Journal of Biotechnology and Bioengineering
Volume 4, Issue 1, 2020, PP 1-13
ISSN 2637-5362

Hepatoprotective Effect of Garlic Aqueous Extract against Hepatotoxicity Induced By Ciprofibrate in Male Albino Rats

Fikry Ali Abushofa[1], Azab Elsayed Azab[2*], Nadia M. Kermani[3], Tahani S. AL-Jelany Sakah[4]

[1, 3] Department of Zoology, Faculty of Science, Zawia University, Libya
[2] Department of Physiology, Faculty of Medicine, Sabratha University, Libya
[4] Department of Biological Sciences, School of Basic Sciences, Libyan Academy of Graduate Studies, Tripoli, Libya

[*]Corresponding author: Azab Elsayed Azab, Department of Physiology, Faculty of Medicine, Sabratha University, Libya, E.mail: azabelsaied@yahoo.com

ABSTRACT

Background: *The liver is exposing too many toxic substances daily either through the environment or form many other sources such as the preservatives or drugs which increase the risk of liver growth. Ciprofibrate is one of the peroxi some proliferators' chemical families that stimulate hepatic cells and the hepatic cell becomes uncontrollably divided, causing liver growth. The hepatic cell is divided through exposure to drugs or other xenobiotics such as hypolipidemic medication that causes cancer by long-term courses in the rat; nevertheless, it has not been established yet in humans.*

Objectives: *The aim of the present study to evaluate the potential beneficial effects of garlic administration against the biochemical and histological alterations induced in the liver by ciprofibrate in male rats.*

Materials and Methods: *In the current study 8 groups of 6 male rats were used (Control group, Oil, Garlic, Ciprofibrate 50 and 100mg/kg body weight, Cipro 50mg/kg body weight with garlic, Cipro 100mg/kg body weight with garlic, and garlic with Cipro 100mg/kg body weight). The rats have been treated daily orally by gavages for 21 days. On the last day of the experiment the animals were killed then blood samples and parts from the liver were collected. Liver function was examined for the enzyme activities; serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline phosphates (ALP), and serum total and direct bilirubin concentration. The histopathological investigation was conducted for the liver tissues of all groups.*

Results: *Treatment of male rats with 50 or 100 mg/kg body weight of ciprofibrate caused a significant increase in serum ALT, AST, and ALP activities, total, and direct bilirubin concentration. Histologically, there were histological changes in central vein area and portal zones, revealed congestion in blood sinusoids, necrosis in hepatic cells, and damage in central vein lining epithelium. Co-administration of garlic aqueous extract with Ciprofibrate significantly improved the structural changes in the liver and the serum ALT, AST, and ALP activities, total, and direct bilirubin concentration were significantly declined.*

Conclusion: *It can be concluded that Ciprofibrate treatment induced elevation in liver function tests and severe histopathological changes and garlic aqueous extract was able to protect the liver against these effects in male rats. So, the patients should be advised to take garlic aqueous extract while they are treated by ciprofibrate.*

Keywords: *Ciprofibrate, Hypolipidemic agent, Garlic aqueous extract, Hepatotoxicity, Hepatic pathophysiological changes*

INTRODUCTION

The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. When the liver works normally, the various metabolic processes within the body are balanced and regular, but the use of certain drugs may cause liver problems. The liver is a specific target for drug toxicity because of its role in the removal and metabolism of chemicals, the drug enters the body by many ways, including oral, intramuscular injection, intra venous, dermal absorption, and nose [1]. It is known that the main function of the liver is elimination of toxins that may enter the body, thus becoming

vulnerable damaged during this mechanism, which can be revealed as bleeding, congestion, necrosis or other conditions of liver injury [2].

Peroxisome proliferators are a class of chemicals that have diverse effects in rats and mice including increased DNA synthesis and cause cell proliferation (peroxisome proliferation). These chemicals act through ligand activation of nuclear membrane receptors termed peroxisome-proliferator-activated receptors' (PPARs), which themselves act as nuclear transcription factors [3, 4]. The PPs induce a cellular process characterised by a dramatic increase in the size and number of peroxisomes correlated with both hepatocyte hypertrophy (an increase in liver cells size) and hyperplasia or increase in the number of liver cells during replicative DNA synthesis and cell division [5]. Peroxisome proliferators are a diverse group of chemicals which differs slightly in structure but all induce characteristic effects in the liver of treated rats and mice [6]. Peroxisome proliferators include several chemicals which are unrelated structurally such as hypolipidemic drugs plasticizers and organic solvents, all of which cause liver carcinogenesis in laboratory rodents by a non-genotoxic mechanism. Ciprofibrate follow the low fat group of lipoproteins, which reduce the production of LDL cholesterol from the high density lipoprotein HDL. It also reduces the production of triglycerides and increases its analysis. These effects reduce the risk of developing sclerosis arterial heart disease and stroke. Although it has been used clinically since 1930, the mechanism of action of ciprofibrate is not entirely clear in 1990. Peroxisome proliferation is accompanied by replicative DNA synthesis and liver growth. Many of these peroxisome proliferators contain acid functions that can modulate fatty acid metabolism [7]. Liver enlargement induced by peroxisome proliferators is due to both hepatocyte hyperplasia (increased replicative DNA synthesis and cell division) and hypertrophy [8]. Peroxisome proliferators cause liver cell proliferation in addition to other pleiotropic effects such as peroxisome proliferation and induction of certain peroxisomal and cytosolic enzymes in liver [9].

Oxidative stress is a disturbance in the balance between the productions of reactive oxygen species (ROS) that named free radicals. The oxidative stress is based on the hypothesis that long term administration of peroxisome proliferators produces a sustained oxidative stress in rodent hepatocytes due to an imbalance in the production and degradation of hydrogen peroxide [10]. One is based on increased production of active oxygen species due to imbalanced production of peroxisomal enzymes; it has been proposed that these reactive oxygen species cause indirect DNA damage with subsequent tumor formation [11].

The trend increased towards antioxidants used in recent decades, they are showed positive effects against some diseases. Here are some of them which used in this study. *Allium sativum* commonly known as garlic is a bulb-forming herb of Lilliaceae family. Garlic is the oldest cultivated plant and has been used as a spice, food and folklore medicine for over 4000 years [12]. Ancient Egyptian records mentioned that use of garlic as a remedy for a variety of diseases [13]. It has been used as a traditional medicine in the treatment of heart diseases, tumors and headaches and exhibits medicinal properties including immunomodulation, hepatoprotection, antioxidant, antimutagenic, antibacterial and anticarcinogenic effects [14]. Moreover, it has also been reported to possess antifungal [15], hypoglycemic [16], hypolipidemic [17], and anti-atherosclerotic properties [18]. Garlic has historically been used to treat earaches, leprosy, deafness, severe diarrhea, constipation and parasitic infections, and to lower fever, fight infections and relieve stomach aches [19].

Garlic contains at least 33 sulfur compounds, several enzymes and the minerals germanium, calcium, copper, iron, potassium, magnesium, selenium and zinc; vitamins A, B1 and C, fiber and water. It also contains 17 amino acids to be found in garlic: lysine, histidine, arginine, aspartic acid threonine, swine, glutamine, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tryptophan and phenylalanine [20]. Garlic mainly contains organosulfur compounds such as allicin, ajoene, diallyl disulphide, diallyltrisulfide. SAC sulfoxide and flavonoids, phenolics and anthocyanins [21-23]. It also contains carbohydrates, proteins, fatty acids, glycolipids, phospholipids, fiber, saponins, glycosides lectins, and vitamin B1, B2, B6, C and E [24].

