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Research Article

The Nephroprotective Potential of the Ethanolic Leaf Extract of *Pterocarpus mildbraedii* on the Histology of Acetaminophene-Induced Kidney Damage in Albino Wistar Rats

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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 and nephroprotective potential in rats. This study was designed to investigate the possible nephroprotective effects of the ethanolic leaf extracts of this plant on the acetaminophen-induced Kidney damage in Wistar albino rats. Twenty adult albino Wister rats were purchased for this study. The rats were fed along-side with distilled water. The animals were grouped into five (5) groups: each group having four (4) animals as follows; Group A was given distilled water only (control group). Group B given 700 mg/kg of Paracetamol; Group C given 700 mg/kg of Paracetamol and treated with 54.8 mg/kg of plant extract. Group D was given 700 mg/kg of Paracetamol and treated with 27.4 mg/kg of plant extract. Group E given 700 mg/kg of Paracetamol and treated with 13.7 mg/kg of plant extract. Group A served as the control group, Group B, C, D, and E were administered with increasing dose of ethanolic extract of *Pterocarpus mildbraedii*. Histopathological evaluation of the kidney was also carried out. 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 in rat. Hence, the plant can be considered safe for use as leafy vegetable, pharmaceutical and nutraceutical formulations.

Keywords: Histopathology; Pharmaceutical *Pterocarpus mildbraedii*; Paracetamol; Nephroprotective

Introduction

Herbs have played an important role in maintaining and improving the quality of human life and well been for many years. Herbal extract from medicinal plants such as *Pterocarpus mildraedii, Alchornea cordifolea, Cassia alata* are used worldwide as a source of drugs or herbal extract for various chemotherapeutic purposes [1]. 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 [2]. 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 [3]. Different authors have reported on chemical studies carried out on the commonly used Nigeria leafy vegetable. *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 [4] and there has been claim that it possesses anti-diabetic properties. *Pterocarpus mildraedii* is found in Sierra leone, Liberia, Cote d' ivoire, Ghana, Benin, Cameroon, Garbon, Nigeria and the *Usambara* and *udzungwe* mountains of Tanzania [5]. It is a widely cultivated plant in the south-western, south-eastern, and south-southern parts of Nigeria. The leaves were discovered to have phenol compounds such as 1,2,3,4 Butanetetrol. Phenol compounds are known to have an anti-oxidant property which can be used to treat diseases such as cancer [6]. The Kidneys are two (2) bean-shaped organs found on the left and right sides of the human body and in most vertebrates (mammals). The Kidneys are located on the posterior (back) aspect of the abdomen, each being about 4-5 inches long [7]. Acetaminophen is a widely used analgesic and antipyretic also known as acetaminophen or APAP, chemically named N-acetyl-p-aminophenol. It was discovered in 1889. Paracetamol is the active metabolite of phenacetin and acetanilide, both are popular analgesic and antipyretics in their own rights [8].

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, Cross River University of Technology where the voucher specimen (CRUT No.112) 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

These animals were housed in well-ventilated cages and kept in the animal room in the Department of Physiology, University of Cross-River, Nigeria. The environmental condition for the animals was suitable, with good ventilation and was always cleaned and disinfected, the cages were also cleaned as well as beddings changed regularly. The rats were made to acclimatize to the animal room condition for two (2) weeks and were fed with grower's mash obtained from the vital feed company, Mission Road, Igholi, Ogoja, Cross River State. The rats were fed along-side with distilled water. Rats were fed (Growers Mesh, Guinea Feed Nigeria PLC) and water ad libitum.

The leaves of *Pterocarpus mildraedii* were locally harvested from a compound located at No 1 Itu Okon Lane, 4 Miles, Calabar Municipal, Cross River State, Nigeria. The leaves were washed and shade dried for two (2) weeks. The leaves were then blended to powder and macerated in 97% ethanol for 72 hours to give the crude ethanolic extract. The dry-crystalline extract was kept in a refrigerator.

Drug procurement, preparation and administration

The Emzor Paracetamol drug were purchased from Adonah Pharmacy, okuku, Yala Local Government Area, Cross River State. The Paracetamol tablets were dissolved in distilled water and administered orally by a daily dose of 700 mg/kg per body weight to groups B, C, D, and E. Followed by the oral administration of *Pterocarpus mildbraedii* leave extract daily concomitantly to groups C, D, and E, for a period of 28 days. At the end of the treatment period, the animals were sacrificed under light chloroform vapors, the kidneys of the animals were surgically removed.

