DEGENERATIVE ASPECTS OBSERVED IN SEMINIFEROUS TUBULES IN QUAILS (*COTURNIX JAPONICA*) TESTIS AFTER MONOSODIUM GLUTAMATE ADMINISTRATION IN FEED

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Abstract

Sodium glutamate, an amino acid used as a flavor enhancer, is a controversial substance being incriminated in the appearance of obesity in human populations among others. Its administration in animal feed increases weight gain, but side effects such as decreased fertility and degenerative changes in liver and kidney have been observed. To study the effects of monosodium glutamate toxicity on the male reproductive system, one hundred thirty-four Japanese quails (Coturnix japonica) were divided into 4 groups. Monosodium glutamate was admninistrated for 30 days in different concentrations in 3 groups: 10g/kg feed for group I, 30g/kg feed for group II, 50 g/kg feed for group III. For the individuals in group II and III, was observed the reduction in the number of germline and sperm cells compared to the control group, the presence of degenerative Sertoli cells and spermatic cells with pyknotic nuclei, the presence of vacuoles among seminiferous tubule cells. The degenerative, necrotic and inflammatory changes observed demonstrate the toxic effects of glutamate on male reproductive system and on fertility.

Keywords*: Monosodium glutamate, quail, testis degeneration*

Introduction

Sodium monoglutamate is a substance used in the food industry as a flavor enhancer. On the Asian continent it has been used for hundreds of years in food, being extracted from the *Laminaria japonica* algae. Being widely used, numerous research has been undertaken to study its effect on the digestive system, but also on other systems and organs.

Glutamate is one of the most widespread amino acids in nature, being also the most abundant amino acid in human breast milk (Davis et al., 1994; Singh et al., 2004). Administration in feed caused significant development of the jejunum villi resulting in better assimilation of nutrients and increasing breast weight in quails (Salmanzadeh and Shahryar, 2013). Glutamate is also the major neurotransmitter of excitation in the central nervous system, and is also a molecule by which cells from non-nerve tissues communicate with each other (Mallick N., 2007; Watkins J.C., 2000; Meldrum B.S., 2000). Studies have also shown the implication of glutamate in neurodegeneration processes (Hynd at al., 2004; Dong et al., 2009) and was detected in high levels in individuals with autism (Shinohe et al., 2006).

The presence of glutamate was noticed in non-nerve cells, thus being detected in various organs and structures including endocrine glands, stomach, intestine, testis (Hayashi et al., 2003).

Studies conducted on male rats have indicated that monoglutamate sodium is involved in male infertility cases and can also cause testicular bleeding, degeneration, and altered morphology of the spermatozoa population (Oforofuo et al., 1997). Thus, oligospermia was observed and along with it an increased number of the spermatozoa with abnormal morphology. The studies showed that the intensity of the changes is directly proportional to the administered dose (Onakewhor et al., 1998).

Administration of monosodium glutamate simultaneous with arginine caused changes in rat testis such as inflammatory, degenerative, and necrotic lesions. Oedema in interstitial tissue in testis and the presence of inflammatory cells, decreased number of sperm cells and moderate degeneration of Leydig interstitial cells were also observed (Cemaluk et al., 2013).

The prooncogenic potential of the sodium monoglutamate was suggested by administrating it in feed in doses of 30-50 g per kg feed in quails. Anemia, serum enzyme and immune dysfunctions, histological lesions of the kidney, atrophy, coagulation, and necrosis has also been observed (Solcan et al., 2018). In certain countries from Africa it is used even as a bleach (Eweka O.E., 2007).

Materials and methods

In order to study the monosodium glutamate effects on testis one hundred thirty-four japanese quails (*Coturnix japonica*) were divided into 4 groups (three experimental groups and a control one). Monosodium glutamate was admninistrated for 30 days in different concentrations for the three experimental groups: 10g/kg feed for group 1; 30g/kg feed for group 2 and 50 g/kg feed for group 3. Food was administrated ad libitum. The quails were slaughter and the testis were collected and prepared for histological examination. The testissamples were dehydrated in alcohol, cleared in xylene and embedded in paraffin wax. Serial sections of 5 μm thick were obtained. The deparaffinised sections were stained with PAS (acid periodic 1% for 10 minutes, Schiff for 10 minutes, Mayer haematoxylin for 3 minutes). Photomicrographs were obtained using an optical microscope (Leica, Germany) and LAS Version 4.9.0.

