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Effects of Aqueous Leaf Extract of *Ipomoea Batatas*(Sweet Potato) on Ammonium Chloride-Induced Kidney Damage in Adult Wistar Rats

Ehimigbai ARO andOkoduwa PO
Department Of Anatomy, Faculty Of Basic Medical Science, College Of Medical Science, University Of Benin, Benin City, Edo State, Nigeria.

Corresponding Author: Ehimigbai, ARO
E-mail:agbonluai.ehimigbai@uniben.edu;+2348060780281.

ABSTRACT
*Ipomoea batatas*aqueous leaves extract is found to have medicinal flavonoids. The aim of this study is to investigate the effect of *Ipomoea batatas*aqueous leaves extract on Ammonium chloride induced kidney damage on adult Wistar rats. Thirty (30) rats were used for this study. The animals were grouped into six (6) groups with five (5) rats each. Group A served as control group, rats in Group B was treated with 100 mg/kg body weight of Ammonium chloride, rats in Group C was treated with 400 mg/kg body weight of *Ipomoea batatas*aqueous leaves extract, rats in Group D was treated with 1200 mg/kg body weight of *Ipomoea batatas*aqueous leaves extract, rats in Group E was treated with 100 mg/kg body weight of Ammonium chloride and 400 mg/kg body weight of *Ipomoea batatas*aqueous leaves extract, rats in Group F was treated with 100 mg/kg body weight of Ammonium chloride and 1200 mg/kg body weight of *Ipomoea batatas*aqueous leaves extract. The rats in all groups were sacrificed after 30 days.Five (5) ml blood samples were collect via direct cardiac puncture and stored in lithium heparin bottles for kidney function test and antioxidant assay analysis. The results showed significant increase ($P<0.05$) in urea and creatininelevels in groups B when compared to control and other groups, Oxidative stress analysis showed statistically significant increase ($P>0.05$) in MDA levels of groups B and F unlike group C, D and E when compared to control.There was no significant difference statistically in groups C, D, E and F SOD and CAT levels when compared with the control group. The levels of SODand CAT significantly decreased ($P < 0.05$) in group B rats when compared to control group (A). Histological studies showed normal architectural structure of kidney tissues in all groups except the group treated with 100 mg/kg body weight of Ammonium chloride. It can be concluded that*Ipomoea batatas*was seen to havenephroprotective and antioxdative potentials.

Keywords:*Ipomoea batatas,* oxidative stress, nephrotoxicity.

INTRODUCTION
Ipomoea batata is an important root vegetable which is large, starchy, and sweet tasting[1]It has been studied that sweet potato leaves contain magnesium (340 mg 100^{-1} g) and phosphorus (37.28 mg 100^{-1} g), with levels for calcium, iron and manganese at 28.44, 16.00, 4.23, 4.05 and 4.65 mg 100^{-1} g, respectively[2]. Sweet potato is usually planted with other staples such as cassava, yam in West African countries where it is effective in reducing weed growth in such fields[3]. The concentration of anthocyanin and beta-carotene combined with the high stability of color extract make sweet potato leaves good alternative to synthetic coloring agents in the food chain operation [4]. The leaves of sweet potato contain the following phytochemicals such as flavonoids, saponins, tannins, and phenolic acids. Sweet potato leaf has been the herbal leaf of choice in the management of anaemic patients in the olden days by the traditional practitioners in a town called Ikhinin Edo state of Nigeria. Sweet potato leaf is called Ebeeyanumen by the native people of Ikhin. Sweet potato is also an important source of vitamin A, thiamin, riboflavin, niacin, ascorbic acid and many other functional compounds[1].

A lot of studies have been done on the effects of I. batatas such as anti-mutagenic[5],hepatoprotective[6], anti-diabetic[7], antioxidant[5], immunomodulatory, anticancer activities[8], wound healing[9],antibacterial and antifungal[10],cardiovascular.[11]and antiulcer.[12]
Ammonium chloride had been reported to cause kidney enlargement.[13,14]The enlargement appears to be from an imbalance between protein synthesis and degradation.[14, 15]

MATERIALS AND METHODS
Preparation of extract: The plant sample was collected from Delta state fresh and it was identified by a plant taxonomist in Plant Biology and Biotechnology at University of Benin as *Ipomoea batatas*. The plant was air dried in a room temperature of 30^0 degrees for a week then pulverized to powder level using the British milling machine, the weight was actualized 800gram and was macerated with distilled water of 1.6litres in a

chromatographic jar for 24 hours with constant shaking and stirring for every 6 hours, then filtration technique was used to separate the residue from the filtrate using filter paper, conical flask. The filtrate was then concentrated to paste level using crucible water bath, the crude extract was then preserved in sample bottles inside the refrigerator.