Tissue processing

This process involves the systematic procedures involved in the preservation of the tissue from the point of acquisition to the point of mounting on the slides to enable them to be viewed under the light microscope. Fixation of the tissue was carried out with 10% neutral buffered formalin; the tissues were left in the fixative for 48 hours before undergoing the process of dehydration. The tissues were dehydrated in ascending grades of alcohol and two (2) changes each for one (1) hour. i.e. from 70%, 95%, and finally 100% (absolute alcohol). Clearing was carried out using Xylene in two (2) consecutive changes. Clearing in xylene was aimed at removing the alcohol in the tissue, since the alcohol is not miscible with paraffin used in embedding and also to improve the optical contrast of the tissues. Infiltration of the tissue was carried out in

two changes of molten paraffin wax in an oven at a temperature of sixty degrees Celsius. Embedding of the tissue was carried out using molten paraffin wax in an embedding mould. Embedding serves to harden the tissue to allow for sectioning using a rotary microtome in a process called microtomy. Sectioning was done with a rotary microtome into ribbon-like sections of five (5) micron thickness each. The cut sections were floated in water bath containing warm water and packed up on slides that have already been inserted or soaked in albumin.

Staining of sections

The sections were first treated in xylene to remove wax for two minutes. The slides were picked up, rinsed thoroughly in absolute alcohol then in tap water. The next step was staining in haematoxylin for 10-15 minutes. The tissue sections were then blued in running tap water for 5-10 minutes. Differentiation was done in 1% hydrochloric acid in 70% alcohol for 15-20 seconds. Bluing for 15 minutes, this was followed up by staining in eosin for two minutes. The tissues were then rinsed in tap water, then rinsed well in absolute alcohol as in ascending grades mentioned earlier and cleared in xylene, mounted and viewed under light microscope. Mounting of the tissue was done on Distrene Trieresy Phosphate Xylene (DPX) photomicrographs were taken at a magnification of X400. The next step was staining in haematoxylin for 10-15 minutes. The tissue sections were then blued in running tap water for 5-10 minutes. Differentiation was done in 1% hydrochloric acid in 70% alcohol for 15-20 seconds. Bluing for 15 minutes, this was followed up by staining in eosin for two minutes. The tissues were then rinsed in tap water, then rinsed well in absolute alcohol as in ascending grades mentioned earlier and cleared in xylene, mounted and viewed under light microscope. Mounting of the tissue was done on Distrene Trieresy Phosphate Xylene (DPX) photomicrographs were taken at a magnification of X400.

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 grouped into five (5) groups: each group having four (4) animals as follows; Group A was given distilled water only (control group). Group D given 700 mg/kg of Paracetamol; Group C given 700 mg/kg of Paracetamol and treated with 54.8 mg/kg of plant extract. Group D was given 700 mg/kg of Paracetamol and treated with 27.4 mg/kg of plant extract. Group E given 700 mg/kg of Paracetamol and treated with 13.7 mg/kg of plant extract. Group A served as the control group, Group B, C, D, and E were administered with increasing dose of ethanolic extract of *Pterocarpus mildbraedii*. It was observed that the weight of the animals was increasing until an average required weight of 190-250 grams were obtained before the administration of the ethanolic extract of Pterocarpus Mildraedii leaves took place from the third (3rd) week.

Results and Discussion

Histological observations

In Plate 1, which is the control group, the section of the kidney showed the cortex with prominent glomerulus and closely packed renal tubules. The Photomicrograph shows the aggregation of proximal convoluted (CT) with their brush borders thereby, giving the tissue parenchyma a spongy appearance. The collecting duct (CD) is seen with adjourning peritubular capillaries (PC). No pathology was observed on the liver.

Plate 1 conforms with a normal typical Histological appearance of the kidney.

