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EVALUATION OF HEPATOPROTECTIVE EFFECTS OF *Setaria megaphylla* (STEUD) T. DUR AND SPHINZ (POACEAE) ROOT EXTRACT ON PARACETAMOL-INDUCED INJURY IN RATS.

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ABSTRACT

Background*:* *Setaria megaphylla* (Steud) T. Dur and Schinz (Poaceae), is a popular medicinal plant used by the indigenes of Niger Delta Nigeria to treat malaria, hemorrhoids, urethritis, inflammation, diabetes, fevers and various pains (Udobang, Okokon and Etuk, 2016).[1] The locals use this plant a lot treat various ailments, so it becomes necessary to investigate the potential toxic or protective effect of *Setaria megaphylla* on the liver. **Objectives**: This work was therefore designed to investigate the hepatoprotective effects of *Setaria megaphylla* ethanol root extract. **Methodology**: *Setaria megaphylla* ethanol root extract (150, 300, 450 mg/kg) was investigated for its biochemical and histological effects in rats liver using standard procedures. **Results:** There was decrease in weight of liver, reductions in liver enzyme levels and in total and conjugated billrubin in animals pretreated with the extract (150 - 450 mg/kg) when compared to the paracetamol group, and significant ($p < 0.05$ - 0.001) increases in the levels of protein and albumin. **Conclusions:** The findings of this study revealed that *S. megaphylla* ethanol root extract possesses hepatoprotective effect.

KEYWORDS: hepatoprotective, *Setaria megaphylla*, medicinal plant.

1.0 INTRODUCTION

Setaria megaphylla is a perennial broad-leafed bristle grass, with robust roots 30 cm diameter at the base (Bromilow, 1995).[2] Its leaves are soft, bluish grey green in colour and 1 m long and 10 cm broad. Its edges are glabrous and scarbrid with compressed and more or less keeled leaf sheaths (Bromilow, 1995).[2] It is usually found besides rivers in low lying areas or forests and in areas with a lot of moisture, like tropical and subtropical areas of Africa, America (Van Oudtshoorn, 1999).[3]

A leaf-decoction is sedative on cough, and is also indicated for oedema (Burkill, 1985)[4]. Ijo in South East Nigeria rub leaves crushed with salt on the forehead for headache, and squeeze the sap on to a sore after it has been cleaned. The grass has a reputation for beneficial action on urino-genital troubles. Pressed juice of *Setaria megaphylla* leaves is used to treat anuria. The plant has anodynal and analgesic properties. Zulus in South Africa apply crushed leaves to bruises. In Republic of the Congo, sap is massaged into areas of pain. For more vigorous action the affected part may be scarified by rubbing with the rough leaf, and ash of the calcined plant applied (Burkill, 1985).[4]

2.0 MATERIALS

2.1 Collection and Identification of Plant Sample

Setaria megaphylla roots were collected from Anwa forest in Uruan, Uruan Local Government Area of Akwa Ibom State, Nigeria. It was identified and authenticated in the Department of Botany and Ecological Studies, University of Uyo and a voucher specimen (FPHUU 221) deposited in the Faculty of Pharmacy Herbarium, University of Uyo, Nigeria.

2.2 Animal Stock

Adult Swiss albino rats were obtained from the Animal House of the University of Uyo, Uyo, Akwa Ibom State, Nigeria and were maintained in the University of Uyo Animal House and fed with growers pellet feed with water given *ad libitum.* Permission for animal studies was obtained from the Animal Ethics Committee of the College of Health Sciences, University of Uyo, Uyo, Nigeria..

