HAEMATOLOGICAL AND RENAL HISTOMORPHOLOGICAL ASSESSMENTS OF RATS PRETREATED WITH METHANOL STEM BARK EXTRACT OF *PARINARI KERSTINGII* AND INTOXICATED WITH ACETAMINOPHEN

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ABSTRACT

Acetaminophen is known to induce acute kidney injury; and medicinal plants have been recorded to be beneficial in management of such injuries. This study investigated the effects of methanol stem bark extract of Parinari kerstingii (MSBEPK) on the haematology and renal histomorphology of acetaminophen-intoxicated rats. Fifty four albino Wistar rats assigned into six groups, replicated thrice with three rats per replicate were used for the study. Groups A and B received 10 ml/kg distilled water to serve as normal and negative controls respectively; C-E were pretreated with 200, 400 and 600 mg/kg of MSBEPK respectively while Group F rats were given 100 mg/kg of Silymarin. All pretreatments lasted for14 days and on the 15th day, 2000 mg/kg of acetaminophen was administered to all the rats except those in Group A. 48 hours post acetaminophen administration, blood samples were collected for Haematology and thereafter, the kidneys were harvested for histopathology following euthanasia. Results showed that red blood cell counts, packed cell volume values and the haemoglobin concentrations of the MSBEPK-pretreated rats were significantly higher than those of the negative control (Group B). The leucocytic profiles did not show any significant variations across the groups. The kidneys of the rats pretreated with the extract showed little or no lesion as opposed to those of the acetaminophen-treated control group that showed degenerative and necrotic lesions. It was concluded that MSBEPK could improve haematological values and protect the kidneys against deleterious effects occasioned by acetaminophen intoxication.

Keywords: Acetaminophen, Haematology, Kidney, *Parinari kerstingii*, Rats

INTRODUCTION

Medicinal plants have been used to treat diseases since ancient times. *Parinari kerstingii* Engl (Malpighiales: Chrysobalanaceae) is indigenous to West Africa and has ovoid-shaped fruits. It is an evergreen plant that can reach heights of up to 20 meters (Burkill, 1997). The plant is called locally as "kaikayi" by Hausas in northern Nigeria, "okpe" by Yorubas in western Nigeria, "Kakyiki" in Ghana, "ningelia" in Togo, and "aramon" by Ivorians (Odu *et al.*, 2018). It is referred to as "koko" by Igbos, a group of people from southern Nigeria. The herb is used in ethnomedicine to treat discomfort, bronchopneumonia, and as a purgative and emetic agent (Odu *et al.*, 2018). The anti-inflammatory, antioxidant and analgesic potentials of *P. kerstingii* extract had earlier been reported by some researchers (Linus *et al.,* 2018; Odu *et al.*, 2018).

Acetaminophen (Paracetamol), commonly known as acetyl-para-aminophenol (APAP), is an over-the-counter painkiller commonly used for pain treatment and inflammatory management (Saleem and Iftikhar, 2019). The medication is processed mostly in the liver and to a lesser extent in the kidney by cytochrome p-450 enzymes, particularly CYP2E1 (Bessems and Vermeulen, 2001). The active metabolite NAPQI has been widely blamed for the negative symptoms associated with drug overdose (Shao *et al*., 2021). Glutathione, a master antioxidant, is charged with mitigating the negative side effects of acetaminophen metabolism, when it (Paracetamol) is provided at the authorized amount (Saleem and Iftikhar, 2019). When misused or at extremely high quantities, the metabolite NAPQI overwhelms the system and causes organ damage (Saleem and Iftikhar, 2019). Aside from the liver, researchers have discovered that an overdose of paracetamol causes severe renal injury.

Haematology refers to the study of blood and blood disorders. Blood is a bodily fluid and tissue which is essential in diagnosis of certain ailments and disorders. Researchers had earlier reported adverse effects of acetaminophen on blood parameters (Swem *et al.,* 2020). At a very normal dose of paracetamol, haematological aberrations were not recorded (Oyedeji *et al.,* 2013). However, when acetaminophen was administered in a very high dose, varying haematological disorders including anaemia were observed (Iweala and Osundiya, 2010). The disorders observed in haematological values following acetaminophen intoxication were secondary to liver damage (Adeneye *et al.,* 2008). It is a general knowledge that glutathione is a master antioxidant which protects the red blood cell (RBC) membranes. During metabolism of acetaminophen overdose, this glutathione is consumed (Saleem and Iftikhar, 2019). This situation renders the RBCs vulnerable to haemolysis.

