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Biochemical and In-vivo Antioxidant Activities of Ethyl Acetate Fractions of *Boswellia Papyrifera* (Del.) Stem Bark on CCl_4-Induced Liver Damage in Wister Rats†

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The efficacy of any hepatocurative drug is dependent on its ability to reduce the harmful effects or in maintaining the normal hepatic physiological mechanism which has been imbalanced by a hepatotoxin compound. This study was aimed at evaluating the liver biochemical parameters and antioxidant activities of ethyl acetate fractions on carbon tetrachloride (CCl_4)-induced liver damage in rats. The ethyl acetate fractions of *Boswellia papyrifera* (E2) stem bark extract were obtained after column chromatography and TLC of ethyl acetate of the stem bark extract. Four (4) major subfractions (E2A, E2B, E2C, and E2D) were obtained. These subfractions were administered to CCl_4-induced liver-damaged rats for four weeks prior to liver function indices and antioxidant analysis. A total of 25 white male albino (Wister) rats were used for each fraction in the study, wherein the rats were divided into five groups of 5 rats each. The rats in Group I were not induced with lipid peroxidation and liver damage but served as the normal control. Groups II to V were injected with a single dose of 120 mg/kg of CCl_4 prior to oral administration of ethyl acetate fractions of *Boswellia papyrifera* to Groups III to V with 20, 30, and 50 mg/kg of the fractions, respectively. The effect of the E2B fraction on the activity of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and serum concentration of albumin, total and direct bilirubin and total protein were significantly ($p<0.05$) found to be no different from that of the normal control group. However, a significant ($p<0.05$) increase in glutathione peroxidase (GPX) and catalase (CAT) activities were recorded in all experimentally treated animals compared to the test and normal controls. Malondialdehyde (MDA) concentration decreased significantly ($p<0.05$) in all experimental groups as compared to the test control. Histopathology showed that liver integrity was consistent with that of biochemical findings. However, no significant effect was observed from the E2A, E2C, and E2D fractions. It can be concluded that E2B ethyl acetate fraction of *Boswellia papyrifera* stem bark possesses potent curative activity against CCl_4-induced liver damage, which may be due to its antioxidant activity.

Key words: *Boswellia papyrifera*; column chromatography; liver enzymes; antioxidants; carbon tetrachloride

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Liver is one of the vital organs in our body and it plays various functions in the maintenance, performance, and regulation of homeostasis of our body [1]. Liver disease has become one of the serious health problems and a major cause of death all over the world. Nearly 20,000 deaths and 250,000 new cases have been reported every year [2]. The percentage of liver toxicity as a result of various exposures is much higher in developing countries (8–30%), such as India, as compared to developed countries (2–3%) [3]. Liver injury can be attributed to various toxic agents including chemicals, alcohol, viruses or by their bio-activation to chemically reactive metabolites. These metabolites are free radicals, which will either elicit an immune response or directly affect the biochemistry of the cells by interacting with cellular macromolecules. Despite advancements made in modern medicine, reliable synthetic liver curative/protective drugs remain scanty. Hence, natural extracts/products from medicinal plants are considered safe and effective for the treatment of liver diseases [4].

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According to Lius *et al* [5], the ingestion of CCl_4 can lead to marked hepatotoxicity. Hepatic diseases or injuries are modally induced experimentally by administration of CCl_4 since it is known that it produces acute hepatocellular injury with centrilobular necrosis and steatosis [6]. The effects of CCl_4 include hepatic injury on biochemical properties such as increased lipid peroxidation [6]. A high dose of CCl_4 produces an animal model that allows for evaluating the curative or protective effects of medicinal plants comparatively than reporting natural therapeutic action [7]. The hepatic effects can be monitored by certain bioactive compounds serving as valuable antioxidants from natural plant resources [8].

Boswellia papyrifera (Del.) *(Ararrabi)* Hochst belongs to a tropical family called Bruceraceae [9] that can be identified by the presence of resin ducts in the bark [10]. *Boswellia papyrifera* is a deciduous tree that can be as tall as 12 meters, with a rounded crown and a straight regular bole. The leaves and roots of the species are used against lymphadenopathy while the resin is used as a febrifuge [9].

The antioxidant activity can be effective through several ways: as inhibitors of free radical oxidation reactions (preventive oxidants) by inhibiting formation of free lipid radicals; by interfering with the circulation of the autoxidation chain reaction (chain-breaking antioxidants); as singlet oxygen quenchers; through synergism with other antioxidants; as reducing agents which convert hydroperoxides into stable compounds; as metal chelators that convert metal pro-oxidants (iron and copper derivatives) into stable products; and finally as inhibitors of pro-oxidative enzymes (lipoxygenases) [11,12]. Both enzymatic and non-enzymatic antioxidant systems are indispensable for cellular response in order to deal with oxidative stress under physiological conditions. Therefore, antioxidant enzymes such as CAT, SOD, and GSH-Px and non-enzymatic electron receptors such as GSH are affected and used as keys to evaluate the level of oxidative stress [12]. This paper is aimed at determining the effect of *Boswellia papyrifera* ethyl acetate fractions on CCl_4-induced liver damage and their antioxidant activities.

MATERIALS AND METHOD

Plant Sample Collection and Preparation of Extract

The stem bark collected was identified by a botanist (Anas Abba) in the Pharmacognosy Department, Bayero University, Kano, Nigeria and was given a Herbarium accession No: BUKHAN 205. The stem bark was washed in clean water and dried at room temperature, after which it was pulverized to a coarse powder using a mechanical grinder. An aqueous extract of the stem bark of *Boswellia papyrifera* was prepared according to Mittal method [13] and Fernando method [14]. One thousand grams (1000 g) of the stem bark powder was mixed and soaked in 2000 cm^3 of distilled water in a two-litre conical flask; the content of the flask was then mixed vigorously. The conical flask was covered with aluminum foil and left for 48 hours. The aqueous extract was then obtained by filtration using Whatman No.1 filter paper and concentrated using a vacuum evaporator at 60^{o}C in a water bath (OSL200 water bath) and a shaker (Grand Instruments, Cambridge). The concentration and total yield of the aqueous stem bark extract of *Boswellia papyrifera* were determined and the extract was stored in an air tight container before further analyses.

