PROTECTIVE EFFECTS OF METHANOL EXTRACT OF *ASYSTASIA GANGETICA* LEAVES ON MONOSODIUM GLUTAMATE (MSG) INDUCED RENAL INJURY IN RATS

[1]UROKO, Robert Ikechukwu, [2]NWEJE-ANYALOWU, Paul Chukwuemeka, [2]IHUOMAH, Victor Emeka, [1]UCHENNA, Nancy Oluomachi and [3]ASADU, Chidimma Lilian

[1]Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

[2]Department of Chemical Sciences, Faculty of Sciences, Clifford University, Owerrinta, Abia State, Nigeria.

[3]Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria Nsukka, Enugu State, Nigeria.

Corresponding Author: Uroko, R. I. Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. **Email**: ir.uroko@mouau.edu.ng **Phone:** +234 806 591 4471

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ABSTRACT

This study evaluated the nephroprotective and curative effects of methanol extract of Asystasia gangetica leaves on monosodium glutamate (MSG) challenged rats. The study adopted a completely randomized experimental design comprising 9 groups of rats. Group 1 served as the normal control, group 2 was MSG control, group 3 was MSG induced treated with silymarin. Groups 4 and 5 received only methanol extract of A. gangetica leaves, groups 6 and 7 (nephroprotective) were pre-treated with methanol extract of A. gangetica leaves for 7 days before receiving 8 g/kg MSG, while groups 8 and 9 (nephrocurative) received 8 g/kg MSG and treated with methanol extract of A. gangetica leaves for 14 days. Each of the treatments was given to the rats orally. The MSG caused significant elevation (p<0.05) of serum creatinine, urea, sodium ion, potassium ion, chloride ion and bicarbonate ion levels in the MSG control when compared with the normal control. The methanol extract of A. gangetica leaves had no significant effects (p>0.05) on the serum urea, creatinine and electrolyte levels of the extract control groups relative to the normal control. The nephroprotective and curative groups treated with graded doses of methanol extract of A. gangetica leaves had significantly reduced (p<0.05) creatinine, urea, sodium, potassium, chloride and bicarbonate levels with mild alterations in the kidney histo-architecture relative to the MSG control. The findings of this study indicated that methanol extract of A. gangetica leaves possesses nephroprotective and curative effects and could protect the kidney from the adverse effects of nephrotoxic agents.

Keywords: *Asystasia gangetica* leaves, Monosodium glutamate, Kidney functions, Serum electrolytes, Nephroprotective

INTRODUCTION

Monosodium glutamate (MSG) is a food additive derived from common amino acids used to enhance food aroma, and taste because it stimulates sensory receptors and increases appetite (Inuwa *et al*., 2011; Ilegbedion *et al*., 2013). It is widely used in almost all Chinese and South-Asian dishes and its consumption has been reported to increase weight gain (Rogers and Blundell, 1990; He *et al*., 2011). In various parts of Nigeria, MSG is referred at as a

bleaching agent and is used together with detergents to remove stains from clothes which are suggestive of severe detrimental effects on some organs and tissues when consumed (Eweka *et al*., 2010). Many studies with animal models have shown that MSG is toxic to various tissues and organs like liver, brain, thymus and kidneys (Diniz *et al*., 2004; Farombi and Onyema, 2006; Pavlovic *et al*., 2009). Due to oxidative stress associated with ingestion of high dose of MSG, pieces of evidence suggest that MSG ingestion has adverse effects on humans because neuroendocrine abnormalities, neuronal degeneration and damage to different organs have been observed in individuals that ingested high doses of MSG (Farombi and Onyema, 2006; Moreno *et al*., 2005; Pavlovic *et al*., 2007). However, many still argue that MSG is safe for human consumption despite the increasing research-based evidences on the toxicity potentials of MGS and the consumer's negative medical responses (Eweka and Om'Iniabohs, 2007).

The kidney is an important excretory organ in humans and other higher animals responsible for the detoxification and excretion of various toxicants in the body via urine and prone to the oxidative attack of all kinds of reactive metabolites. The cell injury and renal damage that may occur from increased reactive oxygen species (ROS), generated from MSG breakdown and increased α-ketoglutarate dehydrogenase activity induced by MSG could impair renal functions and the health status of an individual (Pfaller *et al*., 1990; Ortiz *et al*., 2006). MSG, when ingested could increase the level of intracellular calcium, decrease glutathione (GSH) level and induce increased production of reactive free radicals and consequently lipid peroxidation (Sharma, 2015). Medicinal plant extracts and standard drugs like silymarin with rich antioxidant activities have been reported to protect kidney and liver from oxidative damage by nephrotoxic substances like MSG and carbon tetrachloride (Uroko *et al*., 2019).

Asystasia gangetica (Linn.) also known as Chinese violet is a medicinal plant from the *Acanthaceae* family found across tropical rainforests in Asia and Africa most especially in south-eastern Nigeria (Akah *et al*., 2003). The extract of *A. gangetica* leaves is used to treat asthma, anthelmintic, antihypertensive, pain relief, stomach ache, cough, arthritis, haemorrhage, fever-aches, epilepsy and induction of labour (Akah *et al*., 2003). The phytochemical analysis of *A. gangetica* leaves has revealed the presence of alkaloids, tannins, glycosides, steroidal saponins, flavonoids, phenols and terpenoids (Burkill, 1995; Akah *et al*., 2003).