Animal care: Thirty (30) adult Wistar rats weighing 180 to 250g were used for the experiment. The animals were purchased from the Animal House of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin city, Edo state, Nigeria. They were housed in clean plastic cages with wood chip bedding, under natural day. They were weighed weekly and recorded.

Experimental design:
Thirty (30) adult Wistar rats were randomly selected into a control group (group A) and five treatment groups (B, C, D, E, F) each containing five animals each (n equals 5 per group). The animals in each cage were given Growers mash, manufactured by Premier Feed Mills co Ltd (a subsidiary of flour mills of Nigeria Plc.) and water. GroupA: Control (feed and water), Group B: 100 mg/kg body weight of Ammonium chloride only, Group C: 400 mg/kg body weight of *Ipomoea batatas*leaf extract only (low dose), Group D: 1200 mg/kg body weight of *Ipomoea batatas*leaf extract only (high dose), Group E: 100mg/kg body weight of Ammonium chloride and 400 mg/kg body weight of *Ipomoea batatas*leaf extract. Group F: 100mg/kg body weight of Ammonium chloride and 1200mg/kg body weight of *Ipomoea batatas*. The duration of the experiment lasted for 30 days and the weight of the Wistar rats were taken weekly and recorded. On the 31st day, the animals were sacrificed.
Ammonium chloride was given at a dose of 100 mg / kg body weight [16]four (4) times per week for 30 days intraperitoneally.Extract was given throughorogastric route.

Sacrifice of the animals: At the end of the experimental period, the animals were grossly observed for general physical characteristics, and was weighed using a top loader weighing balance. An abdominal incision was made while the rats were under mild anesthesia using chloroform. Then the pair of kidneys were weighed and fixed in 10% formalin for histological analysis. Blood samples were gotten through cardiac puncture and placed in heparin bottles for analysis.

Histological analysis: The tissues were dehydrated in ascending grades of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. The de-paraffinized sections were stained routinely with Haematoxylin and Eosin.
Assay of lipid peroxidation, SOD and catalase: Lipid peroxidation, SOD and catalase were assayed in the kidney of rats according to the methods of Preuss *et al*. Marklund and Marklund and Cohen *et al*. respectively.[17, 18,19]

The kidney was homogenized in cold phosphate buffered saline (10% w/v) and the homogenates were centrifuged and the clear supernatant collected for the analysis of MDA,SOD and catalase.

Photomicrography: The sections of the kidney were obtained and examined under Leica DM750 research microscope with a digital camera (LeicaCC50) attached. Digital photomicrographs of the tissue sections were taken at ×40 and ×100 magnifications.

Statistical analysis
Data were subjected to statistical analysis using the IBM SPSS statistics software (Statistical Package for Social Science) (Version 25) and relevant statistical values were obtained. One-way analysis of variance (ANOVA) was carried out and data were expressed as mean ± SEM. LSD was used as the post-hoc test. Values of $P < 0.05$ were considered significant.

RESULTS

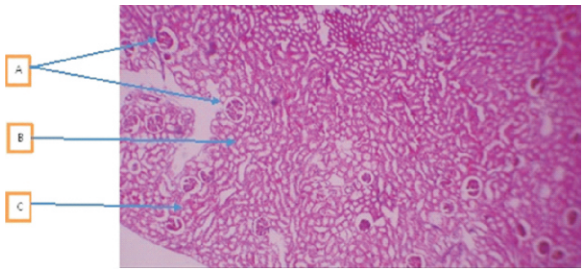

Figure.1a. Photomicrograph of cross section of kidney in Control Group A, showing normal kidney architecture of glomerulus (A), tubules (B), Interstitial space (C) (H&E X 40)

