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Research Article
Toxicological Evaluation of *Psychotria microphylla* Crude Extract on Rats

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Abstract

Background and Objective: *Psychotria microphylla* Elmer is used in South Eastern Nigeria in fish harvesting. Though apparently believed non-toxic to humans, there are no scientific reports available regarding the toxicity of this plant on rats. This study was designed to evaluate the safety of *P. microphylla* leaves extract in wistar albino rats. **Materials and Methods:** The method of Lorke was used to determine the acute toxicity using 14 rats. In the sub-acute phase, twenty five albino rats were randomly divided into five groups (A-E) of 5 rats each. Group A served as control and received 0.5 mL normal saline, Groups B, C, D and E received 300, 500, 1000 and 2000 mg kg^{-1} b.wt., respectively for 21 days orally. The results obtained were analyzed using SPSS for windows version 20. **Results:** The results showed that the crude leave extract of *Psychotria microphylla* did not cause any mortality at all doses up to 5000 mg kg^{-1} b.wt. The extract also did not produce any marked ($p>0.05$) effect on the haematological indices analyzed. However, oral administration of *P. microphylla* leave extract did cause significant reduction at ($p<0.05$) in triacylglycerols, total cholesterol and glucose levels in the rats in dose dependent manner. The extract did not cause significant impact on hepatic and renal functions at lower doses. **Conclusion:** In conclusion, this extract did not elicit significant effect on the immune, hepatic and renal systems and could be useful in the management of lipid related diseases due to its hypolipidemic activities.

Key words: *Psychotria microphylla*, toxicity, mortality, hypolipidemic, haematological

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Psychotria microphyilla is an important promising species in herbal medicine and is commonly used for the management of infections of the female reproductive system, bronchitis, gastrointestinal disturbances, skin infections, tumours and ulcers[1]. The tender fruit of *P. nilgriensis* is consumed along with honey for the management of rheumatism[2,3], reported poisonous effect of fruits and seeds of *P. cuspidate* and the leaves of *P. involucrate* are used as fish poison, while the fruits of *P. poeppigana* are used to kill rats. *P. camptopus* (Nchaing) bark concoction with rhizomes of longisetosa is taken orally to treat nerves and partial paralysis in Cameron[4].

However, many extracts of *Psychotria* species have revealed anti-inflammatory and analgesic activity[5], antibiotic activity[6], antiviral activity in *P. serpens* [7], while the piscicidal activity of *Psychotria microphylla* was reported[8].

Phytochemical investigations of *Psychotria* species have led to the isolation of a pyrrolidinoindoline[9], quinoline and isoquinoline[10], benzoquinones[10], pigments[11] and alkaloids[12].

Psychotria microphylla Elmer is one of the *Psychotria* species found in the Eastern part of Nigeria. It is known as "Akwukwo iyi" (Stream leaf) in Afikpo South Area of Ebonyi State Nigeria because it is normally found along river banks and in a swampy forest. Infusion of the whole plant is used in this area for fishing and prevention of insects from destroying farm crops. Preliminary screening of *P. microphylla* leaves showed alkaloids, flavonoids, tannins, saponins, cardiac glycosides[13]. This plant is reported to be very toxic to *Clarias gariepinus* with LC_{50} of 0.35 mg L^{-1} of the aqueous extract and the fish harvested with the plant are consumed by the local people[8]. This plant is a promising botanical piscicide and pesticides and may even have medicinal applications. The plant has been reported effective against some clinical bacterial isolates. Though apparently believed non-toxic to humans, strict scientific tests are required because this plant may contain a few harmful ingredients in them as secondary metabolites that may have adverse side effects including mutagenic potentials. Presently there are no scientific reports available regarding the toxicity of this plant on rats. This study was therefore designed to evaluate the safety of *P. microphylla* leaves extract by carrying out the acute and sub-chronic toxicity studies in wistar albino rats.

MATERIALS AND METHODS

Research duration: This study was carried out at the Department of Biochemistry, Ebonyi State University, Abakaliki, Nigeria from 2013-2015.

