Research Article [Nandy & Datta, 3(3): Mar., 2012]
ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Acute and sub-acute toxicity studies of methanolic leaves extract of *Pterospermum acerifolium* (L.) Willd in rodents

Subhangkar Nandy[1] and Rana Datta[2]
1, Department of Pharmacology, Vedica College of Pharmacy, RKDF Group, Bhopal, (M.P.) - India
2, Department of Pharmacology, Gupta College of Technological Sciences, Asansol, (W.B.) India

Abstract
Pterospermum acerifolium (L.) Willd is one of the most popular medicinal plants in Eastern regions of India, commonly known as Muchkunda. Traditionally the leaves of the plant have been used as Hemostatic and for its wound healing properties, but to date there is no documented evidence corroborating its safety. This study thus aimed to determine the toxicity profile of the methanolic extract of *Pterospermum acerifolium* (L) Willd (MEPA) by determining its effects after acute and sub-chronic oral administration in female and male rats (Wistar albino). In adult rats, single oral administrations of the MEPA (4–20 g/kg body weight) did not show any mortality upto 20g/kg b.w. p.o. However there were signs of toxicity, namely anorexia and hypoactivity above 12 mg/kg b.w. (p.o.), which was the no-observed-adverse-effect level (NOAEL). In rats, daily single oral doses of MEPA (100 mg/kg b.w., 1g/kg b.w. and 2 g/kg b.w.) were well tolerated behaviorally after 28 days of dosing and induced no significant changes in body and organs weights. However, haematological and biochemical parameters showed significant increases in ALT, AST, cholesterol and creatinine levels suggesting disturbances of liver and kidney functions. Histopathological findings also provided conclusive evidence towards hepatotoxic and nephrotoxic potential of the extract at high dose levels. The extract was tolerated well at low dose levels (100 mg/kg b.w. p.o.) after 28 day repeated administration of the extract. Overall, the findings of this study indicate that MEPA is non-toxic and has, at low dose, a low toxicity potential in acute and chronic oral administrations, respectively. However, at high oral doses, repeated administration (28 days) MEPA has significant hepatotoxic and nephrotoxic potential.

Key-Words: Acute, sub-acute, toxicological, *Pterospermum acerifolium* (L.) Willd, MEPA

Introduction
Medicinal herbs are used for the prevention and treatment of diseases, and have a long history. However, the most commonly used herbal formulae have no indications of quality, safety and efficacy [1]. The plant *Pterospermum acerifolium* (Common name Muchkunda) is used in traditional medicines for its haemostatic and wound healing properties. Initial pharmacological screening also shows the presence of anti-inflammatory, analgesic, antioxidant, antiulcer, wound healing and antipyretic properties [2-7]. In spite of the popular use of *Pterospermum acerifolium* in traditional medicine, no systematic evaluation of its toxic effects has been carried out till date as evident from literature review. [8-11] Therefore the aim of the present study was to investigate the acute and sub-acute toxic effects of a methanolic extract of *Pterospermum acerifolium* in rodents (Wistar albino rats), thereby evaluating the safety of the plant as used in traditional medicine.

*** Corresponding Author**
E-Mail: subhangkarnandy@gmail.com
Tel: +91-07415366404

Material and methods
Plant material
The leaves of *Pterospermum acerifolium* (L) willd were collected from Asansol, West Bengal, India in September 2010. A herbarium sheet was prepared and it was identified and authenticated by the Botanical survey of India, Howrah, and West Bengal, India. The leaves were dried in shade to avoid the deterioration of phytoconstituents and made into a coarse powder by using a grinder. Herbarium sheet was submitted to Shibpur Botanical Garden, Botanical survey of India, Howrah, West Bengal , India, under specimen number CNH/I-I/(289)/2008/Tech.II/331 for authentication. The specimen has been identified and authenticated as *Pterospermum acerifolium* (L) willd.

Preparation of extract
The air dried crushed leaves (1000g) were soaked for 12 hr in Methanol (3L) at room temperature. The residue was extracted with hot Methanol under reflux 3 times (each 1500 ml) after vacuum filtration. All solvent was evaporated under vacuum and extract was then lyophilized, to yield approximately 12% w/w/) of

the residue, which was stored at 20ºC until use. The concentrate was suspended in 5% w/v Tween 80 and given at dose 1ml/100gm body weight.

Treatment of animals

Healthy male and female rats (Wistar albino) of 4-8 weeks old were selected after physical and behavioural veterinary examination from Institutional Animal House of Gupta College of Technological Sciences. The weight range was fall within ± 20% of the mean body for each sex at the time of initiation of treatment. All experiments involving animals complies with the ethical standards of animal handling and approved by Institutional Animal ethics committee (955/A/06/CPCSEA). All the selected animals were kept under acclimatization on the same day. The animals were acclimatized for minimum 5 days before initiation of dosing. The rats were housed in standard polypropylene cages with stainless steel top grill in-group of 6 rats per cage. Clean autoclaved paddy husk was used as bedding. The paddy husk was changed at least thrice in a week. The animals were kept in a clean environment with 12-hour light and 12-hour dark cycles. The air was conditioned at 22±3ºC and the relative humidity was maintained between 30-70% with 100% exhaust. Standard rat pellet feed was provided *ad libitum* throughout the study, except over night fasting prior to blood collection and was offered the feed immediately after completion of blood collection of all the animals. Drinking water was provided *ad libitum* in polypropylene bottles with a stainless steel sipper tube throughout study period.

Acute toxicity study

The *Pterospermum acerifolium* methanol extract was suspended in 5% (v/v) Tween 80 solution. Healthy rats of either sex, weighing between 150-250 were divided in groups of 6 (3male + 3female). The *Pterospermum acerifolium* extract was administered by gavages at doses of 0, 4, 8, 12, 16 and 20g/kg body weight in single doses to both female and male rats.

