The protective role of propolis against the reproductive toxicity of mono-sodium glutamine in male rabbits

Fayrouz A Khaled, Mokhtar I Yousef, Kamel I Kamel

Abstract
The present study was carried out to investigate the possible protective effect of propolis on semen characteristics induced by the flavor enhancers, monosodium glutamate (MSG) in adult male V-line rabbits. Twenty mature male rabbits were randomly divided into four equal groups of 5 rabbits each. Group 1 served as control. However, group 2, 3 and 4 were given propolis (50 mg/kg body weight), mono-sodium glutamine (8 mg/kg body weight) and the combination of propolis (50 mg/BW) and MSG (8 mg/BW), respectively. Animals were orally administered the doses of propolis, MSG and propolis plus MSG every day for 12 weeks. Results showed that semen quality was deteriorated following treatment with MSG. Also, testosterone levels, body weight (BW) and relative weights of testes (RWT) were decreased. Propolis alone significantly increased BW, RTW, testosterone levels and semen characteristics. Furthermore, the presence of propolis with MSG alleviates its toxic effects. From the present study, it can be concluded propolis can be effective in the protection of MSG-induced reproductive toxicity.

Keywords: role of propolis, reproductive toxicity, mono-sodium glutamine, male rabbits

1. Introduction

Either most food additives act as preservatives or enhancer of palatability, monosodium glutamate (MSG) is one of such food additives that generated much controversy locally and globally about its safety usage (Moore, 2003) [23]. Vinodini et al. (2008) [36] defined MSG as a sodium salt of naturally occurring non-essential L-form of glutamic acid, and as one of the main flavour enhancer used as an ingredient in various food products. MSG is the sodium salt of glutamic acid, it is one of the most common amino acids found in nature (Adrienne, 1999) [4]. MSG is also produced in the body and plays an essential role in human metabolism, it is a major component of many proteins such as meat, fish, milk and some vegetables (IFIC, 1994) [15]. MSG showed morphological and despite its taste stimulation and improved appetite enhancement, reports indicated that MSG is toxic to human and experimental animals (Andrew, 2007) [6].

The concentrations of MSG used as food additive vary in different foods (Walker, 2000) [37]. Currently, the safe concentration of MSG in foods and its toxicity in human is still a controversial issue (Beyreuther et al., 2007) [8]. In animals, MSG at higher doses was demonstrated to be a neurotoxic salt that could alter the hypothalamic-pituitary-adrenal axis (HPA) and damage neurons in the hypothalamic nuclei (Seo et al., 2010) [31].

The authors further mentioned that exito toxins are widely distributed in our food supply, and we may not be able to depend on the Food and Drug Administration (FDA) to protect us from these toxins; because of the powerful food lobby known as the Glutamate Association, which counteract any negative reports or publicity of research showing the harmful effects of MSG. Samuels (1999) [30] reported that MSG is a neurotoxic agent i.e. causing damage to brain cells, retinal degeneration, leading to many endocrine disorders and causes renal damage. Many studies reported the implication of (MSG) in cases of male infertility as it causes testicular hemorrhage, degeneration and alteration of sperm cell population and morphology (Andrew, 2007) [6]. The aim of the present study was to go more through the toxic effects of MSG administration on the structure of the testis of albino rats and to determine the possibility of reversibility of these effects. Morphometric changes as decrease in testicular weight, decrease in tubular diameter, reduction in germinal epithelium height, decrease in the spermatid count and abnormalities of sperms morphology.
There was significant gradual improvement after cessation of treatment with MSG. However, the normal structure of the testis was not regained even after six weeks of cessation of treatment.

The influence of reactive oxygen species (ROS) on fertility has become of increasing interest. In patients with asthenozoospermia, an elevated production of ROS in seminal plasma and increased ROS-mediated damage of sperm membranes has been detected. By altering membrane integrity, ROS may impair sperm motility as well as sperm viability. Therefore, protective agents against ROS may be useful therapeutic agents in the treatment of male infertility (Aitken, 1995). Mammalian tissues contain several enzymes scavenging reactive oxygen species (ROS) such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-PX) and glutathione S-transferase (GST), and reduced glutathione (GSH) as controlling system of ROS and protecting cells under stress conditions. Also, there are some natural compounds contribute to the detoxification process from ROS such as propolis (Jasprica et al., 2007; Yousef and Salama, 2009) [17, 39].