Collection and authentication of the plant sample: The fresh samples of *Psychotria microphylla* were collected from the wild at Afikpo South L.G.A of Ebonyi State Nigeria. The plant was identified and authenticated by Mr. Ozioko of the International Bioresources and Research Centre, Nsukka, Nigeria. The sacrificed animals were approved by the Ebonyi State University research ethic committee Ref. No. EBSU/TBR/2015/07.

Acute toxicity study: The acute toxicity study was done according to the method of Lorke[14]. The study was carried out in two phases using a total of fourteen male rats. In the first stage, nine rats were divided into 3 groups of 3 rats each. Groups 1, 2 and 3 animals were given 300, 800 and 2000 mg kg^{-1} b.wt., of the extract, respectively, to possibly establish the range of doses producing any toxic effect. Rats in group 1 were receiving the extract 48 h intervals for 14 days, while those in group 2 and 3 were given a single dose after 7 days of adaptation. In addition, a fourth group of three rats was set up as control group and animals in the group were not given the extract. In the second phase, 3000 and 5000 mg kg^{-1} b.wt., of the extract were administered to 2 groups of 1 rat each to further determine the correct LD_{50} value. The animals were observed continuously for the first 4 h and then each hour for the next 24 h and at 6 hourly intervals for the following 48 h and daily for 14 days after administering the extract, to monitor any death or alterations in general behavior and other physiological activities[15]. All examinations were methodically recorded with individual data being maintained for each animal. Observations included changes in skin and fur, eyes and mucous membranes and behavioral pattern. Attention was given for observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and mortality.

Subchronic test: Twenty five male wistar rats weighing 160±10 g were used. They were allowed to acclimatize to the laboratory conditions for seven days and were maintained on standard animal feeds and provided with water *ad libitum*. The animals were weighed and divided into five groups of five animals each. After fasting the rats overnight the control group received a dose of 0.5 mL of normal saline orally once a day for 21 days. The four treated groups respectively received 300, 500, 1000 and 2000 mg kg^{-1} b.wt., of the aqueous extract orally once daily for 21 days according to the modified method of Pieme *et al.*[16], Joshi *et al.*[17] and Mythilypriya *et al.*[18]. At the end of the treatment, they were anaesthetized with chloroform and blood was collected via cardiac puncture into two tubes: One containing EDTA for analysis of haematological parameters and the other

containing heparin for biochemical estimations. The collected blood was centrifuged within 5 min of collection at 4000 g for 10 min to obtain plasma, which was analyzed for total cholesterol, total triacylglycerols, alanine aminotransferase (ALT), aspartate amino transferase (AST), alkaline phosphates (ALP, U L^{-1}), creatinine, glucose and total protein. After blood collection, the rats were sacrificed and dissected[19]. The liver and kidney tissues were carefully removed and rinsed in water. Plasma glucose, total protein, Aspartic amino transferase (AST, U L^{-1}), alanine amino transferase (ALT, U L^{-1}), alkaline phosphates (ALP, U L^{-1}), plasma glucose (mg dL^{-1}), total cholesterol (mg dL^{-1}), triglycerides (mg dL^{-1}) were determined using assay kits supplied by Randox Laboratories Limited, BT29 4QY, United Kingdom. The organs liver and kidney were prepared for histopathological observation. They were fixed in bouin's fluid for 24 h, washed with 70% ethanol and dehydrated through a graded series of ethanol[20,21]. They were embedded in paraffin, sectioned at 4-5 µm thickness, stained with hematoxylin and eosin and examined using light microscope and photomicrography[22]. The haematological examinations performed were according to standard methods. Haematocrit was determined by the micro-haematocrit method[23]. Erythrocytes and total leucocytes were counted using the improved Neubauer haemocytometer.

Statistical analysis: The basic statistics, means, standard deviation and ranges of the measured parameters were estimated. Data were expressed as Mean±SD of five replicates and were subjected to one way ANOVA followed by Ducan multiple range test to determine significant differences in all parameters using SPSS for windows version 20. Values were considered statistically significant at $p<0.05$[24].

