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Some Biochemical Effect of Cocoa (Theobroma Cacao) Pod Ethanol Extract on Renal Histo-Morphology and Function in Monosodium Glutamate (MSG)- Intoxicated Rats

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Abstract

Utilization of food waste could improve food supply hence; lessen the environmental hazards caused by these wastes. This study investigated some biochemical effect of cocoa (theobroma cacao) pod ethanol extract on renal histo-morphology and function in monosodium glutamate (MSG)- intoxicated rats, using standard protocols. To evaluate the biochemical response, 24 male Wistar rats (weight range 80 – 101g) were grouped into six (n=4). Group 1, the positive control received feed and water while the group 2 (negative control) received MSG alone. The group 3 (extract control) received the extract at 300mg/kg body weight while the group 4 received MSG and 150mg extract. Group 5 received MSG and 300mg of extract while group 6 received MSG and 500mg of the extract. There was a significant ($p<0.05$) decrease in Urea and creatinine concentration characterized by negative change relative to MSG group. There was also a significant ($p<0.05$) increase in RBC in all the treatment groups (groups 4, 5, and 6) compared to the MSG group. The histopathological examination of the kidney indicated a clear central vein with no congestion at 150mg (groups 4), 300mg (5 respectively) in relation to the MSG group seemingly supporting the serum chemistry results on the ameliorative effects of the different extract concentrations on the toxicity induced by MSG. Thus, extract of cocoa pod which is a food waste has ameliorative effect on MSG induced renal assault.

Keywords: Bio-Chemical, Cocoa, Monosodium Glutamate, Intoxicated Rats.

Introduction

Monosodium glutamate (MSG) is a sodium salt of the non essential amino acid, glutamate. It is one of the world's most extensively used food additive and is ingested as part of commercially processed food as it is used to increase the sapidity of food[1] ; and is the most widely used flavor enhancer [2] and used in all Chinese and Japanese, ready to serve foods [3].

In industrialized countries, the estimated average intake of MSG per person is between 0.3g to 1.3g [4]. In Japan, MSG is used to elicit taste described as "umami" which translates to "savory" [5]. MSG has been linked to some conditions including neurotoxic effects, obesity and metabolic effects, Chinese restaurant syndrome and detrimental effects on sex organs [6]. However, Induction of oxidative stress as a consequence of MSG treatment has been reported [2,7].

Monosodium glutamate is a flavor enhancing food additive that may be present in packaged food without appearing on the label. This could lead to intake of high concentrations of MSG and at relatively high concentration, could induce adverse effects in animals, including seizure [8]; liver damage [9] and enhance appetite hence, increased food intake leading to obesity [10].

MSG has a molecular weight of 187.13 and is typically marketed as a white crystalline powder which is readily soluble in water and sparingly soluble in ethanol. It is not hygroscopic and is stable at room temperature. Even though it is not decomposed during normal food processes, MSG is partially dehydrated in acidic condition (2.2- 2.4) and at high temperature, converted to 5-pyrorlidone-2-carboxylate [11].

Since cocoa pod is discarded as waste and constitutes environmental burden [9]; and MSG is used in "ready to eat food" without appearing in labels hence possibility of overdose, harnessing cocoa pod as possible drug could lighten the burden caused by its disposal thus, this study aimed at determining Some Biochemical Effect of Cocoa (Theobroma Cacao) Pod Ethanol Extract on Renal Histo-Morphology and Function in Monosodium Glutamate (MSG)- Intoxicated Rats

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Materials and Methods

Chemical Reagents

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

Collection and Identification of Sample

The *Theobroma cacao* seeds were obtained 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

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 [12].

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 14th day, the animals were starved overnight and were sacrifices by cervical dislocation.

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. Standard ethical use of experimental animals were considered. The animals 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.

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 EDTA containers (plasma for hematological tests) and plain containers for other biochemical assays. The kidney were excised and processed for the histopathological examination.

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.

