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Attenuating Role of Vitamin C on Sperm Toxicity Induced by Monosodium Glutamate in Albino Rats

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Abstract: The toxicity of monosodium glutamate has been on the increase due to increased patronage of fast foods; with its attendant effects. There have also been attempts to attenuate these effects. Hence, the attenuating role of vitamin C on sperm toxicity induced by monosodium glutamate was accessed on the weight of testes and epididymes, epididymal count, motility, viability, semen pH and sperm head abnormality in albino rat as a model. The male rats were divided into five groups of six rats each. The rats were administered with MSG and vitamin C treatments for 65 days. Vitamin C attenuated the MSG induced toxicity on weight of testes and epididymes, sperm motility, count and sperm head abnormality. Vitamin C can actually attenuate the effect of MSG induced toxicity in rats as a model.

Key words: Monosodium glutamate, sperm count, sperm head abnormality, toxicity

INTRODUCTION

Monosodium Glutamate (MSG), a sodium salt of glutamic acid is one of the main flavor enhancer used in various food products associated with food in Chinese restaurants. The flavor enhancer comes in common names such as Vedan, Sasa, Ajinomoto, Miwan and Weichaun, among others (Geha *et al.*, 2000).

MSG gives a feeling of special taste (Leung and Foster, 1996). It stimulates taste, enhances and improves appetite (Eweka, 2007). In large amount it may cause chest pain sensation, facial pressure, headaches, burning sensation, excessive fluid retention and sweating (Xiong *et al.*, 2009), resulting in a condition called "Chinese restaurant syndrome".

Most Nigerians use MSG to remove stains from fabrics (Kondoh and Torii, 2008). Reports indicated that MSG is toxic to human and experimental animals (Eweka, 2007). Harmful or toxic effects of MSG have been reported in experimental animals and man (Hayes, 1982; Eweka, 2007).

According to Igwebuikwe *et al.* (2011), the oral administration of MSG results in lower serum testosterone levels and reduction in the caudal epididymis sperm reserves of male rats. MSG causes testicular hemorrhage, degeneration and alteration of sperm population and morphology (Oforofuo *et al.*, 1997; Das and Ghosh, 2010), hence could lead to infertility. MSG can severely destroy testicular tissues and affect spermatogenesis also causing a decrease in their testicular weight and testicular diameter, reduction in germinal epithelium height,

decrease in sperm count and increase in abnormalities of sperm morphology (Das and Ghosh, 2010; Nosseir *et al.*, 2012).

Vitamin C is needed for many physiological functions (Halliwell, 2001). According to Nayanatara *et al.* (2008) vitamin C is a natural antioxidant that prevents the increased production of free radicals induced by oxidative damage to lipids and lipoproteins in various cellular compartment and tissues. The protective role of vitamin C against oxidative stress and morphological changes has been reported by Karawya and El-Nahas (2006) and El-Sokkary and Awadalla (2011). Also its protection to testicular tissues indicated by increased testicular weight, increases organ weight, reduces sperm abnormalities and significantly increases sperm count in rats (Fernandes *et al.*, 2011; Al-Amoudi, 2012). Restoration of germinal epithelium and partial protection to the genetic material has also been shown by Karawya and El-Nahas (2006). Nashwa and Venes (2008) also showed the protective effects of vitamin C on the genotoxicity induced by deltamethrin.

In view of above finding, this study set out to ascertain whether vitamin C could attenuate the sperm toxicity induced by MSG in albino rats as model; using short-term *in vivo* assays.

MATERIALS AND METHODS

Chemicals: Emvit-C tablets from Emzor pharmaceutical industries limited, Lagos, Nigeria was used as source of vitamin C. Vedan, a concentrated brand of monosodium

glutamate (about 99.9% pure MSG) was procured from Marian market, Calabar, Nigeria. Other chemicals used in the course of the study were of certified analytical grade.

Animals: Thirty healthy and sexually mature male albino rats of 12 weeks old were used in this study. The rats were obtained from the Experimental Animal Unit of Department of Genetics and Biotechnology, University of Calabar, Calabar. The rats were housed in conventional wire mesh cages under standard laboratory conditions. They were allowed free access to water and pellet feed throughout the period of the experiment. Generally, the study was conducted in accordance with the recommendation from the declarations of Helsinki on guiding principles in care and use of animals.

