Original Article
Prophylactic effect of cousts saussurea lappa against liver injury induced by deltamethrin intoxication

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Abstract: Aim: This study was undertaken to investigate the hepatoprotective effect of Coustus saussura lappa versus liver toxicity induced by deltamethrin exposure. Experimental design: sixty adult male albino rats rattus norvegicus (150-180 gm) were divided into 6 groups treated for 28 days as ; G1 control group, G2 (CT = coustus 300 mg/kg), G3 (DH = high dose deltamethrin 1/15 LD50; 4 m/kg), G4 (CTDH), G5 (DL = low dose deltamethrin 1/30 LD50; 2 mg/kg), G6 (CTDL). Results: showed remarkable elevation in plasma liver biomarkers; alanine aminotransferase (ALT), aspartate aminotransferase (AST) alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) in both deltamethrin treated groups, as well as slight elevation total plasma proteins. These results are consequence to the recorded significant reduction in the defence system biomarkers (SH-protein) and antioxidant enzymes; superoxide dismutase (SOD), catalase and detoxifying enzyme glutathione s-transfearse (GST). Degeneration in hepatocyets with congestion in the central veins was recorded in liver tissues. However supplementation with 300 mg/kg aqueoues extract of cousts saussura lappa induced partial counteract in the above tested parameters especially in CTDL group. In conclusion: treatment with aqueous extract of saussura lappa induced early improvement in injured liver that promise better results on longer repeated use.

Keywords: Pyrethroid insecticides, deltamethrin, liver, defense system, antioxidant enzymes, liver enzymes

Introduction

Mosquito acts as one of the most important vectors of some human diseases. The major vectors are members of the Culex, Aedes and Anopheles genera. All these genera are found in Saudi Arabia [1, 2]. Pyrethroid and organo-phosphate insecticides are used extensively to control disease vectors [3, 4]. Deltamethrin [α-cyano-3-phenoxybenzyl-(1R, S)-cis, trans-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate] is one of pyrethroid insecticide used widely in agriculture, disease vector control and home pest control. It is one of the toxic industrial and environmental pollutant to humans and animals [5]. The indoor application of pyrethroids led to their chronic exposure to unaware individuals. It was noted that chronic exposure to this insecticide and its cumulative impact can alter the function of body vital organs and this must be taken seriously in light of the effects obtained in response to exposure of organisms to low doses accumulated during life. Repeated exposure to deltamethrin was found to induce oxidative stress and production of oxidizing species leading to irreversible oxidation of organic molecules and membrane lipid peroxidation, therefore resulted in chemical modification of biological processes that in turn can cause cell damage and in turn, dysfunction of many organs [6]. Endocrine disruption, neurodevelopmental toxicity and adverse immune system effects related to pyrethroids exposure have been reported in numerous studies [7]. Oxidative stress is a harmful process that can mediate damage to cell structures, including lipids, proteins, RNA and DNA which lead to a number of diseases [8]. Deltamethrin is metabolized in the liver through hydrolytic ester cleavage by cytochrome P450’s and the oxidative route [9, 10]. Deltamethrin was found to induce histological alterations in liver, kidney and lungs [5, 11]. The selective neurotoxicity of deltamethrin is attributed to their effect on voltage sensitive sodium channels (VSSCs).
**Hepatoprotective effect of coustus saussurea lappa**

*Saussurea lappa* Clarke [Synonym: *Saussurea costus*) (family Asteraceae) is a well-known medicinal plant growing in the Himalayan region between 2500 to 3000 m above sea level. In view of increasing national and international market demand of *S. lappa*, it is also cultivated in a few states of India, including Uttarakhand and Himanchal Pradesh. *Saussurea lappa* has many active constituents, are sesquiterpene lactones (constunolide and dehydrocostus lactone). Sesquiterpene lactones such as constunolide and dehydrocostus lactone, are major components of the roots, and have been reported to possess various biological activities such as antifungal [12], antidiabetic, antitumor [14] antimicrobial [15], immuno-stimulant [16], antiulcer [17], antiinflammatory [18] and antihepatotoxic [19]. Different extracts of this plant have been found to exhibit anti-inflammatory, hepatoprotective, anti-ulcer, anticancer, immunomodulatory and pesticidal activities [20]. The cynaropicrin, a sesquiterpene isolated from *Saussurea lappa* strongly inhibits tumor necrosis factor TNF-a release from lipopolysaccharide-stimulated macrophage [21]. Two active components, constunolide and dehydrocostus lactone, showed strong suppressive effect on the expression of the hepatitis B surface antigen (HBsAg) in human hepatoma Hep3B cells. Both costunolide and dehydrocostus lactone suppressed the HBsAg production by Hep3B cells in a dose-dependent manner [20].

