ASSESSMENT OF *PARSONSIA STRAMINEA* (R.Br) F. MUEL STEM BARK TOXIOCOLOGICAL IMPACT ON THE LIVER IN MICE

ABSTRACT

***Background**: Toxicity is defined as the degree to which a substance can harm humans or animals. In reality, every entity that makes substance are undoubtedly labelled poisonous except it is quantified to make safety certainfor therapeutic purpose. Herbal medicines are generally considered to be safe and efficacious among people of various ethnic background globally. The plant Parsonsiastraminea has been traditionally claimed to be used in arthritis and seizures, although it is not widely explored. P.straminea a plant used medicinally cannot be said to be free from toxicity owing to fact on its use.**Aim of the study:** The aim of the study is to assess the potential hepatotoxicity of P.straminea stem bark extract in mice **Methodology:** For the study, about thirty rodents (mice) were set into six groups with five (5) mice in each group. The study groups (GPs) include 1=0.2ml/kg as control; 2 to 6=50,100,200,400 and 800 mg/kg of P.straminea extract. The harvested tissues were sent for histopathological examination. **Result:**This study has proven that the extract relatively impacted no significant toxicity on the liver enzymes such as alanine aminotransferase (ALT), alkaline phosphate (ALP), albumin (ALB) aspartate aminotransferase (AST) and total protein (TP). **Conclusion**: The study findings has shown that the ethanolic stem bark of P.straminea possesses relatively no remarkable toxicity impact on the liver as revealed in the liver enzymes and the histology assessment.*

Key word: Toxicity, Liver, P.straminea, liver enzymes, histology.

1. INTRODUCTION

Every substance applicable as food or medicine need to be assessed for its safety in both human and non-human population in order to be accepted as medicine or food.

Thus, this study tends to provide more information about the safety of *Parsonsiastraminea* (R.Br.) F. Muellon the hepatic system that can project short- and long-term use either topically or orally [1].Toxicity is defined as the degree to which a substance can harm humans or animals. It is the quality, relative degree, or specific degree of a substance being grossly averse to physiological events. Every consumable including edible substances are potential poison depending on the dose and range of exposure [2]. It has been considered and accepted by majority of traditional medicine

users that plants with medicinal values are safer, though may be characterised with little toxic potential that must be identified in other further guider users and enhance the alternative medicine practice especially in the developing nations [3].These claimed toxicity related to medicinal plants may differ from specie to specie and from geographical locations as they greatly play phytoconstituent role that defines toxicity profile of individual plants[4]. However safe a plant base traditional medicine maybe the quantification as dose for the right disease state should be prioritised to improve positive treatment outcome and avoid product induced toxicity and mortality [5].The plant *P. straminea* has little or no ethno pharmacological records [6]. The plant was accidently identified by means of local use for seizure control in the Wilberforce Island of Bayelsa State, Nigeria. Thus, this study is aimed at the screening for *P. straminea* stem-bark hydro-ethanol extract possible toxicological impact on the hepatic tissues.*P. straminea,*also known as silk pod or “monkey rope”, known to be a woody vine of the dog-bane family of *Apocynace*, "*P. straminea*" as described from the publication ofAustralian Plant Name Index (APNI, 2009). It occurs in the states of New South Wales and Queensland in Australia. *Parsonsiaspecies* a woody climbing vines in eastern Australia: Family *Apocynaceae.*It has been recorded at some part of the world to be relatively toxic with unknown toxin; some literatures are pointing that the possible toxicity claim could be linked to the presence of cardiac-glycosides in the *Apocynaceae* family. Other species of *Parsonsia*like the *euclyptophylla* also referred to “gargaloo” and “monkey vine” are given as feed to cattle and sheep without any toxicity record observed [7][8]. The claim that*P. straminea,*isresponsible for the death of sheep and cattle [9] is inconsistent with other literatures[7][8] as well our previous phytochemical work *P.straminea* that is yet to be published. However, some other claim of toxicity signs recorded in rominant animals include interstitial-eodema and

haemorrhage of the heart and lung congestion without scientific evidence of the cause in all acclaimed cases. The main constituents responsible for the toxicity of *P. straminea* are pyrrolizidine alkaloids (PAs) particularly lycopsamine and its N-oxide [10]. Theses alkaloids are present primarily as N-oxides in the plant, accounting for up to 99% of the total PAs in the pods [11]. PAs are known to be hepatotoxic, causing liver damage[12]. This study evaluated the single and repetitive (14 days) treatment through the oral route to determine toxicity potential of *P.straminea*in the liver using mice as the subject of interest.

