

The Protective Effect of Ethanolic Extract of Unripe Pulp of *Carica papaya* (Pawpaw) Against Potassium Bromate Induced Tissue Damage in Wistar Rats

¹S.J. Josiah, ¹S.C.O. Nwangwu, ¹A.A. Akintola, ¹T. Allu, ¹U. Usunobun,
¹Njoya Helen and ²B.I. Adejumo

¹Department of Biochemistry, School of Basic Medical Sciences, Igbinedion University Okada,
Edo State, Nigeria

²Department of Medical Laboratory Science, School of Basic Medical Science,
College of Medical Science, University of Benin, Benin City, Nigeria

Abstract: The protective role of ethanolic extract of unripe pulp of *Carica papaya* (fruit), against potassium-bromate induced tissue damage in wistar rats was investigated. The animals were grouped into four groups (A, B, C, D) of five rats. Group A was administered 1 mL of 0.25 M sucrose solution. 60 mg/kg of Potassium bromate $KBrO_3$ was administered orally to rats in groups B, C and D. Groups C and D were pretreated with 250 and 500 mg/kg of the extracts for fourteen (14) days, respectively. The organ to body-weight ratio, total amino acid, total protein and malondialdehyde (MDA) concentration in the brain, spleen, kidney, liver, and heart were colorimetrically measured as an assessment of the level of tissue damage. All the parameters studied increases significantly in all the tissues of rats group B. There was significant decrease in the organ-to-body weight ratio and total protein level in all the tissues investigated ($p < 0.05$), when compared with Group B at both doses of the extract. The total protein level of the stomach had a drastic reduction at 500 mg/kg. Also, the Amino acid level of investigated tissues decreased significantly, while malondialdehyde levels decreased significantly in a dose dependent manner. These findings suggest that ethanolic extract of unripe pulp of *Carica papaya* may be protective against $KBrO_3$ induced tissue damage in Wistar rats.

Key words: *Carica papaya*, malondialdehyde, potassium bromate, tissue damage

INTRODUCTION

The *C. papaya* tree is one of the most distributive plants on the earth. It is generally 45 m tall and unbranched but old plants may be 10 m tall. The trunk looks a little like an upside down, gray carrot (Hunt *et al.*, 1980). This plant has huge leaves up to 75 cm across that are palmately and then prinnately lobed leaves, which have very long petioles, are clustered near the top of the tree, because the lower ones are shed, leaving large circular scars on the bark. (Hunt *et al.*, 1980). Some of these components are responsible for some of the acclaimed medicinal properties of the plant. Additionally, the quality of organic and inorganic acid, and metals, present in the extracts such as sodium, calcium, iron, phosphorous, potassium, zinc, copper; magnesium, manganese, cobalt, also contributes to it nutritional (additional to its carbohydrate, protein, free sugar, starch, fiber ash content and moisture content) and medicinal value. Certain anti-nutrient such as cadmium, lead has also been identified. The plant has found much usefulness in folklore, both for preservative and medicine purpose. That papaya latex is a very effective meat tenderizer (Morton, 1987). Fruits and seed extracts hay pronounced

bactericidal activity against *Staphylococcus awe*, *Bacillus cereus*, *Esherichia coli*, *Psuedomonas aerugmosa* and *Shigella flexneri* (Youdim, 2002). The juice is used for curing warts, cancer, tumors and injurations of the skin (Huxtable, 1992; Youdim, 2002; Davies and Stewart, 1990). Leaves have been poultice into nervous pains, elephantoid growth and it has been smoked for asthma relief in various remote areas (Reed, 1976; Iwu, 1993). The hypoglycemic effect has also been reported by Olagunju *et al.* (1995).

Several chemicals have the ability to induce tissue damage by a free radical mediated reactions. One of such chemicals is potassium bromate. Potassium bromate is extremely toxic to tissues especially those of the central nervous system and kidneys (Youdim, 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). The present study is to evaluate the protective potential of the ethanolic extract of *C. papaya* pulp against the toxic effect of potassium

bromate on tissues, using the extent of lipid peroxidation, total protein and total amino acid level as well as the organ-body weight ratio as an indication of the effect of the toxicant and the extract on the tissues investigated.

MATERIALS AND METHODS

Plant sample collection and identification: Fresh, unripe mature fruits of *Carica papaya* were harvested from a tree in Igbinedion University campus premises. The fruits were identified and authenticated at the Department of Botany, Igbinedion University Okada, Edo state, Nigeria.

Experimental animals: Twenty wistar rats were purchased from the animal house at the University of Ibadan, Oyo State. The rats were housed in well-ventilated cages in the animal house of Igbinedion University. They were acclimatized for two weeks and fed with rat cubes.

Experimental design:

Group A: Base-line control (given 1 mL 0.25 M sucrose solution)

Group B: Second control (oral administration of 60 mg/kg body weight of $KBrO_3$ but not pretreated with the plant extract)

Group C: Test group, Pretreated with extract (250 mg/kg) for 14days, then oral administration of $KBrO_3$ (60 mg/kg)

Group D: Test group, Pre-treated with extract (500 mg/kg) for 14days, then oral administration of $KBrO_3$ (60 mg/kg).