The present study aims to evaluate the effect of coustus *Saussurea lappa* water extract against liver injury induced by deltamethrin intoxication.

**Materials and methods**

**Insecticide**

Deltamethrin insecticide, (S)-cyano-3-phenoxybenzyl (1R, 3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate, in the formulated form Agrodelta 25 EC/ULV. Contain Deltamethrin 2.5% was purchased from Jeddah, KSA market.

**Antioxidant used**

Dried roots of coustus *Saussurea lappa* were purchased from a local herbalist market in Jeddah, KSA. Plant material was cleaned, crushed and 100 gm were soaked into 70% aqueous-methanol solution. It was then filtered through filter paper and concentrated into thick semi solid paste under reduced pressure on a rotary evaporator. The extract was soluble in normal saline and distilled water.

**Animals and experimental design**

Male albino rats *Rattus Norvegicus* (3-4) month’s age, weighing between 150-180 g were used. Animals were supplied by the breeding unit of King Fahd research center in King abdulAziz University, Jeddah, KSA. The animals were housed in plastic cages, fed ad libitum and allowed to adjust to the new environment for two weeks before starting the experiment. The rats were housed at 23 ± 2°C dark/light cycle. All animals were treated according to the standard procedures laid down by OECD guidelines 407 repeated dose 28 days oral toxicity study in rodents [22]. Animals were randomly divided into six experimental groups five animals each as follows:

- **Group I:** (control group): Each animal in this group was given distilled water (1 ml/animal) by gastric intubation every day for 28 days.
- **Group II:** coustus extract (CT): rats were orally given (300 mg coustus extract/kg.bw) dissolved in distilled water every day for 28 days.
- **Group III:** deltamethrin high dose (DH): rats were orally given 1/15 LD50 (4 mg/kg bw) of deltamethrin daily via gastric tube for 28 days.
- **Group IV:** deltamethrin high dose with coustus extract (CTDH): rats were orally given coustus extract (300 mg/kg bw) one hour prior administration of 1/15 LD50 (4 mg/kg bw) of deltamethrin daily for 28 days.
- **Group V:** deltamethrin low dose (DL): rats were orally given 1/30 LD50 (2 mg/kg bw) of deltamethrin daily via gastric tube for 28 days.
- **Group VI:** deltamethrin low dose with coustus extract (CTDL): rats were orally given coustus extract (300 mg/kg bw) one hour prior administration of 1/30 LD50 (2 mg/kg bw) of deltamethrin daily for 28 days.

**Sampling**

Blood collected from the retro-orbital plexus vein according to Schermer [24], on heparinized tubes at 28 days of treatment periods. Plasma samples were separated by centrifugation of the blood samples at 3600 rpm for 15 minutes. Plasma samples were kept at -20°C for subsequent use. At the end of the experiment, animals were sacrificed and samples of
the liver were excised for histopathological studies.

**Histopathology**

Histopathological examination was carried out according to Drury and Wallington [25]. The liver tissue was dissected and the tissue samples were fixed in 10% formalin solution for 14-18 h, passed in a series of graded ethanol and embedded in paraffin. Paraffin sections were cut with at 5 µm thickness and stained with hematoxylin and eosin for light microscopic examination. The sections were examined and photographed on an Olympus light microscope (Olympus BX51, Tokyo, Japan) with attachment photograph machine (Olympus C-5050, Olympus Optical Co. Ltd., Japan).

**Biochemical assay**

Total thiol proteins were determined in plasma the method based on the development of a yellow color when 5,5-dithiobis (2-nitrobenzoic acid) DTNB is added to plasma [26]. Activity of superoxide dismutase (SOD) [27] and catalase [28] and glutathione s-transferase (GST) [29] were measured in plasma respectively. Markers for liver were determined using the commercial diagnostic kit of Stanbio Co., Spain. Plasma transaminases (AST and ALT) activities were determined according to Reitman and Frankel [30]. Plasma total albumin was carried out according to Dumas et al. [31]. Total protein was determined by Biuret method [32]. Y-glutamyl transferase (γ-GT) activity was determined according of Szasz [33] kinetic method (Stanbio Laboratory, Spain). Plasma alkaline phosphatase (ALP) activity was determined by the method of Shephard and Peake [34].

