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(RESEARCH ARTICLE)

Hepatoprotective effect of the ethanol extract of *Detarium senegalense* stem bark in albino Wistar

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Abstract

Aim: The hepatoprotective effect of *Detarium senegalense* ethanol stem bark extract was evaluated against paracetamol induced hepatic damage in albino rats.

Material and methods: The ethanol stem bark extract of *D. senegalense* at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg were orally administered once daily for 3 days.The liver function tests and biochemical investigation such as asparte transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total bilirubin (TB) and direct bilirubin were carried out against paracetamol induced hepatotoxicity in rats. Phytochemical investigation was performed to find active constituents of the plant extracts by the different phytochemical tests.

Results: Pre-treatment with the ethanol stem bark extract significantly prevented the biochemical, histological, and changes induced by paracetamol in the liver. The extract showed significant hepatoprotective effects as evidenced by decreased serum enzyme activities such as AST, ALT,, ALP, TB and DB which was supported by histopatological assessment of the liver.The ethanol stem bark extract exhibited significant hepatoprotective activity comparable with silymarin, the standard drug as well as hepatotoxin agent, paracetamol. The phytochemical screening of the extract revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, cardiac glycosides, resins and balsam.

Conclusion: The results of this study strongly show that ethanol stem bark extract of *Detarium senegalense* possess a potent hepatotoxicity activity against paracetamol induced hepatic damage in rats.

Keywords: *Detarium senegalense*; Stem bark extract; Hepatotoxicity activity; Paracetamol; Rats

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1. Introduction

The liver is of vital importance in intermediary metabolism and in detoxification and elimination of toxic substances. The liver is often affected by a multitude of environmental pollutants and drugs, all of which place a burden on this vital organ and can damage and weaken it, eventually leading to diseases like hepatitis or cirrhosis [1]. Paracetamols hepatotoxicity is caused by its reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which causes oxidative stress and glutathione (GSH) depletion. Paracetamol toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P450 [2]. Introduction of cytochrome or depletion of hepatic glutathione is a prerequisite for paracetamol-induced hepatotoxicity [3, 4, 5]. In spite of tremendous strides in modern medicine, the treatment of liver disorders is inadequate and many formulations containing herbal extracts are used for regeneration of hepatic cells and for protection of the liver against damage [6] Hepatic damage is associated with distortion of its metabolic functions and it is still a major health problem [7] . Unfortunately many synthetic drugs used in the treatment of liver diseases are inadequate and also cause serious side effects [8]. In view of severe undesirable side effects of synthetic agents, there is growing interest in evaluating traditional herbal medicines that are claimed to possess hepatoprotective activity. A single drug cannot be effective for all types of severe liver diseases. Therefore, an effective formulation using indigenous medicinal plants has to be developed with proper pharmacological experiments and clinical trials [9].

Medicinal plants have important contributions in the healthcare system. Use of herbal medicines represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. A number of modern drugs currently in use have been obtained through medicinal plants. Despite the profound therapeutic advantages possessed by some of the medicinal plants, some constituents of medicinal plants have been found to be potentially toxic, mutagenic, carcinogenic and teratogenic. This raises concern about the potential toxic effects of some organs such as the liver, kidneys resulting from the short-term and long-term use of such medicinal plants. Therefore, evaluating the toxicity effects of any medicinal plants extracts intended to be used in humans and animals is of greatest significance.

The medicinal plant *Detarium senegalense* (Family Fabaceae), is used in folk medicine as hypoglycemic, antirhematic, antihelmintic, antiepileptic, antipyretic. It is also used in treating intestinal and hepatic diseases. This plant contains various active principles able to inhibit the growth of mycobacteria. Previous pharmacological studies revealed that extract of *D. senegalense* was found to possess antidiarrhoeal activity [10], antimicrobial activity [11, 12], and antiproliferativ activity [13], anthelminthic [14], antiepileptic [15] and antihyperglycemia [16]. The present study investigates the hepatotoxicity potential of ethanol stem bark extract of *Detarium senegalense* against paracetamol induced hepatotoxicity in albino rats.

2. Material and methods

2.1. Plant Material Collection and Authentication

The stem bark of *D. senegalense* was sourced from Chaza, Niger State, by an ethnobotanist, Mallam Muazam in the department of Medicinal plants and traditional medicine (NIPRD, Abuja). The plant was identified and authenticated in the same department by a taxonomist.

