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Protective effect of silymarin on metiram fungicide-induced hepatotoxicity in albino rats

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ABSTRACT

Metiram is a member of the ethylene bis dithiocarbamate used to prevent crop damage in field crops and fruits. Silymarin, an extract from Silybum marianum and showed many medicinal uses. The present work studied the effect of metiram on the liver of albino rats and the possible protective role of silymarin. Treating animals with metiram induced many histological changes in the liver including congestion of blood vessels, cytoplasmic vacuolization of the hepatocytes, leucocytic infiltrations and fatty degeneration. Moreover, the expression of proliferating cell nuclear antigen (PCNA) was increased in the hepatocytes. The liver enzymes, aspartate aminotransferase (ALT) and alanine aminotransferase (AST) were increased in the sera of treated rats. Treating animals with metiram and silymarin led to an improvement in both the histological and biochemical alterations induced by metalaxyl. Moreover, the expression of proliferating cell nuclear antigen (PCNA) decreased. It is concluded from the present results that protective effect of silymarin against hepatic damage induced by metiram may be due to its antioxidant properties.

Keywords: Metiram, Silymarin, Hepatotoxicity, Transaminase, PCNA

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INTRODUCTION

Fungicides are applied against wide range of fungal diseases of field crops, fruits, nuts and ornamentals. Metiram is a member of the ethylene bis dithiocarbamate group of fungicides, which include the related active ingredients mancozeb and maneb. It is used on apples and ornamental crops to prevent crop damage in the field and to protect harvested crops from deterioration in storage or transport [3]. On the other hand, metiram showed toxicity to mammalian animals. Using alkaline unwinding assay DNA, [9] reported that metiram is one of the pesticides which showed genotoxic effect. Dermal administration of metiram resulted in minimal to moderate exfoliation and ulcerative dermatitis in the skin of rabbits treated at the high-dose level [26, 31] reported follicular hyperplasia in thyroid of female rhesus monkeys treated with metiram. The effect of the fungicides (maneb, metiram, and ziram) on human natural killer (NK) cells cytotoxic function was studied by [33]. The results provide evidence of relative toxic potential for these compounds and the immunomodulatory effects on both T and NK lymphocyte function. Thyroid effects observed in subchronic studies in rats include increased thyroid weights, increased thyroid stimulating hormone (TSH) and decreased T4 (serum thyroxin) values [24, 31] reported that metiram induced histopathological as well as biochemical alterations in the liver of albino mice.

Silymarin, an extract from seeds and fruits of Silybum marianum, is a mixture of flavonoids isomers such as silibinin, isosilibinin, silidianin, and silichristin [14]. Silymarin has been used in the prevention of alcoholic liver disease [21], and it protected against injury from various other hepatotoxicants such as carbon tetrachloride, paracetamol [4, 19] and concanavalin A [25]. It showed activity against lipid peroxidation and oxidative stress [2]. The aim of this study is to evaluate the protective effect of silymarin supplement on liver of rats treated with metiram.

MATERIALS AND METHODS

Sexually mature male albino rats weighing 150 ±5 g were used in the present work. The animals were kept in the laboratory under constant temperature (22±1^{o}C) for at least one week before and along the period of the experimental work. They were maintained on a standard rodent diet composed of 20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitaminzed starch. Water was available ad libitum. Animals were divided into 4 groups:

Group1: Animal of his group (20 rats) were orally given 1/10 LD_{50} (284 mg/kg b.w.) of metiram daily for 8 weeks dissolved in distilled water. It consists of 80% active ingredients [zinc ammoniate ethylenebis (dithiocarbamate)-poly (ethylenethiuram disulfide) and 20% inert ingredients.

Group2: animals in this group (20 rats) were given the same dose of metiram of group 1 followed by silymarin at a daily dose of 25 mg /kg body weight.

Group3: animals (10 rats) were given silymarin.

Group4: These animals (10 rats) were served as control.