Arising from the above background, this study was designed to evaluate the effects of ethanol extract of *A. gangetica* leaves on the renal function indices and kidney histomorphology of MSG challenged rats

MATERIALS AND METHODS

Chemicals and Drug: All the chemicals and reagents employed in this study were of analytical grade obtained from Merck Group, Sigma-Aldrich, St. Louis, Missouri, United States. The MSG was purchased from Arshine Pharmaceutical Company Limited, Changsha, China, while the silymarin (tablets) was obtained from the Micro Labs Limited, India.

Collection and Identification of Plant Material: The *A. gangetica* leaves were collected from the Forestry Research Institute of Nigeria, Eastern Station, Ahia Eke Ndume, Umuahia, Abia State. The plant was identified (Burkill, 1995) and authenticated by a plant taxonomist at the Herbarium Unit of the Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, as *A. gangetica* with a voucher number FH124264.

Preparation of Extract: The plant leaves were carefully handpicked and rinsed in running clean tap water and dried under shade until a constant weight was obtained. The dried leaves were ground into a coarse powder using a mechanized grinder, weighed and 500 g of it extracted with 1.5 Litre of absolute methanol for 72 hours. The cold macerated plant sample was filtered using Whatman No. 1 filter paper and the filtrate was concentrated in a water bath at

50°C until all the methanol has evaporated completely. The concentrated plant extract was weighed, percentage yield calculated and the extract stored in a desiccator for the study.

Toxicity and Phytochemical Assay of *Asystasia gangetica:* The lethal toxicity test of *A. gangetica* was adopted from Akah *et al*. (2003), while the phytochemical screening of *A. gangetica* extract was adopted from Hamid *et al*. (2011) and Gopal *et al*. (2013).

Experimental Animals: Fifty-four male Wistar rats weighing 130 – 140 g were purchased from the Animal Genetics and Breeding Laboratory, Department of Zoology and Environmental Sciences, University of Nigeria, Nsukka and acclimatized for 2 weeks at Biochemistry Department, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike. The rats had free access to standard feed (Vital feed; containing 18% crude protein and 3000 kcal/kg metabolizable energy) and drinking water *ad libitum* throughout the acclimatization period. The experimental study was carried out in accordance to the ethical guidelines of the Research Ethics Committee of Iran for the use of animals in experimental research (Mobasher *et al.*, 2008). Approval for the study (MOUAU/VPP/EC/18/003) was obtained from the Ethical Committee of the Department of Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike.

Experimental Design: The study adopted a completely randomized experimental design comprising 9 treatment groups, replicated thrice with each replicate having two rats. Group 1 served as the normal control that received distilled water (2 ml/kg/d), Group 2 was MSG control that received MSG 8 g/kg on day 1 without any treatment, while Group 3 was the standard control that received MSG 8 g/kg but treated with 100 mg/kg silymarin for 14 days. Groups 4 and 5 were extract treated groups without any MSG administration but received 200 and 500 mg/kg/d methanol extract of *A. gangetica* leaves respectively for 14 days. Groups 6 and 7 were nephroprotective groups pre-treated with 200 and 500 mg/kg/d methanol extract of *A. gangetica* leaves for 7 days respectively but received 8 g/kg MSG on day 8 and treatment with the methanol extract of *A. gangetica* leaves continued until the 14^{th} day. Groups 8 and 9 were the curative groups, administered 8 g/kg MSG on day 1 and treated with 200 and 500 mg/kg/d methanol extract of *A. gangetica* leaves respectively for 14 days. The concentrations of the extract used were obtained by dividing LD_{50} of *A. gangetica* (2150 mg/g Akah *et al.,* 2003) by 10.75 for 200 mg/kg and 4.30 for 500 mg/kg. All the treatments were administered to the rats orally. The rats fasted overnight on the 14^{th} day, anesthetized under chloroform fume, dissected to harvest the kidney and blood samples collected from them via cardiac puncture and allowed to clot for biochemical analyses and histological examination respectively.

Determination of Kidney Function Indices: The clotted blood was centrifuged to remove the clot and blood cells, and the resulting liquid supernatant (serum) was used for the biochemical assay**.** The serum creatinine and urea concentrations were determined according to the methods described by Bartels *et al*. (1972) and Fawcett and Scott (1960) using their respective Randox commercial kits. Also, the serum electrolytes: sodium ion (Na^+) and potassium ion (K^+) were determined using the methods of Berry *et al*. (1998) and Teeri and Sesin (1958) respectively, whereas chloride ion (Cl^-) and bicarbonate concentration (HCO_3^-) concentrations were determined according to the methods of Hamilton (1965) and Forrester *et al*. (1976) respectively.