Propolis is a resinous natural product collected from cracks in the bark of trees and leaf buds which are enriched with the salivary enzymes of honeybees. It has gained popularity and was used extensively in healthy drinks and foods to improve well-being and prevent diseases such as inflammation, heart disease, diabetes and even cancer. Propolis possesses several biological properties such as anti-inflammatory, anticancer, antioxidant, antibiotic and antifungal activities (Banskota et al., 2000) [7]. Propolis contains some minerals such as Mg, Ca, I, K, Na, Cu, Zn, Mn and Fe as well as some vitamins like B1, B2, B6, C and E, and a number of fatty acids. In addition, it contains some enzymes as succinic dehydrogenase, glucose-6-phosphatase, adenosine triphosphatase and acid phosphatase (Tikhonov and Mamontova, 1987) [35]. Propolis, also contains more than 300 biochemical constituents, including mostly a mixture of polyphenols, flavonoid aglycones, phenolic acid and their esters, and phenolic aldehydes and ketones, terpenes, sterols, vitamins, amino acids (Khalil, 2006) [19]. The antioxidant activity of propolis is mainly attributed to its flavonoid content, that is capable of scavenging free radicals and thereby protection against lipid peroxidation (Yousef and Salama, 2009) [39]. Propolis also induces the activation of antioxidant enzymes such as superoxide dismutase (Jasprica et al., 2007) [17] and catalase (CAT) (Sobocanec et al., 2006) [33] against free radicals. It has been demonstrated that propolis provides protection against infertility by improving sperm production, motility, count and quality, and increased the process of steroidogenesis and hence testosterone production (Yousef and Salama, 2009) [39].

Yousef et al. (2010) [38] showed that semen quality was deteriorated following treatment with TPTC. Also, testosterone levels, body weight (BW), relative weights of testes (RWT) and epididymis (RWE) were decreased. Thio Barbir turic acid-reactive substances and lactate dehydrogenase were increased, while glutathione S-transferase, transaminases and phosphatases were decreased in seminal plasma of rabbits treated with TPTC compared to control. They added that treated male rabbits with propolis alone significantly increased testosterone levels, BW, RTW, REW, semen characteristics and seminal plasma enzymes, and decreased the levels of free radicals and lactate dehydrogenase. Furthermore, the presence of propolis with TPTC alleviates its toxic effects. Russo et al. (2006) [28] revealed that propolis protects sperm DNA from the oxidative damage caused by thio barbituric acid-reactive substances (TBARS).

2. Materials and Methods

In this study, the effect of Mono-sodium glutamine (MSG) with or without propolis on the reproductive performance in mature male rabbits were investigated. Mono-sodium glutamine was obtained from El Dawlia for Medical Equipments and Chemicals Co. Egypt. It was dissolved in distilled water before use. Propolis was supplied from California Health Products, Inc. 11577W. Olympic Blvd. Los Angeles, and CA90064.

Male V-line rabbits (age of 6-7 months and initial weight of 3.200±0.083 Kg) were used. The animals were individually housed in stainless steel cages. Feed and water were provided ad libitum. They were fed on a commercial ration pellets consisting of 30% Berseem hay (Trifolium alexandrinum), 25% yellow maize, 26.2% wheat bran, 14% Soybean meal, 3% molass, 1% calcium chloride, 0.4% sodium chloride, 0.3% mixture of minerals and vitamins and 0.1% methionine. The chemical analysis of the pellets [13] showed that they contained 17.5 % crude protein, 14.0 % crude fiber, 2.7 % crude fat and 2200 K cal./kg diet.

To determine the effects of propolis, mono-sodium glutamine (MSG) and/or their combination on reproductive performance and testosterone levels, 20 mature male rabbits were randomly divided into four equal group of 5 rabbits each. Group I served as control. However, group 2, 3 and 4 were given propolis (8 mg/kg body weight), mono-sodium glutamine (50mg/kg body weight) and the combination of propolis and MSG, respectively. The doses of the propolis and MSG were calculated according to the animal’s body weight on the week before dosing. The tested doses for propolis and MSG were given every other day for 12 weeks.

