

## EFFECTS OF AQUEOUS AND ETHANOLIC LEAF EXTRACTS OF *PTEROCARPUS MILDBRAEDII* ON RENAL AND HEART FUNCTIONS OF ALBINO RATS

<sup>1</sup>EZEKWESILI, Chinwe Nonyelum, <sup>1</sup>ADEGBITE, Adesola Victor and  
<sup>2</sup>OKANI, Onyeaghana Chukwudi

<sup>1</sup>Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

<sup>2</sup>Department of Anatomic Pathology, Anambra State University, Awka, Anambra State, Nigeria.

**Corresponding Author:** Adegbite, A. V. Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. **Email:** [adevic16@yahoo.com](mailto:adevic16@yahoo.com) **Phone:** +234 7033267514

### ABSTRACT

*Pterocarpus mildbraedii* leaf is among the commonly consumed leafy vegetables in Nigeria. Ethanol and aqueous extracts of the leaves had been found to exert anti-diabetic effect in rats. This study was designed to investigate the possible toxicological effects of the ethanol and aqueous leaf extracts of this plant on renal and heart functions in Wistar albino rats. Both extracts were administered intraperitoneally to four groups of rats at the doses of 200 and 400 mg/kg body weight for 28 days. The renal and heart function indices were analyzed using standard methods. Histopathological evaluation of the kidney was also carried out. Results showed no remarkable alterations of serum levels of renal and heart function indices estimated. No significant differences ( $p > 0.05$ ) in sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ), chloride ( $\text{Cl}^-$ ), bicarbonate ( $\text{HCO}_3^-$ ), urea and creatinine (CRSC) levels used to assess renal functions were observed at the end of the 28 days-treatment when compared with control group that received saline. Also, serum levels of lactate dehydrogenase (LDH), and aspartate aminotransferase (AST) were insignificantly ( $p > 0.05$ ) affected. Furthermore, histopathological examination of the kidney samples revealed normal cellular architecture in both control and treated groups. These results suggest non-toxic effects of leaf extracts of *P. mildbraedii* on the kidney and heart in rats. Hence, the plant can be considered safe for use as leafy vegetable, pharmaceutical and or nutraceutical formulations.

**Keywords:** *Pterocarpus mildbraedii*, Renal function, Lactate dehydrogenase, Kidney function, Heart, Toxicology

### INTRODUCTION

A wide variety of leafy vegetables are consumed in Nigeria. Plant-derived foods, particular vegetables and fruits, are beneficial components of the human diet. They contribute great importance in daily life by providing wide range of nutrients, vitamins and other substances (Newman *et al.*, 2003). The selection of a particular vegetable for inclusion in the diet depends on a number of factors such as availability, indigenous knowledge and cultural practice (Eyo and Mohme, 2003). Different

authors have reported on chemical studies carried out on the commonly used Nigeria leafy vegetable. Nevertheless, a careful examination of literatures revealed that, there are some less commonly used and inexpensive leafy vegetables whose nutritional potentials as well as their possible toxicological effects on living organism have not been adequately studied. One of such is *Pterocarpus mildbraedii* Harms (Leguminosae) leaf which is locally known as *Ora* in *Ibo* (Akpayung *et al.*, 1995).

*Pterocarpus mildbraedii* belongs to the family Leguminosae. It grows mostly in the

eastern part of Nigeria. The exudations produce gums and resins which have been used for various purposes. In some part of Eastern Nigeria, the young and tender leaves of this plant are used traditionally as vegetable for the preparation of soups (Smith, 1983) and there has been claim that it possesses anti-diabetic properties.

Toxicity is an expression of being poisonous, indicating the state of adverse effects led by the interaction between toxicants and cells. This interaction may vary depending on the chemical properties of the toxicants and the cell membrane. The toxic effects may take place prior to the binding of the toxicants to the vital organs such as liver and kidneys. Hence, evaluation of toxic properties of a substance is crucial when considering the substance for public health consumption. In practice, the evaluation typically includes acute, sub-chronic, chronic, carcinogenic and reproductive effects (Asante-Duah *et al.*, 2002).

