



HEPATOPROTECTIVE ACTIVITY OF AQUEOUS EXTRACT OF *CURCUMA LONGA* IN ETHANOL INDUCED HEPATOTOXICITY IN ALBINO WISTAR RATS

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ABSTRACT

To evaluate the hepatoprotective activity of aqueous extract of *Curcuma longa* in ethanol induced hepatotoxicity in albino wistar rats. Six groups, each group containing of six rats allotted to different treatment groups. Group 1 (control) is treated with normal saline (10 ml/kg bw po) as vehicle only. All other groups received ethanol (10 ml/kg bw po) with group 2 serving as ethanol treated control. After ethanol administration, group 3, 4 and 5 also received *Curcuma longa* at different doses (100 mg/kg, 200 mg/kg, 400 mg/kg bw po) respectively. As for group 6, after ethanol administration rats receive silymarin (200mg/kg bw po). At the 28th day, animals were anaesthetized with ether and blood was collected from the retro orbital plexus and serum is separated by centrifugation. To study the liver function, the transaminase enzymes (AST, ALT, and ALP) were measured in the serum of respective groups. Histopathological study was also conducted to measure the action of *Curcuma longa* on parameters such as hepatic fatty degeneration and centrizonal necrosis of respective groups. Present study suggested that ethanol administration increased the levels of transaminase enzymes. However, the aqueous extract of *Curcuma longa* significantly reduced the elevated serum levels of transaminase enzymes in ethanol intoxicated rats. Apart from that, ethanol also causes hepatic fatty degeneration and centrizonal necrosis in ethanol intoxicated rats. Histopathological studies showed administration of aqueous extract of *Curcuma longa* offered significant protection from these damaging actions as study showed marked reduction in fatty degeneration and centrizonal necrosis in ethanol intoxicated rats. Present study suggests that aqueous extract of *Curcuma longa* has significant hepatoprotective activity against ethanol intoxicated rats.

Key words: *Curcuma longa*, Ethanol, Hepatoprotective, Transaminase enzymes, Histopathological study.

INTRODUCTION

Undeniably, liver is one of the vital organs available in vertebrates (Dierdle A. Kelly, 2008; Tortora G *et al.*, 2008). Liver carry out numerous number of vital functions in human body system and among these functions, three have been known as the most important ones, clearance of damaged red blood cells and bacteria by phagocytosis, remove and excrete body wastes and

hormones as well as drugs and other foreign substances, and synthesize plasma proteins, including those necessary for blood clotting.

Liver disease is a worldwide problem. Liver is prone to many diseases because of its multidimensional functions. Diseases that interfere with liver function will lead to derangement of these processes. The most common diseases includes infections such as hepatitis A, B, C, E, alcohol damage, fatty liver, cirrhosis, cancer, and drug damages (especially by acetaminophen and cancer drugs). However, liver has a great capacity to regenerate and has a large reserve capacity. In most cases, the liver only produces symptoms after an extensive damage.

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Necrosis and apoptosis are the two forms of cell death have become well defined (Reed JC, 2000). Hepatocytes death is the main feature of most liver diseases (Bilodeau M, 2003). Hepatic injury is caused by hepatocytes death and can be identified when there is an increase of more than three times of normal serum transaminase enzymes (AST, ALT and ALP) (Victor J *et al.*, 2006).

Liver disorders are one of the foremost health concerns in human due to various chemicals (carbon tetrachloride, D-galactosamine, thioacetamide, ethanol) including therapeutic agents (acetaminophen and chemotherapeutic drugs) and other environmental toxins that can produce hepatotoxicity which may lead to death (Victor J *et al.*, 2006). Ethanol is a well-known hepatotoxicity that is widely used to induce toxic liver injury in a range of laboratory animals. Damage by ethanol is regarded as the analogue of liver damage caused by a variety of hepatotoxicity in human. Ethanol results an increase in the release of endotoxin from gut bacteria and membrane permeability of the gut to endotoxin, or both. Elevated levels of endotoxin activate Kupffer cells to release substances such as eicosanoids, TNF-alpha, prostaglandins and free radicals. Prostaglandins increase oxygen uptake and most likely are responsible for the hypermetabolic state in the liver. The increase in oxygen demand leads to hypoxia in the liver, and on reperfusion, alpha-hydroxyethyl free radicals are formed which lead to tissue damage in oxygen-poor pericentral regions of the liver lobule (Thurman RG, 2009). When ethanol is consumed chronically, it eventually results in liver scarring or what is known as cirrhosis or end-stage alcoholic liver disease (Longstreth George F *et al.*, 2009). As there is no reliable liver protective drugs in allopathic medical practices, herbs plays a major role in the management of liver diseases.

