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THE EFFECT OF ETHANOL EXTRACT OF *ANARCARDIUM OCCIDENTALE* (CASHEW) STEM BARK ON SOME BIOCHEMICAL PARAMETERS OF ACETAMINOPHEN-INDUCED HEPATOTOXICITY IN RATS

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Abstract
The present study was designed to investigate some biochemical parameters associated with acetaminophen induced liver injury on rats treated with ethanolic stem-bark extract of Anacardium occidentale (EBAO). Qualitative studies on EBAO showed the presence of phytochemicals such as polyuronides, phenolic compounds and reducing sugars. Thirty (30) Wistar rats weighing 168.98 g to 292.91 g were grouped into five (I, II, III, IV and V) of six rats each. The curative model was applied. Liver Enzyme levels were measured prior to induction with acetaminophen in each group (I, II, III, IV and V) and the values were recorded as baseline enzyme or protein levels. Administration of 2500 mg/kg of acetaminophen caused a significant elevation in the levels of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) and decreased level of Albumin (ALB) in all the experimental rats compared to their baseline levels measured. Group I (treatment with silymarin) received 100 mg/kg of silymarin and distilled water respectively. Group II received normal saline as a control, Group III, IV and V were the plant extract treatment groups that were treated with 250 mg/kg, 500 mg/kg and 1000 mg/kg of the extract respectively for a period of five days. The experiment lasted for 10 days. There were significant ($P<0.05$) reduction of ALT, AST and ALP and an increased level of albumin during the five-day treatment of 500 mg/kg and 1000 mg/kg extract compared to their respective levels after induction.

Keywords: Ethanolic stem-bark extract of *Anacardium occidentale,* Acetaminophine, Alanine aminotransferase, Aspartate aminotransferase, Alkaline phosphatase

Introduction

The liver in its activity of metabolizing foreign molecules generate free radicals which if the biological system is not able to scavenge, attack the liver and cause injury to it (Kaplowitz and DeLeve, 2013). Immunologic reactions, inhibition of cellular pathways of drug metabolism; abnormal bile flow resulting from disruption of subcellular actin filaments or interruption of transport pumps, leading to cholestasis and jaundice, sometimes with minimal cell injury and programmed cell death (apoptosis). This occurs through tumor necrosis-factor and inhibition of mitochondrial function, with accumulation of reactive oxygen species and lipid peroxidation, fat accumulation, and cell death (Kaplowitz et al., 2013). Plants and their derivatives play an important role in world health and have long been identified to possess biological activity (Saxena et al., 2013). Plant kingdoms are the rich source of organic compounds, many of which have been used for medicinal purposes. There are many natural crude drugs from plants that have the potential to treat many diseases and disorders (Atanasov et al., 2015; Singh, 2015). *Anacardium occidentale* commonly known as cashew in English, Atiya in Twi, "Kashu" in Hausa, is one of the known members of the Anacardiaceae family comprising of some 60 to 74 genera and 400 to 600 species (Lim, 2012). This plant is a multi-purpose tree whose leaves, stems, and

bark extracts are used extensively for the treatment of certain diseases and ailments (Lim, 2012). In Thailand, the phenolic content and antioxidant activities of young leaves of cashew ranked second out of 13 indigenous vegetables screened by Kongkachuichai et al. (2015). Also the leaves of the plant contain anti-inflammatory properties, and there is a stronger anti-inflammatory activity in the ethanol extract than the aqueous extract (Thomas et al., 2015). Pre-clinical trials using metabolites from the bark extract of *A. occidentale* have been reported to possess antioxidant biological activity and thus have the potential to be used as hepatoprotective agents (Anyaegbu, Ajayi, and Adedapo, 2017). *Anacardium occidentale* stem-bark also possesses phyto-constituents such as saponins, tannins and flavonoids, which have been reported to exert antioxidant activities which may serve as means of scavenging the free radicals generated during the metabolism of drugs by the liver. In addition, sub-chronic administrations of *Anacardium occidentale* inner stem bark extract did not depress the function of hepatocytes in rats (Okonkwo et al., 2010). We set to investigate the effect of ethanolic extract of stem-bark of cashew tree on biochemical indices associated with Acetaminophen-induced hepatic status in rats.

