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Antioxidant Effects of Aqueous Extract of Sweet Potato and Ascorbic Acid on Paracetamol Induced Damage

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The antioxidant effects of aqueous extract of sweet potato (*Ipomoea batata*) and ascorbic acid on paracetamol induced damage in liver and kidney were investigated in female rats by monitoring the enzymatic and non-enzymatic antioxidant profiles as well as lipid peroxidation and serum enzymes activities. The rats were given freshly prepared aqueous extract of sweet potato (100 mg kg⁻¹ body weight) or ascorbic acid (100 mg kg⁻¹ body weight) orally for 4 weeks. These rats were also given paracetamol (4 g kg⁻¹ body weight) orally for 2 days at the last week of treatment. Another group of rats were either given extract (100 mg kg⁻¹ body weight. daily, orally, for 4 weeks) or ascorbic acid (100 mg kg⁻¹ body weight. daily, orally, for 4 weeks) or paracetamol (4 g kg⁻¹ body weight for 2 days) or distilled water. The results show that the level of lipid peroxides in the liver and kidney and serum enzymes GOT and GPT activities were significantly decreased in extract and ascorbic acid pretreated rats when compared to control ($p < 0.05$). Paracetamol however significantly increase the level of these parameters when compared to control. Liver and kidney Superoxide Dismutase (SOD) and Catalase activities significantly increase in extract and ascorbic acid pretreated rats compared to control. Paracetamol significantly reduced the activities of these enzymes in liver but the reduction in SOD activity was not significant in the kidney when compared to control. There were significant increase in reduced Glutathione (GSH) in both organs in ascorbic acid pretreated rat but the increase were not significant in extract pretreated rats. Paracetamol significantly decrease GSH level in the liver when compared to control. The study revealed that sweet potato extract and ascorbic acid have a potential to prevent oxidative damage induced by acute dose of paracetamol in both the liver and the kidney.

Key words: Antioxidants, lipid peroxides, superoxide dismutase, catalase, reduced glutathione

INTRODUCTION

Painkillers are generally available over the counter. The range includes aspirin, paracetamol and non-steroidal anti-inflammatory drugs. Paracetamol, also known as acetaminophen, was first introduced into clinical medicine towards the end of the last century but it attracted little attention and was soon forgotten^[1]. There was a resurgence of interest in paracetamol when it was found to be the major metabolite phenacetine^[2] and it was commonly assumed to be responsible for the therapeutic effect of both of these drugs. Paracetamol has since been used increasingly as a substitute for other analgesics such as aspirin and phenacetine and in the United Kingdom its sale has exceeded those of aspirin for more than a decade. With its increasing use, poisoning with paracetamol has since emerged as a significant problem in many other countries. Paracetamol is a hepatotoxic drug through mechanism involving oxidative stress^[3]. The major target organ in paracetamol poisoning is the liver and the primary lesion is acute centro lobular hepatic necrosis^[4]. Reported complications of paracetamol poisoning include disturbances of coagulation with disseminated intravascular coagulation^[5], acute pancreatitis^[6], impaired carbohydrate tolerance^[7], myocarditis^[8] and hypophosphotemia^[9]. Oliguric renal failure can occur in the absence of significant hepatic injury^[10].

In children, paracetamol is a drug of choice as an analgesic/antipyretic because it is relatively safe and easily accessible. In many instances it is also the preferred drug for adults. Unless properly used and stored such drugs can result in poisoning especially in children.

Sweet potato (*Ipomoea batata* L.) is a dicotyledonous plant with tubers derived from swollen roots. Its crude protein content has been reported to vary between 16 to 29%^[11]. Sporamin, a storage protein in sweet potato tuber, exhibits antioxidant and radical scavenging activity and is an inhibitor of trypsin^[12]. It has been reported that trypsin inhibitor has antioxidant activity^[13,14] and that trypsin inhibitor also had dihydroascorbate reductase and monodihydroascorbate activities^[15,16] associated with intermolecular thiol-disulphide exchange. Sweet potato is high in antioxidant vitamins, which help our bodies to fight ageing, heart diseases and cancer. An average sweet potato has four times the recommended dietary allowance of β -carotene or vitamin A. it is also a good source of vitamin E and they are fat-free. Sweet potato is also high in vitamin C, providing half the day's recommended daily allowance in one medium potato. Previous studies have shown *in vitro* that the tuber and leaf of sweet potato have antioxidant and antiproliferative activities^[17].

