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PROTECTIVE EFFECT OF *NIGELLA SATIVA* ON THE REPRODUCTIVE SYSTEM OF MONOSODIUM GLUTAMATE – CHALLENGED MALE WISTAR RATS

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ABSTRACT

Background: Monosodium glutamate (MSG) is one of the most widely used food additives that might lead to infertility in male. Previous studies established that *Nigella sativa* (NS) extract provides antioxidative and cytoprotective effect.

Aim: This study investigated the male reproductive system potential protective effect of *Nigella sativa* Seed (NSS) aqueous extract on monosodium glutamate- challenged male Wistar rats

Methods: Twenty-five male Wistar rat were randomly divided in to five (5) groups of five rats each. Control Group received normal diet of food and water, Experimental Control received 120mg/kg body weight MSG, Experimental Group 1 received 120mg/kg body weight MSG + 50mg/kg NSS aqueous extract, Experimental Group 2 received 120mg/kg body weight MSG + 100mg/kg NSS aqueous extract and Experimental Group 3 received 120mg/kg MSG + 200mg/kg NSS aqueous extract. The study was for a period of 35 days. The rats were anaesthetized with chloroform by inhalation. The blood sample was collected by cardiac puncture. The Total Body Weight (TBW), Gonadosomatic Index (GSI), Testosterone levels and Semen parameters were evaluated.

Results: There was no significant difference in the TBW and GSI of rat between the control and experimental groups ($p > 0.05$). There was significant decrease in testosterone and sperm parameters in the experimental control group when compared with normal control group ($p< 0.05$) after administration of 120mg/kg MSG. Administration of NSS aqueous extract at different doses significantly increased the level of testosterone and total sperm parameters compared with all experimental groups ($p< 0.05$).

Conclusion: Oral administration of MSG, induced male reproductive system toxicity, which protected by oral administration of NSS aqueous extract in male Wister rats. Therefore, consumption of high dose MSG must be avoided because it may cause damage in male reproductive organs and incorporation of NSS to the processed feed could prevent the cytotoxic effects of MSG.

Key words: *Monosodium Glutamate, Nigella sativa Seed, Gonadosomatic, Testosterone, Sperm count*

INTRODUCTION

Monosodium glutamate (MSG), a glutamic acid salt derivative, is an important but "non-essential" amino acid naturally found in several foods including beef, milk and vegetables, and plays an important role in human metabolism (Bera *et al.,* 2017). In addition to its role in protein synthesis, glutamate is utilized in nitrogen assimilation, biosynthesis of nucleoside, amino acid and co-factor, secondary natural product formation, and in the catabolism of certain amines (Walker and van der Donk, 2016). MSG is used as flavor enhancer commonly in the processing of foods in many homes, restaurants, and in canned and processed foods (David, 2008).

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However, research suggest that consumption of monosodium glutamate has detrimental effect on humans and experimental animals (Shimada *et al.*, 2013). MSG caused oxidative stress, inflammation and neurodegeneration in rats even at low dose (Kesherwani *et al.*, 2024). In addition, MSG may affect the male reproductive system by increasing reactive oxygen species (ROS) in the testicles leading to infertility (Vinodini *et al.,* 2008). According to The World Health Organization - WHO (2023), approximately one in every six people of reproductive age globally experience infertility in their lifetime.

Nigella sativa (NS), an annual herb of the Rununculaceae family is cultivated in many countries of the world including middle eastern, Mediterranean region, south Europe, India, Pakistan, Syria, Turkey, Saudi Arabia. For thousands of years, NS has been used in these countries as a spice, food preservative, and as protective and health remedy in traditional folk medicine for the treatment of numerous disorders (Chopra *et al.*, 1956). Studies have shown that *Nigella sativa* Seed (NSS), commonly known as 'Black seed' contains several active pharmacological ingredients (Harzallah *et al.*, 2011), with thymoquinone (major component of essential oil) having most of the therapeutic properties of the plant. Evidence suggest that black seed has ameliorating effect on deranged parameters of kidney (Abd-Elkareem, *et al.,* 2022), liver (Eshami *et al*, 2015), blood (George and Kumaran, 2016), and central nervous system (Bauchi *et al.,* 2016) among others. Black seed significantly improves sperm, semen, corpus luteum and gonadotropic hormones like testosterone and progesterone (Darand *et al.*, 2019).

