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HEPATOPROTECTIVE ACTIVITY OF DIFFERENT PARTS OF *CHRYSOPHYLLUM ALBIDUM* (AFRICAN STAR APPLE) IN ACETAMINOPHEN-INDUCED HEPATOTOXICITY IN RATS

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Abstract

The oxidative capacity of acetaminophen (N-acetyl-para-aminophenol) metabolite (N-acetyl-p-benzoquinone imine) is believed to be the major cause of hepatotocixity of acetaminophen. This study investigated the protective effects of administration of different extracts of *Chrysophyllum albidum* on acetaminophen-induced liver damage in male wistar albino rats with a single dose (250 mg/kg) of acetaminophen. Serum and tissue γ-glutamyl transferase, alkaline phosphatase activities as well as hepatic expression of Mating type protein-1, Early growth response protein-1, Nuclear-enrinched abundant transcript-1 and Cytochrome-P450 3AF genes were determined. The mechanisms of action of *C. albidum* extracts were examined by measuring the degree of tissue peroxidation and tissue antioxidant status. The result showed that acetaminophen caused liver damage as evident by statistically significant ($p < 0.05$) increase in malondialdehyde levels, GGT and ALP activities in the control when compared with treatment groups both in the serum and liver. Increased in the expression of MAT-1, EGR-1 and NEAT-1 gene were observed in the control when compared with the treatment groups. Administration of the *C. albidum* leaf extract increased the hepatic expression of CYP3AF when compared with the control. Acetaminophen induction significantly reduced the superoxide dismutase, catalase activities, glutathione reduced status and protein concentration in the control. Administration of the two doses of *C. albidum* extracts significantly reduced the degree of tissue peroxidation and increased the antioxidant status at the two doses. The result suggests that *C. albidum* extracts could act as hepatoprotective agent for preventing hepatic tissue from toxicity resulting from acetaminophen-induced hepatotocixityby reducing peroxidation and increasing antioxidant enzyme status.

Keywords: *Acetaminophen, Oxidative stress, Hepatotoxicity, Chrysophyllum albidum, Gene expression.*

Introduction

The toxication or detoxication of xenobiotic substances is controlled by xenobiotic biotransformation in the liver (Watkins and Seeff, 2006). Reactive intermediates produced during biotransformation reaction could interact with important cellular macromolecules and then trigger the events that promote tissue injury cell death and permanent genomic changes, resulting potentially to cancer [1].

Acetaminophen (N-acetyl-para-aminophenol, APAP), is a clinically important analgesic and antipyretic medication. It is regarded as over-the-counter medication generally used by all age group. At therapeutic doses, N-acetyl-para-aminophenol has been reported as a mild analgesic medication, but at high doses, it is regarded as a potent hepatotoxicant in persons with enhanced susceptibility [2]. N-acetyl-para-aminopheno is largely converted to conjugates of glucuronate and sulfate. A minute amount is broken down to an active metabolite, N-acetyl-p-benzoquinone imine (NAPQI) through the action of cytochrome P450 enzyme, which binds rapidly to glutathione (GSH) and other metabolites appear to have no toxicity [3]. When the content of an active metabolite increases above the GSH content, the excess metabolite binds to tissue molecules resulting in toxicity such as necrosis. The toxic metabolite consequently binds to key proteins and the lipid bilayer of hepatocyte membranes, leading to centrilobular liver necrosis and hepatocellular death [4].

Due to technological advances in molecular biology, gene expression profiles may be valuable biomarkers for evaluating toxicity in animal models. Hepatic expression of MAT-1, EGR-1 NEAT-1 and CYP3AF genes were determined for possible mechanism of action of the extract. The expression profiles of some specific gene have been evaluated for several types of toxicological studies including identifying exposure to specific chemical classes [5, 6], identifying toxic end points, [7], predicting or classifying exposures that produce a toxic outcome [8], and identifying mechanisms of toxicity [9].

Liver injury can be classified as hepatocellular, cholestatic or mixed [1]. Due to the functions of the liver, hepatic diseases continue to occur among the principal threats to public health [10]. It has been reported that no completely effective drugs stimulate hepatic function, offer complete protection to the organ, or aid in regenerating hepatic cells despite enormous advances in modern medicine [11].

A large body of evidence has shown immense potential of medicinal plants for treatment and management of various kinds of disease and liver disorder. The use of some plants and the consumption of different fruits have played fundamental roles in human health care.

Chrysophyllum albidum commonly called Africa star apple is an edible tropical fruit that belong to the Sapotaceae family. The fruit is called 'agbalumo' in South-western Nigeria. Different parts of this plant have been reported to have a quite number of therapeutic potentials. *C. albidum* possess natural antioxidants which are known to promote health by preventing oxidative stress related diseases [12]. The bark is used for the treatment of yellow fever and malaria while the leaf is used as an emollient and skin eruption treatment, diarrhea, stomach ache [13]. The seed cotyledon contains hypoglycemic and hypolipidemic effects [14]. In the previous studies by Idowu *et al.* [15], Idowu *et al.* [16] and Adebayo et al. [17], various polyphenolic compounds have been isolated and identified from different parts of C. *albidum* plant (Table 1). Other studies performed on the plant include anti-plasmodial [18], antimicrobial activity [15]. There are few reports in literature as regards the protective effect of different parts of *C. albidum* on acetaminophen toxicity. However, this study aims at investigating the hepatoprotective effect of different parts of *C. albidum* in experimental hepatotoxicity.

Methods

2.1 Chemicals and reagents

Acetaminophen was obtained from Sigma-Aldrich, USA, Ascorbic acid, Acetic acid, methanol, NH_4OH, $FeCl_3$, Potassium ferrocynide, Diethyl ether, NaCl, Sodium carbonate, Hydrogen peroxide, EDTA, TCA, Phosphate buffer, Potassium iodide, Butanol, Potassium acetate, Folin C, Tannic acid, ethanol, Sucrose, Sulfuric acid, HCl, were obtained from BDH Chemicals Ltd.

2.2 Plant material: collection and identification
The plant was collected from in Ado-Ekiti, Ekiti State, Nigeria. The plant including the leaf and fruit were identified at the Department of Plant Science and Biotechnology, Federal University, Oye-Ekiti.

2.3 Preparation of plant extract
C. *albidum* fruits were rinsed thoroughly with water so as to remove dirt particles. The fruits were sliced and the seed were discarded. The juice was then separated from the pulp using a muslin cloth. The leaf and pulp were air-dried at room temperature. The air-dried leaf and pulp were blended separately to powder using electric blender. Blended air-dried leaf and pulp were soaked separately in adequate volume of methanol for 72 h at room temperature. It was stirred continuously after each 24 h. After 72 h, the mixture in each was filtered and the filtrate was concentrated using rotary evaporator at 40̊ C. The concentrate was heated over a water bath also at 40̊ C to obtain a solvent free extract, which was stored in a refrigerator at 4̊ C. The juice extracted was then filled into sterilized glass bottles, kept in a refrigerator and thereafter lyophilised for further preservation.

2.4 Lyophilization
The juice extract was completely frozen at -4̊ C. The frozen juice extract was then placed under a deep vacuum, well below the triple point of water and heat energy was then applied causing it to sublime. It was backfilled and stopped under vacuum and the dried product was removed from freeze dryer [19].

