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Amelioration of high levels of serum kidney function biomarkers by *Vernonia amygdalina* in monosodium glutamate induced rats

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Monosodium Glutamate (MSG), as a food seasoning agent, even at low doses, has been found to exert exceeding toxic effect on the body system when used for a prolonged time. This study was aimed at investigating the effect of varying doses of *Vernonia amygdalina* stem extract on MSG-induced alteration of some kidney function parameters in rats. Twenty (20) adult male Albino rats (weighing 90-180 g), divided into five groups of four rats each, were used for the study. Some kidney function parameters and histological examination of kidney tissues were determined according to standard methods. The mineral and vitamin contents of *V. amygdalina* stem were also determined. In rats administered with MSG (8 mg/kg), there were significant (P <0.05) increases in the serum urea and creatinine but decreased albumin concentrations compared to rats administered with normal saline. MSG co-administration with extract (400 mg/kg) significantly decreased serum urea and creatinine concentrations. Minerals such as zinc, sodium and iron and vitamins A, C and E were found to be present in the stem extract. Histological examination of the kidney tissues of MSG-administered groups showed signs of toxicity but were much lesser in MSG co-treatment with the extract. These findings suggest that *V. amygdalina* ameliorated MSG-induced toxicity on the kidney functionality and this could be attributed to the presence of the antioxidant vitamins and some minerals as well as other bio-compounds present in the plant's stem extract.

Keywords: Ethanol extract, Kidney functionality, Minerals, Monosodium glutamate, *Vernonia amygdalina*, Vitamins.

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Introduction

Monosodium glutamate (MSG) is a sodium salt of the non-essential amino acid, L-glutatamate. It is a natural constituent of many protein-rich foods such as fish, cheese, meat and vegetables and it is used as a flavour enhancer in many processed foods to improve the taste and palatability of the food items. MSG plays important roles in brain functions such as formation and stabilization of synapses, memory, cognition, learning, and cellular metabolism[1-3]. When administered in a small dose (30 mg/kg), MSG did not exert toxic effect on serum C-reactive protein, trimethylamine N-oxide, angiotensin II, interleuckin (IL)-1β, IL-6, tumor necrosis factor-α, secretory IgA, and fecal albumin in mice. Moreover, it promoted intestinal development and regulated intestinal flora[4].

Despite these beneficial effects of MSG, its usage for culinary and other purposes is associated with several health issues/challenges. It is linked with diseases and disorders such as obesity, diabetes, metabolic disorders, Chinese restaurant syndrome, neurotoxic effects, allergic reactions, hepatoxicity, and oxidative stress, among others. In experimental with rats, MSG caused obesity when administered for some days in the experimental animals. It also caused increase in blood glucose, triglycerides and cholesterol and diabetes in general. It has been demonstrated that administration of even the least dose of MSG led to toxic effects on glutamate receptors and released neurotransmitters which play vital roles in normal physiological as well as pathological processes. Administration of this chemical agent resulted in some symptoms of Chinese restaurant syndrome which include tenderness of the pericranial muscle, headache and elevated systolic blood pressure. Several studies conducted with experimental animals revealed that MSG induced some behavioural changes such as screeching, tail stiffness, head nodding and seizures. Some treated

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rats showed cellular degeneration in the hippocampus coupled with the reduction of cyclic-AMPK level in the hippocampus. Results of some clinical trials showed that MSG caused allergic reactions such as urticarial, ventricular arrhythmia, asthma, neuropathy, chest pain, burning sensation, among others. It also caused non-alcoholic fatty liver disease and non-alcoholic liver inflammation in mice[5,6]. In rats treated with MSG, the activities of oxidative stress marker enzymes, glutathione-*S*-transferase, catalase, and superoxide dismutase were significantly increased; an indication of increased generation of free radicals from the chemical agent[5,6].

Other health hazards of MSG include its effects on the cardiovascular system, fertility and fetal development, and immune system[7]. In four different studies, MSG increased the cardiac tissue oxidative stress and some heart disease biomarkers such as lactate dehydrogenase, aspartate aminotransferase, and alanine aminotransferase. There were morphological changes in testes, as well as sperm abnormalities in adult male rats administered with MSG. Similarly, pathological alterations of oocytes and fallopian tubes in adult female rats were observed following dietary exposure to small doses of it. Some negative health effects such as convulsion threshold, impaired Y-maze discrimination learning, increased body weight and lower serum levels of growth hormone and insulin growth factor were observed in the offspring following the administration of this agent. Exposure to increasing MSG concentrations showed a dose-dependent alterations on B cell viability. Neonatal administration of MSG for about ten days to rats led to increased serum levels of IL-1β and IL-12, and decreased levels of IL-4, IL-10 and tumor growth factor-β[3,4,7,8].

