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EFFECT OF SILYMARIN AGAINST DELTAMETHRIN - INDUCED HISTOLOGICAL AND BIOCHEMICAL CHANGES IN LIVER OF ALBINO RATS

ABSTRACT:
Treating male albino rats with the pyrethroid insecticide, deltamethrin induced various histological changes in the liver. These changes included congestion of blood vessels, leucocytic infiltration, cytoplasmic vacuolization of the hepatocytes and fatty infiltration. Deltamethrin also caused significant elevation in serum ALT and AST enzymes after 2 and 3 weeks of treatment. Silymarin, an extract from seeds and fruits of Silybum marianum, is used in prevention of many liver diseases. Treating animals with both deltamethrin and silymarin led to an improvement in the histological changes induced by deltamethrin. Moreover, a significant decrease in ALT and AST activity was detected. The results of the present work indicated that silymarin has an ameliorative effect against liver damage induced by deltamethrin. This effect may attribute to its potent antioxidant activities.

Key words:
Liver, Deltamethrin, Silymarin, Histology, Transaminases.

INTRODUCTION
Pyrethroid insecticides are used extensively in agriculture, forestry, and public health (Casida and Quistad, 1998). Pyrethroids are synthetic analogs of the natural pyrethrins, the insecticidal components of extracts from the pyrethrum flower (Chrysanthemum cinerariaefolium). They modulate nerve axon sodium channels, resulting in neurotoxic effects (Crofton et al., 1995). Traditionally, pyrethroids are divided into two classes (types I and II) based on their structure and toxicological actions. Type I compounds do not contain a cyano group while type II compounds do. Tremor and paresthesias are the major signs of poisoning by type I compounds. Salivation, hyperexcitability, choreoathetosis, and seizures are the major signs of type II poisoning (Soderlund et al., 2002). Deltamethrin, a type II compound, is one of the most potent pyrethroid insecticides (Wolansky et al., 2006). It was reported that animals exposed to these insecticides exhibited disturbance in their physiological activities beside other pathological features (Abu-El Zahab et al., 1993; Sakr, 1999; El-Banhawy et al., 2000).

The potential role of dietary antioxidants to reduce the activity of free radical-induced reactions has drawn increasing attention. Silymarin, an extract from seeds and fruits of Silybum marianum, is a mixture of flavonoid isomers such as silibinin, isosilibinin, silidianin, and silichristin (Morazzoni and Bombardelli, 1995). Silymarin has been recommended in the prevention of alcoholic liver disease (Saller et al., 2001), and it protected liver against injury from various other hepatotoxins such as carbon tetrachloride, paracetamol (Chrungoo et al.1997, Rastogi et al., 2000), and concanavalin A (Schümann et al., 2003). The aim of this study is to evaluate the effect of silymarin supplement on liver of rats treated with deltamethrin.

MATERIALS AND METHODS
Sexually mature male albino rats weighting 125 ± 5 g were used. Animals were kept in the laboratory under constant temperature (24 ± 2°C) for at least one week before and
throughout the experimental work. They were maintained on a standard diet and water was available ad libitum. Animals were divided into 4 groups. Group1: animals of this group (20 rats) were orally given pyrethroid insecticide, deltamethrin [(S)-alpha-cyano-3-phenoxybenzyl- (1R, cis) 2, 2-dimethyl-3-(2, 2-ibromovinyl) - cyclopropane carboxylate] at a dose level of 12 mg/kg body weight, 4 times per week for 3 weeks. Group2: animals in this group (20 rats) were given the same dose of deltamethrin of group 1 followed by silymarin at a daily dose of 25 mg /kg body weight. Animals in the third group (10 rats) were given silymarin and those in group 4 (10 rats) were served as control. The treated animals and their controls were sacrificed by decapitation after 1, 2 and 3 weeks of treatment. Liver was removed and fixed in Bouin’s fluid. Fixed materials were embedded in paraffin wax and sections of 5 microns thickness were cut. Slides were stained with haematoxylin and eosin for histological examination. For biochemical study sera were obtained by centrifugation of the blood Samples and stored at 20°C until assayed for the biochemical parameters. Total proteins, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using a fully automated Hitachi 911 analyzer (Tokyo, Japan). A commercial randox kits (Randox Laboratories, LTD, Ardomre, Crumlin, United Kingdom) were used in these analysis.

Statistical analysis:
The results are given as mean ± standard deviation (X± S.D.). Significance of the differences was tested by the Student “t” test. The levels of significance were taken at P <0.05.

RESULTS
Tables 1-3 show the effect of treatment of deltamethrin or deltamethrin plus silymarin on serum AST, ALT and total proteins. The results revealed a significant increase (P<0.05) in AST (Table 1) and ALT (Table 2) activities after the second and third weeks of treatment with deltamethrin. On the other hand, serum total proteins was significantly decreased after the same periods of treatment (Table 3). An improvement in these parameters was observed in the group of rats treated with deltamethrin and silymarin, where non significant changes were recorded in this group compared to the control group.

