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Therapeutic effects of *Cyperus esculentus* on Monosodium glutamate-induced haemobiochemical and steroidogenic anomalies in male Wistar rats

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ABSTRACT

Monosodium glutamate (MSG) is a well-known food additive linked to anaemia and reproductive/hormonal disorders. The effects of *Cyperus* esculentus against MSG-induced toxicity in male animals have not been reported, therefore, the study aimed to investigate the therapeutic effects of *Cyperus* esculentus on MSG-induced haemo-biochemical and reproductive hormone anomalies in male rats. Forty adult male Wistar rats were randomly assigned into four groups (n=10). Group A served as the control, received 0.5 mL of distilled water orally. Group B, received MSG alone at 2 mg/kg orally, Group C received oral *Cyperus* esculentus at 500 mg/kg while Group D was treated with MSG alone orally at 2 mg/kg for the first 14 days, after which the rats received oral dose of *Cyperus* esculentus at 500 mg/kg for another 14 days. Five rats were sacrificed from each group 14 and 28 days post-treatment, respectively, during which samples were collected for haematological, biochemical and hormones assays. The packed cell volume and testosterone values in Group D were significantly higher than the values in Group B. It was concluded that *Cyperus* esculentus caused increased packed cell volume and testosterone, remarkable therapeutic effect on MSG-induced haematological and testosterone anomalies. *Cyperus* esculentus juice is therefore recommended as a potential haematinic and therapeutic agent for infertility in male rats exhibiting low libido.

Keywords: Tigernut, Monosodium glutamate, Anaemia, Haematinics, Testosterone

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Introduction

Monosodium glutamate (MSG) is an odourless, colourless, pure white crystalline molecule that exists as monohydrate salt and insoluble in organic solvent.¹ MSG provides a unique taste that falls outside of the established primary tastes (bitter, sweet, sour and salty), called umami in Japanese ² that intensifies the savory flavor of food.³ It has been reported to increase food intake by overstimulation of the satiety center, and consequently results in increased body weight.^{4,5} It is a flavor enhancer commonly used in oriental cuisine.⁶ It is a salt of sodium and glutamic acid. It is actively absorbed from the gastrointestinal tract, by the action of several enzymes; it is taken by cells and catabolized in the cytosol and mitochondria.¹ MSG is not only found in processed food but also in many spiced food products.^{2,7,8} The use of MSG has been abused in food due to its flavour enhancing properties. High content of MSG in processed foods are sold to the public without being labeled to avoid suspicion.⁴

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While it is generally considered safe by food safety regulatory agencies, concerns have been raised about its potential health risks, including obesity, asthma, and metabolic disorders.9 Preclinical and clinical studies have also suggested a link between MSG and various adverse effects, such as neurotoxicity, cardiotoxicity, and hepatic and renal disorders, metabolic disorder along with behavioural changes.^{2,10-13} Excessive consumption of MSG at 3 g/kg in Wistar rat for 30 days has been linked to increase cell death in the germinal epithelial of the testicle,14 drop in sperm quality, male reproductive accessory organs at 120mg/kg body weight for 28 days in Wistar rats,¹⁵ also hypertrophy and hyperplasia of mesangial cells in the nephron resulting in renal failure at 15mg/kg body weight in Wistar rats for 30 days.¹⁰ Alterations in the values of haematopoietic parameters have been reported in the toxicity studies of MSG.^{7,16} Production of reactive oxygen species is one the main pathways by which MSG induce damages in tissues and organs.8,17,18 The use of antioxidants such as vitamin C and E have been reported to mitigate the deleterious effects of MSG on body cells, tissues and organs.1,16

Tiger nut, known as *Cyperus esculentus*, is a highly nutritious tuber with a range of health benefits. It is rich in fiber, proteins, and sugars, as well as oleic acid, glucose, phosphorus, potassium, and vitamins C and E, which are important antioxidants.^{19,20} Tigernut has been reported to exhibit therapeutic and biological effects of antioxidant, anti-inflammatory and improvement of general well-being and health conditions.²¹⁻²³ It is rich in flavonoid glycosides.²⁰ The plant is widely grown and can be consumed in various forms, including raw, roasted, or as milk called "Horchata".^{22,24} It has been used in the food industry to produce oil, starch, and protein, and has potential applications in the development of functional foods.²⁵ Despite the nutritional value and potential of *Cyperus esculentus*, there is a need for further research to fully exploit its functional and therapeutic properties.

