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Some of the effects of aqueous leaf extract of *Malabar nightshade* on the kidney and liver of albino wistar rats

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ABSTRACT

Leaves of the Basella rubra plant has been employed in varieties of use by Man. The plant is often used by Man as food supplement because of the nutritional potentials of the plant. The plant has been reported to have an excellent composition of calcium and iron; good source of vitamins A, B, and C, with a high roughage value. The aim of this study was to elucidate the effect of aqueous leaf extract of Basella rubra often used as food supplement on the kidney and liver of Wistar albino rat. Thirty-two adult Wistar albino rats of both sexes were used for this study. They were assigned into three extract treated groups designated as A, B, C, and were administered with 300 mg/kg, 200 mg/kg and 100 mg/kg body weight of the extract respectively, and one control group designated as D, administered with equal volume of phosphate buffered saline (PBS). Administration was done orally (once daily) using an orogastric tube for fourteen days (14d). Twenty-four hours after the last administration, all the animals were sacrificed using cervical dislocation, laparatomy was performed, the kidney and liver were excised, trimmed free of fat, rinsed in cold PBS solution. The liver was quickly fixed in 10% formolsaline, while the kidney was fixed in Bouin's fluid. In the kidneys, there were neither tubular necroses nor interstitial and glomerular hemorrhage. Both the distal and proximal convoluted tubules were free of occlusion. The glomeruli were also devoid of distortion, derangement and degenerative changes. The histology of the liver displayed normal parenchymal architecture with cords of hepatocytes and portal tracts. The central veins were well preserved. There was no ballooning degeneration of the hepatocytes, necrosis or formation of Mallory bodies. The sinusoids were also devoid of congestion and there were no cytoplasmic vacuolations of the hepatocytes. These findings suggest that the consumption of B. rubra has no deleterious implications on the histological profile of the kidney and liver of Wistar albino rats.

Key words: Malabar nightshade, orogastric tube, Hematoxylin and Eosin, Laparatomy, Phosphate buffered saline.

INTRODUCTION

The use of plant for nutritional and therapeutic purposes has been dated back to the 'old world' where trial and error methods were employed in the folklore means to treating ailments and diseases [1]. Diseases that remain most challenging in today's healthcare system tend to be complex involving cascading mechanisms, targets and drugs for effective disease management. In contrast to current combination therapies, plant based drugs contains a mixture of multiple components thereby saving considerable time and expenses [2].

Nowadays, many plants have been studied and some of their attributed empirical properties proved to be true. Most of these plants often display a wide range of biological and medicinal properties such as anti-inflammatory, anti-hemorrhagic, fertility enhancer, laxative, sexual enhancer, anti-microbial, anti-fungal [3-4].

Basella rubra is a native of the East Indies, and found its way to the New World from China. It has spread throughout the tropical world and it is one of the best tropical spinach widely adapted to a variety of soils and climates. It is particularly abundant in India, Malaysia, and the Philippines, but it is also seen throughout tropical Africa, the Caribbean, and tropical South America. The plant is a common market product, a popular leafy and stew vegetable. Both the young shoots and stems are eaten. It is an excellent source of calcium and iron; good source of vitamins A, B, and C, with a high roughage value. The roots are employed as rubefacient. Poultice of the leaves are used to reduce local swelling. Sap from the plant is applied to acne eruptions to reduce inflammation. Pulped leaves of the plant when applied to boils and ulcers, hasten suppuration. Leaf-juice of Malabar nightshade mixed with butter is soothing and cooling when applied to burns and scalds [5].

From the nutritional point of view, it is known that improving dietary intake and dietary habit can delay the onset of many cardiovascular diseases and diseases affecting nervous system. Phytochemical analysis of *B. rubra* shows that plant contains saponins A, B, C, and D, oleanane-type triterpenes oligoglycosides, together with betavulgaroside 1, spinacoside C, and momordins IIb and IIc [5]. Demand for nutritional food supplements and the act of self-medication calls for extensive study into the nutritional and medicinal properties of the plant alongside its possible side effects.

