplementation with *S lappa* extract caused an improvement in the above-mentioned parameters compared with deltamethrin intoxicated counterpart group.

Genotoxicity results,

The potential impact of *S lappa* extract on the DNA damage in kidneys of rat groups ingested the toxic doses of deltamethrin is expressed in Table 1 and Figure 5. Data revealed significant increases in DNA tail length and DNA percentage in rat kidneys ingested the high or the low dose of deltamethrin versus the control group. Supplementation with *S lappa* extract, significantly reduced the levels of DNA damage markers in both intoxicated groups compared with deltamethrin untreated counterpart group ($p<0.05$). This effect was pronounced in rats administered the low deltamethrin dose compared with animals ingested the high dose.

Histopathological observation

Light microscope examination of control rat kidneys (Figure 6 A) as well as kidneys of animals treated with *S lappa* extract only (Figure 6 B) revealed normal glomeruli with a tuft of blood capillaries surrounded by Bowman's capsule. The distal and proximal convoluted tubules also showed normal appearance. Animals treated with the high dose of deltamethrin (Figure 6 C) showed massive hemorrhage in the renal cortex and congestion of some renal glomeruli. Some renal tubules showed swelling of the epithelial lining and necrosis of other renal tubules was also observed. Animals treated with *S lappa* extract one hour prior administration of the high dose of deltamethrin (Figure 6 D), showed some improvements but some changes were still found as observed by degenerative changes in some glomeruli and tubules as shown by a shrinkage in a tuft of Bowman’s capsules capillaries and distortion of the epithelial lining of some tubules. Animals treated with the low dose of deltamethrin (Figure 6 E) showed mild congestion, hemorrhage and necrosis. Distortions of the glomeruli and tubules were obvious. The epithelia of tubules revealed serious damage in the form of edema with rupture of the tubules. Hemorrhage was also found among the damaged tubules and glomeruli. However, a great improvement was observed in rats treated with *S lappa* extract one hour prior the administration of the low dose of deltamethrin (Figure 6 F) as the kidneys of this animals showed slightly congested glomeruli, swelling of the epithelial tubular lining with few areas of hemorrhage between them.