3.0 METHODOLOGY

A total of 36 adult Swiss albino rats of both sexes were weighed and divided into six groups of 6 animals each and treated as follows: Groups A consisted of normal animals that were administered with distilled water (0.2 ml/kg), Group B was administered with vehicle control

(distilled water, 0.2 ml/kg), while groups C, D and E were respectively administered p.o with 150, 300 and 450 mg/kg of *S. megaphylla* extract daily for eight days. Group F was treated with standard drug silymarin (100 mg/kg) also for eight days. Paracetamol, 2 g/kg, was administered to groups B – F on the eighth day. Twenty-four hours after paracetamol administration, the animals were sacrificed under light diethyl ether vapor. Blood was collected by cardiac puncture into sterile centrifuge tubes and centrifuged immediately at 2500 rpm for fifteen minutes to separate the serum at room temperature to avoid hemolysis. This was used to assess for effects of the extract on liver biochemical parameters. Total protein, albumin, total bilirubin, conjugated bilirubin, Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline phosphatase (ALP) and total cholesterol were used to assess liver function. The analyses were done using various diagnostic kits such as Randox Laboratory kits, Dialab diagnostic kits, HUMAN diagnostic kits and TECO analytical kits. The livers of the animals were fixed in 10% formaldehyde, processed, sectioned and stained with heamatoxylin and eosin (H & E) according to standard procedures. Histopathology and chemical pathology were respectively conducted at Departments of Pathology and Chemical Pathology, University of Uyo Teaching Hospital, Nigeria.

4.0 RESULTS

4.1 Effect of Extract on the Weight of Liver.

The liver weights of rats treated with paracetamol were significantly ($p < 0\text{-}05$) increased when compared to the control group, while those treated with the extract (150 - 450 mg/kg) and silymarin showed significant ($p < 0.05$ - 0.001) decreases in weights when compared to the paracetamol and control groups (Table 1).

4.2 Effect of Extract on Liver Function Test in Paracetamol-induced Hepatotoxicity in Rats.

Administration of paracetamol 2 g/kg produced significant ($p < 0\text{-}05$ - 0.001) increases in the levels of total and conjugated bilirubin, AST, ALT and ALP and decreases in protein and albumin when compared to the control group (Table 2). There were significant ($p < 0.05$ - 0.001) reductions in these enzyme levels and in total and conjugated bilirubin in animals pretreated with the extract (150 -450 mg/kg) when compared to the paracetamol group. Also there were significant ($p < 0.05$ - 0.001) increases in the levels of protein and albumin when compared to the paracetamol group. However there was no effect on the level of total

cholesterol. The various effects of the extract were not comparable to that of the standard drug silymarin 100 mg/kg.

4.3 Histology

Liver: The livers of rats in the paracetamol (2 g/kg) treated groups revealed the presence of focal necrosis which showed deleterious hepatotoxic tissue effect. Livers of rats in groups pretreated with extract and silymarin showed congestion and thrombosis of portal tract blood vessels extending into the sinusoids and central veins, with moderate to severe inflammation including the periportal area, but no necrosis suggesting hepatoprotective effects. Rats in control group revealed mild portal inflammation and congestion, showing negligible hepatotoxic effect as evidenced by absence of steatosis (fatty changes), necrosis and fibrosis (Plates I to VI). Besides the paracetamol group which showed hepatotoxicity involving the hepatic parenchymal tissue, the presence of thrombosis in extract and silymarin treated groups is suggestive of mild to moderate vasculogenic damage otherwise referred as acute to subacute extra-hepatic (vascular) injury. This means that the extract and silymarin showed some hepatoprotective effect.

Table 1: Effect of extract on weight of livers of rats with paracetamol-induced hepatotoxicity.

Treatments mg/kg	Weight of Liver(g)
Distilled water	7.29 ± 0.19
Distilled water/ Paracetamol	8.34 ± 1.13[a]
Extract 150	6.16 ± 0.17[af]
Extract 300	6.66 ± 0.46[f]
Extract 450	6.97 ± 0.31[e]
Silymarin 100	6.79 ± 0.26[f]

Data are expressed as mean ± SEM. Significant at [a]$p < 0.05$, when compared to control, [e]$p < 0.01$, [f]$p < 0.001$ when compared to paracetamol, n = 6.

Table 2: Effect of extract on indices of liver function with paracetamol-induced hepatotoxicity in rats.

