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Cytoprotective Effects of *Nigella sativa* Seeds on Monosodium Glutamate Induced Seminal Vesicle Damages: Histological and Immunohistochemical Studies

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Abstract

Monosodium glutamate (MSG) is a worldwide food flavour enhancer commonly used by the food industry. This feed additive may cause male infertility. *Nigella sativa* seeds (NSS) is a widely used in herbal medicine as it has many biological benefits and could provide a solution. This work was designed to investigate the histological effects of NSS on rats ingesting MSG. To achieve this aim, adult male albino rats (2- 3 months old) were randomly and equally assigned into three experimental groups. For a period of 21 days, control group received no treatment, MSG group received MSG as 30 g/kg feed, and MSG + NSS group received MSG as 30 g/kg feed and NSS as 30 g/kg feed. Seminal vesicle histopathology in MSG group showed mild seminal vesiculitis with degeneration of smooth muscle fibers in tunica muscularis. In addition, there was an increase in the amount of connective tissue and apoptotic cells count. Periodic Acid Schiff stain indicated irregular and interrupted epithelial basement membranes. Glutathione reductase (GR), superoxide dismutase 2 (SOD2), and caspase-3 immuno-expressions increased in MSG group. It was found that there was an increase in the number of apoptotic cells, intraepithelial lymphocytes and dendritic cells in MSG group. However, treatment with NSS ameliorated these disturbances. NSS mitigated MSG-induced seminal vesicle damage by its histoprotective, cytoprotective and anti-apoptotic activities.

KEYWORDS
Monosodium glutamate, *Nigella sativa* seeds, Seminal vesicle, Apoptotic cells, Seminal vesiculitis, Dendritic cells, Lymphocytes.

INTRODUCTION

Monosodium Glutamate (MSG) is one of the most commonly used worldwide food flavor enhancer in commercial foods industry. It has a distinctive taste which called "Umami" in Japanese or "Xien Wei" in Chinese or "savory, "broth-like" or "meaty taste" in English (Airaodion *et al*., 2019). In several countries MSG goes by the name "China salt". Although its application had increased over time and it is found in many different ingredients and processed foods, MSG has been associated with various forms of toxicity (Niaz *et al*., 2018). MSG induced male reproductive disturbance (Jubaidi *et al*., 2019; Kayode *et al*., 2020). It induced oxidative, hormonal, histoarchitectural disturbances in rat testis (Alalwani, 2014; Hamza andand AL-Harbi, 2014; Abd-Elkareem *et al*., 2021). Also it induced oxidative stress in accessory reproductive organs (Abu Hanipah *et al*., 2018). Literature showed that MSG was associated with adverse side-effects in other organs particularly in liver (Abd-Elkareem *et al*., 2022b), Kidney (Abd-Elkareem *et al*., 2022a), ovary (Miśkowiak *et al*., 1999; Das and Ghosh, 2011), uterus (Abdulghani *et al*., 2022) and brain (El-Shobaki *et al*., 2016; Farhat, 2021).

The *Nigella sativa* seed (NSS) is commonly known as black seed or black cumin (*Ranunculaceae*). It used in herbal medicine all over the world for the treatment and prevention of many diseases and toxic conditions (Ali and Blunden, 2003). In many literatures, NSS considered as one of the greatest forms of healing medicine. It has been used on regular basis in Tibb-e-Nabwi (Prophetic Medicine). It has been widely used as anti-diarrheal, antihypertensive, diuretics, appetite stimulant, analgesics, anti-bacterial, antidiabetic, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepato-protective, renal protective, gastro-protective, antioxidant and antiapoptotic properties, etc. (Ahmad *et al*., 2013; Abd-Elkareem *et al*., 2021; 2022a; 2022b). NSS had been placed among the top ranked herbal medicines as it has miraculous power of healing. Much of the biological activity of the NSS had been shown to be due to its active ingredient; thymoquinone (Ahmad *et al*., 2013)

Seminal vesicle plays an important role in male fertility. Its secretion is essential for semen coagulation, sperm motility, sperm capacitation, stability of sperm chromatin and suppression of the immune response in the female reproductive tract (Pang *et al*., 1979; Peitz and Olds-Clarke, 1986; Clavert *et al*., 1990; Gonzales, 2001; Balu *et al*., 2022). Seminal vesicle was reported to be sensitive to MSG which may lead to impairment to its functions (Iamsaard *et al*., 2014) that might be cause male infertility especially in long term use. Therefore, the aim of the current study was to investigate the *in vivo* toxic effects of MSG on seminal vesicle of rat and to explore the possible protective effect of NSS.