The animals were observed for general behavioral changes, signs of toxicity and mortality continuously for 1h after treatment, then intermittently for 4h, and there after over a period of 24 h. The Rats were further observed for up to 14 days following treatment for behavioral changes and signs of toxicity /death and the latency of death. The LD_{50} values were determined according to the method of Litchfield and Wilcoxon (1949). [12]

Study of sub-acute toxicity

For the Sub-acute study, *Wister albino* rats of either sex, weighing 100-240g, were divided into 4 groups (I-IV) of 6 rats each (3females and 3males) and weights was recorded. While the first group was maintained as control, groups (II-IV) were administered 100mg/kg body weight/day, 1g/kg body weight/day and 2g/kg body weight/day of the methanolic extract p.o. for 28 days. [13] The doses were selected based on results of acute toxicity studies and OECD 407 Guidelines. [14]

After completion of the treatment period of 28 days, all the animals were sacrificed on day 29 and the biochemical tests were carried out.

Also histopathological study of Liver, Kidney & Stomach was done. All animals belonging to group number I to IV were twice daily upto day 28 of the treatment for any abnormal physical or behavioral change. The time of onset and intensity of such symptom, if any, was recorded. Symptoms noted included changes in skin, fur, eyes and mucous membranes, occurrence of secretions and excretions. Autonomic activity like lacrimation, piloerection, pupil size and unusual respiratory pattern. Changes in gait, posture, response to handling, presence of clonic or tonic movements, stereotypes like excessive grooming and repetitive circling or bizarre behaviour like self-mutilation, walking backwards etc.

Effect of sub-acute oral administration of extract on the body weight and mortality

All animals belonging to group number I to IV were observed daily twice up to day 28 of the treatment morbidity and mortality during observation period up to the termination of the study. All animals were subjected to ophthalmological examination one day prior to initiation of dosing and on one day prior to blood collection exactly before keeping the animal for fasting. The weight of each rat was recorded one day before initiation of treatment and at weekly intervals throughout the period of study. In addition, body weight of found dead animal, if any, was also recorded. The quantity of feed was offered based on the requirement to the group of animals housed in each cage (6 rats) and the same was recorded, the leftover of the feed was measured weekly once.

All live animals of treated dose groups numbered I to IV were euthanized on day 29 of each group of each group using carbon dioxide gas or Inj. Thiopentone sodium at a dose of 80-90 mg/animal intraperitoneally. Necropsy of all animals was carried out and findings were recorded. For all the found dead and/or moribund animals, necropsy was carried out on the same day and findings were recorded. The weights of the following organs were recorded for each animal. The organ weights were recorded as absolute values. Liver, kidney and stomach were weighed for all the animals belonging to all six groups.

ISSN: 0976-7126

Measurement of hematological and biochemical parameters

The hematological parameters [total red cell (RBC), leukocyte (WBC), platelet, hematocrit (HCT), hemoglobin (HGB), MCV (mean RBC volume), MCH (mean RBC hemoglobin) and MCHC (mean RBC hemoglobin concentration)] were determined using a blood automatic analyzer. Serum creatinine-Alkaline Picrate method (CREST BIOSYSTEMS. A Division of Coral Clinical Systems) [15], triglycerides-Enzymatic GPO-Trinder Method (ACCUREX BIOMEDICAL PVT. LTD. G-54, Midc Tarapur, Boisar) [16], cholesterol- Enzymatic CHOD-PAP method (CREST BIOSYSTEMS. A Division of Coral Clinical System) [17], aspartate amino transferase (AST, SGOT) [18], ala-nine amino transferase(ALT,SGPT-DNPH Colorimetric method)(Span Diagnostics Ltd., Surat, India)[19], bilirubin-Jendrassik & Grof Method (SIEMENS HEALTHCARE Diagnostics Pvt. Ltd. 589 Sayajpura)[20] were determined enzymatically using specific kits by measurement of the optical density of the reaction products at the corresponding wavelengths with a spectrophotometer.

Histopathological examination

The liver, kidney and stomach of rat was sampled, immobilized in 10% formaldehyde solution, wrapped with wax, and cut into slices with 4–5m thick. Formalin-fixed specimens were embedded in paraffin and stained with hematoxylin and eosin staining kit for 15 min. After being washed in turn with dimethylbenzene (5 min, twice), 100% ethanol (2 min), 95% ethanol (1 min), 80% ethanol (1 min), 75% ethanol (1 min) and distillated water (1 min), the liver, kidney and stomach slices were detected under a light microscope for conventional morphological evaluation [21].

Statistical analysis

Results are expressed as the mean value ± standard error of mean (S.E.M.). Within group comparisons were performed by the analysis of variance using ANOVA test. Significant difference between control and experimental groups was assessed by student's t-test. A probability level of less than 5 %($P < 0.05$) was considered significant.

Results and Discussion

Acute toxicity of *Pterospermum acerifolium* extract in rats

There were no deaths observed after oral administration of single doses of the *Pterospermum acerifolium* any dose level up to the highest dose tested (20g/kg b.w. p.o.). However there were signs of toxicity, namely anorexia and hypoactivity above 12 mg/kg b.w.p.o., which was the no-observed-adverse-effect level (NOAEL). Some adverse effects, such as hypoactivity and salivation, were seen immediately after feeding, while others (such as anorexia and weight loss) were observed later and were more pronounced at the higher doses (Table 1) [22]

Sub-chronic toxicity studies in rats

The body weights of control and *Pterospermum acerifolium (L)Willd* extract treated rats at various dose levels are presented in Table 4 and 5. No significant differences ($P<0.05$) in body weighs were recorded in the 28 day treatment period.