Although, there hasn't been any report on the harmful effect of this highly consumed plant on the body, it was known from laboratory testing, that high intake of food supplements and additive from certain plants source may have deleterious effect on the body. In view of the reputed efficacies of this vegetable plant, the aim of this study was to elucidate some of the implications of *B. rubra* on the histology of the kidney and liver in albino Wistar rats.

MATERIALS AND METHODS

Collection of plant and preparation of plant extracts

Fresh leaves of *B. rubra* were collected from the Botanical garden of the University of Ilorin, Ilorin, Kwara State, Nigeria and were authenticated at the Department of Plant Science University of Ilorin, Nigeria. The leaves of the plant were air-dried. The dried plant material were measured using Gallenkomp (FA2104A, England) electronic weighing balance and grinded with Blender/Miller III, (model MS -223, China). Sixty grams of the grinded plant sample were later soaked in 600 ml of PBS for 24 hours [6] at room temperature with constant shaking on a shaker (Stuart Scientific Orbital Shaker, UK), and then filtered through silk cloth [7], the filtrate was concentrated using a rotary evaporator (Rotavapor® R-210) at 42- 47°C. The residue of the extract obtained was kept in a capped sample bottle and stored in a refrigerator until used.

Toxicity evaluation:

The method of Lorke (1983) [8] was modified and used to determine the dose of the extract that would be lethal to 50% of the population of animals. Three dose points (550, 500 and 450 mg/kg) were chosen for the pilot experiment, from which, doses of 300 mg/kg, 200 mg/kg and 100 mg/kg respectively were administered to one animal per group in the second phase.

Animal care and experimental design:

The body weights of the rats were taken at 0d prior to *B. rubra* extracts treatment, and on daily basis thereafter for 14d using a digital weighing scales (Saltun® EK5055Max). The rats in the extract treated groups designated as A, B, and C were administered orally once daily (07:00am–07:45am) using plastic syringes attached to metal

oropharyngeal cannula with 300 mg, 200 mg and 100 mg per kilogram body weight of the aqueous leaf extract of *B. rubra* for 14d respectively, while the rats in the control group (group D) were administered with equal volume of PBS.

All the rats were housed in clean cages of dimensions 33.0×20.5×19.0 cm contained in well ventilated standard housing conditions (temperature: 28–31^{o}C; humidity: 50–55%). Their cages were cleaned everyday. The rats were observed for illness, abnormal behavior and physical anomalies. All experimental procedures followed the recommendations provided in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and Published by the National Institutes of Health [9]. The rats were fed with standard rat chow at a recommended dose of 100 g/kg as advised by the International Centre of Diarrheal Disease Research, Bangladesh (ICDDR, B) daily. Drinking water was supplied *ad libitum*.

Twenty-four hour after the last administration, all the experimental animals were sacrificed using cervical dislocation, laparatomy was performed, the kidney and liver were excised, trimmed free of fat, rinsed in cold PBS solution. The liver was quickly fixed in 10% formolsaline, while the kidney was fixed in Bouin's fluid for routine histological study.

Histological procedure:
After fixation, the tissues were embedded in paraffin wax; serial sections of 5µ thick were produced using Leitz Rotary microtome (Leitz 1512 Microtome). The sections were mounted in DPX. After fixing the kidney and liver of both the extract treated and control rats, the liver tissues were processed for Hematoxylin and Eosin, while the kidney tissues were processed for Period Acid Schiff (PAS) [10] staining techniques respectively. The photomicrograph of each slide was taken with a Nikon Digital Camera DXM1200F (Nikon, Japan) for subsequent histological analysis**.**

RESULTS

Microscopic observations of the liver
When the sections obtained from the histological processing of the liver was viewed under the microscope it was observed that the sections conformed to normal histological features. The sinusoids in the sections of the treated rats are devoid of occlusions and are not distorted. The histological outline of the rats in the control group was also preserved (Fig. 1).

Microscopic observations of the kidney;
The histological preparations of kidney from the extract treated and control rats showed that the various histological segments of kidney tubules were well preserved. Abundant glomeruli, nephrons with interspersed blood capillaries were also clearly seen. Various regions of kidney tubules appeared normal without any alteration in mesangial thickening or hyaline deposition. The renal parenchyma showed no evidence of distortion of any kind. The profile of the rats in the control group was also well preserved (Fig. 2).