Efforts have been and are being made to ameliorate the toxic effects accruing from the administration of MSG through the use of some natural products. In MSG-induced obesity model, anthocyanins from *Hibiscus sabdariffa* L. proved anti-obesogenic in rats by reducing the body weight, glycemia, and increasing the activity of alanine aminotransferase[8]. Pentamethylquercetin, a naturally occurring compound, present in *Hippophae rhamnoides* (sea buckthorn) and in the rhizome of *Kaempferia parviflora*, reduced the serum glucose, triglycerides, cholesterol, body weight, waist circumference and adipose tissue mass in mice. However, it improved insulin resistance, activated AMP-activated protein kinase and increased acetyl-coA carboxylase phosphorylation and GLUT4 abundance[9].

A good number of medicinal plants such as *Syzygium cumini*, *Baccharis dracunculifolia*, *Cedrus deodara* and others, with their constituents such as embeline, quercetin and pentamethylquercetin were found to be effective against metabolic syndrome, obesity and diabetic conditions associated with the administration of MSG[10,11]. The ameliorative effects of *Annona muricata* and *Vitis vinifera* on oxidative damage and inflammatory disorders caused by MSG in rats and *Caenorhabditis elegans* respectively have been established[12,13]. *Marsilea crenata* and grape seed extracts and the natural compound quercetin were able to improve the histopathological, ultrastructure, oxidative stress, and biochemical parameters of testicular toxicity induced by MSG in male rats[14-16]. Extracts of *Drymaria cordata*, *Zingiber officinale*, *Allium sativum*, etc., have been found to be potent in the treatment of fibroid; MSG has been reported to be one of the causes of uterne fibroid[17]. In MSG-induced hepato-cardiac toxicity, extract of *Coccinia grandis* was found to be efficacious by its inhibition of NF-kB and caspase 3 mediated signaling in rats[18]. Dietary supplementation with *Spirulina platensis* protected against the damage on blood biochemical parameters such as cholesterol, glucose, alanine aminotransferase, aspartate aminotransferase, triglycerides, and creatinine in MSG-induced mice[19].

Vernonia amygdalina (Delile), belonging to the family *Asteraceae* or *Compositae*, is among the medicinal plants that protect against and/or improve the toxicity posed to the body. It is used ethnomedicinally in the treatment of malaria, dysentery, inflammatory disorder and many other diseases[20,21]. Its extract protected against 2-acetylaminofluorene-induced hepatotoxicity in rats[22]. Also, the extract increased haematological parameters such as white blood cell count and haemoglobin concentration in Wistar rats exposed to stress[23]. Though the protective or ameliorative effects of *V. amygdalina* on monosodium glutamate induced toxicity in some vital body tissues and organs have been demonstrated, little or no scientific documentation has been made on its effect on MSG-induced toxicity in the kidney functions. This study was aimed at investigating the effect of varying doses of *V. amygdalina* stem extract on monosodium glutamate-induced alteration of some kidney function parameters in rats.

Materials and Methods

Chemicals/Reagents

The chemicals used for this study include formalin, picric acid, analytical ethanol (98%), tap water, Bouyer's reagent, monosodium glutamate and chloroform and are all of analytical grade.

Collection, identification, and preparation of plant material

Fresh stems of *V. amygdalina* were collected from a vegetable garden in Amaba, Oboro L.G.A., Umuahia, Abia State, Nigeria. The plant was identified and authenticated by Prof. Hillary Edeoga, a taxonomist of the Department of Botany, Micheal Okpara University of Agriculture, Umudike, where the voucher specimen with the voucher number BTD/MOUA/041 was deposited for future reference. The fresh stems were cut into smaller sizes, weighed and shade-dried for about three weeks under room temperature. The dried stems were weighed and then ground into a coarse powder using a laboratory milling machine. Exactly 400 g of the powdered plant material was macerated in 2000 mL of analytical absolute ethanol in a conical flask and was allowed to stand for 72 h to ensure exhaustive extraction. Thereafter, it was filtered with a muslin cloth and the resulting extract concentrated under 65 ºC in a water bath. The crude extract was stored in an air-tight experimental bottle in a refrigerator until ready for use.