Experimental design and procedure: The thirty male rats were randomly divided into five groups of six rats each. The animals were acclimatized for one week before the commencement of the study. The treatment lasted for 65 days and the protocol is shown on Table 1. The rats were sacrificed under chloroform anaesthesia 24 hours after the last treatment. The epididymes and testes were dissected out and weighed using Scout Pro SPU 601 electronic weighing balance. The epididymes were processed for epididymal sperm motility, viability, count and sperm head abnormality.

Semen pH and sperm motility: Immediately after dissection, a puncture was made in the epididymis with a sterile pin. The semen smeared on the pin was rubbed on a pH paper of range 4.0-10.0. The colour change corresponds to the pH and was read from the paper. Two drops of sperm suspension was put on a microscope slide and cover slip was placed. The number of progressively motile cells was divided by the total number of spermatozoa counted under $\times 40$ lenses was expressed as a percentage.

Sperm viability: The sperm viability test was determined using "Eosin-Nigrosin one-step staining technique" (Bjordahl *et al.*, 2003). A portion of the sperm suspension was mixed with equal volume of Eosin-Nigrosin stain and five air-dried smears were prepared on

glass slides for each sample. The slides were examined for percentage viability. Normal live sperm cells excluded the stain and appeared whitish, whereas dead sperm cells took up stain and appeared pinkish. Percentage viability was calculated based on the number of live sperm cells out of the total number of sperm cells observed.

Sperm count: The epididymal sperm samples were obtained by macerating known weights of cauda epididymes in physiological saline in the ratio of 1:10 weight by volume. After vigorous pipetting to release the sperm cells. The suspension was filtered using an 80 μm stainless mesh. Epididymal sperm count was obtained by cytometry using the improved Neubauer cytometer and was expressed as million mL^{-1} of suspension (Ekaluo *et al.*, 2008).

Sperm head abnormality test: A portion of the sperm suspension was mixed with 1% eosin Y solution (10:1) for 30 min and air-dried smears were prepared on glass slides for the sperm head abnormality test. The slides were examined for percentage sperm head abnormalities in every 200 spermatozoa observed on each slide and five air-dried smears were prepared on glass slides for each sample. The percentage of sperm head abnormality was calculated according to Ekaluo *et al.* (2009).

Statistical analysis: Data from weight of testes and epididymes, epididymal semen pH, motility, viability, count and sperm head abnormality were subjected to the Analyses of Variance (ANOVA) test while differences in means were separated using least significant difference (LSD) test.

RESULTS

Weight of testes and epididymes: The weight of the testes and epididymes of the rats were significantly ($p < 0.05$) reduced in the MSG treatment groups (MSG, MSG+vitamin C and 2MSG+vitamin C) when compared with 1.24 and 0.46 g, respectively for the testes and epididymes in the control group. The values of the control group were also comparable to that of the vitamin C group and not significantly different ($p > 0.05$). Vitamin C attenuated the effect of MSG toxicity on weight of testes and epididymes of the rats as shown in Table 2. The attenuating effect of vitamin C on the epididymes was in a dose-dependent manner as follows: MSG (0.34) < 2MSG+vitamin C (0.38) < MSG+vitamin C (0.42) < control (0.46) < vitamin C (0.48).

Table 1: Protocol for treatment of experimental animals

Treatment groups	Description of treatment
Control	No vitamin C and no MSG
Vitamin C	Vitamin C, 100 mg kg^{-1} body weight (BW) orally only
MSG	MSG intraperitoneally at the dosage of 2 mg kg^{-1} BW only
MSG+vitamin C	MSG intraperitoneally at the dosage of 2 mg kg^{-1} BW and vitamin C, 100 mg kg^{-1} BW orally
2MSG+vitamin C	MSG intraperitoneally at the dosage of 4 mg kg^{-1} BW and vitamin C, 100 mg kg^{-1} BW orally

Table 2: Effect of vitamin C on sperm parameters of rats with MSG-induced toxicity