2.5 Experimental design
The rats were randomly divided into 10 groups of 5 rats per group.
Group I: Animals were given only distilled water throughout the experiment (baseline).
Group II: Animals were induced with acetaminophen (250mg/kg b.w) and serve as control (untreated).
Group III: Acetaminophen + 250mg/kg b.w *C.albidum* leaf extract (A + 250 mg/kg L).
Group IV: Acetaminophen + 500mg/kg b.w *C.albidum* leaf extract (A + 500 mg/kg L).
Group V: Acetaminophen + 250mg/kg b.w *C.albidum* pulp extract (A + 250 mg/kg P).
Group VI: Acetaminophen + 500mg/kg b.w *C.albidum* pulp extract (A + 500 mg/kg P).
Group VII: Acetaminophen + 250mg/kg b.w *C.albidum* juice (A + 250 mg/kg J).
Group VIII: Acetaminophen +500mg/kg b.w *C.albidum* juice (A + 500 mg/kg J).
Group IX: Acetaminophen + 100mg/kg b.w ascorbic acid (A + ASC).
Group X: Animals were administered with 100 mg/kg b.w ascorbic acid only (ASC).
All administration was done orally and the treatment spans for one week. Animals were subsequently anaesthetized using diethyl ether and liver samples were collected for biochemical evaluations.

2.6 Animal handling
A total of 50 male wistar albino rats weighing between 150g and 225g were obtained from Federal Polytechnic Ado-Ekiti, Ekiti State, Nigeria. They were housed in individual cages at the animal house of the Department of Biochemistry, Federal University Oye-Ekiti in a temperature and humidity controlled room and were given rat chow (feed) and clean drinking water. They were acclimatized for seven days and were treated in accordance with the recommendations of National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals [20]. The work was approved by the Departmental Ethical Review Committee.

2.7 Induction of acetaminophen in experimental animals
The animals (male wistar rats) of about 150g to 225g were injected intraperitonially with acetaminophen (APAP) (250mg/kg body weight) dissolved in distilled water.

2.8 Collection and preparation of blood samples
Animals were anaesthetized using diethyl ether. They were allowed to go into an unconscious state after which they were sacrificed by cutting their jugular veins to collect blood sample. Blood was collected in plain bottles and allowed to clot for the preparation of serum. The clot blood was centrifuged at 1000 x *g* for 5 minutes. The clear supernatant was then separated from the pellet and kept frozen at 4^{o}C until required.

2.9 Preparation of tissue homogenates

Animals were immediately dissected and the tissue (liver) were removed, rinsed with sucrose solution and weighed before being stored in ice-cold 0.25M sucrose solution. Liver homogenates were prepared by suspending the tissue in ice-cold 0.25M sucrose buffer, pH 7.4 solution to give a final volume of five times dilution and homogenized in a tefflon homogenizer. The homogenates were later centrifuged at 10000 *g* for 15 minutes at 4°C and the supernatants, were collected and used for biochemical assays.

2.10 Histopathological study

The tissues were observed and cut into small pieces into pre-labeled cassettes. They were fixed in 10% formal saline for 24 hours and processed using automatic tissue processor (Leica TP 1020). The processed tissues were placed in paraffin wax using a semi-automatic tissue embedding center. The tissue block formed was separated from the mold and trimmed to expose the tissue surface using a rotary microtome at 6micrometer after which the surfaces were cool on ice. The tissues were sectioned at 4 micrometer (ribbon section). The sections were floated on water bath at 55°C and these were picked using clean slides. The slides were labeled and dried on a hotplate (Raymond lamb) at 60°c for 1hour. Hematoxylin-Eosin staining technique was used. The assessment of histopathological and photomicrography of the prepared slides was achieved through an Olympus light Microscope attached with a Kodak camera.

2.11 Biochemical analysis

The γ-glutamyl transferase (GGT) activity was determined according to the method of Kaplan and Pesce, [21]. Alkaline phosphatase (ALP) activity was assessed using the method of Wright et al. [22]. Tissue MDA was determined as TBA reacting substance (TBARS) as described by Varshney and Kale [23]. The method of Sun and Zigma [24] was used to determine superoxide dismutase (SOD) activity. Catalase activity was assayed for by the method of Sinha [25]. Reduced glutathione (GSH) was determined by the method of Sedlak and Lindsay [26]. Protein content was estimated by the method of Lowry *et al*. [27] using bovine serum albumin as standard.

2.12 Gene expression

RNA was isolated from rats liver using TRIzol Reagent (ThermoFisher Scientific). DNA contaminant was removed following DNAse I treatment (ThermoFisher Scientific) following manufacturer's protocol. The RNA was quantified using Hitachi-U1900 spectrophotometer at 260nm. Purified DNA-free RNA was converted to cDNA immediately using ProtoScriptFirst Strand cDNA Synthesis Kit (NEB). PCR amplification was done using OneTaq® 2X Master Mix (NEB) using the following primer set: (GAPDH- (control)-Forward primer-5'-AGACAGCCGCATCTTCTTGT-3'/Reverse-primer-5'-CTTGCCGTGGGTAGAGTCAT-3'; EGR-1-Forwardprimer-5'-AGGAGTGATGAACGCAAGAG-3'(Sense)/Reverse-primer-5'GGGTAGGAAGAGAGGGAAGA-3'(Antisense);CYP3A-Fowardprimer-5'-CTGCATTGGCATGAGGTTTG-3'(Sense)/Reverse-primer-5'-CTTACAAGGCTGGAAGGAGAAG-3'(Antisense); MAT-1A-Forward primer-5'-CTCTGGAAAGGACTACACCAAG-3'(Sense)/Reverse-primer-5'- GGCATAGGACACCTGAACAA-3' (Antisense); NEAT-1-Forward primer-5'-GTTCCGTGCTTCCTCTTCTG-3'(Sense)/Reverse-primer-5'- GTGTCCTCCGACTTTACCAG-3' (Antisense). The expression level of the genes studied was normalized by GAPDH. The band density was measured using ImageJ which was plotted as a bar graph (Mean ± SEM).

2.13 Data analysis

Results were analyzed using and one-way ANOVA followed by Dunnett's post-hoc test for comparison between control and test groups. All data were expressed as mean ± standard error of the mean. '*p*' values < 0.05 were considered significant. GraphPad Prism 5 was also used for the analyses. The intensities of the bands form agarose gel electrophoresis were quantified densitometrically using ImageJ software.

Results

3.1 Malondialdehyde levels in the tissues of experimental animals

In figure 1, there was significant ($p < 0.05$) increase in the malondialdehyde levels in the control group (acetaminophen-induced untreated rats) both in the

serum and liver when compared to the extracts treated groups. Administration of the *C.albidum* extracts significantly reduced the MDA level at the two doses (250 and 500mg/kg body weight) in a dose dependent manner. Reduction in the malondialdehyde level by the pulp extract and *C.albidum* juice at 250mg/kg body weight were observed to be higher than 500mg/kg body weight both in the tissues (serum and liver). Reduction in the malondialdehyde level by leaf extract at 500mg/kg body weight was found to be higher than the 250mg/kg body weight treated group in the liver. A significant ($p< 0.05$) decrease in malondialdehyde level was also observed in ascorbic acid treated group, and this reduction was not statistically different from the extract treated groups in both tissues.

