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Received: October 04, 2017; Published: October 27, 2017

Abstract

The effect of ethanolic extract of avocado pear (*Persea americana*) seeds (ASE) on normal and monosodium glutamate (MSG)compromised rats' kidney histology and some serum bio-functional parameters was studied using standard protocols. The serum urea concentration of rats was less marked in the rats exposed to the extract alone (57.14%) as against those in MSG group (82.66%) and the effect following concomitant exposure to MSG and ASE decreased progressively with increasing extract dose. A similar trend of effect was observed for the serum creatinine and bicarbonate ion concentration. However, the higher (p < 0.05) serum chloride ion concentration was more marked in the medium extract co-treated group of rats relative to the control (240.04%) and MSG (30.69%) groups followed by high extract co-administered group (209.96 and 18.99% respectively) whereas the change in the serum potassium concentration of rats in high extract co-administered group relative to either the control or MSG group was significant (p < 0.05) but dose independent. The observations on the blood vessels congestion of rats' kidney histology in the various groups seem to support the serum chemistry results. Thus, the study demonstrated MSG-induced adverse effect, and inconsistent modulation by ASE, on the rats' kidney histology and some serum bio-functional indicators. The apparent synergistic interaction effect of MSG with ASE may result from unusual adverse and complex biochemical response in the rats. Caution in the use of ASE especially together with MSG should be exercised while further studies to elucidate the basis for the observations are warranted and recommended.

Keywords: Urea; Creatinine; Chloride; Potassium; Bicarbonate

Abbreviations

ASE: Ethanolic Extract of Avocado Pear (*Persea americana*) Seeds (ASE); MSG: Monosodium Glutamate; bw: Body Weight; rpm: Rotor Per Minute; SEM: Standard Error of Mean; ANOVA: Analysis of Variance

Introduction

The utilization of agro products results in solid waste generation [1,2] which could over burden the environment on decomposition warranting series of studies that could enhance the utilization of such solid agro wastes [3]. Avocado plant (*Persea americana*) belongs to the family, *Lauraceae* and genus, *Persea* [4]. Avocado pear originated from Guatemala and Mexico and it is now grown throughout the tropical and sub-tropical regions [5]. The fruit, a berry having a round or oval shape and a single seed with thick fleshy pulp, is known as known as "*Igba* or *apoka*" in Yoruba [6] and as *ube oyibo* (loosely translated to 'English as 'foreign pear') in Ojoto and neighbouring com-

munities south east Nigeria. The fruit pulp is edible [7] and rich in mono-unsaturated fatty acids [8]. The seed is essentially discarded as solid agro waste, although consumed in some countries [9] including Nigeria, where (owing to the belief in its possible anti-hypertensive properties) it is milled and incorporated into foods including soup, puddings and paps [10]. Ethno medicinal uses of avocado pear plant parts including the seeds against varied ailments have been reported [11-17]. In particular the use of avocado pear seed in the management of hypertension, diabetes, cancer and inflammation have been reported [11,12,17]. Kidney is among the major organs of the body and could be involved in the patho-biochemistry of these pathologies, thus we therefore speculated that avocado pear seeds could affect the kidney function and histology of especially compromised animals.

Monosodium glutamate (MSG), a sodium salt of glutamic acid produced by fermentation of notably sugarcane starch [18], is a flavour enhancer [19]. Daily MSG consumption ranges from 0.5 mg/kg to 3 g/kg [20] and could be inadvertently abused since its content in packaged may not be indicated on the label [21]. Monosodium glutamate-induced toxic effects in human and experimental animals have been reported [22-29], but its continued use as flavour enhancing food additive was not legislated against. These warranted continued search for natural products that could mitigate the adverse effects of MSG including on renal function of animals, necessitating this study aimed at determining the effect of ethanolic extract of avocado pear seed flour on normal and monosodium glutamate-compromised rats' kidney histology and serum bio-functional parameters. The objectives to achieving this aim were by determining the serum bio-functional indicators of kidney functions including serum creatinine, urea and sodium chloride concentration as well as assessing the kidney histology of the rats exposed as in the study design. Urea is a waste product of protein metabolism in the liver that is excreted by the kidney via urine [30] and is a common bio-indicator of kidney functions and pathologies. Creatinine is also a bio-indicator of renal function. Its clearance indicates glomerular filtration rate [31] while increased creatinine concentration in the serum indicated renal dysfunction following altered ability of the kidney to filter creatinine [28]. And, kidney malfunction affects serum chloride homeostasis, resulting to elevated or reduced serum chloride concentration [32] while changes in organ histology could confirm compromised organ function.