Curcuma longa, a perennial herb and member of the Zingiberaceae (ginger) family which is well known as turmeric and is cultivated extensively in Asia, India, China, and other countries with a tropical climate (Dobelis IN, 1986). Turmeric has been used extensively in traditional Chinese and Ayurvedic medicinal systems (Kapoor LD, 1990). The rhizome, the portion of the plant used medicinally. *Curcuma longa* rhizomes contain approximately 2% volatile oils, composed mainly of alpha and beta-turmerone, monoterpenes (Leung AY *et al.*, 1996), 5% curcuminoids, mainly curcumin (Budavari S, 1996), demethoxycurcumin, bis-demethoxycurcumin and dihydrocurcumin, minerals, carotene and vitamin C (Kapoor LD, 1990). Curcumin is the active constituent of *Curcuma longa*, which is the yellow substance which has been shown to have a wide range of therapeutic effects. Curcumin administration has been shown to be having hepatoprotective activity in hepatotoxicity caused by carbon tetrachloride (Park EJ *et al.*, 2000), trichloroethylene (Watanabe S *et al.*, 2000), endotoxin

(Morikawa T *et al.*, 2002), thioacetamide (Shapiro H *et al.*, 2006), and ethanol (Rukkumani R *et al.*, 2004). Curcumin exhibits multiple anti-carcinogenic effects (Leu TH *et al.*, 2002). *Curcuma longa* or curcumin has also shown to have anti-microbial activity (Vetrivelvan S *et al.*, 2012) and to be protective in diverse diseases such as atherosclerosis (Naito M *et al.*, 2002), ischemia-reperfusion injury (Ghoneim AI *et al.*, 2002), cystic fibrosis (Egan ME *et al.*, 2004), and diabetes mellitus (Arun N *et al.*, 2002). One of the major mechanism underlying *Curcuma longa* disease-modifying effects is its pleiotropic anti-oxidant activity (Miquel J *et al.*, 2002). It scavenges and prevents formation of reactive oxygen species (ROS) (Betancor-Fernandez A *et al.*, 2003) and reactive nitrogen species (RNS) (Kim JE *et al.*, 2003). In addition, curcuma longa was shown to induce several enzymatic anti-oxidants, such as glutathione transferase (Iqbal M *et al.*, 2003), catalase (Iqbal M *et al.*, 2003), and hemeoxygenase-1 (Motterlini R *et al.*, 2000). Also inhibits nuclear binding of hepatic nuclear factor kappa B (NFkB) in a rat model of ethanol-induced hepatotoxicity (Nanji AA *et al.*, 2003) and NFkB-mediated expression of pro-inflammatory molecules, such as inducible nitric oxidized (iNOS) is partially prevented (Singh SS *et al.*, 1995).

Therefore this study has been conducted to evaluate the hepatoprotective activity of aqueous extract of *Curcuma longa* on ethanol induced hepatotoxic in rats.

MATERIALS AND METHODS

Plant material

The plant material of *Curcuma longa* was purchased from local market in the form of dried rhizomes (Narasimhanaidu Kamalakkannan *et al.*, 2005). Further identification has also been done.

Preparation of extract

After drying at 37 degree Celsius for 24 hours the plant material was ground into powder. Exposure to sunlight was avoided to prevent the loss of active components. One liter of double distilled water was mixed with 200 g of powdered *Curcuma longa* rhizomes, filtered with filter paper and the extracted liquid was subjected to water bath evaporation to remove the water. For water bath evaporation, liquid extract material was then placed into a beaker and subjected to water bath evaporation at 70 degree Celsius temperature for 7 to 10 hours daily for 2 to 3 days until a semisolid state of extracted liquid is obtained. The semisolid extract produced was kept in the deep freezer at -20 degree Celsius overnight and then subjected to freeze drying. Extract obtained by this method was then weighed and stored at 22 degree Celsius in desiccators until further use (Mahuya S *et al.*, 2011).