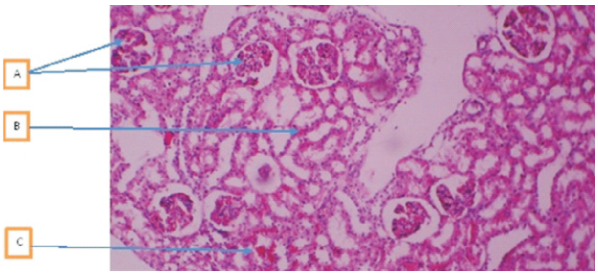

Figure.1b. Photomicrograph of cross section of kidney in Control Group A, showing normal kidney architectureofglomerulus (A), tubules (B), Interstitial space (C) (H&E X 40)

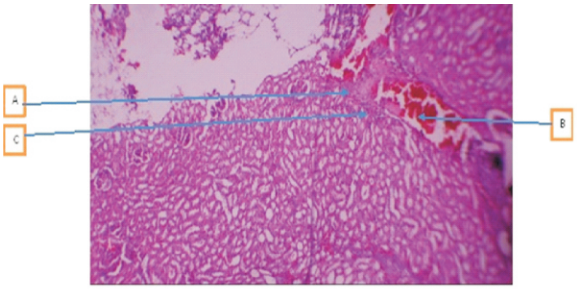

Figure.2a. Photomicrograph of cross section of kidney given 100mg/kg body weight of Ammonium chloride (Group B)showingmoderate vascular congestion, A,haemorrhage, B, inflammatory infiltrates , C (H&E X 40)

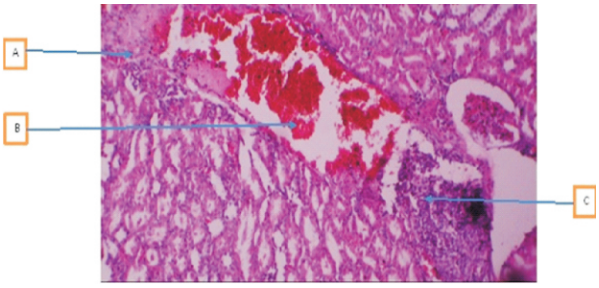

Figure.2b. Photomicrograph of cross section of kidney given 100mg/kg body weight of Ammonium chloride (Group B) showing moderate vascular congestion, A, haemorrhage, B, inflammatory infiltrates , C (H&E X 100)

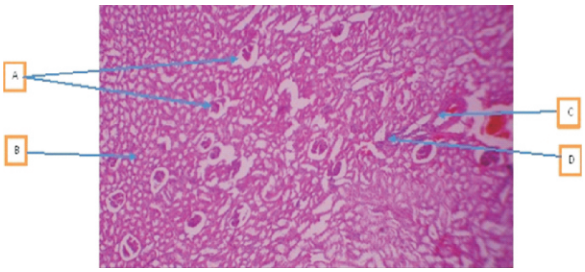

Figure.3a. Photomicrograph of cross section of kidney given 400 mg/kg body weight of *Ipomoea batatas*leaf extract only (Group C)showing normal kidney architecture of glomerulus (A), tubules (B), Interstitial space (C), perivasclar lymphocytes (D) (H&E X 40)

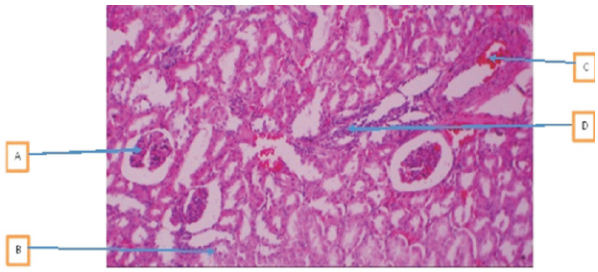

Figure.3b. Photomicrograph of cross section of kidney given 400 mg/kg body weight of *Ipomoea batatas*leaf extract only (Group C)showing normal kidney architecture of glomerulus (A), tubules (B), Interstitial space (C), perivasclar lymphocytes (D) (H&E X100)

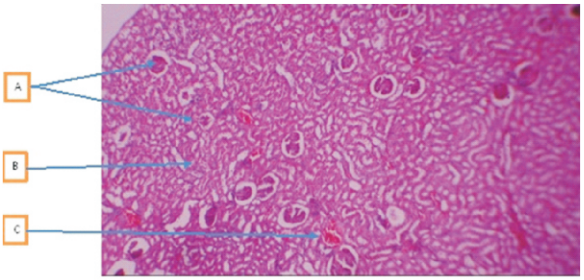

Figure.4a. Photomicrograph of cross section of kidney given 1200 mg/kg body weight of *Ipomoea batatas*leaf extract only (Group D)showing normal kidney architecture of glomerulus (A), tubules (B), Interstitial space (C)(H&E X 40)

