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Effects of ethanol extract of cocoa (*theobroma cacao*) pod on normal and monosodium glutamate-intoxicated rats' hepatic histo-morphology, serum bio-functional parameters and serum antioxidant activities

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Abstract

Improving the use of food wastes could reduce the attendant environmental burden – related health implications, but requires scientific basis. Thus, this study investigated some effects of ethanol extract of cocoa (Theobroma cacao) pod on normal and monosodium glutamate-intoxicated rats' hepatic histo-morphology, serum bio-functional parameters and serum antioxidant activities using standard protocols. Following daily oral exposure of rats to cocoa pod extract (CPE) and monosodium glutamate (MSG) either alone or co-treated CPE for 14 days, changes in the rats' liver histopathology, serum bio-indicators and serum antioxidant enzymes were assayed using standard protocols. Data were analysed by one-way ANOVA using SPSS version 22. Serum chemistry result showed a significant ($p<0.05$) % decrease (-44.98%, -33.04% and -31.33%) in AST activity of co-treated groups relative to MSG group. This trend was followed through ALT, ALP, total bilirubin and in the computed ratios (ALT:AST, AST:ALP and ALT:ALP). Interestingly, the trend followed through in the antioxidant enzyme CAT and in GSH while the SOD remained within comparable range as compared to MSG group. The histopathological examination of the liver indicated normal blood flow without congestion in the normal and CPE group in contrast to full congestion of the central vein as seen in the MSG group. Consequently, reduced congestion as seen in the co-treated groups seemingly confirmed the serum chemistry results as against MSG group suggesting ameriolative effects. Hence, this study showed ameriolative effects of CPE on the histo-morphology, serum bio-functional indicators and antioxidant activities of MSG-intoxicated rats

Keywords: Monosodium glutamate, agricultural waste, cocoa pod, liver enzymes, serum antioxidant.

Introduction

Harnessing food wastes utilization in diets and drugs could improve food supply, health and the environment. [1]. Cocoa *(Theobroma cacao)*, a cash crop mainly produced in the tropical regions including Nigeria, Ghana, Ivory Coast and Cameroun is made up of cocoa beans, pulp and the pod [2].

Monosodium glutamate, the sodium salt of the non essential amino acid, glutamate is a flavor enhancing food additive that may be present in packaged food without appearing on the label. [3]. The safety of monosodium glutamate (MSG) in human is controversial. Some of the observed adverse effects following MSG consumption include the Chinese restaurant syndrome (CRS), which is characterized by palpitation, general weakness and later flushing, dizziness, syncope, and partial pressure [4]; Obesity and metabolic disturbances could result due to impaired glucose tolerance [5]; Neuronal necrosis in the hypoyhalamic arcuate nucleus [6] and neuroinflamation [7]**.** Also reproductive malfunction characterized by lowered testosterone in male and increased follicle in women [8,9]. MSG could be used as laundry bleach [3] and at relatively high concentration, could induce adverse effects in animals, including seizure [10]; liver damage [11] and enhance appetite hence, increased food intake leading to obesity [3].

Cocoa comprises the seed, pulp and pod. The cocoa seed constitute only about 10% (ten percent) of the fresh cocoa weight and when fermented, could have some antioxidant properties [12]. Cocoa by-products (pulp and pod) which constitute about 90% (ninety percent) of the total fresh weight of cocoa, they are being discarded as cocoa waste. In particular, cocoa pod could be a source of beneficial bioactive compounds warranting this study aimed at determining the effects of ethanol extract of cocoa (*Theobroma cacao*) pod on normal and monosodium glutamate-intoxicated rats' hepatic histo-morphology, serum bio-functional parameters and serum antioxidant activities.

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Materials and methods

Chemical Reagents

Ajinomoto, a brand of monosodium glutamate marketed by West African Seasoning Company Limited, was bought from a daily market at Umuahia, Nigeria. Other chemicals for the Liver and renal function test were kits by RANDOX LTD and were of analytical grade. The standards were used without further purification.

Collection and Identification of Sample

The *Theobroma cacao* seeds were bought from a commercial market in Ezenwelike, Eziora Ozubulu in Ekwusigo Local Government Area, Anambra State, Nigeria. It was identified as *Theobroma cacao* by a plant taxonomist in the Department of Plant and Biotechnology science in Michael Okpara University of agriculture, Umudike, Nigeria

Preparation of Extract

The cocoa seed obtained were cut open, the seeds and pulp were removed and the pods were washed, weighed (wet weight = 15000g), cut into pieces with a machete and sundried for 7 days to obtain the corresponding dry weigh (5100g). The dried pods were milled with a local grinder and the flour which directly macerated with ethanol in a beaker for 2 to 3 days with intermittent shaking and turning to facilitate extraction at a ratio of 100grams of sample: 300ml of ethanol. The mixture was first filtered with a muslin cloth and then with a filter paper. The resultant filtrate was dried in a hot water bath at 50^{o}C. Stock solution of the extract was stored in a refrigerator (Thermocool, Nigeria).

Ethical consideration

The animals were kept in rat cages kept in a well ventilated room and allowed free access to standard feed (Vital feed Jos, Nigeria) and clean tap water *ad libitum* at natural room temperature with 12 hours day/night cycle. This study considered and adhered to the standard ethical use of experimental animals 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 ethical committees of Michael Okpara University of Agriculture Umudike, Nigeria.