Chemicals

All reagents used in the study were of high purity. All chemicals such as ethanol, formalin, xylene and DMSO were purchased from Sigma Aldrich Chemical (Malaysia). Silymarin purchased from Sigma Aldrich Chemical (China) were also used in the experiments.

Experimental animals

Experiments were carried out on healthy adult male albino wistar rats weighing 180 ± 20 grams. They were raised in the animal house at the Faculty of Pharmacy of the Masterskill University College of Health Sciences. Animals were housed in polypropylene cages with stainless steel grill top at $25 \pm 2^{\circ}\text{C}$ with 12:12 hours light and dark cycle was followed. They were fed a standard diet of pellets and tapped water ad libitum. Rats were routinely acclimatized to laboratory conditions for 7 days prior to experiments ((Narasimhanaidu Kamalakkannan *et al.*, 2005; Mahuya S *et al.*, 2011; Somchit MN *et al.*, 2005). After acclimation, the animals will be subjected to a gross observation, to ensure that the selected animals are in good state of health. Animals were then randomly selected for final allotment to the study. Prior authorization for the use of laboratory animals in this study was obtained from the University College Animal Ethical Committee (Reg. MUCH/AEC/HS/2012/03). The experimental procedures were carried out in strict compliance with the Animal Ethics Committee's rules and regulations followed in this institute.

Acute toxicity study

Acute toxicity studies (Chan PK *et al.*, 1994) were carried on rats as per the guidelines (OECD NO: 423) given by the Organization for Economic Co-operation and Development. Overnight-fasted albino wistar rats (180 ± 20 g) of male sex were used for the study. The animals were divided into five groups of three animals each. The extracts were administered separately to the all the three animals in each group at starting single dose of 5mg/kg. Animals were observed for the period of 1 hour, occasionally for 3 h for severity of any toxic sign and mortality. If no mortality is observed at this dose, the same procedure will be repeated for dose level of 50 mg/kg, 400 mg/kg, and 2000 mg/kg of extracts of separate newer groups. The LD₅₀ was thus determine, which was selected for the hepatoprotective animals study. The animals were observed up to 7 days after drug administration to find out for any delayed mortality.

Experimental design

Six groups, each group containing of six rats allotted to different treatment groups. Group 1 (control) is treated with normal saline (10 ml/kg bw po) as vehicle

only. All other groups received ethanol (10 ml/kg bw po) with group 2 serving as ethanol treated control. After ethanol administration, group 3, 4 and 5 also received *Curcuma longa* at different doses (100 mg/kg, 200 mg/kg, 400 mg/kg bw po) respectively. As for group 6, after ethanol administration rats receive silymarin (200 mg/kg bw po). This study was carried out continuously for 28 days.

Collection of blood

At the 28th day, animals were anaesthetized under ether and blood was collected from the retro orbital plexus and serum is separated by centrifugation (Mahuya S *et al.*, 2011).

Biochemical estimation

To study the liver function, the transaminase enzymes (AST, ALT, and ALP) levels in the serum were essayed using spectrophotometer with Sigma Diagnostic kits (USA) (Somchit MN *et al.*, 2005).

Histopathological studies

Livers from animals from different groups were isolated and fixed in 10% phosphate buffered formalin for at least 24 hours. Then the paraffin sections were prepared and cut into 5 μm thick section in a rotary microtome and mounted on the slide. The sections were then stained with haematoxylin-eosin dye. After staining, the sections were observed under light microscope for histopathological changes, i.e. necrosis, fatty degeneration, lymphocytes and Kupffer cells infiltration, and photographs were taken (Narasimhanaidu Kamalakkannan *et al.*, 2005).

Statistical Analysis

Data were expressed as the mean \pm standard deviation (SD). The data were analyzed using one way analysis of variance (ANOVA) followed by Tukey's test as post hoc test for multiple comparisons. Data were considered significant at different level of P value; $P < 0.05$, $P < 0.01$ and $P < 0.001$ (Somchit MN *et al.*, 2005).