Most studies highlighting the effect of extracts of NS on the reproductive system focused on ameliorating toxicity induced by substances. With the increasing trend of infertility especially among males globally, it is paramount to focus research on protective effects of substances on the reproductive system. This study attempted to provide information on the protective effect of NSS aqueous extract on MSG- challenged reproductive system of Wister rats.

MATERIALS AND METHODS

Plant Materials and Preparation

The black seed was obtained from shop no 4 Aliya Block, adjacent to Yakasai Primary School, Rimi-Asibiti market, Kano State of Nigeria. Botanical identification and authentication were obtained from Plant Biology Department, Faculty of Life Sciences Bayero University Kano, Nigeria on January 9, 2023 with Herbarium Accession Number (BUKHAN 0275). Aqueous extraction of black seeds by maceration method was performed in Pharmacognosy laboratory, Department of Pharmacology, Faculty of Pharmaceutical Science Bayero University Kano, Nigeria.

Experimental Design

The posttest only control group experimental design was used for this study.

The experimental animals consisted of twenty-five male Wistar rats, 7-8 weeks old, mean weights of 165 grams. The rats were sourced from animal house Faculty of Pharmaceutical Sciences, Bayero University Kano, Nigeria. The rat where housed in metallic cage (38cm×46cm×24cm) with saw dust as bedding, under standard conditions. The rats were acclimatized in the laboratory for period of two weeks before the commencement of study. The rats were maintained under 12:12 LD cycle with rodent chow and water available *ad libitum* throughout the study period.

The rats were randomly assigned into five different groups, five rats in each group, and were administered (according to the groups) distilled water, MSG and NSS aqueous extract as follows:

Group 1 (control) received distilled water

Group 2 (experimental control) received 120mg/kg/day of MSG and distilled water

Group 3 received 120mg/kg body weight MSG + 50mg/kg body weight NSS aqueous extract

Group 4 received 120mg/kg body weight MSG + 100mg/kg body weight NSS aqueous extract
Group 5 received 120mg/kg body weight MSG + 200mg/kg body weight NSS aqueous extract
All interventions were given to the animals by oral gavage once daily for a period of five weeks. The dose of 120mg/kg body weight of MSG was chosen based on allometric extrapolation from human Average daily intake of MSG described by Fatin *et al., (*2019). The dosages of *Nigella sativa* was adopted from the study conducted by Darand *et al.,* (2019).
At the end of the 28th day, the animals were anaesthetized using chloroform by inhalation, and their blood and semen samples collected using procedures described below.
Animal care, handling and use were carried out in accordance with National institute of Health Guide for the care and use of laboratory animals (National Research Council, 2011), and ethical approval was obtained from Animal care and use research ethics committee, Bayero University Kano, Nigeria.

Body Weight Measurement

Each rat has weighted before the sacrifice, the weight of the rats was measured by digital weighting balanced (CAMRY, made in China) and express in kilogram (Kg). The weight obtained is used for the subsequent gonadosomatic index express in percentage.

Blood Samples Collection

At the end of five weeks, the rats were anaesthetized using chloroform by inhalation. The blood sample was collected by a cardiac puncture in the morning between 07-10am; 3ml of the blood sample was collected from each rat into EDTA container and centrifuged at 3000 rpm for 30 minutes (relative centrifugal force=504g). The plasma was collected in a plain container and stored at frozen temperature. The plasma was used for testosterone analysis.

Semen Collection

The testicles were exposed by incising tunica vaginalis. The spermatic cords were exposed, ligated and incised as explain by Akusa *et al.,* (1985). Semen sample were collected from the caudal epididymis for semen analysis (sperm count, sperm motility and sperm morphology). The methods of collecting are similar to that employed by Saba *et al.,* (2009).