OBJECTIVES

The aim of the present study to evaluate the potential beneficial effects of garlic administration against the biochemical and histological alterations induced in the liver by ciprofibrate in male rats.

MATERIALS AND METHODS

Materials

Animals

Male rats were purchased from medical national centre – Zawia. Rats were used for this study aged 14-15 weeks. Animals were housed in plastic cages in biologically clean rooms, 6 rats per cage. Temperature and relative humidity were held at 22 ± 2°C and 50 + 5% respectively and maintained on a 12 hr (light/dark) cycle. Rats were maintained on a standard lab diet and purified water with addition of *ad libitum.*

Chemicals:

Ciprofibrate was purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Ciprofibrate powder was dissolved in 20ml of corn oil and was given at a dose of 50, 100 mg/kg b. wt orally by gavage [25].

Preparation of Garlic Extract:

One kilogram of garlic cloves (*A. sativum* L.) was purchased from the local market peeled and grounded with an electric mincer until an aqueous suspension was obtained. It was diluted in double distilled water at 4 g/mL on the basis of the weight of the starting material [23].

Methods

Experimental Design

The animals were divided into nine groups. Each group of six animals has left a week to acclimatizing. The experiment continued 21 days and the groups were designed as follows:

G1: Control group: Normal diet (feed and tap water).

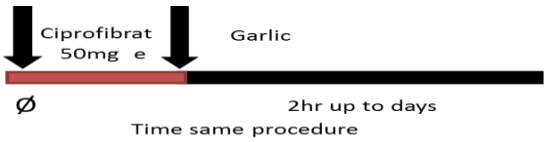
G2: Group of oil vehicle; was given corn oil at same time of other treated groups.

G3: Administrated group with garlic.

G4: Positive control (1): Ciprofibrate 50mg/kg body wt

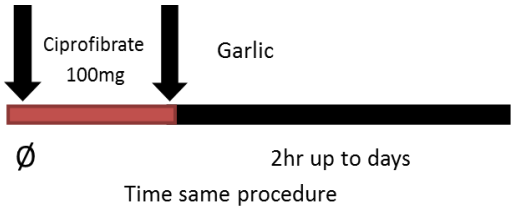
G5: Positive control (2): Ciprofibrate 100mg/ kg body wt

G6: Treated group-1: Ciprofibrate 50mg/kg body wt +Garlic.



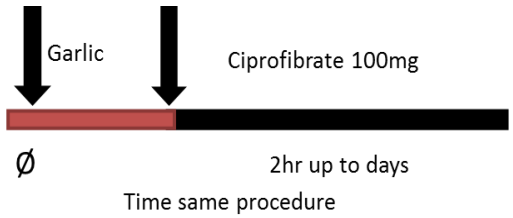
FigureA. *Schematic representation time courses protocol 1 of doses. The schematic diagram describes the two doses protocol of induction hepatic by peroxisome proliferator (ciprofibrate 50mg) then 5% garlic two hours later for two days. The red bar shows the period where the animals were gavage with 50mg ciprofibrate. The black bar shows the period where the animals were gavage with garlic.*

G7: Treated group-2: Ciprofibrate 100mg/kg body wt +Garlic.



FigureB. *Schematic representation of two doses (protocol 2). The schematic diagram describes the two doses for hepatic induction by peroxisome proliferator (ciprofibrate 100mg) and then 5% garlic two hours later for two days. The red bar shows the period of animals gavaged with 100mg ciprofibrate. The black part shows the period of animals' administration with garlic.*

G8: Protective group: Garlic administrated for a week then Garlic + Ciprofibrate 100mg/kg body wt



FigureC. *Schematic representation of two doses (protocol 3). The schematic diagram describes the two doses, the red bar shows gavage with 5% garlic at time Ó. The black bar shows the period of animal's gavage with 100mg ciprofibrate. In the time course used 50mg/kg and 100mg/ kg body wt of ciprofibrate for 10 days, the animals were dosed by gavaged with appropriate dose of ciprofibrate, and garlic. The animals were killed after 10 days.*

Biochemical Analysis

After killed the animals the blood samples were drown out by cardiac puncture and centrifuged at 3000 rpm for 15 minutes to harvest the serum with which the liver function assessment were analyzed. The activities of Alanine amino transferase (ALT), aspartate amino transferase (AST) are measured in serum according to the methods described by Reitman and Frankel, [26]. Serum alkaline phosphatase (ALP) activity was determined according to Kind *et al.*, [27]. Serum total and direct bilirubin levels were determined according to the methods described by Dangerfield and Finlayson, [28].

Tissue Processing

Tissues were freshly collected from the animals. The livers were stored in fixative 10 % (v/v) formalin until use. The tissues were lifted in the fixative not more than 3 days. Fixed tissues were dehydrate and infiltrated with paraffin wax by processing in Shandon citadel 2000 Automated Tissue processor.

Counting of Cell Proliferation

The classic count method was used and the comparison between treated and control groups, instead of labelling index (hepatic DNA synthesis) through BrdU technique. Three slides were examined for each rat of the six rats in all groups. Namely 2000 nuclei were counted by choosing 9 fields randomly of eight views of each slide (the mean was calculated for all rat groups). Nuclei labelled with haematoxylin included necrosis cells. The mean of the labelled nuclei was then calculated. Light microscope at x 100.

Statistical Analysis

All data is represented as mean ± standard deviation. Statistical significance was tested by Dunnett's Multiple Comparison Test with a two ways analysis of variance (ANOVA) was used for multiple comparisons. All date analyzed using Graph-pad Prism 7.0 software. The results were considered statistically significant when $p < 0.05$.

RESULTS

Biochemical Parameters Of The Liver

Treatment of rats with garlic extract, 50 and 100 mg/kg body wt ciprofibrate (gavages) once a day for 21 days, caused a significant (*P<0.01*) increase in serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphates (ALP) activities compared with the control group. In contrast, corn oil gavages once a day for 21 days caused a significant decrease in ALT, AST, and ALP activities compared with the control group. Co-administration of garlic extract with 50 and 100 mg/kg body wt ciprofibrate in all groups were decreased serum ALT, AST, and ALP activities compared with ciprofibrate treated (50 and 100mg/kg body wt) groups (Tables 1& 2, Figures 1& 2).

Table1. *Effect of oil vehicle, garlic and/or ciprofibrate 50mg/kg body wt on serum alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatise activities in male albino rats*

Parameters	**Alanine aminotransferase (ALT, U/L)**	**Aspartate aminotransferase (AST, U/L)**	**Alkaline phosphatase (ALP, U/L)**
Groups	Mean ±SD	Mean ±SD	Mean ±SD
Control	82.6± 0.7	100±0.9	61±0.8
Oil vehicle	64.3± 0.9**	86± 0.8**	141.3± 0.6**
Garlic	92± 0.8**	144± 1.6**	116± 1.7**
Cipro 50mg/ kg body wt	119.3± 1.2**	250± 2.6**	281,3± 2.0**
Cipro 50mg/ kg body wt + garlic	101.6± 0.9**##	142.6± o.8**##	123.6± 0.9**##

*All data are mean of 6 individuals. **: Significant differences as compared with control group (P < 0.01).*

##: Significant differences as compared with Cipro 50mg/ kg body wt group (P < 0.01).