DISCUSSION

A fourteen days study on the effects of *B. rubra* leaf on the kidney and liver was investigated to highlight the possible histological implications that could occur following its consumption. Using the Olympus binocular light microscope (XSZ-107BN, No. 071771), the results obtained from the sections of the kidney and liver stained with H&E and PAS respectively revealed that the plant extract has no adverse effects on the histology of the kidney and liver in Wistar albino rats.

Many herbal preparations have been known to exhibit renal tubular necrosis showing extensive interstitial fibrosis and severe tubular loss which are prominent in the outer cortex. Mengs *et al*. (1982) [11] showed that specific acid from herbal preparation are nephrotoxic in female Wistar rats which rapidly developed renal tubular necrosis and renal failure.

According to Steven and Lowe, (2005) [12], biochemical dysfunctions may result from impaired tubular functions as a result of failure of excretion of H^+ and K^+ ions. This then makes the blood to have an increase in the concentration of H^+ ions (acidosis) and K^+ ions (hyperkalemia), coupled with the retention of nitrogenous waste

materials as a result of impairment in the function of the glomerulus, this may ultimately lead to renal failure (e.g. acute tubular necrosis, acute and chronic renal failure).

Response of cell to toxins has been implicated as one of the major cause of cell death and this may cause apoptotic death pathway within the kidney and liver cells [13]. Genetic mechanism has also been implicated in cell death in that cell response to neurotoxins occurring as controlled events involving cascade and/or succession of activated enzymes [13]. In some instances, the kidney and/or the liver may be damaged as a result of oxidized agents known as free radicals generated in the body by the oxidation of nutrients derived from food substances and other chemical reactions taking place within the cells [14]. Cellular degeneration in many organs of the body has been observed to be one of the major causes of cell death, which may occur either as apoptotic and necrotic cell death.