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 [13]

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

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

Methods

Determination of hematological indices

Hematological indices were determined by standard method described by Dacie and Lewis [14]. Blood (0.02ml) was added to 4ml of diluents in a test tube (1:200). The test tubes were sealed and the content mixed for one minute. The counting chamber was filled by means of capillary tube, counted after 2-5 minutes in the 1/5 square indicated under ×10 magnification objective lens

Calculation

$$\text{Cell count /mm}^3 = \frac{number\ \ of\ cells\ counted \times dilutions\ \times 10}{volume\ \ counted\ \ (\mu l)}$$

$$= \frac{N \times 200 \times 10}{0.02}$$

Determination of PCV

The blood sample collected using capillary tubes coated with anticoagulants EDTA was sealed with a plasticine, centrifuged for 5 minutes resulting to three suspensions; gray layer (mass of erythrocytes), thrombocytes (buffy coat), and plasma from bottom to top in a hematocrit reader to get the percentage of PCV.

CALCULATION

$$\text{PCV} = \frac{red\ \ cells\ \ counted}{whole\ \ blood\ \ volume} \times 100$$

Determination of Serum Electrolytes

Potassium ion

It was estimated by turbidity method based on the principle that Potassium ion present in the specimen reacts with Sodium Tetraphenol Boron (Boron reagent) to produce and insoluble potassium tetra phenyl boron resulting in turbid suspension. The extent of turbidity is measured at 580nm and is proportional to the concentration of potassium ion in the mixture.

Chloride Ions

This was done using colorimetric method based on the principle that Chloride ion form a soluble non-ionized compound with mercury ion and will displace thiocyanide ion from non-ionizes mercury thiocyanide. The released thiocyanide reacts with ferric ion to form colour complex that absorb light at 480nm. The intensity of the color produced is directly proportional to the chloride ion concentration.

Bicarbonate

The titrametric method described by Hodes [15] was used based on the principle that when an excess of acid is added to the serum, CO_2 is evolved. The rest of the acid is retitrated with an alkaline. Bicarbonate concentration is equal to the difference between the acid concentration and alkaline concentration spent titrating the excess acid after CO_2 evolved

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. 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 urea (table 1) and creatinine (table 2) concentration increased significantly in the MSG group, reduced in the extract group and across the co-treated groups when compared to the MSG group. The Hb (table 3) indices result showed a marked reduction in the MSG group (group 2) when compared to the control group (group 1). In comparism, the extract group and groups co-treated MSG with different varying concentrations of the extract groups showed an increase in the hemoglobin concentration through the groups. The white blood cell count (table 4), Red blood cell count (table 5) and packed cell volume (table 6) followed a similar pattern hence, were reduced in MSG group but were increased in all the treatment groups compared to the MSG groups.

The pattern of bicarbonate (table 7) and chloride (table 8) was similar across the groups. The treatment groups and the MSG groups were all higher than the normal groups however; the potassium concentration (table 9) followed a different trend where the co-treated groups were comparatively lower than that of the MSG group. The result of the kidney micrograph shows a normal flow of blood with no congestion on the renal vein of the kidney section of the normal control rats (plate 1). The section of the MSG group (plate 2) shows full congestion of the renal vein while the section for the extract control (plate 3) shows a normal flow of blood on the central vein. The micrograpgh of the group co-treated MSG with 150mg and 300mg (plate 4 and 5) of extract shows reduction in the congestion caused by MSG while the group co-treated with 500mg (plate 6) extract showed exacerbation of the effect**.**

Discussion

Use of phytotherapeutic products has been implicated in cases of renal and hepatic toxicity [16, 17]. Urea and Creatinine are usually measured to evaluate the kidney function. The decrease in urea and creatinine of the treatment groups as implicated in relative change MSG (%) suggests ameliorative potential as against MSG group which elevation signifies renal assault [18]. Significant decrease in serum concentrations of potassium relative to MSG (%) could be attributed to normalization the pump that maintains the constancy of its extracellular concentration which is obstructed in the MSG group leading to its abnormal increase

The five major types of white blood cells are neutrophils, lymphocytes, monocytes, eonisophils and basophils. WBC is decreased in leucopenia and may be due to bone marrow deficiency while levated WBC is called leukocytosis and may be due to anemia or disease condition [19,20]. The WBC was not significantly increased in the extract control and the treatment groups indicating that the immune was not challenged. Decrease in number of RBC, Hemoglobin PCV and increased WBC as shown in MSG group indicated Inflammation and hematological abnormalities. However, concomitant percentage increases of same with respect to MSG re-normalization.