RESULTS AND DISCUSSION

The result of acute toxicity study in wistar albino rats treated with aqueousextract of *P. microphylla* leavesis depicted in Table 1. This revealed thatthere was no mortality in animals at all doses of the extracts up to 5000 mg kg^{-1}. The absence of death at doses up to 5000 mg extract/kg showed that LD_{50} of the aqueous extracts of *P. microphylla* leaves is greater than 5000 mg kg^{-1}, except for behavioral changes like restlessness which disappeared within 24 h of extracts administration. Other clinical signs of toxicity may include salivation, loss of hair, ataxia and changes in animal eye colour, decreased respiratory rate and motor activity as loss of righting reflex and diarrhea[25]. Though *P. microphylla* has been used by rural people of Afikpo south in Ebonyi state, Nigeria for fishing without report of any mortality due to toxicity, this

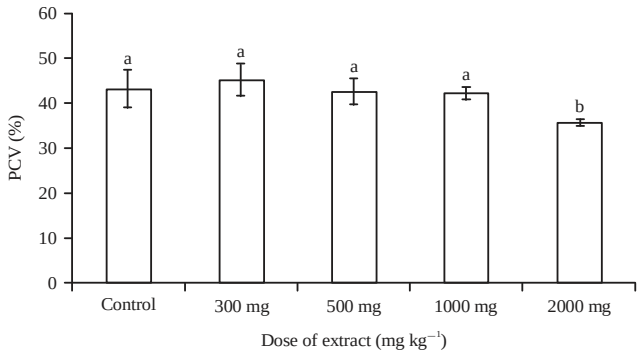

Fig. 1: Effect of aqueous extract of *Psychotria mycrophylla* on packed cell volume (PCV). Results are presented as Mean±SD of five rats. Bars with different letter are statistically significant ($p<0.05$)

Table 1: Acute toxicity study in wistar albino rats with aqueous extract of *P. microphylla* leaves

Group	Number of rats	Doses of extract	Number of dead	Dead (%)
1	3	300	0	0
2	3	800	0	0
3	3	2000	0	0
4	3	0	0	0
5	1	3000	0	0
6	1	5000	0	0

claim is corroborated by the lack of death at oral treatment of over 5000 mg kg^{-1} b.wt., of the extract. The extract did not cause any clinical adverse effect of substance-related toxicity on the rats.Thisindicated that the LD_{50} value of *P. microphylla* leaves is approximately higher than 5000 mg kg^{-1} and is therefore comparatively safe and practically non-toxic to rats.

Toxicity studies in animals are usually employed to evaluate possible health risk in humans caused by intrinsic adverse effects of plant extract[26]. These harmful impacts might perhaps be present in the form of profound variations in the quantities of biomolecules[27]. Biochemicals are the assessable body fluid contents for checking the toxicity of any chemicals[28] which are importance parameters in the evaluation of physiological state of organisms and are often used when clinical diagnosis is needed to determine the effects of external stressors and toxic substances.

The effects of the aqueous extract of *P. microphylla* leaf on haematological parameters of rat are presented on Fig. 1-5. The responses of blood cell are vital signals of alterations in the internal and/or external environment of animals[29]. It is one of the most sensitive targets for toxic compounds[7]. There were no significant ($p>0.05$) changes in packed cell volume, haemoglobin, total white blood cells and its differentials lymphocyte, monocytes, neutrophil and

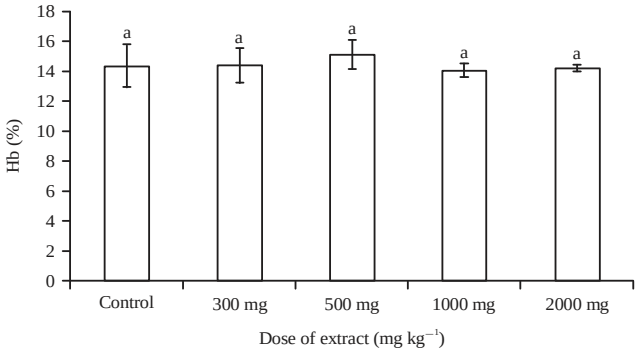

Fig. 2: Effect of aqueous extract of *Psychotria mycrophylla* on haemoglobin (Hb). Results are presented as Mean±SD of five rats. Bars with different letter are statistically significant (p<0.05)