The kidney is a vital organ responsible for many physiologic functions in the body including excretion of metabolic by-products such as urea. Another important function of the kidney is in the regulation of erythropoiesis. It is known that kidney failure results in anaemia of hypoproliferative type due to decreases in erythropoietin secretion (Renner *et al.,* 2020). Erythropoietin or haemopoetin is a glycoprotein produced by the interstitial fibroblast of the kidneys (due to hypoxia) to stimulate erythropoiesis (Renner *et al.,* 2020). The uremic state occasioned by renal failure also results in moderate degree of haemolysis (Corrigan and Boineau, 2001).

This study investigated the possible haematological aberrations and renal histomorphological changes associated with methanol stem bark extract of *P. kerstingii* pretreatment and intoxication with acetaminophen.

MATERIALS AND METHODS

Plant Materials: The *P. kerstingii* stem bark was collected from the wild and identified (Prance and Sothers, 2003) and authenticated by a plant taxonomist from the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The voucher specimen number InterCEDD/16285 was deposited in the herbarium of the International Center for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu State, Nigeria for referral purposes. The stem bark was cut into tiny pieces and shade dried to a constant weight.

Preparation of Plant Extract: The stem barks were collected, cleaned of dust, air dried and pulverized with a mortar and pestle. The powdered *P. kerstingii* stem bark (500 g) was extracted with 80% methanol using cold maceration and vigorous shaking at two hour intervals for 48 hours. The methanol extract was filtered using Whatman filter paper (Number 1) and then concentrated using a rotary evaporator under reduced pressure.

Acute Toxicity of *Parinari kerstingii* Stem Bark Methanol Extract: Thirty (30) albino Wistar rats assigned to six groups (A – F) of five rats per group were used for acute toxicity studies. Groups B – F rats were orally administered with 50, 200, 800, 3200 and 5000 mg/kg of the extract respectively. Group A rats

were given 10 ml/kg of distilled water to serve as normal control. The rats were watched for 48 hours for signs of toxicity such as sedation, excitement, death etc. (Aba *et al.*, 2012).

Animals: Fifty four male Wistar strain rats weighing 130 – 135 g were procured from the Animal House, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were divided into groups and housed in stainless wire mesh cages for two weeks to acclimate to 28 – 32°C prevailing laboratory temperature. During the stabilization period and throughout the trial, Chikun Feed grower pellet (18% crude protein, 2800.0 kcal/kg metabolizable energy) and clean water were fed *ad libitum*.

This study was approved by the Faculty of Veterinary Medicine, University of Nigeria Nsukka Ethical Committee according to the guidelines of the Institutional Animal Care and Use Committee (IACUC) (IACUC, 2020) with the approval number: FVM-UNN-IACUC-2023-09/116b.

Experimental Design: A total of 54 rats were divided into six groups (A – F), replicated three times, with each replicate having three rats. The extracts were given to the rats orally by gastric gavage. Groups A and B acted as normal and negative controls, receiving simply distilled water (10 ml/kg). Groups C – E were given 200, 400 and 600 mg/kg of a methanol extract of *P. kerstingii*, respectively, whereas rats in Group F were given Silymarin (100 mg/kg), a conventional drug. These methanol extract of *P. kerstingii* dosages were 1/25, 1/12.5 and 1/8.33 fractions of the LD_{50} (5000 mg/kg). All therapies were administered once a day for 14 days. On day 15, rats in Groups B – F were given a single dosage of 2000 mg/kg acetaminophen orally. Blood samples were collected from the retrobulbar plexus in the medial canthus of the eye 48 hours after acetaminophen treatment for haematological study. Thereafter, the rats were humanly sacrificed under chloroform fume anesthesia and the kidneys harvested for histopathological study.

Determination of Haematological Parameters: The erythrocyte and leucocyte counts were determined by the haemocytometer method (Thrall and Weiser, 2002). Packed cell volume determination was by microhaematocrit method as described by Thrall and Weiser (2002), while the haemoglobin concentration (Hb) was determined by the cyanmethaemoglobin method (Higgins *et al.* 2008). Differential leucocyte counts were also done using the method of Thrall and Weiser (2002).

