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ASSESSING INDUCED EFFECT OF CURCUMIN ON METHIMAZOLE HEPATIC DAMAGE IN ALBINO RATS: A HISTOLOGICAL AND HISTOCHEMICAL STUDY

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ABSTRACT

The present study investigates the possible protective effect of curcumin on Methimazole (MMZ). It is an anti-thyroid drug induced hepatotoxicity in albino rats. In this study the rats were randomly divided into four experimental groups; first group was used as control treated with a standard diet. The second group was treated with curcumin at dose of 150 mg/kg body weight. The third group was treated with the MMZ dissolved in drinking water at dose of 60mg/kg body weight. The fourth group was treated with a liquid mixture of MMZ and curcumin. The rats were dissected after 6 weeks of treatment and livers of these animal were collected separately and used for histological and histochemical methods. It was clear form the results obtained that the MMZ caused histological alterations in the liver tissue of the MMZ treated rats. These changes appeared as congestion of blood vessels, leucocytic infiltrations and cytoplasmic vaculation of the hepatocytes. The histochemical analysis has revealed a reduction in carbohydrates content, total proteins and DNA. The histochemical results of the same rats treated with MMZ showed an increase in serum level of ALT, AST and ALP. However, the group treated with curcumin showed a remarkable improvement in the histological structure of the liver tissue. The results revealed an increase in carbohydrates level, total proteins and DNA. In addition the Biochemical results of this group showed a noticeable decrease in the hepatic enzymes (ALT, AST and ALP). These finding have confirmed that curcumin is an effective agent with special antioxidant properties that prevent and protect the liver from histological and histochemical damages induced by MMZ.

Keywords. Methimazole, curcumin, hepatotoxicity, histochemistry, rats.

INTRODUCTION

Methimazole (MMZ) (1-methyl-2-mercaptoimidazole) is known as an antithyroid medication used to prevent thyroid hormone synthesis and described medically to treat hyperthyroidism. It has been documented in by Nakamura *et al*. (2007) that MMZ acts by interacting with thyroid peroxidase enzyme (TPO) function. This enzyme is expressed mainly in the thyroid and secreted into colloid. The MMZ function is to lower thyroid hormone levels and minimize the effects of thyroid manipulation by interrupting the iodination of tyrosine residues in thyroglobulin which cause a reduction in hormone secretion (Cooper, 2005). Another study, Aboul-Enein and Al-Badr (1979) have reported a similar observation confirming that when MMZ is absorbed by the thyroid gland it prevents the thyroid hormone production and stops the synthesis of hormone by oxidizing the anion iodide. However, it has been administrated that in 5-7% of patients taking MMZ different symptoms and side effects were reported including fever, skin rashes, gastrointestinal, loss of taste and neuropathy (Chevalley *et al*., 1954) and (Schmidt *et al*., 1986). In addition, agranulocytosis is another uncommon side effect of MMZ. This was occurred in three cases causing a death of one patient (Specht and Boehme, 1952).

Rivkees *et al*., (2010) has revealed that when one hundred consecutive pediatric patients were given MMZ other symptoms such as pruritus and hives were recognized in a few patients. Some other patients were suffered from diffuse arthralgia and joint agony and unusual symptoms known as neutropenia and Stevens-Johnson syndrome were noticed. One kid was suffered from a decrease in bile flow due to impaired secretion by hepatocytes, which is known as Cholestasis (Rivkees *et al*., 2010). It has been substantiated by Mikhail (2004) that MMZ could lead to a liver damage and injury causing intrahepatic cholestasis. While, Epeirier *et al*. (1996) has also reported that one patient had suffered from hyperthyroidism severe inflammation and hepatic necrosis as a result of 6 weeks treatment with MMZ carbimazole and propranolol.

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In some cases, it has been noticed that in a 43-year-old man suffering from hyperthyroidism and hepatitis B surface antigenemia acute hepatic failure was accrued during the treatment with MMZ (Epeirier *et al*., 1996) and (Kang *et al*., 1990). It was also reported that the needle biopsy post mortem examination showed micronodular cirrhosis characterized with mild septal/portal inflammation, cholestasis and scattered acidophilic bodies in liver tissue of some patient (Epeirier *et al*., 1996). Recently, Gallelli *et al*. (2015) has also stated that the MMZ has caused hepatotoxicity in a 54-year-old patient suffered from hyperthyroidism during treatment and biopsy examination of the liver tissue revealed diagnosis of cholestatic hepatitis in this patient. Morover, Tashkandi *et al*. (2014) has showed that hepatorenal toxicity was noticed in the rat's liver as a result of MMZ treatment.

