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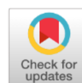

RESEARCH ARTICLE

Ethanol crude leaf extract of weeping fig (*Ficus benjamina*) causes mild hepatotoxicity in *Sprague dawley* rats

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Abstract

Weeping fig (*Ficus benjamina*) leaf extract has diverse medicinal properties but little is reported about its hepatotoxicity. This study determined the mean lethal dose (LD_{50}) and investigated the effects of *F. benjamina* ethanol crude leaf extract on biochemical parameters and liver histology of *Sprague Dawley* rats. Twenty-nine female rats weighing 133-204 g were used. The LD_{50} was determined with nine rats based on Lorke's method. The experimental groups consisted of twenty rats, divided into four groups of five. Each group received treatment as follows: Control (feed and water only) and low, medium, and high doses (500, 1000, 1500 mg/kg respectively) of the extract orally for 21 days. All animals were weighed and sacrificed using Ketamine intra-peritoneal injection. Blood samples were collected for biochemical parameters of total bilirubin, conjugated bilirubin, aspartate aminotransferase (AST) alanine aminotransferase (ALT), and alkaline phosphatase (ALP). Liver tissues were removed and processed by the formalin-fixed-paraffin wax-embedding method. The tissue blocks were sectioned and subjected to Hematoxylin/Eosin and Masson trichrome staining. The extract's LD_{50} was >5000 mg/kg. The rats' body weights did not change statistically ($p=0.985$). Total bilirubin ($p=0.003$), conjugated bilirubin, AST, ALT, and ALP values ($p=0.001$) increased significantly. The AST high-dose group had the highest fold increase (4.8). The liver histology showed mild sinusoidal dilation at 500 mg/kg. There was marked hemorrhage and fibrosis at medium and high doses. Although the extract had relatively low acute toxicity, 1000 and 1500 mg/kg doses were associated with mild hepatotoxicity characterized by veno-occlusion disease. The 500 mg/kg dose is safer for medicinal purposes.

1. INTRODUCTION

Medicinal plant- or herbal-induced liver injury (HILI) has contributed significantly to the increase in hepatotoxicity worldwide (1,2). In the United States, HILI was reported to be the second leading cause of liver injury after antibiotics (3). Recently, 404 cases of HILI were reported between 2003 and 2019 in the United States (4). In the Asia-Pacific region, about 72% of patients with acute liver failure were linked to HILI (5). These HILI have been reported in some plants such as turmeric, kratom, aloe vera, and green tea supplements (5,6).

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F. benjamina is a widely used medicinal plant but, there are limited studies on its hepatotoxic effect or potential to cause HILI.

The *F. benjamina (Linn*) plant also called the weeping fig is in the family, Moraceae (7). Weeping fig is cultivated in many countries of the world including Nigeria. It is a large evergreen shrub and is mostly grown as an ornamental plant in Nigeria. The evergreen leaves are eaten by goats as feed. Its leaves, fruits, and bark are rich in phytochemicals like lactose, serotonin, naringenin, cinnamic acid, quercetin, stigmasterol, and caffeic acid (7,8). Other phytochemicals include flavonoids, palmitic acid, benjaminide, chlorogenic acid, p-coumaric acid, and ferulic acids (9). *F. benjamina* is popular among other medicinal plants due to its diverse properties. The plant is used as a hypotensive, antipyretic, anti-dysentery, and antimicrobial agent due to its rich constituent of flavonoids, phenols, alkaloids, and glycosides (9,10). The extracts from its fruits and latex are rich in isoflavonoids, flavonoids, alkaloids, ursolic acid, and lupeol used in the treatment of cancer, convulsion, inflammation, skin disorders, piles, leprosy, malaria, and vomiting (7,9,10). Other known medicinal plants include *Cassia alata* leaf extracts shown to have antifungal properties (11), *Gongronema latifolium* leaf with anti-diabetic functions (12), and tiger nut which ameliorates male infertility (13).