Experimental animals

Twenty (20) adult male albino rats weighing 120-180 g were procured from the Genetics and Animal breeding Unit of the Department of Zoology and Environmental Biology, Micheal Okpara University of Agriculture, Umudike. Clearance and approval for the humane use and handling of laboratory animals were given by the ethical committee of Biochemistry Department, Micheal Okpara University of Agriculture, Umudike with the approval number BCH/ETH/16/116 (20/10/2016). All the animals were maintained under standard laboratory conditions throughout the experiment and were allowed unhindered access to food and water.

Experimental design

The 20 adult male albino rats were randomly divided into five groups A-E of 4 rats each and housed in separate stainless wire rat cages. The rats were treated as follows; group A: administered with 8 mg/kg of MSG (positive control), group B: administered with 200 mg/kg of the extract, group C: administered with normal saline, groups D and E: co-administered with 8 mg/kg each of MSG, and 200 and 400 mg/kg of the extract respectively. The administration of the MSG and extract, which was orally, lasted for 2 weeks.

Determination of urea

The urea level of the sample was determined using the method of Tietz[24] as described in Randox commercial kit. A certain amount, 10 µL of both the sample and standard were pipette into different tubes labelled sample tube and standard tube respectively. At the same time 10 µL of distilled water was added to a blank tube. The same amount (10 µL) of sodium nitroprusside and urease were added to each of the three tubes. The tubes were thoroughly mixed and incubated at 37 ºC for 15 min. Subsequently, 2.50 mL each of phenol and sodium hypochlorite were added to each of the three tubes one after the other. After mixing, they were incubated at 37 ºC for 15 min and the absorbance read against the reagent blank at 546 nm.

Determination of creatinine

The creatinine level of the sample was determined following the method of Tietz[24] as described in Randox commercial kit. One hundred microlitre (100 µL) of distilled water was pipette into a blank tube. The same amount (100 µL) of the standard and sample were appropriately pipetted into the standard and sample tubes. Equally, the same amount of the working reagent was pipetted into each of the three tubes and the contents were thoroughly mixed. The absorbance of the sample was read at 492 nm against the blank.

Determination of vitamins and minerals

Determination of Vitamin C

The ascorbic acid (vitamin C) content of the sample was determined by the method of Pearson[25]. An aliquot (0.5 g) of the extract was soaked with 20 mL of 0.4% oxalic acid for 10 min after which it was centrifuged for 5 min. The supernatant was transferred into three tubes. Later on, 9 mL of 2,6-dichlorophenol (12 mg/L) was added, mixed and the absorbance taken at 520 nm at 15 and 30 sec intervals.

Determination of Vitamins A

The vitamin A content of *V. amygdalina* stem extract was determined by the method of Pearson[25]. Just 1 g of the extract was soaked with 20 mL of petroleum ether for few min and allowed to stand for 1 h with intermittent shaking after every 10 min. Afterwards, it was centrifuged for 5 min, then transferred into three tubes and evaporated to dryness

in a water bath. To the residue was added 0.2 mL of acetic anhydride and chloroform (1:1). Then 2 mL of 50% trichloroacetic acid in chloroform was added and absorbance taken at 620 nm after 15 and 30 sec.

Determination of Vitamins E

The vitamin E content of the sample was determined by the method of Pearson[25]. Precisely 1 g of the extract was macerated with 20 mL of ethanol and filtered. An aliquot, 1 mL of the filtrate was pipetted into a test tube and 1 mL of 0.2% ferric chloride in ethanol was added. Then 0.5% of 2,2′-bipyridine was added too. Finally, the mixture was made up to 5 mL with distilled water and absorbance taken at 520 nm after 15 sec.

Determination of Sodium

The sodium content of *V. amygdalina* stem extract was determined using the method of Preston *et al*[26]. Stock solution of Na was prepared by dissolving a quantity (0.2542 g) of NaCl in distilled water finally made up to 1000 mL. This solution was diluted to the concentration range of 1-100 mg/L to obtain the working standards. The standard and sample solutions were aspirated into the flame photometer when the operating wavelength has been selected. The Na concentration of the sample was determined by making reference to the constructed calibration curve.