Histopathological examination of the kidney shows full congestion of the renal blood vessels (Plate 2) in the MSG group seemingly supporting the serum chemistry observation, hence confirming the adverse influence on the kidney of the MSG-treated rats. This agrees with the result presented by Egbuonu at al [21]. The Plate 3 which is the extract control shows a normal renal blood flow with no congestion, hence confirming the serum chemistry observation that the extract has no adverse effect at the used concentration. The Plate 4 and Plate 5 shows a relative free blood flow with no congestion, confirming the serum chemistry result of ameliorating effects of the extract at 150 mg and 300mg respectively on the renal malfunction induced by MSG toxicity.

Conclusion

Cacao pod reduced the urea and creatinine concentration when compared to the MSG group, it could be indicative that *cacao* pod ameliorated the MSG-induced renal assault in the rats. This is supported by histopathological examination of the kidney. Hence the adverse influence of MSG on the kidney of the MSG-treated rats was ameliorated by the cocoa pod extract.

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Figures

Photomicrographs of Rat Kidney Section. (Hematoxyline and Eosin) Stained x 400

Figure I. The Photomicrograph of the kidney section of rats showing free blood flow with no congestion of the vessels of the kidney. (control group)

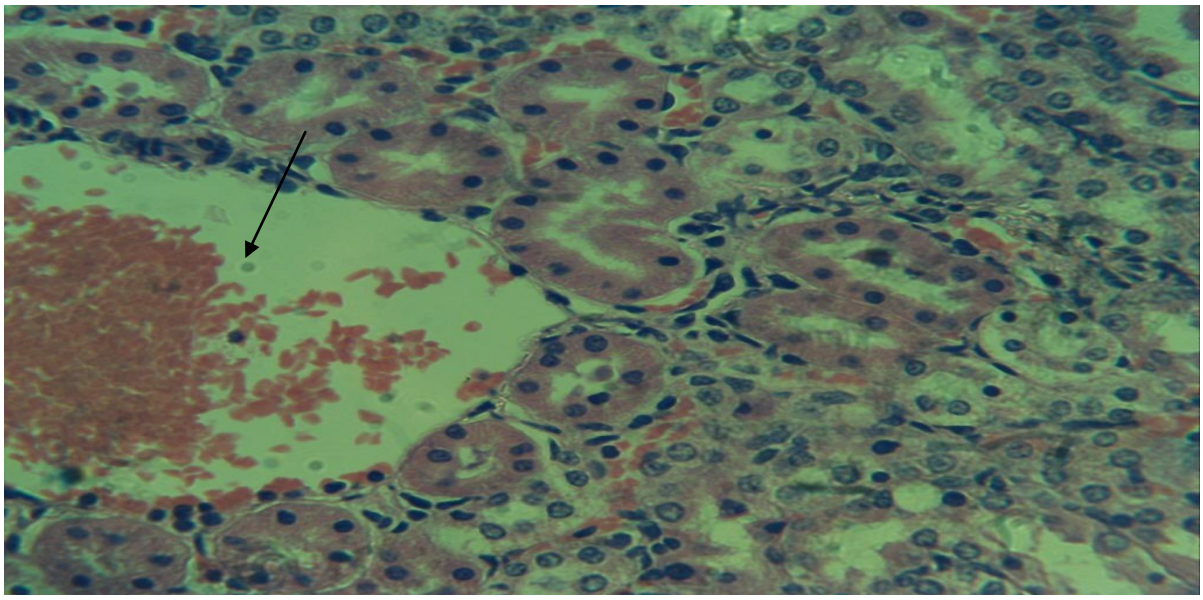


Figure II. The Photomicrograph of the kidney section of rats showing a full congestion of the central vein.(MSG treated)