The effect of sub-chronic oral administration of MEPA on the hematological and the biochemical profiles of the treated and control rats are presented in (Table 2). Repeated oral administration of MEPA (up to a daily dose of 2gm/kg body weight) did not cause significant changes in plasma creatinine, triglyceride and alkaline Phosphatase levels ($P<0.05$). However, the liver marker enzymes (ALT and AST), Bilirubin and cholesterol levels were significantly increased ($P < 0.01$) in rats treated with 1gm/kg and 2gm/kg doses of the MEPA after 28 days of treatment; the effect was sustained until the end of the treatment period (28days). No significant alterations ($P<0.05$) in biochemical or haematological parameters were observed in the animals treated with 100 mg/kg b.w. (p.o.) for 28 days.

Histology of the liver sections of normal control animals (Figure-3a) showed normal hepatic architecture and normal liver lobular structure with well-preserved cytoplasm, prominent neucleous, and nucleolous. The toxicity (hepatic cell damage) was not prominent for the lower dose (100mg/kg b.wt.) group of animals, but the liver sections of the middle and high dose groups(1g/kg b.wt. and 2g/kg b. wt. respectively) of MEPA treated animals(Figure-3b-d) showed hepatic cells with toxicity characterized by centrilobular necrosis, periportal hepatocytic vacoulation with clearing of cytoplasm, heavy pigmentation around central veins, scattered inflammation, and giant cell transformation.

Histopathological photomicrographs of kidney sections from various treatment groups are shown in figure 4a-d. Histopathological examination of sections from rat kidney treated with high dose of MEPA (2g/kg. b.w.) , show severe and generalized tubular epithelial cell necrosis associated with diffuse tubular lumina in (Figure-4d) with the comparison against control (Figure-4a). The toxicity (glomerular cell damage) was not so prominent in lower dose (100mg/kg b.wt.) and cellular architecture could not be much differentiated when compared with the control group.

The mucosa and submucosa of the fundic region of the stomach are illustrated at a higher magnification in Fig. 5a-b. The extension of simple columnar surface epithelium, into the Gastric pits and the opening of the tubular Gastric glands into these pits are clearly seen in treatment groups (Figure-5b) in comparison to control (Figure-5a). Thus there is no significant alteration of gastric mucosa or gastric toxicity after repeated administration of MEPA. [23]

Phototherapy has never stopped gaining in popularity. In low and middle income countries, it often represents the main, if not, only therapeutic system to which majority of people are referred to for their primary health care [24]. Its widespread use is further substantiated by the affordability, knowledge of medicinal plants and the belief that they are harmless [25]. The increase in number of users as oppose to the scarcity of scientific evidences on the safety of the medicinal plants have raised concerns regarding toxicity and detrimental effects of these remedies [26] and the same applies for MEPA. This medicinal plant, just as the rest of these, contains several bioactive principles which have the potential to cause beneficial and/or detrimental effects. To optimize its safe use as plant-based medicine, one should, beside the historical documentation on MEPA, have a toxicity evaluation of this medicinal plant. The assessment of the safety of this dosage form of MEPA appears to be biologically essential [27].

Changes in body weight have been used as an indicator of adverse effects of drugs and chemicals (Table 4,5) and (Fig. 1,2) [28] · Since, no significant ($P<0.05$) changes were observed in the general behavior, body weight and food intake of rats in the treated groups as compared to the control group after 28-day period of daily treatment, it suggested that at the chronic oral doses administered, MEPA had no effect on the normal growth of rats. The hematopoietic system is one of the most sensitive targets for toxic compounds and an important index of physiological and pathological status in man and animal. The hematological parameters (i.e. RBC, haemoglobin, hematocrit, WBC, MCV, MCH and MCHC) showed no significant differences between the control and the treated groups indicating that MEPA had no effects on the circulating blood cells or on their production.

In this study, the biochemical parameters (i.e. AST, ALT, bilirubin and creatinine) also showed significant ($P<0.01$) treatment-related increases in the high dose group as compared to the control group. Indeed, the transaminases (AST and ALT) are well-known enzymes used as good indicators of liver function [29] and as biomarkers predicting possible toxicity [30]. Generally, any damage to the parenchymal liver cells results in elevations of both transaminases in the blood [31]. In addition, AST found in the serum is of both mitochondrial and cytoplasmic origin and any rise can be taken as a first sign of cell damage that leads to the outflow of the enzymes into the serum. Thus, the significant ($P<0.01$) increases observed in ALT and AST activities strongly suggest that the chronic administration of MEPA did alter the hepatocytes and consequently the metabolism of the rats. Equally, there was also a significant rise in creatinine in the high dose (2 g/kg b.w. p.o.) when compared to the control. Indeed, creatinine is known as a good indicator of renal function [32]. Any rise in creatinine levels is only observed if there is marked damage to functional nephrons [33]. Therefore, the results recorded in this study similarly suggest that MEPA did also alter the renal function. Clearly, this only serves as a preliminary test and that for a better estimation of renal function a creatinine clearance test is required.

Kaplan et al. [34] said the liver is the site of cholesterol disposal or degradation and its major site of synthesis. Significant ($P<0.01$) increase in cholesterol at high dose of extract shows hepatic damage and hence decreased cholesterol degradation.

