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Research Article
Effect of Ethanolic Extract of Avocado Pear (*Persea americana*) Seed on Normal and Monosodium Glutamate-compromised Rats' Hepatic Histo-morphology and Serum Bio-functional Parameters

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Abstract

Background and Objective: Continuous search for non-toxic natural products that could mitigate possible MSG-induced effects including on hepatic histo-morphology and functional capacity of animals. This study aimed to determine the effect of ethanolic extract of avocado pear (*Persea americana*) seeds (ASE) on normal and monosodium glutamate (MSG)-compromised rats' liver. **Methodology:** After 14 days daily oral exposure of rats to ASE and MSG either alone or combined, changes in the rats' liver histology and serum bio-indicators were assessed using standard protocols. Data were analyzed by one-way ANOVA using SPSS. **Results:** Serum alanine aminotransferase, ALT, (360.00 and 31.58%) and aspartate aminotransferase, AST, (176.77 and 04.46%) activities in the high-extract co-treated-group increased ($p<0.05$) above the others relative to control or MSG-group but decreased ($p<0.05$) more in ASE-group (07.03%, ALT, (43.50%, AST) relative to MSG-group. A similar trend was noted in the serum alkaline phosphatase (ALP) activity, protein concentration and the computed ratios (ALT:AST, AST:ALP, ALT:ALP and the respective corollary) of the rats while the highest increase and decrease in total bilirubin concentration relative to MSG-group was respectively in the medium-extract co-treated-group and in the ASE alone-group. Preserved hepatic histo-morphology with normal blood flow devoid of central vein congestion noted in the control and ASE alone-group contrasted the full central vein congestion in the MSG-group. Apparently reduced central vein congestion in the liver sections of rats co-exposed to MSG and low ASE dose was not sustained at either medium or high ASE concentration, seemingly confirming the serum chemistry observations. **Conclusion:** Thus, the study demonstrated a non-definitive influence of ASE alone and overriding adverse influence of MSG either alone or together with ASE on the hepatic histo-morphology and serum bio-functional indicators of the liver and perhaps other high metabolic organs.

Key words: Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, histo-morphology, monosodium glutamate

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Earlier, dietary intervention in the management of health dysfunctions was speculated while the utilization of agro products which result in solid waste generation in various forms including peels and seeds to over burden the environment on decomposition could offer dietary and therapeutic benefits[1,2]. Avocado plant (*Persea americana*) that belongs to the family, Lauraceae and genus, *Persea originated* from Guatemala and Mexico, but now grown throughout the tropics and sub-tropics[3]. The fruit and even the plant, is known as "*Igba* or *apoka*" in Yoruba[4] and as ube oyibo (loosely translated to 'English as 'foreign pear') in Ojoto and neighbouring Igbo speaking communities South East Nigeria. The fruit pulp is edible[5]. However, the seed is essentially discarded as solid agro waste, although consumed in some countries including Nigeria, where it is milled and incorporated into foods (owing perhaps to numerous ethno medicinal uses of avocado pear in the management of various ailments including hypertension, diabetes, cancer and inflammation[6-9]. Liver is a major organ of the body and could be involved in the pathobiochemistry of these pathologies, thus speculated that avocado pear seeds could affect the hepatic function and histology of especially compromised animals.

Monosodium glutamate (MSG), a sodium salt of glutamic acid produced by fermentation of notably sugarcane starch[10], is a flavour enhancer[11]. Daily MSG consumption ranges from 0.5 mg kg^{-1} to 3 g kg^{-1} [12] and could be inadvertently abused[13] with possible adverse influence[14-19]. Series of studies that can enhance the utilization of such solid agro wastes[20] and continued search for non-toxic natural products that can mitigate possible MSG-induced effects including on hepatic histo-morphology and functional capacity of animals, warranted this study aimed at determining the effect of ethanolic extract of avocado pear seeds flour on normal and monosodium glutamate-compromised rats' hepatic histo-morphology and serum bio-functional parameters. The objectives to achieving this aim were by determining the serum bio-functional indicators of hepatic functions including serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase activities and calculating the corresponding diagnostic ratios, serum total bilirubin and total protein concentration and assessing the liver histo-morphology of the rats exposed as in the study design. Liver damage or impaired function was associated with deranged histo-morphology and altered serum activity of aminotransferase and alkaline phosphatase and even total bilirubin concentration while changes in organ histology could confirm compromised organ function[21-24]. This study aimed to determine the effect of ethanolic extract of avocado pear (*Persea americana*) seeds (ASE) on normal and monosodium glutamate (MSG)-compromised rats' liver.