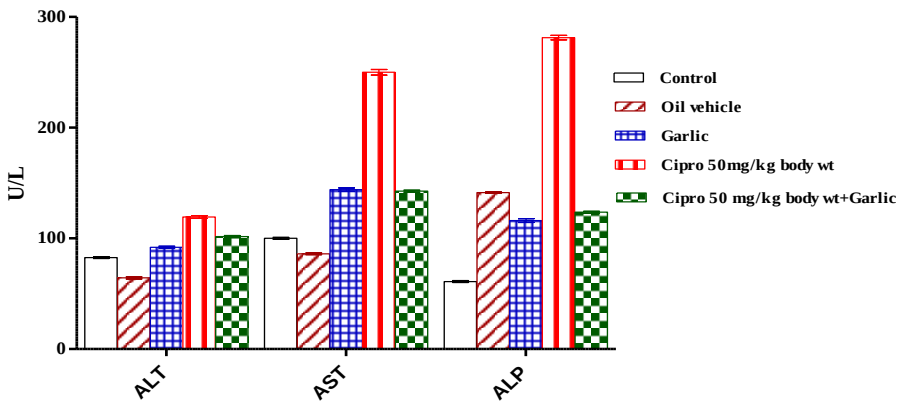


Figure1. *Effect of oil vehicle, garlic and/or ciprofibrate 50mg/kg body wt on serum alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatise activities in male albino rats*

Table2. *Effect of oil vehicle, garlic and/or ciprofibrate 100mg/kg body wt on serum alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatise activities in male albino rats*

Parameters	Alanine aminotransferase (ALT, U/L)	Aspartate aminotransferase (AST, U/L)	Alkaline phosphatase (ALP, U/L)
Groups	Mean ±SD	Mean ±SD	Mean ±SD
Control	82.6± 0.7	100±0.9	61±0.8
Oil vehicle	64.3± 0.9**	86± 0.8**	141.3± 0.6**
Garlic	92± 0.8**	144± 1.6**	116± 1.7**
Cipro 100mg/ kg body wt	174± 1.9**	181.2± 1.4**	263± 2.3**
Cipro 100mg/ kg body wt + garlic	144.6± 0.9**##	163± 0.8**##	135 ± 2.1**##
Garlic + Cipro 100mg/ kg body wt	99.75±1.3**##	121.3±1.5**##	159.5±0.7**##

*All data are mean of 6 individuals. **: Significant differences as compared with control group (P < 0.01). ##: Significant differences as compared with Cipro 100mg/ kg body wt group (P < 0.01).*

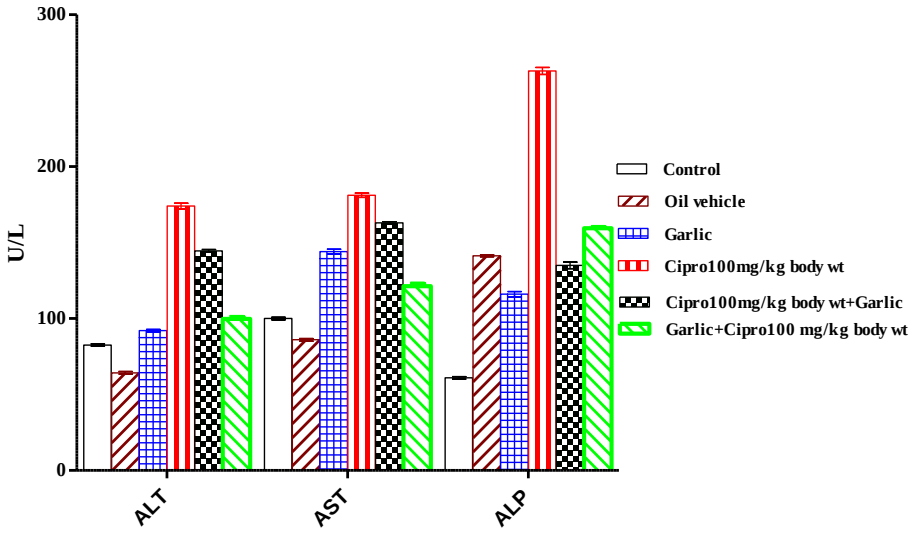


Figure2. *Effect of oil vehicle, garlic and/or ciprofibrate 100mg/kg body wt on serum alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatise activities in male albino rats.*

Treatment of rats with 50 and 100 mg/kg body wt ciprofibrate (gavages) once a day for 21 days, caused a significant (P<0.01) increase in serum total bilirubin and direct bilirubin concentrations compared with the control group. Corn oil gavages once a day for 21 days caused a significant (P<0.01) increase in serum total bilirubin concentration and a decrease in serum direct bilirubin concentration compared with the control group. In all groups, Co-administration of garlic extract with 50 and 100 mg/kg body wt ciprofibrate were decreased serum total bilirubin and direct bilirubin concentrations compared with ciprofibrate treated (50 and 100mg/kg body wt) groups (Tables 3& 4, Figures 3& 4).

Table3.*Effect of oil vehicle, garlic and/or ciprofibrate 50mg/kg body wt on serum total bilirubin and direct bilirubin concentrations in male albino rats*

Parameters	Total bilirubin (g/dl)	Direct bilirubin (g/dl)
Groups	Mean ±SD	Mean ±SD
Control	2.1± 0.040	1.8± 0.010
Oil vehicle	2.9±0.020**	1.1±0.010**
Garlic	2.8±0.040**	0.6±0.020**
Cipro 50mg/ kg body wt	3.13±0.030**	2.17±0.001**
Cipro 50mg/ kg body wt + garlic	2.9± 0.020**##	1.2 ±0.080**##

*All data are mean of 6 individuals. **: Significant differences as compared with control group (P < 0.01).*

##: Significant differences as compared with Cipro 50mg/ kg body wt group (P < 0.01).

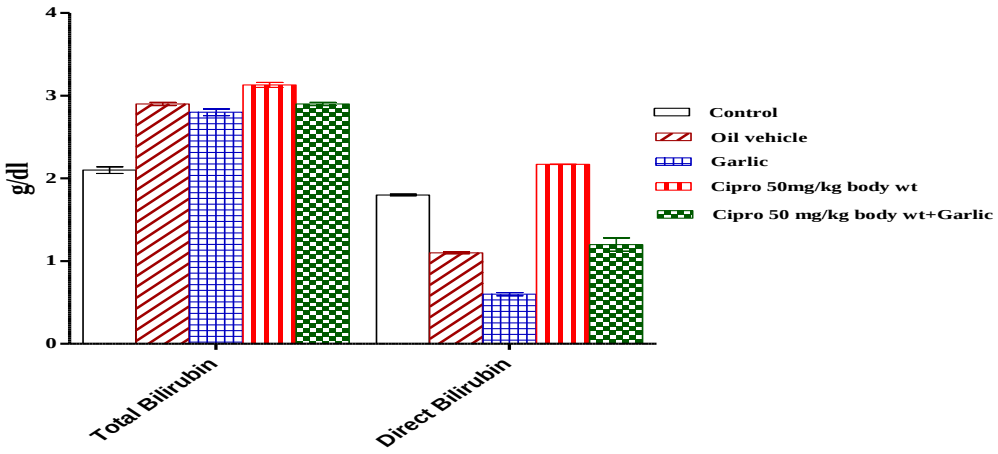


Figure3. *Effect of oil vehicle, garlic and/or ciprofibrate 50mg/kg body wt on serum total bilirubin and direct bilirubin concentrations in male albino rats*