At last, no significant ($P<0.05$) differences were recorded in the weights of the organs (Table 3) indicating that the sub-chronic oral administration of extract did not detrimentally affect the wet weight, organ-to-body weight ratio and the colour of organs. This study is the first to demonstrate that MEPA, which is claimed to be a cure for poor blood circulation and blood purifying agent, is a medicinal plant with potentially detrimental biological properties. If an extrapolation of the above results is to be made to humans, then it may be said that, precautions during use may be necessary, especially, in higher doses and over longer periods of administration. In conclusion, this study provides valuable data on the acute and sub-acute oral toxicity profile of *Pterospermum acerifolium* that should be very useful for any future in vivo and clinical study of this plant medicine. The *Pterospermum acerifolium* aqueous extract is non-toxic and has, at low dose, a low toxicity potential in acute and chronic oral administrations, respectively. However, at high chronic oral doses, *Pterospermum acerifolium* has significant hepatotoxic and nephrotoxic activities. Further studies to determine the effects of this plant on an animal foetus, on pregnant animals, and their reproductive capacity, etc. are needed to complete the safety profile of this drug. Further, aspects on the effects of this plant on haemopoiesis, liver and kidney function need to be gained and

ascertained over longer periods of study. Since the extract was nor not purified for chronic toxicity studies, nor the toxic principles involved in the study isolated, exact toxic nature of the principle involved in hepatic and renal toxicity could not be determined, and further studies need to be done.

Acknowledgement

The authors are thankful to Prof. Debesh Chandra Mazumder (Chairman, Trinity Trust,Asansol,W.B), Dr. Kalyan Kumar Sen (Principal, G.C.T.S., Asansol, W.B.) and Dr. Amalesh Samanta (Professor, Department of Pharmaceutical Sciences, Jadavpur University, Kolkata) for providing the resources to carry out this work and their valuable guidance. This research was supported by Mr. Uttam Kumar Bhattacharya, Mr. Aniruddha Mukherjee , Tanima Sarkar, Mr. Sankhadip Bose, Mr. Bibek Laha, Mr. Biswajit Manna, Mr. Gautam Bhunia, Mr. General Nagesh, Mr. Sadhan Chandra Das, Bodhisattwa Chakraborty and Paoulomi Chatterjee.

References

1. Anonymous, Wealth of India, Raw Materials. (1985). New Delhi: Publication and Information Directorate, P.227-9.
2. Manna Ashis Kumar, Jena Jitendra. (2009). Anti Inflammatory and Analgesic Activity of Bark Extract of *Pterospermum acerifolium, International Journal of Current Pharmaceutical Research*, Vol 1 Issue 1, pp 32-37.
3. Santanu Sannigrahi1, Sambit Parida, V. Jagannath Patro, Uma Shankar Mishra, Ashish Pathak. (2010). Antioxidant and Anti-inflammatory potential of *Pterospermum acerifolium* , *International Journal of Pharmaceutical Sciences Review and Research,* Volume 2, Issue 1, May – June, Article 001, pp 1-5.
4. Ashis kumar manna, Jitendra jena, Alok kumar behera,Dipankar roy, subhas manna, Dr. sanmoy karmakar, Dr. subrat kar. (2009). Effect of *Pterospermum acerifolium* bark extract on oxidative damages in the gastric tissue during alcohol induced ulceration, *International Journal of Pharmacy and Pharmaceutical Sciences*, vol-1`, suppl 1, nov.-dec, pp 51-59.
5. Aswini Kumar Senapati, Ranjan Kumar Giri, Dibya Sundar Panda and Sremantula Satyanarayan, Wound healing potential of *Pterospermum acerifolium* wild. With induction of tumor necrosis factor – α, *Journal of Basic and Clinical Pharmacy,* pp 203-208.
6. Sambit Parida, V. Jagannath Patro, Uma Shankar Mishra, Lucy Mohapatra, Santanu Sannigrahi. (2010). Anthelmintic potential of crude extracts and its various fractions of different parts of *Pterospermum acerifolium* Linn. *International Journal of Pharmaceutical Sciences Review and Research,* Volume 1, Issue 2, March – April, pp 107-111.
7. Shweta Saboo, Deore S. L., Khadabadi S.S., Deokate U. A. (*2007).* Evaluation of Antimitotic and Anticancer activity of the crude extracts of *Pterospermum acerifolium* willd leaves, *Nig. J. Nat. Prod. and Med*. Vol. 11, pp 75-78.
8. Agarwal SS, M. Paridhavi. Herbal Drug Technology. (2007).2nd ed. Hydrabad University Press, p.340-60.
9. Basu KR, Basu BD. Indian Medicinal plants. (1987). 2nd ed. Published by Bishen Sing, Mahendra P Sing, p.372-77.
10. Agarwal VS. (1964). Drug Plants of India. Kalyani Publishers. Vol.II .New Delhi, p.590-91.
11. Chatterjee A, Prakash SC. (1994). The treatise of Indian plants. Vol- III. Publication and Information Directorate, p.16-19.
12. Ghosh MN. (2005). Fundamentals of Experimental Pharmacology. Hilton & Company, Kolkata, p.196-97
13. Wang Q, Yuan B. (1997). Preclinical Safety Evaluation and Practice of New Drug: Military Medical Science Press Inc, p. 47.
14. OECD 407 guideline for the testing of chemicals: adopted on 03rd) October 2008-Repeated dose 28-day oral toxicity study in rodents.
15. Jaffe M. Uber den Niederschlag, welchen Pikrinsäure in normalen Harn erzeugt und über neue Reaktion des Kreatinines. (2003). Hoppe-Seylers Zeitschrift fur Physiologische Chemie 10, 391–400.
16. Cole, T.G., Klotzsch, S.G., McNamara, J. (1997). Measurement of triglyceride con- centration. In: Riafi, N., Warnick, G.R., Dominiczak, M.H. (Eds.), Handbook of Lipoprotein Testing. AACC Press, Washington, pp. 115–126.
17. Deeg, R., Ziegenhorn, J. (1983). Kinetic enzymatic method for automated determina- tion of total cholesterol in serum. Clinical Chemistry 29, 1798–1802.
18. IFCC (International Federation of Clinical Chemistry). (1977).Committee on Standards, IFCC. Revised IFCC method for aspartate amino transferase(l-aspartate: 2-oxoglutarate amino trasferase,EC2.6.1.1).Clinical Chemistry and Laboratory Medicine 15,719–720.
19. IFCC (International Federation of Clinical Chemistry). (1980). IFCC methods for the