Histopathological Examination: Histopathological examination was done following Drury *et al.* (1967). Briefly, a thin section of kidney tissue (2 cm in diameter) was trimmed with a sharp blade. The small pieces of the tissue were placed in the 10% formalin, the container was shaken gently several times to make sure that the fluid had reached all surfaces and that pieces were not sticking to the bottom. This was then incubated at 25^0C for 24 hours, to allow proper fixing. The fixed tissue pieces were washed with running water for 24 hours to free them from excess fixatives. The dehydration was achieved by immersing the thin sections of the tissue in automatic tissue processor containing 12 jars. Solutions of xylene were used for clearing the tissue sections. Clearing was done to remove opacity from dehydrated tissue. A period of 15 minutes was allowed to elapse before the tissue was removed from the solution for infiltration with paraffin. Paraffin wax at 50 to 52◦C was used to infiltrate the tissue. The tissue was transferred directly from the clearer to a bath containing melted paraffin. As soon as the tissue was thoroughly infiltrated with paraffin, it (paraffin) was allowed to solidify around and within the tissue. The embedded blocks were trimmed into squares and fixed in the microtome knives for sectioning after which the sections were floated on a water bath. Glass slides were thoroughly cleaned and a thin smear of albumen fixative was made on the slides. The albumenized slide was used to collect the required section from the rest of the ribbon in the water. The section on the glass slide was kept moist before staining. The slides were passed through a series of jars containing alcohols of decreasing strength and various staining solutions. The slides prepared were mounted on photomicroscope, one after the other and viewed at different magnification

power of the microscope. Photograph of each of the slides was taken.

Data Analysis: Data generated were analyzed with one-way Analysis of Variance (ANOVA) using SPSS version 20. Variant means were separated using Duncan's Multiple Range Test and the results presented as Means ± Standard Error of Mean (SEM) in tables.

RESULTS

There were no signs of toxicity observed after 48 hour duration of the acute toxicity studies across all the groups. The LD_{50} of the extract was above 5000 mg/kg.

The erythrocytic profile results indicated that the mean RBC counts, PCV values and haemoglobin concentrations of the rats in Group B were significantly ($p<0.05$) lower when compared to those of the normal control rats and the extract and Silymarin-treated groups. There were no significant differences ($p>0.05$) in the mean MCV values across the groups. The mean MCHC and MCH values of Group B rats compares very well with that of the Group E rats but were significantly higher ($p<0.05$) when compared to those of Groups A, C, D and F (Table 1).

The results of the leucocytic panel showed that there were no significant differences ($p>0.05$) in white blood cell (WBC) counts of all the groups. In the same vein, the differential leucocyte counts showed no significant differences ($p>0.05$) in the neutrophil, lymphocyte, monocytes, eosinophil and basophil counts across all the groups (Table 2).

The kidney histophotomicrograph indicated that the renal histomorphology of normal rats showed normal Bowman's capsule and renal tubules while the kidney of rats intoxicated with acetaminophen showed necrosis of renal tubular cells and infiltration of inflammatory cells. The rats pretreated with the extract at varying doses showed mild or no lesions and were comparable to those of the normal control rats and to those pretreated with Silymarin especially those pretreated with the extract at the dose of 400 mg/kg (Figure 1).

DISCUSSION

This study evaluated the haematological profile and renal status of rats pretreated with methanol stem bark extract of *P. kerstingii* for 14 days and intoxicated with acetaminophen.

The acute toxicity studies indicated absence of any toxicity signs after 48 hours. This implies that the LD_{50} of the extract was above 5000 mg/kg. Earlier researchers had reported the wide safety margins associated with medicinal plants compared to their synthetic counterparts (Fabricant and Farnsworth, 2001).