3.2 γ-glutamyl transferase and alkaline phosphatase activity

A significant ($p< 0.005$) increase in the activity of GGT and ALP in serum were observed in the control group when compared with extract and ascorbic acid treated groups (Figure 2). In the same manner, the activity of GGT and ALP were observed to be significantly ($p< 0.05$) higher in the control group when compared to the treated groups in the liver (Figure 3).

3.3 Antioxidant enzymes status

There was a significant ($p < 0.05$) decrease in SOD and catalase activities, GSH level and total protein in the control group when compared to extract treated groups. Administration of the *C.albidum* extracts significantly increased all the antioxidant status (Table 2).

3.4 Expression of hepatic genes

Expression of MAT-1, EGR-1 and NEAT-1 gene were observed to be higher in the control group when compared with the extract and ascorbic acid treated groups. Administration of the leaf extract at 500 mg/kg body weight increased the hepatic expression of CYP3AF when compared with the control and other extract treated groups (Figure 4-7).

3.5 Histopathological results

The photomicrograph of liver section of rat induced with acetaminophen and treated with *C. albidum* extracts and ascorbic acid are presented in Figure 8a- c. The cross section of liver of animals with no induction showed normal central venules without congestion as well as normal portal tract, the morphology of the hepatocytes appeared normal, the sinusoids appeared normal and not infiltrated and no pathological lesion seen. The control group induced with acetaminophen showed mild congestion within the portal vein of the portal tract, the liver parenchyma showed areas with degenerated hepatocytes and necrosis (Figure 8a).

The liver parenchyma of the group treated with 250 mg/kg leaf extract showed severe necrosis, hemorrhage and very poor architecture with loss of liver plates but the sinusoids appeared normal and not infiltrated.The group treated with 500 mg/kg leaf extract showed normal central venules without congestion and mildly infiltrated portal tract, the morphology of the hepatocytes and sinusoids appeared normal and not infiltrated. There was a mild congestion within the portal vein of the portal tract in the animals that received 250 mg/kg pulp extract while the 500 mg/kg pulp extract showed moderate congestion within the portal vein of the portal tract, severely degeneratated hepatocytes by fat and also severe to chronic necrosis. The group treated with 250 mg/kg *C. albidum* juice showed normal central venules without congestion, the morphology of the hepatocytes appeared normal but the sinusoids showed very few inflammatory cells. The animals that received 500 mg/kg *C. albidum* juice also showed normal central venules without congestion and moderate periportal infiltration of inflammatory cells, followed by a moderate degeneration of hepatocytes morphology (Figure 8b).

The cross section of liver of animals treated with ascorbic acid showed moderate infiltration of portal tract and focal area of mild lymphocytes aggregates was seen, followed by the presence of moderate cystic area seen, but the sinusoids appeared normal and not infiltrated. The group that only received ascorbic acid without induction of acetaminophen showed poor hepatocytes morphology followed by moderate fat degeneration owing to fat infiltrating the hepatic cytoplasms, the sinusoids appeared normal and not infiltrated,venules and portal tracts appeared normal (Figure 8c).

Discussion

Drug-induced hepatotoxicity is a common cause of liver injury and accounts for approximately half of the cases of acute liver failure while mimicking all forms of acute and chronic liver diseases [28]. The leading drug-related cause has been associated to acetaminophen toxicity (Larson *et al*., 2005). Acetaminophen is conjugated to water soluble metabolites and is excreted in the urine at low doses while at higher doses it is metabolized to toxic intermediates. [29]. At therapeutic doses, acetaminophen is considered a safe medication but when taken in overdose can cause hepatic necrosis, nephrotoxicity, extra hepatic lesions, and even death in humans and animals [2, 30].

The secondary metabolites and polyphenolics compounds in plants have provided sources of compounds in the development of new therapeutics. Some naturally occurring plants have antioxidant properties which made them to be hepatoprotective and therefore can be considered for use in the treatment of acute and chronic diseases [31].

Reactive oxygen species (ROS) are known to be products of normal cellular metabolism. At low concentration, ROS can induce a mitogenic response while at high concentration; it serves as mediators of damage to cell structures, including lipids and membranes, proteins and nucleic acids [32]. From the result of this study, treatment with *C. albidum* extracts reduced the level of malondialdehyde (MDA) in both serum and liver when compared to the control (untreated) group. Administration of the *C.albidum* extracts significantly reduced the MDA level in a dose dependent manner, this observation is in agreement with the work of Lahouel *et al*. [33].

The alkaline phosphatase (ALP) and γ-glutamyl transferase (GGT) activity in the control group increased both in serum and liver when compared with the extract treated groups. Increase in the level of MDA, ALP and GGT in the tissues could be as a result of perturbation in hepatocytes physiology and morphology caused by acetaminophen as well as production of ROS and free radicals. The increase could also be associated with drug induced cholestatis [34]. This is substantiated with the histopathological results which showed a mild congestion within the portal vein of the portal tract with degenerated hepatocytes and necrosis in the liver of untreated control group. Findings from this study support the hypothesis that oxidative stress which is always associated with lipid peroxidation is a crucial step in acetaminophen toxicity.

Antioxidant system maintains the redox homeostasis in the liver. The oxidative stress do not only triggers liver damage by inducing irreversible modification of lipids, proteins and DNA contents but also modulate pathways that control normal biological functions. These pathways regulate genes transcription, protein expression, cell apoptosis and signalling cascades activation [35].

Gene expression profiles are valuable biomarkers for evaluating toxicity in animal models. Gene expression changes have been normally used to obtain specific mechanistic information concerning the type of action of a toxicant. From the result of this study, an up regulation of MAT-1, EGR-1 and NEAT-1 gene were observed in the control group when compared with the extract and ascorbic acid treated groups. Administration of the leaf extract at 500 mg/kg body weight increased the hepatic expression of CYP3AF when compared with the control and other extract treated groups.

The effect of reactive oxygen and nitrogen species is stabilized by the antioxidant action of non-enzymatic antioxidants and antioxidant enzymes. These antioxidants directly get rid of free radicals, thus providing maximal protection for biological sites [36]. Catalase is important in cellular antioxidative defense mechanisms and it is efficient in degrading endogenously produced hydrogen peroxide.

Glutathione is essential in protecting cells against oxidative stress by neutralizing free radicals, scavenging reactive oxygen and nitrogen species and other radicals produced in association with electron transport, inflammatory responses and xenobiotic metabolism [37]. A decrease in SOD and catalase activities, GSH level and total protein were observed in the control group when compared to extract treated groups. The reduction in the activities of these enzymes in the control could be as a result of oxidative stress caused by acetaminophen induction. NAPQI is an oxidative product of acetaminophen metabolism attach covalently to proteins leading to cell necrosis and

lipid peroxidation as a result of decrease in glutathione in the liver causing hepatotoxicity [38]. Alteration in the protein concentration could be due to the effect of NAPQI on the protein metabolizing ability of the liver. Administration of extract restored the declined hepatic antioxidant capacity induced by acetaminophen in the present study. *C.albidum* extracts increased all the antioxidant status and protein concentration when compared with the control.

