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RESEARCH ARTICLE

# EFFECT OF GINGER EXTRACT ONHYPERCHOLESTEROLEMIAINDUCED HEPATOTOXICITY IN ALBINO RATS

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**ABSTRACT**

The purpose of the present study was to investigate the effect of ginger extract on hepatotoxicity of hypercholesterolemia in rats. Four groups of male albino rats were used: group 1,animals fed on normal diet, group 2 animals fed on normal diet and given aqueous ginger extract,group 3: animals fed on hyper cholestrolemic diet, and group 4: animals fed on hypercholestrolemic diet (HCD) and given ginger extract. Experimental animals were subjected to histological, immunohistochemical and biochemical analysis. The results showed that liver of rats of HCD group showed histopathological alterations including congestion of blood vessels, leucocytic infiltrations, cytoplasmic vacuolization of hepatocytes and fatty infiltrations. Expression of PCNA was elevated. Biochemical results revealed an increase in the activity of ALT and AST. Moreover, an increase in cholesterol and triglycerides was recorded in sera of HCD animals. All these changes were reversed in animals given HCD and treated with ginger extract. It is concluded from the present study that the protective effect of ginger against hepatotoxicity may be mediated by the antioxidant activity of its components.



## INTRODUCTION

Hypercholesterolemia is one of the major problems to many countries as well as health professionals because of the close correlation between cardiovascular diseases and lipid abnormalities (Matos *et al*. 2005). Lipids, such ascholesterol and triglycerides, are insoluble inplasma and circulating lipid are carried on bylipoproteins that transport them to various tissuesfor energy utilization, lipid deposition, steroidhormone production, and bile acid production.Lipoprotein consists of esterified and unesterifiedcholesterol, triglycerides, and phospholipids, andprotein, which consist mainly of apolipoproteins,or apoproteins (Rader *et al*. 1994).

Clinical hypercholesterolemia,characterized by increased levels of blood cholesterol, is a major risk factor for developing cardiovascular diseases such as myocardial infarction andhypertension, atherosclerosis and its complications (Gerhardt and Gallo, 1998). Also, there is a strong relationship between increased levels ofcholesterol and steatohepatitis, achronic disease that affects the liver (Burtis *et al*. 1998).Lewington *et al*. (2007) reported that hypercholesterolemia is one of the major risk factors for heart diseases, including in those over the age of 65. Hypercholesterolemia was found to cause focal glomerulosclerosis and proteinuria that progress rapidly to renal failure (Seleim and Abdel-Hafez 2010). Dietary factors such as continuous ingestion of high amounts of saturated fats and cholesterol are believed to be directly related to hypercholesterolemia and susceptibility to atherosclerosis (Asashina *et al*., 2005). Hypercholesterolemia was induced in animal models such as rats (Estadella *et al*. 2004) and mice (Musial *et al*.2013) by high-fat diet.

Natural products and their active principles have attracted attention in recent years for treatment of diseases. Ginger (*Zingiberofficinale*, Roscoe Zingiberaceae) is one of the most widely consumedspices for the flavoring of food worldwide (Li *et al*.,2012). Ginger rhizome has been used in traditional herbal medicine and has enormous health promoting potential effects in number of ailments. Ginger contains active phenolic compounds that have potential effects innumber of diseases including degenerative disorders (arthritis and rheumatism), digestive health (indigestion and constipation), cardiovascular disorders (atherosclerosis and hypertension), diabetes mellitus and cancer. Also it has anti-inflammatory,hypolipidemic, antioxidant and hepatoprotective properties **(**White, 2007, Ali etal., 2008 and Nicoll and Henein, 2009).The present work was

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aimed to study the effect of ginger on hepatotoxicity induced by hypercholesterolemia in albino rats.

## MATERIALS AND METHODS

### *Hypercholesterolemic diet*

The hypercholesterolemic diet consisted of 3% cholesterol , 2% thiouracil, 3% cholic acid, and 15% cocoa butter in addition to standard diet which contained 8.0% moisture, 20.8% crude protein, 4.8% crude fat, 3.2% crude fiber, 5.0% crude ash, 37.2% non-fiber carbohydrate and vitamins and minerals adequate to meet the nutritional needs of a rat.