Curcumin is the principle yellow pigments herbal plant from a species of Curcuma longa. It is a major component of turmeric and widely used in most tropical and subtropical region as a spice and food-coloring agent. It has been commercially used as a cosmetic component and also added into some medical preparations for some of its positive side effects (Menon and Sudheer, 2007). It has been commonly used in treatment of a wide variety of diseases and chronic illness to exhibit any anti-inflammatory, immunomodulatory, and anti-atherogenic effects (Srinivasan, 1952; Joe *et al*., 2004) and also described as a potent inhibitor of many reactive oxygen-generating enzymes (Chainani-Wu, 2003; Borra *et al*., 2013). It has been reported by Manikandan *et al*. (2004) and Venkatesan, (2000) that curcumin has a positive influence against free radical formation in animal rats suffered from myocardial ischemia and in lung toxicity.

Kadasa *et al*. (2015) has revealed in his study that curcumin showed a strong hepatoprotective effects against hepatocarcinogenesis caused by diethylnitrosamine (DENA) in rodents. He has also reported that curcumin improved the liver functions by modulating the hepatic enzymes serum levels and increasing the antioxidant hepatic system which cause a reduction in the pro-inflammatory cytokines (Kadasa *et al*., 2015). Palipoch *et al*. (2014) has demonstrated that a mixed of curcumin and α-tocopherol suppressed the induction of cisplatin and reduce the hepatotoxicity of liver. The present study aimed to investigate the effects of curcumin against the histological, histopathological and histochemical changes of hepatotoxicity induced by MMZ in albino rats.

MATERIALS AND METHODS

Animals

Mature healthy male albino rats were chosen for this experiment, they were weighted between 140-160g. The animals were housed in the animal care center and were treated and kept under normal conditions with minimal diurnal or seasonal variation in temperature, light cycle, or other environmental conditions. They were wire-floored cages under standard laboratory conditions of 12 h/12 h light/dark, 25 ± 2C ^{o} with free access to food and water in accordance with the Ethical Principles for the Care and Use of Laboratory Animals (NRC, 1995).

Experimental design

Animals were divided into four experimental groups:

Group 1. Rats were fed the standard diet and were served as control group.

Group 2. Rats were orally administrated curcumin at a dose of 150 mg/kg body weight daily for 6 weeks. Dry turmeric rhizomes of the plant *Curcuma longa* were purchased from a local market and were crashed into powder. The powder was then dissolved in distilled water and were orally given to the rats.

Group 3. Rats were orally given MMZ at a dose level of 60mg/kg body weight in distilled water. The solution was daily given to these animal for 6 weeks.

Group 4. Rats were given MMZ (60mg/kg body weight) followed by oral administration of curcumin extract dissolved in distilled water a dose level of 150mg/kg daily for 6 weeks.

Histological and Histochemical Examination

The treated animals and their controls were sacrificed by decapitation after 6 weeks of treatment and their liver were carefully separated and collected separately. The liver tissues were then washed in normal saline. Specimens were fixed in 10% phosphate buffered formalin (pH 7.4). Fixed materials were embedded in paraffin wax and then sections were cut at thickness of 5 micrometer. Slides were after stained with haematoxylin and eosin for histological observation. For the analysis of histochemical demonstration the total carbohydrates periodic acid Schiff's technique (PAS) (Kiernan, 1981) was applied. Total proteins were detected using the mercury bromophenol blue method (Pearse, 1972) and DNA was detected using Feulgen reaction (Kiernan, 1981).

Biochemical Assays

For biochemical measures, blood samples were taken and harvested from rats after 6 weeks of treatment. Sera were acquired by centrifugation of the blood samples and kept at -20°C. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were detected colourimetrically as indicated by Reitman and Frankel (1957). Alkalin phosphatase (ALP) activity was measured by the method of Belfield and Goldberg (1971) using reagent kits acquired from Bio-Merieux Chemical Company (France).