The liver is the body's largest internal organ that carries out numerous metabolic activities, detoxification, synthesis of bile and clotting factors; and storage of fats and glycogen. The liver synthesizes enzymes such as aspartate aminotransferase (AST) alanine aminotransferase (ALT), and alkaline phosphatase (ALP). The liver is also responsible for the breakdown of aged erythrocytes to produce bilirubin. The primary role of the liver in detoxification exposes it to toxic metabolites after absorption from the small intestines through the hepatic portal vein (14). Thus, the liver is susceptible to toxicity and may be injured in the process. Several studies have reported the diverse uses of *F. benjamina* as a medicinal plant (7,9,10). Most of these studies are on the hepatoprotective potential of *F. benjamina* with limitations on its hepatotoxic effects. The hepatoprotective potential of *F. benjamina* leaf extract was stated by Kaur *et al.* (10). Another study reported the hepatoprotective potential of *F. benjamina* (at a dose of 400 mg/kg) following carbon tetrachloride (CCl4)-induced liver injury in mice (15). An earlier study by Pilapil *et al.* (16) reported the hepatoprotective effect of crude aqueous leaf extract of *F. benjamina* (at a dose of 500 mg/kg) following ethanol-induced hepatotoxicity in mice. The level of hepato-protection was comparable to the standard drug Silymarin. This hepatoprotective activity of *F. benjamina* leaves has been linked to flavonoids and phenolic compounds (15). These facts are supported by work on other Ficus species by El Ghouizi *et al.* (17) using *F. carica* leaves and buds extracts who reported hepato-protection and ameliorative effects against type I diabetes.

Although there are several studies on the hepatoprotective effects of *F. benjamina* leaf extract, there are limited studies on its effects on herbal-induced liver injury or hepatotoxicity at doses of 500 mg, 1000 mg, and 1500 mg/kg being 10%, 20%, and 30% respectively of the LD_{50} of >5000 mg/kg. It is therefore essential to investigate if it has a toxic effect on the integrity of the liver cells and the histological changes that may arise using *Sprague dawley* rats at these doses. It is also vital to determine the optimum dose with less toxicity required for safe administration. Hence, the rationale of this study is to determine the LD_{50} and investigate the hepatotoxic effect of ethanol crude leaf extract of *F. benjamina* on liver biochemical parameters and histology.

2. MATERIALS AND METHODS

2.1. Chemicals

Ethanol (Sigma-Aldrich, USA) and Ketamine (Pfizer, USA) were purchased from a local chemical store in Calabar. Bilirubin and ALP reagents were Randox kits (Randox, United Kingdom). The ALT and AST determinations used ELITech Clinical Systems (France) reagents. All reagents were of analytical grade.

2.2. Experimental Animals

From the Animal House University of Calabar, twenty-nine (29) female *Sprague Dawley r*ats with body weights ranging from 133-204 g were acquired and included in the study. Nine (9) rats were used for the LD_{50} study while twenty (20) rats were used for the experiment. All rats were housed in well-ventilated cages for a two-weeks acclimatization period. During the study, water and feed (grower) were provided to the rats daily. Animals with low body weights or any visible sign of illness were excluded from the study.

2.3. Collection and Preparation of *F. benjamina* Leaf Extract

F. benjamina leaf samples were obtained from No. 50 Webber Street Calabar, Cross River State. The leaf was registered and authenticated in the Department of Plant and Ecological Sciences, University of Calabar, Calabar by

Dr. Effa B. Effa. It was assigned the identification number Bot/Herb/UCC/764. Fresh leaves of *F. benjamina* plant were rinsed with tap water, allowed to drain, and air-dried for 7 days. Dried leaves were pulverized using a manual blender. Then, 650 g of the pulverized leaves were put in 2000 mL of 95% ethanol for 48 hours.

A homogenous filtrate of the extract was obtained after filtering twice using Chess cloth and Whatman No.1 filter paper. The homogenous filtrate was put in a rotary evaporator and subjected to reduced pressure at 45°C to concentrate and subsequently in a vacuum water bath to dry after which 56 g of the crude extract was obtained. The extract was safely stored at 4°C in a refrigerator throughout the study. This temperature did not allow the formation of molds.

2.4. Mean Lethal Dose (LD_{50}) Determination of The Plant Leaf Extract

The method of Lorke as reported by Udonkang *et al.* (18) was used in the LD_{50} determination where nine rats were used. The rats were grouped into three comprising three animals respectively for each phase. In phase 1, the rats were orally administered 100, 500, and 1000 mg/kg doses per body weight of extract and were monitored for 24 hours. The second phase was 1500, 2500, and 3500 mg/kg and the third phase was 4500, 5500, and 6500 mg/kg, respectively. All rats were monitored for 24 hours for any physical signs of toxicity.

2.5. Experimental Design

The experiment groups comprised twenty rats made up of four groups with each having five rats and given treatment as follows: Control (feed and water only) and low, medium, and high doses (500, 1000, and 1500 mg/kg respectively of the extract orally for 21 days. These doses were determined based on the LD_{50} of the extract by calculating 10%, 20%, and 30% of the value, equivalent to 500, 1000, and 1500 mg/kg, respectively). After 21 days, the body weights were measured and the rats were sacrificed using Ketamine intra-peritoneal injection. Blood sample from each rat was collected by cardiac puncture using a syringe and needle. The collected samples were dispensed inside plain plastic containers and spun in a centrifuge to obtain serum which was used to analyze the biochemical parameters. All the animals were dissected, and the liver from each rat was excised and placed in 10% neutral buffered formalin to fix for 48 hours.