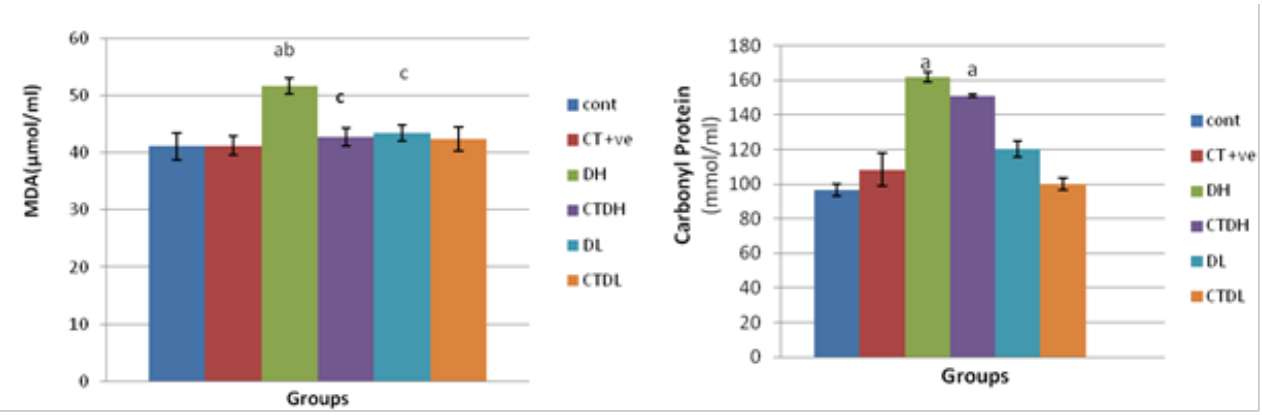

Figure (1), Effect of S *lappa* extract on plasma oxidative stress biomarkers (MDA and carbonyl protein) in rats treated with 1/15 LD50 and 1/30 LD50 deltamethrin. Cont = control, CT=control treated with *S lappa* extract, DH = animals treated with high deltamethrin dose (1/15 LD50), CTDH = animals pre -treated with S *lappa* extract and administered high deltamethrin dose, DL= animals treated with low deltamethrin dose (1/30 LD50), CTDL = animals pre -treated with S lappa extract and administered low deltamethrin dose. Values are expressed as mean ± SD of 5 rats. ap≤ 0.05, compared to Cont group, bp ≤ 0.05 compared to CT group, cp ≤ 0.05 compared to DH group.