STATISTICAL ANALYSIS

The results were expressed as mean ± SD of various groups. The differences between the mean values were assessed by ANOVA followed by Student's "t" test using Minitab 12 PC program (Minitab Inc., State Collage, P. A).

RESULTS AND DISCUSSION

Histological Observations

Histological examination of control sample of the liver tissue showed normal architecture. The hepatocytes were found arranged at normal shape and strands around the central vein, the sinusoids appeared natural and containing normal Kupffer cells as shown in Figure 1A.

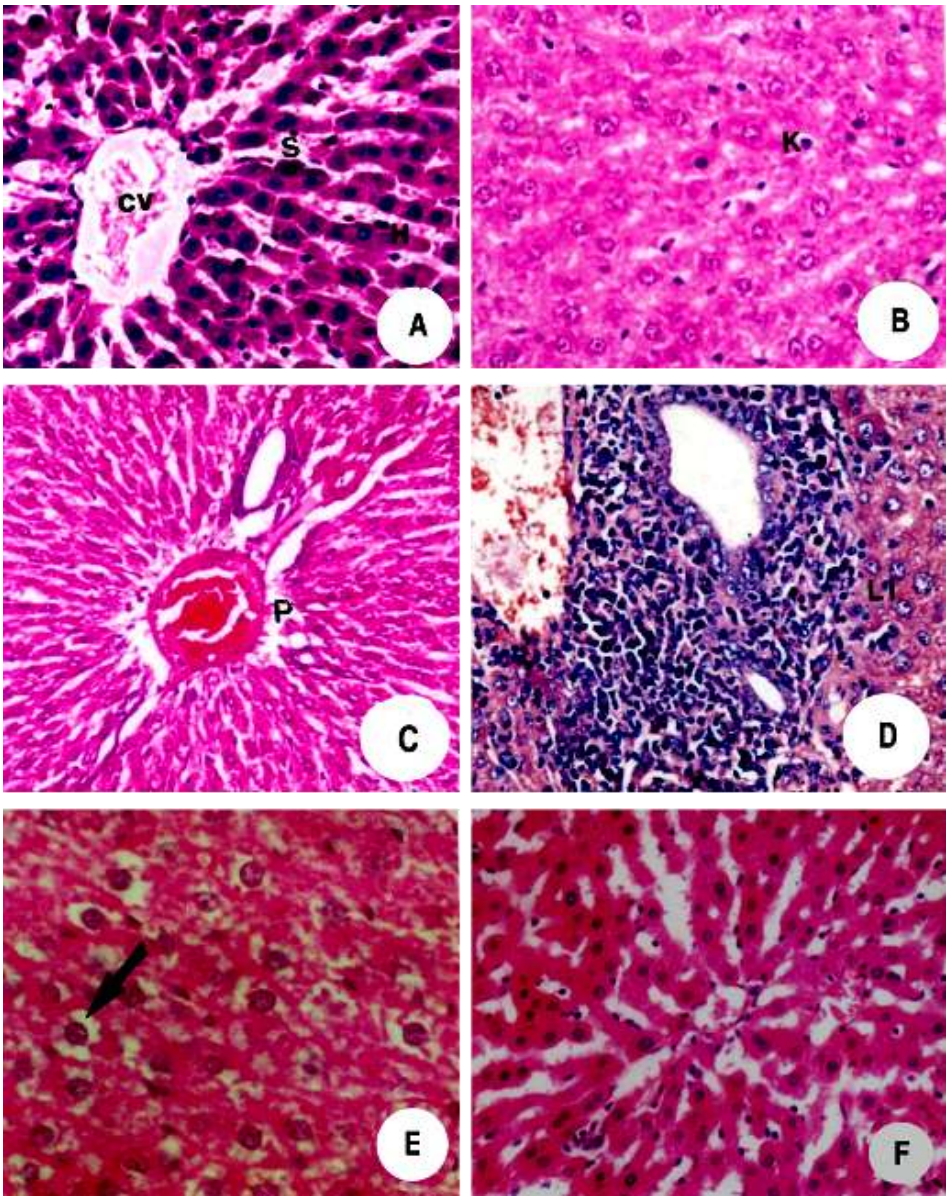

Fig. 1 A. Section in liver of a control rat showing hepatocytes (H), central vein (CV) and sinusoidal spaces(S), X 400.
B. Section in liver of a rat treated with curcumin showing activated kupffer cells (K),X400.
C. Section in liver of a rat treated with MMZ showing congested portal vein (P), X200.
D. Leucocytic infiltrations (LI) in liver of a rat treated with MMZ, X200.
E. Section in liver of a rat treated with MMZ showing cytoplasmic vacuolation of the hepatocytes (arrow), X400.
F. Section in liver of a rat treated with MMZ and curcumin showing an improvement in the liver structure,X200.

Animals treated with the curcumin solution appeared normal and no histopathological alterations were observed (Fig. 1B). On the other hand, animals treated with MMZ appeared to be congested with enlarged central and portal veins as shown in Figure 1C. Leucocytic infiltrations were observed in large areas of the liver tissue (Fig. 1D). The liver tissue treated with MMZ also showed cytoplasmic vacuolization of the hepatocytes with pyknotic nuclei (Fig. 1E). In the liver tissue of rats group treated with curcumin and MMZ different observations were obtained. In these sections there was a histological improvement in the structure of the liver tissue and the hepatocytes appeared to be normal with healthy nucleus and cytoplasm as shown in Figure 1F.

Histochemical Observations