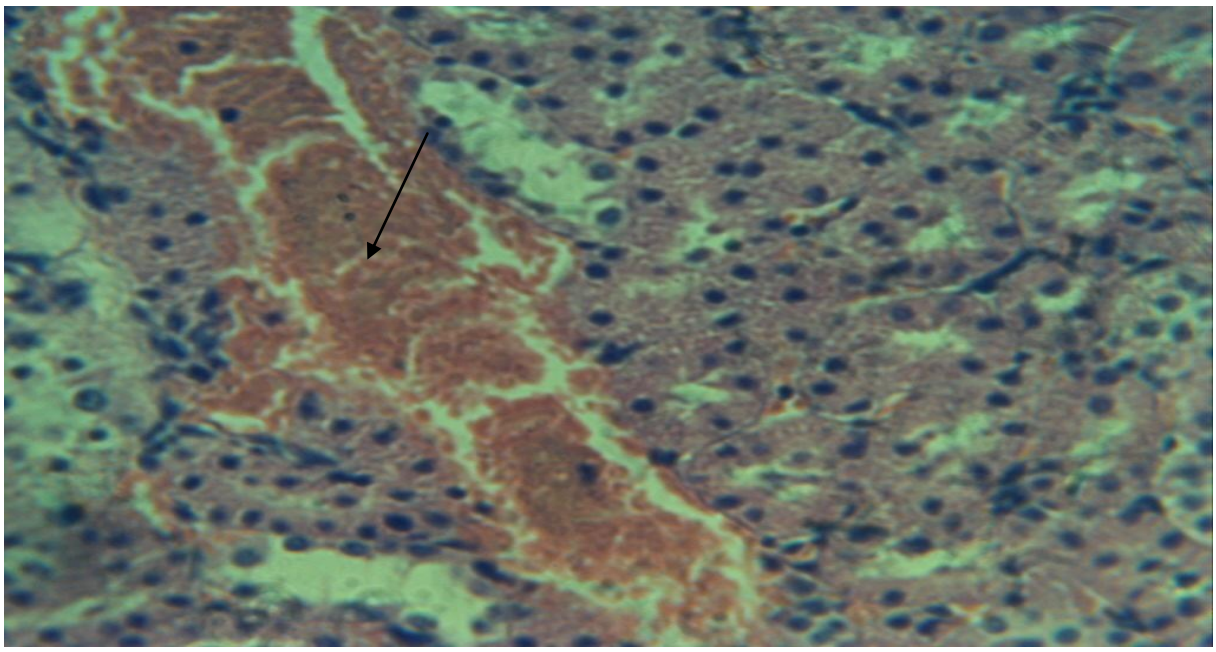


Figure III. The Photomicrograph of the kidney section of rats showing a normal flow of blood with no congestion of the central vein. (Extract group)