Moreover, treatment with the reference drug, ascorbic acid enhanced the antioxidant defense systems. Due to its strong antioxidant effect, ascorbic acid modified liver oxidative status, as revealed by a significant increase in liver SOD, catalase and GSH content along with reduced MDA, GGT and ALP as compared to acetaminophen-intoxicated group.

Finding from this study is in consonance with other previous studies [39]. Antioxidants status typically reduced with the degree of stress conditions [39] which was also observed in this study. The result from this study is also substantiated with the histopathological results. It has been reported that treatment of acetaminophen overdose is based on replenishment of antioxidant thiols to supplement the role of glutathione. Since toxicity of acetaminophen is believed to be due to free radical formation and oxidative stress, the protective effect of *C. albidium* extracts against acetaminophen-induced hepatic oxidative damage may be ascribed to the presence of secondary metabolites and polyphenolic compounds from different parts of *C. albidium* extract which protect against oxidative insult and boost the endogenous enzymatic and nonenzymatic antioxidant defense system as represented in (scheme 1).

Conclusion

The result of this study suggests that *C. albidium* extracts and juice are effective therapeutic agents for preventing hepatic tissue from toxicity resulting from acetaminophen-induced hepatotocixity.

Conflict of interests

The author(s) did not declare any conflict of interest.

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Table 1. Phytoconstituents identified from different parts of *C. albidum*

Chrysophyllum albidum	Isolated/identified phytoconstituents	References
Stem-bark	Stigmasterol, Epicatechin, Epigallocatechin, procyanidin B5	Idowu *et al*., 2016
Seeds	Eleagnine, Tetrahydro-2-methylharman, Skatole	Idowu *et al*., 2003
Leaves	Myricetin-3-rhamnoside	Adebayo *et al*., 2011

Table 2. In vivo antioxidant enzymes status and protein concentration in the liver after administration of *C. albidum* extracts

Group	SOD (U/g protein)	Catalase (U/g protein)	GSH (µmol/g protein)	Protein (g/l)
Baseline	1.60 ± 0.02^{a}	40.35 ± 4.48^{a}	0.61 ± 0.14^{a}	3.06 ± 0.31^{a}
Control	1.28 ± 0.08^{b}	20.49 ± 0.42^{b}	0.22 ± 0.09^{b}	1.66 ± 0.08^{b}
A + 250mg/kg L	1.71 ± 0.02^{ac}	41.84 ± 0.15^{a}	0.74 ± 0.09^{a}	2.39 ± 0.20^{c}
A + 500mg/kg L	1.94 ± 0.03^{ac}	34.44 ± 4.05^{ac}	0.91 ± 0.06^{c}	2.43 ± 0.22^{c}
A + 250mg/kg P	1.82 ± 0.02^{ac}	40.53 ± 0.19^{a}	0.55 ± 0.11^{a}	2.27 ± 0.27^{c}
A + 500mg/kg P	1.88 ± 0.07^{ac}	41.56 ± 0.03^{a}	0.81 ± 0.06^{c}	2.53 ± 0.17^{c}
A + 250mg/kg J	1.69 ± 0.11^{ac}	37.35 ± 5.02^{ac}	0.87 ± 0.03^{c}	2.57 ± 0.45^{c}
A + 500mg/kg J	1.88 ± 0.06^{ac}	40.59 ± 4.87^{a}	0.62 ± 0.14^{a}	2.07 ± 0.28^{c}
A + Asc (100mg/kg)	1.88 ± 0.06^{ac}	40.59 ± 4.87^{a}	0.62 ± 0.14^{a}	2.07 ± 0.28^{c}
Asc (100mg/kg)	1.75 ± 0.13^{a}	37.76 ± 8.12^{ac}	1.20 ± 0.10^{c}	2.21 ± 0.12^{c}

A = Acetaminophen; L = *C. albidum* leaf extract; P = *C. albidum* pulp extract; J = *C. albidum* juice; ASC = Ascorbic acid; SOD = superoxide dismutase and GSH = glutathione.

Results are expressed as Mean ± SEM of 5 determinations. At ($p < 0.05$), values with different superscript are significant statistically

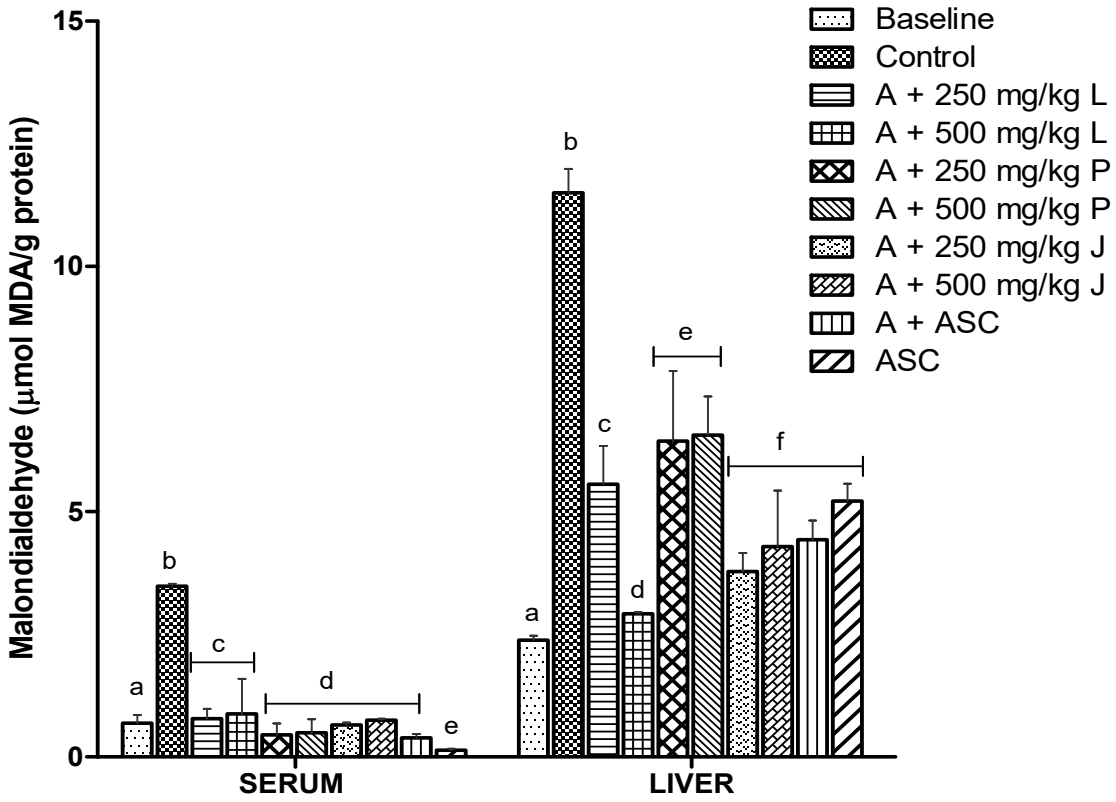


Figure 1. Malondialdehyde level after administration of *C. albidum* extracts
A = Acetaminophen; L = *C. albidum* leaf extract; P = *C. albidum* pulp extract; J = *C. albidum* juice; ASC = Ascorbic acid.
Results are expressed as Mean ± SEM of 5 determinations. At ($p < 0.05$), values with different superscript are significant statistically