Therefore the objective of this study was to evaluate the antioxidant power of aqueous extract of sweet potato tubers on paracetamol-induced oxidative damage in liver and kidney of female albino rats.

MATERIALS AND METHODS

Duration of study: The study was carried out between April and June, 2005

Plant materials: Sweet potato (*Ipomoea batata*) tubers were purchased from a local market in Akure, Ondo State of Nigeria. Extraction was then carried out according to the method of Al-Salkhan *et al.*^[18]. The tubers were chopped into small pieces and homogenized in deionized water (0.25 g tissues/mL of water) for 30 sec using a tissuemizer. Homogenate was filtered through miracloth into centrifuge tubes and centrifuged at 5,000 g for 20 min. Supernatant was filtered through miracloth into test tubes. After capping, extracts in test tubes were boiled five minutes in a water bath to inactivate enzymes. After cooling with tap water, the solution was again filtered through miracloth into centrifuge tubes and centrifuged at 5,000 g for 15 min. Supernatants were capped in tubes at -40°C. For use, the supernatant was evaporated to dryness at 25°C and reconstituted in distilled water

Animals: Adult female albino rats of Wistar strain weighing 160-220 g were purchased from Department of Animal Sciences, University of Ilorin, Ilorin, Nigeria. They were maintained under standard laboratory conditions with standard pelleted diets and were given water *ad libitum*. The animals were allowed to acclimatize for 4 weeks.

Reagent and chemicals: All chemicals used were of analytical grade and are products of Sigma chemical Co. (USA).

Dosage and treatment: Sweet potato extract and ascorbic acid were given at oral dose of 100 mg kg⁻¹ body weight each per day for 4 weeks. Acute oral dose of 4 g kg⁻¹ body weight of paracetamol were give for two days at the last week of treatment. This dose was selected on the basis of acute toxicity study. The rats were sacrificed 24 h after the last dose of paracetamol had been administered.

Experimental procedure: The rats were divided into eight groups of five animals each. Treatments were done accordingly.

- Group 1: Normal control
- Group 2: Animals received extracts of sweet potato 100 mg kg⁻¹ body weight orally
- Group 3: Animals received ascorbic acid 100 mg kg⁻¹ body weight orally
- Group 4: Animals received acute dose of paracetamol 4 g kg⁻¹ body weight orally.
- Group 5: Animals received paracetamol 4 g kg⁻¹ body weight+pretreatment with extract of sweet potato 100 mg kg⁻¹ body weight orally
- Group 6: Animals received paracetamol 4 g kg⁻¹ body weight+pretreatment with ascorbic acid 100 mg kg⁻¹ body weight orally
- Group 7: Animals received ascorbic acid 100 mg kg⁻¹ body weight and extract of sweet potato 100 mg kg⁻¹ body weight orally
- Group 8: Animals received paracetamol 4 g kg⁻¹ body weight+pretreatment with both extract of sweet potato and ascorbic acid 100 mg kg⁻¹ body weight orally.

Biochemical analysis: Protein was estimated by the Biuret method of Gornall *et al.*^[19] using Bovine serum albumin as standard. Lipid peroxidation products were estimated by assaying malondialdehyde (MDA) formation according to the method of Varshney and Kale^[20]. Reduced glutathione level was determined according to the method of Jollow *et al.*^[21]. The auto-oxidation of epinephrine was noted by determining the activity of Superoxide Dismutase (SOD) by the method of Misra and Fridovich^[22]. Catalase activity was measured by following decomposition of H₂O₂ according to the method of Sinha^[23]. Serum enzymes: GOT was assayed by the method of Reitman and Frankel^[24] by monitoring the concentration of oxaloacetate hydrazone formed while GPT was assayed by measuring the concentration of pyruvate hydrazone formed according to the method of Reitman and Frankel^[24].

Statistical analysis: Data are expressed as mean±SEM. Statistical analysis were performed using student t test at p<0.05 significant level.

RESULTS

There was a significant decrease in lipid peroxidation (p<0.05) and increase in antioxidant enzymes in extract and ascorbic acid pretreated rats when compared to control. There were however, significant increase in lipid peroxidation and decrease in antioxidant enzymes, GSH and protein levels in paracetamol treated rats (Group 4) (Table 1).