Materials and Methods

Collection, identification, preparation and extraction of plant materials

Monosodium brand (99% purity) used in this study was procured from *Ubani* market, a daily food condiments market in Umuahia, south east Nigeria. Chemicals and solvents used in this study were products of reputable companies procured from reputable chemical dealers and were used without further purification.

Matured avocado pear fruits were bought in a local market in Umuahia close to Michael Okpara University of Agriculture Umudike, during the fruiting season of June, 2015 and identified as *Persea americana* mill (*Lauraceae*) in the Plant Science Department of Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. Following deseeding, the seeds were washed with clean tap water, crushed into smaller pieces using manual grater and sun-dried for three days. The dried seeds were milled into powder using a laboratory miller (ED-5, U.S.A) and stored in an air tight container until used for the determination of vitamins as in the study design.

The avocado seed flour was extracted with ethanol by cold maceration method. The extraction method involved weighing 700g of the avocado pear seed flour into a volumetric flask, soaking in 1400 ml of 90% ethanol with intermittent shaking and stirring for three days and thereafter filtering with No 1 Whatmann filter paper. The filtrate was concentrated using water bath at 60°C and was further dried in an oven at 50°C. The extract was packed into a sample bottle and stored in a refrigerator until used as in the animal study design to assess the effect on normal and monosodium glutamate-intoxicated rats' kidney histology and serum bio-functional parameters.

Animal experimentation

The MSG-intoxicating dose for the rats was 8000 mg/kg body weight for 14 days according to Mariyamma., *et al.* [22] as supported by other studies [33-36]. The ethanolic extract of avocado pear seed (1g) was dissolved in 10 ml of distilled water as the stock solution and

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three graded doses were selected as follows: low, medium and high doses (100 mg/kg body weight, 300 mg/kg body weight and 500 mg/kg body weight) respectively.

Twenty-four albino rats (*Rattus norvegicus*) of either sex (mean body weight, 96.00 ± 10.00g) used in this study were obtained from the animal breeding unit of the College of Veterinary Medicine, University of Nigeria, Nsukka. The animals were acclimatized for 1 week and then randomized (based on weight) to six experimentation groups with sample size of four rats as described below.

Rats in the normal control group were sham-dosed with distilled water (without either the extract or MSG) while rats in the MSG group (negative control) were fed intoxicating dose (8000 mg/kg body weight) of MSG [22]. Rats in the extract group (Extract control group) were fed ethanolic extract of avocado pear seed flour at 300 mg/kg body weight while rats in the MSG + low extract group were concomitantly exposed to ethanolic extract of avocado pear seed flour (100 mg/kg body weight) and intoxicating dose of MSG (8000 mg/kg body weight) whereas rats in the MSG + medium extract group were co-administered 300 mg/kg body weight of ethanolic extract of avocado pear seed flour (500 mg/kg body weight). Rats in the MSG + high extract group were concomitantly exposed to ethanolic extract of avocado pear seed flour (500 mg/kg body weight). The exposure was per oral using orogastric tube and daily for 2 weeks (14 days).

Ethical consideration

The animals were placed in rat cages kept in a well ventilated room and allowed free access to standard feed and clean tap water throughout the experimentation period. Animals were exposed to natural room temperature with a 12 hour day/night cycle.

This study considered and adhered to the standard ethical use of experimental animals. Throughout out the experimentation (acclimatization and exposure periods), all rats were housed at 25°C in stainless steel cages under normal daylight/dark cycle and humid tropical conditions. The rats were allowed free access to rat feed (Vital feed, Jos Nigeria) and tap water, and generally received humane care in accordance with the guidelines of the National institute of Health, USA for ethical treatment of laboratory animals as approved by the various (departmental and college) ethical committees of Michael Okpara University of Agriculture Umudike, Nigeria.