Table4. *Effect of oil vehicle, garlic and/or ciprofibrate 100mg/kg body wt on serum total bilirubin and direct bilirubin concentrations in male albino rats.*

Parameters	Total bilirubin (g/dl)	Direct bilirubin (g/dl)
Groups	Mean ±SD	Mean ±SD
Control	2.1± 0.040	1.8± 0.010
Oil vehicle	2.9±0.020**	1.1±0.010**
Garlic	2.8±0.040**	0.6±0.020**
Cipro 100mg/ kg body wt	4.6±0.060**	2.9±0.002**
Cipro 100mg/ kg body wt + garlic	4.02± 0.020**##	2.1± 0.400##
Garlic + Cipro 100mg/ kg body wt	3.95 ±0.030**##	1.8± 0.060##

*All data are mean of 6 individuals. **: Significant differences as compared with control group (P < 0.01).*

##: Significant differences as compared with Cipro 100mg/ kg body wt group (P < 0.01).

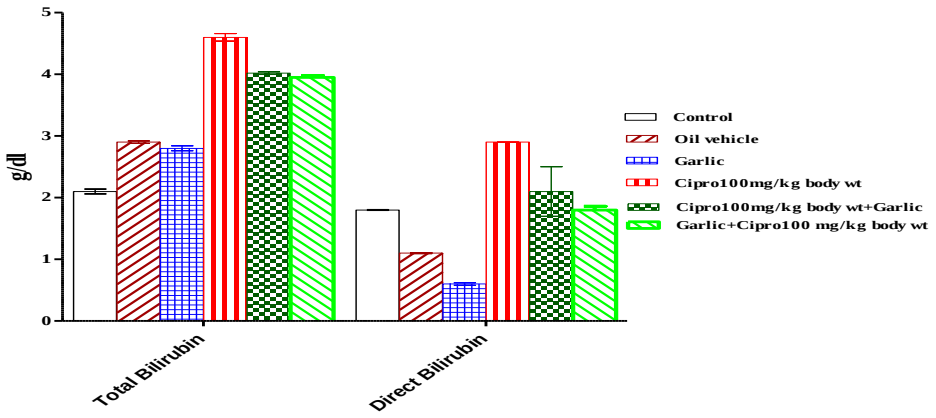


Figure4. *Effect of oil vehicle, garlic and/or ciprofibrate 100mg/kg body wt on serum total bilirubin and direct bilirubin concentrations in male albino rats.*

Cell Proliferation

Cell proliferation is an indicator to the degree of proliferation with taking into account all the cells that have underwent to necrosis and apoptosis. It is a defined by the balance between cell divisions and cell loss through the cell death or differentiation. A counting process for the liver cells division was applied, by compute a 2000 cells using the force of magnification (100 x) in 9 fields per slide (described in figure 4.5).

The table.5 is showing the means of cell proliferation (cell division) in all groups. The liver induction with 50 or 100mg/kg of ciprofibrate were caused increase in liver cell proliferation. The result of the comparison showed conflict in cell divisions between groups.

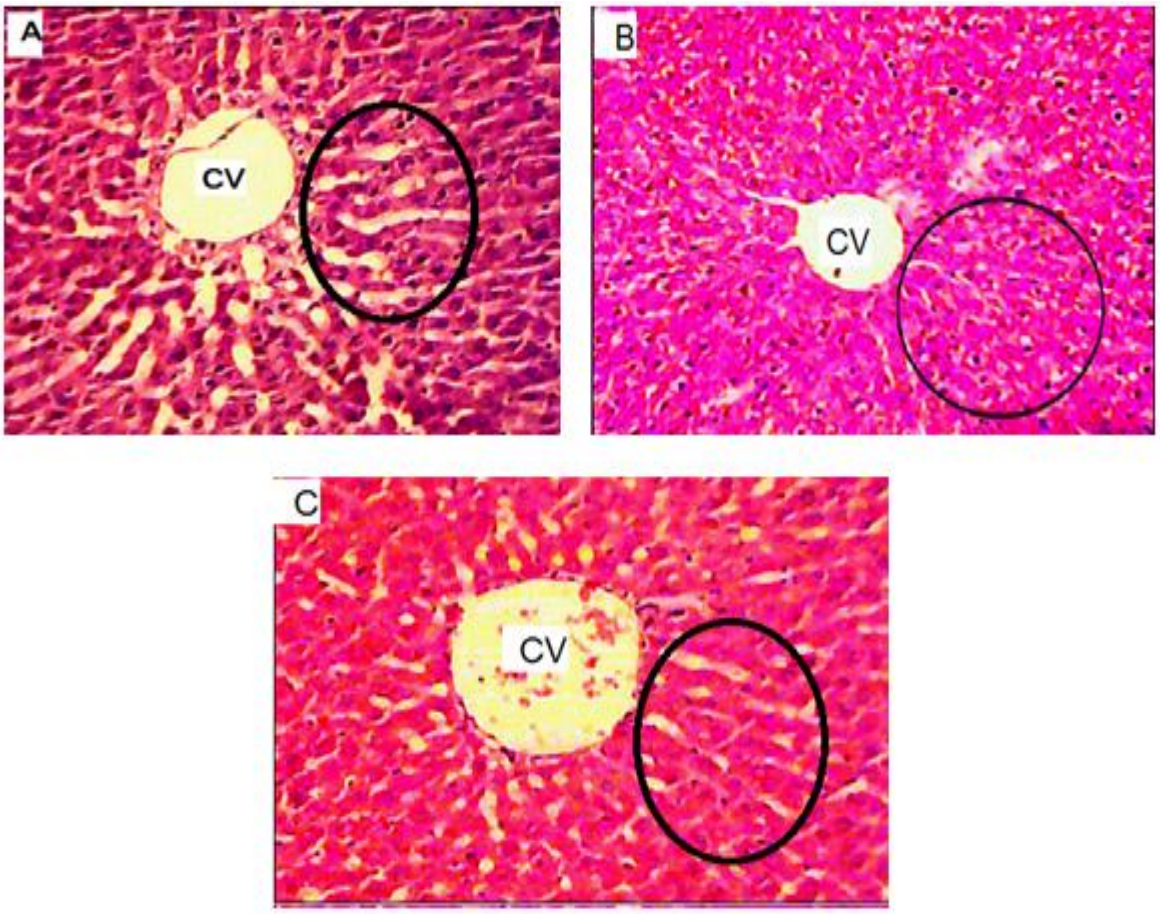


Figure5. *Micrographs of hepatocytes were collected from normal control and induction group with ciprofibrate rats. A; Control group section is showing normal number of hepatocytes in zone 3. Section B; from induced group with ciprofibrate is showing increase in hepatocytes proliferation in zone 3, Section C; showing decrease in hepatocytes proliferation (H&E X400).*

Table3. *The means of liver cells count in experimental groups*

Groups	Means of total cells divisions
Control	1979
Corn oil	2033
Garlic	2049
Ciprofibrate 50mg	2131
Ciprofibrate 100mg	2145
Ciprofibrate 50mg with garlic	2077
Ciprofibrate 100mg with garlic	2001
Garlic with ciprofibrate 100mg	2029

Histopathological Changes of Liver

The Central Vein Alteration

In order to verify the accuracy of the results obtained from the biochemical analysis of liver serum enzymes of all groups. The liver tissue sections were divided into two mains structural units which are: central vein and portal area which were deeply looked. As a result of ciprofibrate induction with 50mg and 100mg showed massive cell division. Figure.6 for central vein (CV) which reveals normal lining endothelial, normal hepatocytes radiating arrangement from central vein and blood sinusoids appear between the hepatocytes. The Variation in the central vein (CV) is shown in micrographs (Figure .6).