This study was designed to scientifically investigate the possible toxicological effects of ethanol and aqueous extracts of *P. mildbraedii* on renal and heart functions in albino rats.

## MATERIALS AND METHODS

**Plants Materials:** The fresh leaves of *P. mildbraedii* were collected from Abagana in Njikoka Local Government Area of Anambra State. The leaves of *P. mildbraedii* were then identified (TPL, 2010) and authenticated by a taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka, where the voucher specimen (NAUH No. 162) was deposited in the herbarium. The leaves were separated from the stalk and air-dried to a constant weight under shade at room temperature after which they were grounded and sieved to obtain a fine powdered form of the leaf.

**Experimental Animals:** Forty male (40) Wistar albino adult rats weighing  $200 \pm 20.35$  grams were purchased from the Animal House of the Veterinary Medicine Department, University of Nigeria, Nsukka. They were acclimated for two weeks in stainless metabolic

rat cages in the Animal House of Applied Biochemistry Department, Nnamdi Azikiwe University, Awka. Rats were fed (Growers Mesh, Guinea Feed Nigeria PLC) and water *ad libitum*.

**Preparation of Crude Extracts:** The extraction was carried out at room temperature with 500 g of the powdered leaves macerated in 2 litres of distilled water for 72 hours to obtain the aqueous extract, while another 500 g of the powdered leaves was macerated in 2 litres of 80 % ethanol to prepare the ethanolic extract. The aqueous extract was filtered through clean muslin cloth and the extraction process was repeated by adding another 2 litres of distilled water. The ethanol extraction was carried out with the use of soxhlet extraction. The filtrate from the extraction of ethanol extract was concentrated by evaporating the excess ethanol to obtain thick slurry of ethanol extract. The same process was applied during aqueous extraction to obtain a thick aqueous extract.

**Study Design:** The animals were fed commercial manufactured Growers Mesh, Guinea Feed Nigeria PLC) and water *ad libitum* for the period of the experiment (28 days). The animals were sorted and divided into five groups (A – E), replicated twice with each replicate having four rats. Group A is the control which was not given the extracts of *P. mildbraedii* but were given 1 ml kg<sup>-1</sup> of normal saline, group B and C received 200 mg kg<sup>-1</sup> and 400 mg kg<sup>-1</sup> of ethanol extract of *P. mildbraedii* intraperitoneally respectively while group D and E were exposed to 200 and 400 mg kg<sup>-1</sup> of aqueous extract of *P. mildbraedii* respectively.

## Study of the Extract's Effects on Renal Functions

**Determination of electrolytes:** The rats were sacrificed on day 0, 14 and 28 post administrations of the extracts. One animal per replicate was sacrificed on day 0, 14 and 28. The blood samples were collected into plain bottle and the serum part of the blood was used to study the possible effects of the extracts on the electrolytes such as Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, HCO<sub>3</sub><sup>-</sup>. The electrolytes were analyzed using Vitros DT60

manufactured by Ortho Clinical diagnostic, Johnson and Johnson, USA. The analyzer estimate electrolytes by principle of direct potentiometry as reported by Sharma and Sharma (2013).

**Determination of urea:** 0.1 ml of the serum was used to examine the possible effects of the extracts on the Urea. Urea was estimated using Vitros DT60 machine which use Vitros Urea DT slides to analyze urea. A drop (10  $\mu$ l) of serum was deposited on the slide and was evenly distributed by the spreading layer to the underlying layers of slide. Water and nonproteinous components then travel to the underlying reagent layer where urease reaction generates ammonia. The semi-permeable membrane allows only ammonia to pass through to the color-forming layer where it reacts with ammonia indicator form a dye. The reflection density of the dye was measured by colorimetric method at 660 nm and is proportional to the concentration of urea in the sample (Vitros DT60, 2003).