Materials and Methods

Plant Collection

The plant *A. occidentale* (stem bark) was collected in January, 2017 from a forest in Wenchi in the Brong-Ahafo region, Ghana and was transported to Navrongo, Upper East Region of Ghana. The plant was identified with the aid of a botanist from the Department of Applied Biology, University for Development Studies.

Sample Preparation and Extraction

Preparation of *A. occidentale* stem bark extract was carried out in the University for Development Studies, Ghana, Chemistry Laboratory and continued at the Department of Pharmacognosy, Centre for Scientific Research into Plant Medicine (CSRPM), Akuapem-Mampong, Ghana.

The stem bark of *Anacardium occidentale* was dried under shade for a period of two (2) weeks. The dried sample was crushed into powder using pestle and mortar. About 140 g of the powder was soaked in 400 ml ethanol (99.6 %) and 20 ml distilled water making 95% ethanol solution (1 in 6 sample to solvent ratio). After 72 hours, the macerate was filtered using Whatman filter paper No 42 with the residue discarded and the filtrate concentrated using rotary evaporator (Heidoph laborota 4001, Canada) into a semi-solid state.

Experimental Animals

Wister rats of either sex of weights between 168.98 g to 292.91 g were used for the experiments. The animals were acclimatized to laboratory conditions for seven days before commencement of experiments. All the procedure described, were reviewed and approved by Institutional Animal Ethical Committee of the Centre for Scientific Research into Plant Medicine animal house in Akuapem-Mampong, Ghana. The animals were housed in standard cages and maintained on a standard pelleted feed (Guinea feed) and water.

Experimental Procedure

The curative model was used for this study. Thirty (30) wistar rats of both sexes were weighed and put into five (5) groups (I, II, III, IV and V) of six animals each. Group I was the group treated with silymarin, a known standard drug, group II normal Saline, Group III, IV and V were labelled as the treatment groups with different concentrations of EBAO. Blood samples were taken from the animals of all groups prior to induction of liver injury with acetaminophen to undertake biochemical analysis (baseline). All the rats were then induced with 1500 mg/kg of acetaminophen with respect to the group average body weight for five (5) days. Two (2) hours after the last induction dose on day five (5), blood sample of the rats were taken for biochemical analysis to confirm liver injury inflicted. One (1) hour after blood sample collection, the groups were treated as follows: Group I were administered with 100 mg/kg of silymarin (standard drug), Group II with normal saline, Group III, IV, and V were administered with 250 mg/kg, 500 mg/kg and 1000 mg/kg of ethanol extract of *A. occidentale* respectively for five (5) days. Blood samples were then collected for further biochemical analysis two hours after the last dose of treatment. The curative effect of the stem-bark extract (250-1000 mg/kg) against acetaminophen-induced liver injury regarding biochemical parameters namely alanine transaminase (ALT), aspartate transaminase (AST),

alkaline phosphatase activities (ALP) and albumin (ALB) of the serum were measured using a biochemical analyzer (Semi-automatic chemistry analyzer TC-84, China). The blood was centrifuged at 3000 rpm for 5 minutes and the supernatant (serum) collected and stored at -20°C in an air-tight bottle until it was used for the biochemical analysis.

Phytochemical Screening

The crude extracts were subjected to qualitative phytochemical screening to identify presence or absence of selected bioactive compounds using standard methods from Ciulei (1982) and Harborne (1998). Secondary metabolites tested included alkaloids, reducing sugars, triterpenoids, flavonoids, phenolics, saponins, anthracenosides, polyuronides and steroids.

Test for Saponins

Two (2) ml of diluted solution (1:1) in a test tube of 1.6 cm diameter was shaken for 15 minutes. The occurrence of a foam column of at least 1 cm in height persisting minimum 15 minutes was not observed indicating a negative result for saponins.

Test for Reducing Compounds

The alcohol extract (1ml) was diluted with water (2 ml) Fehling's (A and B) (1 ml) solutions added followed by heating the mixture obtained. The presence of a brick red precipitate denoted a positive result for reducing compounds.