Histological Examination: The rats were euthanized on the 15^{th} day and tissue sections of the kidneys were taken for histological examinations. The sections of the kidneys were fixed in 10% phosphate-buffered formalin for 48 hours followed with tissue preparation. The kidney sections were trimmed, dehydrated in 70, 80, 90% and absolute alcohol, then cleared in 3 grades of xylene and embedded in molten wax. The solidifying tissue-containing wax blocks were cut with a rotary microtome into 5

µm thick sections, floated in a water bath, mounted on grease free slides and incubated at 60℃ for 30 minutes. The 5 µm thick sectioned tissues were then cleared in 3 grades of xylene and rehydrated in 3 grades of alcohol (90, 80 and 70%). The sections were stained with haematoxylin for 15 minutes, blued with ammonium chloride differentiated with 1% acid alcohol and counterstained with Eosin. Permanent mounts were made on degreased glass slides using DPX as mountant (Bancroft and Gamble, 2008). The slides were viewed using a compound light microscope at x4, x16 and x40 objective lenses. Photomicrographs were taken using a Motic 5.0 megapixels microscope camera at x400 magnifications.

Statistical Analysis: The data obtained from this study were subjected to one-way analysis of variance (ANOVA) and Duncan's multiple range comparison tests with a Statistical Product and Service Solutions (SPSS) version 22. The statistical significance was established at 95% confidence level ($p<0.05$) and the results presented as mean ± standard error of mean.

RESULTS

Effects of Methanol Extract of *Asystasia gangetica* Leaves on Serum Urea Concentrations of Monosodium Glutamate Challenged Rats: The urea concentrations of MSG challenged rats indicated significant increases ($p<0.05$) in the serum urea concentrations of the MSG control (Group 3), silymarin treated (Group 3), nephroprotective (Groups 6 and 7) and curative (Groups 8 and 9) treated with grade doses of methanol extract of *A. gangetica* leaves relative to the normal control (Figure 1). However, the extract controls (Groups 4 and 5) treated with only 200 and 500 mg/kg/d methanol extract of *A. gangetica* leaves respectively had no significant decreases ($p>0.05$) in the serum urea levels when compared with normal control. The silymarin treated (Group 3), extract controls (Groups 4 and 5), nephroprotective and curative groups had significant reductions ($p<0.05$) in the serum urea concentration when compared with the MSG control respectively. Also, the extract controls, nephroprotective and curative groups had significantly reduced ($p<0.05$) serum urea levels in comparison with the silymarin treated (Group 3).

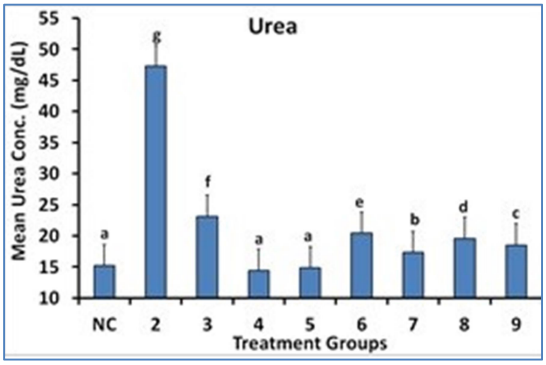

Figure 1: Serum urea concentrations of monosodium glutamate challenged rats treated with methanol extract of *Asystasia gangetica* leaves. *Bars with different superscripts are significantly different at p<0.05*

Effects of Methanol Extract of *Asystasia gangetica* Leaves on Serum Creatinine Concentrations of Monosodium Challenged Rats: The was a significant elevations ($p<0.05$) in the serum creatinine levels of rats in MSG control (Group 2), Group 3 treated with silymarin, nephroprotective treatments (Groups 6 and 7) and nephrocurative groups (Groups 8 and 9) treated with methanol extract of *A. gangetica* leaves when compared with the normal control (Group 1) (Figure 2).

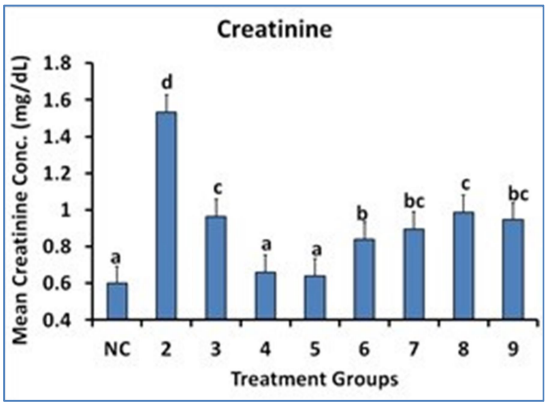

Figure 2: Serum creatinine concentrations of monosodium glutamate challenged rats treated with methanol extract of *Asystasia gangetica* leaves. *Bars with different superscripts are significantly different at p<0.05*

The extract control rats that received only 200 and 500 mg/kg/d methanol extract of *A. gangetica* leaves (Groups 4 and 5) respectively

had no significant increase ($p>0.05$) in the serum creatinine levels in comparison with normal control rats. The MSG challenged rats treated with 100 mg/kg/d silymarin, *A. gangetica* leaves extract (Groups 4 and 5), nephroprotective and curative groups treated with graded doses of methanol extract of *A. gangetica* leaves indicated significant reductions ($p<0.05$) in the serum creatinine levels relative to the MSG control (Group 2). The extract control rats treated with 200 and 500 mg/kg/d methanol extract of *A. gangetica* leaves respectively and the group 6 of the nephroprotective groups treated with 200 mg/kg/d of methanol extract of *A. gangetica* leaves indicated a significant reductions ($p<0.05$) in the serum creatinine levels relative to the group 3 treated with silymarin. However, Group 7 of the nephroprotective groups and curative groups (8 and 9) showed no significant difference ($p>0.05$) with the silymarin treated Group 3.