2.6. Determination of Liver Function Biochemical Parameters

2.6.1. Determination of Bilirubin

Serum total and conjugated bilirubin levels were determined based on the method of Jendrassik and Grof (19) using Randox reagent according to the manufacturer's instructions. The reaction was based on the colorimetric method. The principle is based on the reaction when conjugated bilirubin comes in contact with diazotized sulphanilic acid to form a blue-colored azobilirubin compound under alkaline pH. The total bilirubin reaction is based on the reaction with diazotized sulphanilic acid in the presence of caffeine. The values were read with a colorimeter at 540 nm wavelength after 10 minutes incubation at 25°C using a 1 cm cuvette.

2.6.2. Determination of Serum AST and ALT

The ALT and AST levels were analyzed based on the method of Reitman & Frankel (20) using ELITech Clinical Systems (France) reagents and ELITech autoanalyzer with procedures based on the manufacturer's manual. The principle of ALT determination is based on ALT catalysis of the reaction of L-Alanine with alpha-ketoglutarate to form pyruvate which is acted upon by lactate dehydrogenase to form L-Lactate. The reaction was measured at 340 nm wavelength with a 1 cm cuvette at a temperature of 37°C. The principle of AST determination is based on AST catalysis of the reaction of L-Aspartate with alpha-ketoglutarate to form oxaloacetate which is acted upon by malate dehydrogenase to form L-Malate.

2.6.3. Determination of ALP

Alkaline phosphatase assay was carried out based on the Colorimetric method of Rec (21). The procedure was based on the manufacturer's manual using a Randox kit (Randox, United Kingdom, Catalog number: 2443AP). The method involved the reaction of alkaline phosphatase in the serum with the substrate P-nitrophenyl phosphate in the presence of P-nitrophenol, diethanolamine as a buffer at pH 9.0, and Magnesium chloride. The reaction was read at 405 nm wavelength with a 1 cm cuvette at 37°C.

2.7. Tissue processing and staining

The fixed liver samples were grossed and placed in plastic tissue cassettes for processing via the paraffin wax-embedding method. Thereafter the tissue blocks were cut at 3 µm with a rotary microtome (Leica RM2125,

USA) and stained for general tissue structure with hematoxylin and eosin and Masson trichrome for collagen fiber staining according to the method described by Bancroft *et al.* (22). All stained sections were dehydrated in ascending grades of ethanol, put in xylene to clear, and mounted with a coverslip using DPX (23). All slides were viewed with a light microscope (OMAX, China) at x100 magnification.

2.8. Statistical analysis

Results of the body weights and biochemical parameters were presented as mean values with standard deviations. The chi-square test measured any association between the final and initial body weights. The biochemical parameters were analyzed with ANOVA. Fold change of the biochemical parameters were calculated by dividing the test value by the control value for each parameter. The test of significance was measured with the chi-square test. All results were significant at a probability value ≤ 0.05. Statistical Package of Social Science (SPSS, USA) software version 20 was used to analyze the data.

3. RESULTS AND DISCUSSION

In this study, the mean lethal dose (LD_{50}) of *F. benjamina* was found to be greater than 5000 mg/kg (Table 1). This range of LD_{50} is in Category 5 of acute toxicity hazard categories. This indicates that the plant extract has relatively low acute toxicity and did not result in mortality among the rats. This is similar to the report by Ladaf *et al.* (24) and Chike-Ekwughe *et al.* (25) who worked on *F. pumila* and *Tapinanthus cordifolius* leaf extracts respectively. In both studies, the plants extracts did not cause any mortality in the rats at doses exceeding 5000 mg/kg.

Table 1. LD_{50} determination of *Ficus benjamina* leaf extract

Ethanol leaf Extract	Phase	Dose (mg/kg)	Death	
			After 24 hours	LD_{50}(mg/kg/b.w)
F. benjamina (Crude Extract)	1	100	0	>5000
		500	0	
		1000	0	
	2	1500	0	
		2500	0	
		3500	0	
	3	4500	0	
		5500	0	
		6500	0	

Keys - Category 1: LD_{50} < 5 mg/kg (very high toxic), Category 2: LD_{50} > 5 mg/kg < 50 mg/kg (high toxic), Category 3: LD_{50}> 50 mg/kg < 300 mg/kg (moderate toxicity), Category 4: LD_{50} > 300 mg/kg < 2000 mg/kg (low toxicity), Category 5: LD_{50}> 2000 mg/kg < 5000 mg/kg or >5000 mg/kg (relatively low toxicity or non-toxic) (ESSR (25)).