Determination of Iron

The iron content of the sample was determined using the method of Preston *et al*[26]. By dissolving 0.1 g of Fe in 10 mL of 10 mL of 10% H_2SO_4, a stock solution of Fe standard was prepared. It was allowed to cool and then made up to 1000 mL. The working concentration range of 0-20 mg/L was prepared from the stock. The Atomic Absorption Spectrometer (AAS) was set at the proper operating wavelength for iron. The iron concentration of the sample was obtained from the calibration graph.

Determination of Zinc

The zinc content of the sample was determined using the method of Preston *et al*[26]. A zinc stock solution (100 mg/L) standard was prepared by dissolving a little quantity (0.4398 g) of $ZnSO_4.7H_20$ in distilled H_20 which was finally made up to 1000 mL. The stock was diluted to obtain working standards concentration range of 0-5 mg/l. The sample solution and standards were aspirated in the flame of AAS. From the calibration curve, the zinc concentration in ppm of the sample was determined.

$$\text{Calculation: Zn (mg/kg)} = \frac{(a - b) \times v \times f \times 1000}{1000 \times w}$$

where a = concentration of Zn in the sample extract, b = concentration of element in the blank extract, v = volume of the extract solution, w = weight of the sample, and f = dilution factor.

Determination of Serum Albumin

The serum albumin content of the sample was determined using Bromocresol Green according to Dumas *et al*[27]. Three test tubes labeled Reagent, Standard and Sample were provided. Into the Reagent test tube was pipetted 0.01 mL of distilled water. The volume, 0.01 mL of standard and sample was pipetted into Standard and Sample test tubes appropriately. A known volume (3.0 mL) of 3,3′,5,5- tetrabromo-m-cresosulphonephthelein (BCG) into each of the three test tubes. The contents of the tests were incubated at 25 ºC for 3 min and absorbance taken against reagent blank at 630 nm. The albumin concentration in the sample was calculated from the following formula:

$$\frac{\triangle A_{sample}}{\triangle A_{standard}} X\ C_{standard} = C_{sample}$$

Histopathological examination

The tissue specimens (kidneys) were removed from the sacrificed rats and fixed in 10% formaldehyde buffered saline. After some days, the processing started with the dehydration in graded levels of alcohol in order to remove the water content. After dehydration, the tissues were cleared in xylene impregnated with paraffin wax and sectioned at 5 microns thickness using rotary microtone. The sections were floated on a water bath which was maintained at a temperature range of 2-3 ºC below melting point of the paraffin wax after which the sections were dried on a hot plate maintained at the same temperature range. After drying, the sections were stained and mounted using haematoxylin and eosin such that air bubbles were avoided.

Statistical analysis

The data obtained in this study were subjected to One-way Analysis of Variance (ANOVA). Significant difference was accepted at P <0.05 and the results expressed as mean±standard error of mean (S.E.M.). This analysis was carried out using IBM SPSS Statistics version 18.

Results and Discussion

Serum urea concentration

Fig. 1 shows the effect of *V. amygdalina* stem extract on serum urea of normal and MSG-induced rats. Animals groups co-administered with MSG (8 mg/kg) and the extract (200 and 400 mg/kg) (Groups D and E respectively) showed significant (P <0.05) increase in serum urea concentration compared to the group administered with 8 mg/kg of MSG (Group A). Groups D and E also showed significant (P <0.05) increase in serum urea concentration compared to the group administered with 200 mg/kg b.w. of the extract (Group B) and the group administered with normal saline (Group C).

Despite its taste stimulation and improved appetite enhancement, reports indicate that MSG is toxic to human and experimental animals even at low doses[28]. From the results of this study, the serum urea concentration of rats administered with MSG (positive control) increased significantly compared to that of normal rats (normal control) showing that there is impairment of kidney function; thus it was not able to filter off the urea. The urea concentration of rats treated with 200 mg/kg of the extract increased significantly compared to the normal control. However, there was a reduction in the serum urea concentration of group treated with 400 mg/kg of the extract. The implication is that a higher dose of the extract may be needed to significantly reduce the serum concentration of urea. In agreement with the studies of Achuba[29], *V. amygdalina* extract reduced serum urea concentration in rats induced with crude petroleum.