Haematological parameters are generally used as indicators of wellness in an individual and serve as valid diagnostic markers of certain diseases.^{26,27} Abnormality of haematopoietic parameters may be a reflection of an ongoing disease condition or toxicosis.^{28,29} Determination of blood parameters provides opportunity to clinically detect substances or their metabolites, which aides in diagnosis. Haematopoietic indices are used as biomarkers of diseases and oxidative stress.^{30,31} They are the first invasive line of protection and indicators of the state of the cellular environment.

The prevalence of male infertility has increased globally.³² Studies that will provide ways of protecting and enhancing the functions of both primary and accessory male reproductive organs will significantly contribute to ameliorating this problem. The effects of *Cyperus esculentus* against MSG-induced toxicity has not been reported, therefore the aim of this study was to determine the therapeutic effects of tigernut (*Cyperus esculentus*) on monosodium glutamate-induced haemo-biochemical anomalies in the reproductive indices of Wistar rats.

Materials and Methods

Experimental animals

Forty (40) adult male Wistar rats weighing $120 \pm 10g$ were used in this study. The animals were obtained from the Experimental Animal House of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria. Animals were securely kept in cages ($60 \times 60 \times 50$ cm). All animals were kept under controlled conditions of temperature (37° C) and humidity ($50 \pm 15\%$) and normal photoperiod (12-hour light and 12-hour dark). The rats were fed with standard diets as needed and water was provided *ad libitum*.

Treatment materials

Monosodium glutamate (98%) and *Cyperus esculentus* were purchased from Bodija market, Ibadan, Nigeria. All other chemicals used in this study were of the highest available grades in Nigeria.

Preparation of Cyperus esculentus Juice

The tigernut (*Cyperus esculentus*) tubers were thoroughly washed with clean water to remove dirt and foreign materials. The cleaned tubers were then soaked in potable water at room temperature for 6-12 hours to soften them and enhance juice extraction, as described by Adejuyitan $(2011)^{33}$. After soaking, the tubers were drained and mechanically crushed using a high-speed blender to release the juice. The resulting pulp was manually filtered using a 0.075 mm plastic sieve, ensuring the separation of fibrous residues from the liquid extract. To maximize juice yield, the pulp was gently pressed and squeezed through the sieve, allowing the milk-like liquid to pass through while retaining solid residues.

Ethical consideration

The experiment was conducted in accordance with the current guidelines set forth for the ethical care of laboratory animals by the Animal Care and Use Ethics Committee at the University of Ibadan with approval number NHREC/UIACUREC/05/12/2022A, ensuring adherence to ethical standards throughout the study.

Study design

The forty (40) adult male Wistar rats were randomly assigned into four groups (n=10). Group A served as the control and received 0.5 mL of distilled water orally. Group B, received oral monosodium glutamate (MSG) at 2 mg/kg body weight³⁴ orally, Group C received oral *Cyperus* esculentus juice at 500 mg/kg body weight while Group D was treated with MSG alone at 2 mg/kg for the first 14 days, after which the rats received oral dose of *Cyperus* esculentus alone at 500 mg/kg body weight³⁵ for another 14 days. MSG was dissolved in distilled water for oral administration throughout the treatment period.

Blood sample collection and biochemical assay

The rats were anesthetized using diethyl ether within a desiccator, and blood samples were obtained via peri-orbital venous bleeding (venipuncture), collected into sterile heparinized and non-heparinized sample tubes, and promptly placed on ice. After collection, the blood samples in heparinized tubes were transferred to microfuge tubes. Packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), total and differential white blood cell count and platelets count were determined. Serum was harvested from the nonheparinized blood samples after centrifugation at 3000 rpm for 5 minutes at 25°C. The obtained serum was stored in 1.5 mL Eppendorf tubes at a temperature of -20°C. For the quantification of biochemical assays and serum hormones, commercial kits were utilized, conducting triplicate analyses to minimize errors attributable to inter-assay variability. Specifically, the concentrations of testosterone and estradiol in the serum were determined using ELISA kits (TES 6670- from MP Biomedicals, Ohio, USA, following the manufacturer's instructions. Testosterone and estradiol concentrations were expressed in ng/mL and pg/mL, respectively.