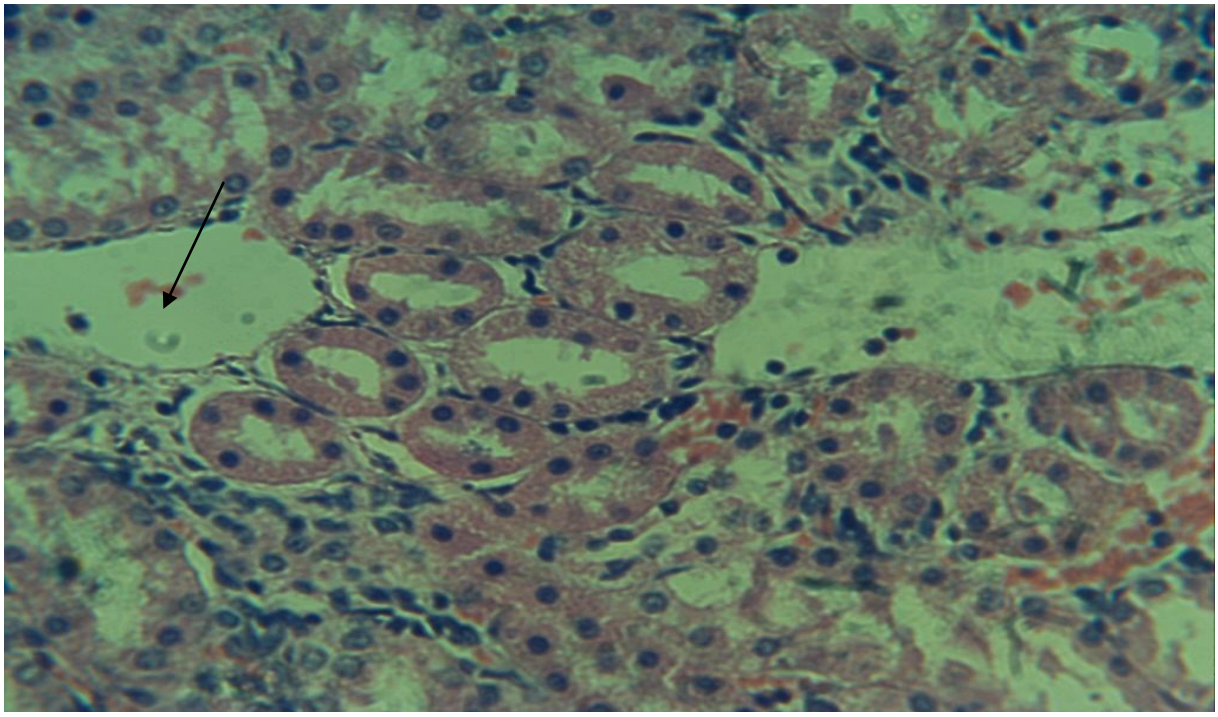


Figure IV. The Photomicrograph of the kidney section of rats showing a normal flow of blood with slight congestion of the central vein. (MSG co-treated with 150mg extract)

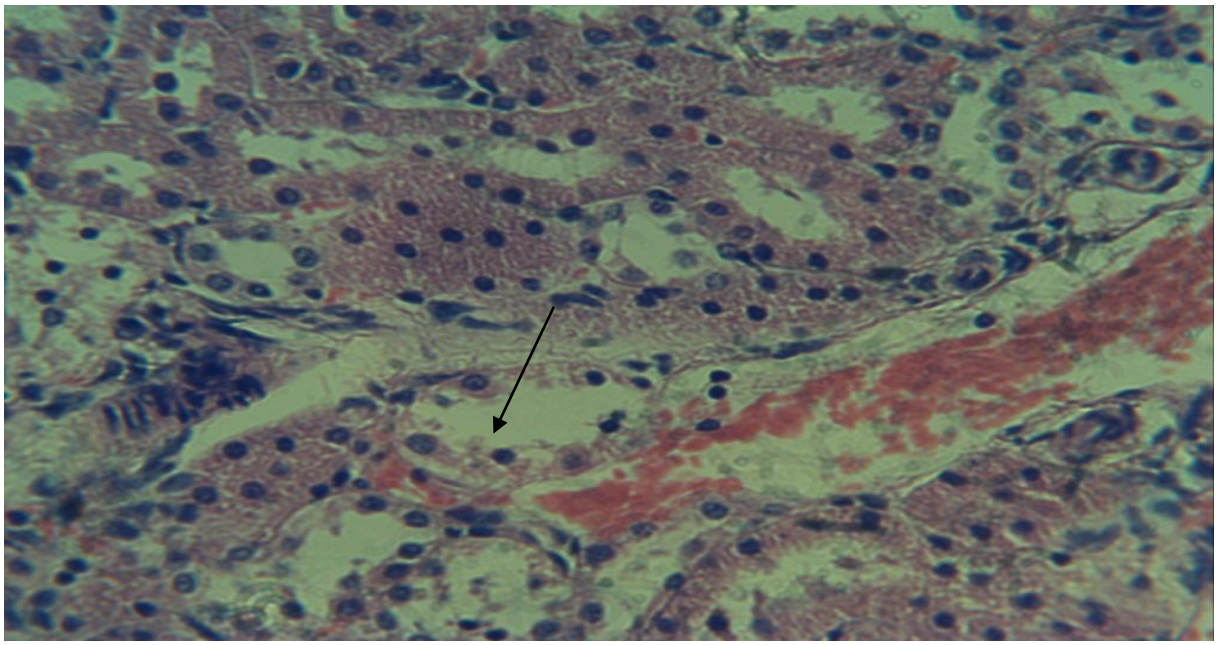


Figure V. The Photomicrograph of the kidney section of rats showing a normal flow of blood with slight congestion of the central vein. (MSG co-treated with 300mg extract)