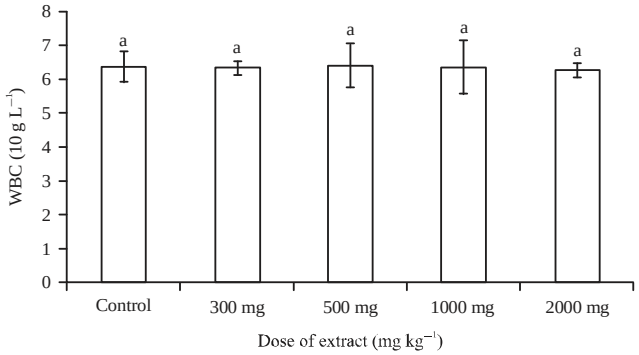

Fig. 3: Effect of aqueous extract of *Psychotria mycrophylla* on white blood cell (WBC). Results are presented as Mean±SD of five rats. Bars with different letter are statistically significant (p<0.05)

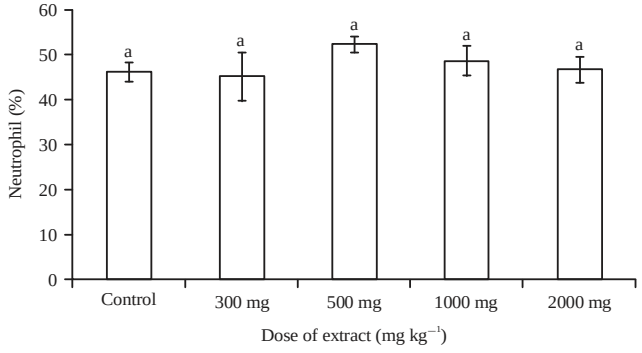

Fig. 4: Effect of aqueous of *P. microphylla* leaves on neutrophils levels of albino rats. Results are presented as Mean±SD of five rats. Bars with different letter are statistically significant (p<0.05)

eosinophils of all the treated groups. Administration of the aqueous extract of *Psychotria microphylla* levels for 21 days

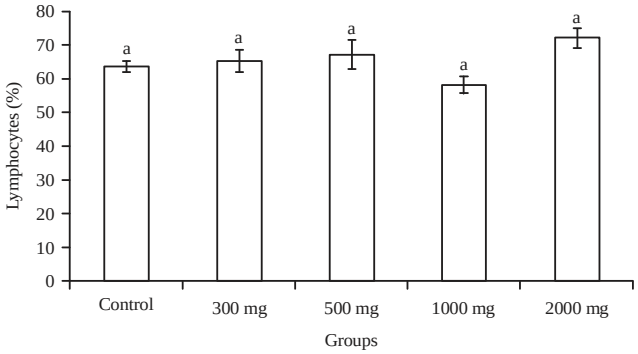

Fig. 5: Effect of aqueous of *P. microphylla* leaves on lymphocytes levels of albino rats. Data are presented as Mean±SD of five rats. Bars with different letter are statistically significant (p<0.05)

had no effects on most of the circulating cells nor disturbed their biosynthesis. The haemoglolobin and packed cell volume (PCV) levels were not altered indicating that haemolytic anemia and polycythemia were not likely to be induced by *P. microphylla* in rats. The levels of white blood cells that obliterate microorganisms at sites of infection, eliminate xenobiotics and remains of dead or injured cells, were not altered indicating that the aqueous extract of *P. microphylla* was not harmful to the immune system and did not affect leukopoiesis. The non-specific effects of the extracts on heamatological parameters of rats in this study might be an indication that it is unlikely to be heamatotoxic.