Determination of packed cell volume (PCV)

The PCV of each blood sample was determined using the microhaematocrit method described by Coles.³⁶

Determination of haemoglobin concentration

Haemoglobin concentration was determined by cyanmethaemoglobin method described by Coles.³⁶ Haemoglobin was oxidized to cyanmethaemoglobin by the addition of cyanide (in Drabkin solution), and the cyanmethaemoglobin was then determined spectrophotometrically.

Determination of red blood cell (RBC) count

The RBC count was determined by the haemocytometer method.

Haematimetric indices

The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the PCV, RBC and HB values obtained earlier.

$$MCV (fl) = \frac{PCV (\%) \times 10}{RBC (X \ 10^{12} / \mu L)}$$
$$MCH (pg) = \frac{Hb (g/dl) \times 10}{RBC (X \ 10^{12} / \mu L)}$$

Total and differential white blood cell count

The total white blood cell (leucocytes) count was determined using the haemocytometer method.

Differential leucocyte count

A fresh smear of each blood sample was prepared, fixed with methanol and then stained with Giemsa. One hundred cells were identified morphologically and counted and the number of each leukocyte type was expressed as a percentage of the total WBC from which the absolute leukocyte values were calculated.

Biochemical parameters determination

Total protein (TP), albumin (ALB), globulin (GLB), creatinine (Creat), serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), alkaline Phosphatase (APT) and blood urea nitrogen (BUN), total bilirubin (T. Bil) were determined by

the method described by Meyer and Harvey.³⁷

Statistical analysis

The data generated were analyzed using SPSS IBM 20. One-way ANOVA was employed to assess significant mean differences, followed by the *post-hoc* Tukey's test to evaluate significant differences within the Groups (P < 0.05 was considered significant).

Results and Discussion

Table 1 and 2 show the results of leukocyte and erythrocytic indices values which indicate that the platelet levels in the MSG treated group $(24.36\pm5.29 \ \mu\text{L})$ and MSG + *Cyperus esculentus* treated group

(23.60±4.32 µL) were significantly (P < 0.05) higher than the levels recorded in the control group (10.56±1.47 µL) and *Cyperus esculentus* treated group (16.88±4.76 µL). Monosodium glutamate administration for 14 and 28 days showed no significant on haematological parameters, this finding is in agreement with the report of Oluwole *et al.*, (2024)¹ that monosodium glutamate does not have significant effects on haematological parameters. However, this finding is at variance with some reports that monosodium glutamate could be toxic to erythrocytes and also cause deleterious changes in haematological parameters.^{38:40} These differences might be attributed to the difference in dosages and route of MSG administered in the different studies.

	Table 1: Leukocytes and erythroc	cytic indices of male Wistar rats in different treatment	groups at 14 days post-treatment
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	А	В	С	D
Parameters	(Control)	(MSG only)	(Tigernut only)	(MSG + Tigernut)
WBC (x10 ⁶ ul)	3.99 <u>+</u> 1.7	3.14 <u>+</u> 1.0	3.39 <u>+</u> 0.6	3.02 <u>+</u> 0.7
PLT (x10 ⁵ ul)	11.98 <u>+</u> 1.11	10.90 <u>+</u> 2.83	9.38 <u>+</u> 1.30	8.84 <u>+</u> 9.81
LYMPH (%)	75.80 <u>+</u> 4.82	74.40 <u>+</u> 1.14	74.00 <u>+</u> 2.35	72.40 <u>+</u> 1.95
NEUT (%)	21.00 <u>+</u> 3.87	21.60 <u>+</u> 1.52	22.60 <u>+</u> 1.34	24.40 <u>+</u> 2.19
MON (%)	1.60 <u>+</u> 1.14	2.00 <u>+</u> 0.71	1.80 <u>+</u> 0.84	2.00 <u>+</u> 1.00
EOS (%)	1.60 <u>+</u> 0.89	1.60 <u>+</u> 0.55	1.60 <u>+</u> 1.14	8.09 <u>+</u> 0.53
MCV (fl)	58.10 <u>+</u> 3.57	61.68 <u>+</u> 1.23	60.34 <u>+</u> 1.81	60.16 <u>+</u> 1.71
MCHC (%)	32.28 <u>+</u> 0.44	32.729 <u>+</u> 0.249	32.329 <u>+</u> 0.606	32.264 <u>+</u> 0.536
MCH (pg)	$18.76\overline{8+1.39}$	20.186+0.43	19.51 ± 0.82	19.41+0.73