2.2. Preparation of Plant and Extraction Procedure

The stem bark of *D. senegalense* was cleaned, cut into pieces and dried under room temperature. The dried leaves were ground into powder with the aid of pestle and mortar and sieved to obtain fine materials. Thereafter, 450 g of the powdered material was soaked in 1.5 litre of ethanol for 48 hours using maceration method. The solution was filtered through Whatmann (No. 25) filter paper into a conical flask. The filtrate was then concentrated to dryness on a water bath set at temperature of 40°C.

2.3. Experimental Animals

One hundred and two (102) mature Swiss mice of both male and female gender weighing between 18-25 g were used for this study. They were sourced from Animal House of the Department of Veterinary Medicine, University of Nigeria, Nsukka. The mice were kept in plastic cages and allowed to acclimatize in laboratory environment for 14 days. During the period of study, animals were given pellets (Guinea Feeds, Plc Nigeria) and provided with clean water ad libitum.

2.4. Phytochemical analysis

The method as described by Inyang et al.,[17], Aziz, [18) was adopted for the phytochemical analysis of the ethanol extract of *D. senegalense* stem bark. The metabolites that were assayed include tannins, saponins, alkaloids, flavonoids, terpenoids, steroids, anthraquinones, glycosides, reducing sugars and resins.

2.5. Acute Toxicity Test

This was determined following the method described by Lorke [19]. The study was carried out in two phases. In the first phase, nine rats were divided into three groups of three rats each. They were given 10 mg/kg, 100 mg/kg and 1000 mg/kg of the tem bark extract respectively. They were then monitored for signs of toxicity initially for first 4 hours, and then for 24 hours. The signs of toxicity that were looked out for include hyperactivity, paw licking, respiratory distress, and mortality. At the end of the first phase, there was no mortality. The study then proceeded to the second phase. In this phase, three rats were grouped into three with one rat in each group, and given 1600 mg/kg, 2900 mg/kg and 5000 mg/kg of the extract respectively, and then monitored for signs of toxicity as stated earlier. The animals were further monitored for 48 and 72 hours for signs of late toxicity.

2.6. Experimental Design

The method as described by Aneje et al, [20] with slight modification was used for this study. Forty-eight (48) albino rats weighing between 150-220 g were grouped into six with 8 rats in each cage. Group1: This group served as normal control which received 10 mL/kg distilled water once daily for 3 days. Group II: Rats in this group received paracetamol 3 g/kg as a single dose on day

3. Group III which served as a reference control received 25 mg/kg silymarin once daily for 3 days also. Group IV – VI were administered graded doses (100 mg/kg, 200 mg/kg and 400 mg/kg) of *D. senegalense* stem bark ethanol extract for 3 days, respectively. All were orally administered. Thirty minutes after last dosing, all animals in groups 3-6 received 3g/kg of paracetamol as a single dose.

2.7. Collection of blood samples

Forty-eight hours after, all animals (rats) were anaesthetized with halothane and blood sample were collected via retro-orbital plexus into lithium heparin sample container for assessment of liver function and biochemical analysis. The blood samples were separated by centrifugation at 2000 rpm for 10 min and subjected to biochemical investigation such as Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), Total bilirubin (TB) and Direct bilirubin (DB) [21, 22]..

2.8. Histopathological studies

One animal from each group was utilized for histopathological study. The livers were fixed in 10% formalin for 24 h. The formalin fixed liver stained with haematoxylin-eosin for microscopic observation.

2.9. Statistical analysis

Results were expressed as Mean ± SD. Statistical analysis of the data was done using one-way analysis of variance (ANOVA) followed by Dunnett's test and significance determined by using $p<0.05$.

3. Results

3.1. Phytochemical analysis

It is important to know the chemical nature of plant products when their pharmacological responses are screened [17]. Phytochemical evaluation of D. senegalense extract showed the presence of the following secondary metabolites; alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, cardiac glycosides, resins and balsam.

3.2. Acute toxicity test

There were no lethality or toxic reactions observed at any of the doses administered. All the rats were healthy and active during and after the period of study. Hence, oral acute toxicity result was greater than 5000 mg/kg in rats.