Test for Polyuronides

Two (2) ml of the ethanol extract was added drop wise in a test tube where 10 ml of alcohol or acetone have already been placed. The presence of a thick precipitate denotes a positive result for polyuronides.

Test for Phenolic Compounds

Two (2) drops of 5 % Ferric Chloride was added to 3 ml of ethanol extract. A sudden change in colour to black, bluish-black or dark green denotes a positive result for phenolic

Test for Alkaloids

The plant extract (0.5 g) was added to 5 ml of dilute sulphuric acid H_2SO_4 (1 %) on a steam bath. The solution was filtered, and the filtrate was treated with few drops of Dragendroffs reagent. Reddish brown turbidity or precipitate indicates a negative result for alkaloids.

Test for Anthracenosides

The ethanol extract (4 ml) was concentrated to 2 ml, then ammonia solution (25%, 1-2 ml) was added and the solution was shaken to observe colour change. A cherish-red colour of the alkaline solution was not observed which indicated a negative result for anthracenosides (Borntrager's reaction).

Test for Triterpenes

The ethanol extract (10 ml) was evaporated to dryness. The residue was then dissolved successively in acetic anhydride (0.5 ml) and chloroform (0.5 ml). The solutions were then transferred to a dry test tube. Concentrated Sulphuric acid (1-2 ml) (Liebermann-Burchard's reaction) was then added at the bottom. A reddish-brown coloration at the interface indicates a negative result for triterpenes.

Test for Flavonoids

The ethanol extract (5 ml) was evaporated to dryness. The residue was then dissolved in methanol (50 %, 1-2 ml) and was heated. Magnesium metal was added and five (5) drops of concentrated hydrochloric acid were added. The absence yellow coloration that disappeared on standing indicated the absence of flavonoid.

Analysis of results

Results obtained were analyzed using the statistical software, statistical package for social scientists (SPSS version 16.0). Values were presented as mean ± Standard error of the Mean (SEM) for each experimental group. Differences within experimental groups were determined by one-way Analysis of Variances (ANOVA) followed by the post hoc Tukey's test whenever necessary and P values were considered as significant when $P < 0.05$.

Results

Qualitative Phytochemical Screening

A qualitative phytochemical screening carried out on the extract revealed the presence of reducing sugars, polyuronides, flavonoids and phenolic compounds as shown in Table 1 below.

Table 1: Phytochemical constituents of the ethanol stem bark extract of *Anacardium occidentale.*

Phytochemical	Ethanol extract
Saponins	-
Alkaloid	-
Steroids	-
Triterpenoids	-
Reducing sugars	+
Flavonoids	+
Phenolic compounds	+
Polyuronides	+
Anthracenosides	+

Key (+) = Present (-) = Absent

Experimental Results

The Wister rats used for this study weighed between 168.98 g and 292.91 g. Enzyme levels were measured prior to induction with acetaminophen in each group (I, II, III, IV and V) and the values were recorded as baseline enzyme or protein levels. Administration of 1500 mg/kg of acetaminophen caused a significant ($P<0.05$) elevation in the levels of AST, ALT and ALP (Fig. 2, Fig. 3 and Fig. 4 respectively) and decreased level of ALB (Fig. 1) in all the experimental rats compared to their baseline levels measured earlier. There were significant ($P<0.05$) reduction of ALT, AST and ALP and an increased level of ALB at significance ($P < 0.05$) for 500 and 1000 mg/kg during the five-day treatment compared with their respective levels after induction.