Induction of toxicity

Toxicity was induced using 8000mg/kg body weight of the monosodium glutamate was orally administered to the rats daily for 14 days according to [13]

Study design

The rats were randomly assigned into six groups of four rats each thus:

GROUP 1 (Control) rats were fed normal rat feed and water only.

GROUP 2 (MSG group) rats were exposed to monosodium glutamate (MSG) (8000mg/kg body weight),

GROUP 3 (extract group) rats received 300mg/kg body weight of extract.

GROUP 4 (treatment group 1) rats were exposed to MSG (8000mg/kg body weight) co treated with extract (150mg/kg body weight)

GROUP 5 (treatment group 2) rats were exposed to MSG (8000mg/kg body weight) co treated with extract (300mg/ kg body weight)

GROUP 6 (treatment group 3) were exposed to MSG (8000mg/kg body weight) co treated with extract (500mg/kg body weight)

The extracts were administered through a gavage and were administered daily for 14 days. The rats in the respective groups were allowed free access to feed. On the 14^{th} day, the animals were starved overnight and were sacrifices by cervical dislocation.

Collection of blood Samples and Organs

The animals were sacrificed by cervical dislocation and the blood was collected through cardiac puncture. Blood samples were collected into plain containers (serum for enzyme assays) for other biochemical assays. The liver were excised and processed for the histopathological examination.

Calculation of diagnostic ratios and change relative to groups

Diagnostic ratios were calculated from the result of corresponding parameters as obtained in this study. The calculation of change relative to any group was developed and used severally. Change relative to either control or MSG- group was calculated using the relationship [14]:

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

Where K represents the constant group hence constant value and V represent the variable groups hence variable values.

Quantitative Estimation of serum Alanine Transaminase (ALT) and Asapartate Transaminase (AST)

The method of [15] was used. The procedure for determining AST and ALT are similar. Test tubes were labeled blank, standard, control and test. Then 0.5 ml of the substrate was introduced into all the test tubes. The serum (0.1ml) was added to the tube labeled test, was mixed gently and was allowed to incubate at 37^{0}C for 30 minutes and 60 minutes for ALT and AST respectively. To both tubes, 0.5ml of the colour reagent was added, mixed and allowed to stand for 20minutes. Then, 0.5ml of NaOH was added after 5 minutes and the absorbance was read against blank at 540nm

Calculate the concentration of enzyme activity using the formula

$$\text{Units (IU/L)} = \frac{OD\ test - OD\ control \times concentration\ \ of\ standard \times 1000}{OD\ standard - OD\ blank \times 5.0 \times time\ (minutes)}$$

Quantitative estimation of serum alkaline phosphatase (ALP)

Alkaline phosphatase in the serum was estimated by the end point colorimetric method [16]. Three test tubes were labeled blank, standard, control or test and 0.5ml of ALP substrate was transferred into all the test tubes and incubated for 3minutes at 37^{o}C . Deionized water (0.1 ml) was used as blank while the sample and standard were added to other test tubes appropriately and was incubated for 10 minutes at 37^{o}C. Then 2.5ml of colour reagent was added to the test tubes and were mixed. The absorbance of the mixture was read at 590nm against reagent blank.

The activity of the ALP in the sample was calculated using the formula

$$\text{Units (IU/L)} = \frac{OD\ test - OD\ control \times concentration\ of\ standard}{OD\ standard}$$

Quantitative Determination of serum Total Bilirubin

The serum total Bilirubin was estimated by the end point colorimetric method [17]. Test tubes were labeled blank, standard, control and test. Total bilirubin was prepared by adding one drop of sodium nitrite (reagent 1) to 0.2 ml of sulfanilic acid and HCL (reagent 2). The solution obtained (1ml) was then transferred to all the test tubes and was then allowed to incubate for ten minutes at room temperature. One milliliter of water, standard and sample were added to the appropriate test tubes. Caffeine reagent (reagent 3) (1ml) was added to the mixture and was incubated for ten minutes. The absorbance of the resulting mixture was measured at 555nm against reagent blank.

Total bilirubin in the sample was calculate using the formula

$$\text{Total bilirubin (mg/dl)} = \frac{OD\ of\ sample}{OD\ of\ standard} \times CONC\ of\ standard$$

Quantitative Estimation of serum Total Protein

The serum total protein was estimated by the biuret method [18]. Test tubes were labeled test, blank and standard. Then, 3ml of the standard solution and water were added to the standard and blank tubes respectively and 0.1 ml of serum was added to the tube labeled test and was made up to 3ml by with distilled water. 3.0ml of Biuret solution were added to all tubes, was allowed to incubate foe 30 minutes at 37^{o}c, and cooled at room temperature. The absorbance was read at 540nm wavelength against reagent blank.