3.3. Biochemical analysis

The ethanol extract of D. senegalense showed a significant decrease in the level of biochemical parameter ALP (Alkaline phosphatase) in group D, E, and F compared to group B. These reductions were comparable to the normal control group as well as the hepatotxin group (paracetamol). The effect of the extract and standard drug was also seen in other groups but was not significant enough, as seen in table 4.7

3.4. Histopathological effects

The histopathology profile of the rats liver in Normal control group 1 revealed normal hepatic tissue with well outlined hepatocyte (H), portal traid (PT) and central vain (CV). Photomicrograph of paracetamol group 2 section of liver induced with paracetamol shows severe degeneration with severe intra hepatic inflammation (IHI) replacing the normal liver parachyma and congestion of the blood vessel (CBV) (cirrhosis and chronic hepatitis), severe portal inflammation (PI),fatty necrosis (FN) (chronic portal hepatitis),intra hepatic hemorrhage (IHH) and infilteration of inflammatory cell (IIC) (hemorrhagic liver). Photomicrograph of group 3 section of a liver administered with silymarin and paracetamol shows moderate protection with mild fatty changes (FC) ,mild protection with moderate intra hepatic inflammation(IHI) and moserate pyknotic hepatocyte(PH), mild portal inflammation(PI) with well enhanced active hepatocyte (H). photomicrograph of group 4 section of a liver administered with paracetamol and low dose of *Detarium senegalense* extract shows moderate protection with mild intra hepatic hemorrhage (IHH), necrosis (N) and pyknotic hepatocyte(PH) with moderately enhanced active hepatocyte (H) .

Photomicrograph of group 5 of a liver administered with paracetamol and medium dose of *Detarium senegalense* extract shows moderate protection with moderate intra hepatic hemorrhage (IHH), mild fatty change (FC) and mild intra hepatic inflammation(IH) with moderate increased number of active hepatocyte (H).

Photomicrograph of group 6 of a liver administered with paracetamol and high dose of *Detarium senegalense* extract shows high protection with moderate intra hepatic fibrosis (IHF), mild intra hepatic inflammation (IHI) and mild intra hepatic hemorrhage (IHH) with highly increased number of well enhanced active hepatocyte (H).

Table 1 Effect of ethanol extract of *D. senegalense stem* bark on liver function and biochemical parameters in paracetamol induced hepatotoxicity in rats

Groups	Treatment	Dose (mg/kg)	TB(Mg/dl)	DB(Mg/dl)	AST(U/L)	ALT(U/L)	ALP(U/L)
A	Distilled water	(10 mL/kg)	79.63±13.42	10.50±1.13	6.13±0.72	4.50±0.38	113.75±6.18
B	Paracetamol (hepatotoxin)	3000	39.50±7.57	7.00±1.15	3.88±0.44	5.75±0.84	121.13±4.61
C	Silymarin	25	75.25±18.31	13.50±2.61	4.38±0.63	5.50±1.32	123.63±7.49
D	*D.Senegalense*	100	27.25±2.83	8.38±1.66	5.88±0.64	5.38±0.65	66.50±2.97**
E	*D.Senegalense*	200	26.50±4.95	8.13±1.78	3.50±0.33	5.75±1.82	70.63±4.12**
F	*D.Senegalense*	400	26.50±4.95	13±1.78	3.50±0.33	5.75±1.82	70.63±4.12**

Data is expressed as mean ± SEM, n=6, One way ANOVA followed by Dunnett's test Compared with normal control; *p< 0.05; ** p< 0.01.

