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Full Length Research Paper

The effect of methanolic extract of *Portulaca oleracea* on potassium bromate induced nephrotoxicity in adult wistar rats

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The objective of this study is to evaluate the effect of methanolic extract of *Portulaca oleracea* on potassium bromate ($KBrO_3$) induced nephrotoxicity in adult wistar rats. Twenty five adult wistar rats weighing 160-280 g were divided into five groups. The negative control group A was orally administered with 1 ml of distilled water daily, whereas the positive control group B was orally administered with 75 mg/kg/body weight for 14 days. Group C received 250 mg/kg/body weight of extract and 75 mg/kg/ body weight of $KBrO_3$ after six hours orally. Group D received 500 mg/kg/body weight of extract and 75 mg/kg/ body weight of $KBrO_3$ after six hours orally, while group E received oral dose of potassium bromate at 75 mg/kg/ body weight and 500 mg/kg/body weight of extract after six hours. Both the control and experimental groups were sacrificed under chloroform anaesthesia at the end of the period of administration. The results show that the body weights were reduced at the end of the period of administration. Histopathological examination indicated that group B (positive control group) showed nephrotoxicity, this is evidenced by the presence of inflammation and tubular dilatation within the renal cortex. Groups C and E showed significant recovery evidenced by the presence of normal kidney. Group D showed area of mild peri-capsular inflammation, that is, within and around the glomerulus. The prevention of nephrotoxicity induced by potassium bromate was observed to be higher in group E, which took the extracts six hours after the induction of potassium bromate when compared with group D, which took the extracts six hours before potassium bromate induction. This study suggests that oral administration of methanolic extract of *P. oleracea* significantly ameliorates potassium bromate induced nephrotoxicity in rats and seem to be useful in controlling kidney injury in drug induced nephrotoxicity.

Key words: Kidney, histopathology, potassium bromate, nephrotoxicity, *Portulaca oleracea*.

INTRODUCTION

Once in a while, one comes across a plant that is so outstanding, that one wonders how on earth it has been overlooked. Purslane (*Portulaca oleracea*) is one of such plants. It is fascinating that a plant so prevalent around the world has achieved almost identical recognition in each culture for its benefits. The use of this plant as a vegetable, spice and medicinal plant has been known since the TIMES of the ancient Egyptians and was popular in England during the Middle Ages; buy why it has fallen into obscurity is quite strange (Lanska, 1992).

Portulaca oleracea is an annual succulent in the family of Portulacaceae which may reach 40 cm in height. Approximately forty varieties currently are cultivated (Prashanth et al., 2005). It is commonly called Purslane in English Language, *ebeehofen* in Edo Language, *Ntioke* in Igbo Language, *babbajibji* in Hausa Language and *esanomode* or *papasan* in Yoruba Language (Burkill, 1997). It has an extensive old world distribution extending from North Africa through the Middle East and the Indian subcontinent to Malaysia and Australia. This half-hardy

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low growing plant has slightly succulent leaves and stems that are used raw or cooked. There are green and yellow leaved forms; the green type has thinner leaves, is more vigorous and possibly better flavoured (Brickell, 1992). It is used as a potherb in the Mediterranean, Central European and Asian countries. It is also referred to as the common Purslane (Lim and Quah, 2007). The species status in the new world is uncertain; in general, it is considered an exotic weed, however, there is evidence that the species was in Crawford Lake deposits (Ontario) in 1430-89 AD, suggesting that it reached North American in the pre-Columbian era (Liu et al., 2000). It is naturalized elsewhere and in some regions, it is considered an invasive weed. It has smooth, reddish, mostly prostrate stems and alternate leaves clustered at stem joints and ends. The yellow flowers have five regular parts and are up to 6 mm wide. Depending upon rainfall, the flowers appear at any time during the year. The flowers open singly at the center of the leaf cluster for only a few hours on sunny mornings. Seeds are formed in a tiny pod, which opens when the seeds are mature. It has a taproot with fibrous secondary roots and is able to tolerate poor compacted soils and drought (Ramesh and Hanumantappa, 2011).