Effects of Methanol Extract of *A. gangetica* Leaves on Serum Sodium Electrolyte Concentrations of Monosodium Glutamate Challenged Rats: The data in Figure 3 indicated significant increases ($p<0.05$) in the serum sodium (Na^+) electrolyte levels in the MSG control (Group 2), silymarin treated (Group 3), nephroprotective (Groups 6 and 7) and curative (Groups 8 and 9) treated with graded doses of methanol extract of *A. gangetica* leaves when compared with the normal control. The extract controls (Groups 4 and 5) had no significant increases ($p>0.05$) in the serum sodium electrolyte levels in comparison with the normal control. The silymarin treated (Group 3), extract controls, nephroprotective and curative groups respectively had significant reductions ($p<0.05$) in the serum sodium electrolyte levels relative to the MSG control. The extract controls and Group 9 of the nephrocurative groups treated with 500 mg/kg/d methanol extract of *A. gangetica* leaves had significant reductions ($p<0.05$) in the serum sodium levels when compared with the silymarin treated Group 3. However, Group 8 rats of the nephrocurative groups treated with 200 mg/kg/d methanol extract of *A. gangetica* leaves had significant increase ($p<0.05$) in the serum sodium level relative to the silymarin treated Group 3 rats.

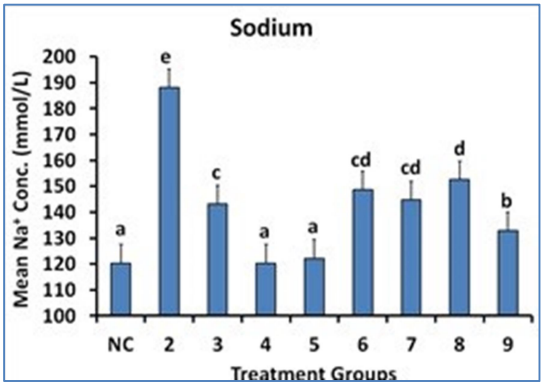

Figure 3: Serum sodium ion concentrations of monosodium glutamate challenged rats treated with methanol extract of *Asystasia gangetica* leaves. *Bars with different superscripts are significantly different at $p<0.05$*

Besides, the rats in the nephroprotective groups (6 and 7) treated with 200 and 500 mg/kg/d methanol extract of *A. gangetica* leaves respectively showed no significant increase ($p>0.05$) in the serum sodium electrolyte levels in comparison with the silymarin treated rats in Group 3.

Effects of Methanol Extract of *Asystasia gangetica* Leaves on Serum Potassium Electrolyte Concentrations of Monosodium Glutamate Challenged Rats: The result in Figure 4 showed a significantly elevated ($p<0.05$) serum potassium ion (K^+) levels in rats in the MSG control (group 2), silymarin treated (Group 3), extract controls (Groups 4 and 5), nephroprotective (Groups 6 and 7) and curative groups (8 and 9) treated with varying doses of methanol extract of *A. gangetica* leaves when compared with the normal control rats. However, rats in the silymarin treated (Group 3), extract controls, nephroprotective and curative groups respectively had significant reductions ($p<0.05$) in the serum potassium electrolyte levels when compared with the MSG control rats. The extract controls had significant reduction ($p<0.05$) in the serum potassium level, while the rats in the nephroprotective and curative groups had significant increase

(p<0.05) in the serum potassium level in comparison with the silymarin treated rats.

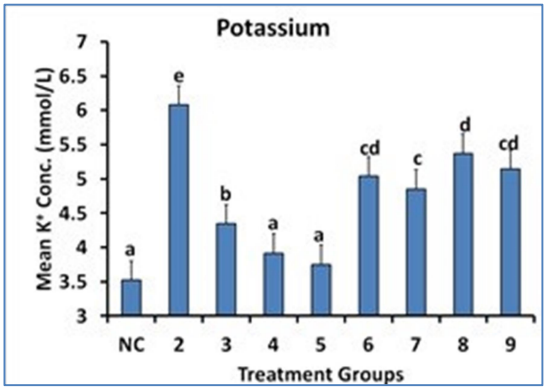

Figure 4: Serum potassium ion concentrations of monosodium glutamate challenged rats treated with methanol extract of *Asystasia gangetica* leaves. *Bars with different superscripts are significantly different at p<0.05*

Rats in the nephroprotective and curative groups treated with methanol extract of *A. gangetica* leaves had significant increases (p<0.05) in serum potassium electrolyte levels when compared with the MSG challenged rats treated with silymarin.