Serum creatinine concentration

Fig. 2 shows the effect of *V. amygdalina* stem extract on serum creatinine of normal and MSG-induced rats.

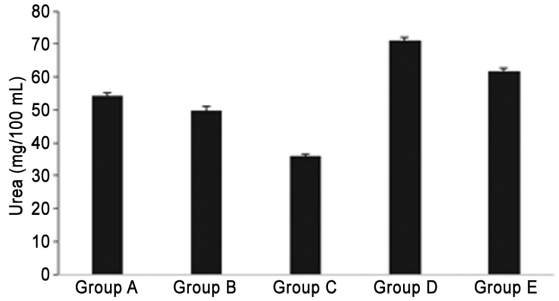

Fig. 1 — Effect of *V. amygdalina* stem extract on serum urea concentration of normal and MSG-dosed rats.
Group A = administered with 8 mg/kg of MSG only (positive control), Group B = administered with 200 mg/kg of the extract, Group C = administered with normal saline (normal control), Group D = administered with 8 mg/kg of MSG and 200 mg/kg of extract, Group E = administered with 8 mg/kg of MSG and 400 mg/kg of extract.

The serum creatinine concentration of Group A was significantly (P <0.05) higher compared other groups.

The serum creatinine concentration of the positive control increased significantly compared to other groups possibly because the Glomerular Filtration Rate (GFR) and excretory function of the kidney has been altered leading to the decreased clearance of creatinine from the serum. This observed increase in serum creatinine concentration has been proven to be a better marker for renal integrity and functionality than increased urea concentration. In a study by Adedoyin *et al.*[30], aqueous extract of *Ocimum gratissimum* reduced the serum creatinine concentration in MGS-induced rats; this agrees with the result of the present study where the serum creatinine concentration was reduced in the treated rats.

Serum albumin concentration

Fig. 3 shows the effect of *V. amygdalina* stem extract on serum albumin of normal and MSG-induced rats. The serum albumin concentration of Group A was significantly (P <0.05) lower compared other groups.

The serum albumin concentration was found to be high in animals administered with both the extract and MSG but was highest in the group administered with the extract only and this could be attributed to the ability of the extract to aid in protein synthesis. The result of the effect of the extract on serum albumin showed a decreased albumin level of the positive control compared to other groups and this agrees with previous report by[30]. The possible mechanism of action could be

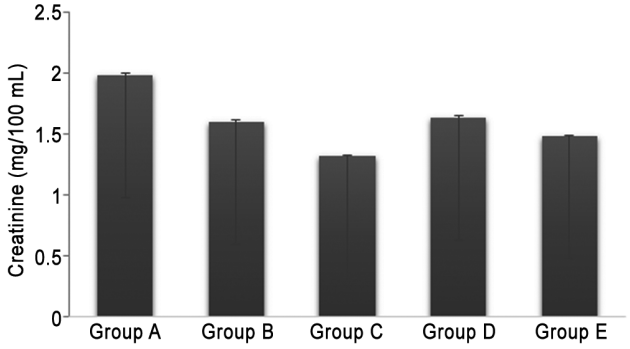

Fig. 2 — Effect of *V. amygdalina* stem extract on serum creatinine concentration of normal and MSG-dosed rats. Group A = administered with 8 mg/kg of MSG only (positive control), Group B = administered with 200 mg/kg of the extract, Group C = administered with normal saline (normal control), Group D = administered with 8 mg/kg of MSG and 200 mg/kg of extract, Group E = administered with 8 mg/kg of MSG and 400 mg/kg of extract.

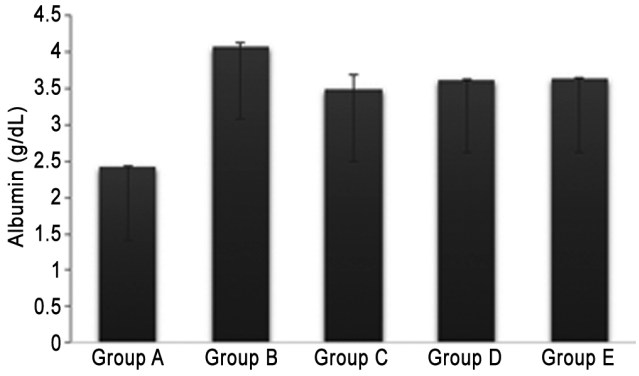

Fig. 3 — Effect of *V. amygdalina* stem extract on serum albumin concentration of normal and MSG-dosed rats. Group A = administered with 8 mg/kg of MSG only (positive control), Group B = administered with 200 mg/kg of the extract, Group C = administered with normal saline (normal control), Group D = administered with 8 mg/kg of MSG and 200 mg/kg of extract, Group E = administered with 8 mg/kg of MSG and 400 mg/kg of extract.