Reductions in the RBC counts, packed cell volume (PCV) values and haemoglobin concentrations of the Group B rats (that were pretreated with distilled water for 14 days and intoxicated with 2000 mg/kg of acetaminophen) is an indication that acetaminophen overdose is capable of causing anaemia. Earlier researchers had reported various erythrocytic disorders associated with acetaminophen overdose (Iweala and Osundiya, 2010; McGill *et al.*, 2012). Metabolism of acetaminophen results in production of an active metabolite known as NAPQI which under normal conditions, will be completely conjugated by glutathione, a master antioxidant responsible for protecting RBC membranes against reactive oxygen species (ROS) and their attendant haemolysis (Aba *et al.,* 2019). Upon intoxication, the excessive production of NAPQI overwhelms glutathione and depletes the antioxidant levels and thus makes the erythrocytes vulnerable to haemolysis. This is thought to be the mechanism of anaemia following intoxication by acetaminophen. The extract-pretreated rats showed improvement in the erythrocytic (RBC, PCV and Hb) values compared to that of the negative control. It is thought that the extract may have protected against haemolysis of RBCs by mopping free radicals or by potentiating the activity of the antioxidant (glutathione) in protecting the blood cells. ROS are continually being produced by the erythrocytes due to high oxygen tension in the arterial blood (Baynes, 2005). Oxidative stress and accumulation of ROS in RBCs can induce haemolysis (Thomsen *et al.,* 2013).

Table 1: The erythrocytic profile of *Parinari kerstingii*-pretreated acetaminophen-intoxicated rats

Group	RBC (x10^{6})	PCV (%)	Hb (g/dl)	MCV (fl)	MCHC(g/dl)	MCH (pg)
A	5.95 ± 0.42^{b}	46.33 ± 0.33^{b}	14.20 ± 0.11^{b}	78.43 ± 5.71^{a}	30.60 ± 0.47^{a}	24.06 ± 1.68^{a}
B	3.75 ± 0.33^{a}	39.66 ± 0.88^{a}	12.87 ± 0.07^{a}	107.66 ± 12.44^{b}	32.47 ± 0.72^{b}	34.83 ± 3.26^{b}
C	4.68 ± 0.54a^{b}	45.33 ± 0.33 ^{b}	13.97 ± 0.29^{b}	99.53 ± 12.23^{ab}	30.80 ± 0.60^{a}	30.56 ± 3.41^{ab}
D	5.46 ± 0.27^{b}	46.33 ± 0.66^{b}	14.27 ± 0.06^{b}	85.23 ± 8.98^{a}	30.80 ± 0.60^{a}	26.20 ± 1.13^{a}
E	5.05 ± 0.46^{ab}	45.33 ± 0.66^{b}	14.23 ± 0.16^{b}	91.23 ± 8.98^{ab}	31.40 ± 0.10^{ab}	28.70 ± 2.76^{ab}
F	6.00 ± 0.50^{b}	47.00 ± 0.57^{b}	14.30 ± 0.15^{b}	79.43 ± 7.21^{a}	30.40 ± 0.11^{a}	24.13 ± 2.19^{a}

The different letter superscripts, a and b across the groups (in the same column) indicate significant differences at $p<0.05$

Table 2: The leucocytic panels of rats pretreated with methanol stem bark of *Parinari kerstingii* and intoxicated with acetaminophen

Group	TWBC (10^{3})	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)
A	7.10 ± 1.68^{ab}	32.66 ± 2.66^{ab}	66.00 ± 3.05^{ab}	1.33 ± 0.66^{ab}	0.00 ± 0.00	0.00 ± 0.00
B	5.90 ± 0.40^{a}	31.67 ± 4.41^{ab}	67.66 ± 4.33^{ab}	0.67 ± 0.67^{a}	0.00 ± 0.00	0.00 ± 0.00
C	7.93 ± 0.84^{b}	38.66 ± 9.33^{b}	60.00 ± 10.00^{a}	1.33 ± 0.66^{ab}	0.00 ± 0.00	0.00 ± 0.00
D	8.30 ± 0.97^{b}	38.00 ± 4.16^{b}	60.66 ± 5.21^{a}	1.33 ± 1.33^{ab}	0.00 ± 0.00	0.00 ± 0.00
E	6.46 ± 0.27^{a}	31.33 ± 5.69^{ab}	66.67 ± 6.66^{ab}	2.00 ± 1.15^{b}	0.00 ± 0.00	0.00 ± 0.00
F	7.43 ± 1.29^{ab}	28.00 ± 4.16^{a}	70.00 ± 5.29^{b}	2.00 ± 1.15^{b}	0.00 ± 0.00	0.00 ± 0.00

The different letter superscripts, a and b across the groups (in the same column) indicate significant differences at $p<0.05$