The kidneys are bean shaped organs that serve several essential regulatory roles in vertebrate animals. They are essential in the urinary system and also serve homeostatic functions such as the regulation of electrolytes, maintenance of acid-base balance, and regulation of blood pressure (via maintaining salt and water balance). They serve the body as a natural filter of the blood, and remove wastes, which are diverted to the urinary bladder. Common clinical conditions involving the kidney include the nephritic and nephrotic syndromes, renal cysts, acute kidney injury, chronic kidney disease, urinary tract infection, nephrolithiasis, and urinary tract obstruction (Cotran et al., 2005).

Potassium bromate ($KBrO_3$) is a bromate of potassium and takes the form of white crystals or powder. It is an oxidizing agent, primarily used as a maturing agent for flour and as a dough conditioner (National Toxicology Program, NTP, 1991). It is also generated as a by-product of ozonization of surface water in treated drinking water (Cavanagh et al., 1992).

Although adverse effects are not evident in animals fed bread-based diets made from flour treated with KBrO3, recent studies have reported that the agent is hepatotoxic (Dimkpa et al., 2013) and several other studies have also reported the nephrotoxicity and neurotoxicity of KBrO3 in man and its carcinogenicity in animals following exposure (International Agency for Research on Cancer, IARC, 1986; Kurokawa et al., 1990; Nakano et al., 1989; Kurokawa et al., 1983), thus demonstrating the danger which potassium bromate poses to health if consumed in food or water. Also, studies have shown that it possesses the potential of inducing deafness, redness and pains of the eye and skin (DeAngelo et al., 1998; Office of Environmental Health Hazard Assessment, OEHHA, 2004). Hence, this study aims to investigate the effect of methanolic extract of *P. oleracea* on potassium bromate induced nephrotoxicity in adult wistar rats.

MATERIALS AND METHODS

Breeding of animals

A total of twenty five Albino wistar rats weighing between 160 and 280 g were obtained in the pre-clinical Animal House of College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. They were acclimatized for a period of 14 days and housed under standard laboratory conditions (29 ± 2°C temperature, 40-55% humidity, good ventilation) and had free access to water and diet (normal rat chow).

Collection of plant material

The fresh specimens of *P. oleracea* were collected from St. Thomas Anglican Church's Compound along Ubiaja Road, Esan North East Local Government Area, Edo State and were authenticated by a botanist, Mr. Orji, in the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State.

Preparation of extracts

Large quantities of the fresh specimens of *P. oleracea* were washed free of soil and debris, and the roots were separated from the leaves and stems. The leaves and stems were air-dried for eight weeks, and the dried specimens were pulverized using mechanical grinder. The pulverized specimen weighed 999 g, approximately 1 kg. This weighted specimen was macerated and extracted with 70% methanol (1:2 wt/vol.) for 72 h at room temperature (26-280°C). The resulting solution was then filtered using a wire-gauze and a sieve with tiny pores (0.25 mm). The 70% methanol was later evaporated using steam bath to give a percentage yield of 10.2% of the starting material. About 26 g of *P. oleracea* extract was dissolved in 500 ml of distilled water and administered to the animals.

Procurement chemical and kits

Potassium bromate (KBrO3) and the biochemical kits for the determination of serum biomarkers of liver were purchased from Cephas Global Resources Limited (A division of Deliving Stone Int'l), E Line 444 (along Fin Bank/Eco Bank), Head Bridge Market, Onitsha, Anambra State. However, 25 g of potassium bromate was dissolved in 1000 ml of distilled water and administered to the animals.

Experimental design

The animals were divided randomly into five groups, each containing five rats. The rats were also separated into male and female in each cage:

- Group A received 1.0 ml of distilled water orally as the negative control group.

Table 1. The mean weights of the animals before and after treatment using T-Test.