Determination of Testosterone levels

Plasma concentration of testosterone was determined by ELISA using a commercially available ELISA kit (CALBIOTECH, USA). The testosterone test kit is a solid phase competitive ELISA. The samples, working testosterone-hoarseradish peroxidase (HRP) conjugate and anti-testosterone biotin solution are added to the wells coated with streptavidin. Testosterone in the plasma competes with the testosterone enzyme (HRP) conjugate for binding sites. 50µL of testosterone standards, control and plasma were added into assigned wells. 100µL of working testosterone-enzyme conjugate reagent was added into each well followed by 50µL biotin reagent and mixed gently for 20 seconds. The plate was covered and incubated for 60 minutes at room temperature. The liquid was removed from all wells and washed three times with 300µL of wash buffer. 100µL of tetramethylbenzidine (TMB) substrate was added to all wells and then incubated for 30 minutes at room temperature (27C). 50µL of stop solution was then added to all wells and rocked gently for 20 seconds. The absorbance was read at 450nm after 15 minutes using microplate reader spectrophotometer (LT-1200, USA). The concentration of testosterone in samples was calculated from the calibration curve of the concentration of standards.

Seminal Fluid Analysis

Sperm sample preparation: Sperm sample was taken from the caudal epididymis, caudal epididymis was separated and then placed in a petridish minced and incubated in 15ml tube for 30 minutes at 37°C in 5% CO_2 incubator to allow the sperm to swim in the medium (Swim up technique) (Alaa-fathi *et al.,* 2022).

Sperm volume: The volume of sperm was determined by reading out the volume in a calibrated measuring cylinder (PYREX, made in Spain) while the color was determined by visual assessment.

Sperm count: 10µL of the prepared semen sample was placed on to a clean microscope slide and covered with 22mm×22mm coverslip. The weight of the coverslip spread the sample accessed the freshly made wet preparation as soon as the dilution of the semen required allowing sperm concentration to be measured accurately and estimate from the number of spermatozoa observed in an entire high magnification microscopic field (wet preparation). The fixation for dilution sperm aliquots was an aqueous solution containing 0.595M sodium bicarbonate and approximately 0.14M formalin. The tube containing the diluted sample is mixed for at least 10sec and immediately charged the Neubauer counting chamber, one chamber consists of the 25 large square (five rows of five large squares each). The large square is the counting chamber and is surrounded by triple line and contains 16 small squares (4 rows × 4 small squares) each counting chamber measured 1mm×1mm and has a depth of 0.1mm. The volume of one counting chamber is 0.1µl, counting was performed with ×20 objective lens. The above procedure is the same as that employed by Alaa-fathi *et al.,* (2022).

Sperm motility: To access the motility, the preparations were examined at a final magnification of ×400. The microscope fields were classified as per the world health organization guideline: active (semen applied at the end of the slide, another slide was used to pull the drop of semen along the progressive motile), slowly progressive, sluggish (non-progressively motile) and non-motile (Immotile). The total sum of the proportions (%) of the four (4) categories of motility should be 100% (Alaa-fathi *et al.*, 2022).

Sperm morphology: two smears were prepared from the fresh semen sample in case there will be problems with staining.10µL aliquot of surface of the slide. The slide was allowed to dry in the air and then fixed using ethanol and stained by the use of Papanicolaou staining which gave the best overall visibility of all regions of the spermatozoa. The slide was mounted with cover slip. Two hundred (200) spermatozoa were evaluated to achieve an acceptable low sampling error. The number of normal spermatozoa and abnormalities in the four regions with the aid of a laboratory counter was tallied (Alaa-fathi *et al.*, 2022).

Gonadosomatic Index (GSI)

Gonadosomatic index of each rat was determined by the equation GSI= weight of gonad/body weight×100 (Howaida *et al*, 1998).

STATISTICAL ANALYSIS

Data obtained from this study were analyzed using IBM Statistical Package for Social Sciences SPSS version 25. Variables were tested for normality using Shapiro-Wilk test and Z values of kurtosis and skewness. Quantitative variables were summarized using mean and standard deviation, frequency and percentage were used to summarize qualitative data. Inferential Statistics of analysis of variance (ANOVA) with *post hoc* test of Bonferroni was computed for the determination of statistically significant differences. P values of $\leq$ 0.05 were considered statistically significant.