**Determination of creatinine:** Another 0.1 ml of the serum was used to examine the possible effects of the extracts on creatinine. Creatinine (CRSC) was estimated using Vitros DT60 machine which use Vitros CRSC DT slide to analyze creatinine. A drop (10  $\mu$ l) of serum was deposited on the slide and was evenly distributed by the spreading layer to the underlying layers. Creatinine diffuses to the reagent layer where it was hydrolyzed to creatine in the rate-determining step. The creatine was converted to sarcosine and urea by creatine amidinohydrolase. The sarcosine in the presence of sarcosine oxidase was oxidized to glycine, formaldehyde and hydrogen peroxide. The final reaction involves the peroxidase-catalyzed oxidation of a leuco dye by hydrogen peroxide to produce a colored product. The intensity of the colored product was measured by the principle of spectrophotometry at 680 nm and is proportional to the concentration of creatinine in the serum (Vitros DT60, 2003).

### **Study of the Extract's Effects on Heart Functions**

**Determination of aspartate aminotransferase activity:** 0.1 ml of the serum was used to examine the possible effects of the extracts on aspartate aminotransferase (AST). The estimation of AST was carried out using Vitros DT 60 machine which is an automated chemistry analyzer. The machine use Vitros AST DT slide which is a multilayered, analytical element coated on a polyester support. A drop (10  $\mu$ l) of serum was deposited on the slide and was evenly distributed by the spreading layer to the underlying layers. The spreading layer contains aspartate and  $\alpha$ -ketoglutarate which are substrates of AST. In the assay for aspartate aminotransferase, the amino group of L-aspartate is transferred to  $\alpha$ -ketoglutarate in the presence of pyridoxal-5-phosphate to produce glutamate and oxaloacetate. Malate dehydrogenase then catalyzes the conversion of oxaloacetate and reduced nicotinamide adenine dinucleotide (NADH) to malate and oxidized nicotinamide adenine dinucleotide (NAD<sup>+</sup>). The rate of oxidation of NADH was monitored by reflectance spectrophotometry at 340 nm. The rate of change in reflection density is proportional to enzyme activity in the sample (Vitros DT60, 2003).

**Determination of lactate dehydrogenase activity:** Another 0.1 ml of the serum was used to examine the possible effects of the extracts on Lactate dehydrogenase (LDH). The estimation of LDH was carried out using Vitros DT 60 machine which is an automated chemistry analyzer. The machine use Vitros LDH DT slide which is a multilayered analytical element coated on a polyester support. A drop (10  $\mu$ l) of serum was deposited on the slide and was evenly distributed by the spreading layer to the underlying layers. Lactate dehydrogenase catalyzes the conversion of pyruvate and NADH to lactate and NAD<sup>+</sup>. The oxidation of NADH which was monitored by reflectance spectrophotometry at 340 nm was used to measure lactate dehydrogenase activity in the serum (Vitros DT60, 2003).

**Histopathology of Kidney:** Kidney samples isolated from each individual rat, macroscopically examined for changes in colour when compared to the untreated rats and later fixed in 10 % normalized buffered formalin, routinely processed and embedded in paraffin wax. Paraffin sections (15  $\mu$ m) were cut using rotary microtome, placed on glass slides and stained with Haematoxylin and Eosin (Bancroft and Stevens, 1982). The slides were examined under a light microscope and the magnified images of the tissue structures were captured for further study (Mcmanus and Mowry, 1984).

**Statistical Analysis:** Statistical analysis involved use of the Statistical Package for Social Sciences (SPSS) for data analysis. Data were analyzed for their central tendencies using descriptive statistics and the results express as mean  $\pm$  standard error of mean. Differences in treatment means were established using analysis of variance (ANOVA) with F-LSD post hoc and probability values of less than 5 % ( $p < 0.05$ ) were considered statistically significant.