### *Preparation of ginger extract*

Ginger (*Zingiberofficinale*) was purchased from local markets, Cairo City, Egypt. A dried powder (10 g) from ginger was mixed with 100 ml organic solvent (ethanol, hexane and ethyl acetate). The mixture was placed at room temperature for 24 h on shaker with 150 rpm. Solution was filtered through muslin cloth and then re-filtered by passing through Whatman Filter No. 1. The filtrate thus obtained was concentrated by complete evaporation of solvent at room temperature to yield the pure extract. Stock solutions of crude extract was prepared by mixing well the appropriate amount of dried ginger extract with respective solvent to obtain a final concentration of 100 mg/ml. The solution was stored at 4°C after collecting in sterilized bottles until further use (Purshotam and Pankaj, 2011).

### *Animals and Treatments*

A total of 40 male albino rats weighing 160±10 gwere used in this study. The rats were placed in the Animal House at the Faculty of Applied Sciences, Um-AlQura University, Makka, KSA. The experiment was designed and conducted according to Bioethics approved by the Animal Care Use Committee (ACUC), Faculty of Applied Sciences, Um-AlQura University. All animals were acclimatized at an ambient temperature of 22 ± 2ºC with 12 hourlight and 12 hour dark cycle for at least one week prior to the start of the experiment.Animals were divided into 4 four groups of 10 rats each:

Group 1: normal control rats were fed on standard diet free from excess fats and free excess of food and water were allowed *ad libitum* for 6 weeks.

Group2 Animals of this group were each orally given 1 ml by stomach tube of the aqueous extract of ginger containing ( 24 mg/ml ) daily for 6 weeks

six weeks. Group 3:Animal group were fed on hypercholesterolemic diet.

Group 4: Animal group were fed on hypercholesterolemic diet and given ginger extract(24 mg/ml, daily for 6 weeks).

### *Histological study*

Ten animals from both the control and treated groups were sacrificed by cervical decapitation after 6 weeks of treatment. Immediately after decapitation animals were dissected, liver was removed andfixed in 10% formalin. After fixation, specimens were dehydrated in an ascending series of alcohol,cleared in two changes of xylene and embedded in molten paraffin wax. Sections of 5 micronsthickness were cut using rotary microtome and mounted on clean slides. For histopathologicalexamination, sections were stained with Ehrlich's haematoxylin and counterstained with eosin.

### *Immunohistochemical study*

Immunohistochemical detection of PCNA was performed using an avidin biotin complex immunoperoxidase technique on paraffin sections.Formalin-fixed slides were deparaffinized and blocked for endogenous peroxidase with 1.75% hydrogen peroxide in methanol for 20 minutes, antigen retrieval for 15 minutes using Biogenex Antigen Retrieval Citra solution in 90°C water bath for 30 minutes. The slides were allowed to cool for 20 minutes before continuing. Slides were then blocked by normal horse serum for 5 minutes at 37°C. Sections were incubated overnight at $4^0$C with the antibodies against PCNApolyclonal rabbit-anti-human (A3533 Ig fraction; DAKO, Glostrup, Denmark).The immunohistochemical reaction was then developed and stained with diaminobenzidine chromogen solution “DAB” (Sigma). The sections were counterstained with hematoxylin, dehydrated, cleared, and mounted with DPX. For the negative controls, PBS was used in place of the primary antibody.

### *Image analysis*

Digital images were analyzed by a semi-quantitative scoring system (Image J software, Java based application for analyzing images). The brown stained immunohistochemical expressions of PCNA positive cells were analyzed by counting the numbers of positive staining cells in five randomly high power fields at magnification of x400.

### *Biochemical studies*

For biochemical assays, blood samples were collected from animals and sera were obtained bycentrifugation of the blood sample and stored at -20°C. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically according to (Reitman and Frankel, 1975). Cholesterol and triglycerides level were estimated according to methods of Finley *et al*. (1978) and Buccolo and David (1973), respectively.