Group	Mean ± SEM	Mean ± SEM	Prob. of Sig.
n=5	Baseline weight	After treatment	
A	1.8500 ± 8.36660	1.8500 ± 8.36660	P<0.05
B	2.3800 ± 11.13553	2.2600 ± 8.71780	P<0.05
C	2.0800 ± 4.89898	1.9100 ± 5.09902	P<0.05
D	2.0000 ± .00000	1.8800 ± 2.54951	P<0.05
E	2.0400 ± 6.78233	1.9520 ± 4.74763	P<0.05

- Group B received oral dose of potassium bromate at 75 mg/kg/ body weight as positive control group.
- Group C received 250 mg/kg/body weight of extract and 75 mg/kg/ body weight of potassium bromate after six hours orally.
- Group D received 500 mg/kg/body weight of extract and 75 mg/kg/ body weight of potassium bromate after six hours orally.
- Group E received oral dose of potassium bromate at 75 mg/kg/ body weight and 500 mg/kg/body weight of extract after six hours orally.

The administration lasted for fourteen days. Twenty four hours (day 15) after the last dosing of the animals, blood samples were collected for determination of serum biomarkers of the liver and histopathological studies were also done.

Tissue processing

For easy study of sections under microscope, the tissues passed through several processes of fixation, dehydration, clearing, infiltration, embedding, sectioning and staining. Fixation was carried out in zenkers fluid. The tissues remained in the fluid for four hours. After fixation, the tissues were washed over night under a stream tap water. Dehydration of the fixed tissues was carried out in different percentages of alcohol: 50%, 70% and 90% absolute. After dehydration, tissues were cleared in xylene for two hours after which infiltration was done in molten paraffin wax at a temperature of 60°C for two hours each in two changes and then sectioned. Haematoxylene and eosine method was used.

RESULTS AND DISCUSSION

Morphometric analysis of body weight

Table 1 shows the mean weights of the animals before and after treatment using T-Test, while Figure 1 shows the histopathological studies on kidney by micrograph plates.

In the present study, we used $KBrO_3$ model for kidney damage induction to investigate whether or not the plant extract could decrease efficiently the toxicity produced by the nephrotoxicant. A reduction in body weights of the rats was observed. The reduction in weight may be due to reduced water intake, which may be secondary to feeling of fullness and loss of appetite after administration of the extract (Joseph et al., 1989; Hassan et al., 2005). In contrast, other studies (Watanabe et al., 2004; Farombi et al., 2002; Abuelgasim et al., 2008) have reported absence of $KBrO_3$ effect on body weights of rats.

In the histopathological examinations, the extent of kidney damage was assessed. The animals in group B (positive control group) showed nephrotoxicity, this is evidenced by the presence of inflammation and tubular dilatation within the renal cortex. Groups C and E showed significant recovery evidenced by the presence of normal kidney. Group D showed the area of mild peri-capsular inflammation, that is, within and around the glomerulus.

The prevention of nephrotoxicity induced by potassium bromate was observed to be higher in group E, which took the extracts six hours after the induction of potassium bromate when compared with group D, which took the extracts six hours before potassium bromate induction.

The efficacy of any nephroprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal renal physiology that has been disturbed by a nephrototoxin. The extracts prevented $KBrO_3$ induced nephrotoxicity in groups C and E and decreased the effect of $KBrO_3$ in group D, indicating the protection of structural integrity of the kidney.

Antioxidants may have protective effect against renal toxicity induced by potassium bromate. Constituents of *P. oleracea* such as flavonoids (quercetin), omega-3, ascorbic acid, β- carotene and glutathione have antioxidant activity (Radhakrishnan et al., 2001; Cai et al., 2004; Simopoulos, 2004), so this plant may inhibit lipid peroxidation by scavenging free radicals and increasing intracellular concentration of glutathione.

Our results suggest that oral administration of *P. oleracea* significantly ameliorates potassium bromate induced nephrotoxicity in adult wistar rats.

Conclusion

Our study suggests that oral administration of methanolic extract of *P. oleracea* significantly ameliorates potassium bromate induced nephrotoxicity in rats and seem to be useful in controlling kidney injury in drug induced nephrotoxicity.