The aqueous stem bark extract of *Boswellia papyrifera* was sequentially fractionated with solvents of varying polarity such as hexane, chloroform, and ethyl acetate. 300 g of the aqueous stem bark extract was mixed with 1000 ml of hexane. The mixture was shaken and allowed to stand overnight with intermittent stirring whereupon the hexane extract was collected. Similar procedures were carried out using chloroform and ethyl acetate, and the total yield of each extract was determined. The solvent was recovered with rotatory evaporator (BuchiRotovapour R-215 Switzland) at a temperature $\leq 40^o$C.

Fractionation of Ethyl acetate Extract using Column Chromatography

On the basis of *in-vitro* radical scavenging capacity studies and biochemical liver function indices evaluation for each of the four solvent extracts, ethyl acetate was found to be the most active extract. Therefore, column chromatography of the ethyl acetate stem bark extract of *Boswellia papyrifera* (E2) was carried out using silica gel [(60-120 mesh) (500 g)]. The silica gel was loaded in a column (58.5 inch in length, 1.2 inch in diameter) in the slurry of n-hexane. The silica gel was washed several times with n-hexane and chloroform to remove oily materials. At the end of the parking, the tap was closed and the column was allowed to stand for 24 hours. The ethyl acetate stem bark extract of *Boswellia papyrifera* (E2) (25 g) was mixed thoroughly with 50 g of silica in a beaker using a spatula until the mixture became homogenous. The mixture was then carefully loaded onto the column that was already packed. Additional silica gel (10 g) was added on the top to serve as a protective layer. The column was eluted gradiently and the eluents were collected in 10 drops per minutes that were aliquots of 15 ml each in beakers. A total of 140 fractions were obtained and evaporated to dryness and monitored using TLC on the different solvent system. Similar fractions were pooled into 4 major subfractions and labeled (E2A, E2B, E2C, and E2D), which were administered to CCl_4-induced rats liver and antioxidant study [15].

Induction of Liver Damage

The liver damage in rats was induced using CCl_4 by the method of Alhassan *et al*. [7]. A stock solution of CCl_4 was prepared in the ratio 1:1 by dissolving 25 cm^3 of CCl_4 in 25 cm^3 of pure olive oil (which was used as a vehicle). The liver damage was induced by a single intraperitoneal injection of CCl_4 (120 mg/kg). The volume of CCl_4 administered was determined by the weight of a rat according to the following relationship:

Volume to be administered (cm^3) =

$$\frac{\text{Weight of rat (kg)} \times 120\ \text{mg/kg}}{\text{Concentration of } CCl_4 \text{ solution } (0.1538\ \text{mg/cm}^3)}$$

Experimental Design

A total of 25 white male albino rats were used in the study for each of the four fractions; as approved by the ethical research committee with ethical clearance no: BUK/CHS/REC/01/0008. The rats were divided into five groups of five rats each. The rats in group I were not induced with lipid peroxidation and liver damage but served as normal control. Group II to V were injected with 120 mg/kg of CCl_4 according to Alhassan method [7] prior to oral administration of ethyl acetate fractions of *Boswellia papyrifera* to groups III to V, which were administered with 20, 30, and 50 mg/kg of the extracts for four weeks, respectively. The dosages were determined using LD_{50} (oral) described by Lorke (1983).

Samples Collection and Biochemical Analysis

All the rats were sacrificed by decapitation. The blood samples collected were allowed to clot and serum was separated for determination of AST, ALP, ALT, total and direct bilirubin, total protein, and albumin. The serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities was analyzed by the method of Reitman and Frankel [16], serum alkaline phosphatase (ALP) activity by the method of Rec [17], serum albumin level by the method of Grant [18], serum total and direct bilirubin level by the method of Jendrassik and Grof [19], and serum total protein level by buiret, Tietz method[20]. The catalase activity was assessed by the method of Luck [21], glutathione-S-transferase (GST) was carried out by Jocelyn method [22], superoxide dismutase activity was determined by Kakkar method [23] and malondialdehyde (MDA) was determined by Wasowich method [24].

Histological Analysis

The liver of the rats that showed significant effects of CCl_4-induced liver damage were removed immediately after being sacrificed and kept in 10% normal saline for histology examination using Nickon Eclipse-E-200, Photomicrograph system [25].

Table 1. Table of Abbreviations

List of Abbreviations	
*CCl_3	Trichloride methyl radicals
ALB	Albumin
ALP	Alkaline phosphatase
ANOVA	One-way analysis of variance
AST	**Serum aspartate aminotransferase**
ALT	**Alanine aminotransferase**
CAT	Catalase
CCl_4	Carbon tetrachloride
DB	Direct bilirubin
E2A, E2B, E2C, E2D	Ethyl acetate fractions of ***Boswellia papyrifera***
EASE	Ethyl acetate stem bark extract
GPX	Glutathione peroxidase
MDA	Malondialdehyde
ROM	Reactive oxygen metabolites
SOD	Superoxide dismutase
TB	Total bilirubin
TP	Total protein

Statistical Analysis

The data were statistically analyzed at p-value ($p<0.05$) significantly accepted and a comparison between the groups was performed using one-way analysis of variance (ANOVA) by GraphPad Instat 3 software (2000) version 3.05 by GraphPad Inc. The data are presented as the mean ± standard deviation.

RESULTS AND DISCUSSION

Effects of E2A Ethyl acetate Fraction of *Boswellia papyrifera* Stem Bark Extract on CCl_4-Induced Liver Damage

The effects of E2A fraction of ethyl acetate of *Boswellia papyrifera* stem bark extract (E2A–EASE) on CCl_4-induced liver damage was assessed after four weeks of daily single oral administration at 20, 30, and 50 mg/kg to groups III, IV, and V, respectively. The mean serum activities of AST, ALT, and ALP, and the concentration of DB, TB, TP, and ALB showed no significant differences ($p<0.05$) when compared with the test control rats in all the three doses administered in the four-week period (Figure 1).