Table 2. The body weights of animals in experimental groups

Group	Mean weight ± SD (mg)		Statistics
	Before	After	
Control	167.6 ± 29.21	156.1 ± 27.64	χ^2=0.152
LD (500 mg/kg)	154.7 ± 15.06	144.3 ± 12.42	p=0.985
MD (1000 mg/kg)	150.9 ± 17.44	143.6 ± 16.11	
HD (1500 mg/kg)	173.2 ± 12.04	163.0 ± 11.09	

Notes: SD – Standard deviation, Low dose- LD, Medium dose- MD, High dose-HD

The body weights of rats in each group before and after the study were as follows: control group (167.6 ± 29.21 g, 156.1 ± 27.64 g), low dose (154.7 ± 15.06 g, 144.3 ± 12.42 g), medium dose (150.9 ± 17.44 g, 143.6 ± 16.11 g), and high dose (173.2 ± 12.04 g, 163.0 ± 11.09 g). There was no statistically significant change in body weights across the groups (χ^2=0.152, p=0.985) (Table 2). The extract did not lead to a reduction in the rats' body weights, indicating that it did not interfere with their feeding habits or ability to digest food. This finding is consistent with the report of El Ghouizi *et al.* (17). which also found no significant reduction in the body weights of the animals.

Table 3 shows the serum liver biochemical parameters of the rats. The total bilirubin values increased significantly in a dose-dependent manner from 2.9 ± 0.51 µmol/L in the control to 5.8 ± 1.01 µmol/L in the HD group (p=0.003). All conjugated bilirubin values increased significantly according to the increased dose from 1.6 ± 0.20 µmol/L in the control group to 3.8 ± 0.40 µmol/L in the HD group (p=0.001). In this study, bilirubin levels were

increased. Bilirubin is a marker that evaluates the excretion and conjugation functions of the liver (26). An increase in total and conjugated bilirubin is an indication of liver damage. This is because bilirubin is a product of hemoglobin catabolism. Increased heme breakdown leads to increased formation of unconjugated bilirubin which when combined with decreased hepatic uptake or conjugation or both leads to an increase in total serum bilirubin (26). Additionally, conjugated bilirubin appears in small amounts in serum because it is rapidly excreted. Increased conjugated bilirubin occurs when the liver has lost at least half of its excretory capacity.

Table 3. Liver biochemical parameters of the animals

Parameter	Control	LD	MD	HD	F	p value
TBIL (µmol/L)	2.9 ± 0.51*	3.6 ± 0.56	4.8 ± 0.41*	5.8 ± 1.01*	10.831	0.003
CBIL (µmol/L)	1.6 ± 0.20*	1.9 ± 0.25*	2.8 ± 0.30*	3.8 ± 0.40*	33.555	0.001
AST (IU/L)	85.6 ± 2.10*	137.7 ± 9.50*	351.0 ± 8.18*	411.7 ± 30.13*	283.784	0.001
ALT (IU/L)	53.7 ± 1.52*	75.7 ± 5.13*	143.7 ± 5.51*	162.7 ± 5.03*	391.530	0.001
ALP (IU/L)	164.3 ± 1.53*	157.3± 15.27	221.3 ± 3.06*	258.3 ± 6.03*	98.815	0.001

Notes: TBIL–Total bilirubin, Conjugated bilirubin-CBIL, Aspartate aminotransferase-AST, Alanine aminotransferase-ALT, Alkaline phosphatase-ALP, SD–Standard deviation, Low dose-LD, Medium dose-MD, High dose-HD, Asterisks (*) – Shows statistical significance

The AST values (IU/L) increased significantly from 85.6 ± 2.10 in the control group l to 411.7 ± 30.13 in high-dose group (p=0.001). This increase in AST activity indicates liver toxicity. This enzyme catalyzes alpha-amino groups transfer from aspartate to alpha-keto group of ketoglutamic acid which forms oxalacetic acid. It functions in the Citric Acid Cycle. AST is a sensitive marker of liver toxicity. It is a cytosolic enzyme released into the plasma when there is an increase in membrane permeability or necrosis (27). Thus, increased AST activity in this study is an indication of acute liver toxicity. This is similar to the report by Hasti *et al.* (28). In this sub-chronic toxicity study, 400 and 800 mg/kg doses of *F. benjamina* leaves ethanol extract caused a significant increase in the serum aspartate aminotransferase activity ($p< 0.05$).