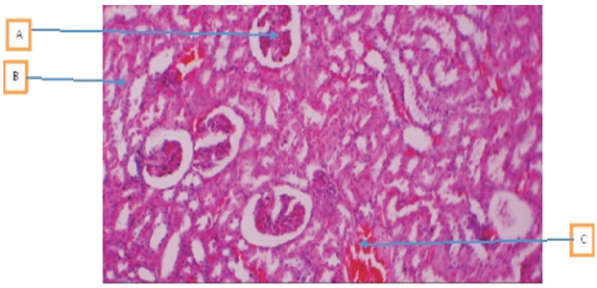

Figure.4b. Photomicrograph of cross section of kidney given 1200 mg/kg body weight of *Ipomoea batatas*leaf extract only (Group D)showing normal kidney architecture of glomerulus (A), tubules (B), Interstitial space (C)(H&E X 100)

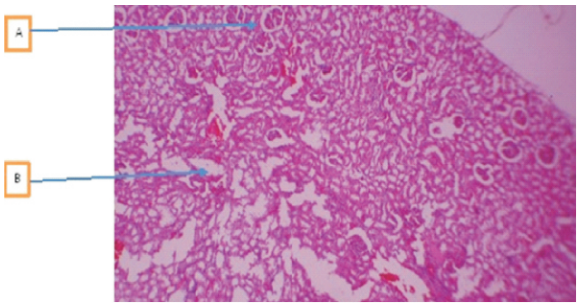

Figure.5a. Photomicrograph of cross section of kidney given 100mg/kg body weight of Ammonium chloride and 400 mg/kg body weight of *Ipomoea batatas*leaf extract (Group E)showing normal kidney architecture with glomerulus (A), tubules (B)(H&E X 40)

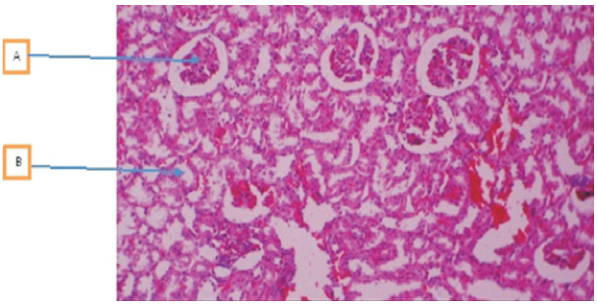

Figure.5b. Photomicrograph of cross section of kidney given 100mg/kg body weight of Ammonium chloride and 400 mg/kg body weight of *Ipomoea batatas*leaf extract (Group E)showing normal kidney architecture of glomerulus (A), tubules (B)(H&E X 100)

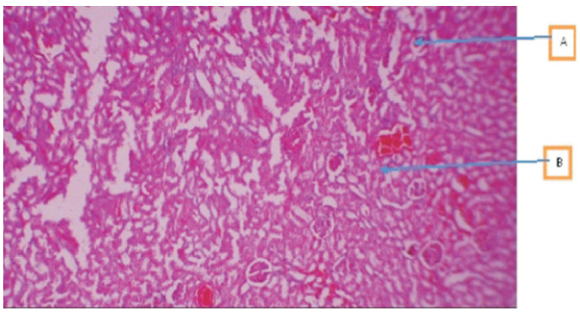

Figure.6a. Photomicrograph of cross section of kidney given 100mg/kg body weight of Ammonium chloride and 1200mg/kg body weight of *Ipomoea batatas*leaf extract (Group F)showing normal kidney architecture of glomerulus (A), tubules (B)(H&E X 40)