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.

Avocado pear fruits were bought in a local market in Umuahia, a major town close to Michael Okpara University of Agriculture Umudike, during the fruiting season 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 the consumption of the edible pulp, the seeds were washed with clean tap water, crushed into small pieces using manual grater and sun-dried for 3 days. The dried seeds were milled into powder using a laboratory miller (ED-5, USA).

The avocado pear seed flour was extracted with ethanol by cold maceration method. The extraction method involved weighing 700 g of the avocado pear seed flour into a volumetric flask, soaking in 1400 mL of 90% ethanol with intermittent shaking and stirring for 3 days and thereafter, filtering with No 1 Whatman 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 kept in 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' hepatic histo-morphology and serum bio-functional parameters.

Animal experimentation: Induction of toxicity in the rats was achieved with MSG at 8000 mg kg^{-1} body weight for 14 days according to Mariyamma *et al.*[14] as supported by other studies[25-28]. The ethanolic extract of avocado pear seed (1 g) was dissolved in 10 mL of distilled water as the stock solution and three graded doses were selected as follows, Low, medium and high doses: 100, 300 and 500 mg kg^{-1} b.wt., respectively. Twenty-four albino rats (*Rattus norvegicus*) of either sex (mean body weight, 96.00±10.00 g) 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 6 experimentation groups with sample size of 4 rats as described below.

Rats in the control-group were sham-dosed with distilled water (without either the extract or MSG) while rats in the MSG-group were fed intoxicating dose (8000 mg kg^{-1} b.wt.,) of MSG[14]. Rats in the extract-group (ASE-group) were fed ethanolic extract of avocado pear seed flour at 300 mg kg^{-1} b.wt., while rats in the MSG + low extract group (Low-extract co-treated group) were concomitantly exposed to ethanolic extract of avocado pear seed flour (100 mg kg^{-1} b.wt.,) and intoxicating dose of MSG (8000 mg kg^{-1} b.wt.,) whereas, rats in the MSG+medium extract group (Medium-extract co-treated group) were co-administered 300 mg kg^{-1} b.wt., of ethanolic extract of avocado pear seed flour and intoxicating dose of MSG (8000 mg kg^{-1} b.wt.,). Rats in the MSG+high extract group (High-extract co-treated group) were concomitantly exposed to ethanolic extract of avocado pear seed flour (500 mg kg^{-1} b.wt.,) and intoxicating dose of MSG (8000 mg kg^{-1} b.wt.,). 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 h day/night cycle. This study considered and adhered to the standard ethical use of experimental animals. Throughout 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) daily oral 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 min and the serum collected and stored in a refrigerator until used.

Determination of studied parameters: Serum activity of alanine aminotransferase (ALT) and aspartate aminotransferase, respectively was determined by the method of Reitman and Frankel[29] based on the principle that ALT and AST activity could be determined by measuring the absorbance of colour intensity of pyruvate hydrazone or oxaloacetate hydrazone respectively formed with 2, 4-Dinitrophenylhydrazine against the blank at 546 nm and reading the activity of ALT from standard curve provided in the assay kit. Serum alkaline phosphatase (ALP) activity was assayed using Randox Enzyme Kit. This was based on the optimized standard method as recommended by the German Society of Clinical Chemistry as described earlier[30].

Total serum protein concentration was determined using the Biuret method as described by Henry *et al.*[31]. This was based on the principle that the carboxyl and the amino ends of peptide bonds of proteins react with cupric ions in moderately alkaline medium to form violet colour with colour intensity proportional to the concentration of protein present in the sample as read using a spectrophotometer set at 540 nm. Total serum bilirubin concentration was determined by a standard colorimetric method as described earlier[24]. The method involved Van den Bergh reaction based on the principle that bilirubin could form a stable complex with diazotized sulfanilic acid in dilute hydrochloric acid to produce blue coloured azobilirubin that was measured at 580 nm.