Effects of the extract on hepatic function parameters: The liver is the site of cholesterol degradation, as well as its major site of synthesis. It controls glucose synthesis and can produce free glucose from hepatic glycogen stores[7]. Various biochemical parameters: aspartate aminotransferase (AST), alanine, aminotransferase (ALT), glucose, cholesterol and creatinine) were to be analyzed after administration to determine if there were any plant-induced alterations in liver and renal functions. The results in Fig. 6-8 showed that oral administration of *P. microphylla* leaf extract did cause significant reduction at (p<0.05) in triacylglycerols, total cholesterol and glucose levels in the treated rats. *Psychotria microphylla* thus did affect lipid and carbohydrate metabolism of the rats. A significant dose dependent decrease in triacylglycerols and total cholesterol levels were noted with the aqueous extract of *P. microphylla* treated rats with maximum changes of 61.29% in plasma. In the same vein a significant dose dependent reduction of total cholesterol was observed with the groups administered the extract in relation to the control groups

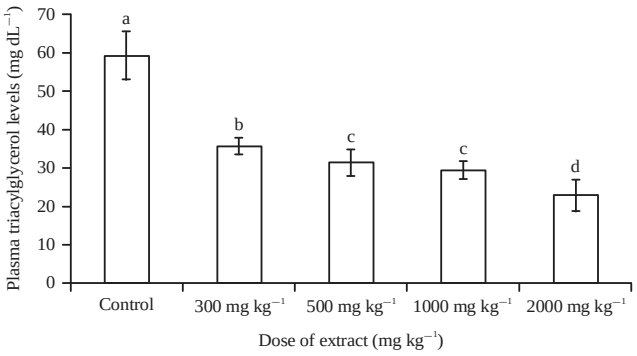

Fig. 6: Effect of aqueous of *P. microphylla* leaves on plasma triacylglycerols levels of albino rats. Data are presented as Mean±SD of five rats. Bars with different letter are statistically significant (p<0.05)

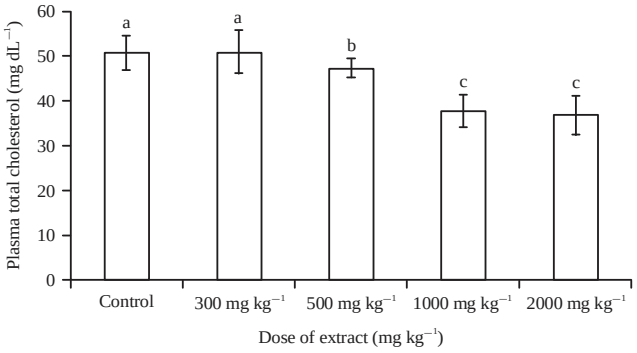

Fig. 7: Effect of aqueous of *P. microphylla* leaves on plasma total cholesterol levels of albino rats. Data are presented as Mean±SD of five rats. Bars with different letter are statistically significant (p<0.05)

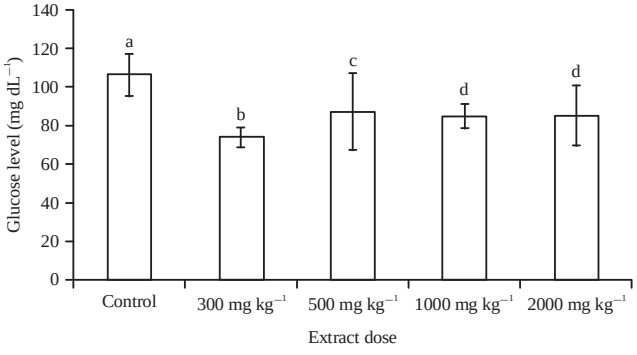

Fig. 8: Effect of aqueous *P. microphylla* leaves on glucose levels of albino rats. Data are presented as Mean±SD of five rats. Bars with different letter are statistically significant (p<0.05)

with maximum changes of 27.74 and 26.95% in plasma. The plant extract possessed an antilipidemic activity.