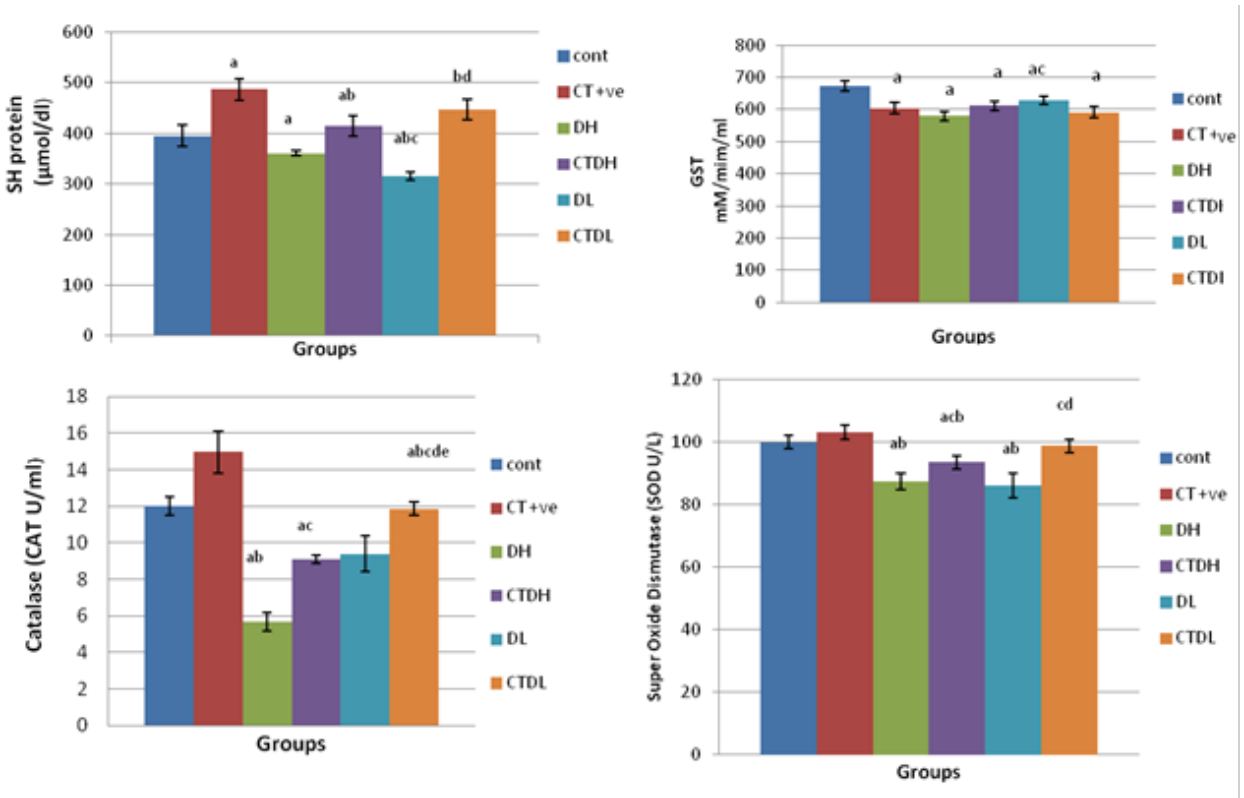

Figure (2), Effect of *S lappa* extract on plasma antioxidant biomarkers (SH-protein,GST, catalase, superoxide dismutase) in rats treated with 1/15 LD50 and 1/30 LD50 deltamethrin. Cont = control, CT=control treated with *S lappa*, DH = animals treated with high deltamethrin dose (1/15 LD50), CTDH = animals pre -treated with S *lappa* extract and administered high deltamethrin dose, DL= animals treated with low deltamethrin dose (1/30 LD50), CTDL = animals pre -treated with S *lappa* extract and administered low deltamethrin dose. Values are expressed as mean ± SD of 5 rats. ap≤ 0.05, compared to Cont group, bp ≤ 0.05 compared to CT group, cp ≤ 0.05 compared to DH group, dp ≤ 0.05 compared to CTDH group, ep ≤ 0.05 compared to DL group.

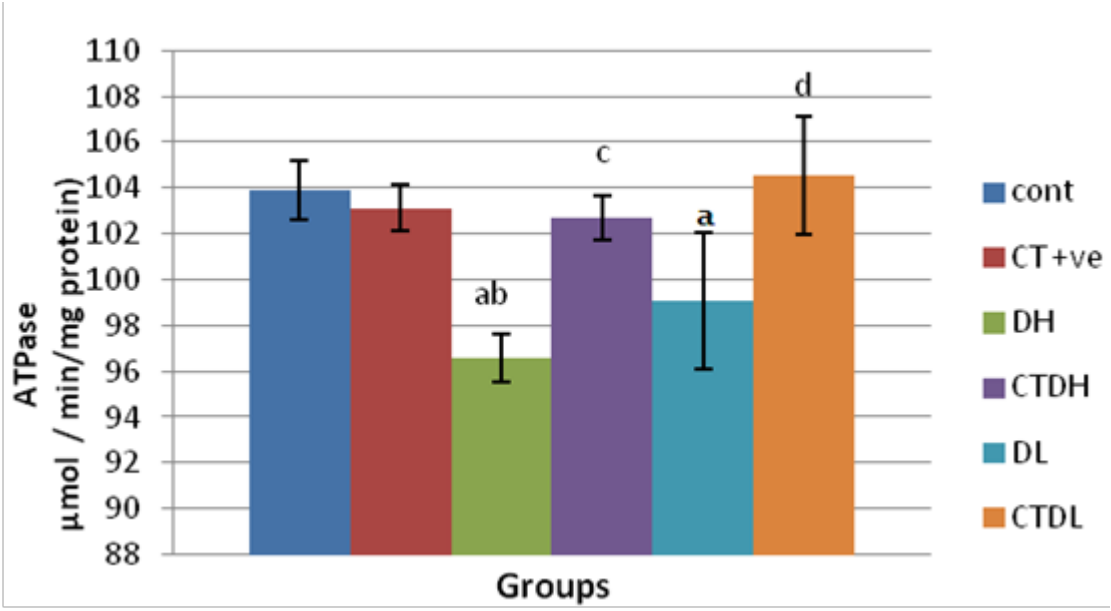

Figure (3), Effect of *S lappa* extract on plasma ATPase in rats treated with 1/15 LD50 and 1/30 LD50 deltamethrin. Cont = control, CT=control treated with *S lappa*, DH = animals treated with high deltamethrin dose (1/15 LD50), CTDH = animals pre -treated with *S lappa* extract and administered high deltamethrin dose , DL= animals treated with low deltamethrin dose (1/30 LD50), CTDL = animals pre -treated with *S lappa* extract and administered low deltamethrin dose. Values are expressed as mean ± SD of 5 rats. ap≤ 0.05, compared to Cont group, bp ≤ 0.05 compared to CT group, cp ≤ 0.05 compared to DH group, dp ≤ 0.05 compared to CTDH group.