There was a significant decrease in lipid peroxidation (p<0.05), increase in antioxidant enzymes and increase in GSH in extract and ascorbic acid pretreated rats compared to control (Table 2). The increase in lipid peroxidation and decrease in Catalase activity and protein level were also significant in presence of paracetamol when compared to control (p<0.05). Serum enzymes GOT and GPT activities were significantly reduced in extract and ascorbic acid pretreated rats when compared to control (Table 3). The increase in activities were however significant in presence of paracetamol compared to control.

DISCUSSION

The present investigation demonstrates that paracetamol treatment alters both the enzymatic and non-enzymatic antioxidant profiles in both the liver and kidney. It also altered GOT and GPT activities. Paracetamol treatment resulted in decrease in GSH and catalase activity and increase in malondialdehyde in the liver and kidney. The drug however increases superoxide dismutase activity in the liver.

Paracetamol is primarily metabolized by the liver. Most of this combined with glucuronides and sulphate which account for about 9% of the dose excreted. About 5% of the dose is oxidized to N-acetyl-p-benzoquinoneimine (NAPQI), which may cause acute hepatic necrosis with toxic dose of paracetamol^[4]. NAPQI causes a depletion of both the mitochondrial and cytosolic pools of reduced glutathione (GSH)^[25]. It has earlier been reported that paracetamol depletes kidney GSH in mice^[26]. This agrees with our finding that paracetamol reduces the level of GSH in both the kidney and liver of treated rats (Table 1 and 2). The increase in liver GSH in the ascorbic acid pretreated rats show that it enhances the reduction of GSSG (Oxidized glutathione). The extract however shows no increase in the presence of paracetamol indicating that its mechanism of action is not through enhancing GSH level. This is in support of the earlier reports by Culley *et al.*^[27] that the scavenging ability of the extract is as a result of antioxidant vitamin A, E and C present in the tuber.

The apparent increase in malondialdehyde (MDA) in both liver and kidney (Table 1 and 2) in the presence of paracetamol is as a result of the oxidative stress due to GSH depletion and decrease in antioxidant enzyme activities. The result is in agreement with the earlier report that paracetamol induced oxidative stress in the liver and causes impairment of renal function by GSH depletion^[10]. The decrease in liver and kidney MDA (Table 1 and 2) in extract and ascorbic acid pretreated rats is an indication of

Table 1: Effect of aqueous extract of sweet potato (*Ipomoea batata*), ascorbic acid and paracetamol on lipid peroxidation, antioxidant enzymes, reduced glutathione and protein levels in liver of rats

Parameters	Groups							
	1	2	3	4	5	6	7	8
Protein (mg mL ⁻¹ homogenate)	12.50±2.60 ^b	15.41±2.28 ^b	13.18±3.51 ^b	6.83±2.13 ^b	14.90±2.02 ^b	11.33±4.58	23.27±1.75 ^{ab}	13.82±1.67 ^b
Lipid peroxide (nmole of MDA/mg protein)	9.55±0.45 ^b	5.61±0.90 ^{ab}	8.12±0.32 ^{ab}	54.12±4.83 ^a	3.43±0.41 ^{ab}	10.24±0.43 ^{ab}	3.97±1.35 ^{ab}	6.33±0.02 ^{ab}
Superoxide dismutase (units/mg protein)	2.06±0.09 ^b	5.78±0.20 ^{ab}	6.84±0.56 ^{ab}	3.67±0.10 ^{ab}	5.51±0.50 ^{ab}	4.11±0.10 ^{ab}	5.07±0.10 ^{ab}	4.05±0.98 ^a
Catalase (µmole of H ₂ O ₂ decompose/min/mg protein)	1.24±0.01 ^b	2.30±0.02 ^{ab}	2.22±0.02 ^{ab}	1.17±0.02 ^a	2.16±0.01 ^{ab}	2.12±0.05 ^{ab}	2.18±0.08 ^{ab}	2.10±0.03 ^{ab}
Reduced glutathione (µg/mi homogenate)	1.05±0.07 ^b	1.38±0.66	1.27±0.21 ^b	0.81±0.06 ^a	0.84±0.33	1.20±0.16 ^b	0.67±0.25 ^a	0.61±0.01 ^{ab}

Values are Mean ±SEM of 5 animals in each group, ^a: significant difference compare to control (p<0.05), ^b: significant difference compared to Paracetamol

Table 2: Effect of Aqueous extract of sweet potato (*Ipomoea batata*), ascorbic acid and paracetamol on lipid peroxidation, antioxidant enzymes, reduced glutathione and protein levels in kidney of rats