Values expressed as Means ± *Standard Deviation (SD).*

Note: WBC (White Blood Cell), PLT (Platelets), LYMPH (Lymphocytes), NEUT (Neutrophils), MON (Monocytes) EOS (Eosinophils), MCV (Mean Corpuscular Volume), MCHC (Mean Corpuscular Hemoglobin), MCH (Mean Corpuscular Hemoglobin).

	А	В	С	D
	(Control)	(MSG only)	(Tigernut only)	(MSG + Tigernut)
Parameters		-		-
WBC (x10 ⁶ ul)	3.60 <u>+</u> 1.11	3.72 <u>+</u> 0.73	3.13 <u>+</u> 0.7	4.00 <u>+</u> 0.7
PLTS (x10 ⁵ ul)	10.56 ± 1.47^{a}	24.36 <u>+</u> 5.29 ^b	16.88 ± 4.76^{a}	23.60 <u>+</u> 4.32 ^b
LYMPH (%)	77.2 <u>+</u> 2.59	74 <u>+</u> 2.55	76.4 <u>+</u> 2.41	75.6 <u>+</u> 3.72
NEUT (%)	21.6 <u>+</u> 4.72	22.8 <u>+</u> 3.49	20 <u>+</u> 3.54	20.8 <u>+</u> 4.38
MON (%)	1.8 <u>+</u> 1.30	1.8 <u>+</u> 1.30	1.8 <u>+</u> 0.84	1.6 <u>+</u> 0.55
EOS (%)	1.4 <u>+</u> 0.55	1.4 <u>+</u> 1.14	1.8 <u>+</u> 1.30	2 <u>+</u> 1.41
MCV (fl)	$60.\overline{86}+1.47$	60.87 <u>+</u> 1.04	60. <u>57+</u> 2.31	60.99 <u>+</u> 1.53
MCHC (%)	32.82 <u>+</u> 0.39	32.39 <u>+</u> 0.70	32.16 <u>+</u> 0.53	32.38 <u>+</u> 0.79
MCH (pg)	19.97 <u>+</u> 0.66	19.72 <u>+</u> 0.46	19.47 <u>+</u> 0.45	19.74 <u>+</u> 0.311

Values expressed as Means \pm Standard Deviation (SD). Means with different superscripts within row are significantly different (P < 0.05).

Note: WBC (White Blood Cell), PLT (Platelets), LYMPH (Lymphocytes), NEUT (Neutrophils), MON (Monocytes) EOS (Eosinophils), MCV (Mean Corpuscular Volume), MCHC (Mean Corpuscular Hemoglobin), MCH (Mean Corpuscular Hemoglobin).

Figures 1-6 show the values of PCV, Hb and RBC recorded after 14 and 28 days of treatments. Figure 1 shows that the value of PCV obtained in all the treatment groups after 14 day were not significantly different. The results of Figure 2 indicated that after 28 days of treatments the PCV obtained in *Cyperus esculentus* treated rats ($53\pm2.17\%$) and MSG + *Cyperus esculentus* treated rats ($48\pm5.34\%$) were significantly (P < 0.05) higher than those obtained in the control ($46.8\pm1.92\%$) and MSG alone treated rats ($45.5\pm5.23\%$), respectively. The Hb concentration value recorded in *Cyperus esculentus* treated rats (17.1 ± 0.43 g/dl) was significantly (P < 0.05) increased when compared to the values obtained in MSG alone treated rats (15.52 ± 1.49 g/dL), respectively (Figure 4). The mild decrease in the mean PCV, Hb, RBC and WBC following exposure to monosodium glutamate relative to control group suggests that a prolonged exposure to MSG for 28 days or more consecutively could