Parameters	Treatment groups				
	Control	Vitamin C	MSG	MSG+Vitamin C	2MSG+Vitamin C
Weight of testes (g)	1.24±0.03 ^a	1.26±0.02 ^a	1.16±0.03 ^b	1.12±0.02 ^b	1.04±0.03 ^c
Weight of Epididymes (g)	0.46±0.02 ^a	0.48±0.03 ^a	0.34±0.03 ^c	0.42±0.02 ^b	0.38±0.02 ^d
pH of semen	7.00±0.03 ^a	6.91±0.03 ^a	6.88±0.03 ^a	6.88±0.03 ^a	6.92±0.03 ^a
Sperm count (×10 ⁶ mL ⁻¹)	7.63±0.03 ^b	8.92±0.39 ^a	5.69±0.02 ^d	6.86±0.13 ^c	6.19±0.04 ^d
Sperm head abnormality (%)	3.60±0.20 ^d	3.52±0.30 ^d	5.54±0.30 ^a	3.75±0.20 ^e	4.47±0.20 ^b
Sperm motility (%)	66.15±0.50 ^b	79.25±0.70 ^a	55.35±0.70 ^e	63.25±0.70 ^e	60.55±0.70 ^d
Sperm viability (%)	79.10±0.40 ^a	79.30±0.40 ^a	76.39±0.50 ^a	78.20±0.50 ^a	78.45±0.50 ^a

Values across the table with similar superscripts are not significantly different at 5% based on ANOVA, MSG: MSG at 2 mg kg⁻¹ BW, 2MSG: MSG at 4 mg kg⁻¹ BW, Vitamin C: Vitamin C at 100 mg kg⁻¹ BW

Semen pH and sperm viability: The semen pH and sperm viability was not significantly ($p>0.05$) affected by MSG and vitamin C treatments shown in Table 2.

Sperm motility: The sperm motility was significantly ($p<0.05$) reduced in the MSG treatment groups (MSG, MSG+vitamin C and 2MSG+vitamin C) when compared with the control (66.16%) and vitamin C (79.25%) groups. The attenuating effect of vitamin C on MSG induced toxicity was also seen on the motility of the sperm cells was also in a dose-dependent manner as follows: MSG (55.35%)<2MSG+vitamin C (60.55%)<MSG+vitamin C (63.25%).

Sperm count: The sperm count was significantly ($p<0.05$) reduced in the MSG treatment groups (MSG, MSG+vitamin C and 2MSG+vitamin C) when compared with 7.63×10^6 mL⁻¹ in the control group. Vitamin C showed attenuating effect on MSG induced toxicity on sperm count by increasing the sperm count from 5.69 to 6.86×10^6 mL⁻¹ as follows: MSG<2MSG+vitamin C<MSG+vitamin C<control; as shown in Table 2.

Sperm head abnormality: The sperm head abnormality was also attenuated by vitamin C with significant ($p<0.05$) reduction from 5.54% in MSG to 3.75% in MSG+vitamin C; compared with 3.60% in the control group.

DISCUSSION

The rats in MSG treatment groups (MSG, MSG+vitamin C and 2MSG+vitamin C) showed significant ($p<0.05$) reduction in weight of the testes and epididymes, sperm count and motility; as well as increase in sperm head abnormalities agree with (Oforofuo *et al.*, 1997; Das and Ghosh, 2010; Igwebuikwe *et al.*, 2011; Nosseir *et al.*, 2012). Vitamin C attenuated the effect of MSG toxicity on weight of testes and epididymes of the rats by increasing their weights over that of the group treated with MSG only. This increases in weight of the testes and epididymes agree with the reports of

(Fernandes *et al.*, 2011; Al-Amoudi, 2012); which can be attributed to protective role of vitamin C against oxidative stress and morphological changes (Karawya and El-Nahas, 2006; Nayanatara *et al.*, 2008; El-Sokkary and Awadalla, 2011). The attenuating effect of vitamin C on MSG induced toxicity on sperm count and motility as well as sperm head abnormality could also be attributed to the protective role of vitamin C which agrees with earlier reports of (Karawya and El-Nahas, 2006; Nashwa and Venes, 2008; Nayanatara *et al.*, 2008; El-Sokkary and Awadalla, 2011; Al-Amoudi, 2012).

CONCLUSION

This study shows that vitamin C administered orally at 100 mg kg⁻¹ BW actually attenuate the effect of MSG (2 and 4 mg kg⁻¹ BW) induced toxicity on weight of testes and epididymes, sperm motility, sperm count and sperm head abnormality in rats as a model in dose-dependent manner.

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