RESULTS

Acute toxicity studies

All the rats that received aqueous extract of *Curcuma longa* either at high dose up to 2000 mg/kg or low dose were found to be safe. No mortality or toxic symptoms were observed during the entire duration of the study. Aqueous extract of *Curcuma longa* showed a stable compliance towards the rats and proved to be safe.

Effect of aqueous extract of *Curcuma longa* on liver enzymes in ethanol induced hepatotoxicity in albino wistar rats

Figure 1 shows the effect of *C. longa* on AST level in serum of ethanol induced hepatotoxicity in male

albino wistar rats. The control had shown the AST level in serum of 43.518 ± 2.414 IU/L but after ethanol treatment, it increased to 369.582 ± 2.212 IU/L. Whereas after administration of *C. longa* at the doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg bw po in ethanol intoxicated rats, the AST level reduced to 340.622 ± 27.911 IU/L, 326.043 ± 39.075 and 81.690 ± 2.132 respectively.

Effect of aqueous extract of *Curcuma longa* on serum ALT level in ethanol induced hepatotoxicity in albino wistar rats

Figure 2 shows the effect of *C. longa* on ALT level in serum of ethanol induced hepatotoxicity in male albino wistar rats. The control had shown the ALT level in serum of 39.885 ± 17.87 IU/L but after ethanol treatment, it increased to 275.403 ± 42.808 IU/L. Whereas after administration of *C. longa* at the doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg bw po in ethanol intoxicated rats, the ALT level reduced to 173.260 ± 10.131 IU/L, 165.738 ± 17.902 IU/L and 99.877 ± 36.681 IU/L respectively.

Effect of aqueous extract of *Curcuma longa* on serum ALP level in ethanol induced hepatotoxicity in albino wistar rats

Figure 3 shows the effect of *C. longa* on ALP level in serum of ethanol induced hepatotoxicity in male albino wistar rats. The control had shown the ALP level in serum of 43.518 ± 2.414 IU/L but after ethanol treatment, it increased to 369.582 ± 2.212 IU/L. Whereas after administration of *C. longa* at the doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg bw po in ethanol intoxicated rats, the ALP level reduced to 340.622 ± 27.911 IU/L, 326.043 ± 39.075 and 81.690 ± 2.132 respectively.

Histopathological studies provided supportive evidence for the biochemical analysis

Histopathological profile of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (Figure 4a). Group II animals exhibited disarrangement of normal hepatic cells with intense centrilobular necrosis, vacuolization of cytoplasm and fatty degeneration (Figure 4b). The liver sections of the rats treated with aqueous extract of *C. longa* followed by ethanol intoxication showed a sign of protection as it was evident by the absence of necrosis and vacuoles (Figure 4c, 4d and 4e). The liver sections of the rats treated with silymarin followed by ethanol intoxication showed a sign of protection as it was evident by the absence of necrosis and vacuoles (Figure 4f).

Table 1. Effect of aqueous extract of *Curcuma longa* on liver enzymes in ethanol induced hepatotoxicity in albino wistar rats

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control	43.518 ± 2.414	39.885 ± 17.870	18.085 ± 0.386^a
Ethanol	369.582 ± 2.212^a	275.403 ± 42.808^a	81.597 ± 1.191^a
Cl 100mg	340.622 ± 27.911^a	$173.260 \pm 10.131^{a,c}$	80.970 ± 1.268^a
Cl 200mg	326.043 ± 39.075^a	$165.738 \pm 17.902^{a,c}$	79.827 ± 3.095^a
Cl 400mg	$81.690 \pm 2.132^{b,c}$	$99.877 \pm 36.681^{b,c}$	$74.623 \pm 2.216^{a,c}$
Silymarin	$79.820 \pm 1.794^{b,c}$	87.553 ± 34.757^c	$71.958 \pm 0.799^{a,c}$

The results are expressed as the Mean \pm SD of six rats/group; One way ANOVA followed by Tukey's multiple test.

a = Results significantly different from Control group, $P < 0.001$

b = Results significantly different from Control group, $P < 0.05$

c = Results significantly different from ethanol group, $P < 0.001$

d = Results significantly different from ethanol group, $P < 0.01$

Figure 1. Effect of aqueous extract of *Curcuma longa* on serum AST level in ethanol induced hepatotoxicity in albino wistar rats