2. METHODS

2.1 Ethical Consideration

The study was approved with reference number, NDU/PHARM/AEC/044a in the Department of Pharmacology and Toxicology, Niger Delta University, Wilberforce Island, Nigeria.

2.2 Plant Identification, Authentication and Crude Drug Preparation

The plant *P. straminea* was, identified by Dr. Gideon Alade and authenticated by Prof. Kola Ajibesin of the Department of Pharmacognosy and Herbal Medicine, Faculty of Pharmacy, Niger Delta University followed by Herbarium deposition number NDUP/21/001. *P.straminea* stem-bark was gotten from the Wilberforce Island rainforest.In preparation for the extraction, the stem bark of *P.straminea* was washed with clean water and air dried under 16^{o}c temperature in a clean and dust free room, afterwards it was reduced to smaller to smaller pieces and grinded. Weight of 200g of the stem bark was taken for maceration in hydro-ethanol of 1000 ml. The maceration stands for 72 hrs with daily manual shaking. After 72 hrs maceration, filtered and concentrated at 45^{o} C using rotary evaporator and water bath to make dry extract.

2.3 Animal

In this study the animal subject of interest was male mice and was gotten from the animal house of the Department of Pharmacology and Toxicology, Niger Delta University, Nigeria with all handling conditions strictly adhered [13].

2.4 Study Design

The study was designed as a sub-acute study that lasted for 21 days. About 30 mice were randomly allotted into six groups (n=5). Group one (1) treated with 0.2 mg/kg daily as normal control. Group (2) treated with 50 mg/kg of *P. straminea* daily; group three (3) was treated with 100 mg/kg *P. straminea* daily; group four (4) 200 mg/kg daily of *P. straminea*; group five (5) treated with 400 mg/kg daily of *P. straminea*; group six (6) was treated with 800 mg/kg daily of *P. straminea*. The animals were sacrificed 18 hours later. At the sacrifice, the blood was collected from the individual animal for liver enzyme assay. The blood was separated using blood centrifuge to obtain the serum. The serum was contained in their respective labelled container with regards to their groups. The serum that was obtained were sent to the laboratory for liver enzyme assay. The liver, kidney and brain were excised and rinsed with normal saline before it was placed into 10% formal saline solution of labelled containers according to their groups.

2.4.1 Determination of liver function biomarkers

Determination of alanine aminotransferase (ALT) activities. The method adopted to quantify the enzyme alanine amino transferase (ALT) in the mice serum in this research was described by Reitman and his colleagues Frankel[14]. The reaction equation illustrates the principle of the technique in expressing the enzyme activities. Alanine + α-ketoglutaric acid; pyruvic acid + glutaric acid.

Each serum in their respective groups were separately prepared and 0.2 mL aliquot of it was dispensed into a test tube in which 1 mL of ALT buffered substrate (Randox) was added. This mixture was incubated for 30 minutes at 37^{o}C. Thereafter, (2, 4-dinitrophenylhydrazine) a colouring agent was introduced to the mixture. This mixture was left to stand at room temperature for 30 minutes and subsequently 10 ml of 0.4N of Sodium Hydroxide was then added left for 5 minutes without disturbance. The above steps were followed replacing the serum with water (0.2 mL) as blank. The absorbance readings were at 500 nm against the blank. The enzyme activities were extrapolated from the standard curve and expressed as unit/ml.

Determination of aspartate aminotransferase (AST) activity. The method adopted to quantify the enzyme aspartate amino transferase (AST) in the mice serum in this

research was described by Reitman and his colleagues Frankel[14]. The procedure is similar to that of ALT except for the replacement of the enzyme ALT with AST substrate buffer (Randox) and the incubation is 60 minutes against 30 minutes as obtained in ALT assay. As obtained in ALT 2, 4-dinitrophylhydrazine is added to generate coloured hydrazine. The enzyme activity was also extrapolated from AST standard curve and expressed as unit/ml.