Concentration of Total protein in the sample was calculate using the formula

$$\text{Total protein (mg/dl)} = \frac{OD\ of\ sample}{OD\ of\ standard} \times CONC\ of\ standard$$

Determination of antioxidant enzymes

Catalase

The catalase activity was determined by the method of [19] based on the principle that catalase split hydrogen peroxide. Assay mixture was composed of hydrogen peroxide,1.0ml of buffer, 0.4ml of distilled water and 0.1ml of dilute liver homogenate (1:10). The reaction started after a period of 60seconds of incubation. 2.0ml of dichromate/ acetic acid reagent was added to stop the reaction. The enzyme source was then introduced into the control tube after the addition of the reagent. The tubes were heated in a water bath for 10 minutes and the colour developed was read at 610nm. Various concentrations of the standard within the range of 20-120 µmoles was used to obtain the calibration curve.

The activity of the catalase was determined by the amount of hydrogen peroxide consumed, expressed in µmoles of H_2O_2 consumed/minute/ml.

Superoxide Dismutase (SOD)

The method of auto oxidation by pyrogallol was used as described by [20]. Liver homogenate was used as the source of enzyme. Absolute ethanol (0.25ml) and 0.15ml of chloroform was vigorously shaken and centrifuged for 30 minutes at 1000g. The supernatant was used for the assay. 0.5ml of the stock solution of pyrogallol was diluted to 50ml with Tris-HCL with final concentration of 0.2mM. The assay mixture for auto oxidation without the enzyme source was composed of 2.0ml buffer containing EDTA and 0.5ml of diluted pyrogallol and water to bring the final volume to 3.0ml which was measured initially after 3 minutes at 420nm. The assay mixture for oxidation with enzymes was composed of 2.oml buffer solution containing EDTA 0.5ml pyrogallol, 0.1ml of liver supernatant bringing the total volume to 3.0ml with distilled water. The absorbance was read at the same wavelength (420mm).

Determination of reduced glutathione

The method of [21] was used for estimating reduced glutathione. To 0,1ml of the homogenate, 2.4ml of EDTA, 1.0ml distilled water and 1.5ml TCA were added and mixed thoroughly. The mixture was centrifuged after 5minutes to obtain the supernatant. To 1.0ml of the supernatant, 1.0ml of DTNB and 1.0ml of buffer were added. The absorbance was read at 412 against reagent blank. The result was expressed in µmol/mg protein.

Histological Examination

The excised organs were rinsed in 0.9% saline solution and preserved in 10% formaldehyde solution. It was embedded in paraffin wax and sectioned into 4-6 microns. The sections were stained with hematoxylin and eosin and photographed.

Statistical analysis

Descriptive statistics and test for significance 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 22 in 2016. The turkey *post hoc* test were 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 of the AST activity shown in table 1 indicated lower ($p<0.05$) serum activity in the rats of the co-treated groups compared to MSG with a relative change of -44.98% -33.04% -31.33% for the low, mid and high doses respectively. However, the change in AST activity of the cp-treated groups relative to the control was highest in the low dose (-23.52%) and least in the high dose group (-4.54%)

The result as shown in table 2 for the ALT activity showed a decrease in ALT activity throughout the co-treated groups with the highest change relative to MSG at the medium dose (-57.83%)

For the table 3, the same trend followed as in table 1 and 2 however, the change relative to MSG was highest in extract group (-17.86%) while the least was the high dose (-5.16%)

For the antioxidant activities, table 10-13, all the co-treated groups showed relatively lower activity relative to MSG with the highest deviation being the low dose (-53.60%)for CAT and (-50.11%) for GSH. However, the reduction in SOD were relatively insignificant since they were all within comparable range as relative to MSG

Discussion

Measurement of activities of various enzymes in tissue and the body fluids play a significant role in disease investigation and diagnosis [22], determination of the extent of toxicity of a drug [23] and aid in determination of cellular damage long before it is picked up by the conventional histological technique [24].

The proliferation of the hepatocytes is followed by leakage of enzymes into the blood system and the activity of these enzymes including AST, ALT, and ALP are usually assayed to determine hepatic assault. [25][26][277].

The ALT, AST and ALP activity activities of the groups that were treated with the extract group were seemingly within comparable range with the normal group and significantly lower than the MSG group suggesting hepatoprotective potentials. This is further buttressed with the enzyme activity of the groups co-treated MSG which showed lower activity following CPE and concomitant MSG exposure indicating possible overriding adverse effect of the MSG assault on the hepatocytes by the extract. Both ALT which is more specific enzyme assay for hepatic assault [28] and AST which is found in the cytoplasm and mitochondria of tissues such as heart, skeletal muscles, liver kidney and erythrocytes [29] are all significantly ($p<0.05$) increased in the MSG group thereby agreeing with the previous reports by [30][31][11] which suggest proliferation, compromised liver integrity hence reduced functional capacity. Importantly, ALP hydrolyses phosphates in alkaline pH and increased activity is attributed to labilization, bile duct dysfunction, myocardial infarction and cancer [32][33]. This same trend which is dose dependent was also seemingly observed in the computed ratios (AST:ALT, AST:ALP ALT: ALP) and confirmed by the hepatic micrograph showing ameriolative conditions of the hepatocytes following concomitant CPE exposure with MSG. Congestion of the hepatic veins causes reduced blood flow to the surrounding hepatic cells leading to anoxia. This anorexic condition causes abnormality of the sodium pump which leads to outflow of fluid and membrane porosity. As the fluids leave the porous permeable membrane, the isoenzymes also dissolve out leading to increased serum isoenzymes concentration.