The Portal Area Alteration

Our target was verifying that the zonal distribution of hepatic induction when dose with (Ciprofibrate 50 and 100mg/kg body wt) around portal areal, and investigation of hepatocytes in the sections of liver acinus how arranged in three areas: Zone 1, 2 and 3. Each zone has a different amount of oxygen which makes it a different response to toxicity. Figure .6 for the Variation in the portal area.

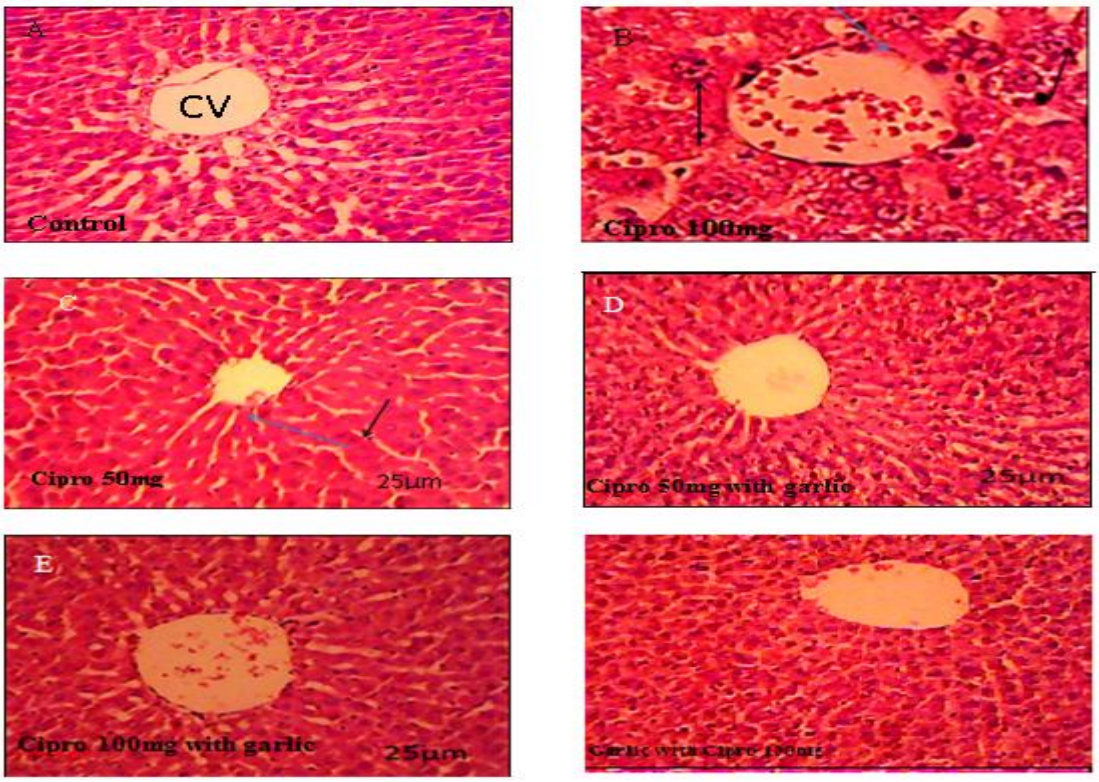


Figure6. *Liver rat sections were gavaged either 50 or 100 mg/kg of ciprofibrate with or without garlic. (A) Liver section from control groups shows normal histology structure of center vein (C.V). (B) Section from induced group with 100 mg/kg body wt ciprofibrate; shows congestion in sinusoids and damage in lining endothelium blue arrow, also destruction in hepatocytes, and necrosis black arrow. (C) Liver section induced with 50 mg/kg body wt ciprofibrate shows damage in lining endothelium blue arrow, destruction in hepatocytes. (D) Section of treated group with 50 mg/kg body wt ciprofibrate and garlic; shows clear improve to central vein. (E) Section of 100 mg/kg body wt ciprofibrate with garlic shows improve to central vein but there was a latte bleeding. (F) Section of garlic with ciprofibrate 100mg/kg body wt group shows hepatocytes were normal and sinusoid as well and (CV) was much better compressing with induced group(H&E X400).*

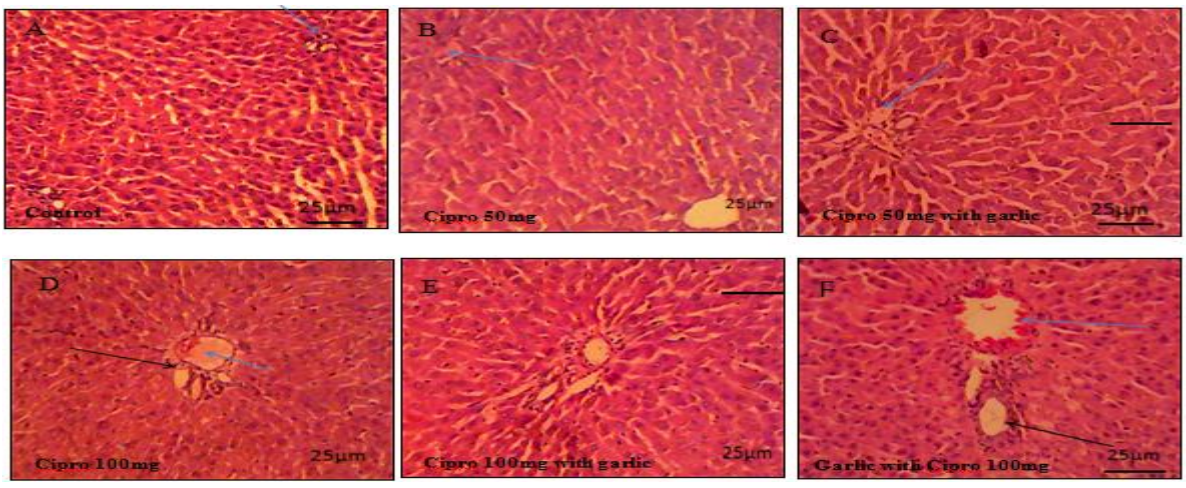


Figure6. *Liver rat sections were gavaged either 50 or 100 mg/kg of ciprofibrate with or without garlic. The liver tissue of control groups shows normal histology structure of portal canal contain branches of portal vein, hepatic artery and bile duct; the triad is clearly bordered by surrounding normal hepatocytes and interlobular septa blue arrow. (B) Section of the liver rat treated with 100mg/kg body wt of ciprofibrate shows congestion in portal areal blue arrow with inflammation around portal canal and distraction in hepatocytes surround portal tract black arrow. (c) Section of the liver rat treated with 50mg/kg body wt ciprofibrate shows congestion in portal area blue arrow. (D) Section of the liver rat of treated group with ciprofibrate 50 mg/kg body wt and garlic; shows improve to portal areal. (E) Section of the liver from treated group with ciprofibrate 100 mg/kg body wt and then garlic shows improve to portal area. (F) Section of treated group with garlic and then ciprofibrate 100mg/kg body wt show mild improve to portal area (H&E X400).*

The micrographs below show disorder in the hepatocytes with gathering to basophilic cells. Apoptosis in liver characterized by scattered single shows cells of necrosis separately in three different phases, which are pyknosis, karyorrhexis, and karyolysis. The slides were harvested.