Rabbits were observed twice daily and weighed weekly in the morning before having access to feed and water. Daily feed intake was recorded weekly and semen collection was carried out weekly and continued throughout the 12 weeks experimental period.

Bucks were subjected to semen collection by taking a female to the buck. Semen was collected in a graduated test tube attached to the artificial vagina. Reaction time was recorded from the moment of subjecting a doe to the buck and completion of erection; it was measured in seconds using a stopwatch. After removal of the gel mass, ejaculate volume was recorded. Initial hydrogen ion concentration (pH) of semen samples was determined just after collection using a pH paper (Universaldikator pH 0-14 Merck, Merek KgaA, 64271 Darmstadt, Germany). Packed sperm volume (PSV) was recorded. A weak eosin solution (Blom, 1950) was used for evaluation of sperm concentration by the improved Neubauer haemocytometer slide (GmbH+Co., Brandstwiete 4, 2000 Hamburg 11, Germany). Total sperm output was calculated by multiplying semen ejaculate volume and semen concentration. Determination of initial fructose concentration in seminal plasma was carried out immediately after collection according to Mann (1948) [21]. Assessment of live, dead, and abnormal spermatozoa were performed using an eosin-nigrosin blue staining mixture. The percentages of motile sperm were estimated by visual examination under low-power magnification (10×40) using a phase-contrast microscope. Total number of motile sperm was calculated by multiplying percentage of motile sperm and total sperm outputs. Total functional sperm fraction (TFSF) parameter was also
calculated as the product of total sperm output by motility (%) by normal morphology (%) (Correa and Zavos, 1996)\(^{[11]}\). Blood samples were collected from the ear vein of all animals every other week and were placed immediately on ice. Heparin was used as anticoagulant and plasma was collected by centrifugation of blood at 860 Xg for 20 minutes, and was stored at -60 °C until used for analyses. Testosterone concentrations in plasma were measured by a simple solid phase enzyme immunoassay utilizing horseradish peroxidase as a tracer (Equipar, Via G. Ferrari, Saronno, Italy). Three rabbits from each group were selected for slaughter at the end of the treatment period. The weight of testes was recorded in the sacrificed rabbits.

Data were analyzed as a randomized design using the General Linear Model procedure of SAS (1996)\(^{[34]}\). The Student–Newman–Keuls test was used for testing the mean differences. P-values < 0.05 were accepted as significant.

3. Results

Effect of Pro, MSG and their mixture on BW, FI, RTW and testosterone level:
The changes in body weight (BW), feed intake (FI), relative testes weight (RTW) and the concentrations of blood plasma testosterone throughout the 12-week experimental period of bucks treated with propolis (Pro), mono-sodium glutamine (MSG) and/or their combination were summarized in Table 1. Treatment with MSG caused a decrease (\(p<0.05\)) in BW, FI, relative testes weights and testosterone levels compared with control group this decrease was only significant in BW and blood plasma testosterone values. While, treatment with propolis alone significant enhancement the pervious parameters, except the RTW which non-significantly change with control group.

Table 1: The overall means (±SE) of body weight\(^1\), feed intake\(^2\), and relative testes\(^3\) weight and blood plasma testosterone concentration during treatment of male rabbits with propolis, mono-sodium glutamine (MSG) and/or their combination.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Propolis</th>
<th>MSG</th>
<th>Propolis+MSG</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW(^1) (kg)</td>
<td>3.39±0.03(^b)</td>
<td>3.45±0.03(^a)</td>
<td>2.83±0.04(^ab)</td>
<td>3.08±0.02(^c)</td>
</tr>
<tr>
<td>FI(^2) (g/kg BW/day)</td>
<td>43.9±0.81(^ab)</td>
<td>46.6±0.61(^a)</td>
<td>41.8±0.98(^bc)</td>
<td>45.0±1.11(^ab)</td>
</tr>
<tr>
<td>RTW(^3) (g/100 g BW)</td>
<td>0.16±0.013(^ab)</td>
<td>0.19±0.010(^b)</td>
<td>0.10±0.013(^ab)</td>
<td>0.14±0.013(^ab)</td>
</tr>
<tr>
<td>Testosterone (ng/mL)</td>
<td>5.46±0.18(^b)</td>
<td>7.26±0.28(^a)</td>
<td>2.80±0.22(^c)</td>
<td>4.76±0.32(^b)</td>
</tr>
</tbody>
</table>

and Within row, means with different superscript letters differ significantly (\(p<0.05\)). Results showed also that propolis counteracted the hazardous effect of MSG on BW, FI, RTW and testosterone concentrations parameters (Table 1).