HISTOLOGICAL ILLUSTRATIONS

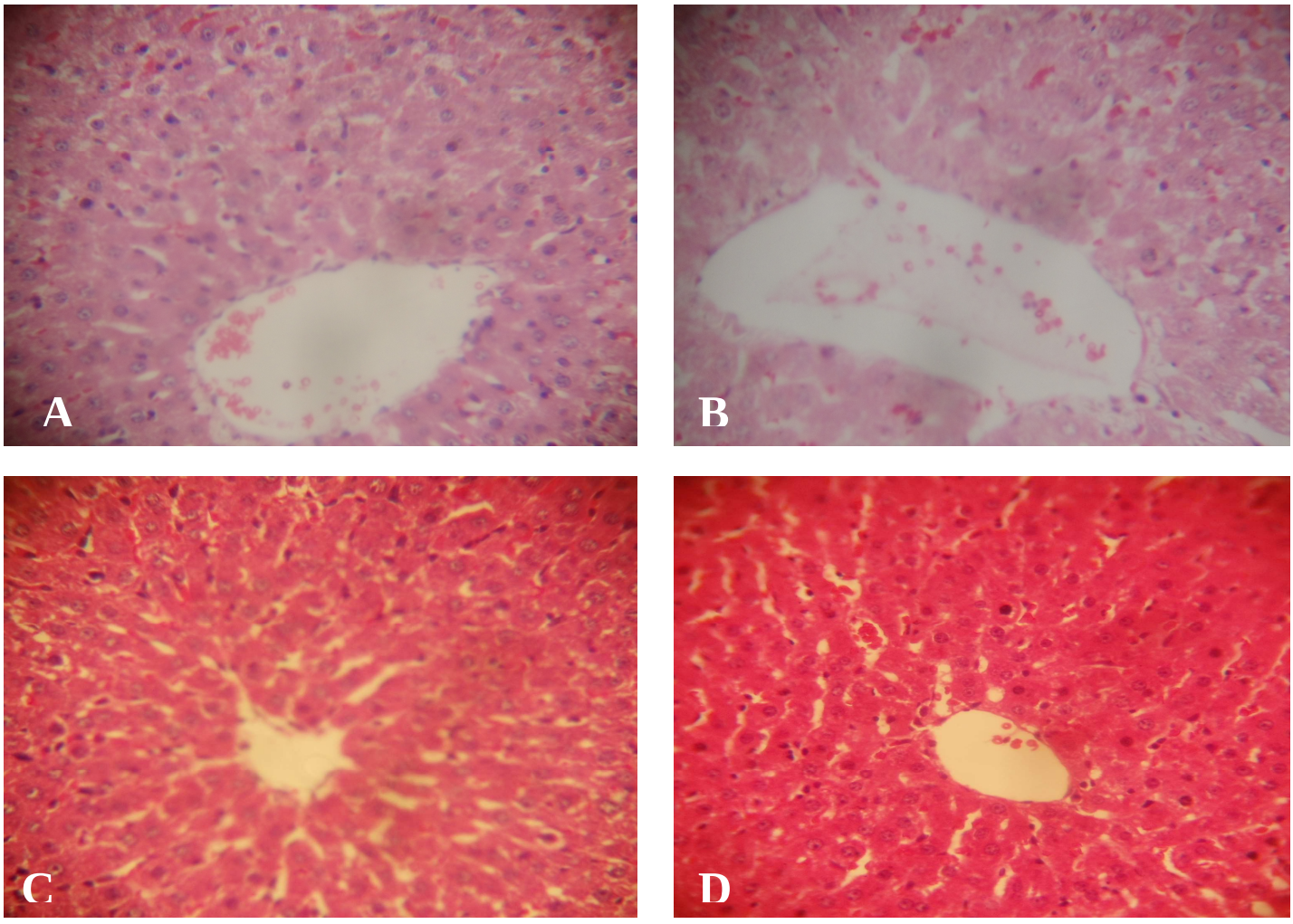

Figure 1; Showing the photomicrograph of the liver in the extract treated groups (A, B, and C) and the control group (D). (H&E x 400)

Processes involved in cellular necrosis which may lead to cell death include compromise and/or disruption of the structural and functional potentials of the various membranes in and within the cell. Necrosis of the cell is not induced by intrinsic stimuli to the cells as observed in programmed cell death, but by an abrupt environmental disturbances and deviation from the normal physiological conditions, factors and functions [15]. Gradual loss of the physiological functions of kidney and/or liver over time can lead to chronic diseases (e.g. acute tubular necrosis, acute and chronic renal failure).

According to Guyton and Hall (2000) [16], one of the main functions of the kidney is to remove metabolic and/or toxic wastes and excess water from the body. In chronic kidney disease, this physiological function is gradually compromised as the clinical condition or state or status of the chronic kidney disease progressively gets worse over time. In the early stages of chronic kidney disease, there may be no observable symptoms; this is because the loss of function (in the kidney) usually takes months or years to occur. At the final stage of chronic kidney disease known as end-stage renal disease, the kidneys no longer function and the individual may require dialysis or a kidney transplant [17].

