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Full Length Research Paper

Protective role of aqueous extract of *Hibiscus sabdariffa* (calyx) against potassium bromate induced tissue damage in wistar rats

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The protective role of aqueous extract of *Hibiscus sabdariffa* (calyx) against potassium bromate induced tissue damage was investigated in rat tissues (brain, kidney, stomach, spleen, heart and liver). The rats were divided into four groups. Group A was administered with 0.25 M sucrose only (base line control), Group B with 60 mg/kg body weight of potassium bromate, and Group C with 250 mg/kg body weight of extracts. Group D was administered 500 mg/kg body weight of extract. Group A and B were used as control groups, while Group C and D were the experimentals. The oral administration of potassium bromate to groups B, C and D were done eight hours before sacrifice. Lipid peroxidation was monitored by colorimetric determination of amino acid, protein and malondialdehyde level in the tissues. The organ-to-body weight ratio was taken as indication for inflammation and necrosis of the tissues investigated. The results of the test groups were statistically ($p < 0.05$) compared with the base line control and the group B. There was no significant difference in the organ-to-body weight ratio in all the tissues investigated at both doses, when compared with base line control, but showed a significant decrease when compared with group B. The protein level of the tissues investigated showed a similar trend but the stomach shows significant increase in the protein level. This may be due to accumulation of the toxicant inducing protein synthesis. Amino acid level decreased significantly when compared with the base-line control and group B. This may be due to the extract ability to reduce proteolysis. Malondialdehyde level in the test groups decreased significantly in a dose dependent manner in all tissues investigated.

Key words: Lipid peroxidation, malondialdehyde, *Hibiscus sabdariffa*, potassium bromate.

INTRODUCTION

Hibiscus sabdariffa L., commonly known as Roselle or red sorrel in English, is native of India and Malaysia, where it is commonly cultivated. It must have been carried at an early date to Africa. It has been widely distributed in the tropics and sub tropics of both hemispheres, and in many areas of the West Indies and Central America as neutralizers (Resendiz-Lopez et al., 1998). It is known as mesta/meshta in India subcontinent. Bissap in Senegal, the Congo and France, wanjo in the Gambia, zobo in Nigeria, Karkade in Egypt, Saudi Arabia and Sudan, omutete in Namibia, sorrel in the Carribean and Jamaican (Wikipedia, 2006). Botanically, it is described as thick red fleshly, and cup shaped calyxes plant. The calyxes are rich in phenolic compounds with marked physiological activities (Rosendiz-Lopez et al., 1988). Hibiscus flowers contains gossypectin, glucoside, bibiscin, hibiscus anthocyann and protocatechuic acid, which may have diuretic and choleretic effects, decreasing the viscosity of the blood, reducing blood pressure, and stimulating intestinal peristalsis (Alli and Salih, 1991). Hibiscus protocatechuic acid (PCA) was shown to significantly decrease the leakage of lactate dehydrogenase (LDH) and alanine transaminase (ALT)

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and the formation of malondialdhyde (MDA) induced by tert-butylhydroperoxide (t-BHP) in rat primary hapatocytes (Tseng et al., 1995). Protocatechuic acid (PCA), isolated from *H. sabdariffa*, present in fruits, vegetables and nuts is an efficacious agent in reducing the carcinogenic action of diethylnitrosamine in the liver (Tanaka et al., 1993), 4-nitroquinone-1-oxide in the oral cavity (Tanaka et al., 1994), and N-methyl-N-nitrosoureas in glandular stomach tissue (Tanaka et al., 1995). Crude extracts of dried flowers of *H. sabdariffa L*. has strong antioxidant potential as they inhibit xanthine oxidase activity, formation of malondialdehyde and scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical most effectively. The extract was reported to inhibit the unscheduled DNA repair synthesis induced by t-BHP in the rat hepatocyte cultures (Tseng et al., 1997). The dried flower extracts protected rat hepatocytes from t-BHP induced cytotoxicity and genotoxicity by different mechanisms.

Anthocyanin pigments contained in its flower, include cyanidin-3-glucoside and delphinidine-3-glucoside (Du and Francis, 1973; Nakamaru et al., 1990), and have been used in food manufacture. It also showed antioxidant activity in a liposomal system (Tsuda et al., 1990). The calyxes are used as food and tea due to the presence of colouring agents present in the structure (Esselen et al., 1995).