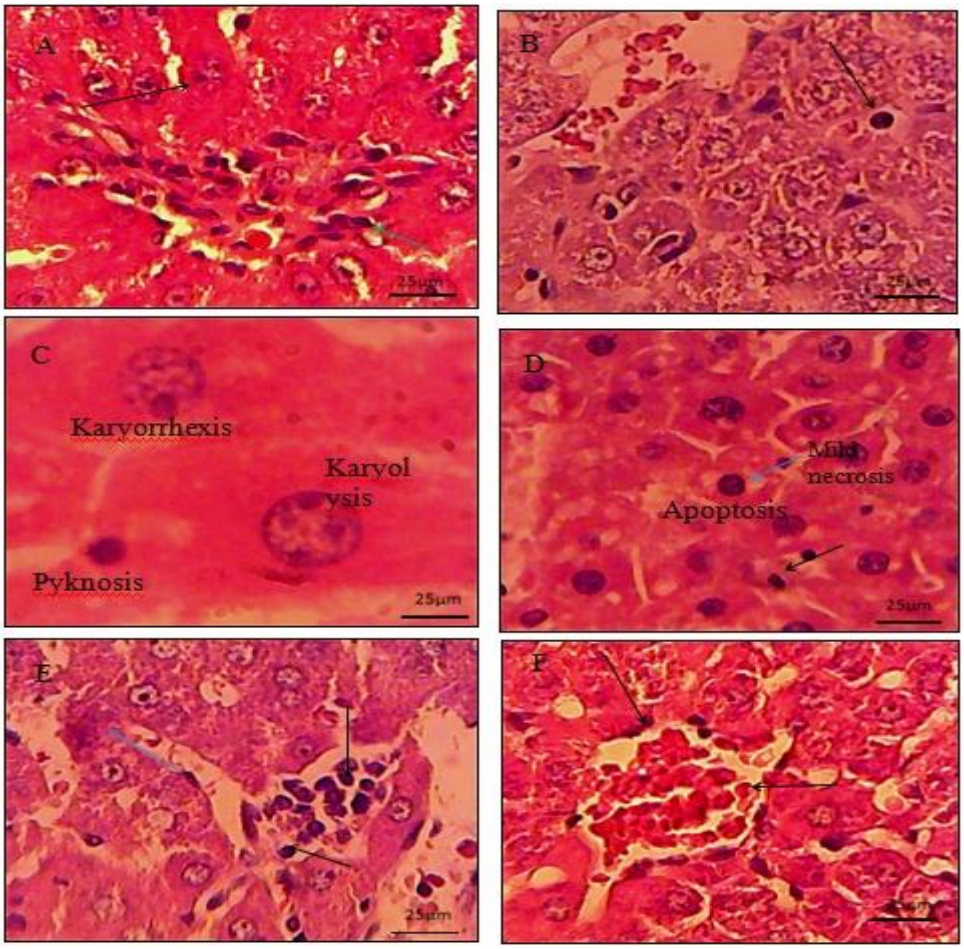


Figure4. *Micrographs of hepatocytes are collected from positive controls that induced and ciprofibrate, ciprofibrate with garlic Histopathological changes can be noticed in sections are focal necrosis with inflammatory cells. A; shows aggregation of inflammatory (green arrow) cells prominent of kupffer cells and apoptotic cells black arrow. B; apoptosis and distraction in hepatocytes and bleeding (RBc). C; shows 3 cells of necrosis separately in three different phases, which are pyknosis, karyorrhexis, and karyolysis. D; shows clear apoptotic hepatocytes blue arrow and necrosis occurring together in the liver cells black arrow. E; aggregation of inflammation in sinusoids black arrow and apoptotic cell blue arrow. F; the black arrow shows cell degeneration and necrosis. Light microscope a magnification are (X40 and X100) H&E stain.*

DISCUSSION

Liver is a principle organ of detoxification, and the major site of intense metabolism in generally, thus undergoing to various disorders because of exposure to the toxins [29]. Liver function test became the most important methods used to check the liver's safety from toxicity at the recent decades. Liver performance indices such as ALT, AST, and ALP are widely used to evaluate the liver injury [30]. Serum aminotransferase activities are known as toxicity markers in the hepatotoxicity studies caused by chemicals and an increase in the activities of these enzymes is termed as the early recognition of toxic hepatitis [31]. Necrosis or cell membrane damage can trigger the release of these enzymes into the blood circulation [30].

Liver function tests showed fluctuate in biochemical parameters. It has emphasized that ciprofibrate 50 and 100 mg had harmful effects on the activities of the serum Alanine aminotransferase, Aspartate aminotransferase, alkaline phosphates, total bilirubin, and direct bilirubin in male albino rats compared with the control group. The current study has found an increase in AST with both concentrations; note it was slightly higher at 50 mg/kg body wt of Cipro. In addition same condition with ALP enzyme, whereas much higher than AST especially at 50 mg/kg concentration [5].

In order to reduce the harmful effect of ciprofibrate, garlic was used to verify their therapeutic and/ or preventive role. It has been reported that the garlic regulates lowering of free radicals improve liver biomarkers, ameliorate hepatic marker enzymes, reduce severity of fibrosis and normalize the hepatocyte architecture. It was known from previous studies *in vivo* and *in vitro*, Wistar and Fischer rats (F-344/NHsd) dosed with peroxisome proliferators (nafenopin and Wyeth-14,643) that the hepatic induction of DNA replication starts as early as 24 hours in male rats [5, 10, 32, 33].

The peroxisome proliferator (ciprofibrate) is known to cause hepatocarcinogenesis in rats, and it strongly increases hepatic DNA replication of male Fischer rats [34]. Liver cells are damaged when exposed to compounds called free radicals, which are formed in the body as a result of exposure to a type of drug such peroxisome proliferators significantly. It is worth mentioning that this free radical's caused weak and harm to the healthy cells and play a large role in the incidence of cancer, but garlic reduces the damage caused by these vehicles as a strong Anti-oxidant. In this study garlic extract has showed positive effect on cell proliferation and significantly reduced serum liver functions [35].