MATERIALS AND METHODS

Treatments

MSG powder was obtained in a sealed bottle from Morgan Chemical Industry, Egypt (purity 99%). NSS were obtained in a

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sealed bottle from Imtenan Health Shop Company, Obour City, Egypt. The chemical constituents of NSS were determined using gas chromatography/mass spectrophotometry in our previous study (Abd-Elkareem *et al*., 2021). All studies of NSS were carried out in accordance with the relevant institutional, national, and international guidelines and regulations.

Animals and experimental design

Eighteen healthy adult male albino rats aged 2–3 months (237.0±32.0 g in weight) were used in this study. Rats were obtained from the Animal House of Faculty of Medicine, Assiut University, Assiut, Egypt. Animals were maintained in metal cages at room temperature with 12 h light: 12 h dark schedule during the period of the experiment. Water and food were allowed ad libitum. The experimental protocol was approved by the Local Ethical Committee and by the Institutional Review Board of Faculty of Medicine, Assiut University (Approval Number: 17300469) and was carried out in accordance with relevant guidelines and regulations. This research was done in compliance with the ARRIVE guidelines and regulations (https://arriveguidelines.org). Rats were randomly and equally divided into three groups, after 1 week of acclimatization. The control group received no treatment, the monosodium glutamate (MSG) group administrated MSG at a concentration of 30 g/kg feed, it was thoroughly mixed with the ration and supplied for 21 days. MSG + NSS group supplemented with MSG at the same previous concentration together with NSS at a concentration of 30 g/kg for the same period as our previously published work (Abd-Elkareem *et al*., 2021).

Sample collection

At the end of the experiment, rats were euthanized by cervical dislocation for tissue specimen collection. The seminal vesicles were fixed in 10% neutral buffered formalin, and then embedded in paraffin.

Histological examination

Transverse serial sections of 5 µm thickness were stained with the hematoxylin and eosin (H and E), Crossmon's trichrome technique, and Periodic Acid Schiff (PAS). Then seminal vesicle tissues were examined by the light microscope.

Flourscent microscopic analysis of acridine orange stain to detect apoptotic cells (Kasibhatla *et al*., 2006; Abd-Elkareem *et al*., 2020).

Negative image analysis was performed to assess the complex color micrographs that were obtained and to give more details (Gross *et al*., 2010; Abd-Elkareem, 2017).

Immunohistochemical detection of apoptosis by caspase-3

For immunohistochemical detection of caspase-3, we used rabbit polyclonal antibody against caspase-3, marker for apoptosis (Abcam, Cambridge, Massachusetts, USA; 1:1000 dilution) and Avidin–Biotin-Peroxidase technique (Bressenot *et al*., 2009).

Immunohistochemical detection of oxidative stress by glutathione reductase (GR) and superoxide dismutase 2 (SOD2)

Immunohistochemical staining of GR and SOD2 in the seminal vesicle were done by using rabbit polyclonal anti-glutathione reductase and anti-superoxide dismutase antibodies, respectively (Chongqing Biospes Co., Ltd, China) and Power-Stain™ 1.0 Poly horseradish peroxidase (HRP) DAB Kit (Genemed Biotechnologies, Inc, 458 Carlton Ct. South San Francisco, CA 94080, USA).

The protocol we used in immunohistochemistry was as the previously published protocol (Abd-Elkareem *et al*., 2021).

All staining preparations were examined with an Olympus BX51 microscope, and photos were taken by an Olympus DP72 camera attached to the microscope (Sayed *et al*., 2019).

RESULTS

The histological organization of seminal vesicle in control group revealed that the mucosa showed thin, branched, anastomosing folds. The lamina epithelialis was formed of columnar epithelium while the lamina propria was formed of loose connective tissue. Tunica muscularis was formed of smooth muscle fibers whereas the adventitia was composed of loose connective tissue. The small lumina and the large central collecting sinus or cavity were communicated and contained homogenous acidophilic secretion (Fig. 1: A, D and G).

The histopathological examination of the seminal vesicle in MSG group showed mild seminal vesiculitis with degenerated muscular layer, congested blood capillaries and leucocytic infiltration in the lamina propria. Apoptotic columnar cells were observed in lamina epithelialis and in some cases the columnar cells became pseudostratified. Also, the seminal vesicle secretion became heterogeneous and violet in color. Proliferation of telocytes and collagen fibers (fibrosis) were also found in MSG group (Fig. 1: B, E and H). While the microscopical examination of the seminal vesicle in MSG + NSS group showed that the architecture of the seminal vesicle was retained to normal. It was formed of folded mucosa consisted of lamina epithelialis of columnar epithelium and lamina propria of loose connective tissue. The muscularis layer was formed of healthy smooth muscle fibers. The lumina were contained homogenous acidophilic secretion (Fig. 1: C, F and I). Herein we found that the number of intraepithelial and interstitial lymphocytes and dendritic cells was increased in MSG group (Fig. 2: B and E) compared to control (Fig. 2: A and D) and MSG + NSS group (Fig. 2: C and F).