Potassium bromate is a very powerful oxidizer used as flour improver, strengthening the dough and allowing higher rising. It is an oxidizing agent, and under the right conditions, will be completely used up in the baking bread. However, if too much is used, or the bread is not cooked long enough or at a high enough temperature, then a residual amount will remain. The mechanism of action of potassium bromate has been investigated by Fujii et al. (1984). When administered orally to rats the bromate was rapidly absorbed from the digestive tract and partly excreted in the urine within two hours of administration. The mechanism of potassium bromate carcinogenicity has been widely investigated and was found to cause lipid peroxidation in the kidney, and subsequently produces 8-hydroxydeoxguanosine (8 0H dG) in kidney DNA (Kurokawa et al., 1987). Several reports showed that potassium bromate is toxic to biological system, capable of generating free radicals and also causing DNA lesion, implicated in carcinogenesis.

The study aims at assessing the protective properties of aqueous extract of *H. sabdariffa* on tissue damage induced by sub-lethal dose of potassium bromate in rats, while the tissue damage was monitored by the level of protein, amino acid and malondialdelyde levels in stomach, kidney, brain, liver, spleen and heart.

MATERIALS AND METHODS

Animals

Twenty (20) adult rats (*Rattus norvegicus*) weighing between 60-90 g were obtained from the animal house of Igbinedion University Okada. They were grouped into four, of 5 rats per group. Group A, the base-line group, was given 1 ml of 0.25 M sucrose solution, while Group B was given distilled water only. Groups C and D were pretreated with 250 and 500 mg/kg body weight of aqueous extract of *Hibiscus sabdariffa* (orally administered), respectively, for 14 days. The rats in Groups B, C and D were administered with 60 mg/kg body weight potassium bromate ($KBrO_3$), 8 h before the rats were sacrificed. Groups A and B served as control, while Groups C and D were the experimental.

The sacrifice was carried out under light anesthesia using chloroform. Blood samples were collected via cardiac puncture, the organs removed and perfused in physiological saline and the weights determined.

Plant materials

Dried flowers of *H. sabdariffa* were purchased from Oba Market, Benin City, Edo State Nigeria. The flowers were identified and authenticated at the department of Botany of Igbinedion University, Okada, Nigeria.

Biochemical assays

Total tissue protein was estimated by Folin-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

The organ-to-body weight ratio can be used as an index for assessing the state of an organ, a significant reduction in the value of the ratio can be traced to organ or tissue necrosis, while a significantly high values is a possible indication of tissue inflammation (Rossi et al., 2003). Table 1 shows the organ-to-body weight ratio, the ratio increa-sed significantly ($p < 0.05$) in all the organs of group B treated with potassium bromate only. This confirmed the reported hepatotoxicity of potassium bromate (Skibola C and Smith M, 2000). The assessment of the organ-to-body weight ratio of the organs at both dosages of treatment showed no significant difference when compared with the baseline control, the observation showed that in the presence of the extracts of *H. sabdariffa*, the cytotoxicity of potassium bromate is inhibited, possibly by free radical scavenging mechanism. The difference in organ weights was indicative of inflammation. The anti-inflammatory activity of this plant has been traced partially to its phenolic acid composition (Rossi et al., 2003). Another potent antioxidant and cytoprotective agent characterized in *H. sabdariffa* is betaine, which can also account for its antioxidant property (Kanbak et al., 2001).

Table 2 showed the amino acid level of all the organs investigated. Except the liver, the amino acid level significantly decreased ($P < 0.05$) in all the organs of the experimental groups treated with the extracts when compared with the base line control, but significantly high in those administered with the toxicant only. The reason

Table 1. Organ to-body weight ratio (mg/g of tissue).