Calculation of diagnostic ratios and change relative to group: Diagnostic ratios were calculated from the result of corresponding parameters as obtained in this study. The calculation of change relative to any group was as developed and severally used[22,32-34]. Change relative to either control or MSG-group was calculated using the relation:

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

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.

For accuracy, ease and to avoid rounding off error, this could be computed as a continuous mathematical operation thus:

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

Histological examination: Liver of the sacrificed rats were identified and harvested. They were fixed in 10% buffered formalin for 72 h. The tissues were then dehydrated in graded concentrations of alcohol and embedded in paraffin. The embedded tissues were cut into sections (5 µm thickness) and stained with haematoxylin and eosin for histo-morphological assessment to obtain the photomicrographs.

Statistical analysis: Descriptive statistics and test for significance difference in mean were carried out on the data generated by one-way analysis of variance (ANOVA) 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.

RESULTS

The result as shown on Table 1 revealed that higher ($p<0.05$) serum ALT activity of rats in the other groups as compared to rats in the control increased with increasing ASE concentration in the groups co-exposed to MSG and ASE (16.73±1.20, 21.33±1.47 and 22.08±2.73 IU L^{-1}). The increase in ALT activity relative to either the control or the MSG group was highest in the high extract co-treated group (360.00 and 31.58 %, respectively) while the decrease relative to the MSG group aside the decrease relative to the control was highest in the rats exposed to ASE alone (07.03%).

The result as shown on Table 2 revealed higher ($p<0.05$) serum AST activity of rats in the various groups compared to rats in the control. The observation increased with increasing ASE concentration in the groups co-exposed to MSG and ASE (61.60±2.00, 64.45±13.06, 85.80±3.93 IU L^{-1}). The increase in AST activity relative to the control was highest in the high extract co-treated group (176.77%) while the decrease in serum AST activity relative to the MSG group, aside the control, was highest in the rats exposed to ASE alone (43.50%).

The result as shown on Table 3 revealed that rats exposed to ASE alone had lower serum ALP activity compared to the control and MSG alone. However, the serum ALP activity of rats in the MSG-group or MSG + ASE co-treated groups were higher ($p<0.05$) than that of the rats in the control. The observed increase in serum ALP activity of rats in MSG + ASE co-treated groups increased with increasing ASE concentration. The increase in ALP activity relative to the control was highest in MSG-treated group of rats (21.39%) followed by that of rats in high extract co-treated group (11.19%). The decrease in serum ALP activity relative to the MSG-treated group was highest in the rats exposed to ASE alone (23.65%).

Table 1: Effect of ethanolic extract of avocado pear seed (ASE) on alanine aminotransferase activity (IU L^{-1}) of normal and monosodium glutamate-compromised rats' serum

Groups	ALT (IU L^{-1})	Change relative to the Control (%)	Change relative to MSG group (%)
Control (feed+water only)	4.80±1.52	0.00	-71.39*
MSG-group (8000 mg kg^{-1} b.wt., MSG)	16.78±3.60	+249.58*	0.00
ASE-group (300 mg kg^{-1} b.wt., extract)	15.60±1.91	+225.00*	-07.03
Low-extract co-treated group (MSG, 8000+100 mg kg^{-1} b.wt., extract)	16.73±1.20	+248.54*	-00.30
Medium-extract co-treated group (MSG, 8000+300 mg kg^{-1} b.wt., extract)	21.33±1.47	+344.37*	+27.11*
High-extract co-treated group (MSG, 8000+500 mg kg^{-1} b.wt., extract)	22.08±2.73	+360.00*	+31.58*

Values are Mean±SEM for n = 4. Difference considered statistically significant at $p<0.05$. $^{+}$Denotes higher by, $^{-}$Denotes lower by

Table 2: Effect of ethanolic extract of avocado pear seed (ASE) on aspartate aminotransferase activity (IU L^{-1}) of normal and monosodium glutamate-compromised rats' serum