### *Statistical analysis*

Data were expressed as mean values±SD and statistical analysis was performed using one way ANOVA to assess significant differences among treatment groups. The criterion for statistical significance was set at $P<0.05$. All statistical analyses were performed using SPSS ver. 16 (SPSS Inc.,Chicago, IL, USA).

## RESULTS

### *Histological observations*

Examination of liver of control rats showed the typical features of hepatic tissue (Fig.1a). animals given ginger extract showed normal structure of the liver (Fig.1b).Animals fed on hypercholesterolemic diet for six weeksdisplayed apparent signs of degenerativechanges. The normal structural organization of the hepatic lobules was impaired and the characteristic cord-like arrangement of the normal liver cells was lost.

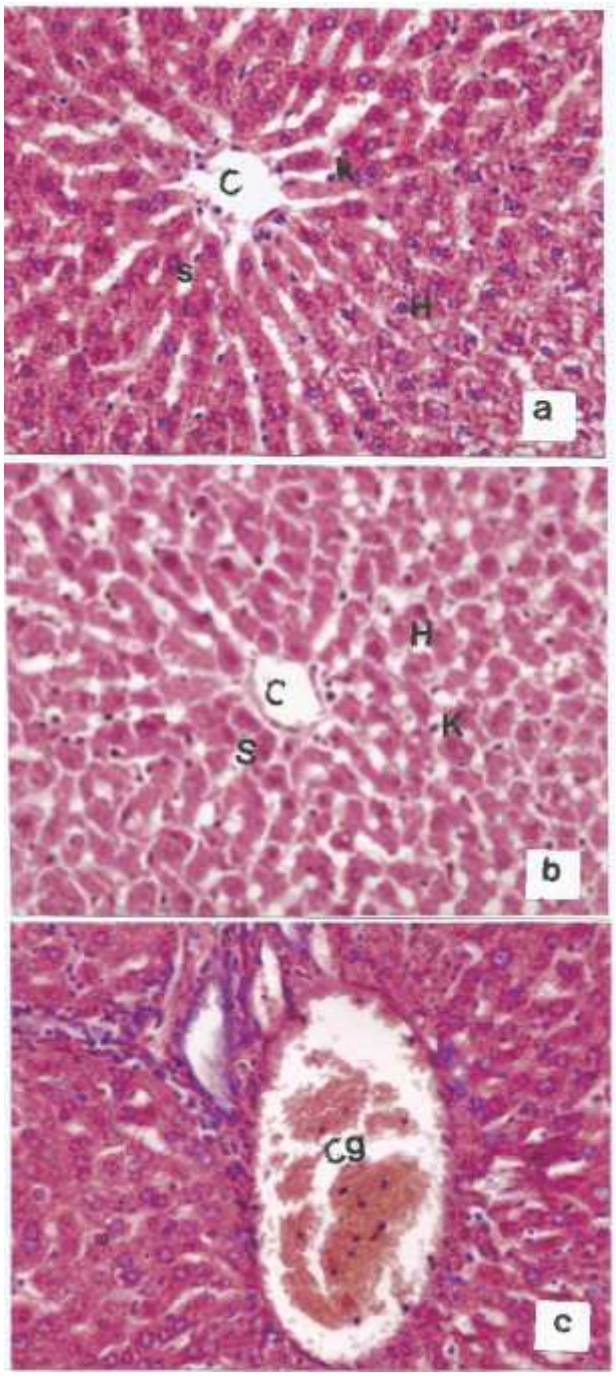


**Fig.1 a.** Liver section of a control rat showing hepatocytes (H), central vein (C) ,sinusoidal spaces(S) with Kupffer cells X 400.
b. section in liver of a rat treated with ginger extract showing showing hepatocytes (H), central vein (C) ,sinusoidal spaces(S) with Kupffer cells X 400.
c. section in liver of a rat given HCD showing congested portal vein (Cg), X400.