Sacrifice and blood sample collection

After 2 weeks (14 days) exposure, the rats were sacrificed the next day after overnight fast by cervical dislocation and the blood sample of the respective rats was collected individually from the heart using a syringe into a clean non-anti-coagulated polystyrene tube, allowed to clot, centrifuged at 3000 rpm for 5 minutes and the serum collected and stored in a refrigerator until used.

Determination of studied parameters

Serum chloride ion concentration was determined based on the colorimetric estimation of red colored complex formation from the reaction of the sample (or the standard chloride) and chloride reagent mixed and incubated at 25°C for 5 min as read at 500 nm and calculated using the formula below:

Serum chloride ion concentration
$$(mmol / l) = \frac{Absorbance of sample}{Absorbance of standard} \times \frac{concentration of std}{1}$$

Serum potassium ion concentration was determined by the turbidometric method as described by Henry., *et al.* [37] based on the principle that the extent of turbidity is proportional to the potassium concentration as measured spectrophotometrically at 578 nm and calculated from the relation:

Potassium ion conc. $(mmol / l) = \frac{Absorbance of test}{Absorbance of standard} \times Conc. of standard$

Serum bicarbonate ion concentration was determined using enzyme spectrophotometric procedures as described by Forrester., *et al* [38]. This was based on the principle that phosphoenol pyruvate carboxylase catalyzes the reaction between phosphoenol pyruvate and bicarbonate to form oxaloacetate and phosphate ion while oxaloacetate is reduced to malate with simultaneous oxidation of an equimolar amount of reduced nicotinamide adenine dinucleotide (NADH) to oxidized nicotinamide adenine dinucleotide (NAD) on catalysis by malate dehydrogenase. This results in a decrease in absorbance at 340 nm that is directly proportional to bicarbonate ion concentration in the serum as could be calculated from the relation:

Serum bicarbonate ion $(mmol / l) = \frac{Absorb of blank - Absorb of test}{Absorb of blank - absorb of std} \times Con of std$

Serum urea concentration was determined based on the principle that urea in serum is hydrolysed to ammonia in the presence of urease and the ammonia measured spectrophotometrically on reacting with hypochlorite and phenol (Berthelot reaction) to form a bluecoloured indophenols compound as could be calculated from the formula:

Urea Concentration =
$$\frac{A_{sample}}{A_{standard}} \times Standard \ concentration \ (mg / dl)$$

Serum creatinine concentration was determined by the method of Henry [37]. This was based on the principle that creatinine reacts with picric acid in alkaline conditions to form a coloured complex, which absorbs at 510 nm and that the intensity of coloured compound is proportional to the creatinine concentration in the sample as calculated from the relation:

Creatinine concentration
$$(mg / dl) = \frac{A_{sample}}{A_{standard}} \times Concentration of standard$$

The calculation of change relative to any group was as developed earlier [39] and severally used [26,28,33,36,40-44]. Change relative to either normal control or negative control (MSG group) was calculated using the relation:

Change relative to
$$K(\%) = \frac{100 \times (V - K)}{K}$$

where K represents the constant group hence constant value and V represents the variable groups hence variable values. It is important that the arrangement order in bracket be adhered to so as to have the accurate sign for the calculated change.

Histological examination

Kidney of the sacrificed rats were identified and harvested. They were fixed in 10% buffered formalin for 72 hours. The tissues were then dehydrated in alcohol of graded concentrations and embedded in paraffin. The embedded tissues were cut into sections of 5 μ m thickness and these were stained with haematoxylin and eosin for photomicroscopic assessment. Photomicrographs of samples were then taken.

Statistical analysis

Descriptive statistics and test for significance difference in mean were carried out on the data generated by analysis of variance (ANO-VA) with the statistical package for social sciences for Windows version 16. The turkey post hoc test was used to identify the means that differ significantly at p<0.05. Results were expressed as mean ± standard error of mean, SEM.

