EFFECT OF SILYMARIN ON HISTOPATHOLOGICAL AND HISTOCHEMICAL LESIONS INDUCED BY THE ANTIMALARIAL DRUG CHLOROQUINE IN THE LIVER OF ALBINO MICE

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ABSTRACT

The hepatoprotective effect of silymarin on histopathological and histochemical alterations induced by the antimalarial drug, chloroquine was studied in albino mice. Administration of chloroquine at a dose level of 10 mg/kg body weight, 4 times per week for 3 weeks induced many histopathological changes in the liver such as leucocytic infiltrations, cytoplasmic vacuolization of hepatocytes and congestion of blood vessels. Histochemical observations revealed marked reduction in total carbohydrates, DNA and total protein contents in the hepatocytes. Treating animals with chloroquine and silymarin led to an improvement in both histopathological and histochemical alterations. The hepatoprotective effect of silymarin was attributed to its antioxidant and free radicals scavenging properties.

Key words: Silymarin, chloroquine, liver, histopathology, histochemistry, mice

INTRODUCTION

Antimalarial drugs are widely used in many countries against different malarial parasites such as Plasmodium malaria and P. ovale (Modell, 1974). Chloroquine is one of the antimalarial drugs which classified as blood schizonticides. Although antimalarial drugs are generally well tolerated at the recommended therapeutic dosage, clinically important side effects were reviewed. Prolonged therapy with chloroquine produced various reactions including alopecia, gray hair, leukopenia, blurred vision, retinitis pigmentosa, and toxic psychosis (Knox and Freeman, 1963). Chloroquine caused decrease in liver weight in offsprings specially in those which were exposed to drug during second and third week of pregnancy (Zahid and Ibidi, 2003a). It also induced histological as well as biochemical changes in the liver. Chloroquine induced considerable endothelial cell injury in the liver of newborn rat with many areas of interahepatic hemorrhage due to complete destruction of endothelial lining of central veins (Zahid and Abidi, 2003b). Deepalakshmi et al. (1994) reported an increase in phospholipid and a decrease in cholesterol in liver mitochondrial membrane after treating rats with Chloroquine. A significant decrease in the activities of mitochondrial inner membrane enzyme such as NADH dehydrogenase, succinate dehydrogenase and cytochrome C oxidase was observed. El-Mofty et al. (1992) reported that chloroquine induced lymphosarcomas in 14% of the toads Bufo regularis.

Silymarin is a seed extract of the milk thistle Silybum marianum (Morazzoni and Bombardelliu, 1995). It is one of the hepatoprotective drugs belong to the group of free-radical scavengers and was useful in treatment of liver damage induced by different agents (Mourelle et al., 1988; Chrunoo et al., 1997; Rastogi et al., 2000) and viral hepatitis (Magluido et al., 1978). The present work was conducted to study the effect of silymarin on histopathological and histochemical alterations induced in the liver of mice by the antimalarial drug chloroquine.

MATERIALS AND METHODS

Sexually mature male Swiss albino mice weighting 20 ± 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 mice) were orally given chloroquine (Egypt Pharmacies) at a dose level of 10 mg/kg body weight, 4 times per week for 3 weeks. Group2: animals in this group (20 mice) were given the same dose of chloroquine of group 1 followed by silymarin at a daily dose of 25 mg /kg body weight. Animals in the third group (10 mice) were given silymarin and those in group 4 (10 mice) 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 alcoholic Bouin's fluid and 10% neutral formalin. 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
histochemical demonstration of total carbohydrates Periodic Acid Schiff's (PAS) technique (Hotchkiss, 1948) was used. Total proteins were detected using the mercury bromophenol blue method (Mazia et al., 1953), and DNA was detected using Feulgen reaction (Stowel, 1945).

