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Protective Role of Ethanolic Extract of *Vernonia amygdalina* Against Potassium Bromate Induced Tissue Damage in Wistar Rats

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Abstract: The protective role of ethanolic extract of *Vernonia amygadalina* against potassium bromate induced tissue damage in Wistar rats was investigated. Twenty rats were divided into four groups of five rats each (A, B, C and D). Group A was administered 1 ml of 0.25 M sucrose solution. Group C and D were pretreated with 250 mg/kg and 500 mg/kg body weight of the aqueous extract of *V. amygdalina* respectively. The oral induction of 60 mg/kg potassium bromate to groups B, C and D were done eight hours before sacrifice. The liver, spleen, brain, kidney, heart and stomach were collected. Organ-body weight ratio, total tissue protein, amino acid level and malondialdehyde level in the tissues were estimated. The result showed a significant increase in all the parameters studied in group B. The organ to body weight ratio, total protein level, MDA concentration significantly lowered at both doses and amino acid level reduction is significant ($p<0.05$) in Group C when compared with Group B. This suggested that ethanolic extract of *Vernonia amygdalina* has a protective potential against tissue damage induced by potassium bromate.

Key words: Potassium bromate, tissue damage, *Vernonia amygdalina*, malondialdehyde

INTRODUCTION

Potassium bromate is typically used as a flour improver, strengthening the dough and allowing higher rising (Vadlamani and Seib, 1999). Over time, it has been discovered that potassium bromate is toxic and is a possible carcinogen in man (Kurokawa *et al*., 1990). It is extremely toxic to tissues especially those of the central nervous system and kidneys (Youdim *et al*., 2002). The pathologic findings include kidney damage and haemolysis (Robert and William, 1996). It is known that potassium bromate induces oxidative stress in tissues (Sai *et al*., 1991; Watanabe *et al*., 1992; Parsons and Chipman, 2000). Indeed, oxidative damage appears to be the basis of bromate-induced tissue damage and carcinogenesis (Chipman *et al*., 2006). In addition experiments aimed at elucidating the mode of carcinogenic action have revealed that $KBrO_3$ is a complete carcinogen, possessing both initiating and promoting activities for rat renal tumor-genesis (De Angelio *et al*., 1998). Bromate was first found to cause tumours in rats in 1982 (Schaur *et al*., 1990). Subsequent studies on rats and mice confirmed that it causes damage of the kidney, thyroid and other organs (Halliwel *et al*., 1990; Schaur *et al*., 1990). These have led to its ban from use in food products in Canada, Nigeria, Brazil, Peru, Sri Lanka and China (IARC, 2001). *Vernonia amygdalina* an edible rainforest plant native to the south Eastern part of Nigeria, has been widely used in folk medicine as anti-malaria, purgative, antiparasitic, treatment of eczema and for maintaining healthy blood glucose levels (Nwanjo and Nwokoro, 2004). The variety of secondary metabolites extracted from *V. amygdalina*, explains well the diversity of the biological function of the plant extracts (Yeap *et al*., 2010). Leaf extract of *V. amygdalina* was found to contain reducing sugar, polyphenolics, terpenoids, saponins, alkaloids, cardiac glycosides, steroids or triterpenes, anthraquinone and coumarins without cyanogenic glycoside (Ayoola *et al*., 2008; Nwanjo, 2005; Otshudi and Foriers, 2000). Total flavonoid and phenolic contents was found to be correlated positively with total antioxidant activity of the plant (Erasto *et al*., 2007). Flavonoids protect the cell as antioxidant against free radicals. The methanol extract of the plant was claimed to protect the liver against damage of Reactive Oxygen Species (ROS) with increase in the protein and glutathione content of animals (Adaramoye *et al*., 2008). This present study attempted to evaluate the toxic effect of potassium bromate on tissues and the protective potential of the ethanolic extract of *Vernonia amygdalina* against the toxicant.

MATERIALS AND METHODS

Experimental animals: Twenty albino rats were purchased from the animal house at the University of Ibadan, Oyo State. They were kept in clean, well ventilated cage under normal condition of light-dark cycle, fed with standard rat cubes and were acclimatized

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Table 1: Effect of potassium bromate and ethanolic extract of *Vernonia amygdalina* on organ-to-body weight ratio of the tissues

Tissue	Group A	Group B	Group C 250 mg/kg b.w	Group D 500 mg/kgb.w
Kidney	0.08±0.004	0.27±0.011[a]	0.764±0.011[b,c]	0.075±0.012[b,c]
Stomach	0.09±0.001	0.15±0.032[a]	0.188±0.032[a,b]	0.1858±0.13[a,b]
Heart	0.05±0.080	0.33±0.027[a]	0.087±0.031[b,c]	0.0731±0.016[b,c]
Spleen	0.07±0.003	0.18±0.012[a]	0.0436±0.005[b,c]	0.0717±0.025[a,c]
Brain	0.11±0.033	0.52±0.011[a]	0.124±0.019[b,c]	0.1380±0.0141[b,c]
Liver	0.32±0.006	1.11±0.22[a]	0.473±0.014[b,c]	0.52790±018[b,c]