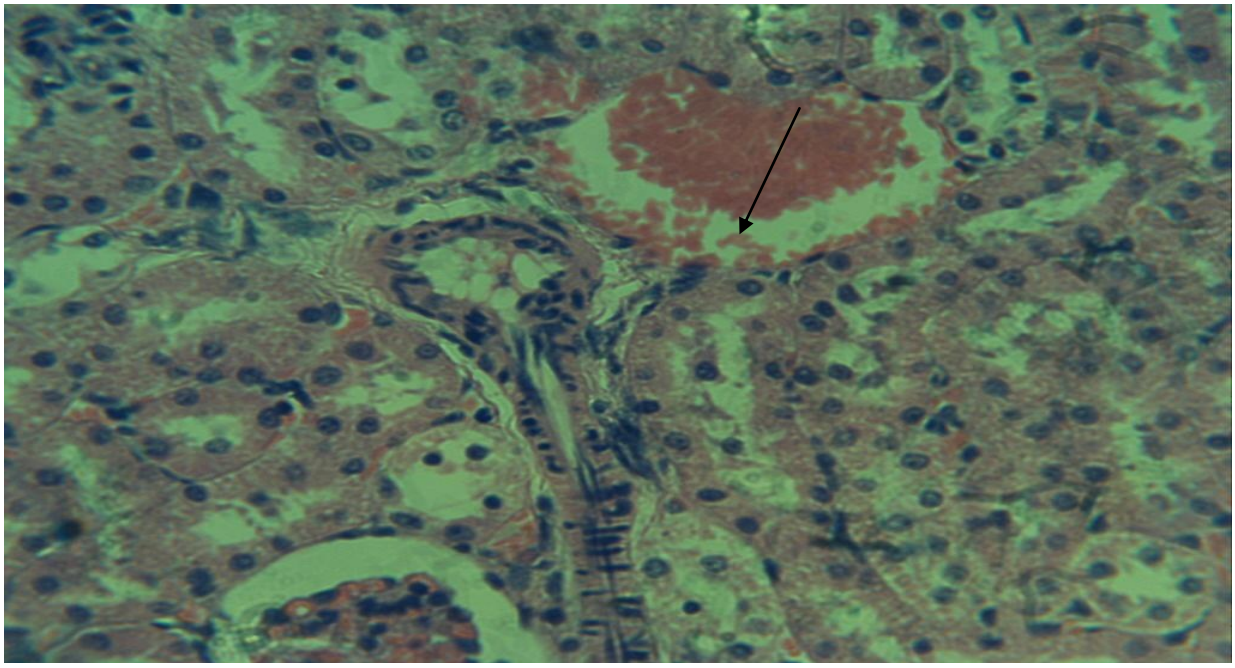
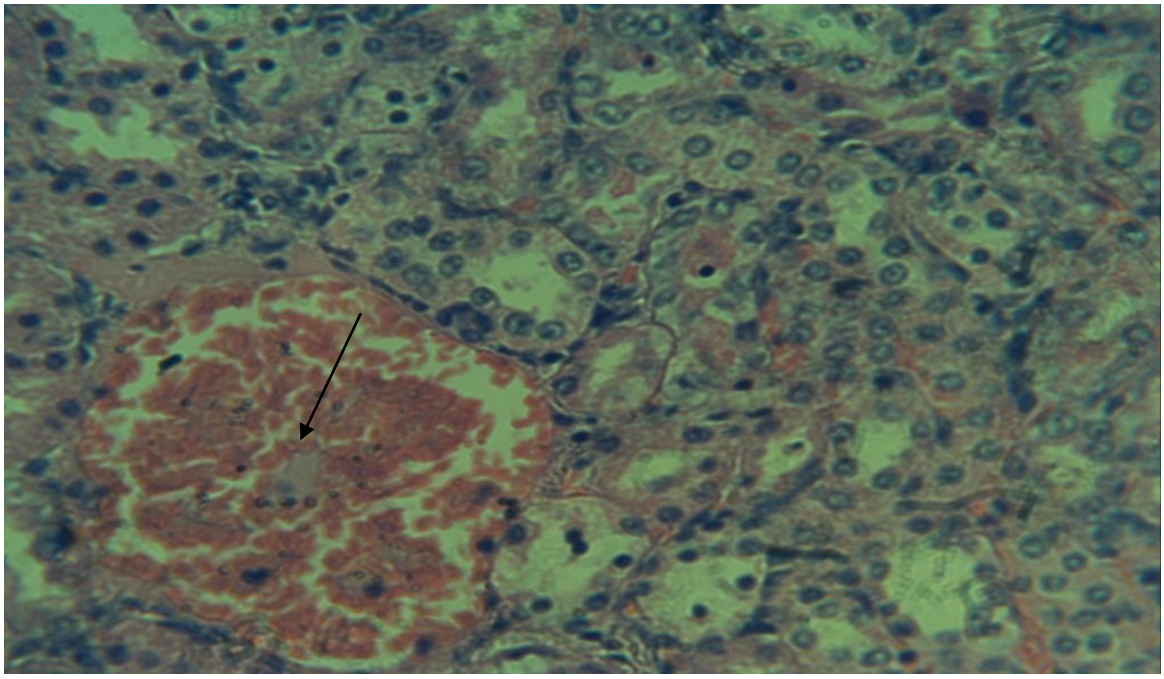