measurement of catalytic concentration of enzymes, Part 3. IFCC Method for alanine aminotransferase(l-alanine: 2-oxoglutarate aminotrasferase,EC2.6.1.2). Clinica ChimicaActa105, 147–154F.
20. Pearlman, F.C., Lee, R.T.Y. (1974). Detection and measurement of total bilirubin in serum, with use of surfactants as solubilizing agents. Clinical Chemistry 20, 447–453.
21. Bustos, M., Beraza, N., Lasarte, J., Baixeras, E., Alzuguren, P., Bordet, T., Prieto, J. (2003). Protection against liver damage by cardiotrophin-1: a hepatocyte survival factor Up-regulated in the regenerating liver in rats. Gastroenterology 125, 192–201.
22. Alexeeff, G.V., Broadwin, R., Liaw, J., Dawson, S.V. (2002). Characterization of the LOAEL-to-NOAEL uncertainty factor for mild adverse effects from acute inhalation exposures. Regulatory Toxicology and Pharmacology, 36: 96–105.
23. Victor P. Eroschenko, Ph.D. (1994). Atlas of Histology with Functional Correlations A Wolters Kluwer Company. Philadelphia, Baltimore, New York, London, Buenos Aires, Hong Kong, Sydney, Tokyo: pp 219,185,249.
24. WHO. (2007). WHO Guidelines for Assessing Quality of Herbal Medicines With Reference to Contaminants and Residues. World Health Organization, Geneva .
25. Springfield, E.P., Eagles, P.K.F., Scott, G. (2005). Quality assessment of South African herbal medicines by means of HPLC fingerprinting. Journal of Ethnopharmacology, 101: 75–83.
26. Saad, B., Azaizeh, H., Abu-Hijleh, G., Said, S. (2006). Safety of traditional Arab herbal medicine. Evidence-based Complementary and Alternative Medicine, 3: pp 433–439.
27. Van Wyk, B., van Oudshoorn, B., Gericke, N. (1997). Medicinal Plants of South Africa, 1st ed. Briza Publications, Pretaria: pp. 196–197.
28. Mukinda, J.T., Syce, J.A. (2007). Acute and chronic toxicity of the aqueous extract of Artemisia afra in rodents. Journal of Ethnopharmacology, 112 : pp 138–144.
29. Hilaly, J.E., Israili, Z.H., Lyouss, B. (2004). Acute and chronic toxicological studies of Ajuva Iva in experimental animals. Journal of Ethnopharmacology, 91: pp 43–50.
30. Mdhluli, M.. (2003). Toxicological and antifertility investigations of oleanolic acid in male vervet monkeys (Chlorocebus aethiops). PhD Thesis. Discipline of Physiological Sciences, University of the Western Cape, Cape Town, South Africa. PathCare, 2009. Laboratory Manual. Parow, Cape Town, South Africa.
31. Slichter, S.J. (2004). Relationship between platelet count and bleeding risk in thrombocytopenic patients. Transfusion Medicine Reviews, 18: pp 153–167.
32. Rahman, M.F., Siddiqui, M.K., Jamil, K. (2001). Effects of Vepacide (Azadirachta indica) on aspartate and alanine aminotransferase profiles in a subchronic study with rats. Human and Experimental Toxicology; 20: pp 243–249.
33. Lameire, N., Van Biesen, W., Vanholder, R. (2005). Acute renal failure. The Lancet, 365: pp 417–430.
34. Kaplan, A., Jack, R., Opheim, K.E., Toivola, B., Lyon, A.W. (1995). Clinical Chemistry Interpretation and Techniques. Williams &Wilkins, USA, 4th ed: pp. 155–333.

Table 1:Acute toxicity study of MEPA by oral administration

Doses (g/kg)	Sex	D/T	Mortality Latency	Symptom of Toxicity
0	M, F	0/3, 0/3	--	None
4	M, F	0/3, 0/3	--	None
8	M, F	0/3, 0/3	--	None
12	M, F	0/3, 0/3	--	None
16	M, F	0/3, 0/3	--	Anorexia, hypoactivity
20	M, F	0/3, 0/3	--	Anorexia, hypoactivity

Table 2: Effect of MEPA on haematological and biochemical blood parameters of rats after 28 day oral administration