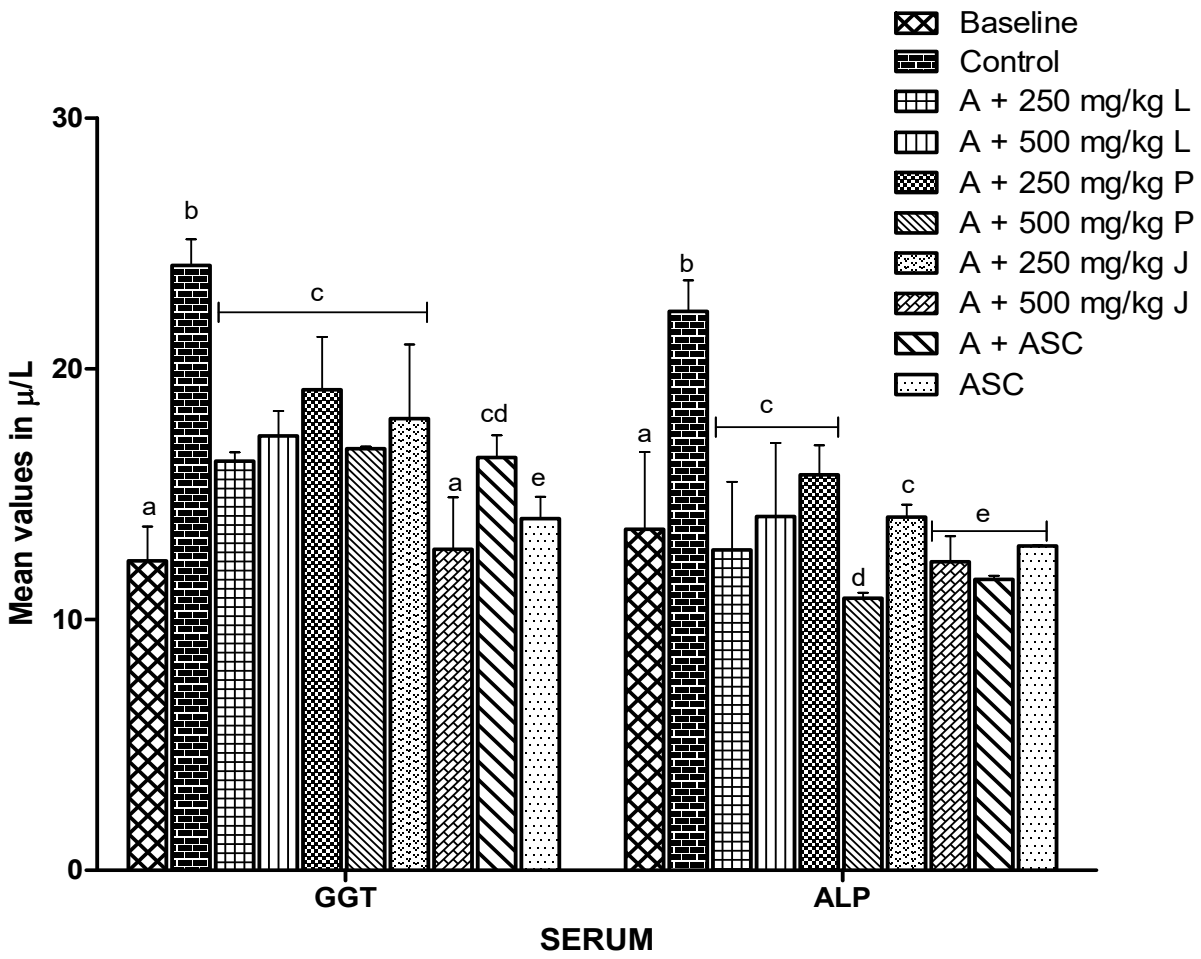


Figure 2. Gamma-glutamyl transferase activity and alkaline phosphatase activity in the serum after treatment with extracts of *C. albidum.*

A = Acetaminophen; L = *C. albidum* leaf extract; P = *C. albidum* pulp extract; J = *C. albidum* juice; ASC = Ascorbic acid.

Results are expressed as Mean ± SEM of 5 determinations. At ($p < 0.05$), values with different superscript are significant statistically

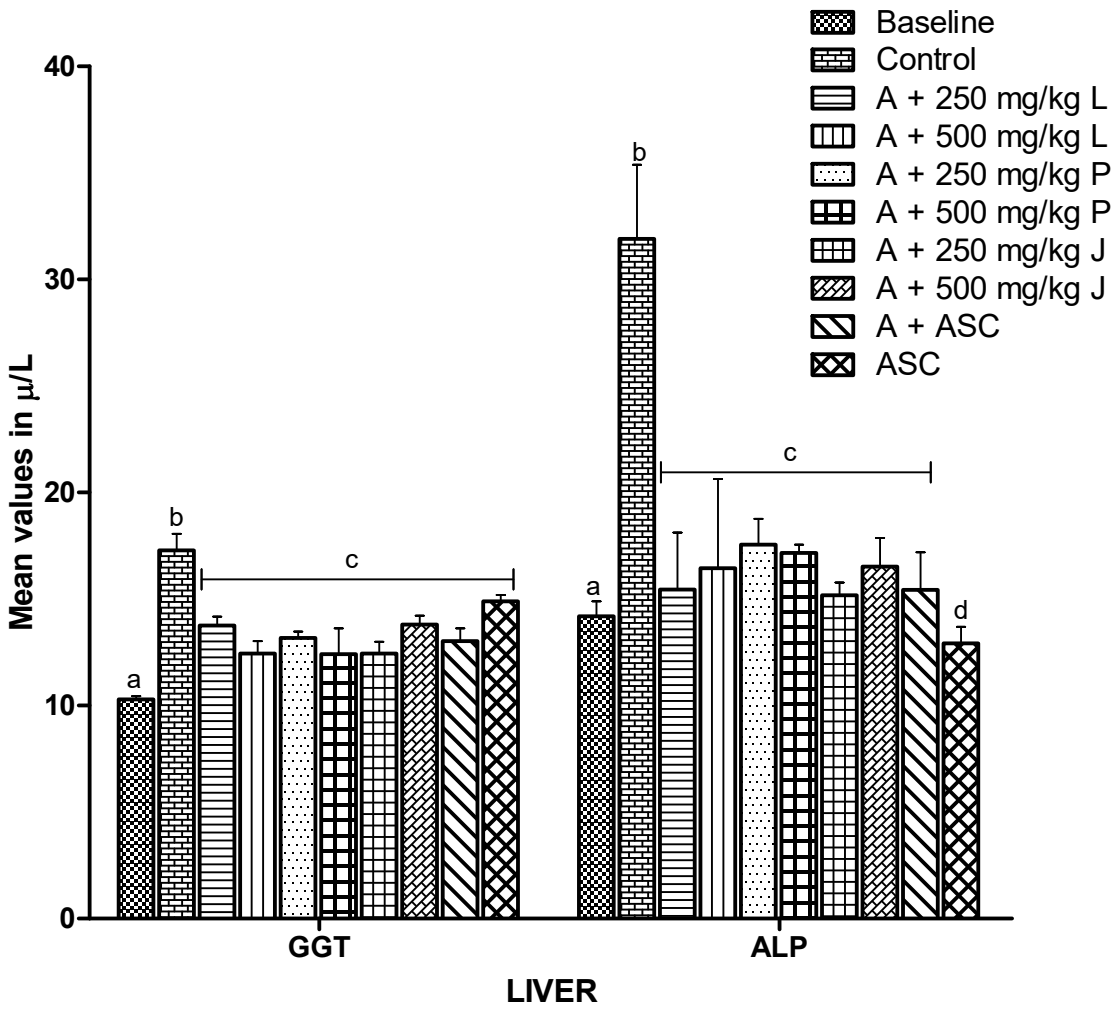


Figure 3. Gamma-glutamyl transferase activity and alkaline phosphatase activity in the liver after treatment with extracts of *C. albidum.*

A = Acetaminophen; L = *C. albidum* leaf extract; P = *C. albidum* pulp extract; J = *C. albidum* juice; ASC = Ascorbic acid.