Groups	AST (IU L^{-1})	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed+water only)	31.00±8.00	0.00	-62.25
MSG-group (8000 mg kg^{-1} b.wt., MSG)	82.13±3.86	+164.93	0.00
ASE-group (300 mg kg^{-1} b.wt., extract)	46.40±7.74	+49.67	-43.50
Low-extract co-treated group (MSG, 8000-100 mg kg^{-1} b.wt., extract)	61.60±2.00	+98.70	-24.98
Medium-extract co-treated group (MSG, 8000-300 mg kg^{-1} b.wt., extract)	64.4513.06	+107.90	-21.52
High-extract co-treated group (MSG, 8000-500 mg kg^{-1} b.wt., extract)	85.80± 3.93	+176.77	+04.46

Values are Mean±SEM for n = 4. Difference considered statistically significant at $p<0.05$. $^{+}$Denotes higher by, $^{-}$Denotes lower by

Table 3: Effect of ethanolic extract of avocado pear seed (ASE) on alkaline phosphatase activity (IU L^{-1}) of normal and monosodium glutamate-compromised rats' serum

Groups	ALP (IU L^{-1})	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed+water only)	23.23±0.61	0.00	-17.62
MSG-group (8000 mg kg^{-1} b.wt., MSG)	28.20±2.28	+21.39	0.00
ASE-group (300 mg kg^{-1} b.wt., extract)	21.53±1.59	-07.31	-23.65
Low-extract co-treated group (MSG, 8000-100 mg kg^{-1} b.wt., extract)	24.18±1.03	+04.08	-14.25
Medium-extract co-treated group (MSG, 8000-300 mg kg^{-1} b.wt., extract)	25.10±0.69	+08.04	-10.99
High-extract co-treated group (MSG, 8000+500 mg kg^{-1} b.wt., extract)	25.83±0.46	+11.19	-08.40

Values are mean ± SEM for n= 4. Difference considered statistically significant at $p<0.05$. $^{+}$Denotes higher by, $^{-}$Denotes lower by

Table 4: Effect of ethanolic extract of avocado pear seed (ASE) on total protein concentration (g dL^{-1}) of normal and monosodium glutamate-compromised rats' serum

Groups	T.Protein (g dL^{-1})	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed+water only)	5.13±0.11	0.00	+13.24
MSG-group (8000 mg kg^{-1} b.wt., MSG)	4.53±0.19	-11.69	0.00
ASE-group (300 mg kg^{-1} b.wt., extract)	4.58±0.39	-10.72	+01.10
Low-extract co-treated group (MSG, 8000+100 mg kg^{-1} b.wt., extract)	4.65±0.30	-9.35	+02.64
Medium-extract co-treated group (MSG, 8000+300 mg kg^{-1} b.wt., extract)	4.73±0.57	-7.79	+04.41
High-extract co-treated group (MSG, 8000+500 mg kg^{-1} b.wt., extract)	4.35±0.79	-15.20	+03.97

Values are Mean±SEM for n = 4. Difference considered statistically significant at $p<0.05$. $^{+}$Denotes higher by, $^{-}$Denotes lower by

Table 5: Effect of ethanolic extract of avocado pear seed (ASE) on total bilirubin concentration (mg dL^{-1}) of normal and monosodium glutamate-compromised rats' serum

Groups	T.Bil (mg dL^{-1})	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed+water only)	0.72±0.33	0.00	-58.13
MSG-group (8000 mg kg^{-1} b.wt., MSG)	1.72±0.22	+138.88	0.00
ASE-group (300 mg kg^{-1} b.wt., extract)	1.24±0.36	+72.22	-27.90
Low-extract co-treated group (MSG, 8000+100 mg kg^{-1} b.wt., extract)	1.79±0.34	+148.61	+04.06
Medium-extract co-treated group (MSG, 8000+300 mg kg^{-1} b.wt., extract)	2.18±0.71	+202.27	+26.74
High-extract co-treated group (MSG, 8000+500 mg kg^{-1} b.wt., extract)	1.49±0.14	+106.94	-13.37

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 4 revealed that the serum total protein concentration of rats in the other groups as compared to rats in the control was lower ($p<0.05$). The observed decrease in serum total protein concentration of rats was dose dependent in the MSG + ASE co-treated groups. The decrease in serum total protein concentration relative to the control group was highest in the high extract co-treated group of rats (15.20%) followed by that in rats exposed to MSG alone (11.69%) and least in the medium extract co-administered group (7.79%). However, the increase relative to the MSG group aside the control (13.24%) was highest in the medium extract co-exposed group (04.41%).