Effects of Methanol Extract of *Asystasia gangetica* Leaves on Serum Chloride Ion Electrolyte Concentration of Monosodium Glutamate Challenged Rats: The result in Figure 5 indicated significant increases (p<0.05) in the serum chloride ion levels of rats in the MSG control (Group 2), MSG challenged rats treated with silymarin (Group 3) and rats in the nephroprotective groups (6 and 7) pre-treated with methanol extract of methanol of *A. gangetica* leaves and challenges with a high dose of MSG when compared with normal control. Rats in the extract controls (Groups 4 and 5) without MSG challenge and nephrocurative groups (8 and 9) challenged with MSG and treated with varied doses of methanol extract of *A. gangetica* leaves respectively had no significant increases (p>0.05) in the serum chloride ion level in comparison with the normal control. On the other hand, rats in the extract controls, silymarin treated, nephroprotective and curative groups respectively had significant reductions (p<0.05) in the serum chloride ion levels when compared with rats in the MSG challenged but untreated (Group 2).

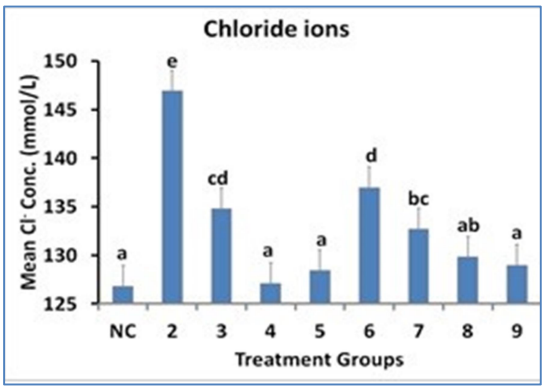

Figure 5: Serum chloride ion concentrations of monosodium glutamate challenged rats treated with methanol extract of *Asystasia gangetica* leaves. *Bars with different superscripts are significantly different at p<0.05*

It was further, observed that only rats in the extract control groups and nephrocurative groups exhibited significant reductions (p<0.05) in the serum chloride ion levels in comparison with the MSG challenged rats treated with silymarin drug.

Effects of Methanol Extract of *Asystasia gangetica* Leaves on Serum Bicarbonate Ion Electrolyte Concentration of Monosodium Glutamate Challenged Rats: The data in Figure 6 indicated significant increases (p<0.05) in the serum bicarbonate (HCO_3^-) levels of MSG challenged and untreated rats (Group 2), silymarin treated (Group 3), nephroprotective groups (6 and 7) and curative groups (8 and 9) rats treated with varying doses of methanol extract of *A. gangetica* leaves respectively, when compared with normal control. However, rats in the extract control groups (4 and 5) had no significant increases (p<0.05) in the serum bicarbonate levels relative to the normal control. Rats in the nephroprotective and curative groups challenge with MSG and treated with graded doses of methanol extract of *A. gangetica* leaves had significant increases (p<0.05) in serum bicarbonate levels in comparison with rats in the MSG challenged group 3 treated with 100 mg/kg/d silymarin.

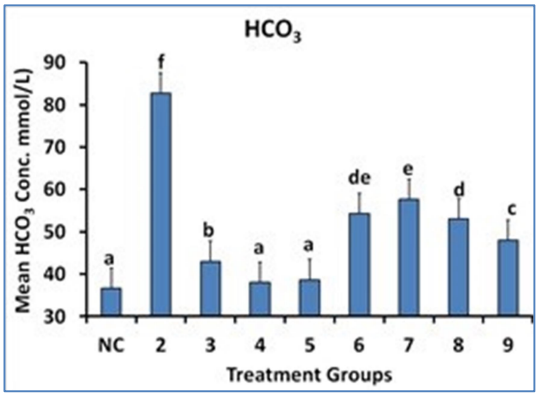

Figure 6: Serum bicarbonate ion concentrations of monosodium glutamate challenged rats treated with methanol extract of *Asystasia gangetica* leaves. *Bars with different superscripts are significantly different at p<0.05*

Besides, the extract control groups 4 and 5 without MSG challenge but treated with 200 and 500 mg/kg/d methanol extract of *A. gangetica* leaves respectively had significant reductions ($p<0.05$) in the serum bicarbonate levels when compared with the MSG challenged rats treated with 100 mg/kg/d silymarin.

Effects of Methanol Extract of *Asystasia gangetica* Leaves on Kidney Histomorphology of Monosodium Glutamate (MSG) Challenged Rats: The kidney histomorphology of rats in the normal control indicated normal renal tissue histomorphology (Figure 7a). Normal glomeruli (G) in their Bowman's capsules (white arrow) were observed surrounded by myriads of normal renal tubules (proximal convoluted tubule, distal convoluted tubule; Pars recta and collecting ducts) suspended in a highly vascularized connective tissue matrix (the renal interstitium). The kidney histomorphology from the MSG challenged untreated group 2 rats (Figure 7b) showed severe multifocal vacuolar degeneration and necrosis of the epithelial lining of the renal tubules. Also, the kidneys of MSG challenged rats treated with 100 mg/kg/d silymarin (Group 3) (Figure 7c), extract control groups (4 and 5) treated with 200 and 500 mg/kg/d methanol extract of *A. gangetica* leaves (Figures 7d and e) respectively showed normal histomorphology similar to the normal control. The histomorphology of kidneys from the nephroprotective groups (6 and 7) (Figures 7f and g) and nephrocurative groups (8 and 9) (Figures 7h and i) respectively showed mild multifocal vacuolar degeneration of the epithelial lining of the renal tubules. The affected cells appear swollen with foamy clear cytoplasm (blue arrow), glomeruli (G), Bowman's capsule (white arrow), blood vessel (BV) and the black arrows indicated renal tubules.