The PAS histochemical examination of seminal vesicle revealed that the seminal vesicle in control group had regular continued PAS positive basement membrane and PAS positive blebs indicated apocrine secretion (Fig. 3A). While in MSG group the seminal vesicle had irregular interrupted PAS positive basement membrane (Fig. 3B). Whereas in MSG + NSS group the seminal vesicle had regular continued PAS positive basement membrane and PAS positive blebs (Fig. 3C).

Crossmon's trichrome technique was used to examine the collagen fibers distribution in different treatment groups. We found that the seminal vesicle in control group showed normal peritubular collagen fibers distribution (Fig. 3D). However, the seminal vesicle in MSG group showed increased in the peritubular collagen fibers (Fig. 3E). While the seminal vesicle in MSG + NSS group showed normal peritubular collagen fibers distribution (Fig. 3F). Fig. 3G-I: negative image for (Fig. 3D-F) images to assess the complex color micrographs and to give more details of the collagen fibers distribution. We also observed that the seminal vesicle in control group had normal collagen fibers distribution in the lamina propria and tunica mucularis (Fig. 4A). Whereas the seminal vesicle in MSG group showed increased in collagen fibers distribution in the lamina propria and tunica mucularis (Fig. 4B). While the seminal vesicle in MSG + NSS group showed normal collagen fibers distribution in the lamina propria and tunica mucularis (Fig. 4C).

To assess the anti-oxidative potential of NSS against MSG im-

muoexpression of GR and SOD2 was performed. This revealed an increased immuoexpression of GR and SOD2 in the epithelium of MSG group (Fig. 5: B and E) compared to control (Fig. 5: A and D) and MSG + NSS (Fig. 5: C and F) groups. While to assess the anti-apoptotic potential of NSS against MSG immuoexpression of Caspase-3 was performed. We found an increased immuostaining of Caspase-3 in the smooth muscle fibers of tunica muscularis of MSG group (Fig. 5H) compared to control (Fig. 5G) and MSG