Figure 1 showed normal liver structure of control mouse. Examination of liver sections obtained from mice treated with chloroquine for one week has displayed slight signs of injury like inflammatory leucocytes infiltration comprised mainly of lymphocytes and sparse eosinophils (Fig. 2). After 2 weeks, the intrahepatic blood vessels, central and portal veins were congested and their lining epithelia were eroded. The liver lobules lost most of its

RESULTS

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structural organization, the hepatocytes showed giant nuclei with vacuolated cytoplasm. The sinusoidal spaces were infiltrated by masses of inflammatory leucocytes. In liver of mice examined after 3 weeks, injurious symptoms were severe, the liver tissue had apparently lost their characteristic architectural organization. The individual hepatocytes were deteriorated and most of them were suffering from cytoplasmic vacuolization (Fig. 3).

Liver of mice maintained on chloroquine and silymarin for one week showed inflammatory leucocytic infiltrations and congestion of blood vessels (Fig. b). After 2 weeks of treatment with chloroquine and silymarin, the liver tissue restored some of its normal structure but some hepatocytes appeared with cytoplasmic vacuolization (Fig. 5). After 3 weeks, the liver tissue indicated an obvious degree of improvement with the appearance of large number of binucleated hepatocytes (Fig. 6).
Histochemical observation of total carbohydrates in control mice showed the deeply stained reddish granules in the cytoplasm and negative reaction nuclei of the hepatic cells as shown by PAS reaction (Fig. 7). Liver of animals treated with chloroquine and examined after 1 week showed slight decrease in carbohydrate in the cytoplasm of most hepatocytes. Such reduction of carbohydrate markedly appeared in the liver of animals examined after 2 weeks. After 3 weeks there was an obvious reduction in total carbohydrate contents of hepatocytes (Fig. 8). Animal treated with chloroquine and silymarin revealed an improvement and marked restoration in carbohydrate contents of the hepatocytes when compared with the same periods of chloroquine group (Fig. 9).

Total proteins in the liver cells of control mice were displayed in the form of small bluish irregular particles which were sometimes closely packed together making blue irregular dense bodies against a weakly to moderately stained ground cytoplasm. The cell membrane, nuclear membrane, chromatin materials and nucleoli were positively stained (Fig. 10). Examination of liver of mice after 1 week of treatment with chloroquine showed a slight reduction of the protein contents in the liver tissue in comparison with normal animals. After 2 weeks, a marked diminution in the amount of protein materials was observed in the liver cells. After 3 weeks of treatment a large number of cells extensively vacuolated and nearly devoid of protein which still as a rim around each hepatocyte (Fig. 11). Animals treated with chloroquine and silymarin for 2 weeks showed that the hepatocytes appeared with noticeable amount of total proteins. After 3 weeks, most of the hepatocytes regained their normal contents of proteins (Fig. 12).

Using Feulgen reaction, DNA - containing particles (chromatin) appeared in the form of densely stained red particles distributed in the nucleoplasm and the peripheral rim of the nuclei (Fig. 13). Hepatic cells of mice examined 1 week after treatment with chloroquine showed a weak Feulgen reaction of their chromatin granules indicating a reduced amount of DNA. The decrease in DNA became obvious in animals examined after 2 and 3 (Fig. 14). Two and three weeks following silymarin treatment in addition to chloroquine, most of the nuclei acquired normal amount of DNA containing particles (Fig. 15).