Effects of E2B Ethyl acetate Fraction of *Boswellia papyrifera* Stem Bark Extract on CCl_4-Induced Liver Damage

The effects of E2B ethyl acetate fraction of stem bark extract of *Boswellia papyrifera* (E2B-EASE) on the mean serum level activities of AST, ALT, and ALP and DB and TB concentrations at 20 mg/kg were significantly lower ($p<0.05$) when compared to test control rats, but still higher than the normal rats. However, the serum concentration of ALB and TP was significantly higher ($p<0.05$) when compared to the test control (Figure 2). At 30 mg/kg daily dose, the mean serum activities of AST, ALT, and ALP, and DB and TB concentrations decrease significantly ($p<0.05$) when compared with test control but still higher than the normal control group, while serum concentration of ALB and TP also increased. But at 50 mg/kg, all the liver function parameters assessed were reduced to normal when compared with the normal control (Group I). The decrease in the mean serum activities of AST, ALT, and ALP, and the concentration of TB and the increase in the mean serum ALB concentration were found to be dose-dependent as it occurred with an increase in the dose of E2B-EASE. Multiple comparison tests after inducement showed that the activities of AST, ALT, ALP, and ALB, and the TB, DB and TP concentrations in Group II were considered significant ($p<0.05$) when compared with the normal group. The activities of AST, ALT, ALP, and ALB, and the concentration of TB and TP of Groups III to V were considered significantly lower ($p<0.05$) while DB was found not significant when compared with the test control (Figure 2).

Effects of E2C Ethyl acetate Fraction of *Boswellia Papyrifera* Stem Bark Extract on CCl_4-Induced Liver Damage

The mean serum activities of AST, ALT, ALP and ALB,

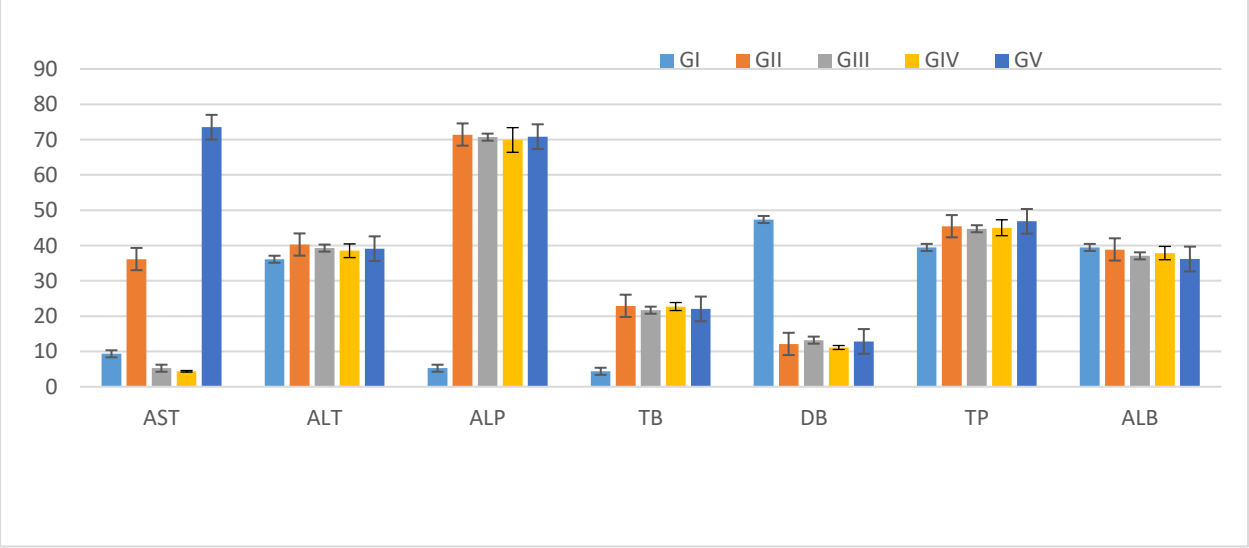

Figure 1. Serum liver function indices of CCl_4-induced liver damaged rats after four weeks oral administration of E2A fraction of ethyl acetate stem bark extract of *Boswellia papyrifera*. GI = Normal control; GII = Test control induced with 120 mg/kg of CCl_4; GIII, IV, and V = administered with 20, 30, and 50 mg/kg of E2A respectively, once daily. Results are expressed as mean ± SD, n = 5 with 95% confidence intervals; AST-Aspartate amino transferase; ALP-Alanine amino transferase; ALP - Alkaline phosphatase; TB - Total bilirubin; DB - Direct bilirubin; TP - Total protein; ALB - Albumin.

and TB, DB, and TP concentrations in Groups III to V showed no significant differences when compared with the test control (Group II), but statistically significant ($p<0.05$) when compared with the normal control (Group I) four weeks after daily oral administration of 20, 30, and 50 mg/kg of E2C ethyl acetate fraction of stem bark extract (E2C-EASE) of *Boswellia papyrifera* (Figure 3).

Effects of E2D Ethyl acetate Fraction of *Boswellia papyrifera* Stem Bark Extract on CCl_4-Induced Liver Damage

The effects of E2D ethyl acetate fraction of stem bark extract (E2D-EASE) of *Boswellia papyrifera* on serum activities of AST, ALT, ALP, and ALB, and TB, DB, and TP concentrations after CCl_4 inducement of liver