The ALT value (IU/L) increased significantly from 53.7 ± 1.52 in the control group to 162.7 ± 5.03 in high - dose group (p=0.001). This elevated ALT activity indicates liver damage, as ALT play key role in gluconeogenesis and catabolism of pyruvate and glutamate to produce adenosine triphosphate (ATP). It is more in the mitochondria than in the cytosol. High ALT activity is seen in reversible injury. An increase in this enzyme activity is equivalent to the number of hepatocytes damaged and leads to liver damage (26). This enzyme is released from hepatocytes due to increased membrane permeability, acute hepatitis, or necrosis. At a dose of 800 mg/kg body weight, a statistically significant increase in activity of alanine aminotransferase ($p< 0.05$) was also reported after 60 days by Hasti *et al.* (28).

The ALP (IU/L) increased significantly from 164.3 ± 1.53 in the control group to 258.3 ± 6.03 in the high dose (p=0.001). ALP is primarily located on the surface of the bile duct epithelium and transports metabolites across the cell membrane. It functions in dephosphorylation reactions and plays a major role in bile production (26). An increase in ALP activity in the liver indicates bile duct obstruction or Cholestasis (28). This cholestasis enhances the synthesis and release of more ALP as accumulated bile salts stimulate its release. The toxic products of metabolism from the phytochemicals notably alkaloids may be responsible for the observed effect on the enzyme levels (29). Singh and Sharma (30) stated that *F. benjamina* has the toxic secondary metabolites called pyrrolizidine alkaloids that are responsible for hepatotoxicity. The pathogenesis occurs when these pyrrolizidine alkaloids are metabolized by the liver's cytochrome p450 monooxygenases (CYP3A and CYP2B) enzymes through oxidation to form pyrrole esters or dehydropyrrolizidine alkaloids (DHPA).

The DHPA formed then binds to cellular proteins and DNA to form adducts leading to generation of reactive oxygen species in the cells. These cellular adducts enter the sinusoidal cells and hepatic vein to cause membrane damage leading to haemorrhage. They also enter into the hepatocytes and cause mitochondria dysfunction, autophagy, and necrosis leading to leakage of enzymes resulting in increased ALT and ALP. The assault also leads to a decrease in bile acid synthesis or disruption and induction of liver bile acid stasis. This cholestasis in turn leads to an increase in ALP levels (30,31). The link between these observations and the LD50 result is that although the extract has relatively low acute toxicity, it might cause hepatic damage on continuous administration beyond the safest dose of 500 mg/kg (25).

The fold change analysis showed a dose-depended fold increase in all biochemical parameters (Figure 1). Total bilirubin increased from 1.2-fold in LD to 2.0-fold in the HD group. Conjugated bilirubin increased from 1.2-fold in LD to 2.4-fold in the HD group. The highest fold increase was with AST which increased from 1.6-fold in the LD to 4.8-fold in the HD group. The second highest increase was with ALT from 1.4-fold in LD to 3.0-fold in the HD group. The least increase was with ALP from 0.9-fold in LD to 1.6-fold in the HD group. These results

indicate that the extract caused mild hepatotoxicity as the fold increases in all parameters were <5 fold as stated by Satué *et al.* (32).

The extract affected majorly hepatocyte function (causing hyperbilirubinemia) and integrity (increasing AST and ALT activities) more than cholestasis (increasing ALP activity). Conjugated hyperbilirubinemia is a sign that the liver has lost its excretory capacity and an increase in AST and ALT indicates hepatocyte membrane damage causing an increase in permeability (26,27,33). These dose-dependent increases in all serum liver biochemical parameters of total bilirubin, conjugated bilirubin, AST, ALT, and ALP are linked to the toxic metabolites found in most Ficus species including *F. benjamina* leaf. A study by Chukwudouro *et al.* (29), also linked the dose-dependent increase in the levels of liver biochemical parameters of 100, 150, and 250 mg/kg of *F. capensis* extract to its toxic metabolites. These metabolites are pyrrolizidine alkaloids found in Ficus species that have been implicated as dose-dependent hepatic toxins (30,31).