The histopathological alterations including enlargement and congestion of interhepatic blood vessels (Fig.1c),leucocytic infiltrations ,cytoplasmic vacuolization of hepatic cells and bile duct proliferation (Fg.2a).Moreover, an obvious fatty degeneration indicated by the large numberof fatty droplets of different size was observed (Fig.2b). Liver sections obtained from animals fed on hypercholesterolemic diet and treated with ginger extract indicatedanobviousdegree of improvement. There was lessprominent histopathologiccaanges as represented by few leucocytic infiltrations and few fat droplets besides the appearance of binucleated cells (Fig.2c).

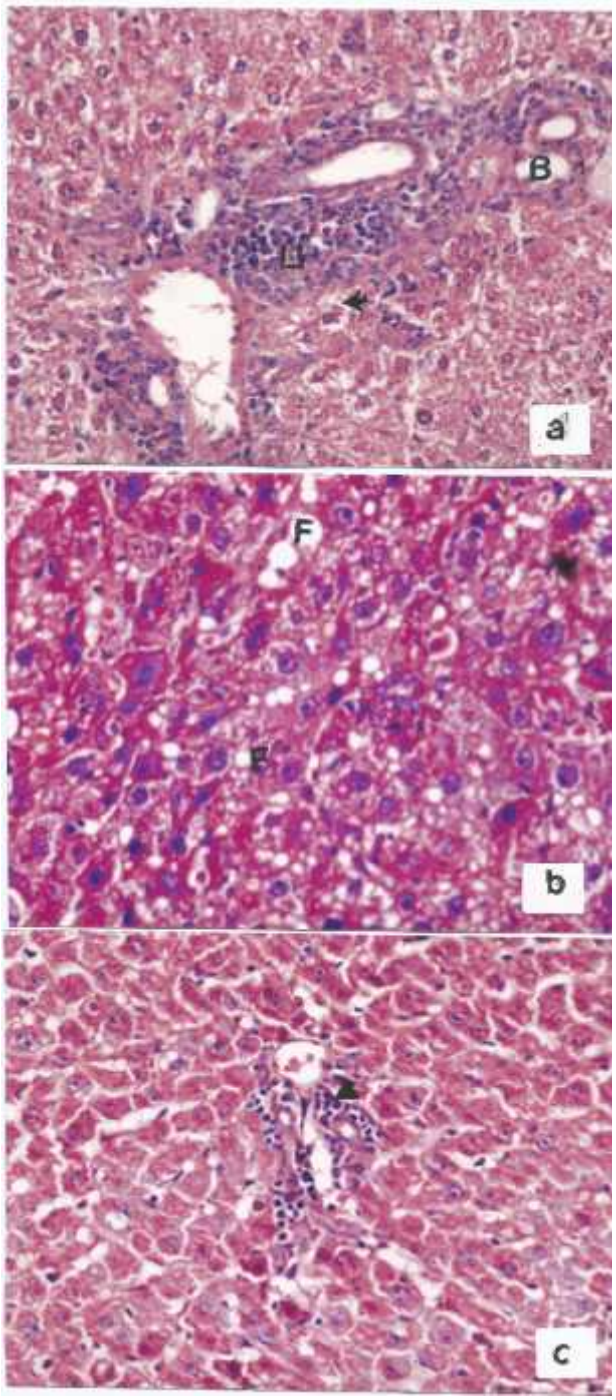


**Fig 2. a.** Section in liver of a rat given HCD showing cytoplasmic vacuolation of the hepatocytes Leucocytic infiltrations (LI) and bile duct proliferation (B), X200.
b. Fatty infiltrations (F) in liver of a rat given HCDX400.
c. section in liver of a rat given HCD and ginger extract showing an improvement in the liver structure with few leucocytic infiltrations,X200.