Table 1 — Vitamins and mineral contents of *V. amygdalina* stem extract

Vitamins (mg/g)	Mean±S.E.M.	Minerals (mg/g)	Mean±S.E.M.
Vitamin A	91.77±4.09	Zinc	185.12±3.41
Vitamin C	461.60±16.55	Iron	123.73±23.83
Vitamin E	198.58±201.76	Sodium	396.33±11.43

S.E.M= Standard error of mean

that there was alteration of re-absorptive function of the kidney. It can also be attributed to stabilization of plasma membrane and protection of kidney cell membrane. This suggests that stem extract of *V. amygdalina* can be used to manage hypoalbuminaemia condition.

Mineral and vitamin contents

From the results presented in Table 1, vitamins A (91.77±4.09), C (461.60±16.55) and E (198.58±201.76) mg/g were identified to be moderately present in ethanol stem extract of *V. amygdalina*. The minerals, zinc (185.12±3.41), iron (127.73±23.83) and sodium (396.33±11.43) mg/g were equally present.

Vitamin C was the most abundant vitamin in this stem extract while vitamin A was the least abundant. Sodium was recorded as the highest mineral contained in it while iron was the least in content. The antioxidant enzymes C and E have been found very helpful in the treatment of MSG-induced toxicity. These vitamins improved the histological architecture of rat liver, improved the germinal epithelial of the testicle, reduced MSG-induced testicular toxicity and oxidative stress in rats[31-34].

Histopathological study of the kidney sections

Sections of the kidney collected from the animals treated with normal saline (Fig. 4a) showed normal Glomeruli (G) in their Bowman's capsules (Black arrow) embedded in a framework of normal renal tubules (proximal convoluted tubules, distal convoluted tubules, pars recta and collecting ducts) and normal renal interstitium. Sections of the kidney collected from the animals treated with MSG (Fig. 4b) showed a mild to moderate multifocal degeneration of the renal tubular epithelial cells (black arrow) in the cortex and inner medulla. Also, multifocal areas of intense infiltration of the interstitium by inflammatory mononuclear leucocytes (white arrow) were observed. Sections of the kidney collected from the animals treated with MSG and different doses of *V. amygdalina* stem extract (Fig. 4c,d) showed normal Glomeruli (G) in their Bowman's capsules embedded in a framework of normal renal tubules (proximal convoluted tubules, distal convoluted tubules, pars recta and collecting ducts) and normal renal interstitium.

From the histological study on the kidney of the experimental animals, the positive control showed a mild to moderate multifocal degeneration of the renal tubular epithelial cells while the rat group administered with the extract only showed normal histoarchitecture, normal Glomeruli in their Bowman's capsules embedded in a framework of normal renal tubules and normal renal interstitium. These findings suggest that *V. amygdalina* stem extract is not toxic at considerably high dosage and this agrees with the report of Ojiako and Nwanjo[35]; the extract may have an ameliorative effect on the MSG induced neurotoxicity. This assertion is based on the preservation of neuronal cell bodies in the cortex of the cerebellum as well as the pyramidal cells of the cerebellum following concurrent administration of MSG and test extract at 200 and 400 mg/g doses.