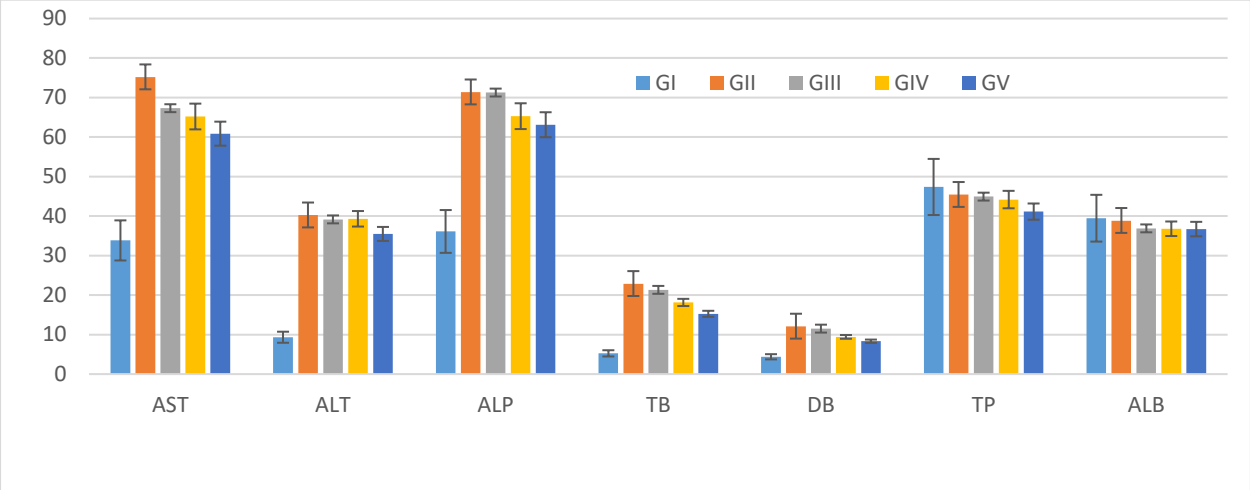

Figure 2. Serum liver function indices of CCl_4-induced liver damaged rats after four weeks oral administration of E2B fraction of ethyl acetate stem bark extract of *Boswellia papyrifera*. GI = Normal control; GII = Test control induced with 120 mg/kg of CCl_4; GIII, IV, and V = administered with 20, 30, and 50 mg/kg of E2B respectively, once daily. Results are expressed as mean ± SD, n = 5 with 95% confident intervals; AST- Aspartate amino transferase; ALP- Alanine amino transferase; ALP - Alkaline phosphatase; TB - Total bilirubin; DB - Direct bilirubin; TP - Total protein; ALB - Albumin.

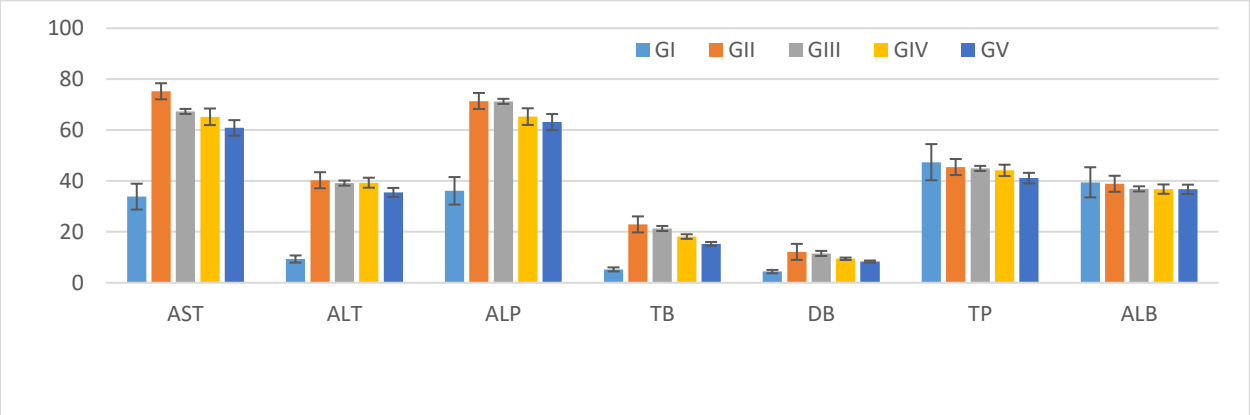

Figure 3. Serum liver function indices of CCl_4-induced liver damaged rats after four weeks oral administration of E2C fraction of ethyl acetate stem bark extract of *Boswellia papyrifera*. GI = Normal control; GII = Test control induced with 120 mg/kg of CCl_4; GIII, IV, and V = administered with 20, 30, and 50 mg/kg of E2B respectively, once daily. Results are expressed as mean ± SD, n = 5 with 95% confident interval; AST- Aspartate amino transferase; ALP- Alanine amino transferase; ALP - Alkaline phosphatase; TB - Total bilirubin; DB - Direct bilirubin; TP - Total protein; ALB - Albumin.