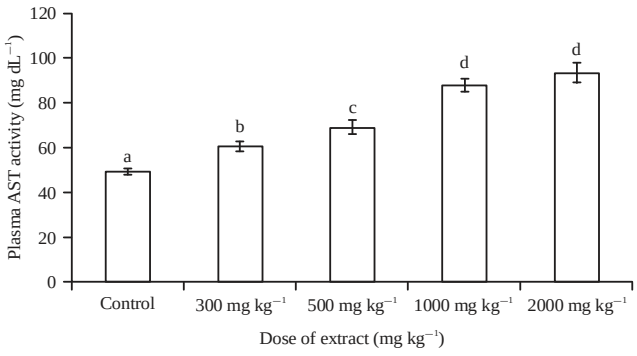

Fig. 9: Plasma AST activity in rats exposed to sub-lethal doses (mg) of aqueous extract of *P. microphylla* leaves. Data are presented as Mean±SD of five rats. Bars with different letter are statistically significant (p<0.05)

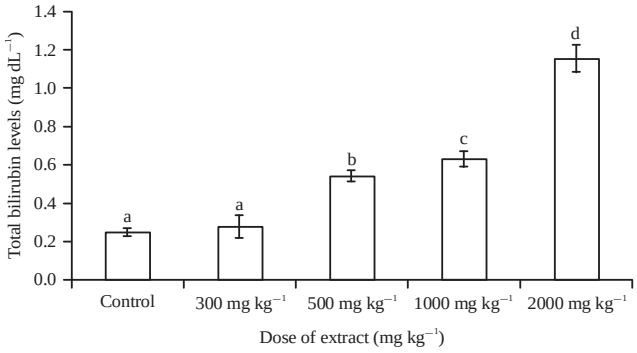

Fig. 10: Effect of aqueous extract *Psychotria microphylla* leaf on the plasma total bilirubin of albino rat. Data are presented as Mean±SD of five rats. Bars with different letter are statistically significant (p<0.05)

The results in Fig. 9-10 produced a significant increase (p<0.05) in the levels of AST and total bilirubin in plasma. These increment were dose dependent. There were no significant changes in the activities of alanine aminotransferase (ALT) in the plasma Fig. 11. The activity of alkaline phosphatase significantly increased at (p<0.05) in the treated groups with high doses of the extract up to a dose of 1000 mg kg^{-1}, activity levels of (48.30, 209.80 and 1579.32 U L^{-1}) as compared with a control values of 24.89 U L^{-1} in plasma. The liver plays a central role in xenobiotic metabolism and the kidneys are the major organs required in drugs removal and thus mainly exposed to the toxic effects of exogenous compounds[30]. This result revealed that the extract did not cause significant effect on the liver and kidney of the treated rats at all doses of 300-500 mg kg^{-1} after 21 days of administration for the sub lethal studies.

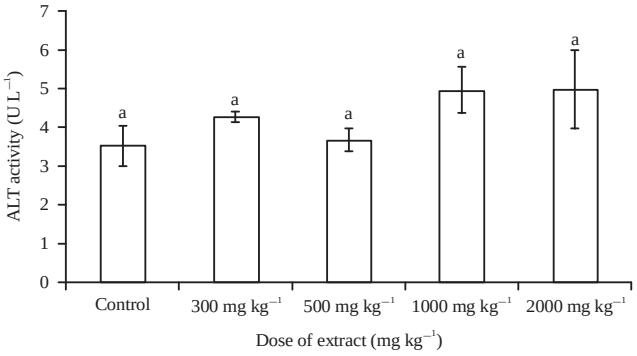

Fig. 11: Plasma ALT activity in rats administered with sub-lethal doses (mg) of aqueous extract of *P. microphylla* leaf. Data are presented as Mean±SD of five rats. Bars with different letter are statistically significant (p<0.05)