DISCUSSION

Methanol extract of *A. gangetica* has been reported to be relatively safe in rodents with LD_{50} value of 2150 mg/kg (Akah *et al.,* 2003). Similarly, the results of previous phytochemical analysis of various extracts of *A. gangetica* have indicated its composition of important pharmacologically active phytochemicals including saponins, reducing sugar, glycoside, flavonoids and anthraquinone (Hamid *et al*., 2011). Gopal *et al*. (2013) have also reported the presence of alkaloids, coumarins, steroids, terpenoids, phenols, and tannins in the whole *A. gangetica* extract. These bioactive metabolites are responsible for the nephroprotective and curative effects of methanol extract of A. gangetica leaves on monosodium glutamate (MSG) challenged rats.

This study evaluated the effects of methanol extract of *A. gangetica* leaves on the kidney function indices and kidney histomorphology of MSG challenged rats. MSG is a well-known seasoning agent that improves the taste, aroma and palatability of food which could enhance the appetite of the consumers but many adverse health effects have been associated with its consumption.

Creatinine is one of the non-protein nitrogenous by-products generated from the metabolic degradation of creatinine and phosphocreatine and serves as a useful marker of renal function status. It is a more reliable marker of kidney function than urea because its blood level is relatively unaffected by dietary intake of food (Price and Finney, 2000; Abireh *et al*., 2020). The elevated serum creatinine level in the MSG challenged untreated rats in comparison with normal control indicated the adverse effects on MSG on kidney and its functions.

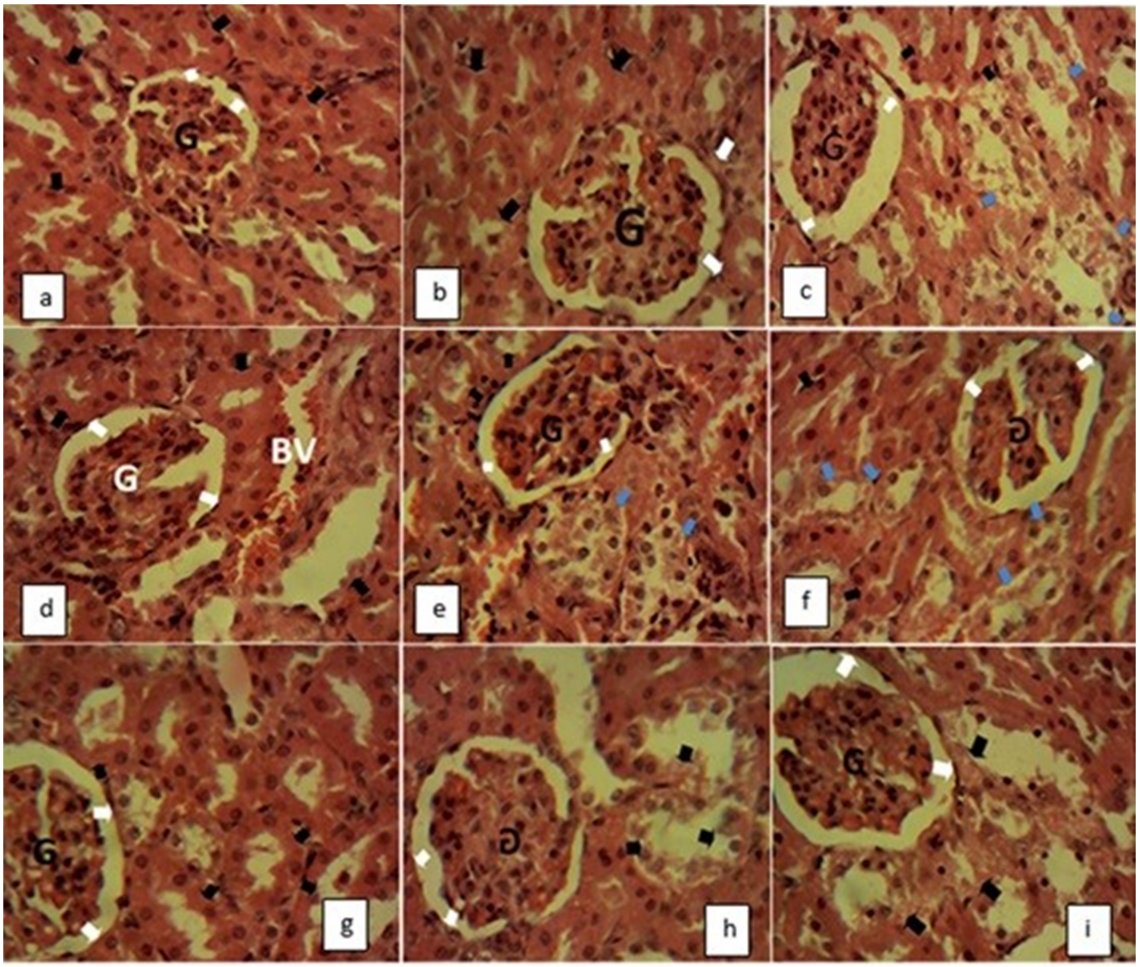

Figure 7 a - i: Kidney histomorphology of monosodium glutamate challenged rats treated with methanol extract of *A. gangetica* leaves. *Key: Cytoplasm (blue arrow), Glomeruli (G), Bowman's capsule (white arrow), Blood vessel (BV), Renal tubules (black arrow). H&E, Mag x400*