Treatment mg/kg	Total bilirubin	Conjugated bilirubin	AST	ALT	ALP	Total cholesterol	Protein	Albumin
Distilled water	13.02 ± 0.34	6.90 ± 0.25	111.0 ± 4.05	45.33 ± 4.39	147.33 ± 12.66	1.70 ±0.19	54.6 ±.70	45.83± 2.96
Distilled water /Paracetamol	19.01 ± 0.54^{c}	9.46 ± 0.70^{a}	152.83 ±7.34^{c}	74.50 ± 5.73^{c}	388.3 ± 12.30^{c}	3.33± 0.87^{b}	40.0 ± 1.20^{c}	36.83± 1.16^{b}
Extract 150	14.53 ± 0.73^{e}	6.86 ± 0.55^{d}	112.16 ± 4.24^{f}	44.5 ± 2.11^{f}	182.62 ±79.54^{e}	2.26 ± 0.17	50.16 ± 2.3^{f}	43.33 ± 0.09^{d}
Extract 300	14.11 ± 1.26^{e}	7.03 ± 0.41^{d}	122.33 ± 3.34^{d}	42.5 ± 2.94^{f}	234.65 ±24.20^{d}	1.91 0.19^{b}	55.0 ± 0.02^{f}	43.66 ± 0.49^{d}
Extract 450	14.18 ± 1.00^{e}	6.86 ± 0.50^{d}	118.0 ± 4.02^{e}	46.16 ± 2.04^{f}	173 ± 12.30^{e}	1.70 0.15c	53.16 0.30^{f}	44.33 ± 0.80^{e}
Silymarin 100	14.51 ± 0.73^{e}	6.01 ± 0.50^{f}	122.16 ± 9.58^{d}	42.66 ± 3.31^{f}	189.43 ±14.28^{e}	1.71 ± 0.09^{c}	53.59 ±0.23^{f}	41.5 ± 0.99

Data are expressed as mean ± SEM. significant at $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ when compared to control, $^{d}p < 0.05$, $^{e}p < 0.01$, $^{f}p < 0.001$ when compared to paracetamol, n = 6.

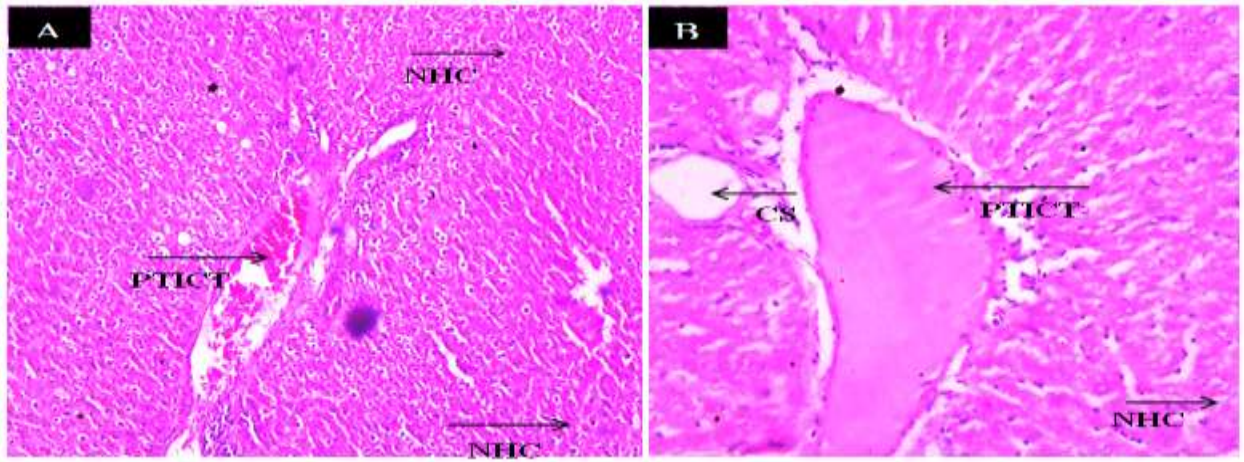

Plate I: Photomicrograph of rat's liver administered with distilled water showed normal architecture with normal hepatocytes (NHC), congested sinusoids (CS) and portal tract with inflammation, congestion and thrombosis (PTICT) H & E x 10 (A) and 40 (B) magnification.