Total carbohydrates of hepatocytes in the liver of control rats appeared as red or magenta color when stained with Schiff's reagent. The carbohydrates in the cells found to be concentrated at one pole, this is termed glycogen flight (Fig. 2A). The nuclei appeared entirely PAS-negative. In the liver cells of curcumin-treated animals the carbohydrates arrangement was natural and appeared normal. However, in the section of rats treated with MMZ the carbohydrates content was reduced as a consequence of MMZ treatment (Fig. 2B). In the section of rats treated with curcumin and MMZ, the observation was different, in this section as shown in Figure 2C, the carbohydrates content of the hepatocyte was increased significantly and appeared to be normal.

Total proteins contents in the control rats section appeared as blue color after staining with bromophenol blue. The proteins appeared as fine granules in the cytoplasm of the hepatocytes (Fig. 3A). Kupffer cells, endothelial lining cells of sinusoids and the walls of blood vessels exhibited strong stainability. In the rats treated with MMZ there was a noticeable dimension in the total protein contents in the liver cells (Fig. 3B). on the other hand, Hepatocytes of rats treated with curcumin and MMZ appeared with an increase in protein contents (Fig. 3C).

The DNA-containing particles (chromatin) appeared in the form of densely stained red particles distributed in the nucleoplasm and the peripheral rim of the nuclei of hepatocytes of control rats (Fig. 4A). Examination of hepatocytes of animals treated with MMZ revealed that most nuclei exhibited a weak Feulgen reaction of their chromatin granules (Fig. 4B). Animals treated with curcumin and MMZ showed that most of the nuclei appeared with normal amount of DNA-containing particles (Fig. 4C).

Biochemical Results

Treatment with MMZ for 6 weeks showed a highly significant increase ($P<0.05$) in the level of ALT and AST compared to the control animals. In contrast, these parameters were restored close to normal values in rats treated with curcumin and MMZ. Both control and curcumin treated rats showed no significant differences in serum content of ALT and AST (Figs. 5 and 6). Similarly, ALP was elevated in MMZ treated rats compared to the control group, and decreased in MMZ and curcumin treated group (Fig.7).