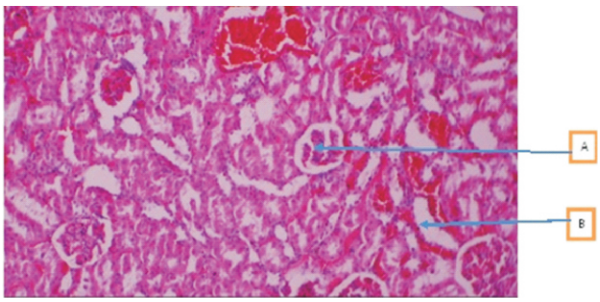

Figure.6b. Photomicrograph of cross section of kidney given 100mg/kg body weight of Ammonium chloride and 1200mg/kg body weight of *Ipomoea batatas*leaf extract (Group F)showing normal kidney architecture of glomerulus (A), tubules (B)(H&E X 100)

STATISTICAL ANALYSIS OF KIDNEY FUNCTION TEST

KIDNEY FUNCTION TEST	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E	GROUP F
Urea	20.90 ± 1.10	80.30 ± 2.30	24.80 ± 1.20	29.65 ± 1.35	30.40 ± 1.60	35.70 ± 0.70
Creatinine	0.35 ± 0.09	1.85 ± 0.07	0.55 ± 0.14	0.65 ± 0. 03	0.85 ± 0.11	0.75 ± 0.05

Statistical analysis showed significantly increase in urea levels (80.30 ± 2.30) in the rats treated with 100 mg/kg body weight of ammonium chloride only when compared to control (20.90 ± 1.10). There was no significant difference in the serum urea and creatininelevels of group (24.80 ± 1.20), (0.55 ± 0.14), group D (29.65 ± 1.35), (0.65 ± 0. 03) group E (30.40 ± 1.60), (0.85 ± 0.11) and group F (35.70 ± 0.70),(0.75 ± 0.05)when compared to control group (20.90 ± 1.10), (0.35 ± 0.09) respectively.

ANTIOXIDANT ANALYSIS

OXIDATIVE STRESS	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E	GROUP F
MDA	5.86 ± 0.45	13.23 ± 1.08	5.31 ± 1.17	6.21 ± 0.68	5.71 ± 1.24	10.90 ± 0.81
SOD	27.87 ± 2.56	4.30 ± 2.18	30.36 ± 4.16	27.01 ± 2.32	26.20 ± 2.80	26.39 ± 3.62
CAT	162.12 ± 10.12	55.07 ± 16.29	163.17 ± 11.83	166.97 ± 11.44	167.20 ± 12.30	169.60 ± 10.60

Malondialdehyde (MDA) level significantly increased (P ˂ 0.05) in group B (13.23 ± 1.08) and group F (10.90 ± 0.68) rats when compared to control group. Rats in group C, D and E showed no significant difference in MDA levels (5.31 ± 1.17), (6.21 ± 0.61), (5.71 ± 1.24)when compared with control group.
There was no significant difference statistically in groups C, D, E and F SOD and CAT (27.87 ± 2.56)(163.17 ± 11.83),(30.36 ± 4.16)(163.17 ± 11.83), (26.20 ± 2.80) and (26.39 ± 3.62) levels when compared with the control group (27.87 ± 2.56)(167.20 ± 12.30)and (162.12 ± 10.12)(169.60 ± 10.60).The level of superoxide dismutase (SOD) (4.30 ± 2.18) and catalase (CAT)(55.07 ± 16.29) significantly decreased (P ˂ 0.05) in group B rats when compared to control group (A) (27.87 ± 2.56) (162.12 ± 10.12).

DISCUSSION

Research done on the aqueous leaves extract of *Ipomoea batatas*on ammonium chloride induced kidney damage in adult Wistar rats showed in the control group (group A,), the presence of normal glomeruli, renal tubules of which the proximal and distal convoluted tubules and the interstitial spaces which are between the glomeruli and tubules in the control group. The proximal convoluted tubules contain brush borders or micro villi seen as fragments in its lumen while the distal convoluted tubule has no brush borders and no fragment in the lumen, making it to appear clearer than the proximal convoluted tubule. Histological examination also showed moderate vascular congestion, hemorrhage and heavy interstitial inflammatory filtrates in ammonium chloride treated group (group B). The lymphocytes appeared to have enhanced the immunity of the tissue. The wall was interrupted and damaged with massive dilated blood vessels making it massively inflamed causing severe ulceration leading to pyelonephritis. Our result is supported by earlier work done [20] where Mousastudied the oxidative effects of ammonium chloride on rats. He discovered that ammonium chloride administered rats showed reactive oxygen species effect on the renal tissue as evidenced by the increased levels of MDA, reduced antioxidant enzymes such as SOD and catalase levels.