The prevention of nephrotoxicity induced by potassium

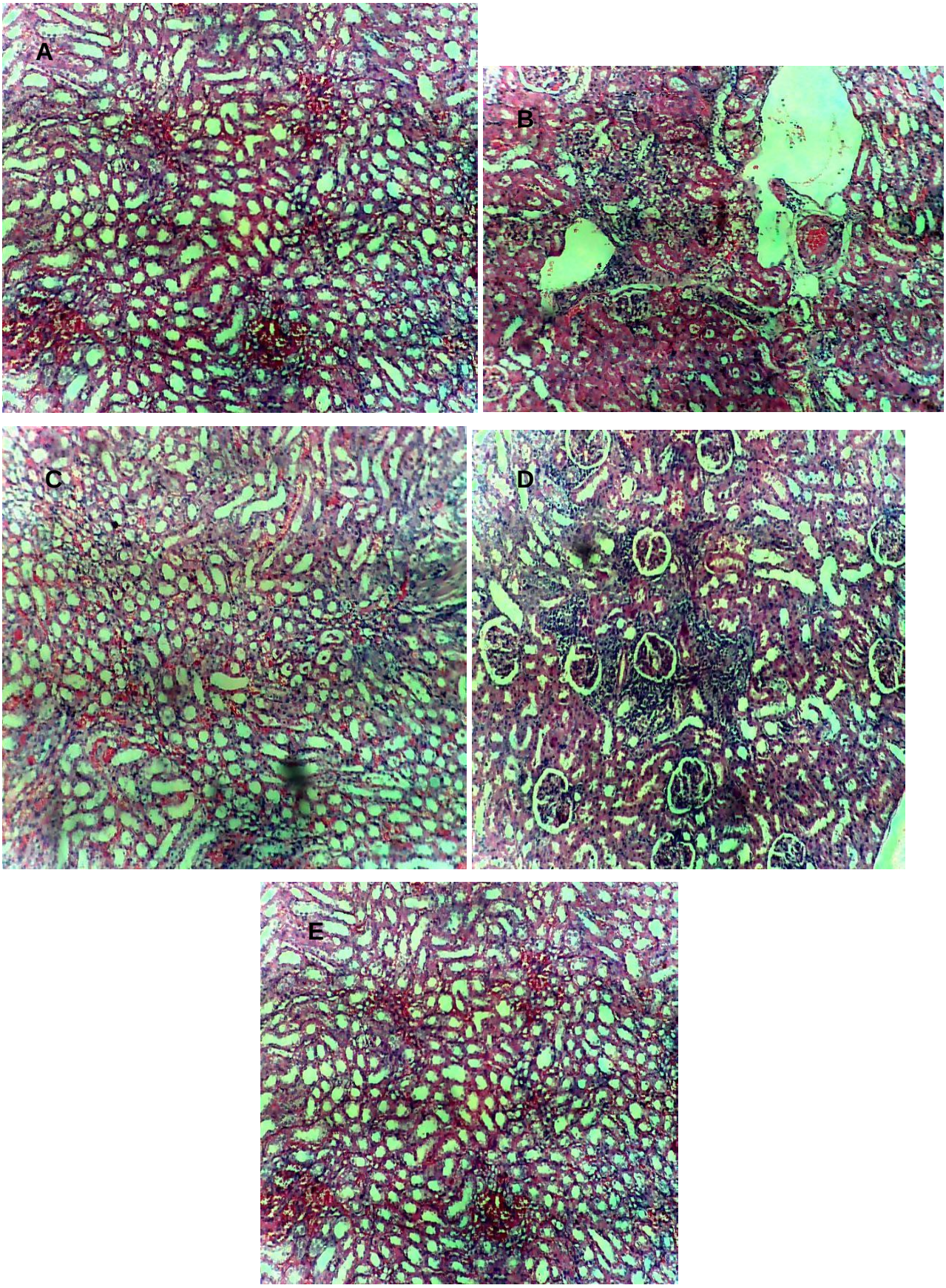

Figure 1. Histopathological studies on kidney. (A) Normal kidney; (B) Showing area of inflammation and tubular dilatation within the renal cortex; (C) Normal renal pelvis, no inflammation and tubular dilatation; (D) Mild peri-capsular inflammation, that is, within and around the glomerulus; (E) Normal kidney.

bromate was observed to be higher in group E, which took the extracts six hours after the induction of potassium bromate when compared with group D, which took the extracts six hours before potassium bromate induction.

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