Results and discussions

For the control and the intoxicated groups the diameter of seminiferous tubules and germinal epithelium height as well as other modifications were studied.

The cross sections in the testis of the control group showed a variety of shapes of the seminiferous tubules, and in the lumen, spermatozoa and spermatide tails (Fig. 1). The seminiferous epithelium with Sertoli cells and spermatogenic cells in different stages of development (Fig. 2) showed the normal histological organization (Shil et al., 2015; Lin et al., 1990).

The group 1, in which quails were administrated 10mg/kg feed, showed several mild modifications in testis. Degenerationof the seminiferous tubule epithelium, vacuolar spaces of various sizes among the Sertoli cells (Fig. 3), and oedema in interstitial space (Fig. 4) were observed. In this group the density and morphology of spermatocytes in the seminiferous tubules were similar to those in the control group.

In group 2, a decrease in sperm cells density was observed. In the seminiferous tubules were present cells with condensed nuclei and a small number of cells in the germinal layers compared to the control group. Intensive oedema was present in the space between the seminiferous tubules (Fig. 5). Some of the tubules contained mature sperm cells in the lumen but other seminiferous tubules presented sloughed spermatogenic cells in lumen and aglomerations of spermatozoa and positive PAS secretion(Fig. 6). Vacuolar degeneration was also noticed in the seminiferous tubules.

In experimental group 3, the regular structure of the seminiferous tubules was maintained. The reduction of cells density in thegerminal epithelium was observed. Oedema in the space between the tubules andin the lumen a PAS positive secretion were present (Fig. 7). In the lumen of seminiferous tubules of third group quails, the number of cells was reduced (Fig. 8). In this group, most cells in the tubules have a dark, condensed appearance, and pyknotic nuclei.

Atrophy of seminiferous tubules was observed focal, thus atrophic seminiferous tubules and vacuolar degeneration were often observed near those with normal spermatogenesis (Fig. 9-10).The lesions consisted of Sertoli cells and cells from the sperm line with large, vesicular nuclei. In severely affected tubules, the changes were characterized by advanced degeneration and necrosis. The number of spermatogenic cells had ranged from 2-7 cells.

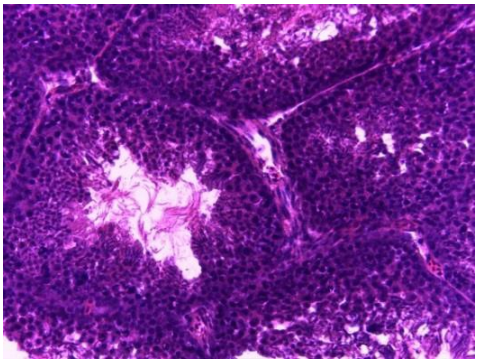

Figure 1. Male quail testis. Control group. PAS, x400
Portions of several seminiferous tubules with visible sperm cells in the lumen.

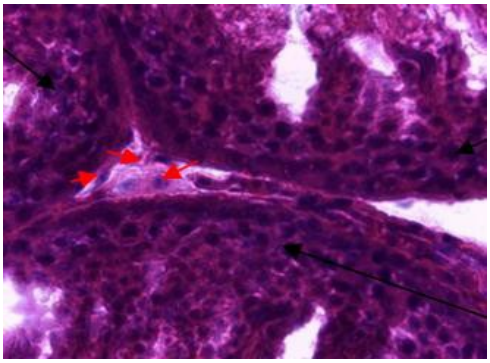

Figure 2. Testis. Control group. PAS, x900
Seminiferous tubules (black arrows) and interstitial (Leydig) cells (red arrow).

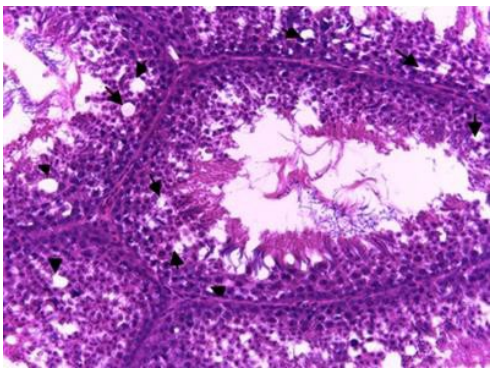

Figure 3. Testis. Group 1. PAS, x400
Vacuolar spaces of various sizes (arrows) in the seminiferous tubules.