The toxic effects of MSG could have affected the renal function of the rats and impaired the glomerular capacity to filter creatinine efficiently from blood to ease its urinary excretion which resulted in the high serum creatinine level common in severe kidney disorder. These findings are in agreement with findings of Abireh *et al*. (2020), that increased serum creatinine levels are associated with kidney injury. Also, the significant reduction in the serum creatinine level of MSG challenged rats treated with silymarin and methanol extract of *A. gangetica* leaves respectively relative to the MSG challenged untreated rats indicated the nephroprotective effect of silymarin and the extract against the toxic effects of MSG on the kidney is in line with the finding of Abireh *et al*. (2020). The silymarin treated rats had improved glomerular filtration of creatinine which promoted its excretion. However, the silymarin treated rats experienced some levels of MSG toxicity and kidney damage as they had impaired excretion compared with normal control.

Urea is a metabolic waste product generated from the catabolism of amino acids, proteins and other nitrogenous compounds and its high blood level is frequently associated with renal disease due to reduced glomerular filtration rate and urinary excretion (Laterza *et al*., 2002). The serum urea level when coupled with the serum creatinine level are very reliable means of assessing kidney function and possible occurrence of renal failure as could give a valid

glomerular filtration rate. The increased serum urea level in the MSG challenged untreated rats is indicative that MSG is nephrotoxic and its administration impaired the kidney function of the rats consequently decreasing glomerular filtration rate and urinary excretion of urea which is consistent with the findings of Nisha *et al*. (2017). The treatment of MSG challenged rats with silymarin decreased the serum urea level drastically relative to the untreated treated MSG challenged rats which indicated the recovery of the rats from the nephrotoxic effects of MSG and improve renal function. However, treatment with silymarin could not fully reverse the toxic effects of MSG on the kidney function as the rats had significantly higher serum urea level than the normal control. The no significant increase in the serum urea levels observed in extract control groups in comparison with the normal control suggest that the methanol extract of *A. gangetica* leaves has no adverse health effect on the renal function. This demonstrated that the control rats have normal glomerular filtration of urea and enhanced excretion from the urine. Furthermore, the significantly reduced serum urea levels in the nephroprotective and curative rats challenged with a high dose of MSG indicated that the methanol extract of *A. gangetica* leaves possesses both nephroprotective and curative effects against MSG toxicity is in line with findings of Abireh *et al*. (2020). The methanol extract of *A. gangetica* leaves possesses better nephroprotective and curative effects than silymarin and could be useful in the management of renal disorders. Although the methanol extract of *A. gangetica* leaves reduced serum urea level in the MSG challenged rats, it was unable to reduce their serum urea level to that of the normal control rats.

The serum sodium ions play a central role in maintaining the normal extracellular fluid level and membrane potentials and as such abnormally high or low levels of serum sodium ions could result to an imbalance which will have negative health consequences (Roumelioti *et al*., 2018). The highly elevated serum sodium ion levels in the MSG challenged untreated rats in comparison with normal control is indicative of the adverse effects of MSG on the kidney most especially the proximal tubule and aldosterone hormone that regulate its serum level which is in agreement with the finding of Olarotimi (2020). MSG could have impaired the urinary excretion of excess sodium and stimulated its increased reabsorption in the proximal tubule which has subjected the rats to hypernatremia and adverse health effects associated with it. However, the significant reduction observed in the serum sodium ion level of the MSG challenged with silymarin is attributed to its nephroprotective effects which possibly prevented excessive reabsorption of sodium ions and increased its urinary excretion. The relatively stable serum sodium level in the extract control groups similar to normal control further showed that methanol extract of *A. gangetica* leaves is not nephrotoxic at the tested concentrations and its use may not impair kidney functions. Also, the much reductions in the serum levels of nephroprotective and curative groups challenged with a dose of MSG but treated with methanol extract of *A. gangetica* leaves could be attributed to the nephroprotective and curative effects of methanol extract of *A. gangetica* leaves. It showed that the methanol extract of *A. gangetica* leaves could relatively maintain serum sodium level at increased doses than lower doses and serve as a better alternative to silymarin in managing a renal injury.