Figure 1 shows histological structure of liver of control rat. Microscopic examination of liver of deltamethrin -treated rats revealed many histopathological alterations which are correlated with the increase of treatment time. After one week post-treatment with the insecticide, liver sections reflected signs of injury as indicated by congestion of intrahepatic veins, central and portal (Fig. 2). Some hepatic cells showed degenerated cytoplasm with pyknotic or karyolysed nuclei. Infiltrations by large mass of leucocytic inflammatory cells were observed (Fig. 3).
After two weeks, most of the hepatocytes appeared with vacuolation of the cytoplasm which is so extensive to the extent that only a very limited portion of it was left (Fig. 4). The nuclei of these cells were pyknotic and the cell membrane was disrupted. Examination of liver sections of animals treated with deltamethrin for 3 weeks reflected more advanced degree of injury as indicated by fatty degeneration with karyolysed nuclei (Fig. 5).

The hepatic sinusoids were invaded with lymphocytic inflammatory cells and the blood vessels showed severe congestion. Liver sections of animals treated with deltamethrin and silymarin showed that the liver tissue acquired some improvement compared with deltamethrin group. Slight congestion of blood vessels and few leucocytic infiltrations were recorded after two weeks of treatment (Fig. 6).

DISCUSSION

The present study demonstrates the adverse effect of deltamethrin on hepatic tissue and its enzymatic activities. Deltamethrin was found to affect serum transaminases (AST, ALT). The activity of these enzymes increased after the second and third weeks of treatment. In this concern, Abu-El-Zahab et al. (1993) reported that serum, GOT, GPT and alkaline phosphatase showed a significant increase in rats that had inhaled mixed pyrethroids (tetramethrin and sumithrin) and that the increase was proportional to the duration time of inhalation. The activity of these enzymes also elevated in rats treated with the pyrethroid, fenvalerate (Foldstorm et al., 1988) and after dermal application of baythroid (El-Elimay, 1986). It was reported that transaminases were considered to be a more sensitive measure in evaluating liver function and damage (Sherlock, 1981). Hatoff and Hardison mentioned (1980) reported that elevations in serum levels of these enzymes were mostly attributed to acute hepatocellular damage or extrahepatic obstruction, or both. Treating rats with deltamethrin caused a decrease of serum total proteins. Similarly, Parker et al. (1983); Saleh et al. (1986) and Abu El- Zahab et al. (1993) stated that the total serum proteins were decreased in animals treated with various pyrethroids. These...
investigators attributed the reduction in protein content partially to the decreased level of protein synthesis. Histopathological results recorded in the present work indicated that deltamethrin induced many alterations. These are cytoplasmic vacuolation of the hepatocytes, necrosis, remarkable abundance of leucocytic infiltrations, blood vessels congestion and fatty infiltrations. Similar observations were observed in liver of rats exposed to fenvalerate (Lamfon, 2007) and tetramethrin (Sakr, 1999). Pyrethroid insecticide, Ezalo, induced many histopathological changes in the liver of adult and embryonic mice (El-Banawy et al., 2000). Goden et al. (2007) reported that while pyrethroid pesticides are used in preference to organophosphates and organochlorines due to their high effectiveness, low toxicity to non-target organisms and easy biodegradability, they may also produce oxidative stress. Deltamethrin was found to induce lipid peroxidation in liver and kidney of male albino mice. The activities of vital antioxidant enzymes glutathione peroxidase, glutathione S-transferase and catalase were also suppressed in both the tissues (Rehman et al., 2006).

Animals treated with deltamethrin and silymarin revealed an improvement in liver enzyme activity and histological changes. This indicated the effectiveness of silymarin in prevention of deltamethrin hepatotoxicity. Silymarin has clinical applications in the treatment of cirrhosis, ischemic injury, and toxic hepatitis induced by various toxins such as ethanol, carbon tetrachloride, acetaminophen, organic solvents, and toxic mushroom (Chrugoo et al., 1997; Rastogi et al., 2000). The pharmacological properties of silymarin involve the regulation of cell membrane permeability and integrity, inhibition of leukotriene, reactive oxygen species scavenging, suppression of NF-κB activity, depression of protein kinases, and collagen production (Saller et al., 2001). It has been recently shown that silymarin can potentiate doxorubicin cytotoxicity by inhibiting P-glycoprotein-mediated drug efflux (Zhang and Morris, 2003). Silymarin have protective effect against acute viral hepatitis and have therapeutic influence on the characteristic increased serum levels of bilirubin, GOT and GPT associated with viral hepatitis (Stickel and Schuppan, 2007). It also has a hepatoprotective effects on cellular immune parameters of patients with histologically proven chronic alcoholic liver disease (Deak et al., 1990). Mourelle et al. (1988) reported that silymarin improved the biochemical indicator of liver damage induced by thallium and this possibly related to the ability of silymarin to scavenge free oxygen radicals. Carini et al. (1992) reported that silyplhe have stimulating effect on hepatic synthesis of RNA and proteins.

The antioxidant and free radical scavenging activities of silymarin were studied by several investigators. Silymarin restored the rifampicin- and/or pyrogalol-induced alterations in the activity of glutathione-S-transferase, glutathione reductase, and glutathione peroxidase, and lipid peroxidation (Upadhayay et al., 2007). It inhibits lipid peroxidation of the hepatocyte (Flora et al., 1998) and increases the activity of antioxidant enzymes, superoxide dismutase and glutathione peroxidase (Altarjay et al., 1992). Thus the present work showed that silymarin improved the liver injury induced by deltamethrin and this effect may attribute to its antioxidant and free radicals scavenging properties.

REFERENCES


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