## RESULTS

**Renal Function:** Figures 1 and 2 showed the effects of aqueous and ethanol extracts of *P. mildbraedii* on the kidney of albino rats. The extracts of *P. mildbraedii* did not have any significant effects ( $p > 0.05$ ) on serum levels of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$ , urea and creatinine used for assessing renal function when compared with the baseline analysis carried out on all the groups before treatment with the extracts of *P. mildbraedii*. Also, there were no significant differences when the results of follow up renal function analysis (i.e. the renal function analysis carried out after the baseline renal function analysis) obtained from extracts treated rats was compared with the results obtained from follow up renal function analysis carried out on the rats in the control group.

**Heart Function:** Figures 3 and 4 showed the effects of aqueous and ethanol extracts of *P. mildbraedii* on the heart of albino rats. The extracts of *P. mildbraedii* used in this study did

not have any significant effects on serum level of lactate dehydrogenase and aspartate aminotransferase ( $p > 0.05$ ) used for assessing heart function when comparing the results obtained from the extracts treated rats with the results obtained from baseline analysis carried out on all the groups before treatment with the extracts of *P. mildbraedii* as shown in the Figures 3 and 4. Also, there were no significant differences when the results of follow up (i.e. analysis carried out on the rats after day 0 be it on the extracts treated rats or rats in the control group) heart function analysis obtained from extracts treated rats was compared with the results obtained from follow up heart function analysis carried out on the rats in the control group.

**Histopathology:** Macroscopic examination of kidney of the rats treated with extract showed no changes in color compared to control. Autopsy at the end of the experiment period revealed no apparent changes in the kidney from both control and extracts treated rats in the histopathological analysis. The microscopic studies of the organs showed unnoticeable differences between the cellular architecture obtained from the rats in control and test groups. The microscopic examination revealed that the kidney cells from the extract treated rats did not show any alteration in cellular structure when viewed under Carl Zeiss Research Microscope (Axioskope 40, Germany) using multiple magnification power with a digital camera attached with the representation areas of the slide digitally photomicrographed (Figures 5 – 7). The structure or coordination of cells in extract treated organ was more or less similar compared with the control organ. Hence, the results of the histopathological analysis correlate with the results obtained from biochemical analysis.

## DISCUSSION

Phytotherapeutic products from medicinal plants have become universally popular in primary healthcare, particularly in developing countries, and some have been mistakenly regarded as safe just because they are a natural source.

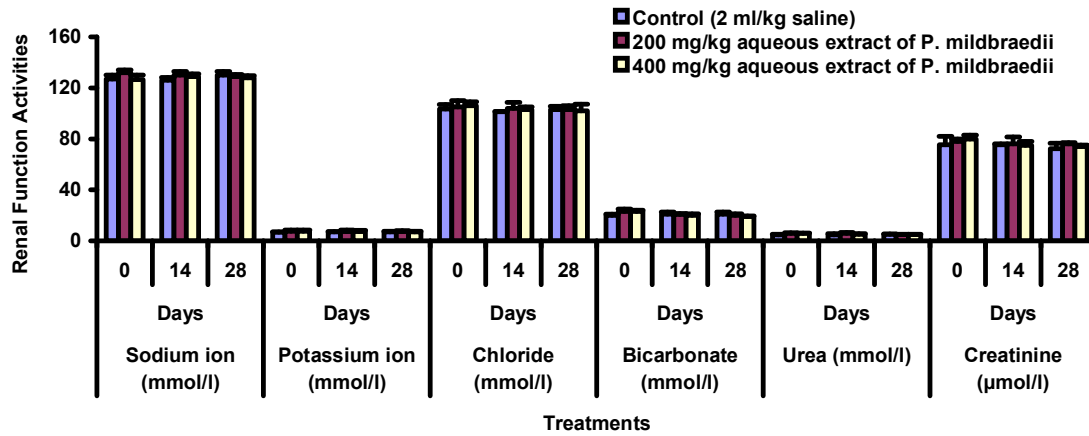


Figure 1: Effects of aqueous extract of *Pterocarpus mildbraedii* on kidney function of male albino rat