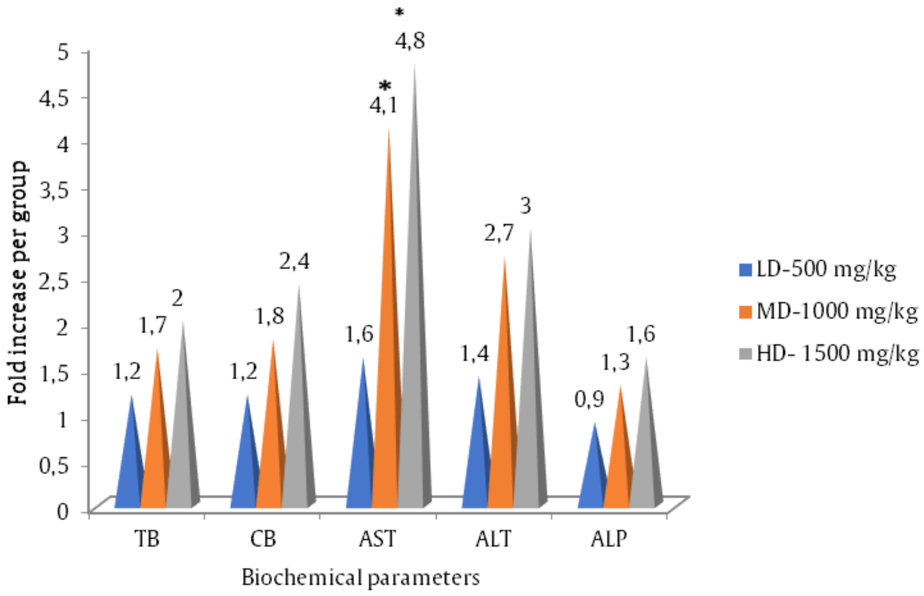

Figure 1. Fold increase in the biochemical parameters in the experimental groups

The histology of the liver showed that at a low dose, there was mild dilation of the sinusoid and at medium and high doses there was hepatocyte nuclei enlargement and marked hemorrhage with an occluded central vein. There was fibrosis around the central vein in both medium and high doses. The mild dilation of the sinusoid indicates the enlargement of the hepatic capillaries. The rats that were given doses of 1000 and 1500 mg/kg of the *F. benjamina* leaf extract showed marked hemorrhage in the central vein and sinusoids as well as hepatocytes arranged in a trabeculae pattern which is most common with non-neoplastic liver parenchyma changes called veno-occlusion disease or hepatic sinusoidal obstruction syndrome (29,31). This veno-occlusion disease is believed to occur when cellular adducts damage the sinusoidal and central vein membranes. The cellular adducts are the end products of metabolism when pyrrolizidine alkaloids are oxidized by cytochrome p450 enzymes to form pyrrole esters. The pyrrole esters then with cellular proteins to form adducts (30,31).

Enlargement and intense collagen staining around the portal vein were found in both medium and high doses in Figure 3. Collagen is a marker of fibrosis (34). This indicates that higher doses of *F. benjamina* extract were able to initiate hepatic fibrosis. This is similar to the work by Wang *et al.* (35) where intense collagen staining was reported in non-alcoholic liver fibrosis. In this study, fibrosis may contribute to hyperbilirubinemia and elevated AST, ALT, and ALP. This is backed by report from Eworo *et al.* (35) which stated elevated liver parameters in liver fibrosis caused by Hepatitis B virus infection. With the wide application of Ficus species as medicinal plants, the findings in this study differ from the report of hepato-protection from *F. exasperata* leaves by Adetuyi *et al.* (36). In this study, *F. exasperata* leaves did not result in increased biochemical parameters or disrupt liver histology in cases of acetaminophen-induced hepatotoxicity. Similarly, the use of *F. caprica* for the treatment of type I diabetes did not cause toxic effects to the liver but exhibited hepatoprotective effects (18). These findings are consistent with report by Hasti *et al.* (28) and Chukwudoruo *et al.* (29), that showed that ethanol extract of *F. benjamina* and *F. capensis* leaves respectively caused significant increases in the serum liver enzymes activities and may cause

hepatotoxicity. Hepatotoxicity is a direct consequence of cellular adducts formed from pyrrole esters after oxidation of pyrrolizidine alkaloids present in *F. benjamina* by cytochrome p450 enzymes (30,31).