**DISCUSSION**

Results obtained in the present work showed that chloroquine induced many histopathological alterations in the liver of mice represented by leucocytic infiltrations, congestion of blood vessels and cytoplasmic vacuolation of the hepatocytes. These results are in agreement with different investigators who reported that chloroquine affected the liver. Dass and Shah (2000) reported that chloroquine caused hepatonecrosis in rats. Ogunbanwo et al. (2001) studied the trypanosomicidal effects of four antiprotozoal drugs including chloroquine in infected rats. Their histopathological findings indicated inflammatory reactions characterized by infiltration to variable degree in the majority of tissues, mostly in the liver and lungs. The most consistent lesions were interstitial pneumonia, multifocal necrosis and oedema. Sharma and Rawat (1989) reported that chloroquine treatment resulted in hepatomegaly and several structural abnormalities in the rats fetus. Chloroquine produced interahepatic hemorrhage and necrosis in albino rats (Zahid and Abidi, 2003a).
It has been reported that hepatotoxicity induced by chloroquine was manifested by alterations of liver enzymes that are associated with hepatocellular damage. Administration of chloroquine to rats caused marked decrease in alkaline phosphatase activity and increase lactate dehydrogenase in liver of rats (Achudume et al., 1998). Jarzyna et al. (1997) studied the effect of chloroquine on glutamate dehydrogenase activity in liver and renal mitochondria. Their results revealed that chloroquine decreased both glutamate synthesis and glutamate deamination. Chloroquine was found to affect antioxidant enzymes, superoxide dismutase and glutathione peroxidase, in liver and kidney (Magwere et al., 1997), and caused an increase in phospholipid and a decrease in cholestrol in liver mitochondrial membrane (Deepalakshmi et al., 1996).

The present investigation reveal that glycogen, total proteins and DNA decreased in liver cells with prolonged treatment with chloroquine. A similar decrease in these materials was reported by some authors. Chloroquine was found to have a hypoglycemic action due to its inhibitory effect on glutamate dehydrogenase activity (Jarzyna et al., 2001). Abdel-Gayoum et al. (1992) reported that chloroquine affected the carbohydrate metabolism and reduced plasma glucose level by 17% in rats. Chloroquine resulted in inhibition of several metabolic pathways in liver and brain of developing rat fetus. This included the inhibition of protein, RNA and DNA metabolism (Sharma and Rawat, 1989). Degradation of low density lipoprotein was induced by chloroquine in rat liver (Stein et al., 1977). Rustan et al. (1989) reported that chloroquine inhibited both cellular protein synthesis and protein secretion. Chloroquine inhibited thymidine incorporation into DNA of rat tissues (Field et al., 1978) and inhibited DNA, RNA and protein biosynthesis in murine thymus cells (Nghiem et al., 1987).

Chloroquine is a lysosomotropic agent. Korolenko et al. (1990) reported an increase in acid hydrolases activity and proteinases after its administration. Thus the decrease in proteins and DNA observed in the present work is related to the effect of chloroquine on the lysosomes which release nucleases and proteinases affecting protein and DNA metabolism.

Animals treated with chloroquine and silymarin revealed an improvement in histopathological and histochemical changes. This indicated the effectiveness of silymarin in prevention of chloroquine hepatotoxicity. Silymarin has been shown to be protective against various liver diseases. 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 (Magliulo et al., 1978). It also have a hepatoprotective effects on cellular immune parameters of patients with histological proven chronic alcoholic liver disease (Deak et al., 1990). Silymarin modulate the hepatotoxicity induced by carbon tetrachloride, paracetamol and D-galactosamine in isolated rat hepatocytes (Chungo et al., 1997). 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.

Silymarin improved the glycogen contents, total proteins and DNA in hepatocytes of chloroquine- treated mice. Similarly, Rastogi et al. (2000) mentioned that silymarin improved glycogen, DNA and RNA in liver of rats injected with aflatoxin-B1. Carini et al. (1992) reported that silipde have stimulating effect on hepatic synthesis of RNA and proteins.

The hepatoprotective action of silymarin rests with a strong antioxidant and free radical scavenging activity, this provided silymarin with the ability to protect the hepatocyte membrane against oxidative damage (Flora et al., 1998). It inhibits lipid peroxidation of the hepatocyte (Bosisio et al., 1992) and increases the activity of antioxidant enzymes, superoxide dismutase and glutathione peroxidase (Altajr et al., 1992). Thus the present work indicated that silymarin improved the liver damage induced by chloroquine and the protective effect of silymarin was attributed to its antioxidant and free radicals scavenging properties.

REFERENCES


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