precipitate anemia.³⁸ This finding is similar to the previous report of ALhamed et al., (2021)⁴⁰ who recorded a significant decrease in PCV, Hb, RBC values after 30 days of consecutive oral dosages of MSG at 1.8 mg/kg body weight in White rats. Continuous exposure to MSG for as long as 28 day at low dose of 2mg/kg could reduce the lifespan of erythrocytes due to the effect of increase sodium concentration in the extracellular fluid from MSG resulting in an hypertonic condition, and consequently causing shrinkage of red blood cells and reduction in function and lifespan. This is further supported by Ashaolu et al., (2011)³⁸ which reported significant effects on RBC, PCV, Hb, MCV and MCH, neutrophil and lymphocyte count, indicative of an anemic conditions and a compromised immune status in rats treated with MSG. Oxidative stress usually associated with the mechanisms of deleterious effects of MSG could have precipitated increased erythrocyte osmotic fragility making the cells vulnerable to changes in the composition of the extracellular fluid, thereby leading to reduced number of erythrocytes.

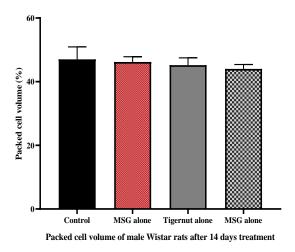


Figure 1: The packed cell volume of male Wistar rats after 14 days of treatments

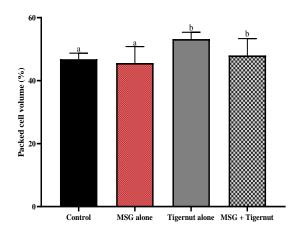


Figure 2: Packed cell volume of male Wistar rats after 28 days of treatments.

 $^{\rm a,b}$ Different superscript alphabets shows significantly different (P<0.05

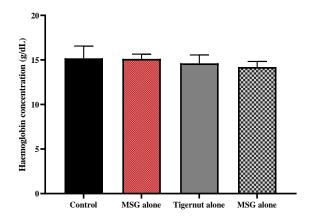


Figure 3: Haemoglobin concentration in male Wistar rats after 14 day of treatments

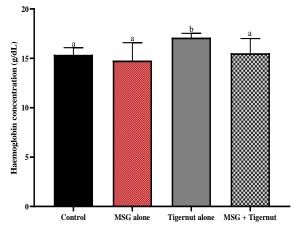
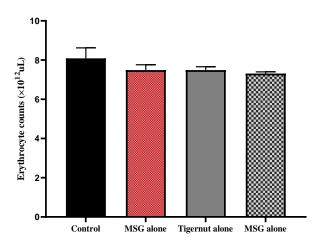
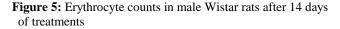


Figure 4: Haemoglobin concentration in male Wistar rats after 28 days of treatments

^{a,b} Different superscript alphabets shows significantly different (P<0.05)





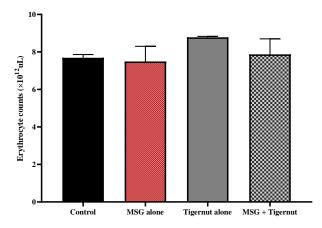


Figure 6: Erythrocyte counts in male Wistar rats after 28 days of treatment

In contrast, administration of Cyperus esculentus for 28 days caused increase in PCV, Hb, and platelet values compared to the Control. Based on the components of Cyperus esculentus, which include oleic acid, glucose, lipid, protein, vitamin C and E, Flavonoid glycosides²⁵, it has been reported for it reactive oxygen species (ROS) scavenging capacity.²⁰ By reducing the generation of ROS, Cyperus esculentus may have induced a protective effect on the erythrocytes, consequently, preserving and prolonging their lifespan, and thereby preventing anaemia. This finding is in agreement with the report of Hassan, 2007⁴¹ that Cyperus esculentus boosts erythrocytic indices in the male Albino rats, his study showed significant increase in hematological parameters after 24 days consecutive administration of 0.5ml/kg of Cyperus esculentus oil intraperitoneally. Furthermore, Group D, which received 14 days of treatment with MSG and Cyperus esculentus for the remaining 14 days, exhibited a significant rise in PCV and platelet levels compared to Group B, which was treated with MSG only. This implies that Cyperus esculentus has haematinic potentials.