Histological and immunohistochemical studies

The treated animals and their controls were sacrificed by decapitation after 4 and8 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.

Immunohistochemical staining of PCNA

Paraffin-embedded rat liver sections were deparaffinized and hydrated. Endogenous peroxidase activity was blocked by incubation using 3% H2O2 for 5 min. The tissue sections were incubated over night with proliferating cell nuclear antigen (PCNA) monoclonal antibody (Dako Corporation, Carpentaria, CA, USA) and washed with phosphate buffer saline (PBS) for 5 min. The monoclonal antibody was then linked with biotinylated goat anti-mouse IgG antibody (Daco, LASB Universal Kit) for 30 min. After being washed with PBS for 5 min, the sections were incubated with streptavidin-conjugated peroxidase for 30 min. A brown coloured reaction was developed by exposing sections to 3, 3-diaminobenzidine (DAB) tetrahydrochloride solution for 5 min and washed in distilled water. Sections were counterstained with haematoxylin and eosin [7]. The number of PCNA-positive cells was counted in 10 randomly selected sections and non overlapping fields and expressed as the number of PCNA positive cells/mm^2.

Biochemical study

For biochemical study sera were obtained by centrifugation of the blood Samples and stored at 2O°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

Histological observations

The histological structure of the liver of control rat is shown in Fig.1a. Animals given silymarin for 8 weeks showed the same histological observations as in the liver of control ones. Examination of liver of metiram-treated rats for 4 weeks appeared with signs of degenerative changes. The normal structural organization of the hepatic lobules was impaired and the

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characteristic cord-like arrangement of the normal liver cells was lost. In addition, areas of leucocytic infiltrations (Fig.1b) and congestion of blood vessel and proliferation of bile duct were observed (Fig.1c). Examination of liver sections of animals treated with metiram for 8 weeks reflected more advanced degree of injury as indicated by cytoplasmic vacuolation of the hepatocytes and activation of kupffer cells (Fig. 2a). Moreover an obvious fatty degeneration indicated by large number of fatty droplets with different size was observed (Fig. 2b).Examination of liver sections obtained from animals treated with metiram and silymarin showed an obvious degree of improvement which is correlated with the increase of treatment time. However, the sinusoidal spaces appear somewhat wide and kupffer cells were activated (Fig.2c).

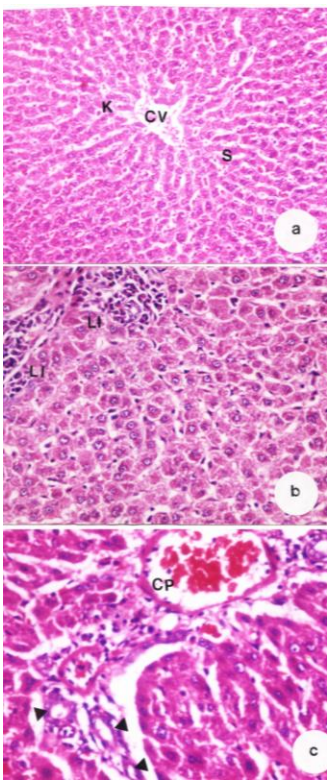

Fig 1(a, b, c)

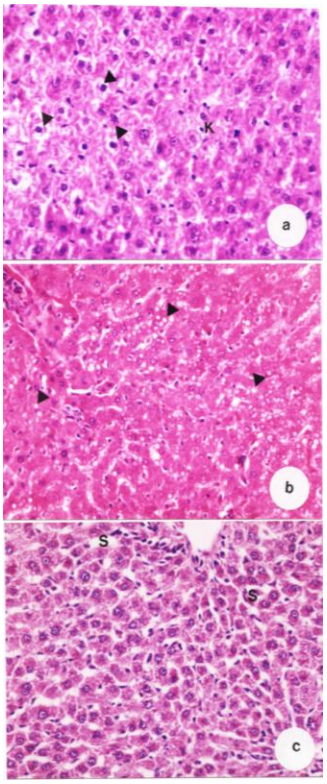

Fig 2(a, b, c)