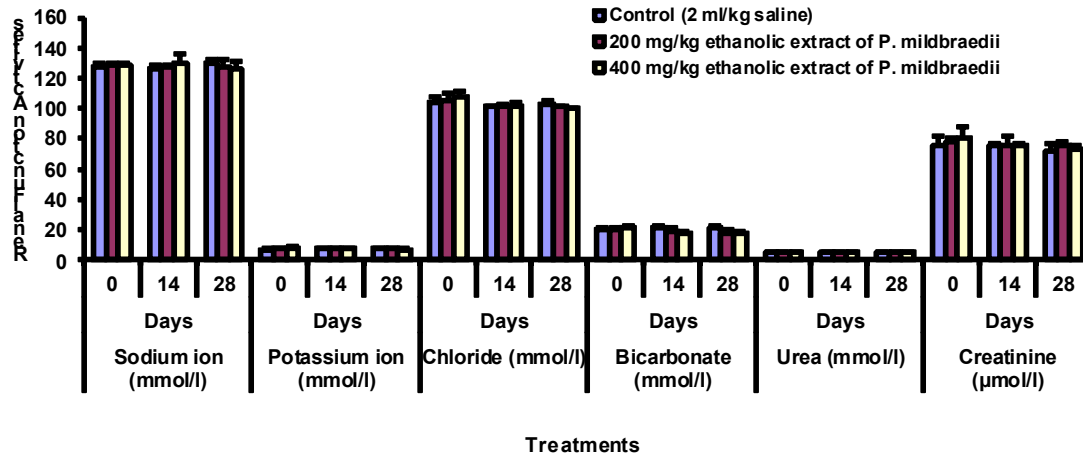


Figure 2: Effects of ethanol extract of *Pterocarpus mildbraedii* on kidney function of male albino rat