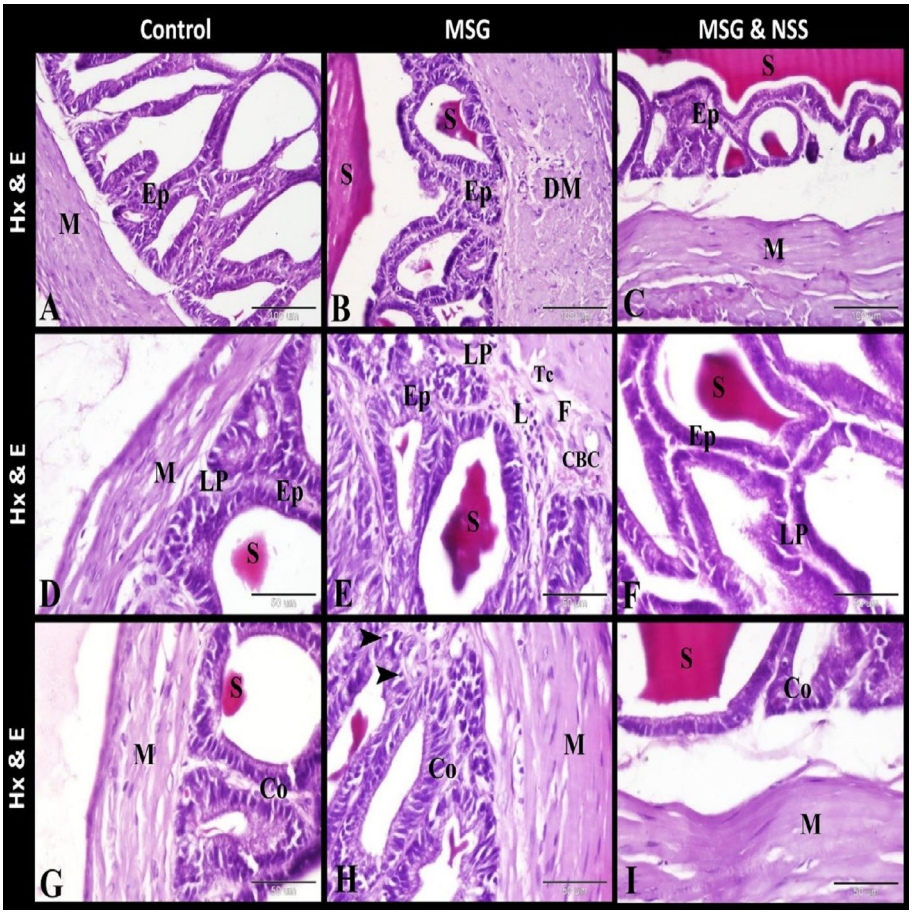

Fig. 1. Photomicrograph of paraffin sections in the rats' seminal vesicles showed the cytoprotective effect of NSS against MSG induced seminal vesicle damages. Control group (A, D and G) showed the normal histology of the seminal glands which formed of folded mucosa consisted of lamina epithelialis (Ep) of columnar epithelium (Co) and lamina propria (LP) of loose connective tissue. Muscularis layer (M) formed of smooth muscle fibers. Note the homogenous acidophilic secretion (S). MSG group (B, E and H) showed degenerated muscular layer (DM) with some degree of fibrosis (F) and congested blood capillaries (CBC), leucocytic infiltration (L) in lamina propria (LP), apoptotic columnar cells (arrowheads) in lamina epithelialis (Ep), proliferation of telocytes (Tc). Note the columnar cells became pseudostratified (Co) and the secretion (S) became heterogeneous and violet in color. MSG + NSS group (C, F and I) showed that the architecture of the seminal vesicle was retained to normal. It was formed of folded mucosa consisted of lamina epithelialis (Ep) of columnar epithelium (Co) and lamina propria (LP) of loose connective tissue. Muscularis layer (M) was formed of healthy smooth muscle fibers. Note the homogenous acidophilic secretion (S). Original magnification; (A, B and C): 200X, scale bar 100 µm; (D-L): 400X, scale bar 50 µm, Hematoxylin and Eosin stain.

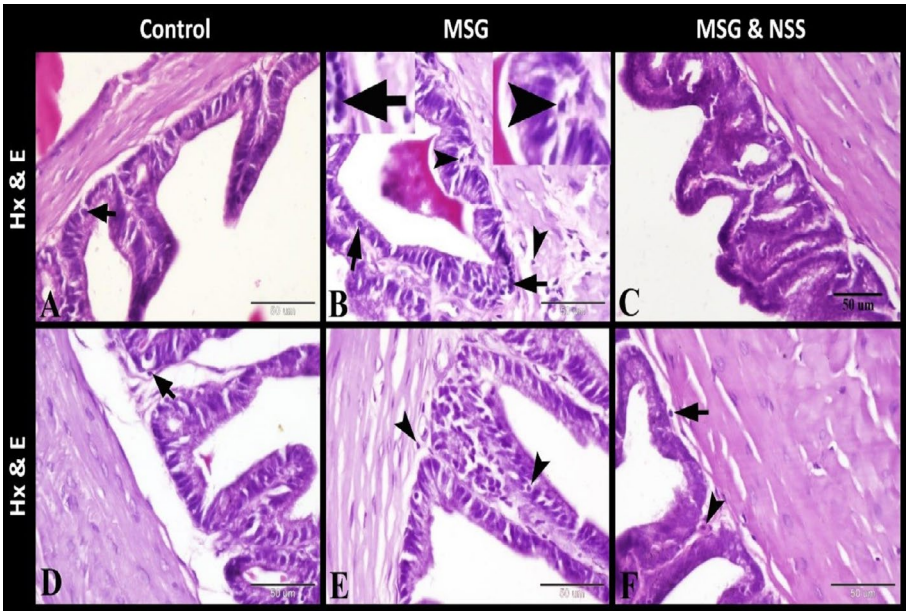

Fig. 2. Photomicrograph of paraffin sections in the rats' seminal vesicles showed the cytoprotective effect of NSS on MSG induced seminal vesicle damages. Note the increased number of intraepithelial and interstitial lymphocytes (arrows) and dendritic cells (arrowheads) in MSG group (B and E) compared to control group (A and D) and MSG + NSS group (C and F). Original magnification: 400X, scale bar 50 µm, Hematoxylin and Eosin stain.

+ NSS (Fig. 5I) groups. Therefore, suggesting a promising anti-oxidant and anti-apoptotic effects of NSS against MSG toxicity.

Further assessment of NSS protective effect was performed through evaluation of apoptosis by fluorescent acridine orang stain. Our findings showed increased in the numbers of apoptotic epithelial cells and smooth muscle fibers in the epithelial and muscular layers respectively in MSG group (Fig. 6B) compared to control (Fig. 6A) and MSG + NSS group (Fig. 6C). The apoptotic nuclei stained with acridine orange appeared as orange spots under a fluorescence microscope.

DISCUSSION

The histological organization of seminal vesicle in control group revealed normal histological structure as described previously (Creasy *et al.*, 2012;Zhang *et al.*, 2019; Maghraoui *et al.*, 2022).