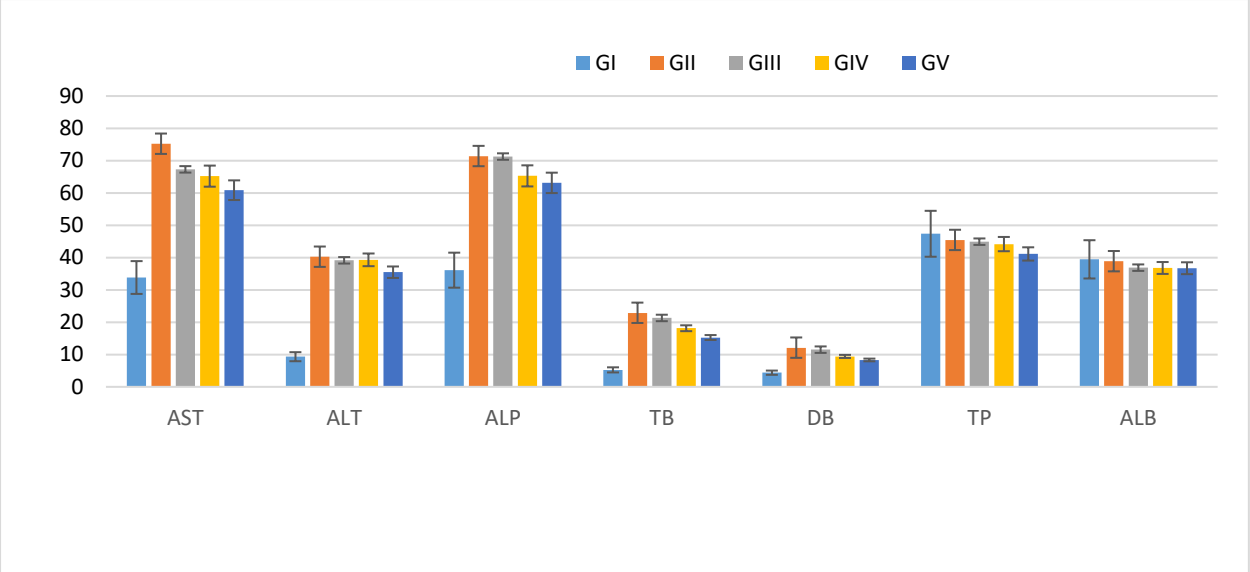

Figure 4. Serum liver function indices of CCl_4-induced liver damaged rats after four weeks oral administration of E2D fraction of ethyl acetate stem bark extract of *Boswellia papyrifera*. GI = Normal control; GII = Test control induced with 120 mg/kg of CCl_4; GIII, IV, and V = administered with 20, 30, and 50 mg/kg of E2B respectively, once daily. Results are expressed as mean ± SD, n = 5 with 95% confident interval; AST- Aspartate amino transferase; ALP- Alanine amino transferase; ALP - Alkaline phosphatase; TB - Total bilirubin; DB - Direct bilirubin; TP - Total protein; ALB - Albumin.

damage in Groups III to V at 20, 30, and 50 mg/kg respectively showed no significant differences after four weeks oral administration when compared with the test control (Group II), but statistically significant ($p>0.05$) when compared with the normal control (Group I) (Figure 4).

Histology of the CCl_4-Induced Liver Damage after Four Weeks Oral Administration of E2B Ethyl acetate Fraction of *Boswellia papyrifera* Stem Bark Extract

Histopathology evaluation of the liver section of a normal rat of Group I (Plate I) showed normal liver architecture with hepatocytes arranged in cord radiating from central venules, while Group II exhibited extensive liver damage with necrosis, vascular damage, and areas of fats cell deposited due to damage caused by CCl_4 administered to the rats (Plate II). However, four weeks oral administration of E2B ethyl acetate fraction of stem bark extract of *Boswellia papyrifera* at doses of 20, 30 and 50 mg/kg to Groups III, IV and V, respectively, the liver showed areas of fibrosis as the initial repair mechanism by the liver hepatocytes (Plate III). Plates IV and V indicate no sign of pathological abnormality four weeks after oral administration of E2B ethyl acetate fraction of stem bark extract of *Boswellia papyrifera*.

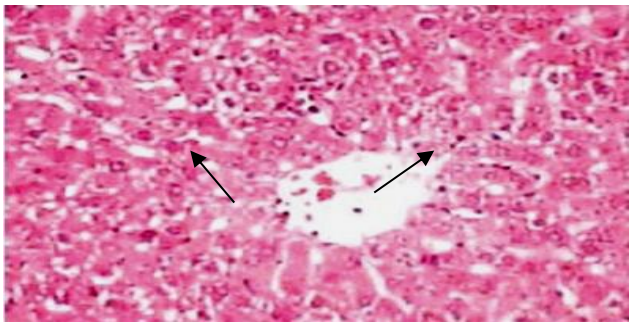

Plate I: The transverse section of the liver of a normal rat with distinct hepatocytes arranged in cord radiating from the venules. H & E Stain X 10

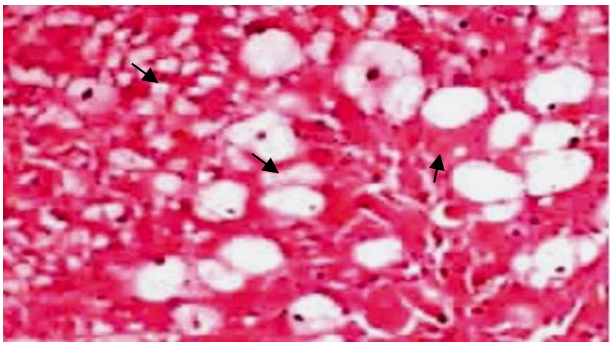

Plate II: The transverse section of the liver of a test control rat; the liver showed extensive damage with necrosis, vascular damage and areas of fats cell deposited due to damage caused by CCl_4 administered to the rats. H & E Stain X 10

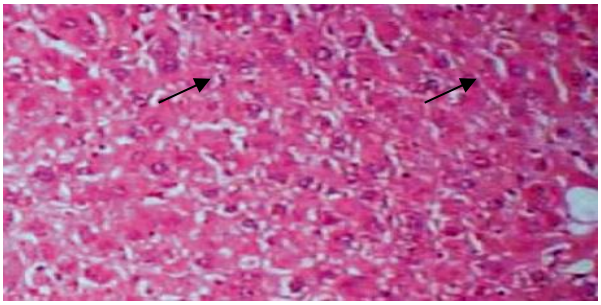

Plate III: The transverse section of the liver of a CCl_4-induced rat of Group III; the liver showed areas of fibrosis after four weeks oral administration with 20 mg/kg of E2B fraction of ethyl acetate stem bark extract of *Boswellia.papyrifera*. H &E stain X10

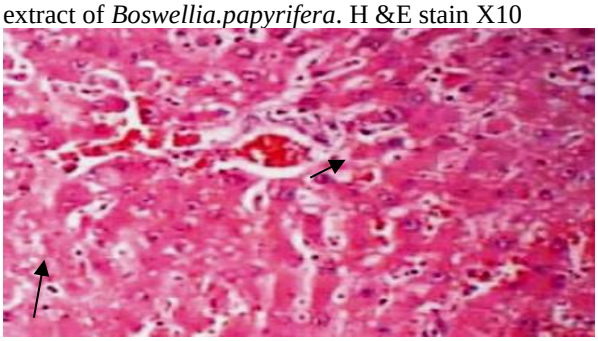

Plate IV: The transverse section of the liver of a CCl_4-induced rat of Group III; the liver showed areas of fibrosis after four weeks oral administration with 20 mg/kg of E2B fraction of ethyl acetate stem bark extract of *Boswellia.papyrifera* . H &E stain X10