Values (meant±SD) of five determinations. a = Significantly higher when compared with Group A ($p<0.05$), b = No significant difference on comparism with Group A ($p<0.05$), c = Significantly lower in comparism with Group B ($p<0.05$), d = Significantly higher in comparism with Group B ($p<0.05$), e = Significantly lower in comparism with Group A ($p<0.05$)

Table 2: Effect of potassium bromate and ethanolic extract of *vernonia amygdalina* on total amino acid (mg/g) of the tissues

Tissue	Group A	Group B	Group C 250 mg/kg	Group d 500 mg/kg
Kidney	72.37±8.242	124.74±8.222[a]	10.00±3.000[c,e]	43.467±4.23[c,e]
Stomach	42.06±11.250	88.77±11.630[a]	16.767±1.018[c,e]	14.823±1.321[c,e]
Heart	156.05±10.131	189.67±9.442[a]	45.71±11.380[c,e]	66.824±2.419[c,e]
Spleen	64.38±6.711	153.44±7.910[a]	45.92±11.460[c,e]	69.29±10.46[b,c]
Brain	97.67±9.333	161.33±15.228[a]	25.90±11.140[c,e]	40.885±1.294[c,e]
Liver	19.266±5.201	77.28±11.232[a]	25.90±.14.000[b,c]	35.094±11.471[b,c]

Value (meant SD) of five determinations. a = Significantly higher when compared to Group A ($p<0.05$), b = No significant difference on comparism with Group A ($p<0.05$), c = Significantly lower on comparism with Group B ($p<0.05$), d = Significantly higher on comparism with Group B ($p<0.05$), e = Significantly lower on comparism with Group A ($p<0.05$)

for two weeks at the animal house of Igbinedion University Okada, Nigeria where the study was carried out.

Plant sample collection and identification: Fresh leaves of *Vernonia amygdalina* were harvested from a tree in Igbinedion University campus premises. The leaves were identified and authenticated at the department of Botany, Igbinedion University Okada, Edo State.

Extraction of juice from fresh sample: Fresh leaves of *Vernonia amygdalina* were dried in an oven. 500g of *Vernonia amygdalina* were weighed and blended. 1.5 litres of ethanol was added for extraction. The extract was filtered using a whatman filter paper 125 mm and concentrated using a rotary evaporator at 45°C.

Experimental design: 20 rats were assigned randomly into four groups, each consisting of 5 rats. The groupings are as follows; Group A (base line control) were given 1 ml of 0.25 M sucrose solution; Group B were fed with standard feed only, for fourteen days following acclimatization then induced with $KBrO_3$ (not pretreated). Group C and D were pretreated with 250 mg/kg and 500 mg/kg body weight of *V. amgdalina* extract respectively for fourteen days before potassium bromate induction.

Induction of tissue damage: Rats in groups B, C and D were orally administered with 60 mg/kg potassium bromate ($KBrO_3$) solution.

Sacrificing of rats: The rats ware sacrificed eight hours (8 hrs) after administration of 60 mg/kg potassium bromate ($KBrO_3$) under light anaesthesia. The tissues were removed and perfused in physiological saline then their weight determined.

Biochemical analysis: Total tissue protein was estimated by follin-ciocalteau lowry method (Lowry *et al.*, 1951), while amino acid assay was carried out using the ninhydrin method of Magne and Larher (1992). Estimation of malondialdehyde level in tissues was measured by the method of Ohokawa *et al.* (1979).

RESULTS AND DISCUSSION

Table 1 shows the effect of potassium bromate and the ethanolic extract of *V. amygdalina* on the tissues. There was significant increase ($p<0.05$) in the organ to body weight ratio of all the tissues studied in group B when compared with group A, an indication of inflammation (Kurokawa *et al.*, 1990). With the exception of stomach that increases in organ-body weight ratio, the tissues pretreated with the ethanolic extract of *Vernonia amygadalina* however, showed a considerable protection against inflammation as was indicated by the significant decrease ($p<0.05$) in the organ-to-body weight ratio at both concentrations of *V. amygdalina* when compared with the group B. The protective property of the plant is possibly due to the luteolin-7-0 beta glucuronide, the linolenic acid and beta carotene (Igile *et al.*, 1994). Increase in the organ to body weight ratio of the stomach at both concentration of the extract when compared with Group B could be traced to accumulation of the potassium bromate in the organ (Josiah *et al.*, 2010).