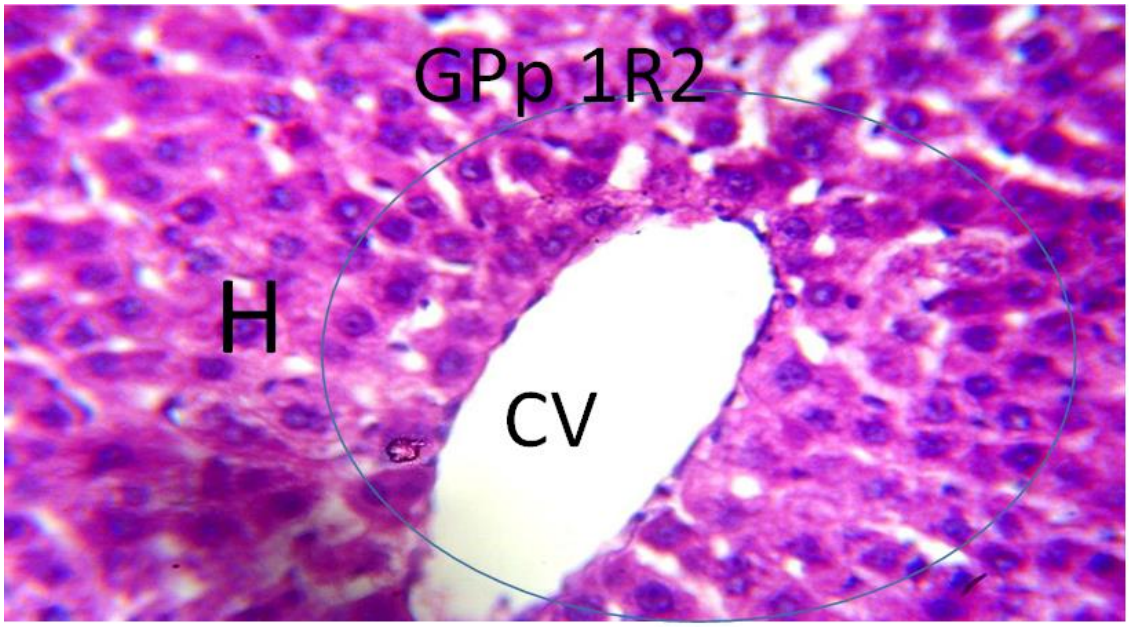

Figure 1 Photomicrograph of group GP1r1 section of liver (x400) (H/E) shows normal hepatic tissue with well outlined active hepatocyte (H) and central vain (CV)

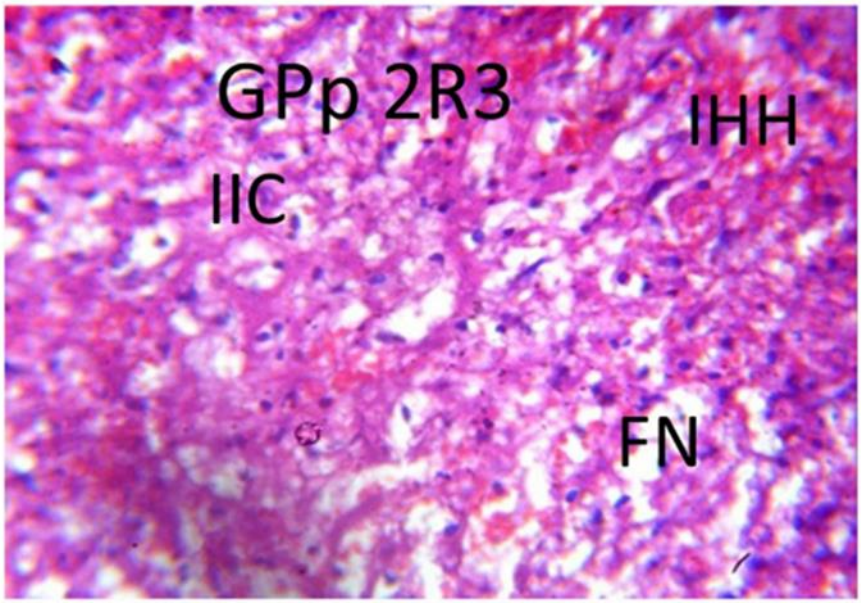

Figure 2 Photomicrograph of group 2r3 section of liver induced with paracetamol (x400) (H/E) shows severe degeneration with severe intra hepatic hemorrhage (IHH). Infiteration of inflammatory cell (IIC) and severe fatty necrosis (FN) the overall feature are consistent with (HEMORRHAGIC LIVER)