Figure VI. The Photomicrograph of the kidney section of rats showing congestion of the central vein. (MSG co-treated with 500mg extract)



Tables

Table 1. *Effects of ethanol extract of Theobroma cacao pod (CPE) on Urea concentration of normal and rats co-treated with mono-sodium glutamate.*

Groups	Urea (mg/dl)	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	13.25±0.85	0.00	-11.67
MSG group (8000 mg kg^{-1} b.w.t MSG)	15.00±0.82	13.21	0.00
CPE group (300 mg kg^{-1} b.w.t extract)	11.00±0.41	-16.98	-26.67
Low extract co-treated group group (MSG, 8000 + 100 mg kg^{-1} b.w.t extract)	12.50±0.50	-5.66	-16.67
medium extract co-treated group group (MSG, 8000 + 300 mg kg^{-1} b.w.t extract)	14.75±1.11	11.32	-1.67
High extract co-treated group group (MSG, 8000 + 500 mg kg^{-1} b.w.t extract)	15.50±1.44	16.98	3.33

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 creatinine concentration of normal and rats co-treated with mono-sodium glutamate*.

Groups	Creatinine (mg/dl)	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	0.49±0.02	0.00	-37.18
MSG group (8000 mg kg^{-1} b.w.t MSG)	0.78±0.23	59.18	0.00
CPE group (300 mg kg^{-1} b.w.t extract)	0.41±0.01	-16.33	-47.44
Low extract co-treated group group (MSG, 8000 + 100 mg kg^{-1} b.w.t extract)	0.50±0.02	2.04	-35.90
medium extract co-treated group group (MSG, 8000 + 300 mg kg^{-1} b.w.t extract)	0.55±0.04	12.24	-29.49
High extract co-treated group group (MSG, 8000 + 500 mg kg^{-1} b.w.t extract)	0.59±0.05	20.41	-24.36

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 Hemoglobin concentration of normal and rats co-treated with mono-sodium glutamate.*

Groups	Hemoglobin (g/dl)	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	10.38±0.77	0.00	46.20
MSG group (8000 mg kg^{-1} b.w.t MSG)	7.10±0.60	-31.60	0.00
CPE group (300 mg kg^{-1} b.w.t extract)	9.55±0.43	-8.00	34.50
Low extract co-treated group group (MSG, 8000 + 100 mg kg^{-1} b.w.t extract)	8.53±1.13	-17.82	20.14
medium extract co-treated group group (MSG, 8000 + 300 mg kg^{-1} b.w.t extract)	7.68±0.13	-26.01	8.17
High extract co-treated group group (MSG, 8000 + 500 mg kg^{-1} b.w.t extract)	8.00±1.26	-22.93	21.95

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 WBC Count of normal and rats co-treated with mono-sodium glutamate.*

Groups	WBC($\times 10^3$/L)	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	2400±234.50	0.00	108.70
MSG group (8000 mg kg^{-1} b.w.t MSG)	1150±170.75	-52.08	0.00
CPE group (300 mg kg^{-1} b.w.t extract)	2000±100.74	-16.67	73.91
Low extract co-treated group group (MSG, 8000 + 100 mg kg^{-1} b.w.t extract)	2600±100.65	8.33	126.09
medium extract co-treated group group (MSG, 8000 + 300 mg kg^{-1} b.w.t extract)	2300±100.67	-4.17	100.00
High extract co-treated group group (MSG, 8000 + 500 mg kg^{-1} b.w.t extract)	2150±150.00	-10.42	86.96

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 RBC Count of normal and rats co-treated with mono-sodium glutamate*.