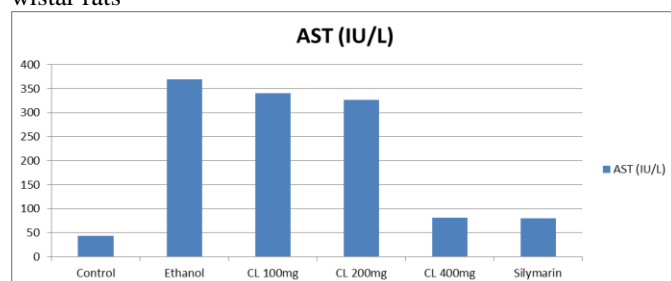


Figure 2. Effect of aqueous extract of *Curcuma longa* on serum ALT level in ethanol induced hepatotoxicity in albino wistar rats

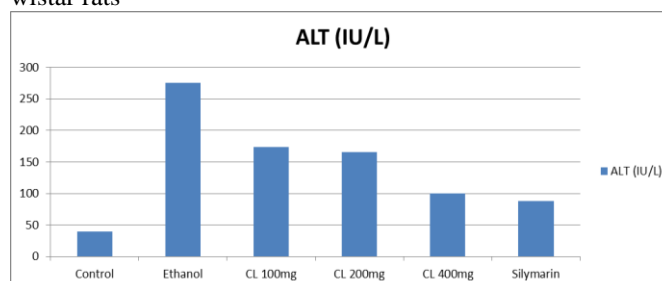


Figure 3. Effect of aqueous extract of *Curcuma longa* on serum ALT level in ethanol induced hepatotoxicity in albino wistar rats

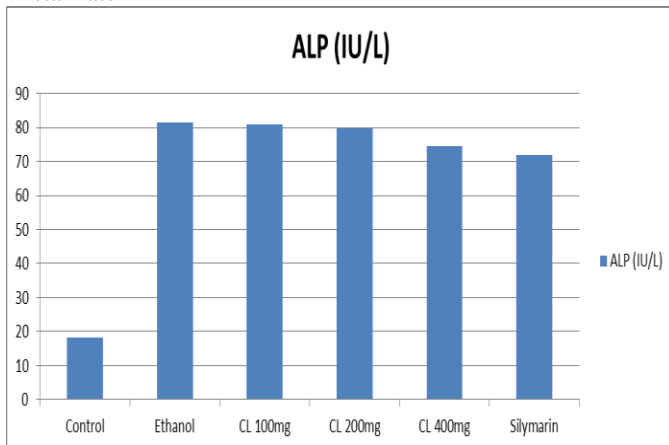


Figure 4b. Ethanol control group (10 ml/kg ethanol) liver section showed intense necrotic hepatitis in the diseased control revealing nuclear pyknosis, karyolysis/karyorhexis and intense cellular infiltration. 400x

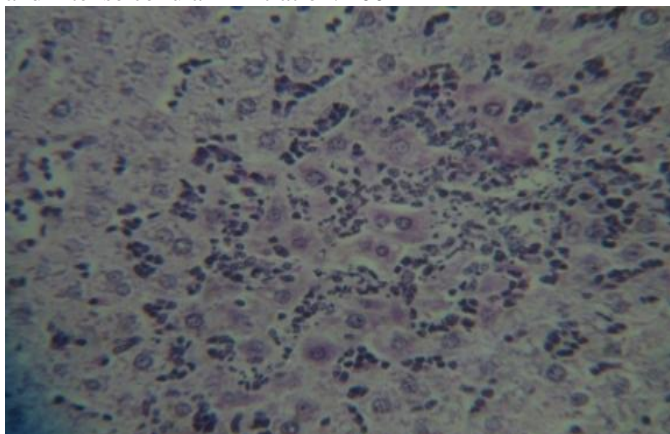


Figure 4d. Treated group (Ethanol 10 ml/kg + *C. longa* 200 mg/kg) liver section revealing swollen hepatocytes with decreased sinusoidal spaces. 400x