Table 3 and 4 show the values of serum chemistry obtained in MSGinduced male Wistar rats treated with *Cyperus esculentus* juice. At the end of 14 days treatment there was no significant (P > 0.05) difference in the values of serum parameters obtained in all the treated groups. The total bilirubin values significantly (P < 0.05) decreased in *Cyperus esculentus* treated group (0.32 ± 0.05 mg/dL) and MSG + *Cyperus esculentus* treated group (0.32 ± 0.05 mg/dL) when compared to the values recorded MSG alone treated group (0.38 ± 0.08 mg/dL).

 Table 3: Serum chemistry of the male Wistar rats in different

 treatment groups at 14 days post

 treatment

t	reatment			
	A (Control)	B (MSG	C (Tigernut	D (MSG +
Paramete		only)	only)	Tigernut)
rs				
TP (g/dl)	7.66 <u>+</u> 0.7 2	7.38 <u>+</u> 0.4 1	7.34 <u>+</u> 0.3 4	7.46 <u>+</u> 0.3 4
ALB	3.24 <u>+</u> 0.3 1	3.14 <u>+</u> 0.2 4	3.04 <u>+</u> 0.2 3	3.26 <u>+</u> 0.1 7
(g/dl)				
GLB	4.46 <u>+</u> 0.4 3	4.24 <u>+</u> 0.2 1	4.30 <u>+</u> 0.1 9	4.20 <u>+</u> 0.1 9
(g/dl)				
ALB/GL	0.72 <u>+</u> 0.0 4	0.74 <u>+</u> 0.0 4	0.71 <u>+</u> 0.0 5	0.77 <u>+</u> 0.0 4
B ratio				
AST (ul)	42.6 <u>+</u> 2.4 1	41.4 <u>+</u> 1.8 2	41.4 <u>+</u> 1.1 4	41.8 <u>+</u> 1.6 4
ALT (ul)	33.4 <u>+</u> 6.2 7	29.8 <u>+</u> 1.3 0	30 <u>+</u> 1.58	30.6 <u>+</u> 2.3 0
ALP (ul)	105.6 <u>+</u> 6. 11	94.2 <u>+</u> 7.5 3	95 <u>+</u> 9.14	98 <u>+</u> 11.20
BUN	15.36 <u>+</u> 0. 69	14.24 <u>+</u> 0. 37	14.38 <u>+</u> 0. 53	14.48 <u>+</u> 0. 51
(mg/dl)				
Creat	0.66 <u>+</u> 0.0 9	0.64 <u>+</u> 0.0 6	0.62 <u>+</u> 0.0 5	0.68 <u>+</u> 0.0 5
(mg/dl)				
T. Bil	0.3 <u>+</u> 0.1	0.26 <u>+</u> 0.0 6	0.26 <u>+</u> 0.0 6	0.26 <u>+</u> 0.0 9
(mg/dl)		-		-
Values orne	accad as Maa	ng + Standar	d Deviation	(50)

Values expressed as Means ± Standard Deviation (SD). Note: ALP (Alkaline Phosphatase), ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), BUN (Blood Urea Nitrogen), ALB (Albumin), GLB (Globulin), TP (Total protein), T. Bil (Total Bilirubin), Creat (Creatinine).

Table 4: Serum chemistry of the male Wistar rats in different
treatment groups at 28 days post-treatment