Potassium ion is the principal cation in the intracellular fluid and its serum level is reflective of the overall potassium status in the body. Na^+-K^+ ATPase helps to maintain a balance between the extracellular and intracellular sodium and potassium levels in the body. However, kidney plays a vital role in maintaining normal serum potassium ion level as it is filtered by the glomerular in the kidney during excretion, while its reabsorption occurs at the proximal convoluted tubule of the kidney (Gumz *et al*., 2015). The significantly increased level of serum potassium ions in the MSG challenged untreated rats in comparison with the normal control may be attributed to the toxic effects of MSG on the kidney. The MSG administration may have induced increased release of potassium from cells, reduced potassium excretion due to kidney injury and

decrease aldosterone secretion and sensitivity is in line with the findings of Viera and Wouk (2015). The reduced serum potassium ion levels in the MSG challenged rats treated with silymarin relative the MSG challenged untreated showed that rats were recovering from toxic effects of MSG and had improved kidney function and the ability to excrete potassium ions. The extract control rats had normal serum potassium ion levels and showed that the rats suffered no impaired kidney function suggesting that the methanol extract of *A. gangetica* leaves may have no adverse health effects on the kidney within the tested doses. The relatively reduced serum potassium ion levels in the nephroprotective and curative groups, when compared with the MSG challenged and untreated group suggest that the rats experienced minimal adverse effects from MSG toxicity due to the nephroprotective and curative effects of methanol extract of *A. gangetica* leaves that resulted to the improved kidney function and excretion of potassium ions. Rats in the nephroprotective and curative groups treated with methanol extract of *A. gangetica* leaves had high serum potassium ion level in comparison with the normal control which may be attributed to the reduced kidney function and potassium ion excretion in the rats (De Nicola and Zoccali, 2016).

Renal injury with resultant impaired kidney function is associated with the accumulation of serum electrolytes like chloride ions due to insufficient blood circulation to the nephrons, and decline in the glomerular filtration rate (Gounden *et al.*, 2021). Chloride ion is a major anion present in the extracellular fluid, it is reabsorbed in the proximal and distal tubules of the kidney and its serum level serves as a good indicator of glomerular filtration rate (Planelles, 2004). The elevated serum chloride ion concentration in the MSG challenged rats indicated that the rats experienced a loss of ability to concentrate urine and passage of large volume of fluid devoid of electrolyte. The stable serum chloride ion concentrations in the extract control groups similar to the normal control suggest that the extract has no toxic effects on the kidney and were able to maintain serum chloride ion level via effective glomerular filtration and reabsorption at the proximal tubules. The significantly reduced serum chloride ion levels in the nephroprotective and curative groups challenged with MSG but treated with methanol extract of *A. gangetica* leaves relative to the MSG challenged untreated group could be attributed to the nephroprotective effects of the *A. gangetica* leaves. The MSG challenged rats treated with the *A. gangetica* leaves had improved renal functions and prevented the accumulation of excess chloride ions (hyperchloremia) in the blood of the rats. These findings are consistent with the reports of Yunos *et al.* (2020), that hypernatremia as observed in this study may induce elevated levels of serum chloride ion.

The much-observed serum bicarbonate levels in the MSG challenged rats untreated could be an indication of impaired glomerular filtration in the rats and increased proximal reabsorption of bicarbonate triggered by the toxic effects of MSG on the kidney. The MSG could have induced alkalosis in the challenged rats possibly via excessive excretion of hydrogen ions or retention of bicarbonate ions in the blood which is in line findings of Gillion *et al*. (2019), that reduction in the glomerular filtration rate is a major factor responsible for elevated serum bicarbonate level.. The no significant differences observed in the serum bicarbonate levels of normal rats (extract controls) treated with methanol extract of *A. gangetica* leaves relative to normal control further showed that the extract of *A. gangetica* leaves has no toxic effects on the kidney functions and serum electrolyte levels. However, the marked reductions in the serum bicarbonate levels in the nephroprotective and curative groups challenged with a high dose of MSG but treated with a methanol extract of *A. gangetica* leaves in comparison with the MSG challenged untreated rats indicated protective effects of the plant extract on the kidney. The extract has both nephroprotective and curative activities that helped rats maintained normal kidney functions including effective glomerular filtration rate and reabsorption of bicarbonate ions in the proximal tubule which is necessary to regulate acid-base balance in the body.

The alterations in the kidney histomorphology such as severe multifocal vacuolar degeneration and necrosis of the epithelial lining of the renal tubules observed in the rats that received a high dose of MSG without any treatment showed that MSG has adverse health effects on the kidney histo-architecture which could greatly impair kidney functions. This is in agreement with the findings of Eweka and Om'Iniabohs (2007). However, the mild multifocal vacuolar degeneration of the epithelial lining of the renal tubules of the nephroprotective and curative groups challenged with a high dose of MSG but treated with methanol extract of *A. gangetica* leaves indicated improved kidney histo-architecture suggesting that rats suffered minimal kidney injury due to MSG toxicity and agrees with the finding of Elbassuoni *et al*. (2018). The improved kidney histo-architecture observed in the nephroprotective and curative groups further showed that the rats could carry out their kidney functions including the concentration of urine and reabsorption serum electrolytes effectively than the untreated MSG challenged untreated rats.

Conclusion: The findings of this study showed that the methanol extract of *A. gangetica* leaves possesses both nephroprotective and curative properties that could prevent or reverse kidney injury and maintain normal kidney functions. However, further studies are required to ascertain the bioactive components responsible for its nephroprotective activities and their mechanism of actions.

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