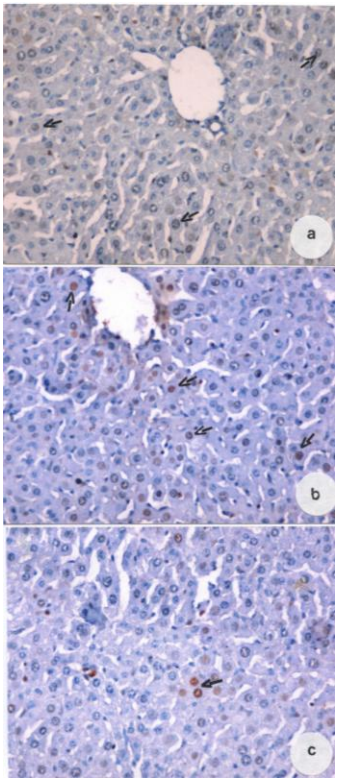

Fig 3(a, b, c)

Fig 1:Sections in liver of (a): a control rat showing central vein (CV), Kupffer cells (K) and Sinusoids (S) (X120).(b): a rat treated with metiram for 4 weeks showing leucocytic infiltrations (LI) (X 300).(c): a treated rat showing congested portal vein (CP) and bile duct proliferation (arrow heads) (X 300).

Fig 2: Section in liver of (a): a rat treated with metiram for 8 weeks showing hepatocytes with cytoplasmic vaculation (arrow heads) and activated Kupffer cells (K), (X 300). (b): a treated rat showing fatty infiltrations (X300). (c): a rat treated with metiram and silymarin showing normal arrangement of hepatocytes and wide sinusoids (S) (X300).

Fig 3: (a): Section of liver of a control rat showing few cells with PCNA expression (arrows). (b): after treatment with metiram showing large number of cells with PCNA expression (arrows). (c): after treatment with metiram and silymarin showing few cells with PCNA expression (PCNA immunohistochemical stain, X300).

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Immunohistochemical results

Few hepatocytes nuclei of liver of control animals and those given silymarin displayed faint stain of PCNA (Fig. 3a). Animals treated with metiram showed an increase in the number of hepatocytes stained PCNA as compared with control group (Fig. 3b). Treating animals with metiram and silymarin for 8 weeks showed stimulation of DNA synthesis and increased PCNA expression in the hepatocytes (Fig. 3c). Figure (4) showed PCNA-labeling index in different groups after 8 weeks of different treatments. This index is significantly higher ($P<0.05$) in rats treated with metiram and silymarin compared with those treated with metiram.

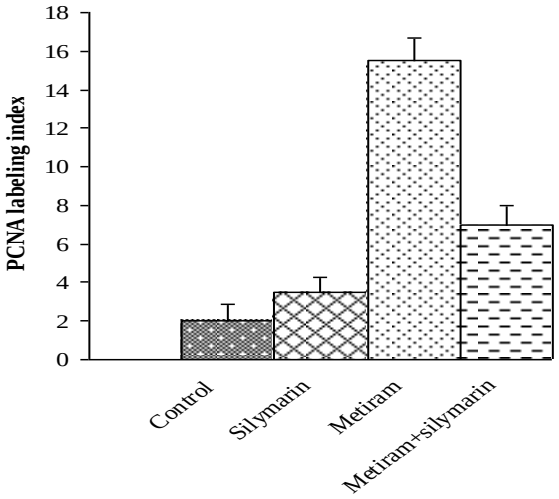

Fig 4: PCNA labeling index in hepatic cells of experimental groups

Biochemical results

Figure (5a) showed the effect of different treatments on serum ALT activity. There was non-significant ($P<0.05$) difference in serum ALT activity in rats treated with silymarin in comparison with control group. Animals with metiram for 4 and 8 weeks showed significantly increase in ALT. On the other hand, a significant decrease in ALT activity was recorded in animals given metiram and silymarin when compared with metiram group. Data in figure (5b) revealed that treating animals with metiram for 4 and 8 weeks revealed significant increase in AST activity. On the other hand animals treated with metiram and silymarin revealed a significant decrease in AST activity. There was non-significant ($P<0.05$) difference in serum AST activity in animals treated with silymarin in comparison with control.