The antioxidant parameters showed improved function especially the among the group exposed to mid dose of CPE showing a significant ($p<0.05$) increased GSH suggesting that *Theobroma cacao* could possibly initiate its hepatoprotective potential by replenishing GSH. GSH reduced oxidative stress and lipid peroxidation either by protecting the detoxifying enzymes by increasing the efficacy of nicotine amide dinucleated phosphate (NADPH), or by helping in the elimination of compounds which produce peroxidation in the cell membranes [34]

CONCLUSION

Cacao pod extract significantly reduced toxic MSG assault on the hepatocytes when co treated suggesting total override of the adverse condition. This is confirmed by the improved histo morphology of the hepatocytes in the co treated groups. Hence, this study showed ameriolative effects of CPE on the histo-morphology, serum bio-functional indicators and antioxidant activities of MSG-intoxicated rats.

Tables

Table 1

Effects of ethanol extract of *Theobroma cacao* pod (CPE) on AST activity of normal and rats co-treated with mono-sodium glutamate.

Groups	AST (IUL^{-1})	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	84.05±2.46	0.00	-28.06
MSG group (8000 mg kg^{-1} b.w.t MSG)	116.83±8.23	39.00*	0.00
CPE group (300 mg kg^{-1} b.w.t extract)	61.25±4.27	-27.13	-47.57
Low extract co-treated group group (MSG, 8000 + 100 mg kg^{-1} b.w.t extract)	64.28±1.91	-23.52	-44.98
medium extract co-treated group group (MSG, 8000 + 300 mg kg^{-1} b.w.t extract)	78.23±4.13	-6.92	-33.04
High extract co-treated group group (MSG, 8000 + 500 mg kg^{-1} b.w.t extract)	80.23±3.67	-4.54	-31.33

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

MSG = monosodium Glutamate CPE = cocoa pod extract

Table 2

Effects of ethanol extract of *Theobroma cacao* pod (CPE) on ALT activity of normal and rats co-treated with mono-sodium glutamate.

Groups	ALT (IUL^{-1})	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	15.50±0.74	0.00	-39.92
Msg group (8000 mg kg^{-1} b.w.t MSG)	25.80±5.62	66.45	0.00
CPE group group (300 mg kg^{-1} b.w.t extract)	19.13±1.73	23.42	-25.85
Low extract co-treated group group (MSG, 8000 + 100 mg kg^{-1} b.w.t extract)	14.75±0.75	-4.84	-48.83
medium extract co-treated group group (MSG, 8000 + 300 mg kg^{-1} b.w.t extract)	10.88±1.01	-29.81	-57.83
High extract co-treated group group (MSG, 8000 + 500 mg kg^{-1} b.w.t extract)	16.00±1.78	3.23	-37.98

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

MSG = monosodium Glutamate CPE = cocoa pod extract

Table 3

Effects of ethanol extract of *Theobroma cacao* pod (CPE) on ALP activity of normal and rats co-treated with mono-sodium glutamate.

Groups	ALP (IUL^{-1})	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	22.75±0.98	0.00	-16.21
Msg group (8000 mg kg^{-1} b.w.t MSG)	27.15±1.17	19.34	0.00
CPE group group (300 mg kg^{-1} b.w.t extract)	22.30±1.40	-1.98	-17.86
Low extract co-treated group group (MSG, 8000 + 100 mg kg^{-1} b.w.t extract)	26.25±1.60	15.38	-3.31
medium extract co-treated group group (MSG, 8000 + 300 mg kg^{-1} b.w.t extract)	25.82±1.73	13.49	-4.90
High extract co-treated group group (MSG, 8000 + 500 mg kg^{-1} b.w.t extract)	25.75±1.78	13.19	-5.16

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

MSG = monosodium Glutamate CPE = cocoa pod extract

Table 4
Effects of ethanol extract of *Theobroma cacao* pod (CPE) on ALB concentration of normal and rats co-treated with mono-sodium glutamate.

Groups	ALB (mg/dl)	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	2.60±0.24	0.00	-12.75
Msg group (8000 mg kg^{-1} b.w.t MSG)	2.98±0.23	14.62	0.00
CPE group group (300 mg kg^{-1} b.w.t extract)	2.40±0.23	-7.69	-19.46
Low extract co-treated group group (MSG, 8000 + 100 mg kg^{-1} b.w.t extract)	3.05±0.12	17.31	2.35
medium extract co-treated group group (MSG, 8000 + 300 mg kg^{-1} b.w.t extract)	2.66±0.25	2.31	-10.74
High extract co-treated group group (MSG, 8000 + 500 mg kg^{-1} b.w.t extract)	2.83±0.47	8.85	-5.03

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

Table 5
Effects of ethanol extract of *Theobroma cacao* pod (CPE) on total protein concentration of normal and rats co-treated with mono-sodium glutamate.

Groups	TOTAL PROTEIN (g/dl)	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	5.08±0.50	0.00	22.41
Msg group (8000 mg kg^{-1} b.w.t MSG)	4.15±0.34	-18.31	0.00
CPE group group (300 mg kg^{-1} b.w.t extract)	3.95±0.41	-22.24	-4.82
Low extract co-treated group group (MSG, 8000 + 100 mg kg^{-1} b.w.t extract)	5.43±0.47	6.89	30.84
medium extract co-treated group group (MSG, 8000 + 300 mg kg^{-1} b.w.t extract)	4.50±0.45	-11.42	8.43
High extract co-treated group group (MSG, 8000 + 500 mg kg^{-1} b.w.t extract)	4.33±0.47	-14.76	4.34

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

Table 6
Effects of ethanol extract of *Theobroma cacao* pod (CPE) on TBil concentration of normal and rats co-treated with mono-sodium glutamate.