treatment giv	A	B	C	D
	(Control)	(MSG	(Tigernut	(MSG +
Paramete		only)	only)	Tigernut)
rs				
TP (g/dl)	8.46 <u>+</u> 0.1 1	8.14 <u>+</u> 0.4 4	8.12 <u>+</u> 0.3 9	8.22 <u>+</u> 0.4 4
ALB	3.3 <u>+</u> 0.16	3.06 <u>+</u> 0.2 9	3.12 <u>+</u> 0.2 3	3.18 <u>+</u> 0.2 9
(g/dl)			-	
GLB	0.64 <u>+</u> 0.0 6	0.60 <u>+</u> 0.2 8	0.62 <u>+</u> 0.2 2	0.61 <u>+</u> 0.3 9
(g/dl)				
ALB/GL	46.2 <u>+</u> 0.0 4	46.8 <u>+</u> 0.0 6	45.8 <u>+</u> 0.0 4	45.6 <u>+</u> 0.0 6
B ratio				
AST (ul)	46.2 <u>+</u> 1.3 0	46.8 <u>+</u> 2.3 9	45.8 <u>+</u> 2.3 9	45.6 <u>+</u> 2.7 0
ALT (ul)	35.4 <u>+</u> 2.3 0	34.6 <u>+</u> 1.6 7	33.6 <u>+</u> 2.6 1	33.6 <u>+</u> 1.6 7
ALP (ul)	125.6 <u>+</u> 4. 16	125.0 <u>+</u> 4. 95	125.6 <u>+</u> 5. 18	123.2 <u>+</u> 5. 68
BUN	16.48 <u>+</u> 0. 31	15.8 <u>+</u> 0.4 6	15.8 <u>+</u> 0.6 5	15.9 <u>+</u> 0.5 1
(mg/dl)	51	0	5	1
Creat	0.76 <u>+</u> 0.0 6	0.72 <u>+</u> 0.0 5	0.8 <u>+</u> 0.22	0.72 <u>+</u> 0.0 5
(mg/dl)				
T. Bil	0.48 <u>+</u> 0.0 5 ^a	0.38 <u>+</u> 0.0 8 ^a	0.32 <u>+</u> 0.0 5 ^b	0.32 <u>+</u> 0.0 5 ^b
(mg/dl)				

Values expressed as Means \pm Standard Deviation (SD). Means with different superscripts within row are significantly different (P<0.05).

Note: ALP (Alkaline Phosphatase), ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), BUN (Blood Urea Nitrogen), ALB (Albumin), GLB (Globulin), TP (Total protein), T. Bil (Total Bilirubin), Creat (Creatinine).

There were no significant variations in the mean values of total protein, albumin, globulin, creatinine, serum aspartate aminotransferase, serum alanine aminotransferase, alkaline Phosphatase and blood urea nitrogen among the groups 14 days and 28 days post-treatment with both MSG and Cyperus esculentus. This connotes that MSG and Cyperus esculentus did not alter the biochemical parameters of the male Wistar rat. It was opined by Enemali et al., $(2015)^{42}$ that MSG did not have significant effects on the biochemical parameters in the male Wistar rats, except at high doses. The study's findings align with previous research on the effects of monosodium glutamate (MSG) on biochemical parameters, which have shown non-significant decreases.43 The current study revealed a noticeable yet non-statistically significant decrease in the mean biochemical parameters values of rats following MSG exposure. However, these findings diverge from the report by Abdel-Reheim et al., (2014)⁴⁴ which suggest that monosodium glutamate increases certain liver enzymes such as ALT, AST, and ALP. The findings of Al-Mousawi et al., (2017)⁴⁵ also observed a statistically significant elevation in AST, ALT, and ALP levels (indicating liver function impairment) in animals treated with monosodium glutamate. A significant increase in blood urea and creatinine levels in the monosodium glutamate-treated animal group compared to the control group was previously reported Al-Mousawi et al., (2017)⁴⁵ concluding that monosodium glutamate may pose health risks. Discrepancies between studies may stem from variations in experimental conditions, such as the concurrent treatment of rats with both monosodium glutamate and sodium nitrite (NaNO₃), as well as differences in the rat populations utilized in experiment.⁴⁵ However, the treatment with *Cyperus esculentus* juice did not significantly alter biochemical parameters.⁴⁶

The results in Tables 5 and 6 show the concentrations of testosterone and estradiol in MSG-induced male Wistar rats treated with *Cyperus esculentus* juice. After 14 days treatment the concentration of testosterone was significantly (P < 0.05) lower in MSG alone treated rats (0.01 ± 0.01 ng/mL) when compared with the other treated groups. The testosterone concentration recorded in *Cyperus esculentus* treated group (0.44 ± 0.39 ng/mL) was significantly (P < 0.05) higher in comparison to other treatment groups. The level of estradiol was significantly (P < 0.05) higher in MSG alone treated rats (217.87 ± 19.43 pg/mL) than the other treatment groups (Table 5). After day 28 of treatments, the concentration of testosterone in *Cyperus esculentus* treated rats (2.19 ± 0.10 ng/mL) was significantly (P < 0.05) increased when compared with other treatment groups. The value of estradiol obtained in MSG treated group (203.57 ± 2.43 pg/mL) was significantly (P < 0.05) higher than the values recorded in other treatment groups (Table 6).