The result as shown on Table 5 revealed that higher ($p<0.05$) serum total bilirubin concentration of rats in the other groups as compared to rats in the control was dose dependent in the groups co-exposed to MSG and ASE. The increase in serum total bilirubin concentration of rats relative to either the control was highest in the medium extract co-treated group of rats (202.27%) while the decrease in serum total bilirubin concentration relative to the MSG-treated group was highest in the control (58.13%) followed by that in the rats exposed to ASE alone (27.90%).

The result as shown on Table 6 revealed higher ($p<0.05$) computed serum ALT:AST ratio but lower ($p<0.05$) computed serum AST:ALT ratio in rats in the various groups compared to rats in the control. The observation relative to rats in either the control or the MSG-group was highest in group of rats exposed to ASE alone and also in the group of rats exposed to MSG+300 mg kg^{-1} b.wt., of ASE.

Table 6: Effect of ethanolic extract of avocado pear seed (ASE) on ALT:AST and (AST:ALT) ratios of normal and monosodium glutamate-compromised rats' serum

Groups	ALT:AST (AST:ALT)	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	0.15 (6.45)	0.00(0.00)	−25.00 (31.90)
MSG-group (8000 mg kg^{-1} b.wt., MSG)	0.20 (4.89)	+33.33 (−24.18)	0.00(0.00)
ASE-group (300 mg kg^{-1} b.wt., extract)	0.33 (2.97)	+120.00 (−53.95)	+65.00 (−39.26)
Low-extract co-treated group (MSG, 8000+100 mg kg^{-1} b.wt., extract)	0.27 (3.68)	+80.00 (−42.94)	+35.00 (−24.74)
Medium-extract co-treated group (MSG, 8000+300 mg kg^{-1} b.wt., extract)	0.33 (3.02)	+120.00 (−53.17)	+65.00 (−38.24)
High-extract co-treated group (MSG, 8000+500 mg kg^{-1} b.wt., extract)	0.25 (3.88)	+66.66 (−39.84)	+25.00 (−20.65)

Values are simple ratio of the mean of the corresponding parameters. Relative change above 10% considered significant. $^{+}$Denotes higher by, $^{-}$Denotes lower by

Table 7: Effect of ethanolic extract of avocado pear seed (ASE) on AST:ALP and (ALP:AST) ratios of normal and monosodium glutamate-compromised rats' serum

Groups	AST:ALP (ALP:AST)	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	1.33 (0.74)	0.00 (0.00)	−54.29 (117.64)
MSG-group (8000 mg kg^{-1} b.wt., MSG)	2.91 (0.34)	+118.79 (−54.05)	0.00 (0.00)
ASE-group (300 mg kg^{-1} b.wt., extract)	2.15 (0.46)	+61.00 (−37.83)	−26.11 (35.29)
Low-extract co-treated group (MSG, 8000+100 mg kg^{-1} b.wt., extract)	2.54 (0.39)	+90.97 (−47.29)	−12.71 (14.70)
Medium-extract co-treated group (MSG, 8000+300 mg kg^{-1} b.wt., extract)	2.56 (0.38)	+92.48 (−48.64)	−12.02 (11.76)
High-extract co-treated group (MSG, 8000+500 mg kg^{-1} b.wt., extract)	3.32 (0.30)	+149.62 (−59.45)	+14.08 (−11.76)

Values are simple ratio of the mean of the corresponding parameters. Relative change above 10 % considered significant. $^{+}$Denotes higher by, $^{-}$Denotes lower by

Table 8: Effect of ethanolic extract of avocado pear seed (ASE) on ALT:ALP and (ALP:ALT) ratios of normal and monosodium glutamate-compromised rats' serum

Groups	ALT:ALP (ALP:ALT)	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	0.20 (4.83)	0.00 (0.00)	−66.10 (187.50)
MSG-group (8000 mg kg^{-1} b.wt., MSG)	0.59 (1.68)	+195.00 (−65.21)	0.00 (0.00)
ASE-group (300 mg kg^{-1} b.wt., extract)	0.72 (1.38)	+260.00 (−71.42)	22.03 (−17.85)
Low-extract co-treated group (MSG, 8000+100 mg kg^{-1} b.wt., extract)	0.69 (1.44)	+245.00 (−70.18)	16.94 (−14.28)
Medium-extract co-treated group (MSG, 8000+300 mg kg^{-1} b.wt., extract)	0.84 (1.17)	+320.00 (−75.77)	42.37 (−30.35)
High-extract co-treated group (MSG, 8000+500 mg kg^{-1} b.wt., extract)	0.85 (1.16)	+325.00 (−75.98)	44.06 (−30.95)