Tissue	Group A	Group B	Group C	Group D
Kidney	0.0797 ± 0.002	0.138 ± 0.013[a]	0.0876 ± 0.003[b,c]	0.0721 ± 0.001[b,c]
Stomach	0.0122 ± 0.001	0.1188 ± 0.024[a]	0.0118 ± 0.001[b,c]	0.0201 ± 0.018[b,c]
Heart	0.0605 ± 0.015	0.1652 ± 0.041[a]	0.0679 ± 0.012[b,c]	0.0593 ± 0.013[b,c]
Spleen	0.0514 ± 0.003	0.1187 ± 0.073[a]	0.0619 ± 0.008[b,c]	0.00552 ± 0.002[b,c]
Brain	0.1063 ± 0.030	0.3124 ± 0.011[a]	0.1286 ± 0.021[b,c]	0.1136 ± 0.023[b,c]
Liver	0.4342 ± 0.008	0.8789 ± 0.103[a]	0.5215 ± 0.119[b,c]	0.4461 ± 0.004[b,c]

Group A, the base-line group, was given 1 ml of 0.25 M sucrose solution, while Group B was given distilled water only. Groups C and D were pretreated with 250 and 500 mg/kg body weight of aqueous extract of *Hibiscus sabdariffa* (orally administered), respectively, for 14 days. The rats in Groups B, C and D were administered with 60 mg/kg body weight potassium bromate ($KBrO_3$), 8 h before the rats were sacrificed. Groups A and B served as control, while Groups C and D were the experimental.
Values are mean± SD for five determinations.
[a] Significantly higher ($p < 0.05$) than group A.
[b] No significant difference ($p < 0.05$) when compared with A.
[c] Significantly lower ($p < 0.05$) when compared with B.
[d] Significantly higher ($p < 0.05$ when compared with B.
[e] Significantly lower ($p < 0.05$) when compared with A.

Table 2. Total amino acid (mg/g of tissue).

Tissue	Group A	Group B	Group C	Group D
Kidney	90.2007 ± 5.620	112.863 ± 11.118[a]	56.987 ± 6.098 [c,c]	40.560 ± 12.05[c,c]
Stomach	33.015 ± 2.315	75/768 ± 4.713[a]	20.561 ± 6.642 [c,c]	14.984 ± 3.674[c,c]
Heart	181.0324 ± 10.131	208.813 ± 15.183[a]	132.987 ± 21.907[c,c]	86.564 ± 12.0 [c,c]
Spleen	57.387 ± 6.711	116.785 ± 12.138[a]	32.543 ± 10.985 [c,c]	25.875 ± 8.216 [b,c]
Brain	101.2123 ± 12.133	140.413 ± 11.438[a]	70.432 ± 16.985 [c,c]	60.094 ± 12.019[c,c]
Liver	16.2602 ± 2.152	65.659 ± 6.715[a]	25.986 ± 10.785 [b,c]	21.907 ± 9.341[c,c]

Group A, the base-line group, was given 1 ml of 0.25 M sucrose solution, while Group B was given distilled water only. Groups C and D were pretreated with 250 and 500 mg/kg body weight of aqueous extract of *Hibiscus sabdariffa* (orally administered), respectively, for 14 days. The rats in Groups B, C and D were administered with 60 mg/kg body weight potassium bromate ($KBrO_3$), 8 h before the rats were sacrificed. Groups A and B served as control, while Groups C and D were the experimental.
Values are mean± SD for five determinations.
[a] Significantly higher ($p < 0.05$) than group A[a].
[b] No significant difference ($p < 0.05$) when compared with A.
[c] Significantly lower ($p < 0.05$) when compared with B.
[d] Significantly higher ($p < 0.05$ when compared with B.
[e] Significantly lower ($p < 0.05$) when compared with A.

for the significant reduction could either be due to an increased protein synthesis or a reduction in the proteolytic activity which, shows that the protein of these organs are protected from excessive proteolytic cleavage induced by free radicals generated by potassium bromate (Wang et al., 2000).

An increase in protein synthesis during chemical injury is protective, especially with the induction of the synthesis of detoxifying protein (Rushmore and Pickeh, 1993). Table 3 showed the total protein of tissues investigated, at both 250 and 500 mg/kg body weight. The protein composition of all the tissues were significantly lower ($p < 0.05$) than those administered potassium bromate only, while when com-pared with the base line group, the protein levels were statistically similar ($p < 0.05$), excepts for the stomach which was significantly higher. This is possible due to induction of enzyme synthesis triggered by the accumu-lation of potassium bromate in this organ prior to detoxi-fication, absorption into the blood stream or excretion. The similarity in the level of proteins of both groups as compared to the control is possibly explained by the ability of flavonoids to scavenge the free radicals generated without reinforcing the antioxidant enzymes in the cell (Youdim, 2000).