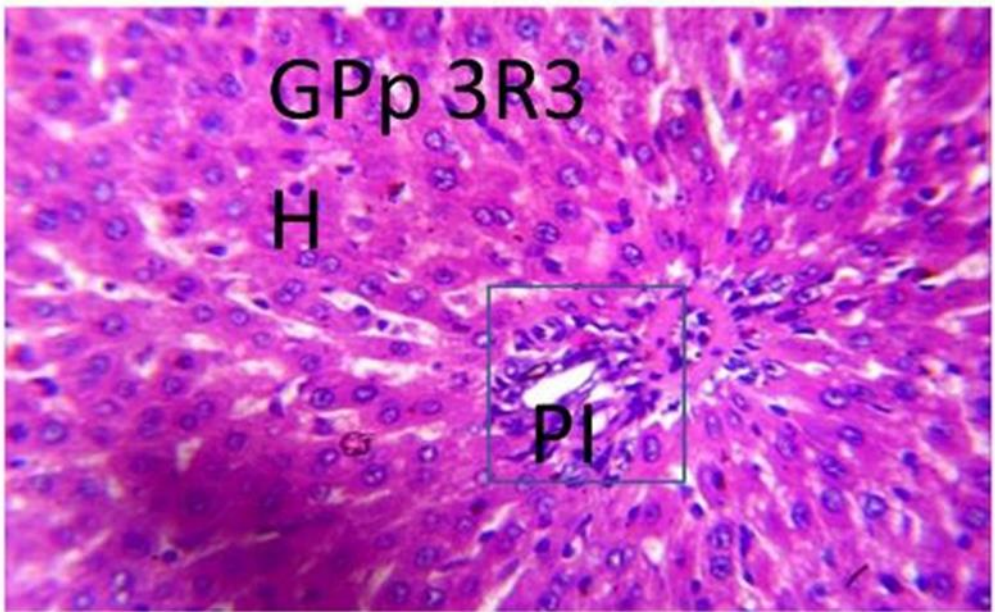

Figure 3 Photomicrograph of group 3r3 section of liver administered silmarin drug and paracetamol (x400) (H/E) shows moderate protection with mild portal inflammation (PI) with well increased active hepatocyte (H)

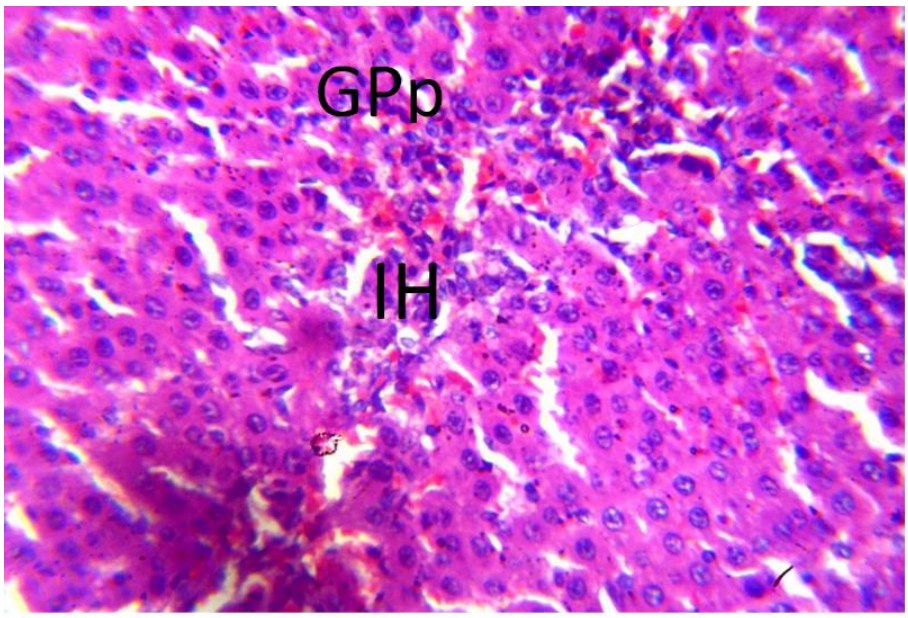

Figure 4 Photomicrograph of group 4R1 section of liver administered with paracetamol drug and 100mg/kg dose extract of D.Senegalense (x400) (H/E) shows mild protection with mild intra hepatic hemorrhage (IHH) with mild enhanced active hepatocyte (H)