Determination of alkaline phosphatase (ALP) activity. Using spectrophotometric method, the commercial kit (Randox) for ALP determination was adopted. The serum contains the enzyme alkaline phosphatase hydrolyses in which phenolphthalein monophosphate is a substrate. Both reacts to produce phosphoric acid and phenolphthalein. This reaction changes from colourless to pink in an alkaline pH environment which can be determine photometrically. Expressed in unit/mL. A unit ALP is required to catalyse the reaction, 1M phenolphthalein monophosphate to yielding 1M phosphoric acid and phenolphthalein.

Determination of albumin (ALB). Albumin determination is principle on its capacity to bind in a direct proportional fashion with the substrate indicator 3,3,5,5, -tetra Bromo-m-cresol-sulphoepthalein (Bromocresol Green, BCG) which maximally absorbed at 578 nm. Three dry and clean test tubes were used to prepare the blank, standard and assay samples, containing 3.0 mL of BCG reagent of all test tubes and distilled water (0.01mL), standard and sample respectively. These mixtures were then left for 5 minutes at room temperature, absorbance reading was subsequently taken at 578nm against the blank [15]. Calculation: Concentration of standard = 4.5g/dL.

Determination of total protein (TP). Three dry and clean test tubes are used to prepare the blank, standard and assay samples, containing 1.0 mL of R1 reagent in all test tubes and distilled water (0.02 mL), standard and sample respectively. These mixtures were then left for 5 minutes at room temperature, subsequently absorbance was read at 520nm against the blank [16]. Calculation: Concentration of standard = 5.98 g/dL.

2.4.2 Histological examinations

The liver tissue that excised from the animals in this study were taken to the histology laboratory for histological processing and microscopic viewing of the slides according

to the groups for proper interpretation. The histo-pathologist applied method according to the laboratory provision [17].

2.5 Statistical analysis

The animals were analysed using Graph Pad Prism 10.2, Two-way ANOVA an\d comparison was done using Dunnett's test, the analysed results were presented either as graph or table with $P<0.05$ is considered significant.

3. RESULTS

3.1 Biochemical Evaluations

This study evaluates the toxicological potential of *P. straminea* stem bark ethanol extract, which revealed that the extract relatively impacted no significant toxicity on the liver enzymes such as alanine aminotransaminase (ALT), alkaline phosphatase (ALP), albumin (ALB), aspartate aminotransferase (AST), and total protein as shown in table 1.

Table 1. Sub-Acute Biochemical (Liver) Parameters Evaluation

Parameters	VEH	50 mg/kgPSE	100 mg/kgPSE	200 mg/kgPSE	400 mg/kgPSE	800 mg/kgPSE
ALT (U/L)	15.0±0.4	15.6±0.0	15.2±0.8	14.8±1.7	15.6±1.0	14.0±0.5
ALP (U/L)	21.0±0.3	20.3±0.2	21.5±1.5	20.9±0.5	19.0±1.2.0	18.0±3.0
ALB(g/dL)	3.2±0.4	3.6±0.2	2.8±0.3	3.0±0.2	3.1±0.2	3.1±0.2
AST (U/L)	67.1±5.8	65.7±1.2	70.8±0.8	78.7±1.7*	80.7±0.4*	76.6±2.9*
TP (g/dL)	26.6±0.8	24.6±0.8	27.2±0.8	27.5±3.7	25.5±3.1	27.0±3.4

VEH=Vehicle/control, PSE=*P.Straminea* Stem Bark Extract. Tisue enzymes measured with TP **(g/dl)** = total protein; ALT **(U/L)** = alanine amino transferase; ALP **(U/L)** = alanine amino phosphate; ALB **(mg/dl)** = albumin; AST **(U/L)** = aspartate amino transferase; *=Significant (P<0.038).

3.2Histological Evaluations

The histological evaluation of *P.straminea* stem bark ethanolic extract showed no remarkable toxicity impact on the cellular structure formation of the liver tissues in the various study groups as it is in figure 2 to 6.