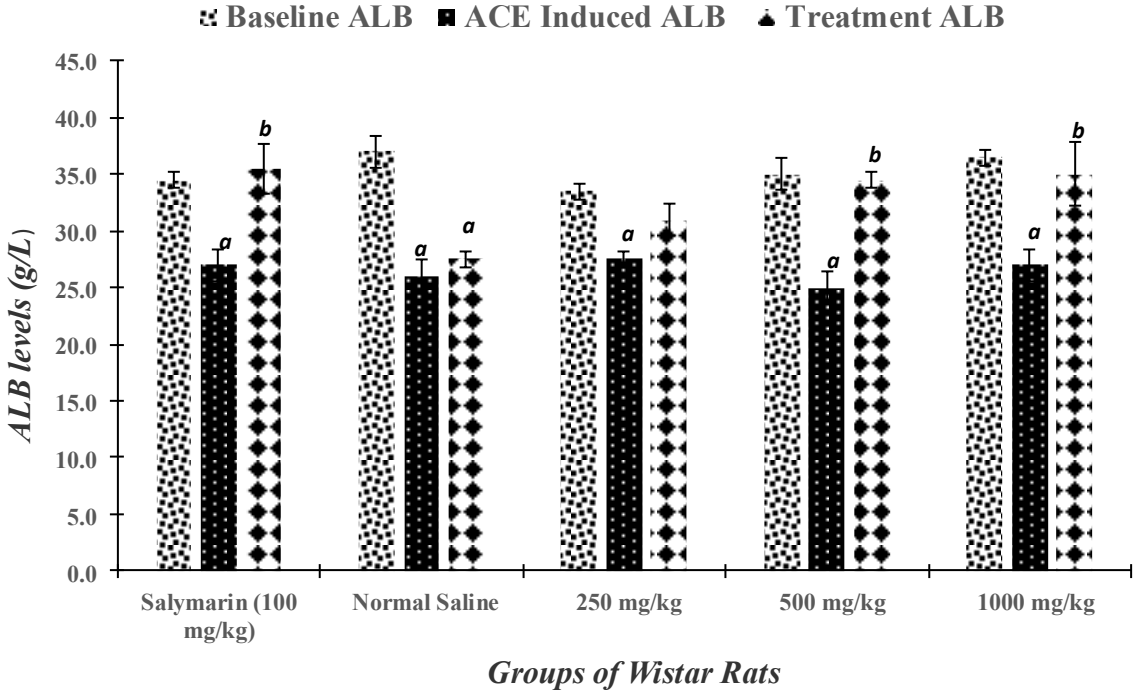

Fig. 1: Effect of ethanolic stem bark extract of *Anacardium occidentale* (EBAO) on ALB level against acetaminophen-induced liver toxicity in adult Wistar rats. Values are means ± SEM. [a]$P < 0.05$; significant difference when compared with the Baseline ALB level; [b] $P < 0.05$; significant difference when compared with acetaminophen induced albumin level. SEM: Standard error of mean, ALB: Albumin, ACE: Acetaminophen.