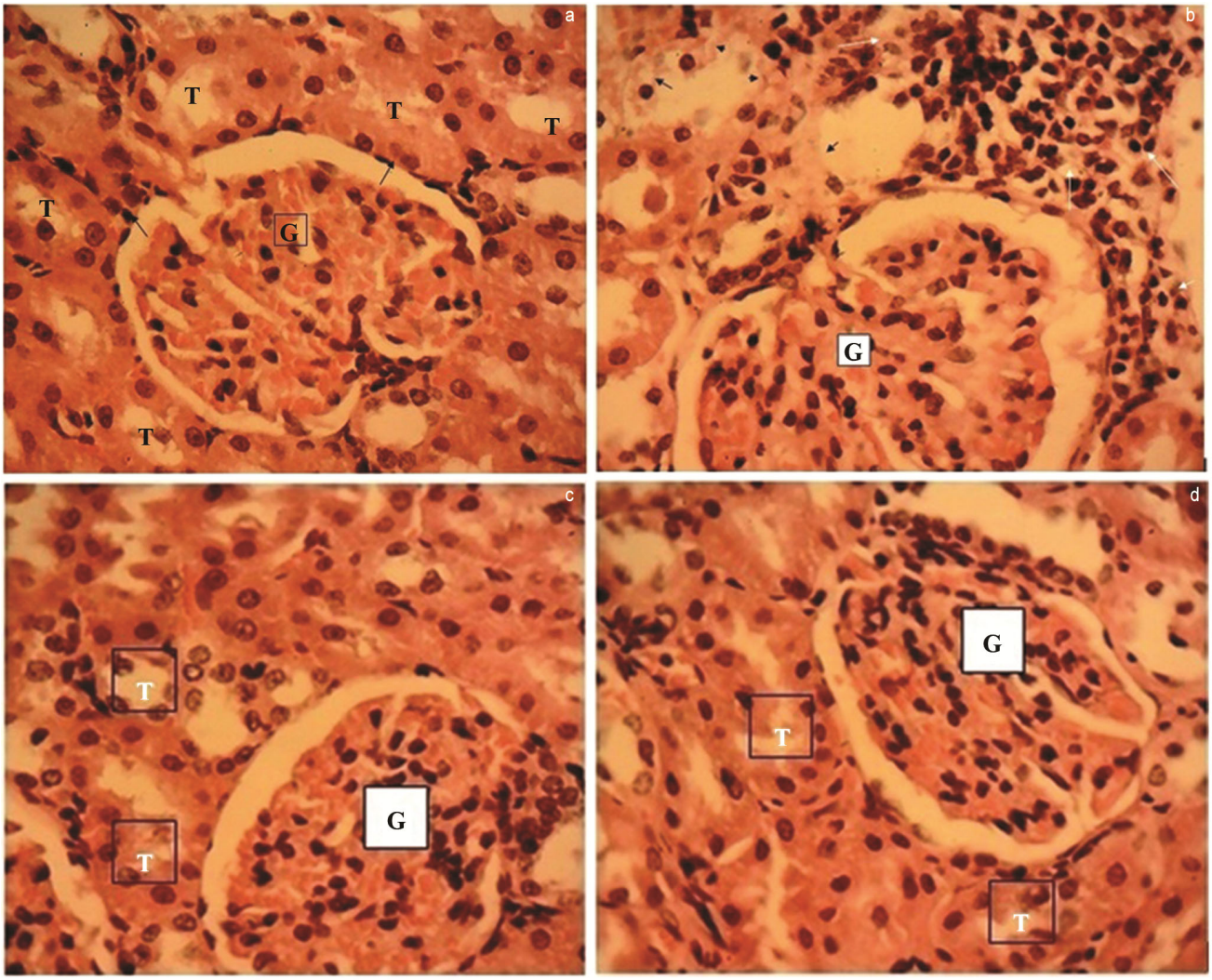

Fig. 4 — a) Photomicrograph of kidney section of rat administered with normal saline (Renal tubules (T); H&E x400), b) Photomicrograph of kidney section of rat administered with MSG (Glomerulus (G); H&E x400), c) Photomicrographs of kidney sections of rat with co-administration of MSG and 200 mg/kg b.w. of the extract (Renal tubules (T); H&E x400), d) Photomicrographs of kidney sections of rat with co-administration of MSG and 400 mg/kg b.w. of the extract (Renal tubules (T); H&E x400).

Conclusion

In conclusion, the extract under study was found to be renal protective since it was able to bring down the increased serum creatinine and urea levels caused by MSG administration and also was able to increase the level of serum albumin. However, it was observed to possess better effect on serum urea at higher doses. Vitamins A, C and E and the minerals zinc, sodium, and iron were observed to be present in the extract. These vitamins and minerals present in this extract may have played a vital role in maintaining the integrity and functionality of the kidney through their free radicals scavenging effect, in which the amount of damage done to tissues and organs in the body is greatly reduced.

Conflict of interest

The authors declare that there is no conflict interest

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