Parameter	Dose group (expressed in mean ± S.E.M., n=6)				
	Control D0 (0mg/kg)	Control D28 (0mg/kg)	Low dose D28 (100mg/kg)	Mid dose D28 (1g/kg)	High dose D28 (2g/kg)
WBC($\times 10^3/\mu L$)	11.5±3.2	11.9±3.1	12.6±1.2	10.5±0.6	11.2±0.4
RBC($\times 10^{12}/l$)	7.1±0.1	7.17±0.24	6.84±0.12	6.92±0.13	6.84±0.24
Haemoglobin(/g/dl)	14.2±0.2	13.74±0.33	12.98±0.33	12.90±0.32	12.80±1.03
Haematocrit(%)					
MCV(fl)	0.44±0.02	0.36±0.01	0.35±0.01	0.36±0.02	0.38±0.03
MCHC(g/dl)	58.5±0.5	53±0.7	51.8±0.2	52.8±1.3	53.5±1.2
MCH(pg)	35±0.1	37.34±0.5	36.8±0.4	35.5±1.1	34.7±1.0
Platelet count($\times 10^9/l$)	21±0.1	20±0.2	20±0.1	20±0.3	20±0.4
	798.4±20.5	880.67±50.2	890.8±90.2	850.2±150.5	835.2±170.6
Bilirubin					
	1.73± 0.2	1.73± 0.2	0.47±0.02**	11.02±1.1**	13.39±1.3**
ALT(μ/l)					
AST(μ/l)	71.3 ± 2.2	75.38 ± 3.2	82.7 ± 7.2	105 ± 2.2**	168.75±3.2**
Creatinine(μ/l)	80.9 ± 3.2	84.98 ± 5.5	93.37 ± 2.5	129.54±2.3**	196.90± 3.1**
Cholesterol(mmol/l)	29.38±2.2	31.38 ± 3.3	28.01 ±2.01	29.19 ± 5.2	32.98 ± 6
ALP(Alkaline phosphate)	31.22±1.8	33.83±3	37.45±10	45.92±9	58.61±2.2**
Triglyceride `	70.22±2.2	74.58±3.1	158.68±3.9**	90.85±2.1*	81.35±3.2
	25.3±4.2	27.33±5.2	28.94±4	30.75±4.2	20.50±2.2

The data are expressed as mean ±S.E.M. Significant differences in each group versus the control were as follows * P < 0.05. ** P < 0.01

Table 3: Weight of liver and kidney in 28 day sub acute toxicity study

Dose group	Wet Weight (gm; mean ± S.E.M.; n=6)	
	Kidney	Liver
ControlD0 (0g/kg)	1.2 ± 0.06	5.1 ± 0.02
Control D28 (0g/kg)	1.5 ± 0.12*	5.6 ± 0.53
Low dose D28 (100mg/kg)	2 ± 0.53	6 ± 0.59
Mid dose D28 (1g/kg)	2.2 ± 0.57	6.3 ± 0.65
High dose D28 (2g/kg)	2.5 ± 0.59	1.8 ± 1.49

The data are expressed as mean ±S.E.M. Significant differences in each group versus the control were as follows * P < 0.05. ** P < 0.01.

Table 4: Body weight of male rats treated with MEPA

Days	Group			
	Control	MEPA 100mg/kg b.w.	MEPA 1g/kg b.w.	MEPA 2g/kg b.w.
0	150.2 ±5.6	140.2 ±4.8	150.1±4.8	170.4 ±5.1
7	152.4 ±4.8	142.6 ±5.0	148.3 ±5.2	165.2 ±3.7
14	155.4 ± 5.2	135.2 ±3.6	145.6 ±3.9	164.8 ±4.6
28	156.5 ±6.6	135.6 ±3.6	143.2 ±5.4	160.2 ±4.4

The data are expressed as mean ±S.E.M. Significant differences in each group versus the control were as follows * P < 0.05. ** P < 0.01.

Table 5: Body weight of female rats treated with MEPA

Days	Group			
	Control	MEPA 100mg/kg b.w.	MEPA 1g/kg b.w.	MEPA 2g/kg b.w.
0	142.6 ±2.8	135.6 ±3.6	160.2 ±6.8	165.2 ±3.3
7	144.7 ±4.2	134.6 ±4.6	158.4 ±4.1	164.0 ±4.8
14	142.8 ±2.9	134.8 ±5.6	156.5 ±2.2	160.4 ±3.9
28	144.6 ±3.2	132.4 ±4.1	155.4 ±3.2	155.2 ±2.5

The data are expressed as mean ±S.E.M. Significant differences in each group versus the control were as follows * P < 0.05. ** P < 0.01.