In the current study, co administration of garlic extract with of 50 or 100 mg/kg body wt of ciprofibrate caused a significant decrease in serum ALT, AST, and ALP. Similar study had found that administration of (therapeutic and preventive garlic) significantly reduced the liver toxicity induced in rats by ciprofibrate [36]. Also; Banerjee *et al.* [1] had reported that many clinical trials showed a positive effect of garlic [37]. As well as, the garlic caused a significant decrease in total bilirubin. The hepatoprotective effect of garlic demonstrated in this study may enhance its therapeutic benefits as a potential preventive intervention for free radical-mediated liver injury [38]. Shaarawy *et al.* [13] reported that administration of garlic significantly reduced the liver toxicity induced in rats by N-nitrosodiethylamine (NDEA) and carbon tetrachloride (CCL_4). Nasr *et al.* [39] reported that aged garlic extract (250 mg/kg once for 21 days), pretreated rats revealed significant reduction in serum levels of AST, ALT induced by cisplatin (7.5 mg/kg, once intraperitoneal) administration. Additionally, histopathological revealed markedly ameliorated cisplatin induced toxicity on liver structure. Aged garlic extract has antioxidant and protective effects against cisplatin induced oxidative stress and liver structure in rats. Thus, it could be used as a dietary supplementation to reduce toxic side effects of anticancer drugs. Akinyemiju *et al.* [23] found that the intra-gastric administration of crude extract of garlic significantly decreased the circulating activities of AST, ALT, and ALP. Garlic extract was found to prevent and normalize oxidative stress generated by immobilization stress, which was evident by the reversal of deranged antioxidant enzymatic activities towards their normal values. This is possibly due to the organ sulfur contents in the garlic like allicin, alliin, and two major organ sulfur compounds SAC and S-allylmercaptocysteine which are potent free radical scavengers [40].

The process that results in an increase in the number of cells, known as proliferation, whereas defined by the balance between cell divisions and cell loss through the cell death or differentiation [41].

In the present study, in order to find out whether the cell division is normal or as a result of ciprofibrate stimulation, 9 fields in each slide were investigated under microscope for each rat in all groups, where the mean count in the control group reached approximately 2000 cells for the 9 fields. In comparison with the stimulus group, there were statistically significant differences; with a maximum of 2145 cells. This method of counting was used as an alternative to labeling method by BrdU used by Abushofa, [5] who has found the same mean approximately. The experiments were designed to establish if the replicating hepatocytes are found mostly in the periportal (PA) or central vein regions (CV) for F-344NHsd rats. In the current study, portal area has showed high liver induction comparing with CV area. This result was obtained by the counting of slide fields. This finding agreed with Kholaifi *et al.*, [42] who used BrdU stain to determine the zonation, he found that liver cells replication are high in the periportal zones.

The histological examination had conducted alongside with liver function tests. Our results revealed liver injury including; bleeding, damage in lining endothelium of central vein and portal area congestion in hepatic sinusoids, distraction in hepatocytes surround portal tract and hepatic necrosis associated with inflammatory cells and focal necrosis. Interestingly, three phases of necrosis were detected nuclear pyknosis, karyorrhexis, and

karyolysis for hepatocytes. Hepatocellular necrosis with some specific components of the metabolic pathways leading to the alteration of their structure and function [43]. In addition, the hepatocytes response to the toxic lesion was also reflected by the irregular shape of nuclei and nuclear condensation. Similar results were established in rats by Allen, *et al.* [44]. In this study the effect of administered ciprofibrate led to the injury of the liver in different zones.

In the present work, the focal necrosis and congestion occurred between central vein and portal zone in conditions of deficiency oxygen supply in liver cells and oxidative stress, all above histopathological changes those a resulting of induce liver with ciprofibrate. We investigated the hepatoprotective effects of therapeutically and preventively with garlic extract on liver injuries induced by ciprofibrate. It had a positive effects on liver injury of ciprofibrate treated rats. Garlic has role on regularizing the oxygen utilization in the cells, it appears that the protective effect of garlic extract involves the maintenance of antioxidant capacity in protecting the hepatic tissue against oxidative stress [45, 46].

CONCLUSION

The current study investigated the beneficial effects of garlic on liver injuries induced by peroxisome proliferators (ciprofibrate). This study provides accurate analyses of the potential effects of ciprofibrate on liver enzymes and the histological effects. The study showed valued results through study of biochemistry parameters and histological changes. Previous studies have addressed the effects of peroxisome proliferators, while the current study confirmed most of the previous results. In addition, in this experiment, garlic was used to reduce the toxicological effects of the drug. Garlic has shown inhibition to hepatotoxicity induced liver injuries in rats.

REFERENCES

[1] Banerjee, R. R. et al. (2004) Regulation of fasted blood glucose by resistin', Science. American Association for the Advancement of Science, 303(5661), pp. 1195–1198.

[2] Hyder, M. A., Hasan, M. and Mohieldein, A. H. (2013) 'Comparative levels of ALT, AST, ALP and GGT in liver associated diseases', European journal of experimental biology, 3(2), pp. 280–284.

[3] Vanden Heuvel, J. P. et al. (2006) 'Differential activation of nuclear receptors by per fluorinated fatty acid analogs and natural fatty acids: a comparison of human, mouse, and rat peroxisome proliferators-activated receptor-α,-β, and-γ, liver X receptor-β, and retinoid X receptor-α', Toxicological Sciences. Oxford University Press, 92(2), pp. 476–489.

[4] Darnell Jr, J. E. (2002) 'Transcription factors as targets for cancer therapy', Nature Reviews Cancer. Nature Publishing Group, 2(10), p. 740.

[5] Abushofa, F. A. A. (2014) 'Studies on the role of peroxisome proliferators: in liver growth and neurodegenerative disorders'. University of Nottingham.

[6] Vanhove, G. F. et al. (1993) 'The CoA esters of 2-methyl-branched chain fatty acids and of the bile acid intermediates di-and trihydroxy-coprostanic acids are oxidized by one single peroxisomal branched chain acyl-CoA oxidase in human liver and kidney.', Journal of Biological Chemistry. ASBMB, 268(14), pp. 10335–10344.

[7] Kliewer, S. A. et al. (1997) 'Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ', Proceedings of the National Academy of Sciences. National Acad Sciences, 94(9), pp. 4318–4323.

[8] Mannaerts, G. P. and Van Veldhoven, P. P. (1993) 'Metabolic pathways in mammalian peroxisomes', Biochimie. Elsevier, 75(3–4), pp. 147–158.

[9] Rao, M. S. and Subbarao, V. (1997) 'Effect of dexamethasone on ciprofibrate-induced cell proliferation and peroxisome proliferation', Toxicological Sciences. Oxford University Press, 35(1), pp. 78–83.

[10] Al Kholaifi, A. et al. (2008) 'Species-specific kinetics and zonation of hepatic DNA synthesis induced by ligands of PPARα', Toxicological sciences. Oxford University Press, 104(1), pp. 74–85.

[11] Kawanishi, S. et al. (2002) 'The role of metals in site-specific DNA damage with reference to carcinogenesis', Free Radical Biology and Medicine. Elsevier, 32(9), pp. 822–832.

[12] Tripathi, K. (2009) 'A review-Garlic, the spice of life-(Part-I)', Asian J Res Chem, 2(1), pp. 8–13.

[13] Shaarawy, S. M. et al. (2009) 'Protective effects of garlic and silymarin on NDEA-induced rats hepatotoxicity', International journal of biological sciences. Ivyspring International Publisher, 5(6), p. 549.

[14] Agarwal, K. C. (1996) 'Therapeutic actions of garlic constituents', Medicinal research reviews. Wiley Online Library, 16(1), pp. 111–124.