**Statistical analysis**

Statistical analysis was based on comparing the values between the treated groups. The results are expressed as Mean ± SD of 5 animals/group. The statistical significance of the data has been determined using one way analysis of variance (ANOVA-LSD) using SPSS statistical software package version 10. The level of significance taken as P<0.05.

**Results**

**Biochemical results**

Data expressed in Table 1 revealed that rats intoxicated with both doses of 1/30 and 1/15 LD50 deltamethrin induced slight elevation in total plasma protein significant versus control in 1/30 LD50 treated group at P<0.05. Meanwhile, supplementation with aqueous extract of coustus (300 mg/Kg) to deltamethrin intoxicated animals induced remarkable elevation in plasma total protein in both supplemented groups significant versus other groups. It should note that there is no change in plasma albumin level in all treated groups. Remarkable elevation in activities of each of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) liver biomarker enzymes was recorded in both deltamethrin intoxicated groups. This elevation was significant versus control and + ve control groups at P<0.05. On the other hand, supplementation with aqueous extract of coustus Saussurea lappa reduced this elevation to be nearly reached to control levels. Significant Reduction in defense system (SH-protein) and antioxidant enzymes; catalase (CAT), superoxide dismutase

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**Table 1. Effect of Supplementation with Aqueous Extract Coustus Saussurea lappa (300 mg/Kg) on Plasma liver Biomarkers of Rats Intoxicated with (1/15 LD50 and 1/30 LD50) Deltamethrin**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>T.P (g/dl)</th>
<th>ALB (g/dl)</th>
<th>ALT (U/l)</th>
<th>AST (U/l)</th>
<th>ALP (U/l)</th>
<th>γ GT (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont</td>
<td></td>
<td>6.44 ± 0.19</td>
<td>4.46 ± 0.15</td>
<td>18.02 ± 0.50</td>
<td>15.38 ± 1.28</td>
<td>67.57 ± 1.43</td>
<td>8.45 ± 0.82</td>
</tr>
<tr>
<td>CT</td>
<td></td>
<td>7.28 ± 0.35</td>
<td>4.00 ± 0.18a</td>
<td>18.2 ± 0.59</td>
<td>18.88 ± 1.24</td>
<td>63.44 ± 1.53</td>
<td>8.42 ± 0.35a</td>
</tr>
<tr>
<td>DH</td>
<td></td>
<td>7.241 ± 0.42</td>
<td>4.44 ± 0.048</td>
<td>23.31 ± 0.29ab</td>
<td>20.47 ± 1.13ab</td>
<td>79.05 ± 1.15ab</td>
<td>11.56 ± 0.51ab</td>
</tr>
<tr>
<td>CTDH</td>
<td></td>
<td>8.80 ± 0.3abc</td>
<td>4.05 ± 0.21</td>
<td>17.95 ± 0.43c</td>
<td>15.86 ± 1.00c</td>
<td>58.78 ± 2.10abc</td>
<td>9.71 ± 0.21abc</td>
</tr>
<tr>
<td>DL</td>
<td></td>
<td>7.42 ± 0.26a</td>
<td>4.25 ± 0.26a</td>
<td>24.51 ± 0.58ab</td>
<td>20.86 ± 1.01</td>
<td>72.96 ± 1.15abc</td>
<td>10.93 ± 0.19ab</td>
</tr>
<tr>
<td>CTDL</td>
<td></td>
<td>8.42 ± 0.38abc</td>
<td>4.75 ± 0.27</td>
<td>19.19 ± 0.35ce</td>
<td>17.38 ± 1.1d</td>
<td>54.14 ± 1.69abc</td>
<td>7.83 ± 0.11abc</td>
</tr>
</tbody>
</table>