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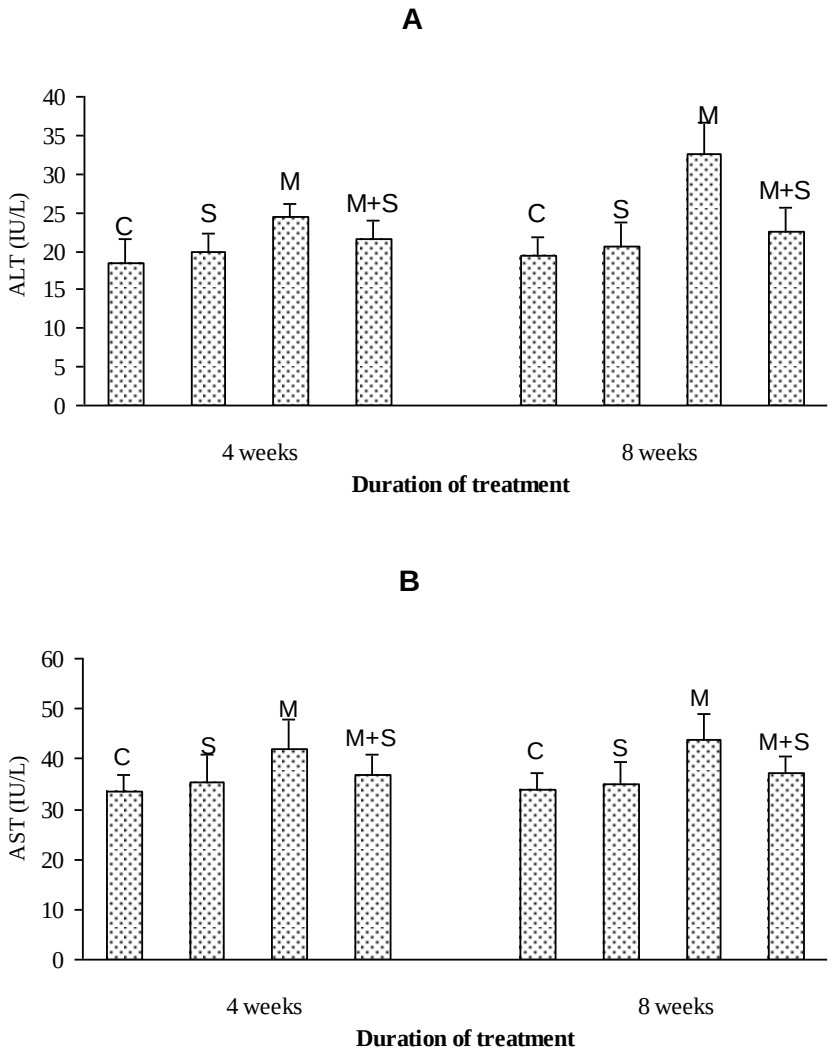

Fig 5: Change in ALT (A) and AST (B) in different animal Groups, C: control, M: metiram, S: Silymarin.