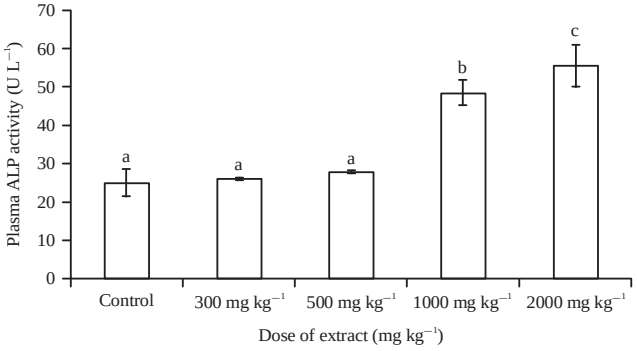

Fig. 12: Plasma ALP activity in rats exposed to sub-lethal doses (mg) of aqueous extract of *P. microphylla* leaves. Data are presented as Mean±SD of five rats. Bars with different letter are statistically significant (p<0.05)

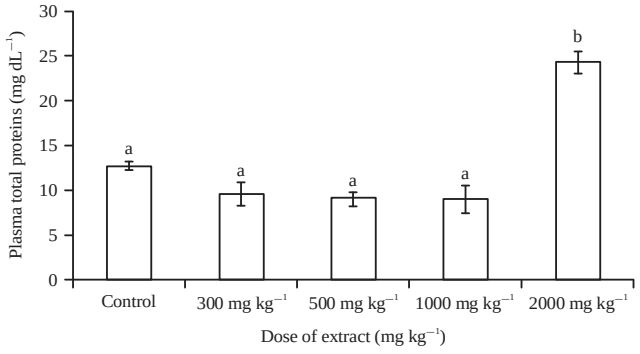

Fig. 13: Effect aqueous extract of *Psychotria microphylla* leaf on the plasma total protein of albino rat. Data are presented as Mean±SD of five rats. Bars with different letter are statistically significant (p<0.05)

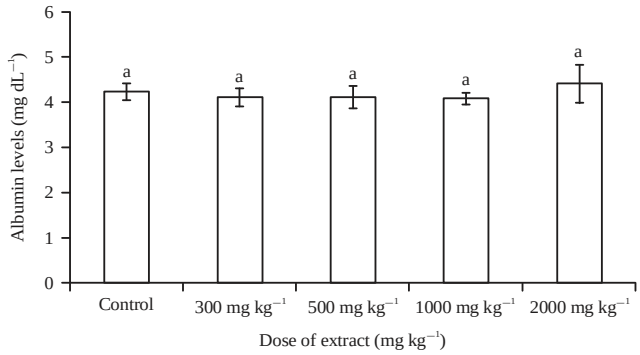

Fig. 14: Effect of aqueous *Psychotria microphylla* on the plasma albumin of albino rat. Data are presented as Mean±SD of five rats. Bars with different letter are statistically significant (p<0.05)

Measurement of plasma enzyme activity is a useful instrument in clinical diagnosis because it offers information on the effect and nature of pathological damages in tissues[31]. The results from this study have shown that there was no significant effect in ALP activity except for slight changes at 1000 and 2000 mg kg^{-1} which could suggest that the extract did not cause damage to plasma membrane Fig. 12. This implies that the membrane integrity may not been compromised and this could be attributed to stabilization of the enzyme molecule[25]. The transaminases (AST and ALT) are valuable enzymes, as biomarkers there signal any likely hood of toxicity[32] and any injure to the parenchymal liver cells will cause an elevations in both transaminases[33]. Elevation in plasma activity of enzymes like ALP, AST and ALT possibly will imply there is injury to cell membrane which could bring about compromise of membrane integrity[26]. ALT and AST are cytosolic enzymes and are typically contained inside the cells of the liver, heart, gill, kidney, muscles and other organs[34]. These enzymes are employed to assess the wholeness and damage to the liver and heart[35]. The non significant effect of the extract on plasma ALT and significant effect on AST might signal limited disruption of the cell membrane of the organs of the rats and hence might perhaps not have consequential impact on the metabolism and regulation of certain enzymes in the liver. The differences observed between the control and treated groups in the levels of plasma total proteins, albumin, creatinine and urea were not statistically significant (p>0.05) Fig. 13-17.

Furthermore, there were no effect on the levels of transaminases ALT and creatinine, which is a good indicators of liver and kidney functions[36].