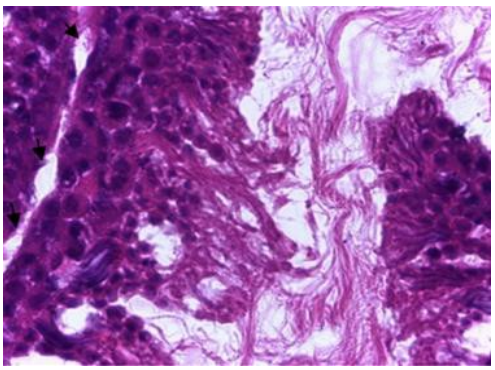

Figure 4. Testis. Group 1. PAS, x900
Oedema in interstitial space (arrows).

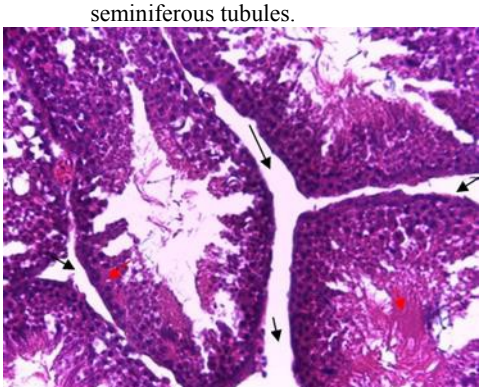

Figure 5. Testis. Group 2. PAS, x400
Intensive oedema in the space between the tubules (black arrows). PAS positive secretion (red arrow).

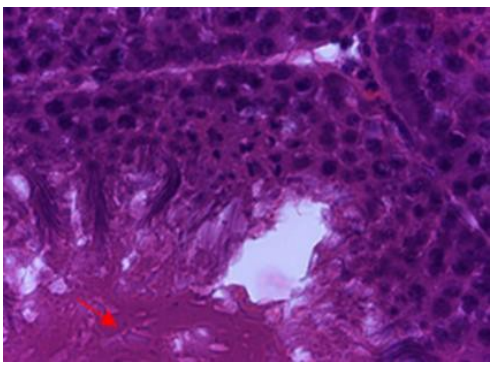

Figure 6. Testis. Group 2. PAS, x900
PAS positive secretion in the lumen of a seminiferous tubule (red arrow).

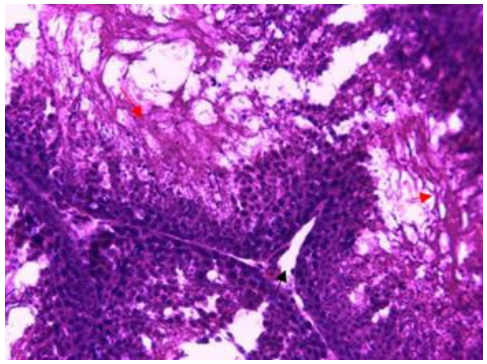

Figure 7. Testis. Group 3. PAS, x400
Oedema in the space between the tubules (black arrow). PAS positive secretion (red arrows).

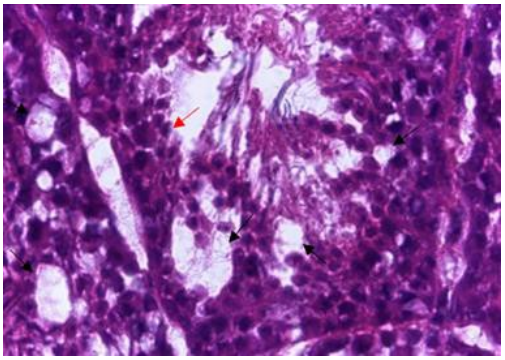

Figure 8. Testis. Group 3. PAS, x900
Presence of vacuoles among cells (black arrows) and decreased number of spermatogenic lineage cells (red arrow).

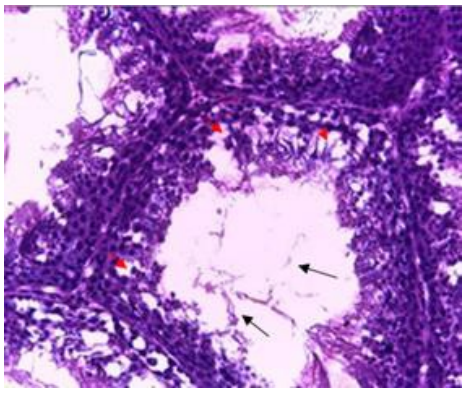

Figure 9. Testis. Group 3. PAS, x400
Presence of atrophy and vacuolar degeneration in the seminiferous tubules (red arrows), numerical reduction of sperm cells in the lumen (black arrows).