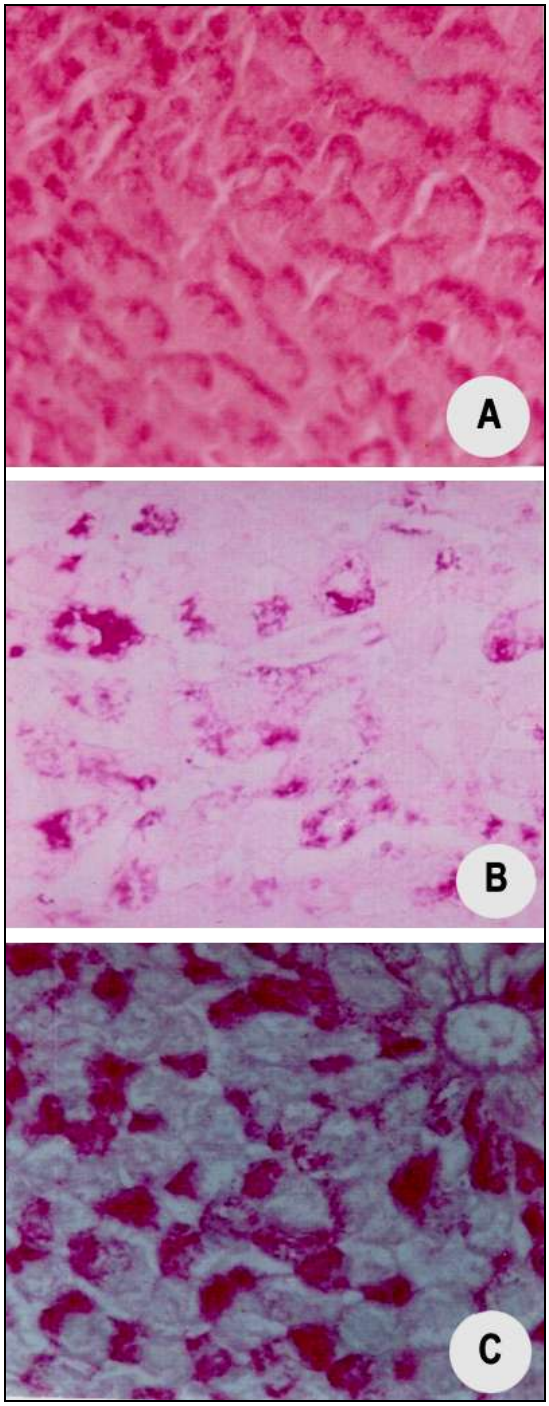

Fig. 2A. Section in liver of a control rat showing distribution of carbohydrates in the cytoplasm, X400. B. Section in liver of a rat treated with MMZ showing decrease of carbohydrates, X 400.