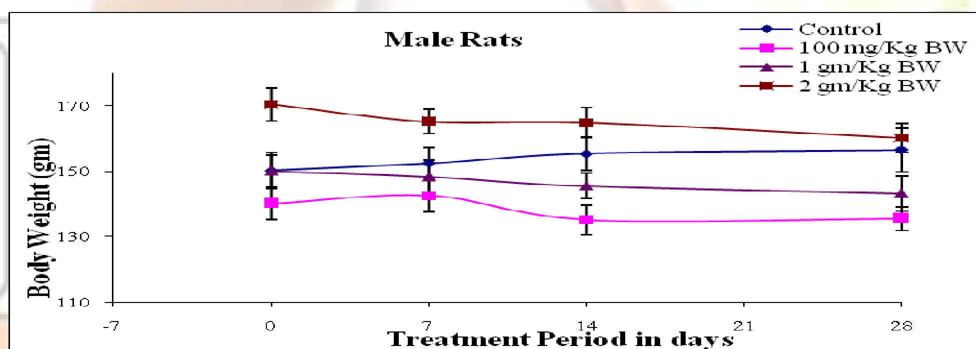


Fig 1: Body weight of male rats Vs treatment period in days

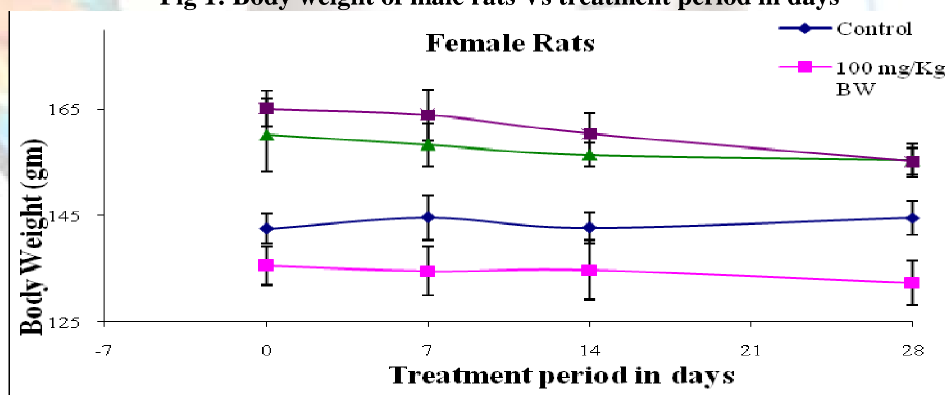


Fig 2: Body weight of female rats Vs treatment period in days

ISSN: 0976-7126

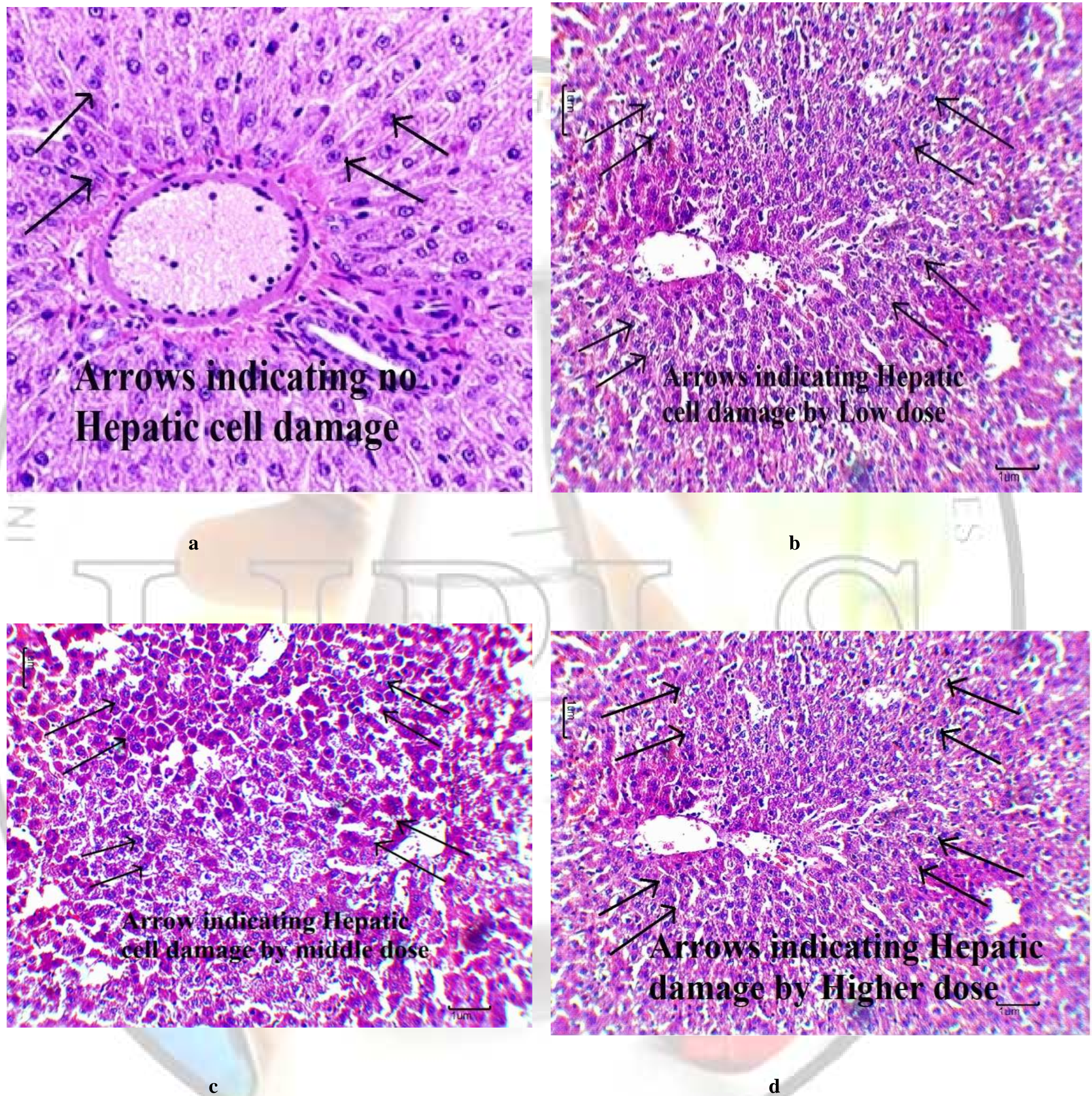


Fig. 3: (a-d): Effects of the *Pterospermum acerifolium* on liver histomorphology in rat.
Fig. 3a: Control, Fig. 3b: Treatment with 100mg/kg b.w. MEPA. Fig. 3c: treatment with 1g/kg b.w. MEPA, Fig. 3d: Treatment with 2g/kg b.w. of MEPA (40X)