Values are simple ratio of the mean of the corresponding parameters. Relative change above 10% considered significant. $^{+}$Denotes higher by, $^{-}$Denotes lower by

The result as shown on Table 7 revealed higher ($p<0.05$) computed serum AST:ALP ratio but lower ($p<0.05$) ALP:AST ratio in rats in the various groups compared to rats in the control. The observation relative to rats in either the control or the MSG-group was highest in the group of rats exposed to MSG+500 mg kg^{-1} b.wt., of ASE.

The result as shown on Table 8 revealed higher ($p<0.05$) computed serum ALT:ALP ratio but lower ($p<0.05$) ALP:ALT ratio in rats in the various groups compared to rats in the control. The observation on ALT:ALP ratio relative to rats in either the control or the MSG-group was highest in the group of rats exposed to MSG+500 mg kg^{-1} b.wt., of ASE. However, the observation on ALP:ALT ratio relative to rats in either the control or the MSG-group was highest in the group of rats exposed to MSG+300 mg kg^{-1} b.wt., of ASE.

Photomicrographs of the liver sections of rats exposed as in the study design: Photomicrograph of the liver sections from rats in the control showed preserved hepatic histo-morphology with normal blood flow devoid of central vein congestion (Fig. 1) while those from rats in MSG-group showed a full congestion of the central vein (Fig. 2). Similar to the control, photomicrograph of the liver sections from rats treated with ASE alone showed normal blood flow with no central vein congestion (Fig. 3).

As compared to the MSG-group, photomicrograph of the liver section from rats co-treated with MSG and 100 mg kg^{-1} body weight of ASE showed reduced congestion of the central vein (Fig. 4). However, photomicrograph of the liver section from rats co-treated with MSG and 300 mg kg^{-1} b.wt., of ASE

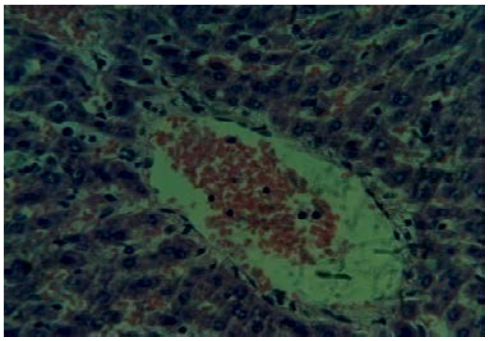

Fig. 1: Photomicrograph of the liver sections from rats in the control showing preserved hepatic histo-morphology, normal flow of blood with no congestion of the central vein. (H&E, ×400)

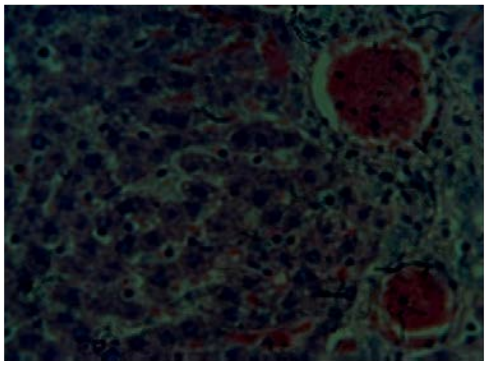

Fig. 2: Photomicrograph of the liver sections from rats treated with MSG alone showing compromised hepatic histo-morphology with a full congestion of the central vein. (H&E, ×400)

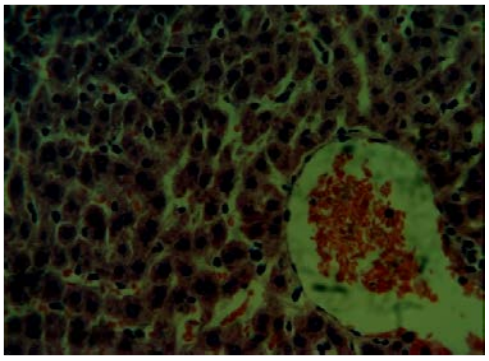

Fig. 3: Photomicrograph of the liver sections from rats treated with ASE alone showing normal flow of blood with no congestion of the central vein. (H&E, ×400)

(Fig. 5) or co-treated with MSG and 500 mg kg^{-1} b.wt., of ASE (Fig. 6) showed congested central vein similar to that of rats in MSG-group.