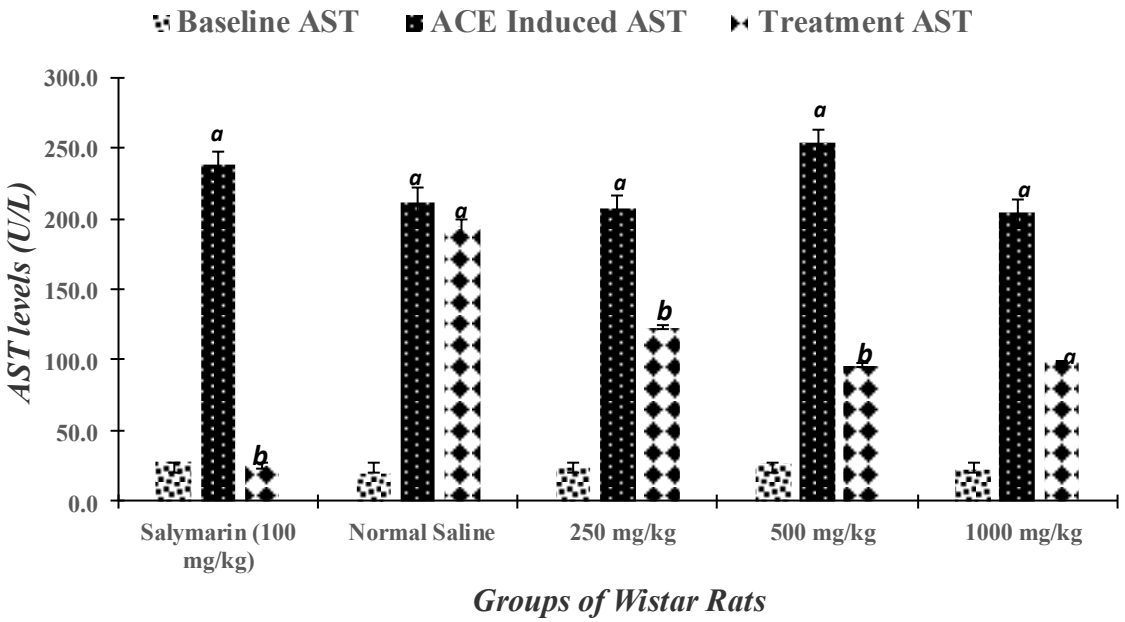

Fig. 2: Effect of ethanolic stem bark extract of *Anacardium occidentale* (BEAO) on AST level against acetaminophen-induced liver toxicity in adult Wistar rats. Values are means ± SEM. $^{a}P < 0.05$; significant difference when compared with the Baseline AST level; ^{b} $P < 0.05$; significant difference when compared with acetaminophen induction. SEM: Standard error of mean, AST: Aspartate Aminotransferase; ACE: Acetaminophen.

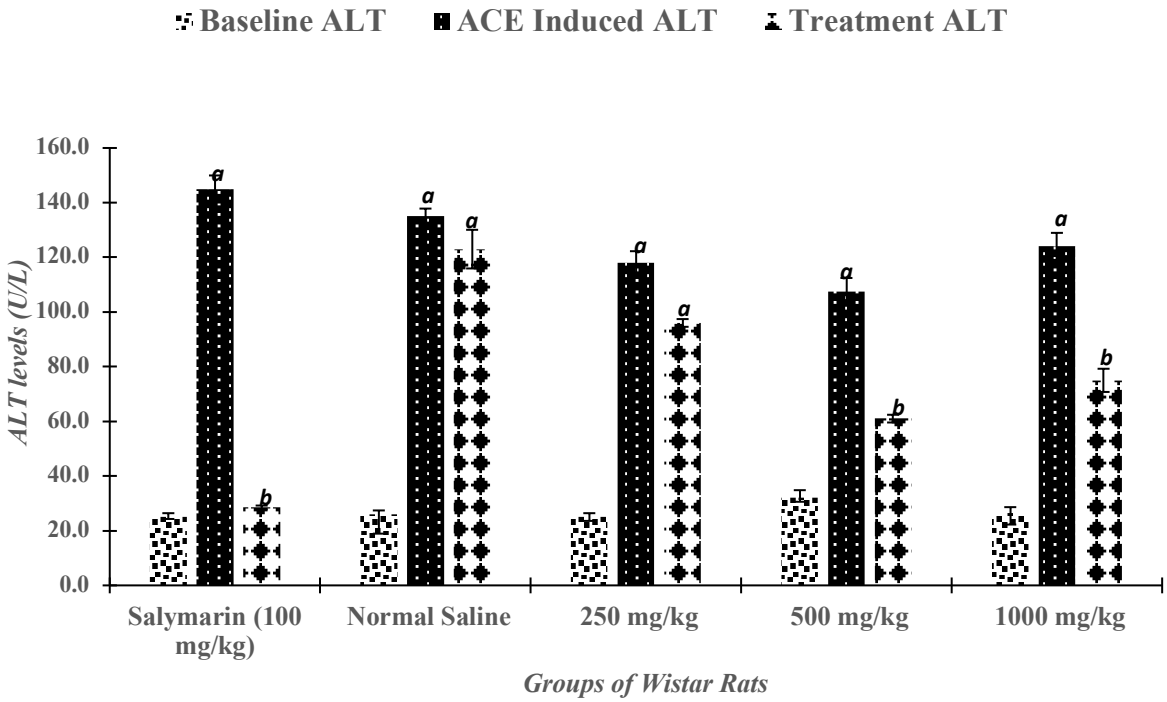

Fig. 3: Effect of ethanolic stem bark extract of *Anacardium occidentale* (EBAO) on ALT levels against acetaminophen-induced liver toxicity in adult Wistar rats. Values are means ± SEM. $^{a}P < 0.05$; significant difference when compared with

the Baseline ALT level; [b] $P < 0.05$; significant difference when compared with acetaminophen induction. SEM: Standard error of mean, ALT: Alanine Aminotransferase; ACE: Acetaminophen.