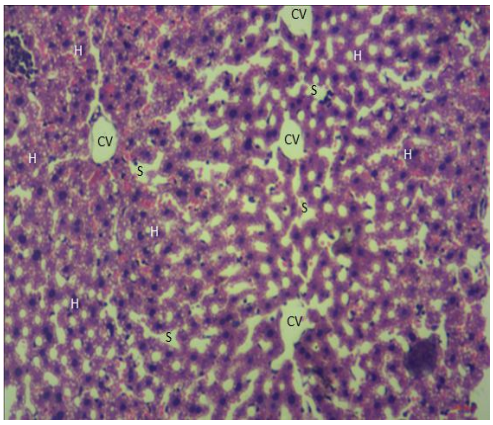

Fig 1: Group 1. VEH**.** Control of toxicity study group of histology showing normal liver architectural intergrity. Patent central vein (**CV**), intact hepatocytes (H), Sinusoids (s) containing kupffer cells & capillaries. Photomicrographs of Liver, Mag X 400 H & E

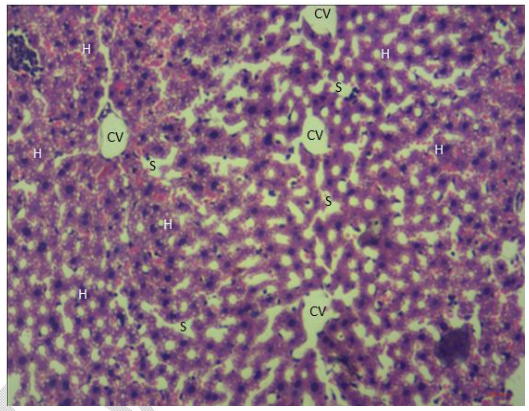

Fig 2: Group 2. 50 mg/kg PS.Extract toxicity study group of histology showing normal liver architectural intergrity. Patent central vein (**CV**), intact hepatocytes (H), Sinusoids (s) containing kupffer cells & capillaries. Photomicrographs of Liver, Mag X 400 H & E

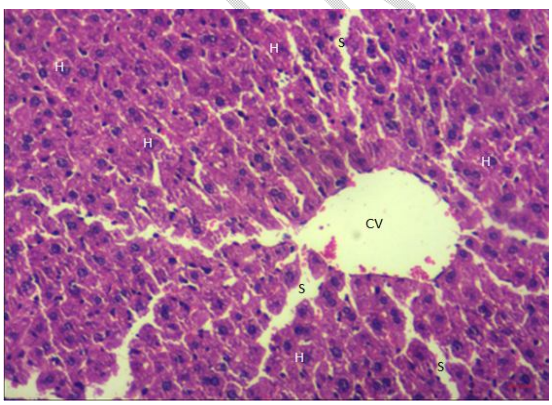

Fig 3: Group 3. 100 mg/kg PS.Extract toxicity study group of histology showing normal liver architectural intergrity. Patent central vein (**CV**), intact hepatocytes (**H**), Sinusoids (**s**) containing kupffer cells & capillaries. Photomicrographs of Liver, Mag X 400 H & E

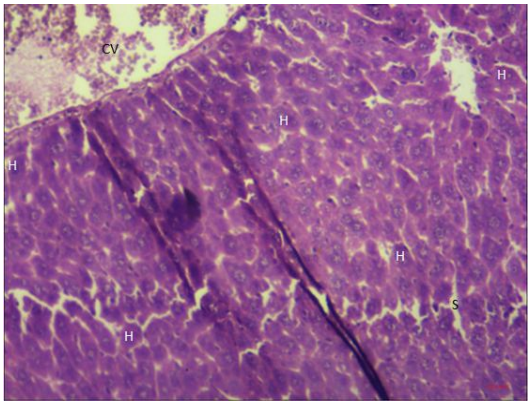

Fig 4: Group 4. 200 mg/kg PS**.** Extract toxicity study group of histology showing normal liver architectural intergrity. Patent central vein (CV), intact hepatocytes (H), Sinusoids (s) containing kupffer cells & capillaries. Photomicrographs of Liver, Mag X 400 H & E