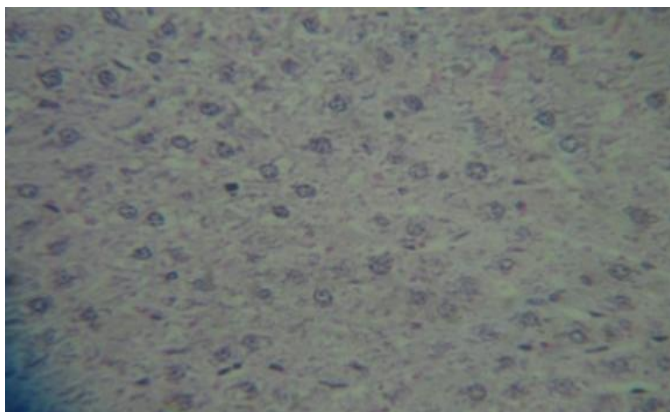


Figure 4a. Control group (10 ml/kg normal saline) liver section revealing normal hepatic parenchyma with a central vein at the top corner. 100x

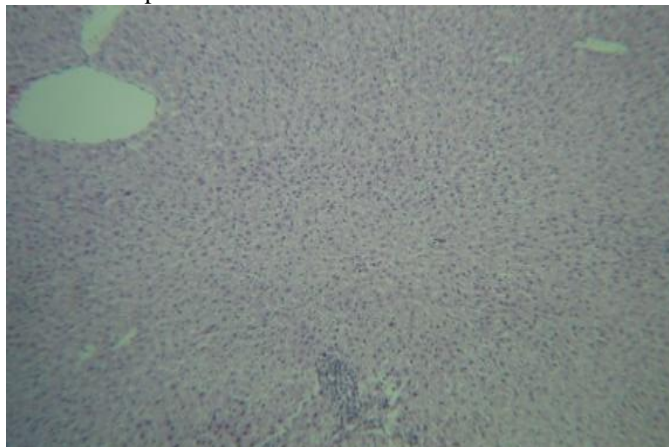


Figure 4c. Treated group (Ethanol 10 ml/kg + *C. longa* 100 mg/kg) liver section revealing swollen hepatocytes with decreased sinusoidal spaces. 400x

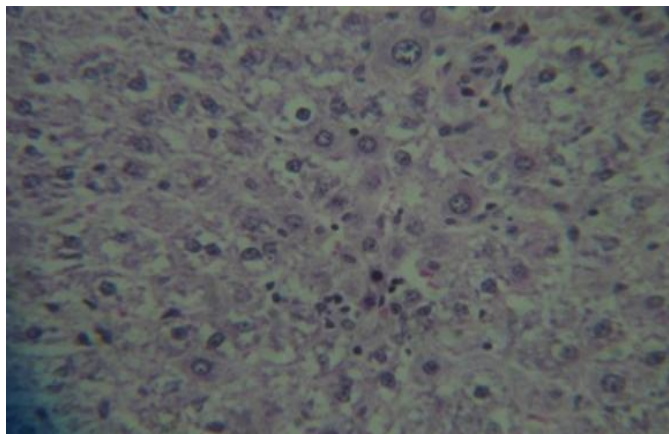


Figure 4e. Treated group (Ethanol 10ml/kg + *C. longa* 400mg/kg) liver section revealing comparatively normal hepatic parenchyma with a single focus of spotty necrosis. 400x