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Results and Discussion

Results

The result as shown on table 1 revealed that higher (p < 0.05) serum urea concentration of rats in the other groups as compared to rats in the control was less marked in the rats exposed to the extract alone (57.14%) as against in the rats exposed to intoxicating dose of MSG (82.66%). The change relative to either the control or the MSG group was highest in the low extract co-treated group but decreased progressively with increasing extract dose.

Groups	Urea (mg/dl)	Change relative to the Control (%)	Change relative to MSG group (%)
Normal control (feed + water only)	8.75 ± 1.60	0.00	-45.31*
MSG/Negative control (8000 mg/kg bw MSG)	16.00 ± 0.91	+82.66*	0.00
Extract group (300 mg/kg bw extract)	+13.75 ± 0.75	+57.14*	-14.06*
Low extract co-treated group (MSG, 8000 mg/kg bw +100mg/kg bw extract)	16.75 ± 2.14	+91.44*	+04.69
Medium extract co-treated group (MSG, 8000 mg/ kg bw +300 mg/kg bw extract)	14.50 ± 1.56	+65.71*	-09.38
High extract co-treated group (MSG, 8000 mg/kg bw +500mg/kg bw extract)	12.75 ± 0.25	+45.71*	-20.31*

Table 1: Effect of ethanolic extract of avocado pear seed (ASE) on urea concentration (mg/dl) of normal and monosodium

 glutamate-compromised rats' serum.

Values are mean ± SEM for n= 4. Difference considered statistically significant at p < 0.05 + denotes higher by; – denotes lower by.

The result as shown on table 2 revealed that higher (p < 0.05) serum creatinine concentration of rats in the other groups as compared to rats in the control was marked in the rats co-exposed to the low extract and intoxicating dose of MSG (118.52%). The change relative to either the control or the MSG group which was highest in the low extract co-treated group decreased progressively with increasing extract dose.

Groups	Creatinine (mg/dl)	Change relative to the Control (%)	Change relative to MSG group (%)
Normal control (feed + water only)	0.27 ± 0.05	0.00	-44.89*
MSG/Negative control (8000 mg/kg bw MSG)	0.49 ± 0.10	+81.48*	0.00
Extract group (300 mg/kg bw extract)	0.51 ± 0.02	+88.89*	+04.08
Low extract co-treated group (MSG, 8000 mg/ kg bw +100mg/kg bw extract)	0.59 ± 0.06	+118.52*	+20.41*
Medium extract co-treated group (MSG, 8000 mg/kg bw +300 mg/kg bw extract)	0.52 ± 0.06	+92.59*	+06.12
High extract co-treated group (MSG, 8000 mg/kg bw +500mg/kg bw extract)	0.46 ± 0.10	70.37*	-06.12

 Table 2: Effect of ethanolic extract of avocado pear seed (ASE) on creatinine concentration (mg/dl) of normal and

 monosodium glutamate-compromised rats' serum.

Values are mean \pm SEM for n = 4. Difference considered statistically significant at p < 0.05 + denotes higher by; - denotes lower by

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The result as shown on table 3 revealed that the significant (p < 0.05) change in the serum bicarbonate concentration of the low extract co-treated group of rats relative to either the control or the MSG-treated rats decreased in the medium extract co-treated group and was reversed in the high extract co-treated group. However, the change in serum bicarbonate concentration of the rats exposed to either MSG or extract alone was similar and non-significant (p > 0.05) as compared to the control or to each group (MSG group and Extract group).

Groups	Bicarbonaate ion (mEq/L)	Change relative to the Control (%)	Change relative to MSG group (%)
Normal control (feed + water only)	30.03 ± 4.07	0.00	+06.87
MSG/Negative control (8000 mg/kg bw MSG)	28.10 ± 5.15	-06.43	0.00
Extract group (300 mg/kg bw extract)	29.40 ± 4.98	-02.09	+04.65
Low extract co-treated group (MSG, 8000 mg/ kg bw +100mg/kg bw extract)	38.43 ± 1.58	+27.97*	+36.75*
Medium extract co-treated group (MSG, 8000 mg/kg bw +300 mg/kg bw extract)	31.48 ± 5.15	+04.83	+12.03*
High extract co-treated group (MSG, 8000 mg/ kg bw +500mg/kg bw extract)	20.15 ± 7.70	-32.90*	-28.29*

 Table 3: Effect of ethanolic extract of avocado pear seed (ASE) on bicarbonate ion concentration (mEq/L) of normal and

 monosodium glutamate-compromised rats' serum.