DISCUSSION

Results obtained in the present study showed that metiram induced histopathological as well as biochemical alterations in the liver of rats. Destruction of liver architecture, inflammatory leucocytic infiltration and fatty degeneration were the most pathological features observed in the liver of metiram-treated rats. Moreover, transaminases, ALT and AST were elevated in sera of these animals. Similar results were obtained by Sakr [24] in liver of mice given metiram. The harmful effects of fungicides were studied in different animals. [28] reported that dithiocarbamates (DTCs) fungicides (e.g.metiram) have toxic effects on liver, kidney and testis. [16] studied the pathological changes in male and female mice given the fungicides, maneb and zineb. Internal organs (liver and kidneys) of the experimental mice were found to be heavier and darker in color than those of the controls. Vein congestion and mononuclear inflammatory cell infiltrations were observed in these organs. [29 reported that

metam sodium fungicide caused liver injury and inflammation. [22] reported that treating albino rats with mancozeb fungicide induce various histological changes in the liver. These changes include congestion of blood vessels, leucocytic infiltration, cytoplasmic vacuolization of the hepatocytes and pyknosis.

Biochemical results showed that treatment with metiram induced a significant increase in activity of serum ALT and AST. This result is in agreement with that of [20, 22] who reported that oral administration of mancozeb to male rats induced changes in the activities of ALT and AST throughout the period of the study in a dose- dependent manner. [10] reported that the fungicide bithionol sulfoxide at high doses (50,500 and 1000 mg/kg) caused hepatotoxicity including an increase in serum AST. [23] observed that metalaxyl fungicide induced many histological changes in liver of mice and increased liver enzymes (transaminases). The increased value of ALT and AST in sera of metiram-treated animals is an indicator of hepatic damage.

Proliferating cell nuclear antigen (PCNA) is a well-known 36-kDa nuclear matrix protein, which is essential for multiple cell cycle pathways, including DNA replication, DNA elongation (leading strand synthesis), and DNA excision repair [11]. The immunohistochemical result indicated that treatment with metiram increased PCNA expression in liver hepatocytes. This result is in egreement with [12, 24] reported that proliferating cell nuclear antigen (PCNA) elevated in hepatocytes of male Sprague-Dawley rats injected intraperitioneally with a 12-fold dose range of thioacetamide fungicide. [32] showed that in folpet- treated animals a greater degree of PCNA staining was recorded in the duodenum than control. [8] reported the PCNA-labelling indices of renal tubule cells were elevated in rats treated with captafol.

Treating animals with metiram and silymarin revealed restoration of most of the damaged tissues to normal status and the values of ALT and AST appeared normal. This indicated the effectiveness of silymarin in prevention of metiram induced- hepatotoxicity. It was reported that silymarin has beneficial effects 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 [4, 19]. The pharmacological properties of silymarin involve the regulation of cell membrane permeability and integrity, inhibition of leukotriene, reactive oxygen species scavenging, suppression of NF-B activity, depression of protein kinases, and collagen production [21, 34] reported that silymarin can potentiate doxorubicin cytotoxicity by inhibiting P-glycoprotein–mediated drug efflux. 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 [27]. It also has a hepatoprotective effects on cellular immune parameters of patients with histological proven chronic alcoholic liver disease [5, 15] 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. [2] reported that silipde have stimulating effect on hepatic synthesis of RNA and proteins. [18] reported that silymarin has protective effect against hepatotoxicity and cardiotoxicity of doxorubicin. Silymarin prevents liver damage by maintaining the integrity of the plasma membrane, thereby suppressing the leakage of enzymes [17].

Silymarin is a free-radical scavenger and a member stabilizer which prevents lipoperoxidation and its associated cell damage. It restored the rifampicin- and/or pyrogallol-induced alterations in the activity of glutathione-S-transferase, glutathione reductase, and glutathione peroxidase, and lipid peroxidation [6, 30] reported that silymarin inhibited lipid peroxidation of the hepatocyte. It also increased the activity of antioxidant enzymes, superoxide dismutase and glutathion peroxidase [1]. It is suggested from the present results that metiram may generate free radicals that play a role in pathogenesis of metiram and silymarin, as antioxidant, has protective effect against hepatotoxicity of metiram.

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