Groups	TBIL (mg/dl)	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	1.69±0.18	0.00	-28.09
Msg group (8000 mg kg^{-1} b.w.t MSG)	2.35±0.38	39.08	0.00
CPE group group (300 mg kg^{-1} b.w.t extract)	2.03±0.44	20.12	-12.77
Low extract co-treated group group (MSG, 8000 + 100 mg kg^{-1} b.w.t extract)	2.16±0.66	27.81	-8.09
medium extract co-treated group group (MSG, 8000 + 300 mg kg^{-1} b.w.t extract)	2.28±0.64	34.91	-2.98
High extract co-treated group group (MSG, 8000 + 500 mg kg^{-1} b.w.t extract)	1.16±0.37	-31.36	-50.64

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

Table 7
Effects of ethanol extract of *Theobroma cacao* pod (CPE) on ALT:AST and (AST:ALT) ratios of normal and rats co-treated with mono-sodium glutamate.

Groups	ALT:AST (AST:ALT)	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	0.18 (5.42)	0.00 (0.00)	-18.18 (19.65)
Msg group (8000 mg kg^{-1} b.w.t MSG)	0.22 (4.53)	22.22 (-16.42)	0.00 (0.00)
CPE group group (300 mg kg^{-1} b.w.t extract)	0.31 (3.20)	72.22 (-40.96)	40.91 (-29.36)
Low extract co-treated group group (MSG, 8000 + 100 mg kg^{-1} b.w.t extract)	0.23 (4.36)	27.78 (-19.56)	4.55 (-3.75)
medium extract co-treated group group (MSG, 8000 + 300 mg kg^{-1} b.w.t extract)	0.14 (7.19)	-22.22 (32.66)	-36.36 (58.72)
High extract co-treated group group (MSG, 8000 + 500 mg kg^{-1} b.w.t extract)	0.20 (5.01)	11.11 (-7.56)	-9.09 (10.60)

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

Table 8
Effects of ethanol extract of *Theobroma cacao* pod (CPE) on AST: ALP and (ALP:AST) ratios of normal and rats co-treated with mono-sodium glutamate.

Groups	AST:ALP (ALP:AST)	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	3.69 (0.27)	0.00 (0.00)	-14.19 (17.39)
Msg group (8000 mg kg^{-1} b.w.t MSG)	4.30 (0.23)	16.53 (-14.81)	0.00 (0.00)
CPE group group (300 mg kg^{-1} b.w.t extract)	2.75 (0.36)	-25.43 (33.33)	-47.21 (56.52)
Low extract co-treated group group (MSG, 8000 + 100 mg kg^{-1} b.w.t extract)	2.45 (0.41)	-33.60 (51.85)	-43.02 (78.26)
medium extract co-treated group group (MSG, 8000 + 300 mg kg^{-1} b.w.t extract)	3.03 (0.33)	-17.87 (22.22)	-29.53 (43.48)
High extract co-treated group group (MSG, 8000 + 500 mg kg^{-1} b.w.t extract)	3.12 (0.32)	-15.45 (18.52)	-27.45 (39.13)

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

Table 9
Effects of ethanol extract of *Theobroma cacao* pod (CPE) on ALT:ALP and (ALP:ALT) ratios of normal and rats co-treated with mono-sodium glutamate.

Groups	ALT:ALP (ALP:ALT)	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	0.68 (1.47)	0.00 (0.00)	-28.42 (40.00)
Msg group (8000 mg kg^{-1} b.w.t MSG)	0.95 (1.05)	39.71 (-28.57)	0.00 (0.00)
CPE group group (300 mg kg^{-1} b.w.t extract)	0.86 (1.17)	26.47 (-20.41)	-9.41 (11.43)
Low extract co-treated group group (MSG, 8000 + 100 mg kg^{-1} b.w.t extract)	0.56 (1.78)	17.65 (21.09)	-41.05 (69.52)
medium extract co-treated group group (MSG, 8000 + 300 mg kg^{-1} b.w.t extract)	0.42 (2.37)	-38.24 (61.22)	-55.79 (125.71)
High extract co-treated group group (MSG, 8000 + 500 mg kg^{-1} b.w.t extract)	0.62 (1.61)	-8.82 (58.50)	-34.74 (53.33)

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

Table 10
Effects of ethanol extract of *Theobroma cacao* pod (CPE) on CAT activity of normal and rats co-treated with mono-sodium glutamate.

Groups	CAT (IUL^{-1})	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	1.73±0.79	0.00	-75.51
Msg group (8000 mg kg^{-1} b.w.t MSG)	6.53±0.31	277.46	0.00
CPE group group (300 mg kg^{-1} b.w.t extract)	4.05±1.01	134.10	-37.98
Low extract co-treated group group (MSG, 8000 + 100 mg kg^{-1} b.w.t extract)	3.03±0.39	75.14	-53.60
medium extract co-treated group group (MSG, 8000 + 300 mg kg^{-1} b.w.t extract)	3.35±0.53	93.64	-48.70
High extract co-treated group group (MSG, 8000 + 500 mg kg^{-1} b.w.t extract)	3.80±0.38	119.65	-41.81

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

Table 11
Effects of ethanol extract of *Theobroma cacao* pod (CPE) on SOD activity of normal and rats co-treated with mono-sodium glutamate.