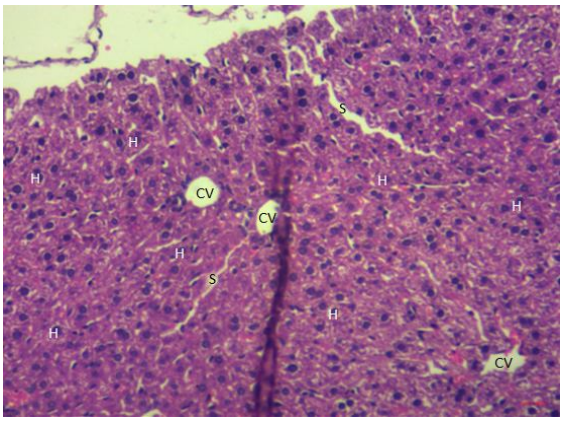

Fig. 5: Group 5. 400 mg/kg PS. Extract toxicity study group of histology showing normal liver architectural intergrity. Patent central vein (CV), intact hepatocytes (H), Sinusoids (s) containing kupffer cells & capillaries. Photomicrographs of Liver, Mag X 400 H & E

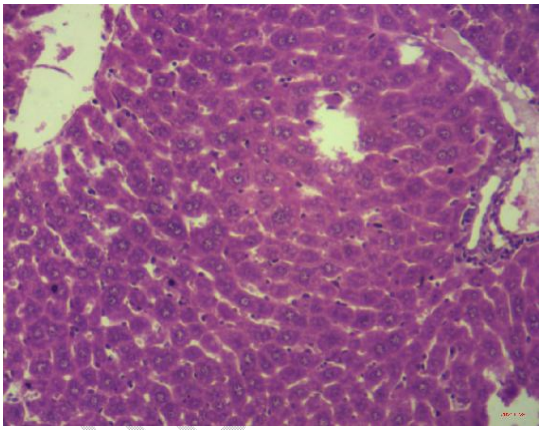

Fig. 6: Group 6. 800 mg/kg PS**.** Extract toxicity study group of histology showing normal liver architectural integrity. Photomicrographs of Liver, Mag X 400 H & E

4. DISCUSSION

The metabolic enzymes and proteins of interest in this study are -ALT,-AST, ALP and ALB of the organ liver which a pivotal role in metabolism especially in the hepatic system. If the liver enzymes are elevated, it could indicate liver injury or damage. The implication of elevated liver enzyme level depends on the treatment doses and the clinical presentations. For instance, a moderate increase in the level of the liver enzymes may be an early sign of liver injury, while a marked or remarkable increase could indicate acute or chronic liver disease. On the other hand, decreased liver enzymes level may suggest impaired liver function and could be due to a number of factors including pharmacologically treatment agents including extracts, genetic variations, among others [18].

From the result recorded in the table 1, the biochemical evaluation indicates no toxicity potential of the stem bark extract of *P.straminea* in the sub-acute treatment duration of the study. This finding suggest that *P.straminea* may not alter the metabolic role of the liver or cause any liver damage, which could lead to elevated

levels of liver enzymes in the tissues [6]. Also the lack of hepatotoxicity may indicate that *P.straminea* extract does not relatively consists any toxic constituents that are harmful to the liver. This is supported by the finding that the plant has antioxidant and antimicrobial properties, which suggest that it contains beneficial compounds such as flavonoids and saponins which have strong antioxidants and antimicrobial properties [19][20].Another reason could be due to species-specific differences, soil, environmental factors. The absence of hepatotoxicity in mice may be due to species-specific differences in the metabolism or sensitivity of the cytochrome enzymes in the liver. Mice may have different enzymes or transporter proteins that metabolize or transport compounds differently than humans, which could result in a different toxicological response [21].Based on the non-hepatotoxicity findings, it can be said that these doses may not be high enough to cause hepatotoxicity in mice. The lack of hepatotoxicity at these doses suggests that *P. straminea* may be relatively safe for use as a traditional remedy.

Histo-pathological examination of the liver tissue from the control group and the treated group showed no remarkable changes in the tissue architecture in any of the groups.The results obtained shows little or no deleterious effect on the liver and liver enzyme because of the vital phytochemical constituents of the stem bark of *P. straminea* [22] that presents bio-protective potential which proves right in the normal architectural integrity of the liver assessment even in the relatively long period of exposure (sub-acute). The results obtained in this have proven contrary to the claim that *Parsonsia*speciesare toxic [9]. However, the toxic claim was made on goats and sheep and not rodents like mice.

5. CONCLUSION

The study findings have shown that the ethanolic stem bark of *P.straminea* possesses relatively no remarkable toxicity impact on the liver as revealed in the liver enzymes and the histology assessment.

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