Parameters	Groups							
	1	2	3	4	5	6	7	8
Protein (mg mL ⁻¹ homogenate)	11.57±1.51 ^b	13.75±7.02	10.89±4.98	8.70±0.72 ^b	11.05±4.94	9.62±1.51	9.37±0.37 ^a	9.05±0.35
Lipid peroxide (nmole of MDA/mg protein)	15.57±0.42 ^b	8.81±0.68 ^{ab}	6.00±0.10 ^{ab}	35.00±1.88 ^a	7.83±0.07 ^{ab}	18.75±0.59 ^{ab}	9.97±0.82 ^{ab}	11.58±0.30 ^{ab}
Superoxide dismutase (units/mg protein)	2.16±0.36	2.19±0.01	3.93±0.50 ^{ab}	2.01±0.65	2.61±0.20 ^a	2.97±0.14 ^{ab}	13.72±0.40 ^{ab}	5.92±0.79 ^{ab}
Catalase (µmole of H ₂ O ₂ decompose/min/mg protein)	1.62±0.01 ^a	2.11±0.10 ^{ab}	2.09±0.18 ^{ab}	1.08±0.20 ^a	1.90±0.10 ^{ab}	1.60±0.01 ^{ab}	1.99±0.05 ^{ab}	1.63±0.13 ^b
Reduced glutathione (µg/mi homogenate)	1.08±0.04	2.44±0.31 ^{ab}	1.11±0.81	1.07±0.11	1.72±0.67	1.69±0.19 ^{ab}	1.73±0.01 ^{ab}	2.11±1.45

Values are Mean ±SEM of 5 animals in each group, ^a: significant difference compare to control (p<0.05), ^b: significant difference compared to paracetamol

Table 3: Effect of aqueous extract of sweet potato (*Ipomoea batata*), ascorbic acid and paracetamol on serum enzymes activities in rats

Groups	Serum enzymes (IU L ⁻¹)	
	GOT	GPT
1	29.94 ^b ±0.89	55.50 ^b ±1.41
2	30.60 ^b ±1.73	43.89 ^{ab} ±0.86
3	26.16 ^{ab} ±0.52	42.27 ^{ab} ±1.65
4	84.99 ^a ±5.41	96.30 ^a ±2.11
5	48.15 ^{ab} ±2.15	65.94 ^{ab} ±0.93
6	45.30 ^{ab} ±9.53	49.35 ^{ab} ±2.07
7	26.94 ^b ±5.92	36.09 ^{ab} ±2.69
8	44.70 ^{ab} ±5.77	45.00 ^{ab} ±2.86

Values are mean ±SEM of 5 animals in each group, ^a: significant difference compare to control (p<0.05), ^b: significant difference compared to paracetamol

their scavenging ability. This is in agreement with earlier reports that sweet potato have high amount of polyphenolic compounds which have an important role in stabilizing lipid oxidation and are associated with antioxidant activity^[28] and that the phytochemicals from sweet potato have significant effects *in vitro* on antioxidant and anticancer activities^[17]. Shewry^[12] has also shown that sporamin, the storage protein, in sweet potato also exhibit antioxidant and radical scavenging activity.

The notable increase in liver and kidney catalase and SOD activities in the extract and ascorbic acid pretreated

rats (Table 1 and 2) is in agreement with the report that oxygen handling cells have antioxidants SOD and catalase that acts synergistically for the complete detoxification of superoxide and hydrogen peroxide^[29]. The oxidation stress caused by paracetamol is therefore prevented by the extract and ascorbic acid.

Cellular necrosis occurs as a result of an injurious environment and has been referred to as murder^[30]. It is characterized by swelling with loss of membrane integrity and intracellular enzymes are released leading to an increase in aminotransferases enzymes in the serum. The significant reduction in the activities of serum GOT and GPT (Table 3) in the extract and ascorbic acid pretreated rats are also indicative of the effectiveness of the extract in preventing necrosis of the liver and kidney by paracetamol toxicity. The significant increase in serum GOT and GPT (table 3) in the presence of paracetamol was in agreement with earlier findings that paracetamol metabolite, NAPQI, causes acute hepatic necrosis with toxic dose of paracetamol^[4, 25].

In conclusion, though it has been demonstrated that the damage caused by paracetamol can be reduced or prevented by pretreatment with N-acetylcystein and other sulfhydryl compounds^[31], the present study has shown that sweet potato and ascorbic acid can also compare

favourably with these compounds in treatment of paracetamol toxicity. However, further studies could elucidate the exact mechanism of action in the treatment of paracetamol toxicity.

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