Table 2 showed the amino acid level of the tissues, the result showed that there was a significant increase in

Table 3: Effect of potassium bromate and ethanolic extract of *vernonia amygdalina* on total protein (mg/g) level of the tissues

Tissue	Group A	Group B	Group C 250 mg/kg	Group D 500 mg/kg
Kidney	172.32±3.420	328.24±6.333[a]	180.25±12.613[b,c]	186.42±23.291[b,c]
Stomach	33.44±5.326	428.67±21.667[a]	105.83±10.971[a,c]	171.08±11.816[c,a]
Heart	133.20±2.232	542.44±34.223[a]	150.25±25.617[c,e]	17192±16718[b,c]
Spleen	57.10±0.891	529.20±9.830[a]	106.92±29.718[a,c]	152.50±23.184[a,c]
Brain	175.63±11.367	442.33±21.698[a]	162.50±14.139[c,e]	115.25±15.613[c,a]
Liver	267.33±14.244	413.24±12.227[a]	189.42±14.443[c,e]	245.83±14.616[b,c]

Values (mean ± SEM) for 5 determinations. a = Significantly higher (p<005) when compared with group A, b = No significant difference (p<0.05) when compared with group A, c = Significantly lower (p<0.05) when compared with group B, d = Significantly higher (p<0.05) when compared with group B, e = Significantly lower (p<0.05) when compared with group A

Table 4: Effect of potassium bromate and ethanolic extract of *vernonia amygdalina* on malondialdehyde levels (mg/g) of the tissues

Tissue	Group A	Group B	Group C 250 mg/kg	Group D 300 mg/kg
Kidney	0.50±0.004	18.98±5.67[a]	0.995±0.127[a,c]	0.186±0.045[b,c]
Stomach	4.58±0.010	21.24±4.220[a]	0.994±0.241[e,c]	1.833±0.1611[e,c]
Heart	3.12±0.050	14.13±3.333[a]	0.249±0.015[e,c]	0.338±0.043[e,c]
Spleen	1.34±0.024	3.27±0.010[a]	0.494±0.018[e,c]	0.234±0.017[e,c]
Brain	1.20±0.000	13.32±4.227[a]	0.290±0.0l6[e,c]	0.4105±0.061[e,c]
Liver	0.51±0.001	17.28±3.89[a]	0.995±0.147[a,c]	0.442±0.126[b,c]

Values (mean±SD) of 5 determinations. a = Significantly higher when compared with group A (p<0.05), b = No significant difference on comparison with group A (p<0.05), c = Significantly lower in comparison with group B (p<0.05), d = Significantly higher on comparison with group B (p<0.05), e = Significantly lower on comparison with group A (p<0.05)

the amino acid level of all the tissues in Group B when compared with the baseline group, an indication of the cytotoxic properties of potassium bromate. The cytoprotective properties of the plant are further strengthened by a significant decrease in the amino acid of all tissues (Chipman *et al*., 1998) investigated. Kidney, stomach, heart and brain showed a significant decrease in their amino acid level at both concentrations when compared with the controls, an indication that the plant stimulated protein synthesis a property typical of flavonoids (Middleton *et al*., 2000). Flavonoids have been characterized in the extract of *Vernonia amygdalina* (Igile *et al*., 1994).

Proteins are synthesized in response to environmental insults with exogenous or endogenous and they adapt the cells to fight back, thus proteins are synthesized to protect the cells, tissues and organs and to rebuild worn out ones. Table 3 showed the tissues' total protein level. At both concentration of the extract, the protein level had no significant variation (p<0.05) when compared with the baseline group for the kidney and the heart. The protein level of the liver and the brain were significantly lowered (p>0.05) when compared with group B especially at 250 mg/kg body weight, this showed that these organs are better protected at 250 mg/kg body weight of the extract. At 250 mg/kg body weight, the protein composition of all tissues were significantly higher (p<0.05) than the base line control, this may be due to induction of the free radical metabolizing enzymes, a property reported for flavonoid (Youdim *et al*., 2002).

Malondialdehyde level showed the extent of lipid peroxidation in biological samples (Ohokawa *et al*., 1979). The level of this chemical agent had been positively correlated to cellular damages and cytotoxicity (Omotuyi *et al*., 2005) as measured by the malondialdehyde level in all tissues investigated. Table 4 showed that malondiadehyde level of all tissues in group B were significantly higher (p<0.05) when compared with the baseline. However, the malondialdehyde level of the tissues were significantly low (p>0.05) at both concentration of the extract when compared with Group A and B, indicating that the cells were protected against peroxidation.

Conclusion: Ethanolic extract of *Vernonia amygdalina* leaves has a protective effect against tissue damage induced by free radicals. However the protection offered by ethanolic extract of *V. amygdalina* was seen to be effective at higher concentration of the extract. Further study would evaluate the toxicity of the extract on the rats.

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