Values are mean \pm SEM for n= 4. Difference considered statistically significant at p < 0.05 + denotes higher by; – denotes lower by

The result as shown on table 4 revealed that the higher (p < 0.05) serum chloride ion concentration of rats in the other groups compared to the control was more marked in the medium extract co-treated group relative to the control (240.04%) and MSG (30.69%) groups followed by high extract co-administered group (209.96 and 18.99%, respectively).

Groups	Chloride ion (mEq/L)	Change relative to the Control (%)	Change relative to MSG group (%)
Normal control (feed + water only)	18.63 ± 2.90	0.00	-61.59*
MSG/Negative control (8000 mg/kg bw MSG)	48.53 ± 2.41	+160.47*	0.00
Extract group (300 mg/kg bw extract)	32.20 ± 4.44	+72.83*	-33.64*
Low extract co-treated group (MSG, 8000 mg/ kg bw +100mg/kg bw extract)	37.38 ± 7.89	+100.63*	-22.97*
Medium extract co-treated group (MSG, 8000 mg/kg bw +300 mg/kg bw extract)	63.43 ± 4.56	+240.04*	+30.69*
High extract co-treated group (MSG, 8000 mg/kg bw +500mg/kg bw extract)	57.75 ± 8.63	+209.96*	+18.99*

Table 4: Effect of ethanolic extract of avocado pear seed (ASE) on chloride ion concentration (mEq/L) of normal and

 monosodium glutamate-compromised rats' serum.

Values are mean ± SEM for n= 4. Difference considered statistically significant at p < 0.05 + denotes higher by; – denotes lower by

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The result as shown on table 5 revealed that the change in the serum potassium ion concentration of the rats in either MSG or extract alone groups was not significant (p > 0.05) compared to the control. However, the change in the serum potassium concentration of rats in high extract co-administered group relative to either the control or MSG group was significant (p < 0.05) but dose independent.

Groups	Potassium ion (mEq/L)	Change relative to the Control (%)	Change relative to MSG group (%)
Normal control (feed + water only)	4.05 ± 1.74	0.00	-04.26
MSG/Negative control (8000 mg/kg bw MSG)	4.23 ± 1.25	+04.44	0.00
Extract group (300 mg/kg bw extract)	3.58 ± 0.62	-11.60	-15.37
Low extract co-treated group (MSG, 8000 mg/ kg bw +100mg/kg bw extract)	3.70 ± 0.24	-08.64	-12.53*
Medium extract co-treated group (MSG, 8000 mg/kg bw +300 mg/kg bw extract)	3.03 ± 0.32	-25.18*	-28.37*
High extract co-treated group (MSG, 8000 mg/kg bw +500mg/kg bw extract)	5.13 ± 0.30	+26.67*	+21.28*

Table 5: Effect of ethanolic extract of avocado pear seed (ASE) on potassium ion concentration (mEq/L) of normal and

 monosodium glutamate-compromised rats' serum.

Values are mean ± SEM for n= 4. Difference considered statistically significant at p < 0.05 + denotes higher by; – denotes lower by

Photomicrograph of the kidney section from rats in the normal control group showed normal kidney without congestion of the blood vessels of the kidney (Plate 1) while that from rats treated with MSG showed full congestion of the central blood vessels of the kidney (Plate 2). Photomicrograph of the kidney section from rats treated with ASE showed normal blood flow in the veins of the kidney with no congestion (Plate 3) whereas photomicrograph of the kidney section from rats co-treated with MSG and low dose (100 mg/kg bw) ASE showed reduced congestion in the blood vessels of the kidney compared to that for MSG group (Plate 4). Photomicrograph of the kidney section from rats co-treated with MSG and high extract dose (500 mg/kg bw) showed further but slight reduction in the congestion of the blood vessel of the kidney (Plate 5 and Plate 6).