Results are expressed as Mean ± SEM of 5 determinations. At ($p < 0.05$), values with different superscript are significant statistically

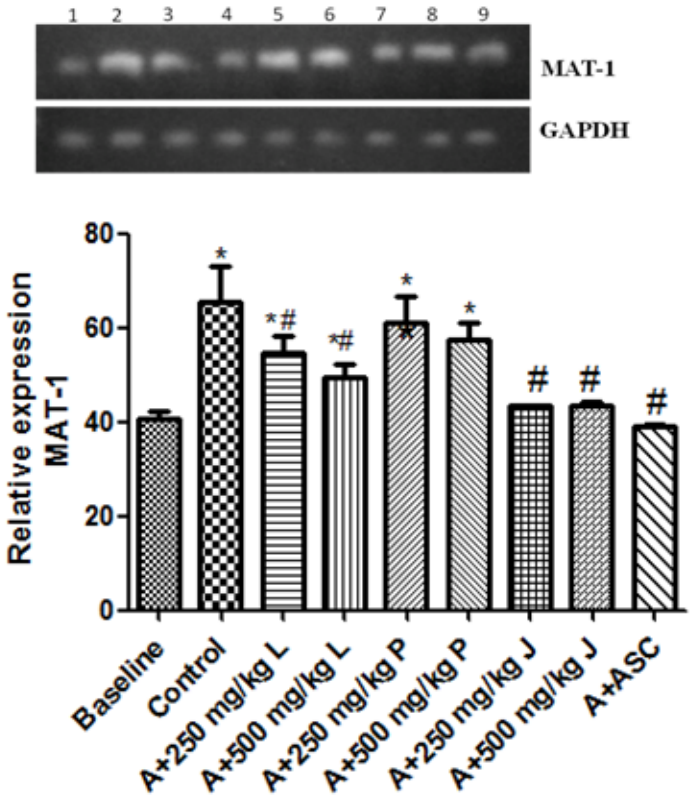


Figure 4. Qualitative-PCR analysis of MAT-1 expression after administration of *C. albidum* extracts. Snapshot representation of RT-PCR and chain reaction-agarose gel electrophoresis was carried on MAT-1 gene followed by desitometric analysis.
Results are expressed as Mean ± standard error of mean (SEM). Values with different superscript are statistically significant at ($p < 0.05$), but values with similar superscript are not statistically different.

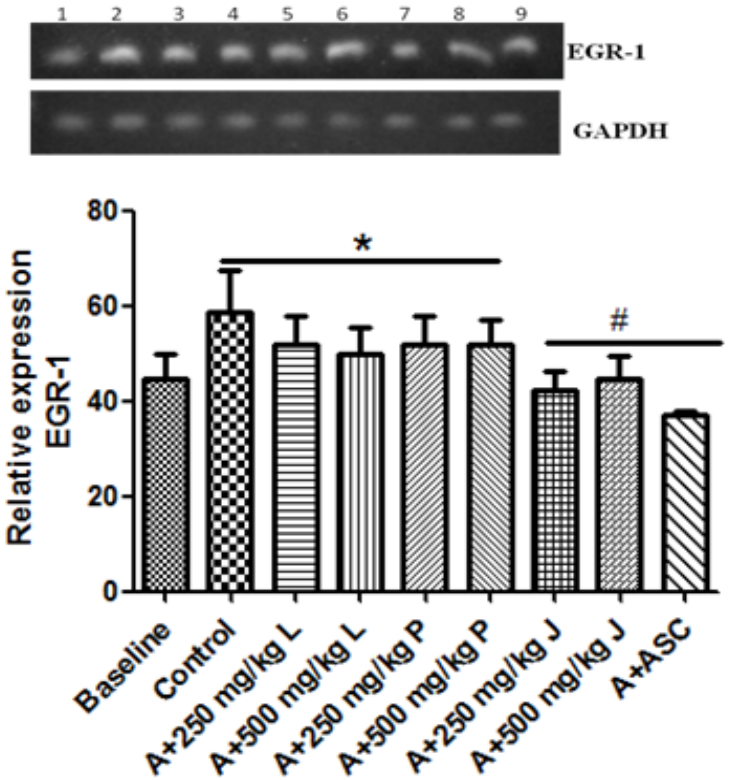


Figure 5. Qualitative-PCR analysis of EGR-1 expression after administration of *C. albidum* extracts. Snapshot representation of RT-PCR and chain reaction-agarose gel electrophoresis was carried on EGR-1 gene followed by desitometric analysis.
Results are expressed as Mean ± SEM of 5 determinations. At ($p < 0.05$), values with different superscript are significant statistically

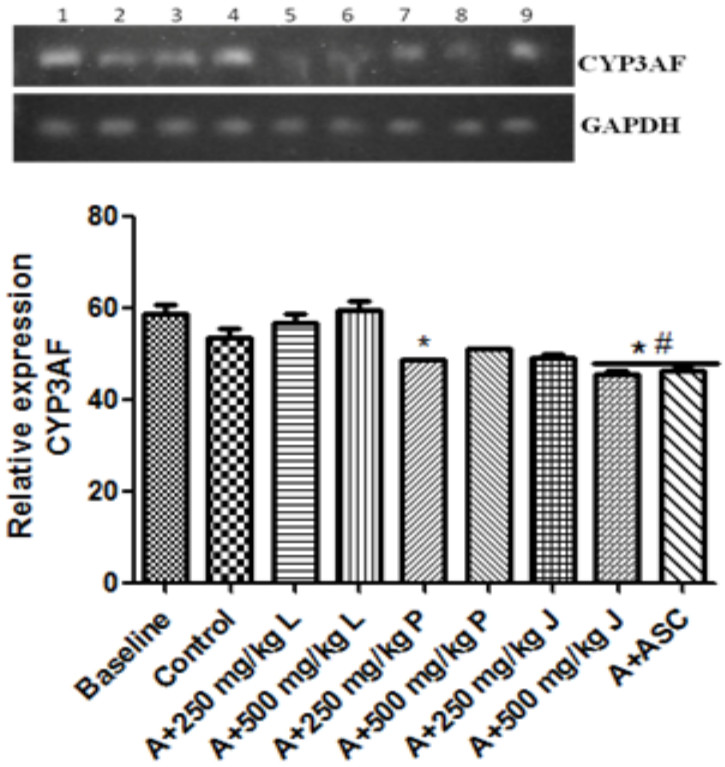


Figure 6. Qualitative-PCR analysis of CYP3AF expression after administration of *C. albidum* extracts. Snapshot representation of RT-PCR and chain reaction-agarose gel electrophoresis was carried on CYP3AF gene followed by desitometric analysis.
Results are expressed as Mean ± SEM of 5 determinations. At ($p < 0.05$), values with different superscript are significant statistically