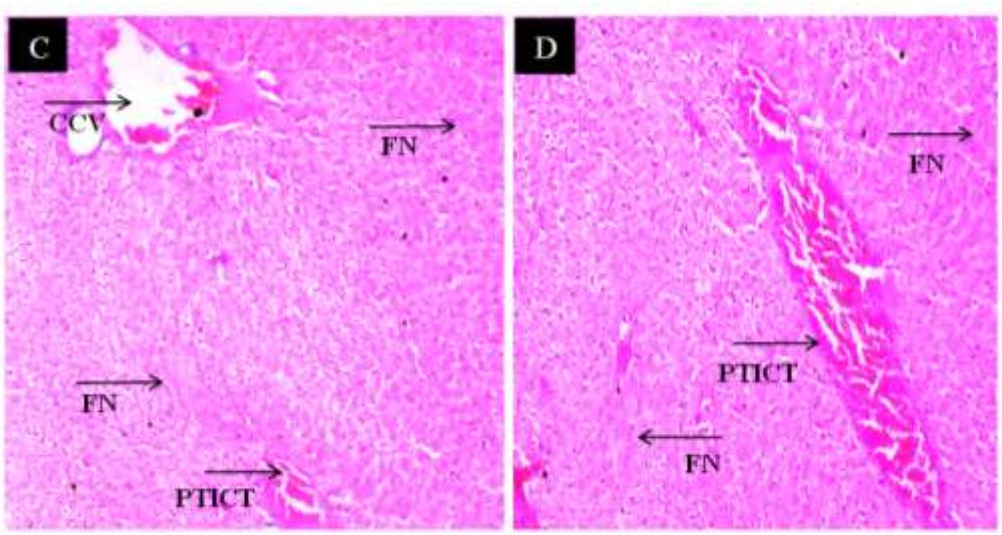

Plate II: Photomicrograph of rat's liver administered with distilled water and paracetamol, (2 g/kg), showed slightly distorted architecture with focal necrosis (FN) and portal tract with inflammation, congestion and thrombosis (PTICT) as well as congested central vein (CCV), H & Ex 10 (C) and) x 40 (D) magnification.

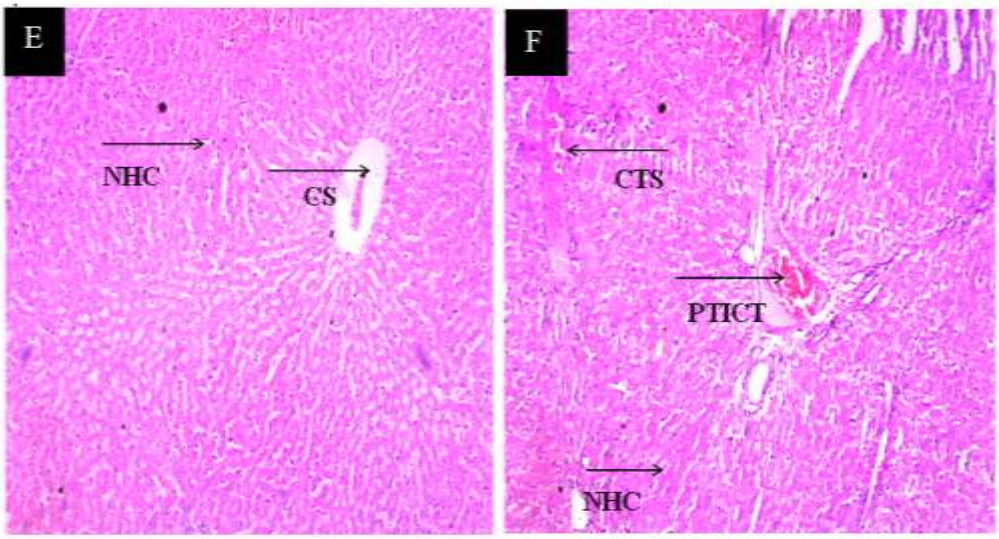

Plate III: Photomicrograph of rat's liver administered with extract,(150 mg/kg), showed normal architecture with normal hepatocytes (NHC), congested sinusoids (CS) and congested to thrombosed sinusoids (CTS) and portal tract with inflammation, congestion and thrombosis (PTICT), H & E x 10 (E) and x 40 (F) magnification.