Groups	SOD (IUL^{-1})	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	11.35±0.09	0.00	0.79
Msg group (8000 mg kg^{-1} b.w.t MSG)	11.44±0.03	0.79	0.00
CPE group group (300 mg kg^{-1} b.w.t extract)	11.41±0.03	0.53	0.26
Low extract co-treated group group (MSG, 8000 + 100 mg kg^{-1} b.w.t extract)	11.44±0.03	0.79	0.00
medium extract co-treated group group (MSG, 8000 + 300 mg kg^{-1} b.w.t extract)	11.46±0.01	0.97	0.17
High extract co-treated group group (MSG, 8000 + 500 mg kg^{-1} b.w.t extract)	11.44±0.01	0.79	0.00

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

Table 12
Effects of ethanol extract of *Theobroma cacao* pod (CPE) on GSH concentration of normal and rats co-treated with mono-sodium glutamate.

Groups	GSH (mg/dl)	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	2.39±0.40	0.00	-47.24
Msg group (8000 mg kg^{-1} b.w.t MSG)	4.53±0.82	89.54	0.00
CPE group group (300 mg kg^{-1} b.w.t extract)	4.28±0.53	79.08	-5.52
Low extract co-treated group group (MSG, 8000 + 100 mg kg^{-1} b.w.t extract)	2.62±0.67	-5.44	-50.11
medium extract co-treated group group (MSG, 8000 + 300 mg kg^{-1} b.w.t extract)	2.50±0.39	4.60	-4.60
High extract co-treated group group (MSG, 8000 + 500 mg kg^{-1} b.w.t extract)	2.46±0.26	2.92	-45.70

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

Figure 1
The Photomicrograph of the liver section showing a normal flow of blood with no congestion of the central vein (group1/control)

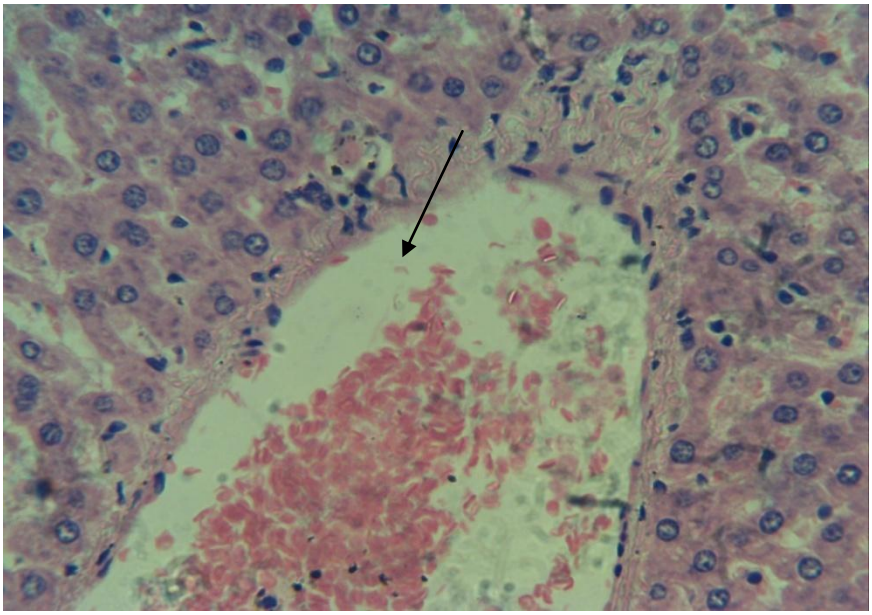


Figure 2
The Photomicrograph of the liver section of rats showing a full congestion of the central vein (MSG treated group)

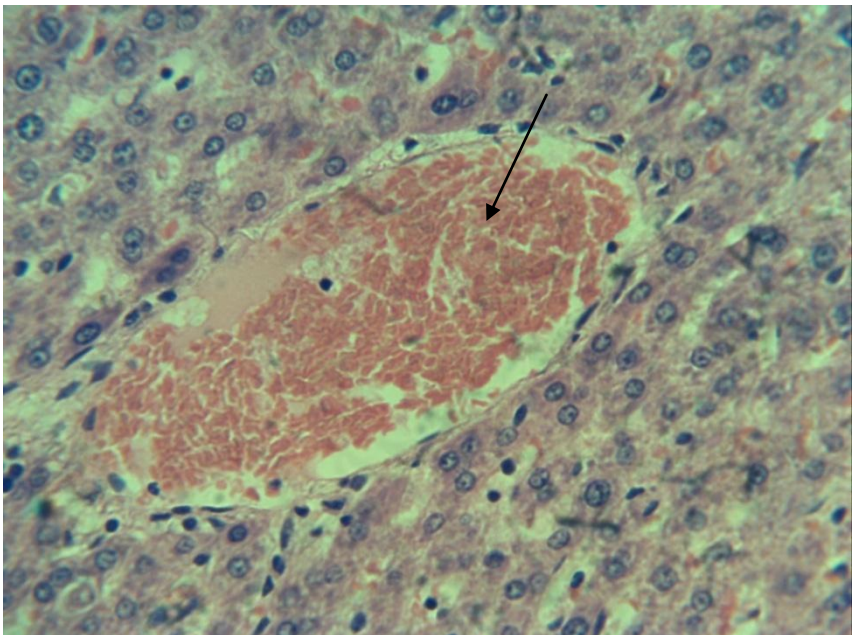


Figure 3
The Photomicrograph of the liver section of rats showing a normal flow of blood with no congestion of the central vein (extract group)