RESULTS

The result showed no significant difference in GSI (p=0.644) and TBW (p=0.912) between control and experimental groups (Table 1). The table also observed that oral administration of 120mg/kg MSG to experimental group significantly reduced serum testosterone level and total sperm count compared to the normal control (p=0.001).

Oral administration of NSS *at* 50mg/kg, 100mh/kg and 200mg/kg aqueous extract in monosodium glutamate-challenged male Wistar rat significantly increased the level of testosterone and total sperm count compared to experimental control group.

Increase in the level of testosterone and total sperm count were shown to be dose dependent. The level of testosterone and total sperm count in Experimental Group 1 (EG1) ware significantly higher compared to experimental control (EC) which received (120mg/kg MSG) only. Likewise, Experimental Group 2 (EG2) has significantly higher testosterone level and total sperm count compared to experimental group 1 (EG1). Similarly, experimental group 3 (EG3) has significantly higher testosterone level and total sperm count than EG2 (p=0.001) (Table 1).

As depicted in table 2, administration of 120mg/kg MSG reduced the percentage of sperm cells that are motile and increased the percentage of sperm cell that are immotile. However, oral administration of NSS aqueous extract on monosodium glutamate-challenged male Wistar rat at doses of 50mg/kg, 100mg/kg and 200mg/kg body weight increased the percentage of sperm cells that are motile and decreased the percentage of sperm cell that are immotile compared to experimental control.

Administration of NSS aqueous extract at a dose of 50mg/kg body weight, 100mg/kg body weight and 200mg/kg body weight in monosodium glutamate-challenged rat increased the percentage of cell with normal morphology and decrease the number of cell with abnormal morphology compared to experimental control.

Table 1: Effect of NSS Aqueous Extract on WG, GSI, TST and TSC in Monosodium Glutamate-Challenged Male Wistar rats

Variable	Group 1 (CG) n=5	Group 2 (EC) (120mg/kg MSG) n=5	Group 3 (EG 1) (120mg/kg MSG+50mg/kg NSS) n=5	Group 4 (EG 2) (120mg/kg MSG+100mg/kg NSS) n=5	Group 5 (EG 3) (120mg/kg MSG+200mg/kg NSS) n=5	P - value
BWG (g)	186.46±5.38	184.66±12.16	179.82±14.35	193.84±17.32	179.85±3.10	0.912
GSI (%)	0.5±0.03	0.52±0.01	0.51±0.04	0.49±0.01	0.55±0.01	0.664
TST (ng/ml)	6.46±0.43	1.19±0.23[a]	2.41±0.32[b]	3.40±0.32[b,c]	5.02±0.51[b,c,d.]	0.001
TSC	220.20±4.22	155.20±7.53[a]	175.80±4.66[b]	195.00±3.60[b,c]	201.80±4.52[b,c,d]	0.001

Key: CG= Control Group, EC= Experimental Control, EG1=Experimental Group 1, EG2= Experimental Group 2, EG3=Experimental Group 3, MSG=Monosodium Glutamate, NSS= *Nigella Sativa* aqueous extract, BWG= Body Weight, GSI= Gonadosomatic Index, TST=Testosterone, TSC= Total Sperm Count, n= number of Wistar rats per group.

The values were presented as mean ± standard deviation. [a] Significant difference when compared with group 1, [b] Significant difference when compared with group 2, [c] Significant difference when compared with group 3, [d] Significant difference when compared with group 4.