A peroxidized lipid yields many products especially malondialdehyde. Table 4 showed the MDA level of rats administered 250 g/kg body weight. The MDA level showed no significant difference ($p < 0.05$) when compared

Table 3. Total soluble tissue protein (mg/g of tissue).

Tissue	Group A	Group B	Group C	Group D
Kidney	133.33 ± 8.113	411.18 ± 14.178[a]	143.89 ± 11.211[bc]	129.986 ± 10.325[bc]
Stomach	16.85 ± 2.175	532.19 ± 35.578[a]	119.64 ± 14.987 [bc]	204.57 ± 32.238 [bc]
Heart	144.17 ± 10.135	369.83 ± 25.117[a]	153.90 ± 18.064 [bc]	14987 ± 12.986 [bc]
Spleen	46.391 ± 2.176	477.16 ± 28.178[a]	56.34 ± 6.876 [bc]	40.456 ± 8.453 [bc]
Brain	209.17 ± 21.113	301.75 ± 13.125[a]	200.54 ± 12.432 [bc]	21297 ± 15.542 [bc]
Liver	256.33 ± 28.117	565.499 ± 4.123[a]	250.32 ± 14.453 [bc]	264.75 ± 13.152 [bc]

Group A, the base-line group, was given 1 ml of 0.25 M sucrose solution, while Group B was given distilled water only. Groups C and D were pretreated with 250 and 500 mg/kg body weight of aqueous extract of *Hibiscus sabdariffa* (orally administered), respectively, for 14 days. The rats in Groups B, C and D were administered with 60 mg/kg body weight potassium bromate ($KBrO_3$), 8 h before the rats were sacrificed. Groups A and B served as control, while Groups C and D were the experimental.
Values are mean± SD for five determinations.
[a] Significantly higher ($p < 0.05$) than group A.
[b] No significant difference ($p < 0.05$) when compared with A[a].
[c] Significantly lower ($p < 0.05$) when compared with B.
[d] Significantly higher ($p < 0.05$ when compared with B.
[e] Significantly lower ($p < 0.05$) when compared with A.

Table 4. Malondialdehyde level of tissue (mg/g of tissue).

Tissue	Group A	Group B	Group C	Group D
Kidney	0.50457 ± 0.121	29.328 ± 3.198[a]	0.495 ± 0.052 [bc]	0.114 ± 0.025 [ce]
Stomach	3.254 ± 0.539	18.135 ± 1.391[a]	4.021 ± 1.211 [bc]	1.121 ± 0.214 [ce]
Heart	4.0612 ± 1.312	17.787 ± 2.138[a]	3.996 ± 0.986 [bc]	1.934 ± 0.012 [ce]
Spleen	3.8234 ± 0.324	15.897 ± 1.181[a]	3.674 ± 0.428 [bc]	1.176 ± 0.174 [ce]
Brain	0.7120 ± 0.128	10.5134 ± 1.132[a]	0.7268 ± 0.075 [bc]	0.352 ± 0.07 [ce]
Liver	0.4817 ± 0.132	13.499 ± 0.123[a]	0.523 ± 0.115 [bc]	0.119 ± 0.004 [ce]

Group A, the base-line group, was given 1 ml of 0.25 M sucrose solution, while Group B was given distilled water only. Groups C and D were pretreated with 250 and 500 mg/kg body weight of aqueous extract of *Hibiscus sabdariffa* (orally administered), respectively, for 14 days. The rats in Groups B, C and D were administered with 60 mg/kg body weight potassium bromate ($KBrO_3$), 8 h before the rats were sacrificed. Groups A and B served as control, while Groups C and D were the experimental.
Values are mean± SD for five determinations.
[a] Significantly higher ($p < 0.05$) than group A[a].
[b] No significant difference ($p < 0.05$) when compared with A.
[c] Significantly lower ($p < 0.05$) when compared with B.
[d] Significantly higher ($p < 0.05$ when compared with B.
[e] Significantly lower ($p < 0.05$) when compared with A.

with the baseline control. At higher dose of 500 mg/kg, there was an observed significant reduction when compared with the base line control. This result showed that this extract does not only prevent lipid peroxidation induced by the toxicant but also those of endogenous origin such as those released during incomplete transfer of electron on the electron transport chain. There is a dose-dependent relationship between lipid peroxidation induced by potassium bromate and the protection offered by the extract of *H. sabdariffa*.

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