[15] Aruoma, O. I. et al. (1992) 'Antioxidant and pro-oxidant properties of active rosemary constituents: carnosol and carnosic acid', Xenobiotica. Taylor &

Francis, 22(2), pp. 257–268.

[16] Kita, T. et al. (1987) 'Probucol prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbit, an animal model for familial hypercholesterolemia', Proceedings of the National Academy of Sciences. National Acad Sciences, 84(16), pp. 5928–5931.

[17] Forman, B. M., Chen, J. and Evans, R. M. (1997) 'Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors α and δ', Proceedings of the National Academy of Sciences. National Acad Sciences, 94(9), pp. 4312–4317.

[18] Bordia, A. (1981) 'Effect of garlic on blood lipids in patients with coronary heart disease', The American Journal of Clinical Nutrition. Oxford University Press, 34(10), pp. 2100–2103.

[19] Omar, S. H. and Al-Wabel, N. A. (2010) 'Organosulfur compounds and possible mechanism of garlic in cancer', Saudi Pharmaceutical Journal. Elsevier, 18(1), pp. 51–58.

[20] Oosthuizen, C. B., Reid, A.-M. and Lall, N. (2018) 'Garlic (Allium sativum) and Its Associated Molecules, as Medicine', in Medicinal Plants for Holistic Health and Well-Being. Elsevier, pp. 277–295.

[21] Augusti, K. T. (1996) 'Therapeutic values of onion (Allium cepa L.) and garlic (Allium sativum L.).', Indian journal of experimental biology, 34(7), pp. 634–640.

[22] Krishnaveni, M. et al. (2010) 'Antidiabetic and antihyperlipidemic properties of Phyllanthus emblica Linn. (Euphorbiaceae) on streptoz-otocin induced diabetic rats', Pak J Nutr, 9(1), pp. 43–51.

[23] Akinyemiju, T. et al. (2017) 'the burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: results from the Global Burden of Disease Study 2015', JAMA oncology. American Medical Association, 3(12), pp. 1683–1691.

[24] Hosseini, A. and Hosseinzadeh, H. (2015) 'A review on the effects of Allium sativum (Garlic) in metabolic syndrome', Journal of Endocrinological Investigation. Springer, 38(11), pp. 1147–1157.

[25] Abushofa, F. A., Bell, D. R. and Dyer, P. S. (2015) 'Mechanism of action of liver growth induced by non-genotoxic carcinogens (peroxisome proliferators)'.

[26] Reitman S, and Frankel A. (1957). Colorimetric method for determination of serum glutamate oxaloaectate and glutamic pyruvate transaminase. Amer J Clin Pathol., 28: 56-58.

[27] Kind PRN, King EJ, Varley H, Gowenlock AH, Bell M. (1980). Method of practical clinical biochemistry. Heinman, London, , pp. 899-900.

[28] Dangerfield WG and Finlayson R. (1953). Estimation of bilirubin in serum. J Clin Pathol., 6(3):173-177

[29] Rasgele, P. and Kaymak F. (2013). EffEcts of food preservative natamycin on liver enzymes and total protein in Mus Musculus. Bulgarian J Agric Sci., 19(2): 298-302.

[30] Drotman, R. B., & Lawhorn, G. T. (1978). Serum enzymes as indicators of chemically induced liver damage. Drug and chemical toxicology, 1(2), 163-171.

[31] AL-Shinnawy, M. S. (2009). "Physiological effect of a food additive on some haematological and biochemical parameters of male albino rats." Egypt. Acad. J. Biol. Sci 2(1): 143-151.

[32] Muenchen, H. J. and Pienta, K. J. (1999) 'The role of the nuclear matrix in cancer chemotherapy', Critical ReviewsTM in Eukaryotic Gene Expression. Begel House Inc., 9(3–4).

[33] Ledda-Columbano, G. M. et al. (1998) 'In vivo hepatocyte proliferation is inducible through a TNF and IL-6-independent pathway', Oncogene. Nature Publishing Group, 17(8), p. 1039.

[34] Bursch, W. et al. (1984) 'Controlled death (apoptosis) of normal and putative preneoplastic cells in rat liver following withdrawal of tumor promoters', Carcinogenesis. Oxford University Press, 5(4), pp. 453–458.

[35] Harris, J. C. et al. (2001) 'Antimicrobial properties of Allium sativum (garlic)', Applied microbiology and biotechnology. Springer, 57(3), pp. 282–286.

[36] Makowska, J. M. et al. (1990) 'Characterization of the hepatic responses to the short-term administration of ciprofibrate in several rat strains: Co-induction of microsomal cytochrome P-450 IVA1 and peroxisome proliferation', Biochemical pharmacology. Elsevier, 40(5), pp. 1083–1093.

[37] Ohaeri, O. C. (2001) 'Effect of garlic oil on the levels of various enzymes in the serum and tissue of streptozotocin diabetic rats', Bioscience reports. Springer, 21(1), pp. 19–24.

[38] Albano, E. (2006) 'Alcohol, oxidative stress and free radical damage', Proceedings of the nutrition society. Cambridge University Press, 65(3), pp. 278–290.

[39] Nasr, T., Bondock, S. and Youns, M. (2014) 'Anticancer activity of new coumarin substituted hydrazide–hydrazone derivatives', European journal of medicinal chemistry. Elsevier, 76, pp. 539–548.

[40] Asdaq, S. M. and Inamdar, M. N. (2010) 'Potential of garlic and its active constituent, S-allyl cysteine, as antihypertensive and cardioprotective in

presence of captopril', Phytomedicine. Elsevier, 17(13), pp. 1016–1026.

[41] Kroemer, G. et al. (2009) 'Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009', Cell death and differentiation. Nature Publishing Group, 16(1), p. 3.

[42] Al Kholaifi, A. et al. (2008) 'Species-specific kinetics and zonation of hepatic DNA synthesis induced by ligands of PPARα', Toxicological sciences. Oxford University Press, 104(1), pp. 74–85.

[43] Suzanne, M. et al. (1984) 'Midzonal necrosis as a pattern of hepatocellular injury after shock', Gastroenterology. Elsevier, 86(4), pp. 627–631.

[44] Allen, J. W., Khetani, S. R. and Bhatia, S. N. (2004) 'In vitro zonation and toxicity in a hepatocyte bioreactor', Toxicological sciences. Oxford University Press, 84(1), pp. 110–119.

[45] Pratibha, R. et al. (2006) 'Enzymatic studies of cisplatin induced oxidative stress in hepatic tissue of rats', European journal of pharmacology. Elsevier, 532(3), pp. 290–293.

[46] Chandra-Kuntal, K., Lee, J. and Singh, S. V (2013) 'Critical role for reactive oxygen species in apoptosis induction and cell migration inhibition by diallyl trisulfide, a cancer chemopreventive component of garlic', Breast cancer research and treatment. Springer, 138(1), pp. 69–79.

Citation: *Fikry Ali Abushofa, Azab Elsayed Azab, Nadia M. Kermani, Tahani S. AL-Jelany Sakah, "Hepatoprotective Effect of Garlic Aqueous Extract against Hepatotoxicity Induced By Ciprofibrate in Male Albino Rats", Journal of Biotechnology and Bioengineering, 4(1), 2020, pp 1-13.*