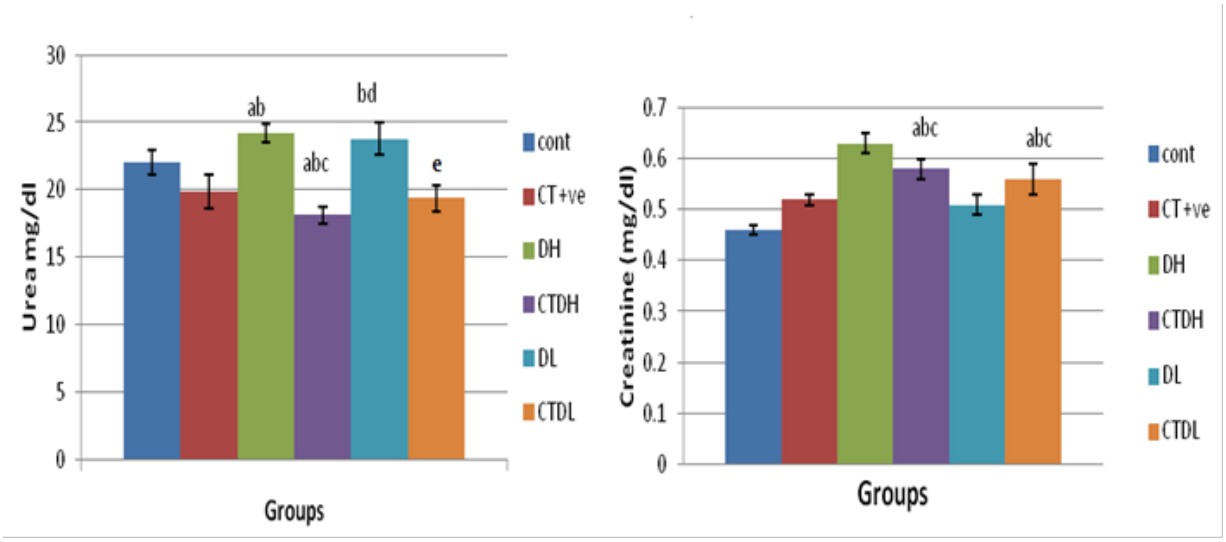

Figure (4), Effect of *S lappa* extract on plasma kidney function biomarkers (urea and creatinine) in rats treated with 1/15 LD50 and 1/30 LD50 deltamethrin. Cont = control, CT=control treated with *S lappa*, DH = animals treated with high deltamethrin dose (1/15 LD50), CTDH = animals pre -treated with S *lappa* extract and administered high deltamethrin dose, DL= animals treated with low deltamethrin dose (1/30 LD50), CTDL = animals pre -treated with S lappa extract and administered low deltamethrin dose. Values are expressed as mean ± SD of 5 rats. ap≤ 0.05, compared to Cont group, bp ≤ 0.05 compared to CT group, cp ≤ 0.05 compared to DH group, dp ≤ 0.05 compared to CTDH group, ep ≤ 0.05 compared to DL group.

Table (1), Effect of S lappaextract on DNA damage markers in kidney tissues in rats treated with 1/15 LD50 and 1/30 LD50 deltamethrin.

Groups	***Tail length (µm)***	***DNA %***
Cont.	2.08±0.06	2.01±0.07
CT	1.05±0.17	0.83±0.21
DH	8.37±0.81[a]	8.83±0.36 [a]
CTDH	3.37±0.34 [a b c]	3.60±0.34 [a b c]
DL	5.55 ± 0.86 [a b c]	6.25±0.64 [a b c]
CTDL	2.83±0.28 [a b d e]	2.70±0.47 [a b d e]

CTDH = animals pre -treated with S lappa extract and administered high deltamethrin dose, DL= animals treated with low deltamethrin dose (1/30 LD50), CTDL = animals pre -treated with S lappa extract and administered low deltamethrin dose. Values are expressed as mean ± SD. ap≤ 0.05, compared to Cont group, bp ≤ 0.05 compared to CT group, cp ≤ 0.05 compared to DH group, dp ≤ 0.05 compared to CTDH group, ep ≤ 0.05 compared to DL group.