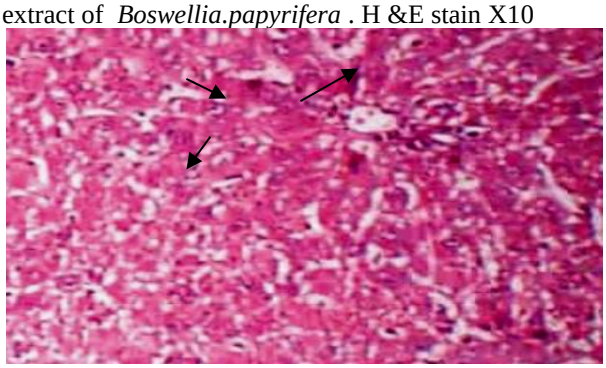

Plate V: The transverse section of the liver of a CCl_4-induced rat of Group V; showing no significant pathological abnormalities after four weeks oral administration of 30 mg/kg of E2B fraction of ethyl acetate stem bark extract of *Boswellia papyrifera*. The liver showed normal architecture with distinct hepatocytes arranged in cord radiating from the venules. H & E stain X 10

Effects of Four Weeks Oral Administration of E2B Ethyl acetate Fraction of *Boswellia papyrifera* Stem Bark Extract on Antioxidant Levels in CCl_4-Induced Hepatotoxicity in Rats

CCl_4 treatment caused a significant ($p<0.05$) decrease in the levels of SOD, catalase, and GST in liver tissues while the level of MDA was significantly increased when compared with the control group (Table 2). The oral administration of E2B ethyl acetate fraction of *Boswellia papyrifera* stem bark extract at doses of 20, 30, and 50 mg/kg for four weeks resulted in a significant ($p<0.05$) increase in the antioxidants SOD, CAT, GST and a significant ($p<0.05$) decrease in MDA level when compared to CCl_4-treated control group (Group II) (Table 2).

DISCUSSION

The efficacy of any hepatocurative drug is indeed dependent on its capability of either reducing the harmful effects or in maintaining the normal hepatic physiological mechanism which has been imbalanced by a hepatotoxin compound. The remarkable elevation in the rats' liver marker enzymes ALT, AST, ALP, and total and direct bilirubin, and the decrease in levels of total protein and albumin in CCl_4-administered rats (Figure 2) served as confirmation of hepatotoxicity of CCl_4 [7]. The hepatotoxic effects induced by a single dose of 120 mg/kg of CCl_4 arise from its metabolite $*CCl_3$, a free radical that alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids in the presence of oxygen, to produce lipid peroxides that result in liver damage [26].

The elevation of the rats' liver marker enzymes administered with CCl_4 alone in this study was similar to the findings of Prakash *et al*. [26], who observed significant hepatic damage in rats treated with a single dose of CCl_4. In particular, the increase in the serum level of ALT is an indicator of liver damage. These enzymes are located in the cell cytoplasm and are emptied into the circulation once the cellular membrane is damaged [4].

However, the reduction in the levels of AST, ALT, and ALP activities, and total and direct bilirubin, as well as the albumin and total protein concentrations elevation in rats treated with E2B ethyl acetate fraction of *Boswellia papyrifera* stem bark extract at all the three doses (Figure 2) was also in agreement with the view that serum levels of transaminases return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes. It is also evident that an increase in bilirubin concentration in the serum or tissue is indicative of obstruction in the excretion of bile. Thus, the increased level of bilirubin observed in rats administered with CCl_4 alone (Group 2) could be attributed to liver damage. However, the decrease in bilirubin levels in treated rats served as an indication of reversal of liver damage by the E2B ethyl acetate fraction of *Boswellia papyrifera* stem bark extract.

Table 2. Effects of Four Weeks Oral Administration of E2B Ethyl acetate Fraction of *Boswellia papyrifera* Stem Bark Extract on Antioxidant Levels in CCl_4-induced Hepatotoxicity in Rats

Groups/ Treatments	CAT (µ /mg protein)	SOD (µ /mg protein)	GST (µ /mg protein)	MDA (nmol/g protein)
I No CCl_4 administered (NORMAL)	75.26±3.12	9.12±0.23	7.24±1.23	451.12±9.23
II 120 mg/kg of CCl_4 (LIVER CONTROL RATS)	25.45±1.12*	2.23±0.11*	3.11±0.56*	613.03±7.41*
III 20 mg/kg of E2B fraction of EASE *B. papyrifera* administered	53.11±6.17^c	4.12±0.67^d	6.23±0.87^e	523.11±7.11^f
IV 30 mg/kg of E2B fraction of EASE *B. papyrifera* administered	65.23±4.11^c	8.11±0.14^d	6.71±0.23^e	501.03±5.48^f
V 50 mg/kg of E2B fraction of EASE ***B***. *papyrifera* administered	85.87±6.27^c	11.42±1.22^d	7.87±1.45^e	487.62±8.21^f

Results are expressed as mean ±SD, n = 5
Values with asterisks are significantly different at $p<0.05$ when compared with the normal rats.
Values in the same column with the same superscript are significantly different at $p<0.05$ when compared with the test control.
EASE- Ethyl acetate stem bark extract; CAT-Catalases; SOD- superoxide dismutase; GST- Glutathione-S-transferase; MDA- Malondialdehyde

CCl_4 induction was also associated with a significant decrease in the serum levels of albumin and total protein. However, treatment with E2B ethyl acetate fraction of *Boswellia papyrifera* stem bark extract cured the liver from the deleterious effect of the toxin by improving the decrease in the circulatory levels of albumin and total protein in a dose-dependent manner. CCl_4 induction causes degeneration of hepatocytes and blockage of the bile ducts which result in significant increase in the serum levels of total bilirubin and direct bilirubin [28]. Treatment with E2B ethyl acetate fraction of *Boswellia papyrifera* stem bark extract normalized the elevated serum levels of total bilirubin and direct bilirubin. Thus, a reduction in the levels of ALT and AST towards the normal values is an indication of the regeneration process. Reduction in the levels of ALP, total bilirubin and direct bilirubin suggests the stabilization of the biliary function. An increase in the serum levels of total protein and albumin suggests the stabilization of endoplasmic reticulum, leading to protein synthesis.