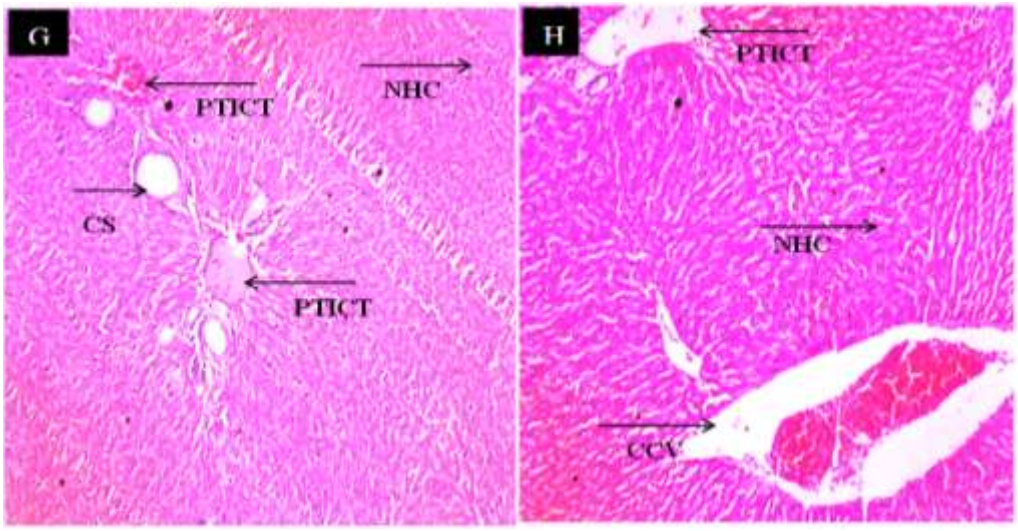

Plate IV: Photomicrograph of rat's liver administered with extract, (300 mg/kg), showed normal architecture with normal hepatocytes (NHC) and portal tract with inflammation, congestion and thrombosis (PTICT) with congested central vein (CCV), H & E x 10 (G) and 40 (H) magnification.

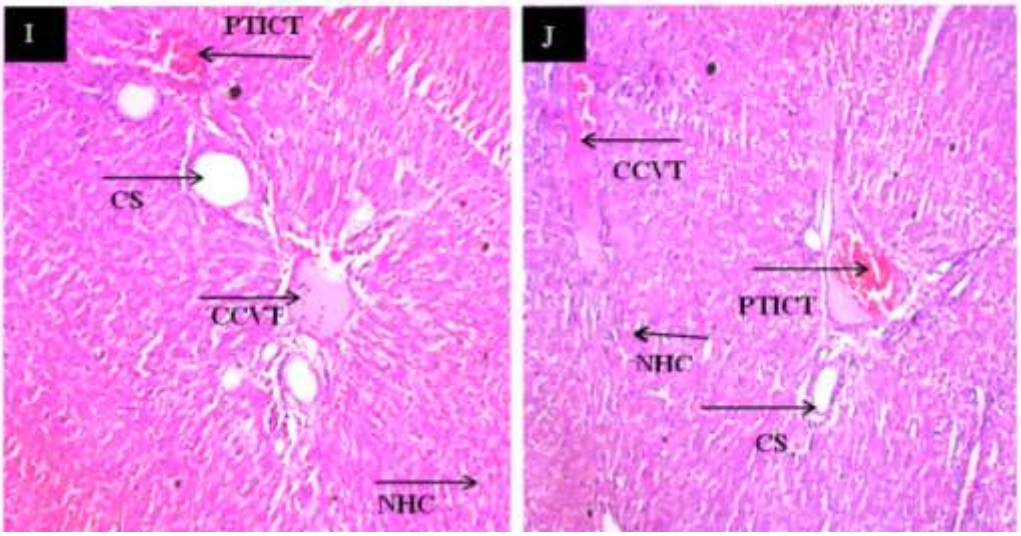

Plate V: Photomicrograph of rat's liver administered with extract, (450 mg/kg), showed normal architecture with normal hepatocytes (NHC), and portal tract with inflammation, congestion and thrombosis (PTICT) as well as congested central vein with thrombosis (CCVT) and congested sinusoid (CS), H & E x 10 (I) and 40 (J) magnification.