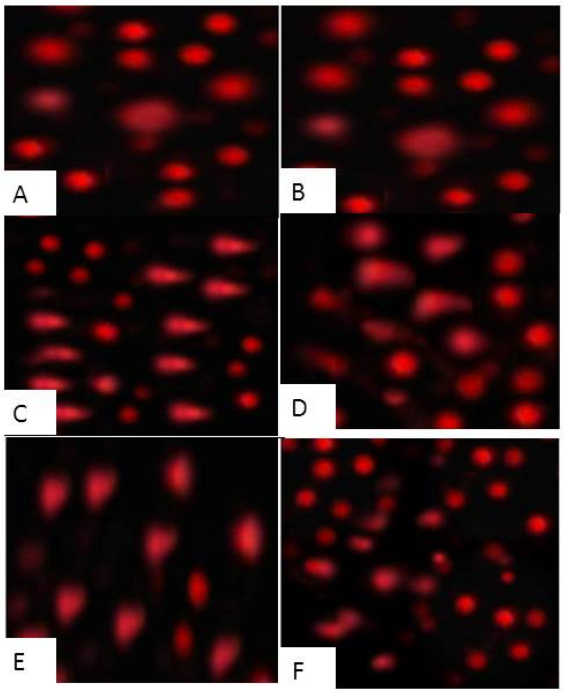

Figure (5), Comet assay reveled the level of DNA damage in the kidney tissues of rats administered deltamethrin and treated with S *lappa* extract. (A), healthy rat group (B), animal group treated with *S lappa* extract. (C) animal group intoxicated with DH.(D), animal group treated with DH and pre- supplemented with *S lappa* extract. (E), animal group treated with DL. (F), animal group treated with DL and pre- supplemented with *S lappa* extract.