On contrary, histopathological examination of the seminal vesicle in MSG group showed mild seminal vesiculitis. This seminal vesiculitis was characterized by the presence of degeneration of the muscular layer, congestion of blood capillaries and leucocytic infiltration in the lamina propria (Zhang *et al.*, 2019). The importance of seminal vesicle damage is the possibility of

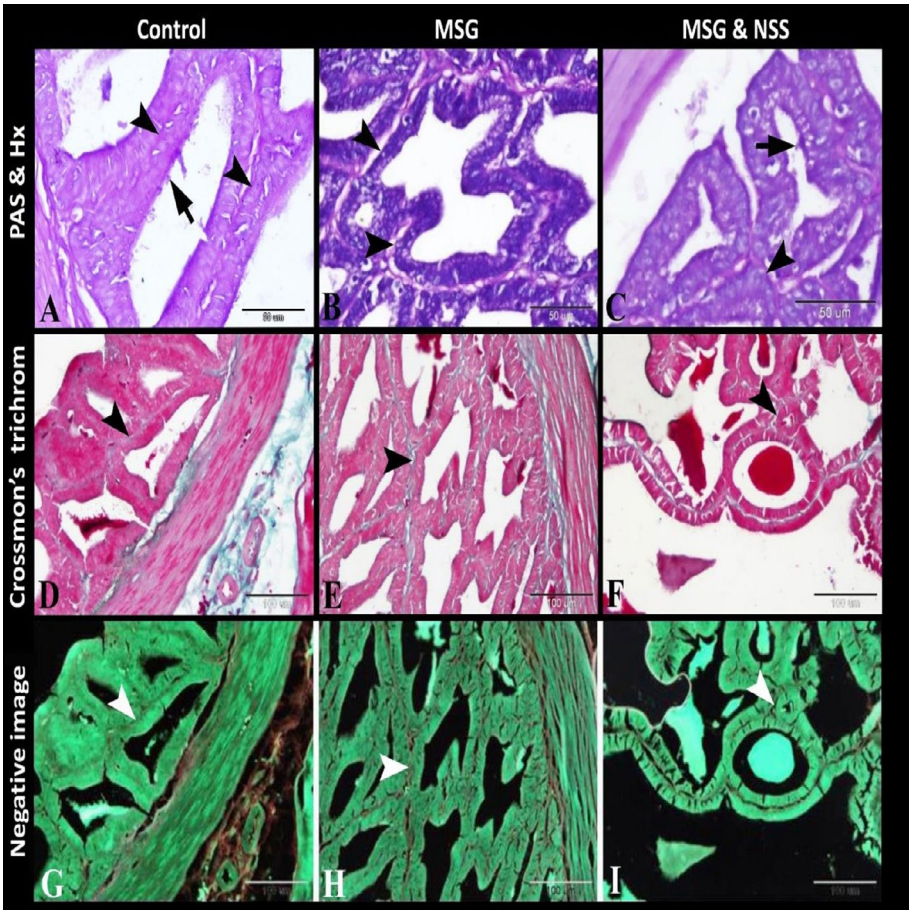

Fig. 3. Photomicrograph of paraffin sections in the rats' seminal vesicles showed the cytoprotective effect of NSS on MSG induced seminal vesicle damages. Control group (A) showed the seminal vesicle with regular continued PAS positive basement membrane (arrowheads) and PAS positive blebs (arrow). MSG group (B) showed the seminal vesicle with irregular interrupted PAS positive basement membrane (arrowhead). MSG + NSS group (C) showed the seminal vesicle with regular continued PAS positive basement membrane (arrowhead) and PAS positive blebs (arrow). Control group (D) showed the seminal vesicle with normal peritubular collagen fibers (arrowhead). MSG group (E) showed the seminal vesicle with increased peritubular collagen fibers (arrowhead). MSG + NSS group (F) showed the seminal vesicle with normal peritubular collagen fibers (arrowhead). G-I: negative image for (D-F) images to assess the complex color micrographs and to give more details. Original magnification; (A-C): 400X, scale bar 50 µm, periodic acid-Schiff (PAS) and hematoxylin, (D-F): 200X, scale bar 100 µm, Crossmon's trichrome technique.