C. Restoration of carbohydrates in liver of a rat treated with MMZ and curcumin, X 400.

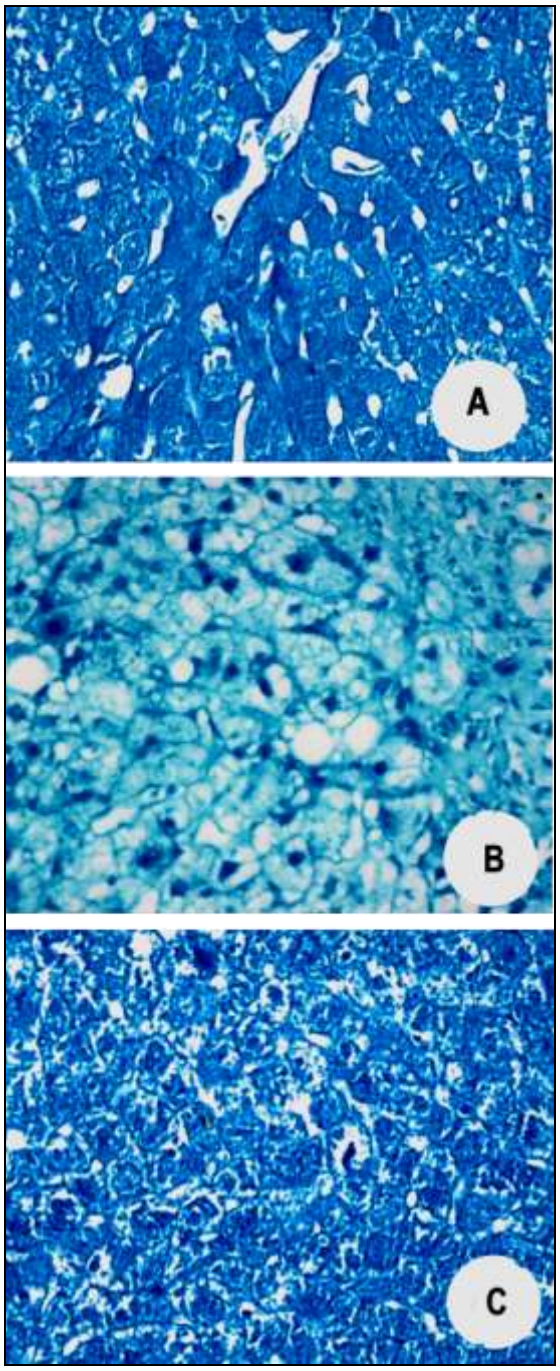

Fig. 3A. Section in liver of a control rat showing normal protein contents, X400
B. Reduction of proteins in liver of a rat treated with MMZ, X 400
C. An increase in protein contents in liver of a rat treated with MMZ and curcumin, X400.

Results our study showed that Methimazole caused histological, histochemical and biochemical changes in the liver of treated rats and induced hepatotoxicity and damage to the cells structure. Findings of our study is similar to the results reported by Tashkandi *et al*. (2014) and Zhao *et al*. (2014), when it has reported that MMZ leads to degenerative changes such as congestion of blood vessels, appearance of inflammatory infiltrative cells, cytoplasmic vacuolization of the hepatocytes and cell death. Moreover, ALT and AST levels were elevated in sera of the treated animals. It is well known that the normal level of AST and ALT serum play an essential role to maintain a normal liver function and any changes in these enzymes level cause a sever liver damage and could lead to dysfunction if the organ. Also in some studies done by (Cano-Europa *et al*., 2011; Gallelli *et al*., 2015), it has been documented that MMZ treatment has induced liver damage and showed abnormality in the enzymes liver, in accordance with this statement the obtained results have confirmed that MMZ is a hazard material that cause damage and abnormality to the liver function and structure.

Histochemical results showed that carbohydrates, total proteins and DNA are drained in liver rats treated with MMZ. This finding is similar to those reported by, Ram and Waxman (1992), it has stated that treatment with MMZ had caused a remarkable reduction of proteins at percentage of 75-85% in hepatic microsomal P450 reductase activity in both male and female rats. Also, Meisami *et al*. (1994) has reported that testicular weight and DNA content were significantly decreased in rat pups treated with propylthiouracil from birth. Snedecor *et al*. (1972) has administrated that some histochemical and histological changes were found in the animal exposed to MMZ such as reduction in liver weight and glycogen content. Moreover, Sakr *et al*. (2012) has reported that treating rats with carbimazole caused alterations in prostate gland tissue including decrease in the polysaccharide, total protein and nucleic acids contents.

There are some evidences that antithyroid drugs had used as a medication that lead to cause cellular damage and produced oxidative stress to cells and organs (Bruck *et al*., 2007). Another study, Valko *et al*. (2007) reported that MMZ can cause oxidant species that lead to lipid peroxidation, nitration, carbonylation or glutathionylation of proteins, and interrupt the hepatocytes DNA. This statement is consistent with the results obtained from this investigation. It also has been mentioned that MMZ is responsible for the cell damage (Ortiz-Butron *et al*., 2011), it has been made clear that the cell damage was associated with an increase of reactive oxygen species (ROS), lipid peroxidation and reduction of catalase activity. It has been reported that MMZ promoted oxidative stress causing an increase in liver malondialdehyde levels, and led to a reduction in glutathione, nonprotein thiols and vitamin C level (Sefi *et al*., 2014). The results revealed from this study is