Histopathology evaluation of the liver section from a normal rat of Group I (Plate I) showed normal liver architecture with hepatocytes arranged in cord radiating from central venules while Group II exhibited extensive damage with necrosis, vascular damage, and areas of fats cell deposited due to the damage caused by CCl_4 administered to the rats (Plate II). However, four weeks oral administration of E2B ethyl acetate fraction of stem bark extract of *Boswellia papyrifera* ahe doses of 20, 30 and 50 mg/kg to Groups III, IV and V, respectively, the liver showed areas of fibrosis as the initial repair mechanism by the liver hepatocytes (Plate III). Plates IV and V indicated no sign of pathological abnormality four weeks after oral administration of E2B ethyl acetate fraction of stem bark extract of *Boswellia papyrifera*. The marked decrease in the levels of serum marker enzymes in treated rats was the indication of the ability of E2B ethyl acetate fraction of *Boswellia papyrifera* stem bark extract to cure the liver against CCl_4 poisoning. This was validated by the cytoarchitecture of the livers of the rats that received E2B ethyl acetate fraction of *Boswellia papyrifera* stem bark extract four weeks after CCl_4 administration in a dose-dependent manner.

The decrease in the activity of superoxide dismutase (SOD) is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury [29]. SOD has been reported as one of the most important enzymes in the enzymatic antioxidant

defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. Oral administration of E2B ethyl acetate fraction of *Boswellia papyrifera* stem bark extract at the doses of 20, 30, and 50 mg/kg caused a significant increase in hepatic SOD activity and thus reduced reactive free radical-induced oxidative damage to the liver in a dose-dependent order (Table 2).

CAT is an enzymatic antioxidant widely distributed in all animal tissues, and the highest activity is found in the red cells and liver. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals [30]. Therefore, reduction in the activity of CAT may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide produced after CCl_4 administration. However, oral administration for four weeks of E2B ethyl acetate fraction of *Boswellia papyrifera* stem bark extract at the doses of 20, 30, and 50 mg/kg increased the level of CAT (Table 2).

Glutathione is one of the most abundant tripeptides, a non-enzymatic biological antioxidants present in the liver. It removes free radical species such as hydrogen peroxide and superoxide radicals and maintains membrane protein thiols. Also it is a substrate for glutathione peroxidase (GPx) [31]. The decreased level of GSH was associated with enhanced lipid peroxidation in CCl_4-administered rats (Group II) (Table 2). However, oral administration of E2B ethyl acetate fraction of *Boswellia papyrifera* stem bark extract significantly ($p<0.05$) increased the level of GST in a dose-dependent manner. Administration of different doses of E2B ethyl acetate fraction of *Boswellia papyrifera* stem bark extract produced higher degenerative changes and absence of centrilobular necrosis, which are indications of the hepatocurative efficiency of the extract.

Products of lipid peroxidation may lead to changes in biological membranes, therefore, these changes result in serious cellular injury. An increase was observed in the formation of MDA in the hepatocytes of rats which were exposed to CCl_4 (Table 2). It is suggested that reactive oxygen metabolites (ROM) play a critical role in the accumulation of neutrophils in tissues after ischemia, whereas activated neutrophils are also a potential source for ROMs. Microsomal mixed-function oxidase plays a basic role in the production of oxidants by neutrophils. Neutrophils are an important source of free oxygen radicals and therefore are considered as a major effector in the tissue damage that occurs in many inflammatory disorders [32].

In this study, hepatic MDA in groups administered with 20, 30, and 50 mg/kg of E2B fraction of ethyl acetate of *Boswellia papyrifera* was found to be significantly lower than the CCl_4-administered control group. However, the declining values of MDA in the group administered with 20 mg/kg was not significant. This observation may be originated from inadequate serum bioavailability of E2B fraction of ethyl acetate stem bark of *Boswellia papyrifera*. However, at the doses of 30 and 50 mg/kg, the MDA values were found close to the MDA values of the control group when compared with other groups.

CONCLUSION

The finding in this study indicated that an ethyl acetate fraction (E2B) of *Boswellia papyrifera* stem bark possesses potent curative activity against CCl_4-induced liver damage, most probably due to high antioxidant activity of the fraction and thus cured the metabolic complications of the liver disease. E2B ethyl acetate fraction of *Boswellia papyrifera* possesses the ability to decrease the level of MDA and increase the antioxidants SOD, CAT and GST activities four weeks after oral administration of the fraction. The reduction in the levels of markers AST, ALT, ALP activities, and total and direct bilirubin as well as albumin and total protein concentration elevation in rats treated with E2B ethyl acetate fraction of *Boswellia papyrifera* stem bark extract at all the three doses ascertain the fraction to possess potent curative effect in CCl_4-induced liver damage in Wister rats in a dose-dependent manner.

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REFERENCES

1. Adekomi, D. A, Musa, A. A., Tijjani, A. A., Adeniyi, T. D. and Usman, B. (2011) Exposure to smoke extract of *Datura Stramonium* Leaf: Some of its Effects on The Heart, Liver, Lungs, Kidneys and Testes of Male Sprague Dawley Rats. *J.Pharmacog, Phytother.*, **3,** 67-75

2. Bourogaa, E., Neri R., Mezghani-Jarraya, R., Racaud-Sultan, C., Damak, M., el Feki, A (2013) Antioxidant activity and hepatoprotective potential of Hammada scoparia against ethanol-induced liver injury in rats. *J. Physiol. Biochem.*, **69,** 227-237.

3. Ahmed S., Rahman A., Alam A., Saleem M., Athar M and Sultana S. (2000) Evaluation of the efficacy of *Lawsonia alba* in the alleviation of carbon tetrachloride-induced oxidative stress, *JEthano-pharmacol*., **69,** 159-164.