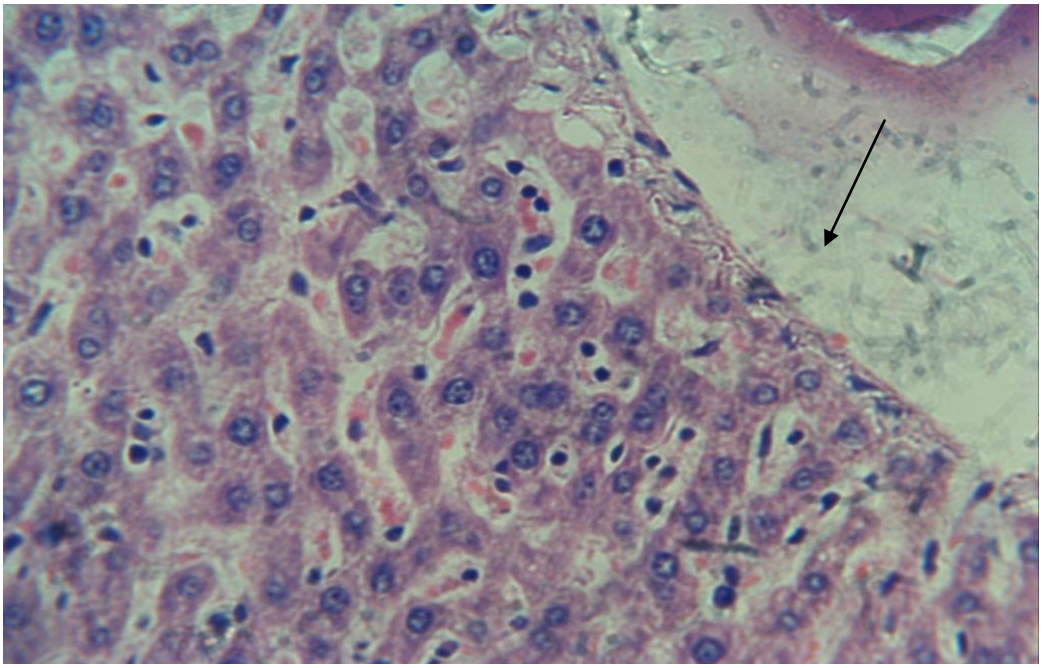


Figure 4
The Photomicrograph of the liver section of rats showing a normal flow of blood with no congestion of the central vein (MSG co-treated with 150mg extract)

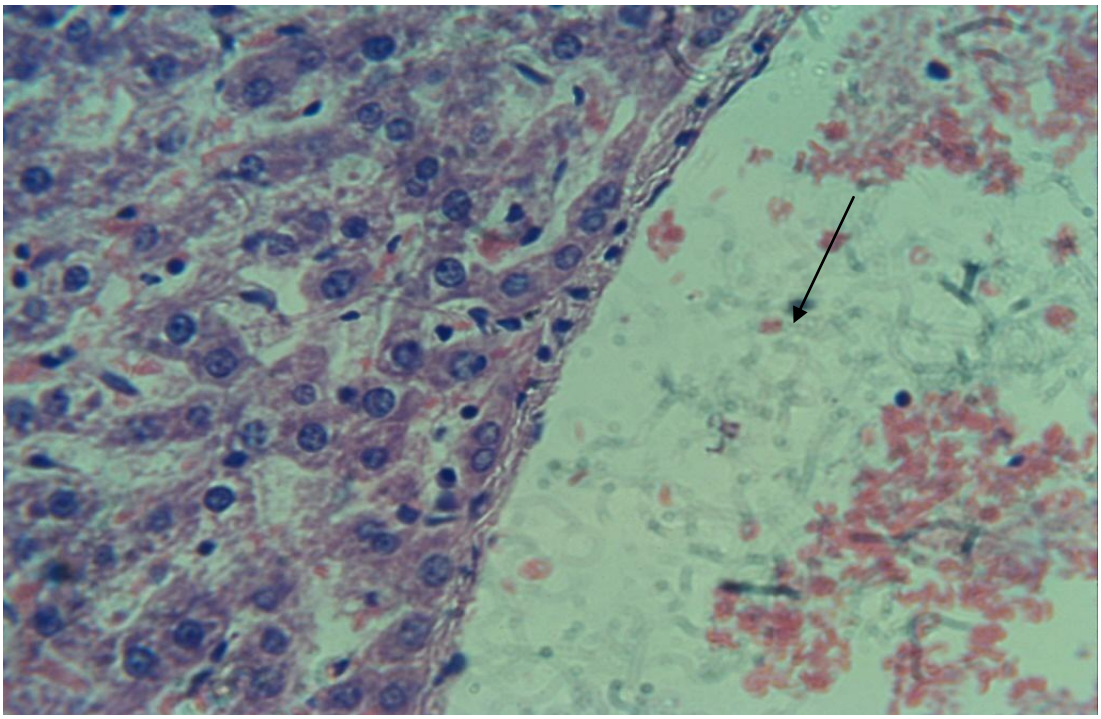


Figure 5
The Photomicrograph of the liver section of rats showing a normal flow of blood with no congestion of the central vein (MSG co-treated with 300mg extract)