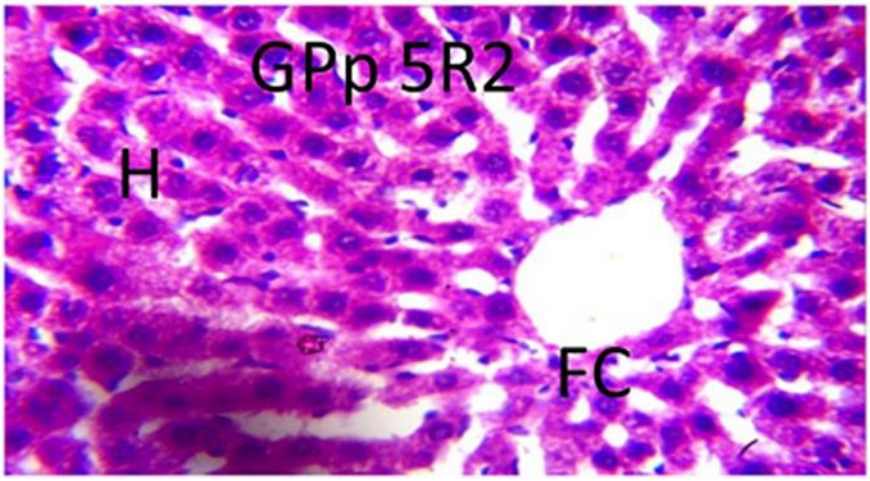

Figure 4 Photomicrograph of group 5R2 section of liver administered with paracetamol drug and 200mg/kg dose extract of D.Senegalense (x400) (H/E) shows moderate protection with mild fatty change (FC) and moderate increase number of active hepatocyte (H)

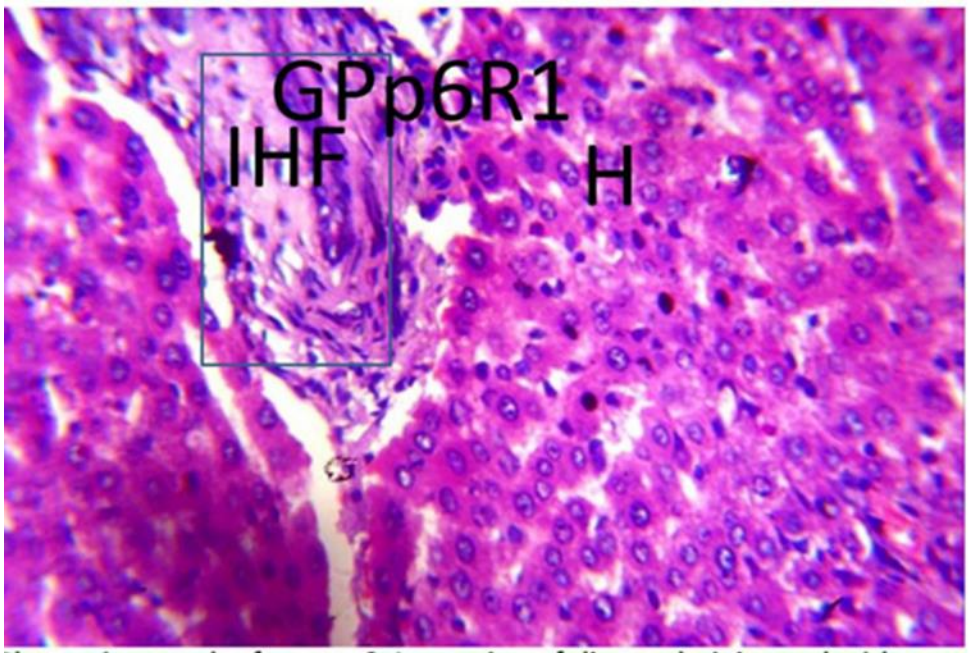

Figure 5 Photomicrograph of group 6r1 section of liver administered with paracetamol drug and 400mg/kg dose extract of D.Senegalense (x400) (H/E) shows high protection with intra hepatic fibrosis (IHF) and high increase number of active hepatocyte (H)

4. Discussion

The study was designed to evaluate the hepatoprotective effect of ethanol extract of *Detarium senegalense* stem bark on the liver of albino rats following paracetamol induced hepatic damage.