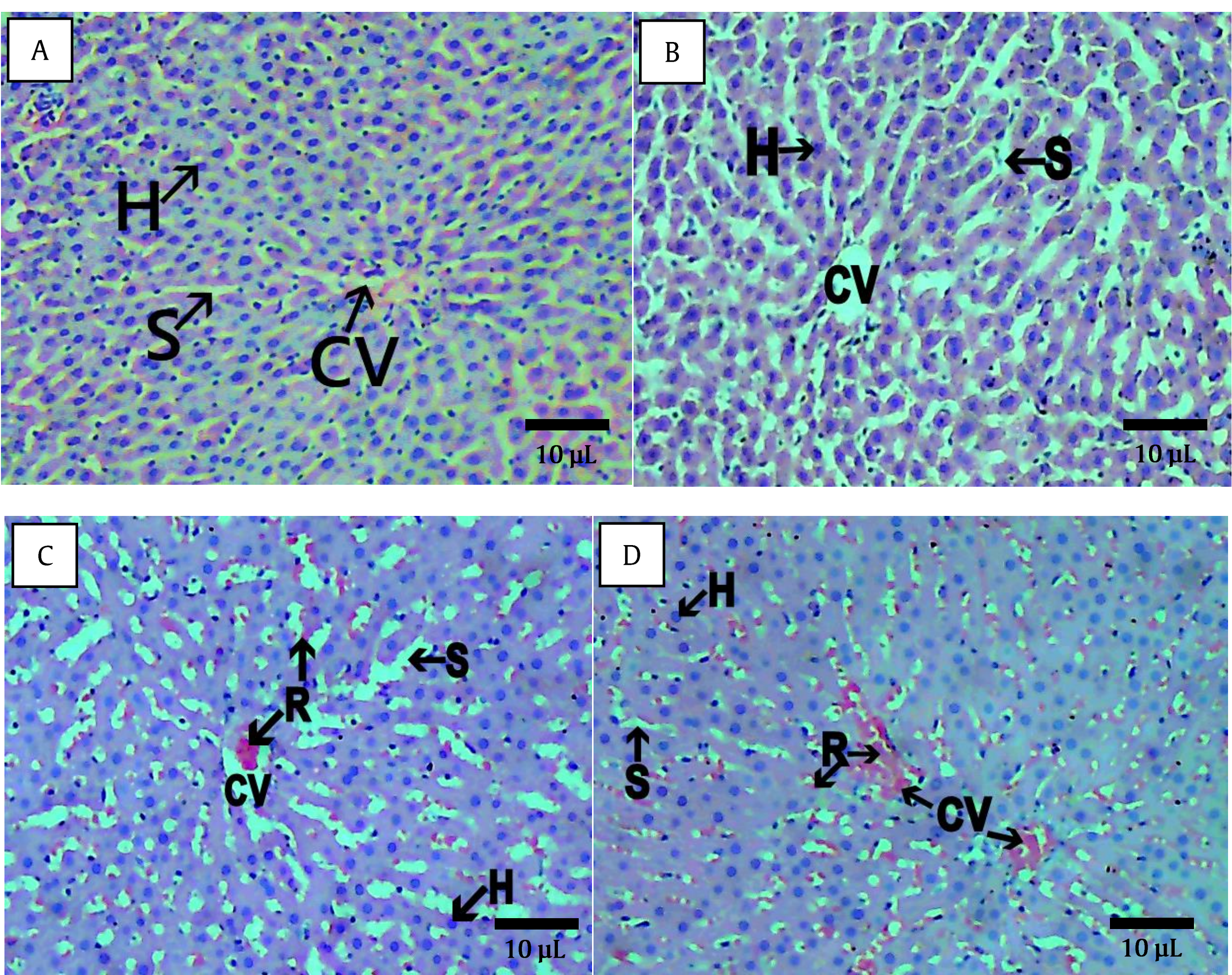

Figure 2. Liver of rats stained with H&E. (**a**) The rat liver of the control group (no treatment) shows normal hepatocyte (H), central vein (CV), and sinusoid (S); (**b**) In low dose group (500 mg/kg), the liver shows normal central vein (CV), mild dilation of sinusoid (S) and hepatocytes (H) arranged in a trabeculae pattern; (**c**) The rat liver in medium dose group (1000 mg/kg) shows central vein (CV) and mild dilation of sinusoid (S) with marked hemorrhage of red blood cells (R). The hepatocytes (H) are arranged in a trabeculae pattern; (**d**) The rat liver in the high-dose group (1500 mg/kg) shows two occluded central veins (CV) with hemorrhage (R). There is mild dilation of the sinusoid (S) filled with hemorrhage and hepatocyte (H) with an atrophic nucleus. H&E X100 magnification.

This hyperbilirubinemia accompanied by elevated activities of AST, ALT, and ALP activities, sinusoidal dilation, hemorrhage, and fibrosis in this study shows that the use of *F. benjamina* plant extract must be at dose ≤500 mg/kg. This is because it has the potential to cause liver toxicity and acute hepatic damage when used at higher doses as the pyrrolizidine alkaloids present cause dose-dependent liver toxicity (30). The strength of this study lies in its ability to contribute to knowledge by revealing the biochemical and histopathological changes that may result when *F. benjamina* is used at high doses of 1000 mg/kg and 1500 mg/kg. This study has reported that *F. benjamina* can cause hepatotoxicity characterized by hepatic sinusoidal obstruction syndrome when administered as a crude extract. The limitation of this study was the unavailability of antibodies to carry out enzyme histochemical staining of the liver tissues for AST, ALT, and ALP. Further study to isolate the alkaloids responsible for the hepatotoxic effects is recommended to ascertain its major role and mechanisms of pathogenesis.