4. Lin C. C. and Huang P. C. (2000) Antioxidant and hepatoprotective effects of *Acathopanax senticosus.*

Phytother. Res., **14,** 489-494

5. Lius S. L., Espoti, S. O., Yao T., Diehl A. M. and Zern M. A. (1995) Vitamin E therapy of acute CCI_4- induced hepatic injury in mice as associated with inhibition of nuclear factor. *Kappa B Binding. J. Hepatol.,* **22,** 1474-1481.

6. Recknagel, R. O., Glende, E. A., Dolak, J. A., Waller, R. L. (2009) Mechanisms of carbon tetra-chloride toxicity. *Pharmacol. Ther*., **43,** 139-154.

7. Alhassan, A. J., Sule, M. S., Hassan, J. A., Baba, B. A. and Aliyu, M. D. (2009) Ideal Hepatotoxicity model using CCl_4. *Bayero J. Pure and Appl.Sci.,* **2,** 185-187.

8. Akhtar, M. S., and Ali, M. R. (1984) Study of Antidiabetic effect of a compound medicinal plant prescription in normal and diabetic rabbits. *J. Pakistan. Med. Assoc.,* **34,** 239-244.

9. Fitchl, R. and Admasu A. (2004) Honeybee Flora of Ethiopia. Margraf Verlag, Weikersheim, Germany, **28,** 510-518

10. Groom, N. (2011) Frankincense and myrrh: a study of the Arabian incense trade. Longman, London, **285,** 221-227.

11. Pokorny, J. (2007) Are natural antioxidants better – and safer – that synthetic antioxidants? *Eur. J. Lipid Sci. Technol*., **109,** 629-642.

12. Kancheva, V. D. (2009) Phenolic antioxidants – radical-scavenging and chain-breaking activity: A comparative study. *Eur. J. Lipid. Sci. Technol*., **111,** 1072-1089.

13. Mittal G. C., Aguwa C. N., Ezeiru V. U. and Abuke P. I. (1981) Preliminary pharmacological studies on anti-venom action of Diodia scandens leave. *The Niger. J. Pharmacol*., **12,** 432-436.

14. Fernando M. R., Wichcramasinghe S. M. D., Thabrew M. I., Karnayaka E. K. (1989) Preliminary investigation of the possible hypoglycemic activity of *Astera-canthus longifolia. J. Ethnopharmacol*., **27,** 7-14.

15. Acharya C. R., Sharma A. K. and Kantharia N. D. (2015) Involvement of oxidative stress in patients of gout and antioxidant effect of allopurinol. *Int J Med Sci Public Health*, **4,** 168-172

16. Reitman, S. and Frankel, S. (1957) A colorimetric method for the determination of serum glutamic oxaloacetate and glutamic pyruvic transaminase. *Amer. J.clin.Patho.,* **28,** 56-62.

17. Rec, G. S. C. C. (1972) Colorimetric method for serum alkaline phosphatase determination. *J. Clin.Biochem.,* **10,** 182-189.

18. Grant, G.H. (1987) *Amino acids and Proteins*; Fundamentals of Clinical Chemistry, Tietz N. W. Editor, 3rd Edition, WB Saunders Company Philadelphia USA, 328-329.

19. Jendrassik, L. and Grof, P. (1938) Colorimetric Method for Determination of Serum Bilurubin. *Biochem. Z.*, **297,** 81-89

19. Tietz N. W. (1995) *Clinical Guide to Laboratory Tests.* 3rd Edition W. B. Saunders Company, PA, **46,** 518-519.

20. Luck H. (1963) Catalase. In, Methods of enzymatic analysis. (Ed.Begmeyer HU) Academic Press, New York, 895-897.

21. Jocelyn, P. C. (1972) Biochemistry of the SH Group. *Academic Press*, London, **10**.

22. Kakkar, P., Das, B. and Viswanathan., P. N. (1984) A modified spectrophotometric assay of superoxide dismutase. *Ind. J. Biochem. Biophys.*, **21,** 131-132.

23. Wasowich W., Neve J. and Peretz A. (1993) Optimized steps in the fluorometric determination of thiobarbituric acit-revtive substances in serum: the importance of extraction and influence of sample preservation and storage. *Clin Chem.*; **39,** 252-262

24. Auwiro, O. G. (2010) *Histochemistry and tissue pathology, principles and techniques* 2nd Edition, **4,** 561-568

25. Patrick-iwuanyanwu K. C., Wegwu M. O. and Okiyi J. K. (2010) Hepatoprotective effects of African locust bean and negro pepper in CCl4 induced liver damage on wistar albino rats. *Int. J. Pharmacol.,* **6,** 744-749

26. Prakash T., Faladu S. D., Sharma U. R., Surendra, V., Goli, D., Stamina P. and Kotresha, D. (2008) Hepatoprotective activity of leaves of *Rhododendron arboretum* in CCl_4induced hepatotoxicity in rats. *Journal of Medicinal Plants Research*, **2,** 315-320.

27. Saraswat B., Visen P. K., Patnaik G. K. and Dhawan, B. N. (2013) Anticholestic effect of picroliv, the active hepatoprotective principle of Picrorhiza kurroa, against carbon tetrachloride-induced cholestatis. *Indian Journal of Experimental Biology*, **31,** 316-371

28. Curtis J. and Mortiz M. (2009) Serum enzymes derived from liver cell fraction and response to carbon tetrachloride intoxication in rats. *Gastroenterol*; **62,** 84-92.

29. Chance B. and Greenstein D. S. (2007) The mechanism of catalase actions-steady state analysis. *Arch Biochem. Biophys*., 1992; **37,** 301-339.

30. Prakash J., Gupta S. K. and Singh N. (2001) Chemopreventive activity of *Withania somnifera* in experimentally induced fibrosarcoma tumors in Swiss albino rats. *Phytother Res*; **15,** 200-204.

31. Sener G., Tosun O. and Şehirli A. Ö. (2003) Melatonin and N-acetylcysteine have beneficial effects during hepatic ischemia and reperfusion. *Life Sciences,* **72,** 2707-2718