CT = coustus + ve cont. DH = delta 1/15 LD50. CTDH = coustus + delta 1/15 LD50. DL = 1/30 LD50. CTDL = coustus + delta 1/30 LD50. All data are expressed as means ± SE five rats. *Significant differences versus control group at p < 0.05. #Significant difference versus CT group at p < 0.05. 
| Significant difference versus DH group at p < 0.05. ▲Significant difference versus CTDH group at p < 0.05. ▼Significant difference versus DL group at p < 0.05.
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(SOD) and glutathione S transferase (GST) was recorded in both deltamethrin intoxicated groups pronounced in (1/30 LD50) versus control and other treated groups as presented in Table 2. However, activities of these enzymes were improved in intoxicated animals supplemented with aqueous extract of coustus saussurea lappa (300 mg/kg). A remarkable counteract in the enzymes activities was recorded in coustus +1/30 LD50 treated group (Table 2).

Histopathological findings

Histopathological results of liver of rats intoxicated with deltamethrin in presence or absence of coustus aqueous extract were depicted in Figure 1. Photomicrograph (A) showed normal liver architecture with the central vein and radiating cords of normal hepatocytes with central rounded nuclei. Normal blood sinusoids appeared between the liver cords. However, supplementation with coustus aqueous extract extract (300 mg/kg) to normal rats as a positive control showed that liver cells appeared normal with mild vacuolization noticed in the cytoplasm of the hepatocytes photomicrograph (B). On the other hand, liver of rats intoxicated with 1/15 LD50 deltamethrin showed vacuolar degeneration in the hepatocytes surrounding the central vein associated with congestion in the central vein and sinusoids photomicrograph (C). There were dilatation and congestion in the central veins and sinusoids in liver tissue of rats intoxicated 1/15 LD50 deltamethrin with and supplemented with aqueous extract of coustus photomicrograph (D). However, Vacuolar degeneration was detected in the hepatocytes surrounding the dilated central vein. The portal veins showed also sever congestion associated with inflammatory cells infiltration and fibrosis in the periportal tissue at the portal rats intoxicated with 1/30 LD50 deltamethrin photomicrograph (E). Whereas, hepatocytes showed degenerative change associated with dilatation in the central veins in 1/30 LD50 deltamethrin supplemented with aqueous extract of coustus photomicrograph (F).

Discussion

Deltamethrin has very broad-spectrum control and is considered the most powerful of the synthetic pyrethroids [35]. Deltamethrin is readily absorbed from the gastrointestinal tract after oral administration in rats and mice. Studies in rats have shown that deltamethrin, an ester, is rapidly metabolised by tissue esterases, which are widely distributed including in the gut wall and liver [36, 37]. Our results revealed that repeated exposure to different doses of deltamethrin to albino rats induced remarkable injury in the liver expressed by elevation in plasma total protein and elevation in the activities of liver biomarker enzymes ALT, AST, γGT and ALP these findings concurrent with significant reduction in defense system (SH-protein) and antioxidant enzymes; catalase (CAT), superoxide dismutase (SOD) and glutathione S transferase (GST). The above results were confirmed by histopathological studies where vacuolar degeneration in the hepatocytes as well as congestion in the central vein and sinusoids was recorded in both treated doses, remarkable in 1/15 LD50 intoxicated animals. These findings could be explained through understanding that Deltamethrin is metabolized in the liver through

Table 2. Effect of Supplementation with Aqueous Extract Coustus Saussurea lappa (300 mg/Kg) on Plasma Antioxidant Biomarkers of Rats Intoxicated with (1/15 LD50 and 1/30 LD50) Deltamethrin

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameters</th>
<th>SH protein (μmol/dl)</th>
<th>GST (mM/min/ml)</th>
<th>CAT (U/ml)</th>
<th>SOD (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont.</td>
<td></td>
<td>395.22 ± 20.76</td>
<td>674.37 ± 16.09</td>
<td>12.02 ± 0.49</td>
<td>99.85 ± 2.16</td>
</tr>
<tr>
<td>CT</td>
<td></td>
<td>486.77 ± 22.12a</td>
<td>603.66 ± 17.62a</td>
<td>14.97 ± 1.15</td>
<td>103.07 ± 2.40</td>
</tr>
<tr>
<td>DH</td>
<td></td>
<td>360.66 ± 5.10a</td>
<td>580.15 ± 13.88a</td>
<td>5.67 ± 0.51ab</td>
<td>87.35 ± 2.55ab</td>
</tr>
<tr>
<td>CTDH</td>
<td></td>
<td>415.42 ± 20.52a,b,c</td>
<td>611.98 ± 14.66a</td>
<td>9.10 ± 0.22ab,c</td>
<td>93.38 ± 2.13ab,c</td>
</tr>
<tr>
<td>DL</td>
<td></td>
<td>316.32 ± 8.21a,c</td>
<td>629.22 ± 12.6c</td>
<td>9.39 ± 0.98bc</td>
<td>85.99 ± 3.84bc</td>
</tr>
<tr>
<td>CTDL</td>
<td></td>
<td>447.43 ± 20.42b,c,d</td>
<td>591.56 ± 16.82a</td>
<td>11.86 ± 0.36abcd</td>
<td>98.71 ± 2.08abcd</td>
</tr>
</tbody>
</table>