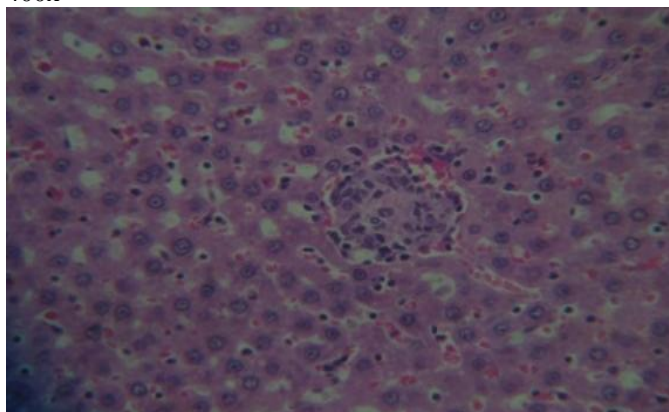
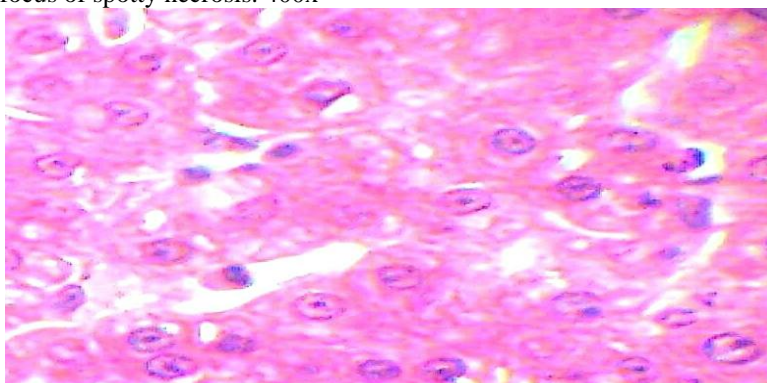


Figure 4f. Standard group (Ethanol 10 ml/kg + Silymarin 200 mg/kg) liver section revealing comparatively normal hepatic parenchyma with a single focus of spotty necrosis. 400x



DISCUSSION

This study was undertaken to evaluate the hepatoprotective activity of aqueous extract of *C. longa* on ethanol induced hepatotoxic in male albino wistar rats. Addition to this, this study was also to compare the hepatoprotective activity of aqueous extract of *C. longa* and standard drug silymarin and determining the dose that is producing almost similar activity to silymarin. The LD₅₀ of *C. longa* was found to be safe up to 2000 mg/kg. Thus, it would be safe to use this extract as a hepatoprotective agent.

The significant ($P < 0.01$) increase in levels of serum AST (369.582 ± 2.212), ALT (275.403 ± 42.808) and ALP (81.597 ± 1.191) confirmed the hepatotoxicity in the group of rats administered with ethanol as shown in table 1. Pretreatment group of rats with aqueous extract of *C. longa* at dose level of 100 mg/kg and 200 mg/kg showed significant ($P < 0.001$) different from control group, which proves at this doses the extract has hepatoprotective activity but not sufficient activity by restoring at the levels of AST (340.622 ± 27.911 , 326.043 ± 39.075), ALT (173.260 ± 10.131 , 165.738 ± 17.902) and ALP (80.970 ± 1.268 , 79.827 ± 3.095) respectively.

Groups of rats treated with aqueous extract of *C. longa* at dose level of 400 mg/kg showed more significant ($P < 0.001$) different from ethanol control group proved by improvement in levels of the AST (81.690 ± 2.132), ALT (99.877 ± 36.681) and ALP (74.623 ± 2.216) respectively. The animals treated with the silymarin (200 mg/kg) showed slightly higher significant ($P < 0.001$) reduction in rise in the serum enzymes level, AST (79.820 ± 1.794), ALT (87.553 ± 34.757) and ALP (71.958 ± 0.799) in comparison to ethanol control group.

The aqueous extract of *C. longa* used in the study preserved the structural integrity of the hepatocellular membrane in a dose dependent manner as

evident from the protection provided as compared to the enzyme levels in ethanol control group rats. The aqueous extract of *C. longa* at dose level 400 mg/kg showed prominent hepatoprotection in comparison to the ethanol control group and silymarin pretreated group rats.

CONCLUSION

In this study, hepatoprotective activity of the aqueous extract of *C. longa* was studied. The aqueous extract of *C. longa* at the dose of 400 mg/kg showed very prominent and similar to silymarin hepatoprotective activity as demonstrated by significant ($P < 0.001$) decrease in transaminase enzyme levels and preserved the structural integrity of the hepatocellular membrane as evident from the protection provided as compared to the ethanol control group rats.

Identification of natural compound of plant will help to develop new therapeutically agents. The results obtained from present study shows that this plant is a good natural source for hepatoprotective activity. As this plant is easily available and the aqueous extract is showing better activity, this suggests that this plant is a cost effective natural treatment available in market. Further clinical trials should be done in order to develop a prominent formulation that will be useful for public. As the cost of the treatment is rising, developing a cost effective remedies will definitely give a better option and opportunities to treat chronic diseases.

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