The liver injuries induced by paracetamol is one of the characterized system of xenobiotic-induced hepatotoxicity and it is a commonly used model for the screening of hepatoprotective activities of drugs [23]. Hepatotoxic drugs, like paracetamol, are known to cause marked elevation in serum level of enzymes, such as AST, ALT, ALP and bilirubin, showing significant hepatocellular injury. Increased activity of serum transaminases in intoxicated rats as seen in the

present study, can be attributed to the damaged structural integrity of the liver because these are cytoplasmic in nature and are released into the circulation after cellular damage. Decrease in total serum protein was seen in rats treated with paracetamol and may be associated with the decrease in the number of hepatocytes, which in turn may result in the decreased hepatic capacity to produce protein and consequently reduce liver weight. Silymarin is a polyphenolic flavonoid isolated from the fruit and seeds of the milk thistle (*Silybum marianum*) [24]. Various studies have shown that silymarin exhibits strong antioxidant activity [24], and also show protective effects against hepatic toxicity induced by so many agents by inhibiting lipid peroxidation. [25, 26]

In this present study it was seen that there was a significant decrease in the level of ALP enzyme (alkaline phosphates) in animals in group D, E, and F treated with paracetamol and ethanol extract of *Detarium senegalense* stem bark compared to animals in group B that were administered with paracetamol. The effect of the extract of *D. senegalense* and silymarin drug were also seen in the other animals in the other groups but were not significant enough. These parameters were maintained at normal levels in the *D. senegalense* treated animals. *D. senegalense* treatment showed a protection against the injurious effects of paracetamol that may result from the interference with cytochrome p450, resulting in the hindrance of the formation of hepatoxic free radicals [27]. The elevated level of these marker enzymes observed in the group 2 paracetamol treated rats in this study corresponded to the extensive liver damage induced by toxin. The reduced concentrations of ALP as a result of plant extract administration observed during the present study might probably be in part, due to the presence of Alkaloid and Flavonoids in the extract [28]. The tendency of these marker enzymes, to return to near normalcy would be owing to the anti-hepatoxic effect of extract of *D.senegalense* stem bark. These results were found comparable to silymarin.

Histopathologic studies also supported the evidence of biochemical analysis. Histological examination of rat liver treated with paracetamol shows significant hepatoxicity characterized by severe intra hepatic inflammation and congestion of the blood vessel (cirrhosis and chronic hepatitis), severe portal inflammation (PI), fatty necrosis (FN) (chronic portal hepatitis), intra hepatic hemorrhage (IHH), infiteration of inflammatory cell (IIC) (hemorrhagic liver) and increased in number of Kupffer cells around the central vein and loss of cellular boundaries.

However, in animals treated with ethanol extract of *D. senegalense* stem bark (100 mg/kg, 200 mg/kg and 400 mg/kg) revealed that the severity of hepatic damage was decreased when compared with the hepatic damage observed in animals administered with paracetamol. Extract of *D. senegalense* stem bark reduced the hypertrophy of hepatocytes and the number of neutrophils in the liver were also seen to have increased significantly, which further indicate it's significant hepatoprotective effect.

5. Conclusion

in conclusion, the extract of *D. senegalense* stem bark offered protection from paracetamol- induced liver damage. The protection against liver damage by the extract of *D. senegalense* stem bark were found comparable to silymarin. The possible mechanism responsible for the protection of paracetamol-induced liver damage by the extract of *D. senegalense* stem bark mighty be that it could act as a free radical scavenger intercepting the radicals involved in paracetamol metabolism by microsomal enzymes. By trapping oxygen-related free radicals, the extract could stop their interaction with polyunsaturated fatty acids and would destroy the enhancement of lipid peroxidative processes. It is well known that flavonoids and glycosides are strong antioxidants. Antioxidants principles from medicinal plants are multifaceted in their effects and provide so many ways in correcting the imbalance through regular intake of a proper diet. Thus, from this present work, it were observed that the extract of *D.Senegalense* stem bark is a promising hepatoprotective agent and this hepatoprotective activity of the extract of *D. senegalense* stem bark may be due to the antioxidant chemicals present in it.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There is no conflict of interest associated with the authors of this paper.

Statement of ethical approval

The study protocol was carried out as per the rules and regulations of the Institutional Animal Ethical Committee, Faculty of Basic Medical Sciences**,** Alex Ekwueme Federal University Ndufu Alike Ebonyi state Nigeria, as well the National Institute of Health Guide for the care and use of Laboratory Animals.

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