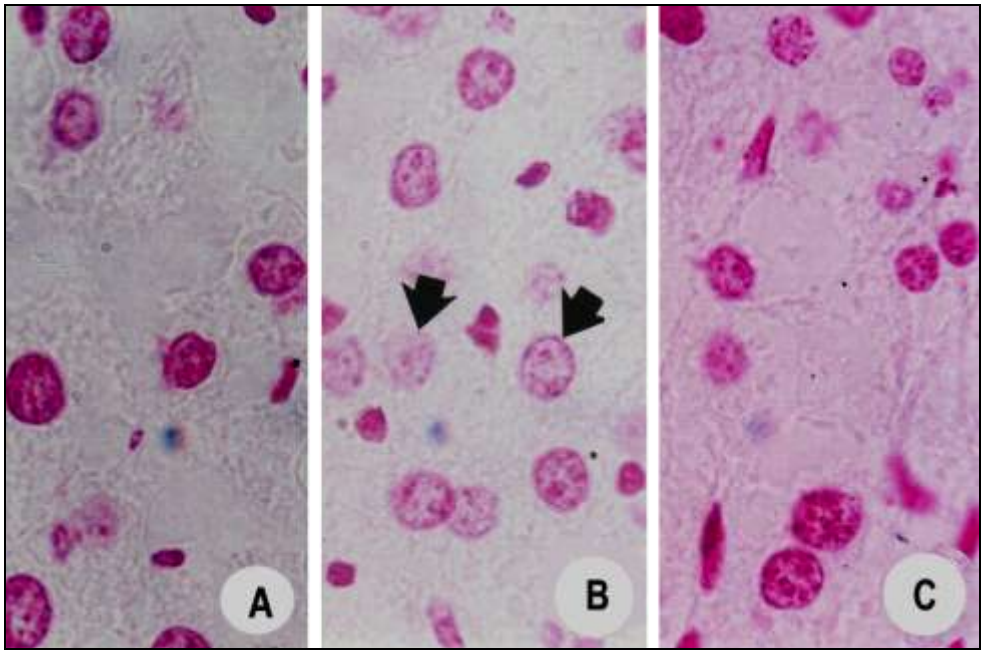

Fig. 4A. Nuclei of hepatocytes of a control rat showing normal content of DNA, X 1000
B. Decrease of DNA in hepatocytes of a rat treated with MMZ, X1000.
C. An increase in DNA hepatocytes of a rat treated with MMZ and curcumin, X1000

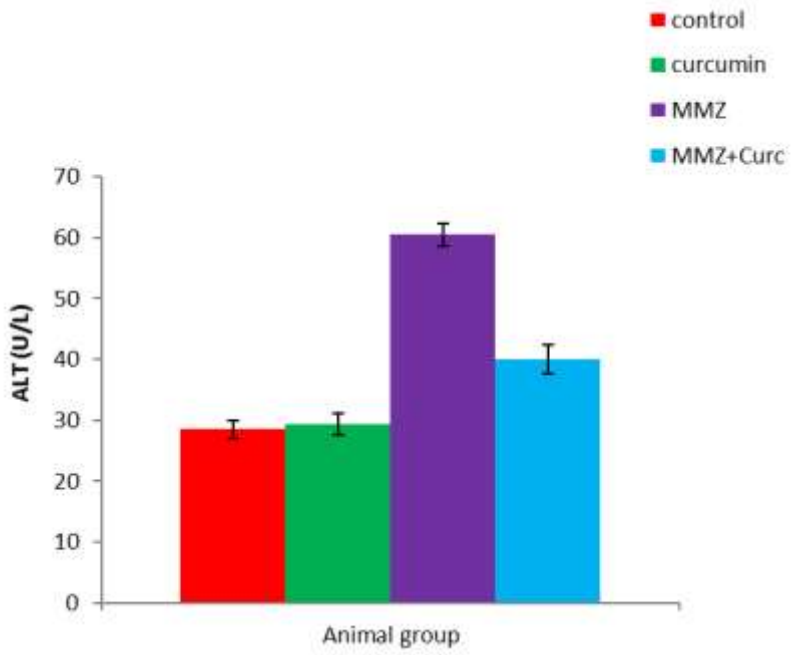

Fig. 5. Change in ALT in different groups.

contestant whit those reported by other researchers. The histochemical analysis showed in this study confirm that MMZ is a harm chemical that interact and create damage to the liver tissue.