Extraction of juice from fresh samples: Fresh unripe fruits of *Carica papaya* were peeled, seeds removed and the pulp cut into small pieces. 500 g of the small pieces were weighed and blended. 1.5 L of ethanol was then used for extraction. The extract was filtered using a whatman filter paper 125 mm and concentrated using a rotary evaporator.

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 the toxicant and the extract on organ to body weight ratio of all tissues investigated as an assessment of inflammation and necrosis in all the groups. Rats in group B showed a significantly high organ-body weight ratio, indicating that potassium bromate is capable of causing inflammation of tissues as reported by Kurokawa *et al.* (1990). The kidney, Liver and the heart showed the highest level of protection as shown by significant decrease ($p < 0.05$) at both 250 and 500 mg/kg body weight treated extract when compared with the base - line control and group B. The brain was only protected at 500 mg/kg body weight of extract. This may be partially due to selective permeability of the antioxidant composition of the extract by the blood - brain barrier (Takeshi *et al.*, 1996). The spleen showed inflammation at 250mg/kg body weight of but at 500 mg/kg body weight, it was protected against the cytotoxic action of potassium bromate. The anti-inflammatory activity of this plant has been traced partially to its linoleic acid composition (Feretti and Flanagan, 1996) an agent that is strongly soluble in polar solvent like ethanol. This explains the reason for the dose dependent protection of some of the organs. Ascorbate composition can also account for the cytoprotection against potassium bromated induced free radicals generation (Duke, 1992b). In the stomach at 250 mg/kg body weight, the ratio is higher when compared to the base - line control, this is possibly due to accumulation of the toxicant and poor detoxification of xenobiotic agents by the stomach (Robert, 2001), an effect which is overcome by increased concentration of the extracts.

The amino acid level was estimated (Table 2) in all tissues studied. The amino acids level was significantly higher in all the tissues investigated ($p < 0.05$) except the heart at 250 mg/kg body weight when compared with the base line group. This may be due to excessive proteolytic process as induced by the toxicant and the utilization of energy derivable from amino acid oxidation to drive the biochemical process of detoxification of free radical induced by the action of the toxicant. Another possible

Table 1: Effect of Potassium Bromate and ethanolic extract of *Carica papaya* pulp on organ-to-body weight ratio of the tissues

Tissues	Group A	Group B	Group C 250 mg/Kg	Group D 500 mg/Kg
Kidney	0.07±0.001	0.12 ^a ±0.040	0.08 ^{bc} ±0.0180	0.07 ^{bc} ±0.021
Stomach	0.01±0.003	0.96 ^a ±0.080	0.21 ^a ±0.0220	0.19 ^{bc} ±0.013
Heart	0.05±0.020	0.13 ^a ±0.000	0.05 ^{bc} ± 0.036	0.04 ^{bc} ±0.012
Spleen	0.06±0.006	0.14 ^a ±0.0120	0.64 ^{ad} ±0.1350	0.06 ^b ±0.011
Brain	0.91±0.010	0.27 ^a ±0.011	0.24 ^a ±0.0220	0.13 ^{bc} ±0.018
Liver	0.42±0.004	0.85 ^a ±0.600	0.47 ^{bc} ±0.0160	0.41 ^{bc} ±0.113

Values (mean±SEM) for 5 determinations; a: Significantly higher ($p < 0.05$) when compared with group A; b: No significant difference ($p < 0.05$) on comparison with group B; c: Significantly lower ($p < 0.05$) on comparison with group B; d: Significantly higher ($p < 0.05$) on comparison with group B; e: Significantly lower ($p < 0.05$) on comparison with group B

Table 2: Effect of Potassium Bromate and ethanolic extract of *Carica papaya* pulp 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	84.13±8.244	106.92 ^a ±10.010	106.76 ^a ±10.113	92.99 ^{b,c} ±5.198
Stomach	39.22±5.667	76.24 ^a ±7.270	73.91 ^a ±8.139	26.15 ^{b,c} ±8.125
Heart	193.10±12.220	204.18 ^a ±15.200	92.60 ^{e,c} ±1.928	36.69 ^{e,c} ±8.181
Spleen	46.24±8.323	160.01 ^a ±9.360	95.39 ^{e,c} ±8.397	26.46 ^{e,c} ±7.821
Brain	101.57±15.270	167.20 ^a ±8.224	135.51 ^a ±10.263	105.18 ^{b,c} ±11.152
Liver	19.43±2.620	84.22 ^a ±7.342	52.28 ^a ±3.388	8.34 ^{b,c} ±3.388

Values (mean±SEM) for 5 determinations; a: Significantly higher (p<0.05) when compared with group A; b: No significant difference (p<0.05) on comparison with group B; c: Significantly lower (p<0.05) on comparison with group B; d: Significantly higher (p<0.05) on comparison with group B; e: Significantly lower (p<0.05) on comparison with group B