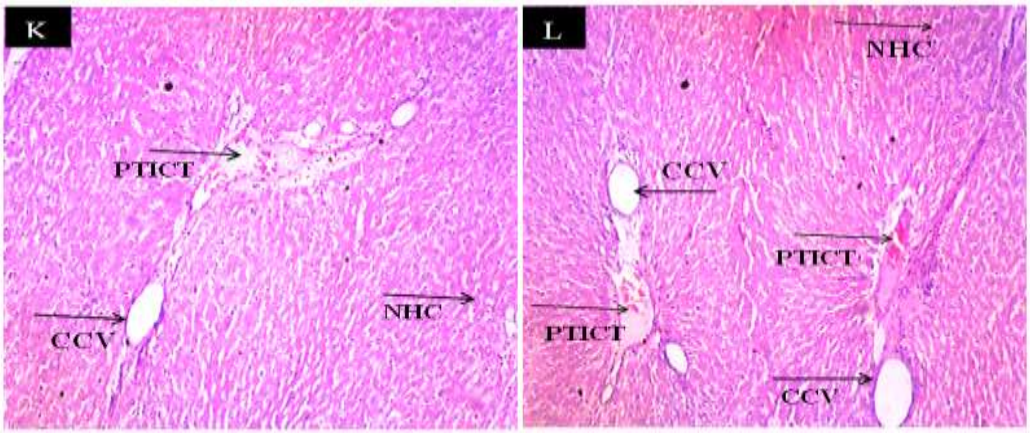

Plate VI: Photomicrograph of rat's liver administered with silymarin, (100 mg/kg), showed normal architecture with normal hepatocytes (NHC), congested central vein (CCV) and portal tract with inflammation, congestion and thrombosis (PTICT) x 10 (K) and x 40 (L) magnification.

5.0 DISCUSSSION

The results of the evaluation of hepatoprotective effect of the *Setaria megaphylla* root extract on rats with paracetamol-induced liver injury showed that the administration of paracetamol increased the levels of serum marker enzymes AST, ALT, ALP, total and conjugated bilirubin significantly ($p< 0.05 - 0.001$), and decreased the levels of protein and albumin, which collectively shows evidence of liver toxicity (Table 2). There was a significant ($p < 0.05 - 0.001$) reversal of these enzyme levels, protein and albumin on administration of the extract and silymarin which indicates protection against paracetamol-induced hepatotoxicity (Mandade, 2011).[5]

The liver is involved in the maintenance of metabolic functions and detoxification from exogenous and endogenous challenges such as xenobiotics, drugs, viral infections and chronic alcoholism. Ample supply of blood and the presence of many redox systems (e.g. cytochromes and various enzymes) enable the liver to convert these substances into different kinds of inactive, active or even toxic metabolites. In addition, serum levels of many biochemical markers like AST, ALT, ALP, triglycerides, cholesterol, bilirubin, are elevated (Mossa *et al*., 1991; Mascolo, Sharma, Jain and Capasso, 1988)[6][7] by release from the cytoplasm into the circulation due to cellular damage to the liver.

Paracetamol usage in the experimental evaluation of hepatoprotective activity of medicinal plants and drugs is well established (Muriel, Garciapina, Perez-Alverez and Mourelle,