Plate 1: Photomicrograph of the kidney section from rats of the normal control showing normal kidney without congestion of the blood vessels of the kidney.

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Plate 2: Photomicrograph of the kidney section from rats treated with MSG (Group 2) showing full congestion of the central blood vessels of the kidney.



Plate 3: Photomicrograph of the kidney section from rats treated with ASE showing normal blood flow in the veins of the kidney with no congestion.

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Plate 4: Photomicrograph of the kidney section from rats treated with MSG and 100 mg/kg body weight of ASE showing reduced congestion in the blood vessels of the kidney.



Plate 5: Photomicrograph of the kidney section from rats treated with MSG and 300 mg/kg body weight of ASE showing slight reduction in the congested blood vessel of the kidney.



Plate 6: Photomicrograph of the kidney section from rats treated with MSG and 500 mg/kg body weight of ASE showing slight reduction in the congested blood vessel of the kidney.

Discussion

The higher (p < 0.05) serum urea concentration of rats in the other groups as compared to rats in the control was less marked in the rats exposed to the extract alone (57.14%) as against in the rats exposed to intoxicating dose of MSG (82.66%) confirmed the overriding adverse influence of MSG at the tested dose on the serum urea homeostasis of rats that could be indicative of underlying renal dysfunction. It further demonstrated adverse influence, but diminished as compared to MSG group, on the serum urea homeostasis of the rats following exposure to ASE and apparent non-definite modulation attempt by ASE against MSG-induced adverse influence on the serum urea concentration of the rats. In particular, the MSG-induced effect on the serum urea concentration of the rats was as expected [23] and indicated MSG-induced compromised kidney function and perhaps pathological state [29] in the MSG-intoxicated rats. A similar trend of effect was observed for the serum creatinine and bicarbonate ion concentration seemingly confirming the adverse effect of the intoxicating dose of MSG or ASE alone on the kidney serum functional parameters while suggesting synergistic interaction adverse effect of MSG with low dose ASE but a promising capacity of ASE to modulate MSG-intoxication notably with increasing ASE concentration.

Serum electrolyte concentration indicates impaired kidney function [33,45] or renal function-related diseased states [19]. The higher (p < 0.05) serum chloride ion concentration was more marked in the medium extract co-treated group of rats relative to the control (240.04%) and MSG (30.69%) groups followed by high extract co-administered group (209.96 and 18.99% respectively) whereas the change in the serum potassium concentration of rats in high extract co-administered group relative to either the control or MSG group was significant (p < 0.05) but dose independent (Table 4 and 5). This demonstrated adverse effect of either MSG or the extract on the serum chloride concentration of the rats that worsened following interaction with MSG even at higher extract dose. In particular, it suggested that co-exposure of MSG with ASE to rats could elicit inconsistent adverse effect related to chloride metabolism and possible pathologies in the rats.

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Photomicrograph of the kidney section from rats in the normal control group showed normal kidney without congestion of the blood vessels of the kidney (Plate 1) and similar to that of rats treated with ASE alone (Plate 3), indicating preserved histo-architecture and function while that from rats treated with MSG showed full congestion of the central blood vessels of the kidney (Plate 2) which is indicative of compromised histo-architecture and function. The slight reduction in blood congestion observed in the kidney sections of rats exposed to MSG and varying concentration of ASE concomitantly indicated non-definite modulation capacity of ASE against MSG-intoxication of the rats' kidney histology and bio-functions in apparent confirmation of the present seric chemistry results.

Conclusion

Thus, the study demonstrated MSG-induced adverse effect, and inconsistent modulation by ASE, on the rats' kidney histology and some serum bio-functional indicators. The apparent synergistic interaction effect of MSG with ASE may result from unusual adverse and complex biochemical response in the rats. Caution in the use of ASE especially together with MSG should be exercised while further studies to elucidate the basis for the observations are warranted and recommended.

Conflict of Interest

None exists.

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