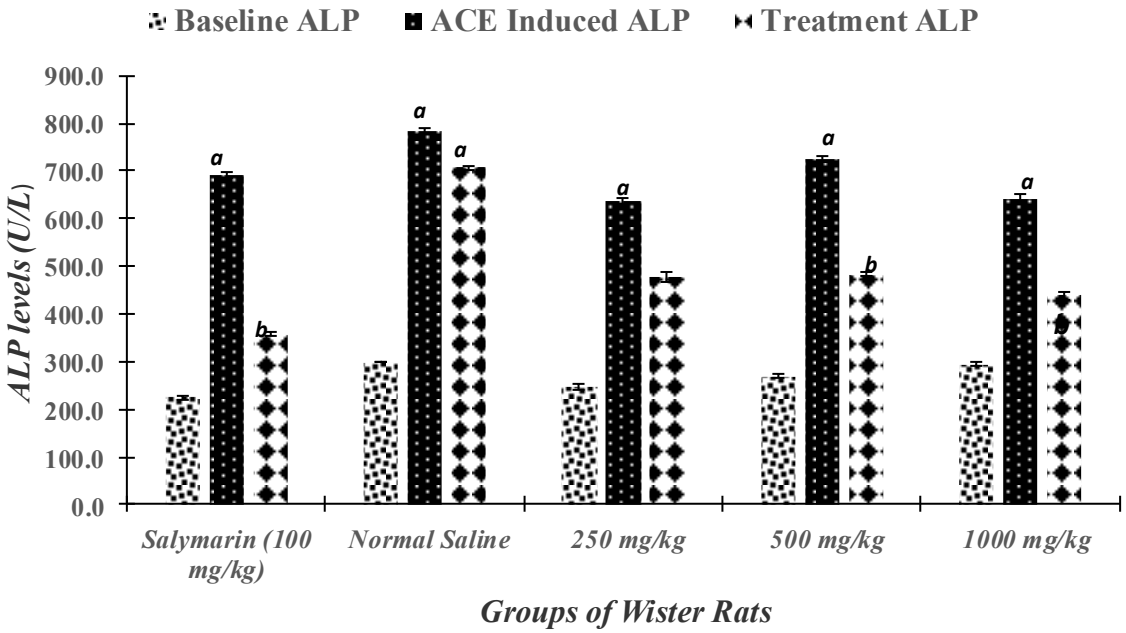

Fig. 4: Effect of ethanolic stem bark extract of *Anacardium occidentale* (EBAO) on ALP level against acetaminophen-induced liver toxicity in adult Wistar rats. Values are means ± SEM. [a]$P < 0.05$; significant difference when compared with the Baseline ALP level; [b] $P < 0.05$; significant difference when compared with acetaminophen induction. SEM: Standard error of mean; ALP: Alkaline Phosphatases; ACE: Acetaminophen.

Discussion

Anacardium occidentale stem bark is widely used as traditional medicine in the treatment of various ailments in Africa (Oyesomi and Ajao, 2011). Various studies have reported the presence of some important phytochemicals such as phenolic compounds, reducing sugars in the stem bark extract and the extract have no significant toxic effect to rats' liver (Okonkwo et al., 2010). The present study qualitatively determined the presence of phytochemicals such as polyuronides, phenolic compounds and reducing sugars shown in Table 1. For 140 g of powdered stem-bark of *Anacardium occidentale* dissolved in 420 ml of a mixture ethanol/water (95:5), the yield of the crude extract was 32.6 g (23.34 % yield).