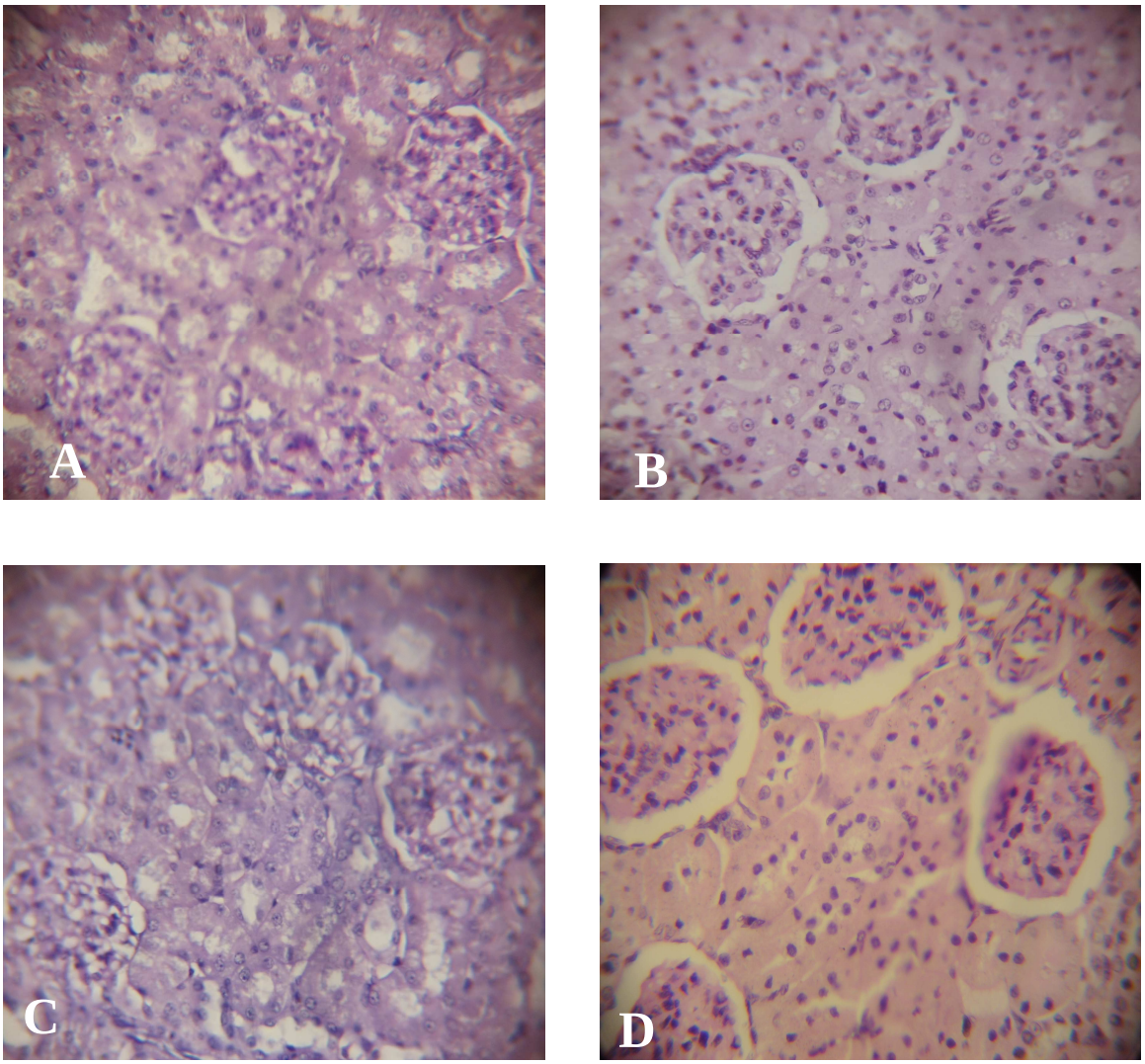

Figure 2; **Showing the photomicrograph of the cortical portion of the kidney in the extract treated groups (A, B, and C) and the control group (D). (PAS x 400)**

Estimation from the use of the modified method of Lorke (1983) [8] provide a tolerable dose of the aqueous extract of *B. rubra* leaves by the extract treated rats. Dapar *et al* (2007) [18] suggested that, the estimation of tolerable doses of plant extract is of immense importance, in view of the large-scale human consumption of plants either in culinary use or in managing or combating certain ailments and diseases and should be a matter of concern.

It was observed that the plant has the potentials of enhancing the histological functions of the kidney and liver as the sections obtained from this study showed a better histological organization in the kidneys and livers of the extract

treated rats. The preservation of the histological components of the kidney and liver observed in the sections obtained from the extract treated rats could be as a result of the phytochemical components of the plant extract.

CONCLUSION

In this study, the oral administration of the aqueous extract of *Basella rubra* significantly increased the body weight of the extract treated rats. The histological outline of the aqueous leaf extract of *B. rubra* on the kidney and liver as seen in the sections obtained from the extract treated rats support this claim as the histology of the studied organs were well preserved. Further studies should be directed towards isolating the specific component(s) of the plant responsible for the positive enhancing effects in order to standardize the plant preparation for maximum culinary and therapeutic benefit.

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