A remarkable drop in mean testosterone values and a marked increase in estradiol following MSG exposure imply that MSG has estrogenic activity and is capable of reducing libido and altered spermatogenesis in the male rats by decreasing the serum testosterone thereby precipitating male infertility.47 This finding is in agreement with Udefa et al., (2020)³⁴ who demonstrated that blood testosterone levels were remarkably lowered in both young and adult rats treated with MSG. Another previous study by Abdou et al., (2020)⁴⁸ affirmed that the administration of MSG caused neuronal damage in the hypothalamus in rats and mice, and such neuronal losses in the hypothalamus could result in disturbance of the hypothalamic-pituitary-testis regulatory axis that governs steroidogenesis of testicular Leydig cells. Decrease in testosterone level can directly affect fertility by causing decreased sperm production and indirectly affect infertility by reducing sexual drive and causing erectile dysfunction.⁴⁷ Conversely, the administration of Cyperus esculentus offered therapeutic effects against monosodium glutamate-induced steroidogenic anomalies in the male Wistar rats used in this study. In addition, a study conducted by Ekaluo et al., (2015)⁴⁹, revealed that the aqueous extract of Cyperus esculentus caused a rise in serum testosterone levels in male rats. Studies carried out by Saad et al., (2008)⁵⁰ and Chiang et al., (2007)⁵¹ have provided evidence that Cyperus esculentus treatment has a substantial impact on elevating testosterone levels in the male. Additionally, the report of Saad et al., $(2008)^{50}$ showed positive enhancements in sexual function as a result of administration of Cyperus esculentus in the rats. The results in this study provide evidence that Cyperus esculentus would have significant impact on the estrogenic activities of MSG thereby boosting the male fertility.

Table 5: Reproductive hormones of the male Wistar rats in different treatment groups at 14 days post-treatment

Parameters	А	В	С	D
	(Control)	(MSG only)	(Tigernut only)	(MSG + Tigernut)
Testosterone (ng/mL)	0.15 ± 0.04^{a}	0.01 ± 0.01^{b}	0.44 <u>+</u> 0.39 ^c	0.14 ± 0.06^{a}
Estradiol (pg/mL)	145.13 <u>+</u> 38.11 ^a	217.87 <u>+</u> 19.43 ^b	163.77 <u>+</u> 28.98 ^a	155.44 <u>+</u> 42.88 ^a

Values expressed as Means \pm Standard Deviation (SD). Means with different superscripts within row are significantly different (P < 0.05).

	A (Control)	B (MSG only)	C (Tigernut only)	D (MSG + Tigernut)
Parameters	, , ,	•		
Testosterone (ng/mL)	0.74 <u>+</u> 0.57 ^a	0.24 <u>+</u> 0.10 ^a	2.19 <u>+</u> 0.10 ^b	0.20 <u>+</u> 0.06 ^a
Estradiol (pg/mL)	175.66 <u>+</u> 5.82 °	203.57 <u>+</u> 2.43 ^b	182.97 <u>+</u> 2.69 ^a	177.43 <u>+</u> 26.71 ª

Values expressed as Means \pm Standard Deviation (SD). Means with different superscripts within row are significantly different (P < 0.05).

Conclusion

It was concluded in this study that treatment of adult male Wistar rats with *Cyperus esculentus* caused a significant increase in Packed Cell Volume and a marked increase in serum testosterone level in contrast to the effect of Monosodium glutamate. Therefore, *Cyperus esculentus* juice administration is recommended as a potential haematinic and therapeutic agent for monosodium glutamate-induced infertility in male breeding stock exhibiting reduced testosterone level.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original. Any liability for claims relating to this article will be borne by us.

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