In the study, protective effect of ethanol extract of *A. Occidentale* against aectaminiphen induced liver injury in rats has been shown. Acetaminophen is one of the most commonly used hepatoxins in the experimental study of liver damages (Black, 1984; Jaeschke, Xie, and McGill, 2014). High doses of acetaminophen changes the function of the liver, ultimately leading to the destruction of hepatocellular membranes (Hinson, Roberts, and James, 2010; Ramachandran and Jaeschke, 2017). In this study, acetaminophen produced a severe liver damage as indicated by a marked increase in the serum levels of AST by an average of 89 %, ALT by an average of 81 %, and ALP by an average of 62 % and a decrease in albumin by an average of 26 % in all the groups of the rats, after five days of oral administration of the acetaminophen. The hepatotoxic effect of acetaminophen in this study could be due to the oxidative toxicity by free radical generation (Du, Ramachandran, and Jaeschke, 2016). Damage to the structural integrity of the liver was reflected by the increase in the levels of serum transaminases AST, ALT and ALP in Fig. 2, Fig. 3 and Fig. 4 respectively, because they are cytoplasmic in location and are released into circulation after

cellular damage. By estimating the levels of serum ALT, AST and ALP which are originally present in the cytoplasm of liver cells makes it possible to assess liver damage (Manokaran et al., 2008). Increase in the aminotransferases (ALT and AST) are the most normally used specific indicators of hepatic necrosis (Kew, 2000; Ozer et al., 2008). Elevated levels of ALP suggest biliary damage or an obstruction of the biliary tract, which disrupts the flow of blood to the liver (Woreta and Alqahtani, 2014; Poupon, 2015). The reduction in albumin levels observed in acetaminophen-induced rats may be linked with the decrease in the number of hepatocytes which in turn may result in a decrease in hepatic capacity to synthesize albumin. Therefore decreased levels of albumin as recorded in acetaminophen-induced rats revealed the severity of the liver injury and may be attributed to the damage produced and localized in the endoplasmic reticulum leading to decrease in protein synthesis (Kanchana and Sadiq, 2010). In the treatment groups, treatment with silymarin in Group I, a known standard drug for treatment of hepatic disorder (Solhi et al., 2014; Hellerbrand et al., 2016), for five days, the silymarin reduced the levels of AST by 81 %, ALT by 78 %, and ALP by 48 % and increased the level ALB by 22 % to their respective levels when intoxicated with acetaminophen. Silymarin doses up to 100 mg/kg has been used as a standard drug in numerous studies (preventive and curative models) and it exerts hepatoprotective effect due to its antioxidant and scavenging properties (Surai, 2015; Ferenci, 2016). The groups treated with normal saline had their AST, ALT and ALP levels decreased by 8%, 7%, and 8% respectively and ALB levels increased by 5%. The normal saline did not have treatment effect on the animals since the changes did not differ significantly ($P<0.05$) from their respective induction levels.

Treatment with ethanolic extracts produced significant reduction of the levels of serum enzymes markers; ALT levels were reduced by 43% and 45% for 500 mg/kg and 1000 mg/kg respectively. ALP levels were reduced by 31% and 33% for 500 mg/kg and 1000 mg/kg respectively by percentages. AST levels however reduced significantly for all concentrations by 40%, 52% and 55% for 250 mg/kg, 500 mg/kg and 1000 mg/kg respectively. The antioxidant constituents polyphenols and reducing sugars present in the extracts might have been responsible for their ability in the reduction of the acetaminophen induced liver injury (Li et al., 2015). The reactive species mediated hepatotoxicity can be treated upon administration of agents possessing anti-oxidant and free radical scavenger and *A. Occidentale* bark extract is reported to have antioxidant properties (da Silva et al., 2016).

The level of the aminotransferases in Fig. 2 and Fig. 3 in all the groups were found to have fallen after the period of treatment with EBAO (500 mg/kg and 1000 mg/kg) indicating that the injury inflicted was an acute one, thereby healing within the shortest period but not as effective as the standard drug, however 500 mg/kg and 1000 mg/kg of EBAO reduced ALT and ALP significantly after treatment but showed no significance ($P<0.05$) difference between baseline and after treatment.

Conclusion

The phytochemical and treatment effect investigations carried out on the ethanolic stem bark extract *A. occidentale* has further strengthened its immense potential in the treatment of numerous diseases in Africa however the method employed is the curative model.

Competing interests

The authors declare that they have no competing interests

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