In Plate 2, the animals were administered with 700 mg/kg of paracetamol only. The photomicrograph of this plate shows few distal convoluted tubules with a central area of focal tubular

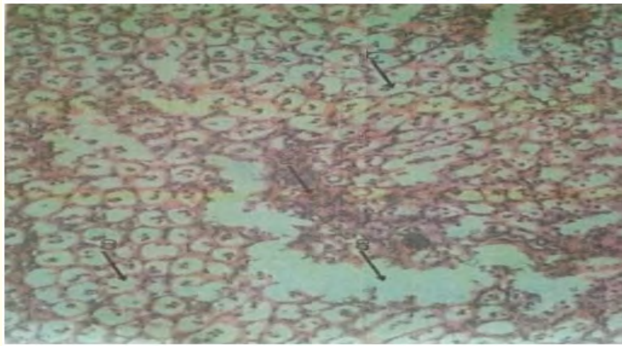

Plate 1

necrosis (encircled area), a mononuclear inflammation. This section of kidney shows variable degrees of distortion of the cortex, the mesangium is loosely packed and there is loss of glomerular architecture.

Plate 2

Plate 3, the animal was administered with 700 mg/kg of paracetamol and 60kg/mg of Pterocarpus Mildraedii extract. The photomicrograph of this plate shows the collecting tubules (CT), convoluted tubules indicating an insignificant tubulointerstitial distortions. A urinary/corpuscular space (CP) can also be seen. Therefore, no pathology is seen or observed.

Plate 4, the animals were administered with 700 mg/kg of paracetamol and 30 mg/kg of *Pterocarpus mildraedii* leaves extract. The Histological photomicrograph of this plate shows

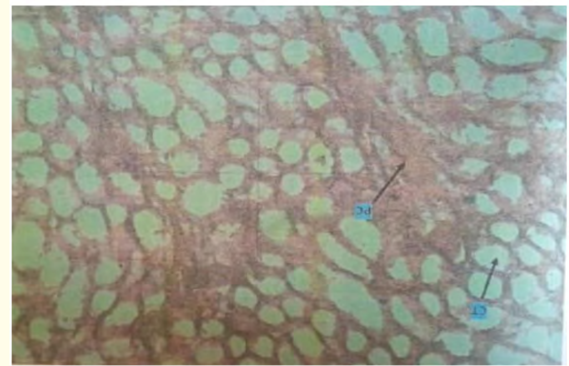

Plate 3

numerous convoluted tubules (CT), peritubular capillaries are also adjourning the tubules. Therefore, no pathology is seen.

Plate 4

Plate 5, the animals were administered with 700 mg/kg of paracetamol and 15 mg/kg of *Pterocarpus mildraedii* extracts. The Histological photomicrograph of this plate shows convoluted Tubules (CT) and Peritubular Capillaries interspersing them. Some Glomeruli are also seen with their vascular pole (VP). No pathology is seen.

Twenty-five adult albino wistar rats were used for this research. The research was carried out for 21 days and was establishing

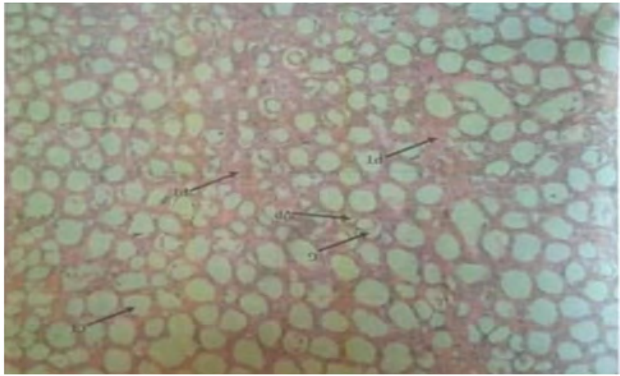

Plate 5

the possible effects of ethanolic extract of *Pterocarpus milbreadii* on the Histology of Paracetamol induced Nephrotoxicity in the Kidney of Wistar Rats. The rats were grouped in five groups; each containing five rats. The extract was administered to rats in groups C, D and E orally using orogastric tubes in three different dosages 60 mg/kg, 30 mg/kg and 15 mg/kg body weights of animals for a period of 14 days. The treatment of the extract followed the oral administration of 700 mg/kg paracetamol to groups B, C, D, and E. The animals were sacrificed after 14 days of administration through cervical dislocation and the kidneys were surgically removed for Histological analysis. From the photomicrographs taken, it could be deduced that the extract (in a low and moderate dose) protects the rats against paracetamol induced kidney cell degeneration.

Conclusion

Findings from this study suggest that the oral administration of *Pterocarpus mildbraedii* leaf extract can pose a protective effect on the kidney at a low and moderate dose. It also suggests that extract can pose a toxic effect on the kidney if administered in high dose therefore, the administration of the extract is dose dependent.

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