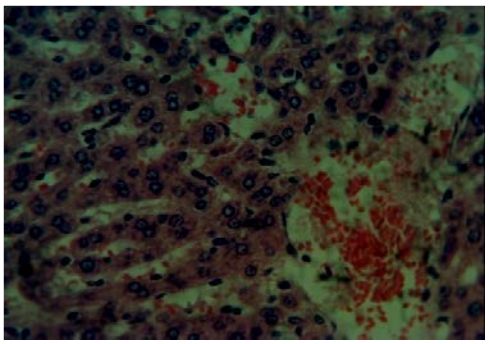

Fig. 4: Photomicrograph of the liver sections from rats co-treated with MSG and 100 mg kg^{-1} b.wt., of ASE showing reduced congestion of the central vein. (H&E, ×400)

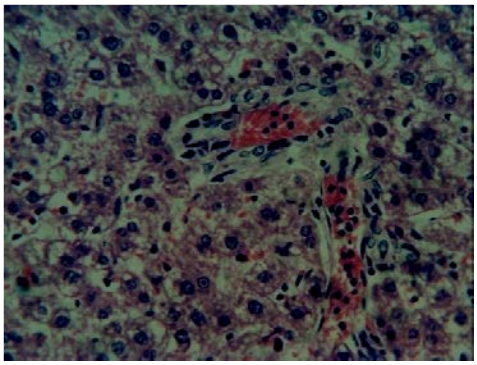

Fig. 5: Photomicrograph of the liver sections from rats co-treated with MSG and 300 mg kg^{-1} b.wt., of ASE showing congested central vein. (H&E, ×400)

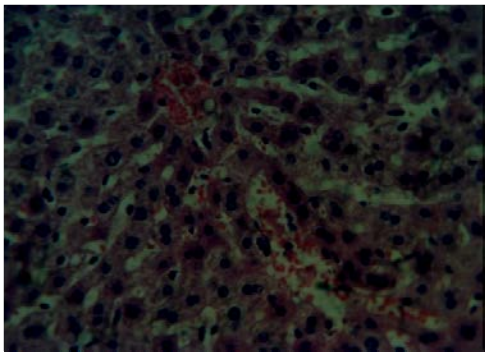

Fig. 6: Photomicrograph of the liver sections from rats co-treated with MSG and 500 mg kg^{-1} b.wt., of ASE showing congestion of central vein. (H&E, ×400)

DISCUSSION

The effect of ethanolic extract of avocado pear (*Persea americana*) seeds (ASE) on normal and monosodium

glutamate (MSG) compromised rats' liver was studied in continuation of this search for non-toxic natural products that could mitigate possible MSG-induced effects including on hepatic histo-morphology and functional capacity of animals. The study was necessary because functional impairment of organs resulted from or to alteration in some biochemical indices hence functional integrity of organs could be assessed by monitoring the concentration of biochemical indicators in the serum and assessing the functional integrity of organs following exposure to xenobiotics remain fundamental to novel diet and drug search research involving natural products[35,36].

Serum alanine aminotransferase, ALT and aspartate aminotransferase, AST, activities of rats in high-extract co-treated-group increased ($p<0.05$) above the others relative to control or MSG-group but decreased ($p<0.05$) more in ASE-group relative to MSG-group (Table 1, 2), probably indicating less severe adverse influence following ASE-exposure, as compared to either MSG-exposure or MSG and ASE co-exposure, on the functional integrity of rats' liver and other high metabolic organs. The result further indicated the overriding adverse influence on the rats' liver and related organs following concomitant exposure of rats to MSG and high concentration of ASE. The observation on the serum ALT and AST activities of rats in MSG-group agreed with previous reports[14,17,37,11], seemingly confirming compromised liver integrity and functional capacity following MSG intoxication that probably resulted to enhanced leakage of ALT and AST enzymes to the blood stream as detected in the serum. Serum ALT and AST activities were bio-indicators of liver function, though ALT is specific while AST which is not could in addition indicate dysfunction of other high metabolic organs, including the heart, kidney, skeletal muscle and brain[30,23,21].