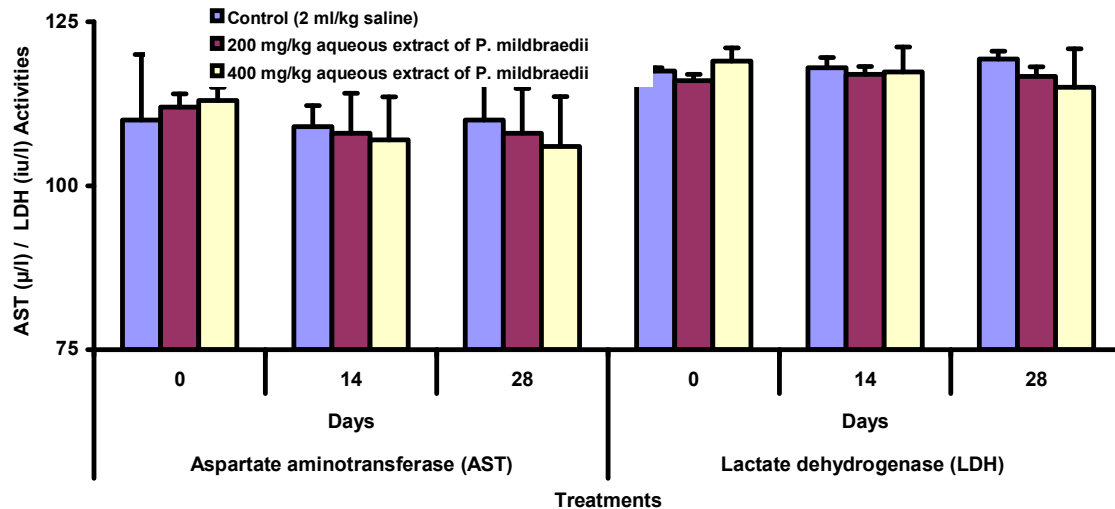
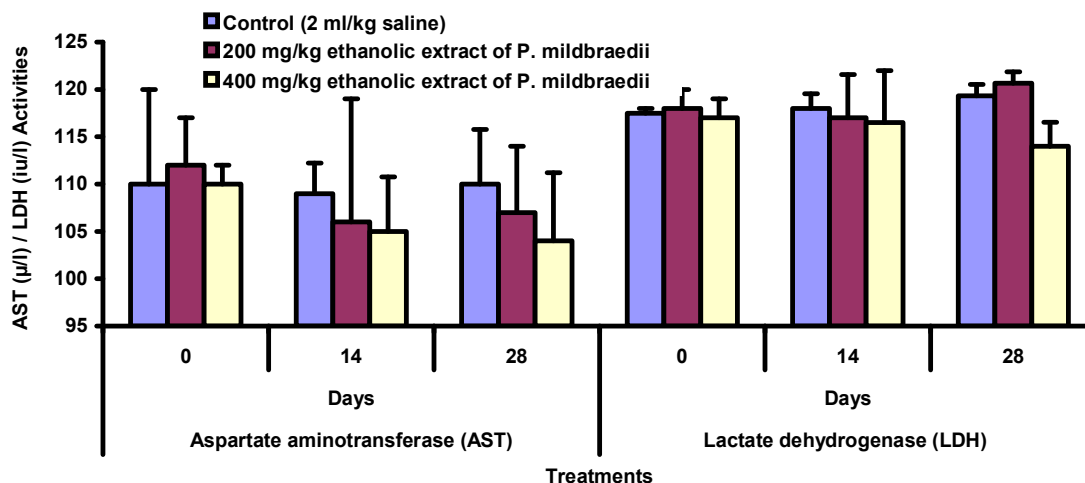
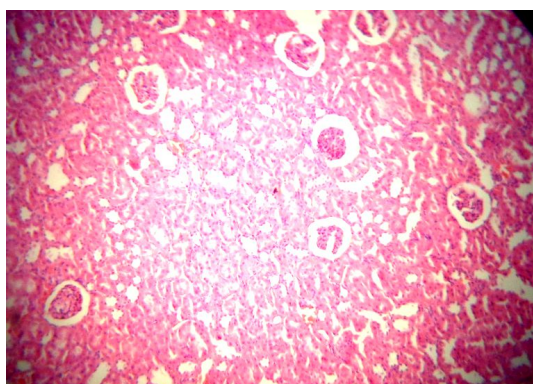


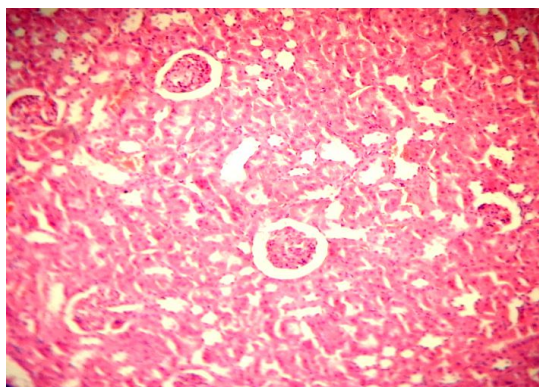
Figure 3: Effects of aqueous extract of *Pterocarpus mildbraedii* on heart function of male albino rat



**Figure 4: Effects of ethanolic extract of *Pterocarpus mildbraedii* on heart function of male albino rat**



**Figure 5: Histopathological photomicrograph of kidney of rats not treated with extract of *Pterocarpus mildbraedii* (H&E, x200)**



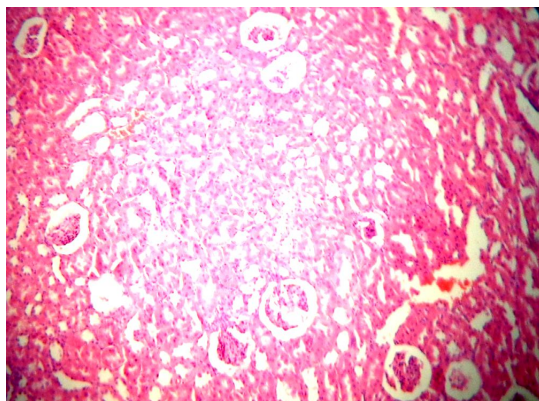
**Figure 6: Histopathological photomicrograph of kidney of rats treated with aqueous extract of *Pterocarpus mildbraedii* (H&E, x200)**

Nevertheless, these bioactive products from medicinal plants are presumed to be safe without any compromising health effect, and thus widely used as self medication (Vaghasiya *et al.*, 2011).