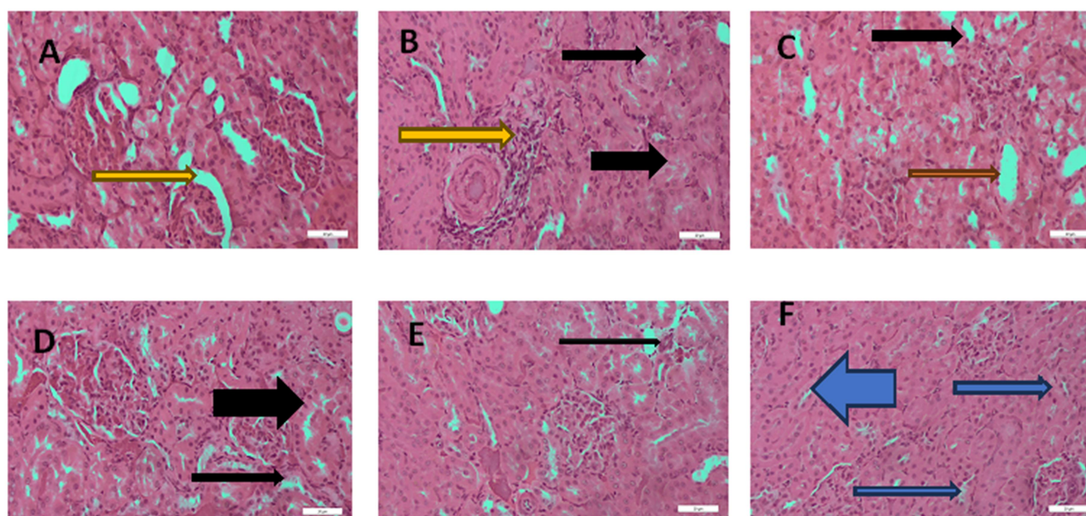

Figure 1: The kidney histophotomicrograph (H & E x400). ***Key:*** *A: The renal histomorphology of normal rats with Bowman's capsule (yellow arrow) and the renal tubules; B: The kidney of rats intoxicated with acetaminophen with necrosis of renal tubular cells (black arrows) and infiltration of inflammatory cells(yellow arrow); C: rats pretreated with 200 mg/kg of P. kerstingii extract and intoxicated with acetaminophen showing mild degenerative lesions of the renal tubular epithelial cells (arrows); D: rats pretreated with 400 mg/kg of P. kerstingii extract and intoxicated with acetaminophen showing normal renal histoarchitecture and intact renal epithelial cells (arrow); E: rats pretreated with 600 mg/kg of P. kerstingii extract and intoxicated with acetaminophen showing mild infiltration by inflammatory cells (arrow); F: rats pretreated with 100 mg/kg of Silymarin and intoxicated with acetaminophen showing normal histoarchitecture and intact renal tubular epithelial cells (arrows)*

The antioxidant activities of *P. kerstingii* had also been reported (Linus *et al.*, 2018).

The results of the total leucocyte counts and the differential leucocyte profiles did not show any significant variations across the groups. Similarly, the erythrocytic indices such as MCH, MCHC and MCV did not reveal any significant findings in the study. This observation is in agreement with submissions of some researchers who reported non-significant changes in the erythrocytic indices such as MCV, MCH and MCHC (Payasi *et al.,* 2010). However,

our finding is not in alignment with the reports of Iweala and Osundiya (2010) who reported significant variations in total leucocytes counts following acetaminophen intoxication.

Histopathology results indicated severe damages to the kidney with marked degenerative and necrotic lesions in the nephrons of Group B rats (Figure 1). The Group B rats are the negative control rats that were pretreated with distilled water for 14 days and then intoxicated with acetaminophen on the 15th day. Renal injuries occasioned by acetaminophen intoxication have earlier been reported by several researchers (Akakpo *et al.,* 2020). The photomicrograph of the kidneys of the rats that were pretreated with the extract prior to acetaminophen intoxication indicated mild or no lesions especially the group pretreated with 400 mg/kg of the extract. This is an indication that the extract may have ameliorated acetaminophen-induced acute kidney injury.

Conclusion: It was concluded that the methanol stem bark extract of *P. kerstingii* (MSBEPK) ameliorated haematological aberrations associated with acetaminophen overdose and protected the kidney against acetaminophen-induced acute kidney injuries especially in the rats administered with 400 mg/kg of MSBEPK.

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