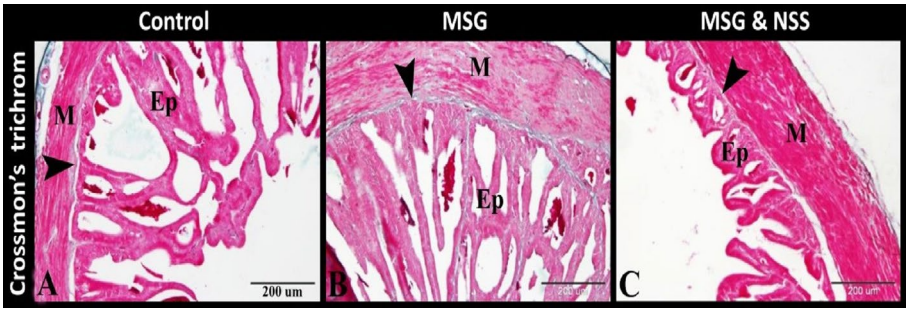

Fig. 4. Photomicrograph of paraffin sections in the rats' seminal vesicles showed the cytoprotective effect of NSS on MSG induced seminal vesicle damages. Control group (A) showed the seminal vesicle with normal collagen fibers (arrowhead) in lamina propria under the epithelium (Ep) and mucularis (M). MSG group in (B) showed the seminal vesicle with increased collagen fibers (arrowhead) in lamina propria under the epithelium (Ep) and mucularis (M). MSG + NSS group in (D) showed the seminal vesicle with normal collagen fibers (arrowhead) in lamina propria under the epithelium (Ep) and mucularis (M). Note the loose connective tissue of the adventitia. (A-D): 100X, scale bar 200 µm, Crossmon's trichrome technique.

impairment of sperms function and the cause of male infertility through multiple pathophysiological mechanisms, such as oxidative stress, and proinflammatory cytokines (Castiglione *et al*., 2014; Zhang *et al*., 2019)

The columnar epithelial cells of seminal gland are concerned with intense protein synthesis. The viscous, gel-like nature of the vesicular glands secretion and formation of copulatory plug at the time of mating are products of complex biochemical interaction that involves the coagulation of a specific protein produced by the seminal vesicles (Adebayo *et al*., 2014). These seminal proteins interact with the sperms leading to changes in their physiology to facilitate capacitation for the fertilization to occur (Aumüller and Riva, 1992; Clavert *et al*., 1990; Balu *et al*., 2022). In addition to secretion of the seminal fluid, epithelial cells of seminal gland are capable of reabsorption of fluids or dissolved substances, performing spermatophagy (ingestion and degradation of damaged spermatozoa), modification of sperm functions (motility, capacitation), and immunosuppression within the female genital system (Aumüller and Riva, 1992). Seminal vesicles secretions have a high concentration of prostaglandins which have antibacterial function in the male genital tract and can modify the contraction of the smooth muscle (Clavert *et al*., 1990).

Moreover, 60-70 % of the ejaculate was formed of seminal vesicles secretion. It is a strongly acidophilic proteinous secretion secreted by the columnar epithelial cells and present in the lumen and central collecting sinus. It has many properties and components that are important for semen function and sperm survival. It is slightly alkaline, to increases the pH of the semen as a whole and counteracts the acidic environment of the vagina. It contains fructose which provides an energy source for sperms. It also contains other constituents, such as proteins, enzymes, vitamin C, flavins and prostaglandins. Semenogelin is an important seminal vesical protein which forms a gel-like matrix that preventing immediate capacitation by coating ejaculated spermatozoa (Creasy *et al*., 2012; McKay *et al*., 2022). We observed that the seminal vesicles secretion in MSG became heterogeneous and violet in color and incorporation of NSS in feed could retain this change to normal. Thus, indicating that NSS could protect the glandular function of seminal vesicles.

The PAS histochemical examination of seminal vesicles revealed that the seminal vesicles in MSG group had irregular interrupted PAS positive basement membrane and decrease in the PAS positive blebs indicated decrease in apocrine activity of the gland. While NSS could retain the changes to normal state comparable to control.

Carbohydrates in seminal vesicles secretion are essential for many functions. Fructose which is a major saccharide present in seminal plasma is synthesized from blood glucose by epithelial cells of seminal vesicles stimulated by testosterone. It plays an important role in production of ATP for sperms (Tsukise and Yamada, 1982). Glucose, another carbohydrates in seminal vesicles has been reported to be beneficial to human sperm for optimal motility, capacitation and fertilization, (Sánchez-Partida *et al*., 1999; Williams and Ford, 2001). Fibronectin which is an extracellular matrix protein, is secreted by apocrine activity from the seminal vesicles and participates in the formation of the seminal clot (Aumüller *et al*., 1997). Collectively, the main functions of the seminal glands are formation of the sperm coagulum, regulation of sperm motility and capacitation and the suppression of immune function in the female genital tract.