Table 2: Effect of NSS Aqueous Extract on SM and SMP in Monosodium Glutamate-Challenged Wistar rats

Variable	Group 1 (CG) n(5)	Group 2 (EC) (120mg/kg MSG) n(5)	Group 3 (EG 1) (120mg/kg MSG+50mg/kg NSS) n(5)	Group 4 (EG 2) (120mg/kg MSG+100mg/kg NSS) n(5)	Group 5 (EG 3) (120mg/kg MSG+200mg/kg NSS) n(5)
Sperm Motility					
% of motile cells	92.55%	14.26%	70.64%	60.10%	75.02%
% of immotile cells	7.42%	85.73%	29.35%	39.89%	24.97%
Sperm Morphology					
% of normal morphology	96.54%	8.22%	35.09%	36.30%	78.39%
% of abnormal morphology	3.45%	91.77%	65.02%	65.69%	27.84%

Key: CG= Control Group, EC= Experimental Control, EG1=Experimental Group 1, EG2= Experimental Group 2, EG3=Experimental Group 3, MSG=Monosodium Glutamate, NSS= *Nigella Sativa*, n= number of Wistar rats per group

DISCUSSION

The present study demonstrated the protective effect of NSS aqueous extract on the MSG challenged reproductive system in male Wistar rats. This study observed that MSG oral administration did not significantly affect the body weight and GSI of male Wistar rats. This corresponds with findings from the study by Vladmila *et al.,* (2020) who reported that there was no significant difference in body weight and GSI of rats injected with MSG for five consecutive days subcutaneously. It is however, contrary to the study by Nosseir *et al.,* (2012) who reported that two weeks' intraperitoneal injection of MSG resulted in significant increase in the body weight of the rats compared with their age matched control. The difference was probably due to difference in the route of administration of the MSG.

The results from this study showed that oral administration of MSG significantly reduced level of testosterone in male Wistar rats as found in previous researches (EL-sawy *et al.,* 2018; Hanipha *et a.l,* 2018). These studies proposed that it is as a result of oxidative stress on leydig cells. Despite the low oxygen tension that is associated with the testicular microenvironment, studies have shown that the testicular tissue is subjected to oxidative stress due to the availability of highly unsaturated fatty acid in the tissue and hence the presence of potential reactive oxygen species (ROS). ROS is released in the form of O_2 and then converted to H_2O_2, which will react with Fe^{2+} through a fenton reaction that forms OH^- and lipid peroxidation. Lipid peroxidation causes damage to cell membrane especially the content of phospholipids, DNA damage, and protein molecules (Szydlowska & Tymianski, 2010). These can lead to rapture of cell membrane and necrosis, causing a decrease in the number of leydig cells and hence testosterone levels (Turner & Lysiak, 2008).

The study also observed that NSS aqueous extract significantly improved the level of testosterone attenuated by oral administration of MSG in male Wistar rate as found in the study conducted by Abd-Elkareem *et al.*, (2021). This is probably due to antioxidative properties of NSS (Ouattar *et al.*, 2022). *Nigella sativa* contains many anti-oxidant compounds which include thymoquinone Carvacol, T-anethoe and 4-terpineo (Sharieatzadeh *et al.,* 2011).

Thymoquinone is the most active ingredient anti-oxidant properties which directly reduces the production of reactive oxygen species and prevent lipid peroxidation (Burits & Bucar 2000). The extract inhibits the non-enzymatic lipid peroxidation, leading to decrease oxidative stress and protection of the anti-oxidant enzymes of testes (Danladi *et al.,* 2013).
The present study established that oral administration of NSS aqueous extract in monosodium glutamate-challenged male Wistar rat has significant effect of increase in the sperm count that are motile with normal morphology compared to experimental control group (120mg/kg MSG). This is probably due to antioxidative and cytoprotective effect of NSS on spermatozoa as stated in previous studies that it has cytoprotection on other cells (Abd-Elkareem *et al.*, 2021; Abd-Elkareem *et al.*, 2023). The thymoquinone in NSS compounds act as immunodulators and antioxidants by activating antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione-Stransferase (GST) (Mehdi *et a*l. 2020).

CONCLUSION

Oral administration of MSG induced male reproductive system toxicity which protected by oral administration of NSS aqueous extract in male Wister rats. Therefore, consumption of high dose MSG must be avoided because it may cause damage in male reproductive organs and incorporation of NSS to the processed feed could mitigate the cytotoxic effects of MSG.
These finding are of major important in presenting *Nigella sativa* extract as a potent testicular protectant in the field of herbal remedy especially in the light of wide spread utilization of food preservatives and high cost bioactive food preservatives in the food industry.

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