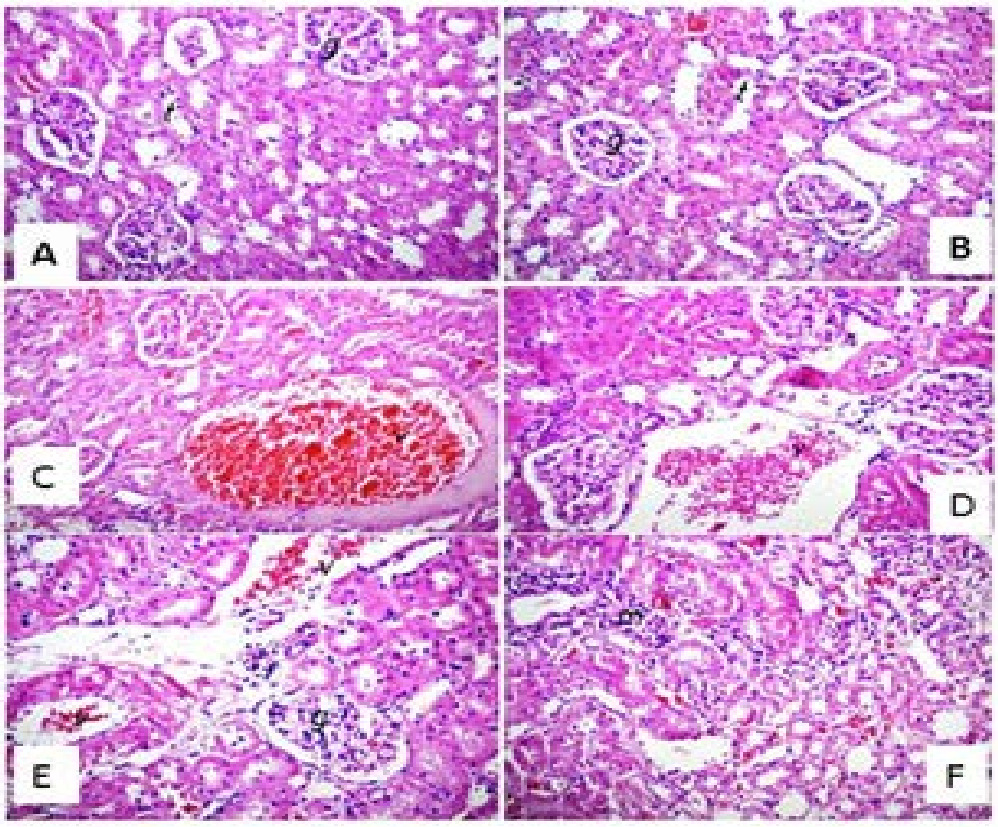

Figure (6), Light micrograph of the kidney tissue of intoxicated rats administered the high and the low dose of deltamethrin and the effect of *S lappa* extract on the histomorphology of rat kidneys. (A & B) Kidney sections of control rats and rats treated with *S lappa* extract only, showing normal appearance of glomeruli with their tuft of capillaries surrounded by bowman's capsules. Kidney tubules are also appeared normal. (C) Kidney section of a rat intoxicated with the high dose of deltamethrin showing massive hemorrhage in the renal cortex, congestion of some renal glomeruli and swelling of the epithelial lining of the tubules. (D) Kidney section of a rat intoxicated with the high dose of deltamethrin and pretreated with *S lappa* extract showing some improvements, but some degenerative changes in some glomeruli and tubules were still found. (E) Kidney section of a rat intoxicated with the low dose of deltamethrin, showing mild congestion, hemorrhage and necrosis. (F) Kidney section of rats intoxicated with the low dose of deltamethrin and pretreated with *S lappa* extract showing a great improvement in kidney architecture (H&E 500).

DISCUSSION

Pyrethroids are lipophilic pesticides and easily can be absorbed through oral and dermal routs. Deltamethrin is easily absorbed from the digestive tract after oral ingestion in experimental animals and rapidly metabolized by liver esterases and microsomal oxidases (28-29). The present results showed that administration of the high or the low dose of deltamethrin to rats, markedly induced elevation in oxidative stress biomarkers, malondialdehyde (MDA) and carbonyl protein (CP), in plasma of intoxicated rats compared with the normal control animals. This deleterious impact of this toxin was concomitant with marked decreases in plasma antioxidant markers, including protein thiol, GST, CAT and SOD in rats intoxicated with either of the two doses. The induction of free radical generation, stimulation of lipid peroxidation, and upset of the antioxidant ability of body vital organs including kidney, are considered mechanisms of pyrethroids toxicity (14). Exposure of organic molecules to free radical species can cause irreversible oxidation of these molecules and cause alterations of many metabolic reactions that in turn can lead to cellular dysfunction (30-31). Reduction in SH-protein has been reported in rats intoxicated with Lambda cyhalothrin for 90 days (32). Also, previous authors showed that significant decreases in CAT, GST and SOD enzyme activities in rats exposed to low doses of deltamethrin (33). In addition, cypermethrin, as one of pyrethroids, has been found to induce lipid peroxidation and suppress the abilities of antioxidant defense systems, namely glutathione, SOD, CAT, GST, glutathione reductase and glutathione peroxidase (12).

Ingestion of S *lappa* extract to deltamethrin intoxicated rat groups, markedly reduced the oxidative stress markers and improved the alteration in the antioxidant defense markers compared with intoxicated counterpart animal group. This result may indicate the potential antioxidant capability of *S lappa* extract. Few reports have investigated the antioxidant impact of *S. lappa* extracts. The present study suggests that the antioxidant beneficial impact of this plant extract may relate to its active phytocompounds including anthraquinone, flavonoids and various terpenes (3, 34).