There are several studies that explored the negative effects of MSG on different parts of the hypothalamic-pituitary-gonadal (HPG) axis. This may be due to the ability of MSG to inhibit secretion of many reproductive neuropeptides, neurotrophic factors, and hormones such as GnRH, LH and testosterone, all of which are regulated through the interaction between the hypothala-

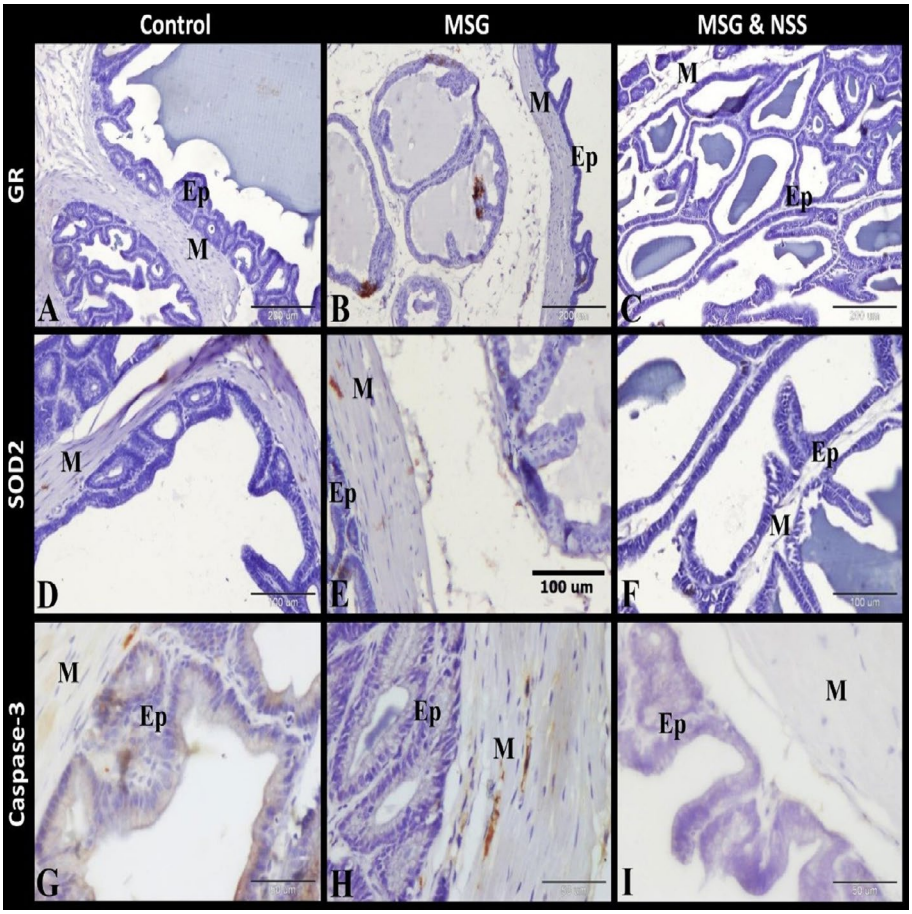

Fig. 5. Photomicrograph of GR (A, B and C), SOD2 (D, E and F) and caspase-3 (G, H and I) immunostaining in the rats' seminal vesicles showed the cytoprotective effect of NSS on MSG induced seminal vesicle damages. Note the immuoexpression of GR and SOD2 in the epithelium of MSG group (B and E respectively) compared to control (A and D respectively) and MSG + NSS (C and F respectively) groups. Also note the immuoexpression of Caspase-3 in the smooth muscle fibers of tunica muscularis of MSG group (H) compared to control (G) and MSG + NSS (I) groups. Ep = Epithelium, M = Muscularis. Original magnification; (A, B and C): 100X, scale bar = 200 µm; (D, E and F): 200X, scale bar = 100, (G, H and I): 400X, scale bar 50 µm.

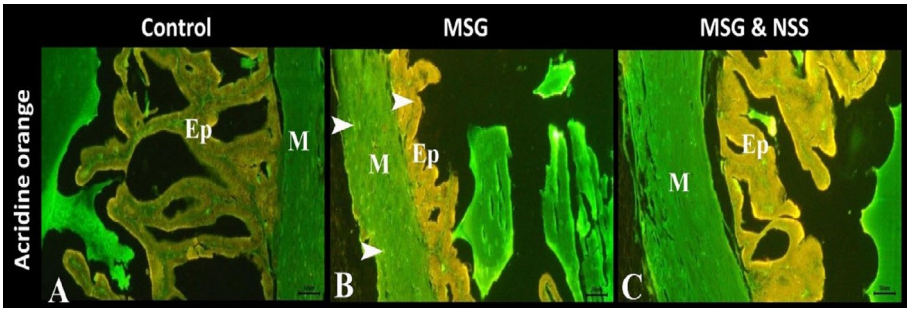

Fig. 6. Fluorescence photomicrograph of paraffin-embedded sections in the rats' seminal vesicles illustrating the anti-apoptotic effect of NSS on MSG induced seminal vesicle damages. Note the increased in the numbers of apoptotic epithelial cells and smooth muscle fibers (arrowheads) in the epithelial and muscular layers respectively in MSG group (B) compared to control (A) and MSG + NSS group (C). The apoptotic nuclei stained with acridine orange appeared as orange spots under a fluorescence microscope. Scale bar = 50 µm, acridine orange stain.