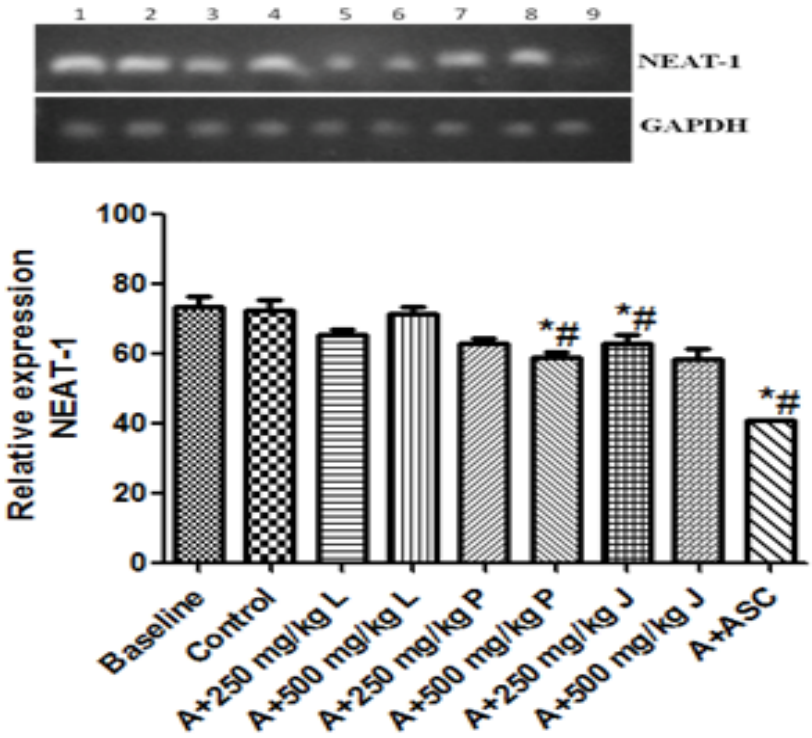


Figure 7. Qualitative-PCR analysis of NEAT-1 expression after administration of *C. albidum* extracts. Snapshot representation of RT-PCR and chain reaction-agarose gel electrophoresis was carried on NEAT-1 gene followed by desitometric analysis.
Results are expressed as Mean ± SEM of 5 determinations. At ($p < 0.05$), values with different superscript are significant statistically

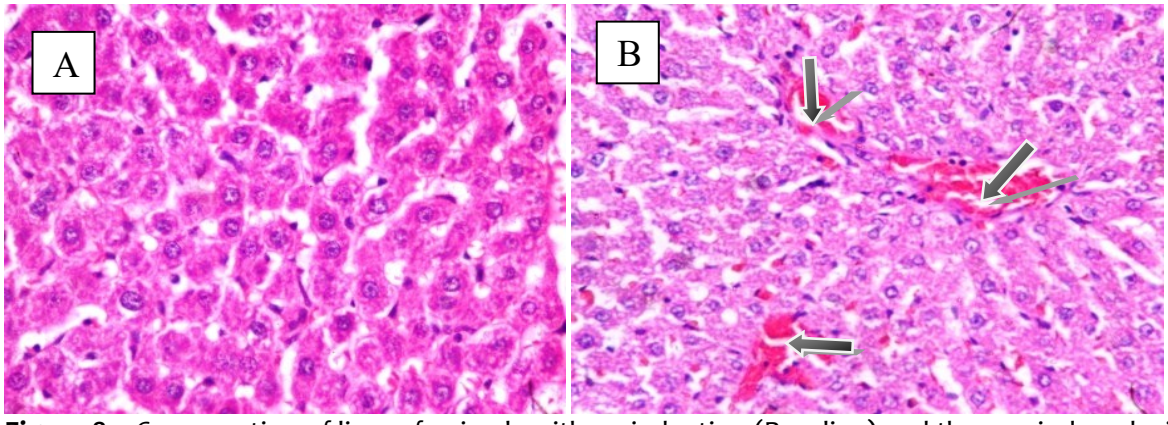


Figure 8a. Cross section of liver of animals with no induction (Baseline) and the one induced with acetaminophen (control).
Baseline ***A:*** *showed normal central venules without congestion as well as normal portal tract, the morphology of the hepatocytes appeared normal, the sinusoids appeared normal and not infiltrated and no pathological lesion seen. Control (Untreated)* ***B:*** *with mild congestion within the portal vein of the portal tract , the liver parenchyma showed areas with degenerated hepatocytes and necrosis.*