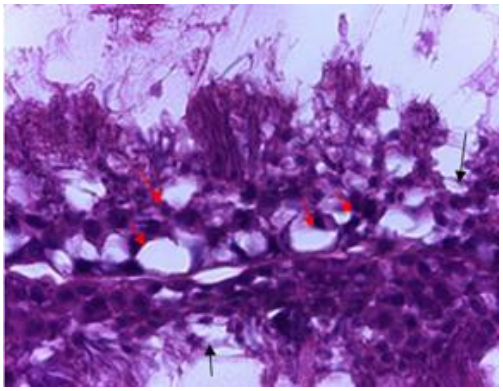

Figure 9. Testis. Group 3. PAS, x900
The reduction in seminiferous tubules hight (black arrows) and vacuolar degeneration (red arrows) in the seminiferous tubules.

The gravity of the lesions and their frequency were increased in a direct relation regarding the administrated dose. The toxic effect of sodium monoglutamate on spermatogenic cells was nonspecific and equally harmful to spermatogonia, primary spermatocytes, and spermatids. In affected seminiferous tubules, the changes were characterized by degeneration, germinative cell line and Sertoli cells atrophy, vacuolar degeneration, and numerical reduction of sperm cells in the lumen. These changes are due to a decrease in androgen levels and / or an increase in oxidative stress in the tissue.

The decrease of the seminiferous tubules height can be explained by the increased oxidative stress produced by monoglutamate that affects the testosteron producing Leydig cells. Spermiogenesis is influenced by testosteron produced by interstitial Leydig cells and is affected when this cells are damaged due to increased lipid peroxidation level (Mondal et al., 2014). Supplementation with different substances during the monosodium glutamate administration showed protective effects against the oxidative stress of MSG. Studies conducted on male mice showed that monosodium glutamate produced an elevation in lipid peroxidation level in the testis but administration of selenium or vitamin E ameliorated the MSG induced testicular toxicity to great extent and reduce the oxidative stress on testis tissues (Hamzaand AL-Harbi, 2014). The

protective effect of α-tocopherol against oxidative stress related to nephrotoxicity by monosodium glutamate was observed in rats (Paul et al., 2012).

Other substances can enhace the lesions produced by monosodium glutamate when administrated simultaneously. Oral administration of arginine and MSG in rats enhanced the lesions observed in testis comparated with that observed in the subjects that were orally administrated only MSG (Cemaluk et al., 2013).

The lesions described in quails testis were also described in other species after monosodium glutamate administration. Studies conducted on rats showed degeneration and alteration of sperm cell population and modifications in sperm cells morphology. Thus it was suggested that MSG may be implicated in cases of male infertility (Oforofuo et al., 1997). The toxic effect of MSG administrated in rats caused significant oligozoospermia and increase abnormal sperm morphology. The number of lesions depended on the quantity of MSG that has been used for the male rats intoxication (Onakewhor et al., 1998). The administration of MSG in young and adult male rats determined histomorphological changes in the testis characterized by degenerative/necrotic changes, decreased number of sperm cells, and the interstitial (Leydig) cells showed moderate degeneration suggesting that exposure to MSG may consequently impair spermatogenesis or testosterone production in the rats (Igwebuike et al., 2011; Iamsaard et al., 2014).

The degenerative lesions observed in quails testis after monosodium glutamate administration are nonspecific. The degenerative, vacuolation and atrophic changes compared with the ones observed in our study were noticed in testis of quails exposed to di(n-butyl) phthalate (DBP) also (Bello et al., 2014).

Conclusions

Gravity and frequency of the injuries in testis increased proportionally to the administered dose. The alterations consisted of different heights in the seminiferous tubule epithelium, pyknotic nuclei in sperm cell line and the presence of a PAS positive secretion in the lumen of the seminiferous tubules. The alterations induced by the sodium monoglutamate in doses of 50g/kg feed may determined serious deleterious reproductive consequences.

The toxic effect of sodium monoglutamate on spermatogenic cells was nonspecific and equally harmful to spermatogonia, primary spermatocyte, and sperm cells.

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