Rats treated with 400 mg/kg body weight of *Ipomoea batatas*aqueous leaves extract (group C) and rats treated with 1200 mg/kg body weight of *Ipomoea batatas*aqueous leaves extract (group D) showed the presence of normal glomeruli, tubules and interstitial spaces. Significant reversal of the damage caused by ammonium chloride was observed in rats treated with 400 mg/kg body weight *Ipomoea batatas*aqueous leaves extract following ammonium chloride administration (group E) and rats treated with 1200 mg/kg body weight *Ipomoea batatas*aqueous leaves extract following ammonium chloride administration (group F). There was increased blood flow which is a desirable effect as it shows blood is being pumped from the heart efficiently.It's evident from the study that aqueous *Ipomoea batatas*leaf extracts from 400 mg / kg btw was able to prevent renal damage due to its antioxidant activities as evidenced by earlier work done [21]. Hue et al., (2012) stated that leaf extracts of sweet potato contained flavonoids, phenolics, reducing activities, and free radical scavenging effects.

The leaf is also an excellent source of beta-carotene, thiamine (vitamin B1), folic acid and ascorbic acid[22,1].
The consumption of sweet potato leaves warrants further and more extensive research study [23]. It hasbeen discovered that the water extracts of sweet potato leaves had potent antioxidant effects[24]. Also, itsrevealed that the leaves had the highest content of phenolic acid when compared with the root of sweet potatoes[25].

For the biochemical results, statistical analysis showed significantly increase in urea levels in the rats treated with 100 mg/kg body weight of ammonium chloride only when compared to control. There was no significant difference in the serum urea level of group C, group D, group E and group F when compared to control group respectively.

It was observed that there was significant increase ($P < 0.05$) in creatinine level in rats treated with 100 mg/kg body weight of ammonium chloride only compared to control. There was no significant difference in the serum creatinine level of group C, group D, group E and group F when compared to control group respectively.

For the oxidative parameters, the malondialdehyde (MDA) level significantly increased ($P < 0.05$) in rats treated with 100 mg/kg body weight of ammonium chloride only, and also in the group that was given 100 mg/kg body weight of ammonium chloride plus 1200 mg/kg body weight of *Ipomoea batatas*when compared to control group. Rats treated with 400 mg/kg body weight of *Ipomoea batatas only,* 1200 mg/kg body weight of *Ipomoea batatas only,*with 100 mg/kg body weight of ammonium chloride plus400 mg/kg body weight of *Ipomoea batatas only*showed no significant difference in MDA levels when compared with control group
There was no significant difference in the rats treated with combination of 100 mg/kg body weight of ammonium chloride and 400 mg/kg body weight of *Ipomoea batataswhen*compared to control The level of superoxide dismutase (SOD) and catalase (CAT) significantly decreased ($P < 0.05$) in rats treated with 100 mg/kg body weight of ammonium chloride only when compared to control group.

Rats treated with 400 mg/kg body weight of *Ipomoea batatas only,* 1200 mg/kg body weight of *Ipomoea batatas only,* with 100 mg/kg body weight of ammonium chloride plus400 mg/kg body weight of *Ipomoea batatas only* and with 100 mg/kg body weight of ammonium chloride plus 1200 mg/kg body weight of *Ipomoea batatas only* showed no significant differences in levels of superoxide dismutase (SOD) and catalase (CAT) levels when compared with control group.

Our findingsare in agreement with previous work done[26,27].Essa and Subramanian, 2006 reported that ammonium chloride induce ammonia toxicity via oxidative stress, which leads to lipid peroxidation and free-radical generation in the cells. While Ishida et al (2000) discovered that the contents of minerals, vitamins, and polyphenols were high in the leaf of sweet potatoes. The leaves contain appreciable amount of vitamin A, vitamin C,zinc, sodium, and iron, and may be included in mealsas nutritional supplement[2].

CONCLUSION

In this study, we ascertained that aqueous leaf extract of *Ipomoea batatas*possessed protective as well as anti-

oxidative properties against ammonium chloride induced kidney damage. Its possibility is attributed to the anti-oxidant properties of the aqueous extract. The low dose of aqueous extract of *Ipomoea batatas*was preferable to the high dose.

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