1992).[8] Paracetamol is metabolized in the liver via glucuronidation, sulfonation and oxidation (Hinson, Bucci, Irwin, Michael and Mayeux, 2002)[9], but oxidation, the main cause of toxicity (Jollow *et al.*, 1973)[10], is primarily catalyzed by cytochrome P-$_{450}$ (Potter *et al.*, 1973)[11] and produces a highly reactive arylating compound called *N*-acetyl-*p*-benzoquinoneimine (NAPQI). As long as the rate of formation of NAPQI is not greater than the maximal rate of synthesis of glutathione (GSH) there will be no deleterious influence to the cell or organ (Mitchell, Jollow, Potter, Gillette and Brodie, 1973).[12] Hepatic synthesis of GSH, which is directly suppressed within the first few hours following ingestion of hepatotoxic dose of paracetamol, is overwhelmed and manifestations of toxicity appear when GSH level falls below 30 % of normal (Makin and Williams, 1997).[13] When more NAPQI is formed than the available GSH for conjugation, the unbound NAPQI becomes toxic by binding to macromolecules, including cellular proteins and DNA (Vermeulen, Bassems and Van de Straat, 1992).[14] NAPQI which is eliminated by conjugation with glutathione (GSH) is further metabolized to a mercapturic acid and excreted in the urine (Thomas, 1993).[15]

The reversal of increased serum enzymes in paracetamol-induced liver damage by the extract may be due to the prevention of leakage of intracellular enzymes by its membrane stabilizing activity. The possible mechanism by which the extract exhibited significant protection against paracetamol-induced hepatotoxicity may be due to the active constituents present such as flavonoids, alkaloids, etc and its free radical scavenging activity (Cheedella, Alluri and Ghanta, 2013).[16] The antioxidant and free radical scavenging activities of borneol **(**Kumar, Kumar and Raja, 2010)[17], astaxanthin (Fassett and Coombes, 2009)[18], cervacrol (Kamimura, Santosa, Hill, and Gomes, 2014)[19], menthone (Vimal, Vijaya, Mumtaz and Farhath, 2013)[20] and hexadecanoic acid (Rajeswari, Murugan and Mohan, 2012)[21] which are all constituents of this extract (Udobang, okokon and Etuk, 2016)[1] has been documented. Astaxanthin protects liver damage induced by CCl_4 by inhibiting lipid peroxidation and stimulating the cellular antioxidant system (Kang, Kim and Kim, 2001)[22] and has a large capacity to neutralize free radical or other oxidant activities in the nonpolar (“hydrophobic”) zones of phospholipid aggregates, as well as along their polar (hydrophilic) boundary zones (McNulty, Byun, Lockwood, Jacob and Mason, 2007)[23] and is used to treat high cholesterol (Iwamoto *et al.*, 2000).[24] Carvacrol is responsible for the biological activities of oregano (*Origanum vulgare*) with many diverse activities including anti-hepatotoxic and hepato-protective effects (Baser, 2008)[25] and is used to treat high cholesterol (De Sousa, Júnior

Andrade and Batista, 2011).[26] These constituents may be responsible for the observed hepatoprotective activity of this extract.

Histopathological studies revealed that the rats in the paracetamol group showed hepatotoxity (focal necrosis) involving the hepatic parenchymal tissue with congestion and thrombosis in the portal tract blood vessels extending into the sinusoids and central veins, and moderate to severe inflammation including the periportal area which is suggestive of mild to moderate vasculogenic damage otherwise referred as acute to subacute extra-hepatic (vascular) injury. The liver in extract and silymarin treated groups showed presence of congestion and thrombosis in the portal tract blood vessels extending into the sinusoids and central veins, with moderate to severe inflammation including the periportal referred to as acute to subacute extra-hepatic (vascular) injury. The absence of steatosis (fatty changes), necrosis and fibrosis in extract and silymarin groups are indicative of negligible hepatotoxic tissue effect and can be ascribed to the protective effect of the extract and silymarin, while the vasculogenic damage could be taken as terminal response to stress of death. These findings corroborate the biochemical findings of negligible hepatotoxicity in this study, thereby confirming the hepatoprotective potential of this extract.

6.0 CONCLUSION

The results of this research work reveals that *Setaria megaphylla* ethanol root extract through its phytochemical constituents possess significant hepatoprotective activity and also validates its ethnomedicinal use.. Further investigation to identify, elucidate and isolate the active components with their possible mechanisms of actions in order to standardize them is recommended to be carried out.

7.0 ACKNOWLEDGEMENT

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