### *Immunohistochemical results*

Examination of liver sections of control rats or after treatment with ginger extractsrevealed that the nuclei of few hepatocytes showed expression of PCNA (Figs.3a&b). Animals fed on hypercholesterolemic diet showed an increase in the number of

hepatocyte-stained PCNA as compared with the control group (Figure 3c). Liver of animals fed on hypercholesterolemic diet and treated with ginger extract showed a decrease in PCNA expression (Fig.3d). Figure 4 shows percentage area of PCNA expression in different groups after 6 weeks of different treatments.

***Biochemical results***

Animals fed onhypercholesterolemic diet revealed a significant increase ($P$<0.05) in the serum activity of ALT and AST as compared with control and ginger-treated group.

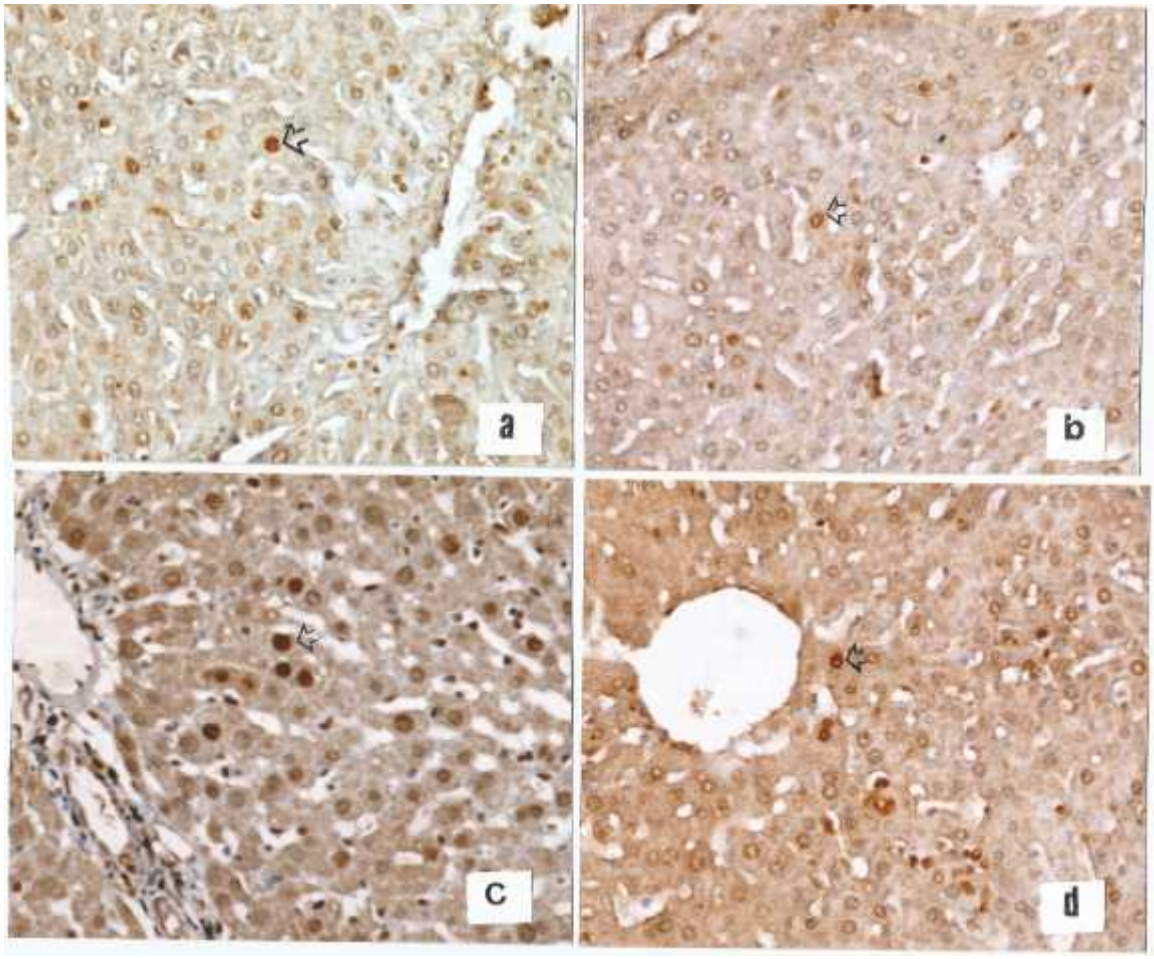


**Fig.3.a** photomicrograph obtained from liver section of a control rat showing few expression of PCNA (arrow) (Immunostain,X400)
b. Few expression of PCNA in hepatocytes of a rat treated with ginger extract (arrow), (Immunostain,X400)
c. photomicrograph obtained from liver section of a rat given HCD showing an increase in expression of PCNA (arrow) (Immunostain,X400)
d. A decrease in expression of PCNA in hepatocytes of a rat given HCD and treated with ginger extract (arrow), (Immunostain,X400)