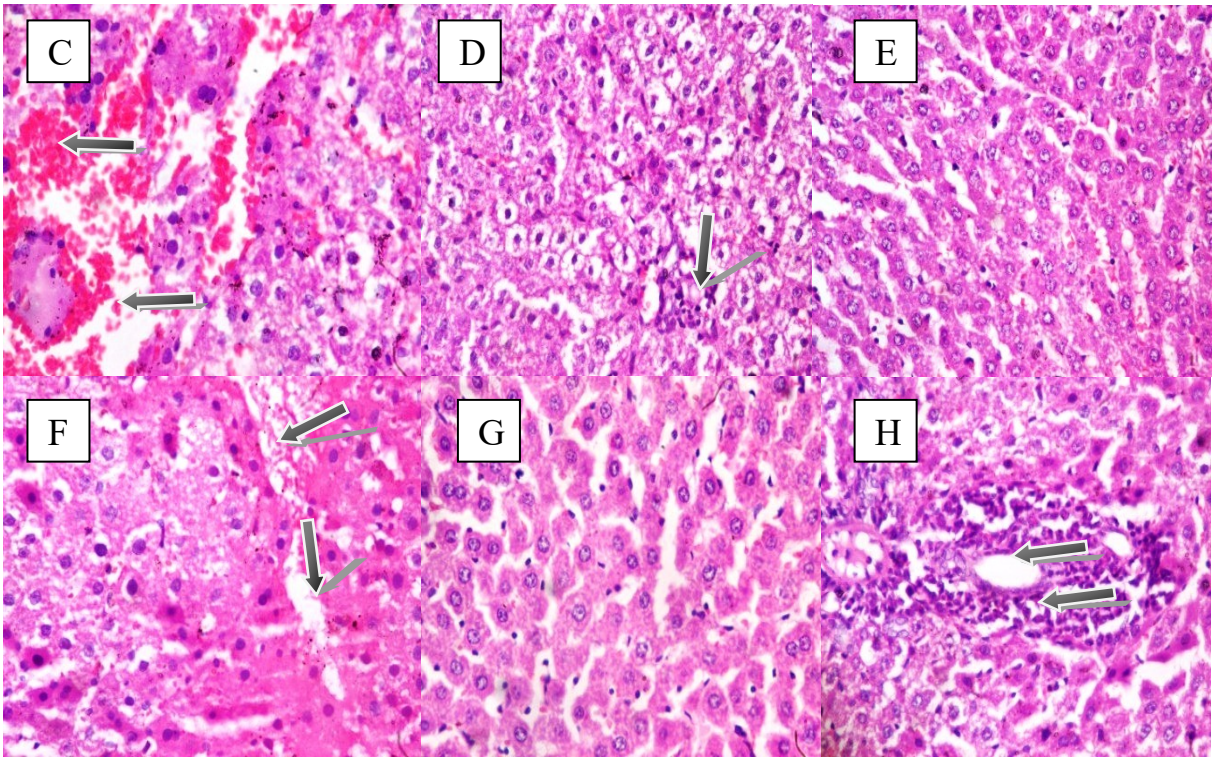


Figure 8b. Cross section of liver of animals induced with acetaminophen and treated with *C. albidum* extracts.
(A + 250 mg/kg L) ***C:*** *with normal central venules without congestion, the liver parenchyma showed severe necrosis, hemorrhage and very poor architecture with loss of liver plates. The sinusoids appeared normal and not infiltrated.*
(A + 500 mg/kg L) ***D:*** *with normal central venules without congestion and mildly infiltrated portal tract, the morphology of the hepatocytes appeared normal, the sinusoids appeared normal and not infiltrated.*
(A + 250 mg/kg P) ***E:*** *with mild congestion within the portal vein of the portal tract, the morphology of the hepatocytes appeared normal, the sinusoids appeared normal and not infiltrated.*
(A + 500 mg/kg P) ***F:*** *with moderate congestion within the portal vein of the portal tract, the hepatocytes appeared severely degeneratated by fat and showed severe to chronic necrosis, the sinusoids appeared normal and not infiltrated.*
(A + 250 mg/kg J) ***G:*** *with normal central venules without congestion, the morphology of the hepatocytes appeared normal, the sinusoids showed very few inflammatory cells.*
(A + 500 mg/kg J) ***H:*** *with normal central venules without congestion and moderate periportal infiltration of inflammatory cells, followed by a moderate degeneration of hepatocytes morphology, the sinusoids appeared normal and not infiltrated.*

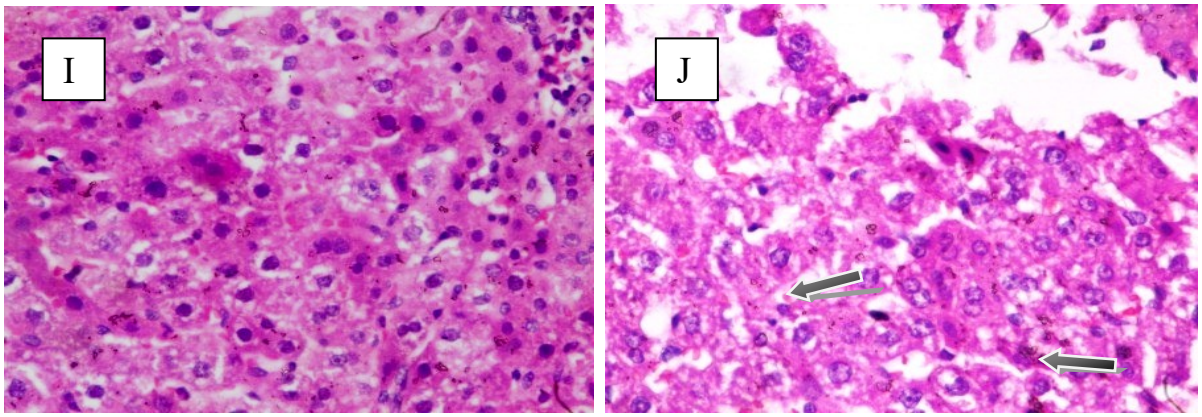
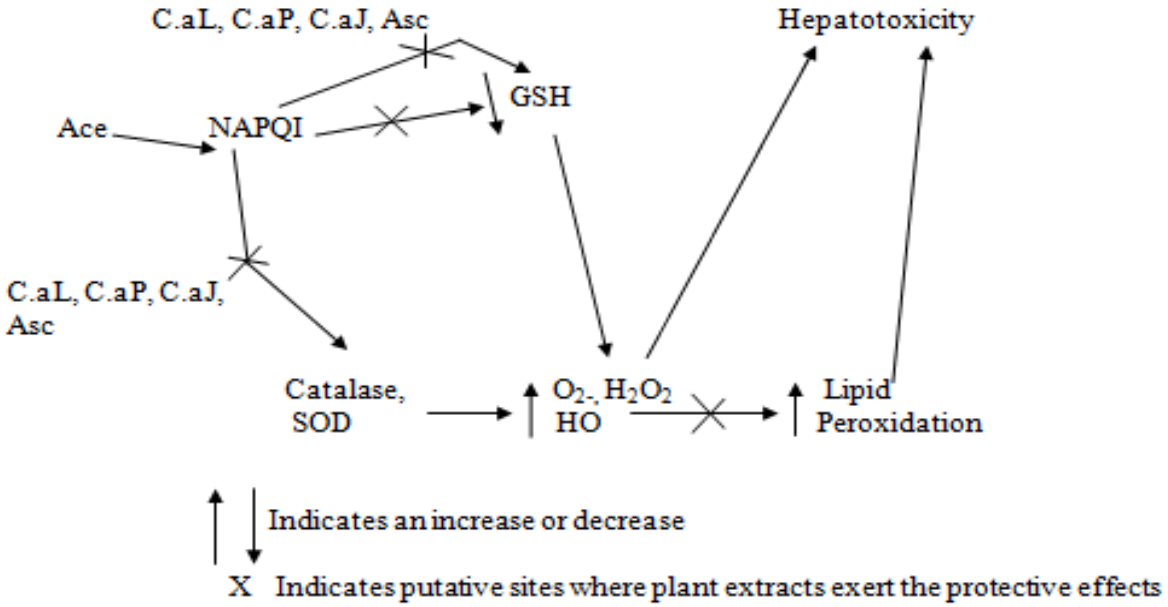


Figure 8c. Cross section of liver of animals induced with acetaminophen and treated with ascorbic acid and the animals administered with ascorbic acid only.
(A + ASC) ***I:*** *with moderate infiltration of portal tract and focal area of mild lymphocytes aggregates was seen, followed by the presence of moderate cystic area seen, the sinusoids appeared normal and not infiltrated .*
(ASC) ***J:*** *with poor hepatocytes morphology followed by moderate fat degeneration owing to fat infiltrating the hepatic cytoplasms, the sinusoids appeared normal and not infiltrated,venules and portal tracts appeared normal.*



Scheme 1. Proposed hepatoprotective scheme of *C. albidum* extracts and ascorbic acid against acetaminophen-induced hepatotoxicity.
Ace = acetaminophen; C.aL = C. albidum leaf extract; C.aP = C. albidum Pulp extract; C.aL = C. albidum juice and Asc = Ascorbic acid
C. albidum is proposed to prevent NAPQ1 from depleting GSH, catalase or SOD. Decreased in the levels of these antioxidant enzymes will actually lead to increase in free radicals and lipid peroxidation and ultimately results in toxicity.