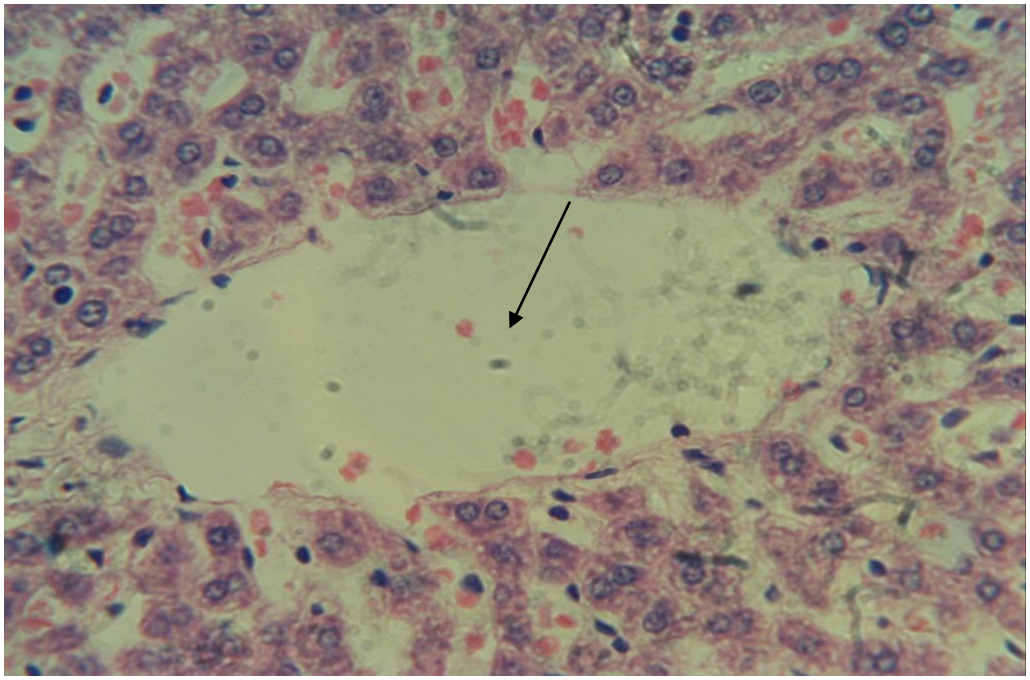
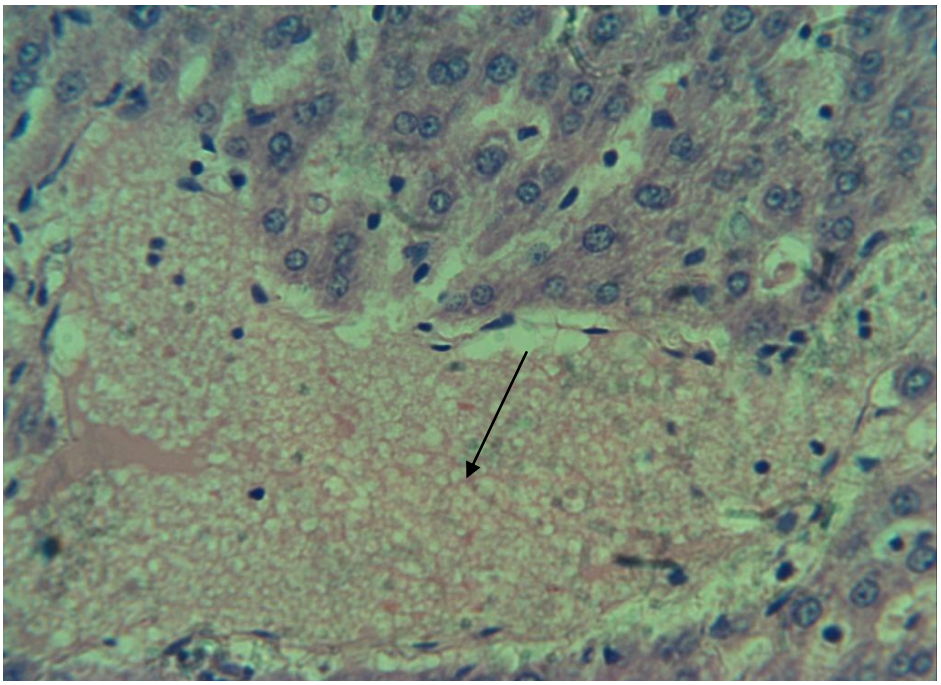


Figure 6
The Photomicrograph of the liver section of rats showing congestion of the central vein coupled with intravascular coagulation (MSG co-treated with 500mg extract)



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