Curcumin is a traditional herb used widely world wised. The results showed in this study have indicated that curcumin ameliorates and control the damage caused by MMZ and prevents the damaged appeared in the hepatotoxicity of rat liver. This result is consistent with

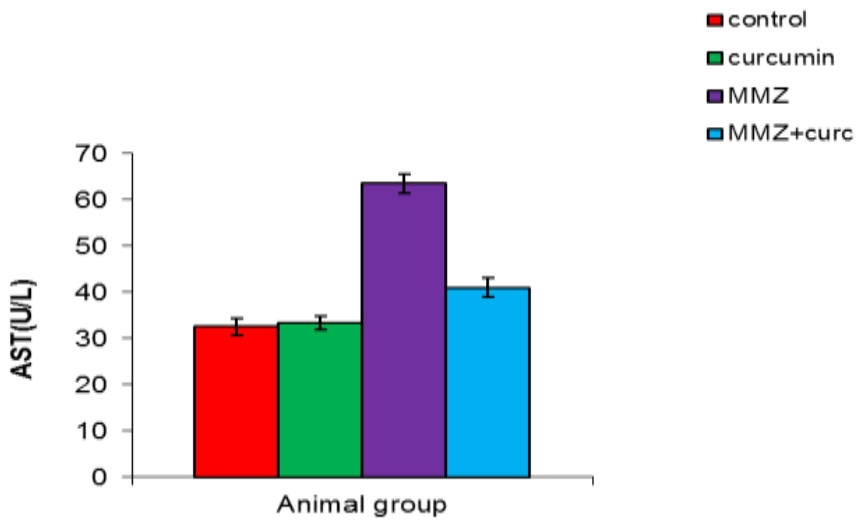

Fig. 6. Change in AST in different groups.

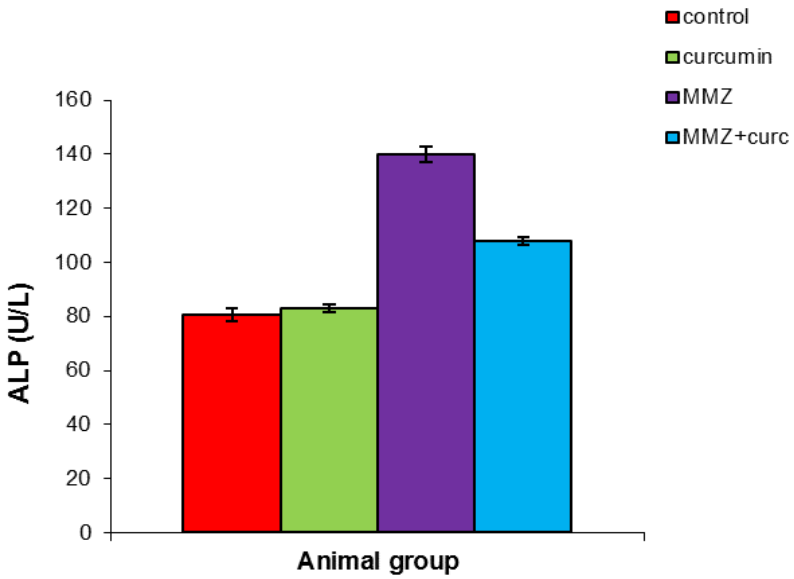

Fig. 7. Change in ALP in different groups.

the other results mentioned in previous study (Singh *et al*., 2013). It has been demonstrated that curcumin could prevent several hepatotoxin. Zhang *et al*. (2014) reported that pretreatment with curcumin has a protective effect against D-galactosamine (D-GalN), lipopolysaccharide (LPS)-induced acute liver damage in mice and rats feed with acetaminophen. Singh *et al*. (2013) and Kalpana *et al*. (2005) have noticed that Curcumin was found to modulate the elevated activities of the biochemical marker enzymes AST, ALP, ALT and of plasma lipid profiles in nicotine treated rats. Curcumin has been found to have some hepatoprotective properties against CCl_4 (Sengupta *et al*., 2011), ethanol (Singh *et al*., 2012), thioacetamide (Salama *et al*., 2013) and lead acetate (Baxla *et al*., 2013).

Curcumin considered as a class of antioxidants agents. It was documented to induce a noticeable increase in the activity of detoxifying enzymes such as glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase and catalase in liver (Iqbal *et al*., 2003), and hemeoxygenase-1 (Motterlini *et al*., 2000). It also was found to reduce lipid peroxidation in different organs (Okada *et al*., 2001; Kempaiah and Srinivasan, 2004). In conclusion, the results of this study indicate that the hepatotoxicity and oxidative stress caused by MMZ can be controlled and prevented by the curcumin antioxidant activity. In future more investigation may reveled more antioxidant properties for curcumin against other hyperthyroidism and antithyroid drugs.

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