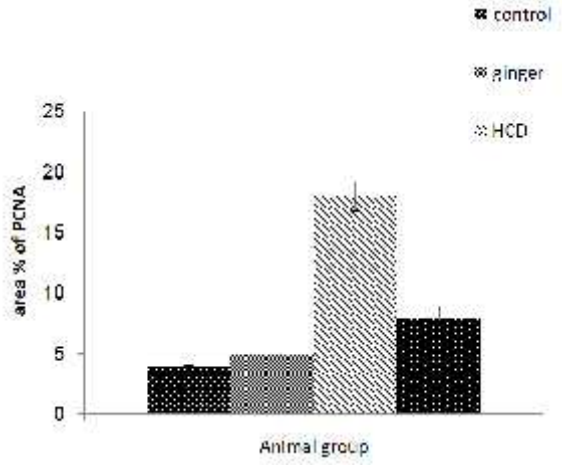


**Fig.4** The mean area percentage of PCNA expression in rat liver of the different experimental groups.

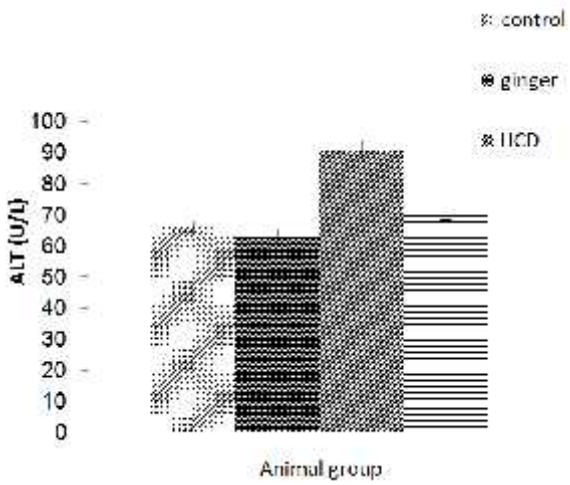


**Fig. 5** Serum ALT activity in different experimental groups.

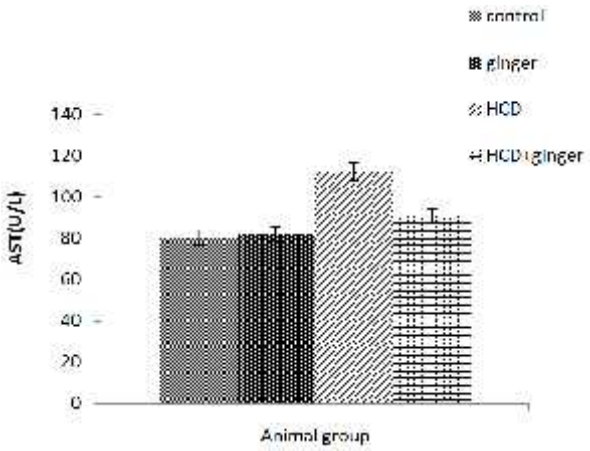


**Fig. 6** Serum AST activity in different experimental groups.

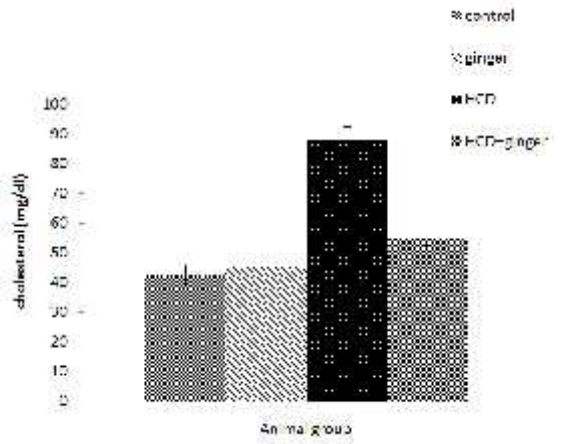


**Fig. 7** Serum level of cholesterol in different experimental groups.

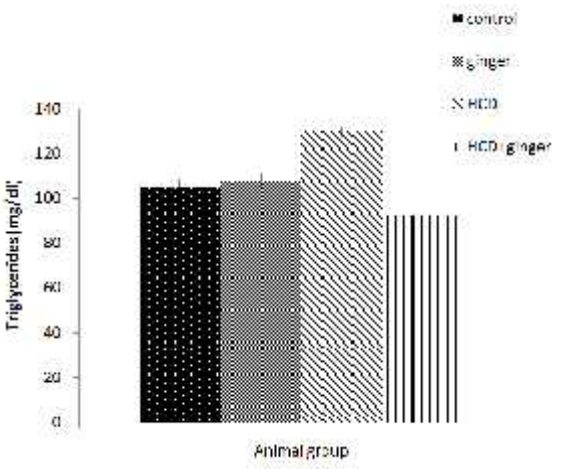


**Fig. 8** Serum level of triglycerides in different experimental groups.

On the other hand,rats fed hypercholesterolemic diet and given ginger extract showed a decrease in the activity of these enzymes (Figs.5&6).Similarly, Cholesterol and triglycerides elevated in sera of animals fed on hypercholesterolemic diet and decreased after treatment with ginger extract (Figs.7&8).

## DISCUSSION

Hypercholesterolemia is one of the leading cause of heart diseases. Results obtained in the present work revealed that liver of rats fed onhypercholesterolemic diet showed histopathological alterations including congestion of blood vessels, leucocytic infiltrations, cytoplasmic vacuolization of hepatocytes and fatty infiltrations. These results are in agreement of some investigators who found that animals fed on hypercholesterolemic diet caused different histopathological alterations in the liver and other organs (Seleim *et al*.2010, Kengkoom*et al*.2013, Musial *et al*.2013). Cytoplasmic vacuolation was observed in the cytoplasm of hepatocytes which may be attributed to the oxidative stress that generate superoxide anions which cause lipid peroxidation. Lipid accumulation leads to alteration and damage of cellular lipid membranes with paralysis of Na-K pump and hepatocytes edema. Congestion in the blood vessels might be due to loss of fluid from the blood and vessels engorged with red blood corpuscles (Mohany*et al*., 2011). Leukocytic infiltration was noticed in the liver of hypercholesterolemic rats. Sakr and Al-Amoudi (2012) explain the appearance of cellular infiltration to the production of ROS which indirectly regulate chemokine receptor expression and promote cytokine interlukin-6 and interlukin-8 which are key modulators of inflammatory response.

The current study revealed a significant increase in theactivity of ALT and AST in the serum of rats fed on hypercholesterolemic diet which may be a sign of impaired liverfunction.Similarly, Bolanle (2011) recorded an increase in ALT and AST in serum of rats fed onhypercholesterolemic diet.It was reported that measurement of the activities of marker enzymes, like AST and ALT can be used in the assessment of liver function (Ulican*et al*.2003). Transaminases are intracellular enzymes and the mostsensitive biomarkers, released into the circulation afterdamage and necrosis of hepatocytes.