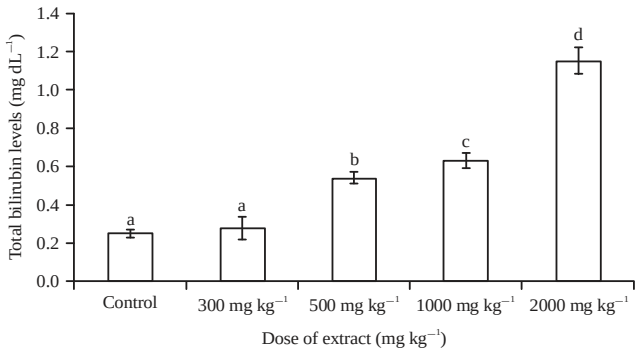

Fig. 15: Effect aqueous extract of *Psychotria microphylla* leaf on the plasma total bilirubin of albino rat. Data are presented as Mean±SD of five rats. Bars with different letter are statistically significant ($p<0.05$)

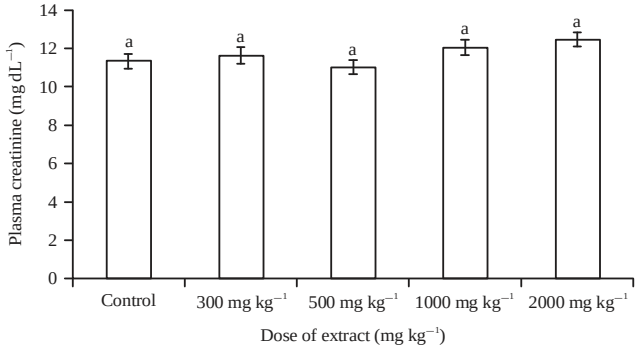

Fig. 16: Effect of aqueous extract of *Psychotria microphylla* leaf on the plasma creatinine of albino rat. Data are presented as Mean±SD of five rats. Bars with different letter are statistically significant ($p<0.05$)

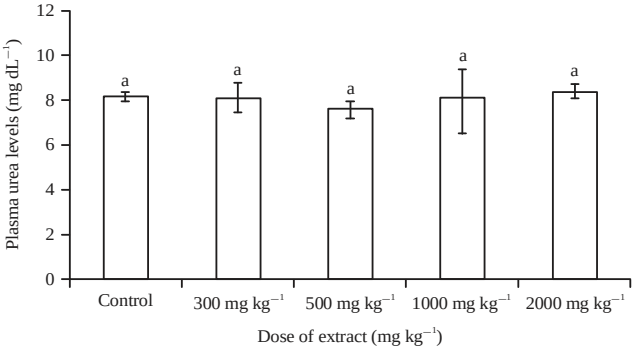

Fig. 17: Effect of aqueous extract of *Psychotria microphylla* leaf on the plasma urea level of albino rat. Data are presented as Mean±SD of five rats. Bars with different letter are statistically significant ($p<0.05$)

SIGNIFICANCE STATEMENT

This study for the first time has provided information on the safety and nontoxic effect of the plant extract on mammals (rats). Since the plant is non-toxic to mammals, the use of it for fishing in rural areas should be encouraged instead of using synthetic chemicals in fishing that are not eco-friendly. Though it was reported to be toxic to fishes, this property could be further investigated to unveil the chemistry of the phytochemical that is responsible for the lethal effect on fishes. Also further studies would be aimed at isolation, purification of the bioactive compounds present and possibly testing their activities on cell lines instead using animals.

CONCLUSION

The results revealed that the ingestion of the aqueous extract of *Psychotria microphylla* did not cause any death in all the treated rats. The extract after 21 days of administration was not hepatotoxic, heamatotoxic and nephrotoxic and did not alter the immune system of the rats. Also since the extract caused significant reduction of triacylglycerols, it could be useful in the management of lipid related disease such as obesity, diabetes and cardiovascular dysfunctions. Overall, the results of this study provide valuable data on the toxicity profile of *P. microphylla* leaf extract that should be useful for the planning of future application of this plant as botanical piscicide and studies of this.

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