Therefore, toxicity study is vitally needed not only to identify the range of doses that could be harmful to human health but also to reveal the possible clinical signs elicited by the substances under investigation (Rang *et al.*, 2001). Toxicity results from animal studies will be crucial in definitively judging the safety of plants (medicinal and foods) if they are found to have sufficient nutritional potential as well as therapeutic potentials for development into pharmacological products (Moshi, 2007).

The 24 hour acute toxicity study of the intraperitoneally administered ethanolic and aqueous extracts of *P. mildbraedii* leaves revealed that the median lethal dose ( $LD_{50}$ ) lies within  $1258 \text{ mg kg}^{-1}$  and  $1778 \text{ mg kg}^{-1}$  body weights respectively. Finding from our study revealed that, all the used doses of ethanol and aqueous extracts of *P. mildbraedii* leaves did not have any significant effects on the biochemical parameters analyzed for the assessment of renal function. When the results obtained from baseline renal function analysis carried out for control group (A) was compared to the one obtained from the baseline analysis carried on other groups (B, C, D, E), there were no significant changes or effects which showed that





**Figure 7: Histopathological photomicrograph of kidney of rats treated with ethanolic extract of *Pterocarpus mildbraedii* (H&E, x200)**

all the rats were almost in the same or similar physiological conditions. There were no significance differences when results obtained from follow up (i.e. analysis carried out after the exposure of the rats to the extracts or subsequent to the analysis carried out on day 0 i.e. at day 14 and 28) renal function analysis carried out on the animals that received the extracts was compared with those obtained from animals in control group.

These observations are in agreement with earlier report (Mishra *et al.*, 2013) that ethanol extract of *P. marsupium* does not have toxicological effects on renal function but instead improved renal functions in rats with altered renal function after treatment with the extract for 28 consecutive days.

The observations are also in agreement with the earlier report (Dutta *et al.*, 2004) that aqueous extract of *P. santalinus* does not have toxicological effects on renal function but instead have a protective effect against nephron-toxicity induced in rats by sub-chronic exposure to coragen. These observations therefore suggest safety of extracts of the *Pterocarpus* species of vegetables.

After 28 days of treatment, no significant changes ( $p > 0.05$ ) were observed in serum levels of LDH and AST used for the assessment of heart function in extracts treated rats at all the doses administered compared to rats in control groups. This also indicates the non-toxic nature of the leaf extracts on heart function. These observations are in agreement

with earlier report (Mohire *et al.*, 2007) that at a higher dilution, the aqueous extract of *P. marsupium* possessed an excellent cardiotoxic activity.

Apart from biochemical analyses, histological analyses were carried out to further confirm the absence of alterations in cell structure of the organs. The histological examination is the golden standard for evaluating treatment related pathological changes in tissues and organs (OECD, 1995).

In this study, the kidney and heart micrograph shows that no adverse effects were observed in all groups. The glomeruli and capsules appeared normal and the Bowman's space are also marked clearly.

**Conclusion:** The present results show that leaf extracts of *P. mildbraedii* do not cause any apparent *in vivo* toxicity on the kidney and heart when studied in rat model since no death or signs of toxicity were observed in rats treated with both ethanol and aqueous extracts at doses 200 and 400 mg kg<sup>-1</sup>. Thus establishing safety in the use of *P. mildbraedii* leaf extracts. Hence, the use of *P. mildbraedii* can be encouraged both as a leafy vegetable and as a medicinal agent in known dosages, especially in rural communities where conventional drugs are unaffordable because of their high cost. A detailed experimental analysis of its chronic toxicity is essential.