An increase in expression of PCNA was observed in hepatocytes of rats fed onhypercholesterolemic diet . Proliferating cell nuclear antigen (PCNA) is an essential regulator of the cell cycle and serves as a co-factor for DNA polymerase delta in S-phase and is involved in DNA repair during DNA synthesis (Bravo, and Macdonald-Bravo,1987). The temporal pattern of PCNA expression makes it a useful tool to study cellproliferation. It starts to accumulate in the G1 phase of the cell cycle, reaches the highest level during the S phase and decreases during the G2/M phase (Tan *et al*.1987).Qualitative analysis of hepatic PCNA levels demonstrated an increased expression of this cell-cycle protein in hypercholestrolemic livers, suggesting an intrinsic effect of this diet in enhancing hepatocyte growth (deOgburn *et al*.2012).

A significant increase was observed in cholesterol and triglycerides of the rats fed hypercholesterolemic diets compared to the control. This result is in accordance with the observations of Laleye*et al*.(2007) and Bolanle (2011).Ohta *et al*. (2003)reported that hypercholesterolemia is brought about by the abnormal lipoprotein metabolism. It has been reported that oxidants and oxidative modifications do play a major role in permanent tissue damage (Kajikawa *et al*., 2011). It has been reported that hypercholesterolemia increased generation of oxygen free radicals, which contributed to the deleterious effects on the organ tissues,including blood vessels, liver, and kidney (Scheuer *et al*.,2000; Zou *et al*., 2003).Thus, the hepatotoxicity recorded in rats fed on hypercholesterolemic diet

in the present investigation may be attributed the oxidative stress induced by this diet.

Several studies revealed the benefits of medical plants like ginger. Animals fed on hypercholesterolemic diet and given ginger extract showed an improvement in the histological structures with minimal alterations.Moreover, the activities of liver enzyme function, ALT and AST, were decreased. Immunohistochemical results showed decrease expression of PCNA in liver of ginger treated rats. Similarly, AbdulazizBardi *et al*.(2013) showed that ginger supplementation can reduce the expression of PCNA and prevent the intensification of liver fibrosis.

The protective effect of ginger against different hepatotoxins was studied. Ginger extract was found to have a protective effect against $CCl_4$and acetaminophen-induced hepatotoxicity (Yemitan and Izegbu,2006). Ginger administration was found to lower the enhanced activity of AST and ALT in cisplatin treated animals (Attyah and Ismail,2012).Khattab *et al*.(2013)reported that ginger improve the liver pathological changes and reduced the congestion andinflammation when compared with diabetic untreatedrats. Result Kalaiselvi*et al.* (2015) demonstrates that ginger extract lowered the elevated levels of the serum AST, ALT and ALP in ginger administered group when compared to aluminium treated group.

Results of this work showed that cholesterol and triglycerides decreased in sera of animals given ginger extract and fed on hypercholesterolemic diet.This observation is in agreement with previous studies in which aqueous extracts ofginger *Z. officinale* significantly reduced serumcholesterol as well as platelet thromboxane B2 andprostaglandin E2 (El-Rokh *et al*.2010). Ginger was found to have hypocholesterolaemic effects and cause decrease in body weight, glucose in blood, serum total cholesterol and serum alkalinephosphatase in adult male rats (Bhandari *et al*., 2005). Nammi *et al*.(2009) and Ramudu *et al*. (2011) mentioned thatthe hypocholesterolemic effect of ginger may beattributed to inhibition of cellular cholesterolsynthesis, results in augmenting the LDL receptor activity, leading to the elimination of LDL from plasma thus modifying lipoprotein metabolism.

Oxidants and oxidative modifications do play a major role in permanent tissue damage.Hypocholesterolemia led to a rise in the generation of reactive oxygen species (ROS), leading to an increased oxidant stress(Zou *et al*.2003).Ginger extract alleviated Hypocholesterolemia-induced hepatotoxicity in rats.Ginger is a strong antioxidant and oxygen radical scavenger (Abdel-Azeem*et al*.2013). The antioxidant effect ofginger is attributed to its bioactive substances, including gingerols and shogaols and some related phenolic ketone derivatives ( Ajith*et al*.2007).It is concluded from the present study that the protective effect of ginger against hepatotoxicity recorded in tpresent work may be mediated by the antioxidant activity of its components.

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