A similar trend was noted in the serum alkaline phosphatase (ALP) activity, protein concentration and the computed ratios (ALT:AST, AST:ALP, ALT:ALP and the respective corollary) of the rats while the highest and lowest serum total bilirubin concentration relative to MSG-group was respectively recorded in the medium-extract co-treated-group and in the ASE-group. These results seemed to support the present observation on the serum ALT and AST activities and the implications there to, while further indicating the over-riding adverse influence of MSG together with even medium and low concentrations of ASE on the serum ALP activity, total protein and total bilirubin concentration of rats. In particular, the higher serum ALP activity of rats in MSG-group as compared with the control agreed with previous report[38]. Generally, alkaline phosphatase, ALP, which hydrolyze phosphates at an alkaline pH, are present in all tissues of the body but particularly concentrated in high metabolic organs including the liver, kidney and bone. Thus, aside possible liver damage, high serum alkaline phosphatase activity as observed in this study could be indicating other organs dysfunction, including bile duct and bone, as well as pathologies, including cancer and myocardial infarction[23]. Further to this, higher serum bilirubin concentration as noted in this study could be indicating diminished uptake by the liver while the diminished serum total protein could be a pointer to diminished protein synthesis due perhaps to treatment-related assault on particularly the liver and consequent collapse in its functional capacities. Generally, liver is an important organ for protein synthesis while bilirubin, a catabolic product of haem-containing proteins is rapidly taken up by hepatocytes via carrier mediated active transport process and rapidly converted (via conjugation with glucuronic acid) to bilirubin mono-and di-glucuronide which are rapidly excreted[23].

It was particularly noteworthy that the present observation on the various parameters was dose dependent in the groups of rats co-exposed to MSG with increasing ASE concentration, indicating persistent effect on the parameters and perhaps associated consequences following concomitant exposure of MSG and ASE to rats irrespective of the concentration of ASE. The implication is worrisome warranting a follow up and cautious concomitant intake of MSG with ASE that could occur with ethno-medication involving avocado pear seed. Preserved hepatic histo-morphology with normal blood flow devoid of central vein congestion noted in the control and ASE alone-group contrasted the full central vein congestion in the MSG-group while the apparently reduced central vein congestion in the liver sections of rats co-exposed to MSG and low ASE dose was not sustained at either medium or high ASE concentration. Thus, the rats' liver histo-morphology results seemingly confirmed a definitive adverse influence of MSG either alone or together with ASE while suggesting that the observed apparent adverse influences of ASE alone were probably not significant enough to derange the rats' histo-morphology and that ASE, irrespective of concentration, could not ameliorate but could aggravate MSG-induced adverse effect on the rats' liver histo-morphology and associated functions.

CONCLUSION

Thus, the study demonstrated a non-definitive influence of ASE alone and overriding adverse influence of MSG either alone or together with ASE on the hepatic histo-morphology and serum bio-functional indicators of the liver and perhaps

other high metabolic organs. The study further demonstrated that ASE irrespective of concentration, could not ameliorate but could aggravate MSG-induced adverse effect on the rats' liver histo-morphology and associated functions, underscoring cautious concomitant use of MSG with ASE in animals while warranting further studies.

SIGNIFICANCE STATEMENTS

This study discovered the non-definite effect of ethanolic extract of avocado pear seeds (ASE) alone, implying that ASE could be either beneficial or not for the rats. It also discovered the over-riding adverse effect of monosodium glutamate (MSG) either alone or in combination with the ethanolic extract of avocado pear seeds on the hepatic histology. Function in the rats, implying that ASE irrespective concentration could not ameliorate but could aggravate MSG-induced adverse effect on the rats' liver histo-morphology and associated functions, This study will help the researchers to uncover the hepatic health implications of exposing rats to ASE either alone or combined with monosodium glutamate that many researchers have hitherto neither been considered nor explored. Thus, a new insight on the possible hepatic implications of using ASE either alone or together with MSG by humans may be provided for further studies.

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