The significant decreased in body weight in our results is agreement with Abd- El-Aziz et al., (2014)\(^{[1]}\) who indicate that prolonged administration of MSG causes an initial increase in adult male rats weight gain followed by terminal suppression, independent of food consumption. They explained that by the induced gastric mucosal damage, therefore it appears that prolonged intake of MSG induces gastric damage which, consequently, leads to decreased body weight. On the other hand, Sharma et al., (2013)\(^{[32]}\) reported that no significant different between MSG-treated rats and control groups. Khadiga et al., (2009)\(^{[18]}\) revealed that total feed intake was significantly higher (\(P<0.05\)) in chicks fed 1% MSG while final body weight gain was significantly (\(P<0.05\)) lower in chicks fed 0.25 and 0.5% MSG compared to control group. Relative testis weight showed significant decrease in the present study are in agreement with Nosseir et al., (2012)\(^{[25]}\) who mentioned that treatment with MSG caused decrease in testicular weight, decrease in tubular diameter, reduction in germinal epithelium height. Also, Nayatara et al., (2008)\(^{[24]}\) reported that MSG induced reduction in testicular weight and decrease in the sperm count.

Ochiogu et al., (2015)\(^{[26]}\) recorded a significant lower serum testosterone in the MSG-treated groups at Days 14 and 28 of MSG administration. This result may be due to reduce gonadotrophin-releasing hormone (GnRH) associated with the lesions on the arcuate nucleus of the hypothalamus that occurs in animals given MSG (Igwebuike et al. 2011)\(^{[16]}\). The significantly lower levels of serum testosterone, however, may be attributed to the low serum LH and total cholesterol recorded in the study by Ochiogu et al., (2015)\(^{[26]}\) as the main action of LH is the conversion of cholesterol to pregnenolone – a rate-limiting step in the biosynthesis of steroid hormones, of which testosterone is one (Hinshelwood, 1998)\(^{[14]}\). It is possible that the effects of MSG administration on testosterone may be as a result of accumulated toxic effects (Samuels, 1999)\(^{[30]}\).

The propolis counteracted the hazardous effect of MSG on BW, FI, RTW and testosterone concentrations parameters are in agreement with El-Masoudy et al. (2011)\(^{[12]}\) who found that propolis increased the relative testis weight and alleviated the negative effects of chlorpyrifos. Also, Yousef et al. (2010)\(^{[38]}\) found that the propolis significantly increases body weight and relative testis weight. Testicular weight was reported to have a high correlation with sperm reserve in the testis or epididymis and therefore a reflection of sperm production (Adeyemo et al., 2007)\(^{[3]}\).

The propolis increased the level of testosterone in male rats exposed to chlorpyrifos toxicity (El-Masoudy et al., 2011)\(^{[12]}\) in male rabbits exposed to triphenyltin toxicity (Yousef et al., 2010)\(^{[38]}\), in male rats exposed to aluminium chloride toxicity (Yousef and Salama, 2009)\(^{[39]}\), and in male rats exposed to profenofos toxicity (Abu-Aita et al., 2012)\(^{[2]}\).

Effect of Pro, MSG and their mixture on EV, pH, RT, PSV, SC and TSO:

Data on semen ejaculate volume (EV), initial hydrogen ion concentration (pH), reaction time (RT), packed sperm volume (PSV), sperm concentration (SC), total sperm output (TSO) of rabbits treated with propolis, MSG and their combination are presented in Table 2. Treatment of rabbits with MSG significantly decreased (\(P < 0.05\)) the EV, PSV, SC and TSO values compared to control group. On the other hand, the values of pH and RT were increased significantly as an administration with MSG compared with control group. The propolis treatment enhanced the semen volume, RT, pH, sperm concentration and total sperm output in male rabbit bucks, and counteracted the hazardous effect of MSG on the previous parameters (Table 2).