Groups	RBC ($\times 10^9$)	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	437.50±23.94	0.00	29.63
MSG group (8000 mg kg^{-1} b.w.t MSG)	337.50±23.94	-22.86	0.00
CPE group (300 mg kg^{-1} b.w.t extract)	387.50±31.46	-11.43	14.81
Low extract co-treated group group (MSG, 8000 + 100 mg kg^{-1} b.w.t extract)	512.50±12.50	17.14	51.85
medium extract co-treated group group (MSG, 8000 + 300 mg kg^{-1} b.w.t extract)	475.50±25.00	8.69	40.90
High extract co-treated group group (MSG, 8000 + 500 mg kg^{-1} b.w.t extract)	525.00±25.00	20.00	55.60

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

Groups	PCV (%)	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	33.00±1.29	0.0	13.79
MSG group (8000 mg kg^{-1} b.w.t MSG)	29.00±1.08	-12.12	0.00
CPE group (300 mg kg^{-1} b.w.t extract)	32.50±0.96	1.52	12.07
Low extract co-treated group group (MSG, 8000 + 100 mg kg^{-1} b.w.t extract)	29.50±0.96	10.61	1.72
medium extract co-treated group group (MSG, 8000 + 300 mg kg^{-1} b.w.t extract)	30.00±3.44	9.09	3.43
High extract co-treated group group (MSG, 8000 + 500 mg kg^{-1} b.w.t extract)	27.50±1.71	-16.67	-5.15

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 bicarbonate concentration of normal and rats co-treated with mono-sodium glutamate.*

Groups	Bicarbonate	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	30.15±3.12	0.00	-4.38
MSG group (8000 mg kg^{-1} b.w.t MSG)	31.53±6.30	3.38	0.00
CPE group (300 mg kg^{-1} b.w.t extract)	35.10±3.15	16.42	11.32
Low extract co-treated group group (MSG, 8000 + 100 mg kg^{-1} b.w.t extract)	33.88±2.60	12.37	7.45
medium extract co-treated group group (MSG, 8000 + 300 mg kg^{-1} b.w.t extract)	39.45±1.25	30.85	25.12
High extract co-treated group group (MSG, 8000 + 500 mg kg^{-1} b.w.t extract)	30.46±5.69	1.03	-3.39

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 chloride concentration of normal and rats co-treated with mono-sodium glutamate*

Groups	Chloride	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	55.60±9.99	0.00	-9.49
MSG group (8000 mg kg^{-1} b.w.t MSG)	61.43±5.22	10.48	0.00
CPE group (300 mg kg^{-1} b.w.t extract)	61.15±7.49	9.98	-0.46
Low extract co-treated group group (MSG, 8000 + 100 mg kg^{-1} b.w.t extract)	69.78±0.88	25.50	13.59
medium extract co-treated group group (MSG, 8000 + 300 mg kg^{-1} b.w.t extract)	63.63±2.81	14.44	3.58
High extract co-treated group group (MSG, 8000 + 500 mg kg^{-1} b.w.t extract)	69.38±2.41	24.78	12.94

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 potassium concentration of normal and rats co-treated with mono-sodium glutamate*

Groups	Potassium	Change relative to the control (%)	Change relative to MSG group (%)
Control (feed + water only)	3.80±0.79	0.00	-41.80
MSG group (8000 mg kg^{-1} b.w.t MSG)	6.53±0.31	71.84	0.00
CPE group (300 mg kg^{-1} b.w.t extract)	4.04±1.01	6.31	-38.13
Low extract co-treated group group (MSG, 8000 + 100 mg kg^{-1} b.w.t extract)	3.03±0.39	-20.26	-53.60
medium extract co-treated group group (MSG, 8000 + 300 mg kg^{-1} b.w.t extract)	3.35±0.53	-11.84	-48.70
High extract co-treated group group (MSG, 8000 + 500 mg kg^{-1} b.w.t extract)	3.80±0.38	0.00	-41.80

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