## REFERENCES

- AKPAYUNG, I. O., UDOH, A. P. and AKPAN, E. J. (1995). Chemical composition of the edible leaves of *Pterocarpus mildbraedii*. *Plant Foods for Human Nutrition*, 48: 209 – 215.
- ASANTE-DUAH, K. (2002). *Public Health Risk Assessment for Human Exposure to Chemicals*. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- BANCROFT, J. D. and STEVENS, A. (1982). *Bancroft's Theory and Practice of Histological Techniques*. Churchill Livingstone, New York.

- DUTTA, K., ALI, M., NAJAM, A., KUMAR, R. and KUMAR, A. (2004). Ameliorative effect of seed extract of *Pterocarpus santalinus* on coragen induced haematological alterations and serum biochemical changes in rats, *Journal of Toxicology and Environmental Health Sciences*, 6(10): 194 – 202.
- EYO, E. S. and MOHME, A. N. (2003). Chemical composition and amino acid content of *Gnetum africanum*. *Nigerian Journal of Nutritional Science*, 4: 57 – 62.
- MCMANUS, J. G. A. and MOWRY, R. W. (1984). *Staining Methods: Histological and Histochemical*. Harper and Row, New York, USA.
- MISHRA, A., SRIVASTAVA, R., STRIVASTAVA, S. P., GAUTAM, S., TAMRAKAR, A. K., MAURYA, R. and STRIVASTAVA, A. K. (2013). Antidiabetic activity of heart wood of *Pterocarpus marsupium* Roxb. and analysis of phytoconstituents. *Indian Journal of Experimental Biology*, 51(5): 363 – 374.
- MOHIRE, N. C., SALUNKE, V. R., BHINSE, S. B. and YADAV, A. V. (2007). Cardiotonic activity of aqueous extract of heartwood of *Pterocarpus marsupium*. *Indian Journal of Experimental Biology*, 45(6): 532 – 537.
- MOSHI, M. J. (2007). Brine shrimp toxicity evaluation of some Tanzanian plants used traditionally for the treatment of fungal infections. *African Journal of Traditional, Complementary and Alternative Medicine*, 4: 219 – 225.
- NEWMAN, D. J., CRAGG, G. M. and SNADER, K. M. (2003). Natural products as sources of new drugs. *Journal of Natural Products*, 66: 1022 – 1103.
- OECD (1995). 28-Day repeated dose oral toxicity study in rodents. Pages 32 – 40. In: *OECD Guideline for Testing of Chemicals*. 2<sup>nd</sup> Edition, Organization for Economic Co-operation and Development, Paris, France.
- RANG, H. P., DALE, M. and RITTER, J. (2001). *Pharmacology*. 4<sup>th</sup> Edition, Churchill Livingstone, New York, USA.
- SHARMA, B. and SHARMA, D. (2013). A comparative evaluation of sodium and potassium measurements by flame photometer and by direct ISE methods. *International Journal of Health Sciences and Research*, 3(6): 59 – 65.
- SMITH, I. F. (1983). Use of Nigerian leafy vegetables for modified nitrogen, sodium and potassium. *Nigerian Journal of Nutritional Sciences*, 4: 21 – 27.
- THE PLANT LIST (2010). *A Working List of All Known Plant Species*. The Plant List (TPL). <http://www.theplantlist.org/>. Accessed July 28, 2015.
- VAGHASIYA, Y. K., SHUKLA, V. J. and CHANDA, S. V. (2011). Acute oral toxicity study of *Pluchea arguta boiss* extract in mice. *Journal of Pharmacological Toxicology*, 6: 113 – 123.
- VITROS DT60 (2003). *Operator's Manual for Vitros DT60*. Ortho-Clinical Diagnostics Incorporated, Johnson and Johnson, USA.