mus and pituitary gland and play a key roles in the regulation of reproductive function (Sun *et al*., 1991; Ochiogu *et al*., 2015; Abd-Elkareem *et al*., 2021; Haddad *et al*., 2021). Another explanation for the reduction of reproductive hormone and factors output may be due to increased damage to GnRH Neurons and apoptotic cell death in Leydig cell by the MSG induced free radicals generations (Abd-Elkareem *et al*., 2021; Wang *et al*., 2021). Whereas NSS addition to food of treated rats normalized this hormonal profile by its anti-apoptotic and anti-oxidants bioactive constituents (Abd-Elkareem *et al*., 2021).

Seminal vesicles depend on androgen-driven stromal-epithelial interactions for normal development, structure, and function (Welsh *et al*., 2010). Healthy smooth muscle fibers play a vital role in this interaction. The main function of the seminal vesicles is to synthesize proteins of the seminal plasma and this is also androgen dependent (Welsh *et al*., 2010). Damage or apoptosis of smooth muscle of the seminal vesicles might impair its secretory function and alters its responsiveness to testosterone.

In this study, an increase in the numbers of apoptotic epithelial cells and smooth muscle fibers in MSG group was demonstrated and this may be attributed to direct effect of MSG on proapoptotic gene and free radicals generation (Abu Hanipah *et al*., 2018; Abd-Elkareem *et al*., 2021) or as a result of reduction in testosterone level (Welsh *et al*., 2010). This could suggest that MSG might have affected the secretary function of the seminal vesicles of rats received MSG by either direct damage of the smooth muscles layer or by altering its responsiveness to testosterone. In another study, MSG showed high-affinity for acetylcholine receptors which disrupted the normal nerve signal (Abdulghani *et al*., 2022). This potentially could mean that MSG as cholinergic neurotransmitter acting through muscarinic receptors in the epithelial and smooth muscle layers may be a key factor in controlling protein secretion and contractility of seminal vesicles respectively (Hamamura *et al*., 2006). Also, MSG was found to slightly affect the rat hypothalamic centers involved in regulation of the pituitary-thyroid axis (Miśkowiak and Partyka, 1993). Hypo and hyperthyroidism have always linked to low semen volume due to reduction in seminal vesicles secretions(La Vignera and Vita, 2018). By looking at the presented evidence and similar work done in this matter, it clear that the ability of MSG to impair male reproductive system and especially seminal vesicles is manifested through complex separate or combined mode of actions. This could be summarized into that MSG might potentially affect hypothalamus centers and its communication with pituitary gland, disrupt the contractility function of the smooth muscle layer of seminal vesicles and make it less responsive to testosterone. Therefore, possibly resulting in the change of the color of the seminal vesicles glandular secretions. Interestingly, the addition of NSS might return these changes to normal state.

Herein we found that the number of intraepithelial and interstitial lymphocytes and dendritic cells was increased in MSG group compared to control group and MSG + NSS group. The presence of an intraglandular immune cells such as mast cells, T lymphocytes, and macrophages dendritic cells may be involved in the maintenance of male reproductive tract sterility and in the prevention of autoimmune reactions (Wang and Duan, 2016; Abd-Elhafeez *et al*., 2021). Intraepithelial leucocytes exhibit various cytotoxic activities as an immune surveillance or first line of defense. When they are activated they may secrete a variety of cytokines which facilitate B cells function and regulate epithelial cell function (Beagley and Husband, 1998; Cheroutre *et al*., 2011).

Our results also revealed that there was an increase in oxidative stress markers; GR and SOD2 in the epithelium of MSG group compared to control and MSG + NSS groups. The antioxidant effect of NSS plays an essential role in protecting against the toxic effects of MSG. This achieved by ameliorating glutathione redox cycle to block lipid peroxidation, activation and up-regulation of expression of enzymatic antioxidants genes and scavenging free radical by thymoquinone (Abd-Elkareem *et al*., 2021). The protective cytological modifications found in MSG + NSS groups as shown by decrease of immuno-expression of GR and SOD2 in the epithelial cells following dietary inclusion of NSS supports the antioxidant effects of NSS.

CONCLUSION

The consumption of high dose MSG must be avoided because it may cause potential damage in male reproductive organs and incorporation of NSS to the processed feed could mitigates the cytotoxic effects of MSG.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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