The current study also showed that oral ingestion of the high or the low dose of deltamethrin to rats caused a remarkable reduction in plasma ATPase activity. The reduction in this enzyme activity was more severe in rats intoxicated with the high dose of deltamethrin compared with ones administered the low dose. Similarly, Medrano *et al*. (35) reported that deltamethrin could inhibit Ca^{2+}-ATPase of plasma membrane and endoplasmic reticulum. In addition, pyrethroids are well known to suppress the ATPase activity from rats (36). The toxic mechanism of this pesticide on ATPase activity may attribute to its lipophilic property, thus it affects membrane fluidity and function and in turn affecting the enzymes associated with the membrane such as ATPase (37). On the other hand, enhancement of lipid peroxidation by deltamethrin presented in the current study may have the major cause in membrane damage that leads to alteration in membrane bound enzymes such as ATPase. This is supported by some authors reported that induction of lipid peroxidation can damage the plasma membranes and inhibit the membrane-bound enzymes as ATPase (38). Protective ingestion of *S lappa* extract to rats intoxicated with the high or the low dose of deltamethrin, effectively modulated the plasma ATPase activity. The modulating ability of the plant extract on ATPase may attribute to its potential impact in stimulating the antioxidant defense system and suppressing lipid peroxidation as confirmed in the present study.

Comet assay is a method for detecting DNA damage that appears as a fluorescing material around the nuclei, making a tail with variable length along the electric field. Our results showed increases in the tail length and DNA % of rat kidneys intoxicated with the high or the low dose of deltamethrin compared with the normal control ones. These results come in line with the result reported by some authors revealed that pesticides could damage DNA in hepatocytes, lymphocytes, and other cells in the body (7, 39). Damage in DNA rat kidneys treated with cypermethrin pyrethroid insecticide expressed by increases in DNA % and tail length was also reported (40). The damaging impact of deltamethrin on DNA of rat kidneys may be due to its accumulation in kidney tissue, causing increases in the generation of free radicals that lead to oxidative stress and apoptosis. Accumulation of free radicals is the major reason that causes oxidative DNA damage (41).

Supplementation of *S. lappa* extract to deltamethrin intoxicated rat groups, successfully reduced the level of DNA oxidative damage markers in both intoxicated groups compared with intoxicated untreated counterpart group. This beneficial impact of the plant extract was pronounced in rats intoxicated with the low deltamethrin dose compared with the animals intoxicated with the high dose. The attenuating effect of the plant extract against deltamethrin induced kidney oxidative DNA oxidative damage may indicate that the phytochemical compounds of the plant extract have potential antioxidant activities. Also, it has been reported that S. lappaposses many diverse terpenes that mainly have anti-inflammatory and antitumor properties, such as costunolide, dihydrocostunolide 12-methoxydihydrocostunolide, dihydrocostuslactone, dehydrocostus lactone 7. The plant contains many compounds with antioxidant medicinal activities (42). The plant contains costunolide (CE) and dehydrocostuslactone (DE), two natural sesquiterpenelactones. The pivotal roles of CE and DE are through the conjugation with mercapto (SH)-groups of target proteins to take part in some key metabolic reactions inside the cells (43). Various biological activities of these compounds were investigated including anti-inflammatory (44), anticancer (45), antioxidant (46), and antidiabetic (47).

The toxic impacts of deltamethrin on rat kidney tissues presented in the current study were reflected by increases in renal function biomarkers, including urea and creatinine in deltamethrin intoxicated rats compared with the control rats. The impairment in kidney function by deltamethrin toxicity was documented by histopathological observation as demonstrated by disorganization of renal tissue. The renal tubules lost their characteristic appearance and their lumens were filled with amorphous cellular derbis. Degeneration of some glomeruli and congestion of the renal blood vessels were also observed in response to deltamethrin toxicity. Similarly, some authors (12, 48) reported that cypermethrin caused histological and degenerative effects on kidney of rats.

The alterations in renal function biomarkers and its histopathological picture of deltamethrin intoxicated rats were greatly improved by pre-ingestion of *S lappa* extract.

Conclusion, the modulating effect of *S lappa* extract on renal function biomarkers and its histomorphological picture may suggest the reno-protective effect of this plant extract against deltamethrin induced oxidative renal damage. This beneficial effect of the plant extract may ascribe to the ability of its active compounds to reduce the uncontrolled generation of reactive species, which cause protein damage, lipid peroxidation, and DNA fragmentation (49).

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