Ochiogu et al. (2015)\(^{[26]}\) reported that the mean libido scores of the MSG-treated groups were significantly (\(P < 0.05\)) lower than that of the untreated control group on Day 26 of MSG administration when libido scores were evaluated. Nosseir et al. (2012)\(^{[25]}\) mentioned that treatment rats with MSG caused decrease in the spermatic count and abnormalities of sperms morphology. Also, Nayatara et al. (2008)\(^{[24]}\) reported that MSG induced reduction in the rats sperm count.

With co-administration of propolis, the sperm concentration was significantly ameliorated as compared to control animals (Table 2). This is in accordance with previous studies which...
mentioned that the administration of propolis caused significant improvement in sperm characteristics and male fertility of rats exposed to chlorpyrifos toxicity (El-Mazoudy et al., 2011) [12]. Also, propolis could provide protection against infertility by improving sperm production, motility, sperm count and quality in male rats exposed to aluminium chloride toxicity (Yousef and Salama, 2009) [39]. This may be due to the free radical scavenging activity of propolis that protects sperm membrane from the deleterious action of oxidative attacks and reduces their barbituric acid reactive substances formation (Russo et al., 2006) [28]. Also, propolis induces a significant increase in the level of antioxidant enzymes (Yousef and Salama, 2009) [39]. Co-administration of curcumin to monosodium glutamate treated rats increased the sperm count (Sakr and Badawy, 2013) [29].

The treated male rabbits with propolis only caused significant increase in SM, TMS, TFSF and IF values and significant decrease in the spermatic count and increase the incidences of abnormal sperm. Igwebuike et al., (2008) [24] reported that treat in rats with MSG reduced the sperm count and increase the incidences of abnormal sperm. Igwebuike et al. (2011) [16] showed that a reduction of sperm counts was observed in the MSG-treated rats. Nosseir et al. (2012) [25] mentioned that treatment rats with MSG caused decrease in the spermatic count and abnormalities of sperms morphology.

The treated male rabbits with propolis only caused significant increase in SM, TMS, TFSF and IF values and significant decrease in the values of DS and AbS compared with control group. Propolis treatment enhanced the previous parameters and counteracted the hazardous effect of MSG on male rabbit bucks (Table 3).

With co-administration of propolis, the semen characteristics were significantly ameliorated as compared to control animals (Table 3). This is in accordance with previous studies which mentioned that the administration of propolis caused significant improvement in sperm characteristics and male fertility of rats exposed to chlorpyrifos toxicity (El-Mazoudy et al., 2011) [12]. Also, propolis could provide protection against infertility by improving motility, sperm count and quality in male rats exposed to aluminium chloride toxicity (Yousef and Salama, 2009) [39]. This may be due to the free radical scavenging activity of propolis that protects sperm membrane from the deleterious action of oxidative attacks and reduces their barbituric acid reactive substances formation (Russo et al., 2006) [28]. Also, propolis induces a significant increase in the level of antioxidant enzymes (Yousef and Salama, 2009) [39].

Our results showed that MSG may have some deleterious effects on semen characteristics and this may be related with reduction in blood plasma testosterone in rabbits given MSG (Table 1). It was reported that MSG destroyed neurons of the hypothalamus in animals (Burde et al., 1971) [10]. Such neuronal losses in the hypothalamus can result in disruption of the hypothalamic-pituitary-testis regulatory axis that controls the stereoidogenesis of testicular Leydig cells (Melchlan et al., 1996) [22].

Antioxidants play a major role in preventing the formation of free radicals, which are responsible for many oxidative processes leading to cell damage. Many studies showed that propolis possesses antioxidant activity. This may be due to the free radical scavenging activity of propolis that protects sperm membrane from the deleterious action of oxidative attacks and reduces their barbituric acid reactive substances formation (Russo et al., 2006) [28]. Also, Yousef and Salama, (2009) [39] reported that propolis induces a significant increase in the level of antioxidant enzymes. Previous studies reported that MSG was associated with the production of oxygen free radicals and oxidative stress in different tissues of experimental animals (Onyema et al., 2012; Kumar and Bhandari, 2013) [27, 20]. The results showed that propolis exerted a protective effect on MSG-induced testicular damage and this is probably due to its antioxidants properties.

4. References

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