cousts + ve cont. DH = delta 1/15 LD50. CTDH = cousts + delta 1/15 LD50. DL = 1/30 LD50. CTDL = cousts + delta 1/30 LD50. All data are expressed as means ± SE five rats. *Significant differences versus control group at P < 0.05. †Significant difference versus CT group at P < 0.05. ‡Significant difference versus DH group at P < 0.05. §Significant difference versus CTDH group at P < 0.05. ¶Significant difference versus DL group at P < 0.05.
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hydrolytic ester cleavage by cytochrome P450’s and the oxidative route [38] to produce acid and alcohol moieties, oxidation of various parts of the molecule before or after cleavage of the ester link, and conjugation with sulfuric acid, glycine or glucuronic acid of the products of oxidation of both moieties [36, 37]. Activities of SOD and CAT and GSH and MDA levels in the liver reflect the oxidative status and the serum enzymes like AST, ALT, ALP represent the func-

Figure 1. A. Liver section of male rats control group. There were no histopathological findings and the normal histological structure of the central vein (cv) and surrounding hepatocytes. B. Liver section of male rats group (+ve cont) showing mild vacuolization in hepatocytes (h). C. Liver section of male rats pesticide high dose group. Showing vacuolar degeneration in hepatocytes (d) is surrounding the central vein. D. Liver section of male rats group 4. Showing vacuolar degeneration in hepatocytes (d) is surrounding the dilated central vein (cv). E. Liver section of male rats group 5. Showing congestion and dilatation in central vein (v) and sinusoids (s). F. Liver section of male rats group 6. Showing degeneration in hepatocytes (d) surrounding the dilatated in central vein (cv).
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...tional status of the liver [39]. Increase of transaminase activity along with the decreased of content of free radical (O2-) scavengers are probably the consequence of deltamethrin induced pathological changes in liver and other visceral organs. On the other hand, animals supplemented with (300/animal) aqueous extract of coustus Saussurea lappa in presence or absence of different doses of deltamethrin counteract the toxic effect of deltamethrin on the previous liver biomarkers to be more or less nearly reach to the control level. However, tissue architecture needs long supplementation with coustus Saussurea lappa for complete recovery. Natural antioxidants play a major role in reducing the oxidative stress by scavenging the excess free radicals [40]. Saussurea lappa is one of the antioxidant-rich medicinal plants. Many authors have reported that the roots of this plant possess cortisollowering effect [41]. Costunolide (CE) and dehydrocostuslactone (DE), two natural sesquiterpene lactones, present in Saussurea lappa. CE and DE may play some pivotal roles through conjugation with mercapto (SH)-groups of target proteins to intervene in some key biological processes in cells [42, 43]. Therefore, these two compounds possess various biological activities, including anti-inflammatory [44, 45], anticancer [46, 47], antiviral [48], antimicrobial [49, 50] antifungal [51], antioxidant [52, 53], antidiabetic [54], antiulcer [55], hepatoprotective effects [41] and anthelmintic activities [56].

Conclusions

In conclusion intoxication with deltamethrin at different dose level induced serious injury in liver of rats represented by elevation in plasma liver biomarkers. (ALT, AST, ALP, GGT) concomitant with reduction in activates of antioxidant enzymes (catalase, SOD, GST and SH-protein) as well as destruction in liver tissues. Supplementation with aqueous extract of saussura lappa counteract changes in the above mentioned parameters but needs more times for liver tissues repair.

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Disclosure of conflict of interest

None.

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