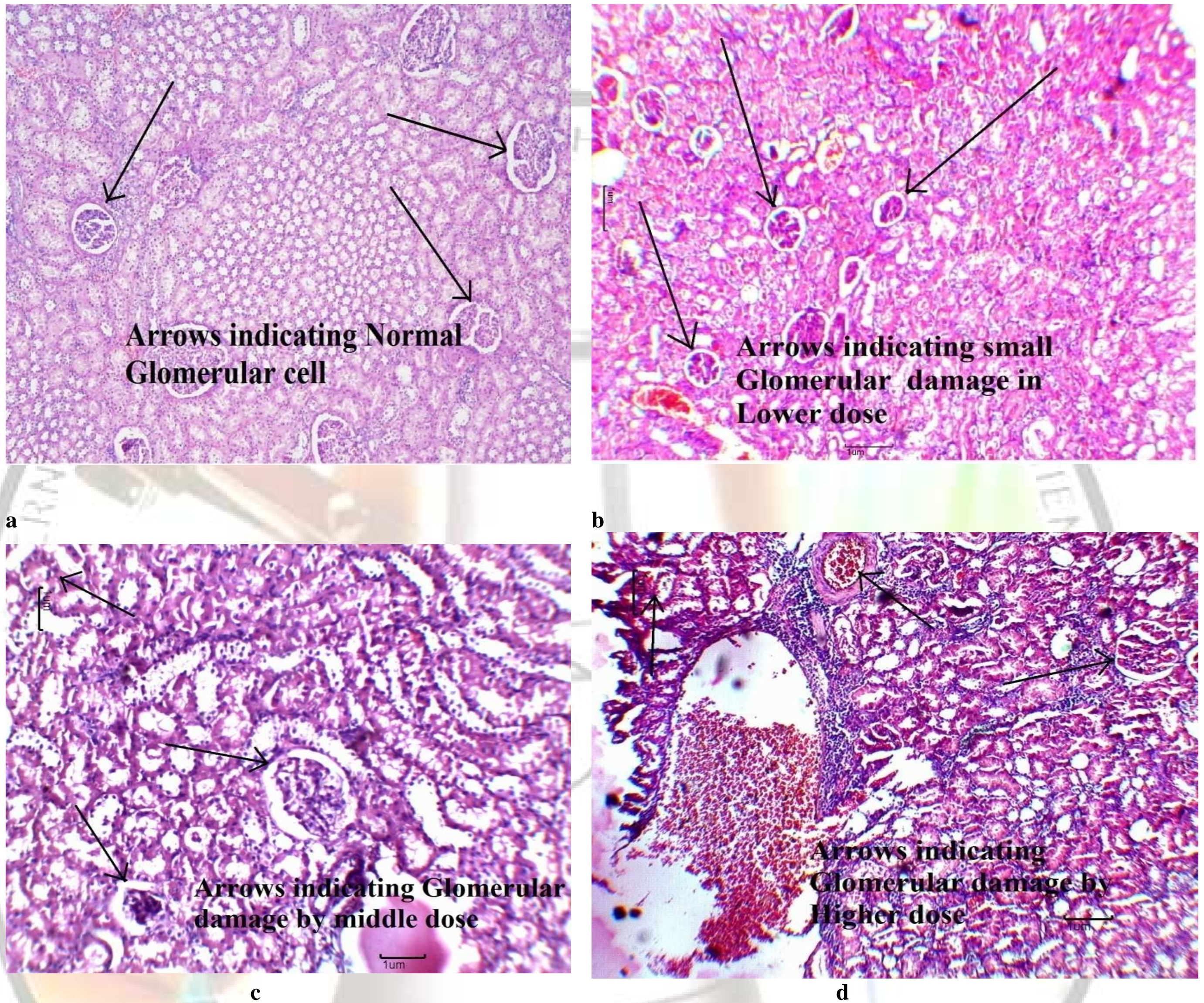
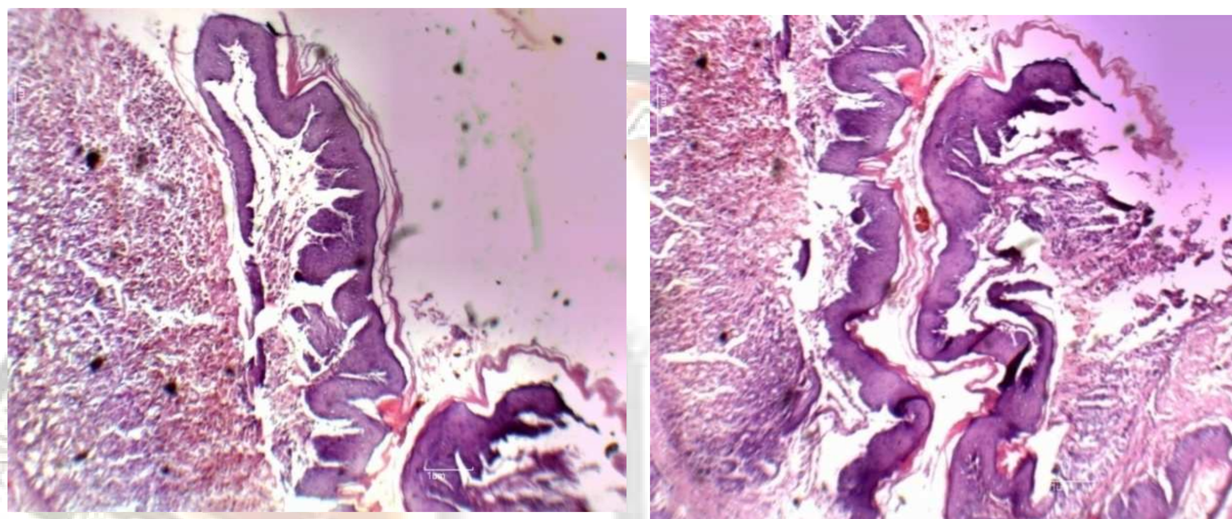


Fig. 4 (a-d): Effects of the *Pterospermum acerifolium* on Kidney histomorphology in rat. Fig. 3a: Control, Fig. 3b: Treatment with 100mg/kg b.w. MEPA. Fig. 3c: treatment with 1g/kg b.w. MEPA, Fig. 3d: Treatment with 2g/kg b.w. of MEPA (40X)



a b

Fig. 5(a-b): Effects of the *Pterospermum acerifolium* on Stomach histomorphology in rat. Fig. 5a: Control. Fig. 5b: Treatment with 2g/kg b.w. MEPA (40X)

IJPLS