Table 3: Effect of Potassium Bromate and ethanolic extract of *Carica papaya* pulp 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	178.20±10.230	516.42 ^a ±17.230	207.57 ^a ±11.716	191.20 ^b ±18.173
Stomach	20.74±4.280	496.18 ^a ±26.455	479.16 ^{b,c} ±14.471	23.23 ^{a,c} ±5.138
Heart	150.12±12.342	402.34 ^a ±14.260	278.33 ^{b,c} ±18.181	146.67 ^{b,c} ±12.197
Spleen	38.44±3.333	469.21 ^a ±29.330	425.87 ^{b,c} ±23.251	52.03 ^{b,c} ±5.072
Brain	205.17±16.227	311.75 ^a ±9.001	235.39 ^{b,c} ±28.811	201.88 ^{b,c} ±12.703
Liver	267.67±22.667	541.18 ^a ±25.228	456.73 ^{b,c} ±27.182	269.67 ^{b,c} ±18.671

Values (mean±SEM) for 5 determinations; a: Significantly higher (p<0.05) when compared with group A; b: no significant difference (p<0.05) on comparison with group B; c: Significantly lower (p<0.05) on comparison with group B; d: Significantly higher (p<0.05) on comparison with group B; e: Significantly lower (p<0.05) on comparison with group B

Table 4: Effect of Potassium Bromate and ethanolic extract of *Carica papaya* pulp on malondialdehyde levels (mg/g) of the tissues

Tissue	Group A	Group B	Group C 250 mg/Kg	Group D 500 mg/kg
Kidney	0.78±0.000	24.67 ^a ±2.333	18.70 ^{a,c} ±1.785	10.28 ^{a,c} ±2.175
Stomach	4.24±1.220	21.36 ^a ±1.112	10.89 ^a ±2.117	5.18 ^b ±1.181
Heart	2.96±0.567	19.77 ^a ±3.667	3.60 ^b ±0.143	11.97 ^a ±5.132
Spleen	4.06±0.040	4.90 ^a ±0.730	1.98 ^{a,c} ±0.128	1.96 ^c ±0.175
Brain	1.02±0.128	14.11 ^a ±3.270	1.98 ^{a,c} ±0.128	0.81 ^{b,c} ±1.318
Liver	0.27±0.000	16.30 ^a ±5.002	4.76 ^{a,c} ±1.113	6.58 ^{a,c} ±1.217

Values (mean±SEM) for 5 determinations; a: Significantly higher (p<0.05) when compared with group A; b: No significant difference (p<0.05) on comparison with group B; c: Significantly lower (p<0.05) on comparison with group B; d: Significantly higher (p<0.05) on comparison with group B; e: Significantly lower (p<0.05) on comparison with group B

explanation is the accumulation of amino acids derived from the plant extracts which was previously been reported to be rich in alanine (Duke, 1992a), arginine (Huxtable, 1992), methionine (Davies and Stewart, 1990), glycine, aspartate and tryptophan (Duke, 1992b). The second possibility of plant derived amino acids and its accumulation is discarded on the basis that stomach should have been the primary organ where amino acids should accumulate as shown in 250 mg/kg body weight. However at 500 mg/kg body weight, the amino acid is not comparable with the base line control. This shows that amino acids in the tissues were derived from proteolytic cleavage of the cellular proteins and the cells were protected at higher dosage of the extract. Similar results were obtained for the spleen.

The protective function of protein is dual; they protect the cells by maintaining the cellular integrity (cytoskeletal proteins), while some protect the cells against chemical injuries, free radical species, xenobiotic agents metabolizing proteins (Porter and Coom, 1991). An increase in protein synthesis during chemical injury is protective, especially with the induction of the synthesis of detoxifying protein (Rush More and Pickeh, 1983).

Table 3 showed the total protein level of tissues investigated, 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 enzymes, a property that has been reported for flavonoid (Youdim *et al.*, 2002). Flavonoid have been isolated and extensively characterized in *C. papaya* (Duke, 1992a). At 500 mg/kg body weight, there were no significant differences between the protein level of most tissues studied and the base line group (p<0.05). This may be due to the detoxification of free radicals generated by the potassium bromate without inducing the formation of enzymes. The ability of *C. papaya* to scavenge free radicals is traced to the gamma alanine, alpha linolenic acid, lycopene, methyl salicylate component of *C. papaya* which accumulates with increasing concentration of the extract (Iwu, 1993; Josiah *et al.*, 2010). Lycopene, linolenic acid and flavonoids have been reported to be the inhibitors of lipid peroxidation, which reduces the formation of malondialdehyde, the major product of lipid peroxidation.

Table 4 showed the malondialdehyde levels. At 250 mg/kg body weight, the MDA level is significantly higher (p<0.05) in all organs studied when compared with the base line group with the exception of the heart, but significantly lower when compared with those groups induced with potassium bromate only. This shows

protection by the extract. The same results were obtained for 500 mg/kg body weight treated kidney and liver but the spleen and the brain were well protected at 500 mg/kg body weight of the extract.

CONCLUSION

In conclusion, potassium bromate has been proven to be cytotoxic, causing tissue damage, and the ethanolic extract of *Carica papaya* can offer protection against damage that may be caused by this chemical. However the protection offered by *Carica papaya* was seen to be effective at higher concentrations of the extract.

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