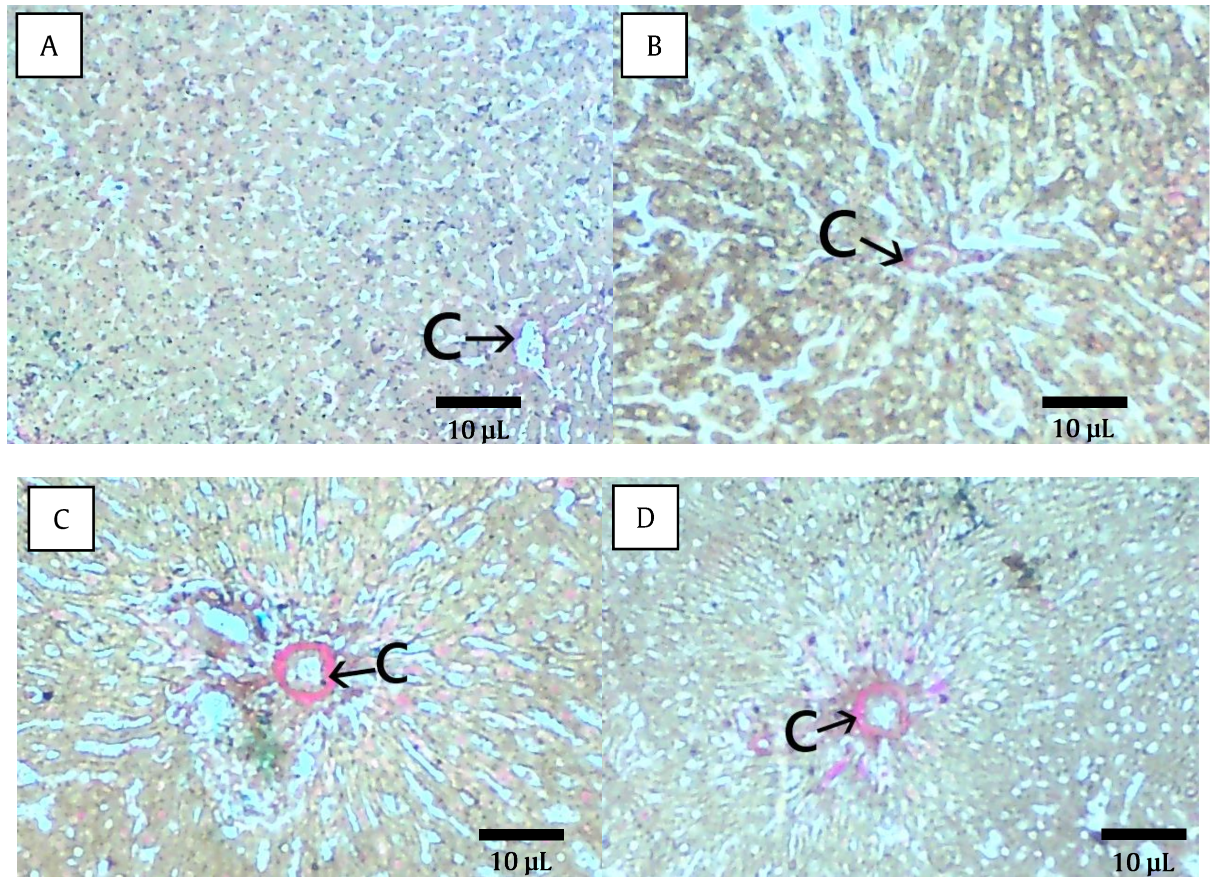

Figure 3. Liver of rats stained for collagen. (**a**) The liver of the rat in the control group (no treatment) had lightly stained collagen (C). (**b**) In the low-dose group (500 mg/kg), the liver showed lightly stained collagen (**c**) In the medium-dose group (1000 mg/kg), the liver shows intensely stained collagen; (**d**) In the high-dose group (1500 mg/kg), the liver showed intensely stained collagen in the portal triad. Masson trichrome X100 magnification.

4. CONCLUSIONS

F. benjamina is an important medicinal plant reported to have antioxidant and hepatoprotective properties. The acute toxicity and effects of crude ethanol extract of *F. benjamina* leaf on the integrity of the liver cells and the histological changes were investigated. The extract is non-toxic after LD_{50} testing and did not affect the mean body weight of the animals. However, the 1000 mg/kg and 1500 mg/kg extracts caused veno-occlusion disease or hepatic sinusoidal obstruction syndrome characterized by mild conjugated hyperbilirubinemia and concomitant mild elevated AST and ALT activities by affecting bilirubin excretion and increasing hepatocyte membrane permeability. Mild dilation of the sinusoid, marked haemorrhage, and fibrosis were observed at the end of the study. These findings indicate that the extract caused mild hepatotoxicity. A low dose of ≤500 mg/kg is suggested to be safer for medicinal and therapeutic purposes.

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Conflict of interest: There is no conflict of interest to disclose.

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