International Journal of Chemical Studies

P-ISSN 2349–8528
E-ISSN 2321–4902
IJCS 2016; 4(2): 04-09

Received: 11-12-2015
Accepted: 11-01-2016

Fayrouz A Khaled
Omar Al-Mukhtar University, Al Bayda, Libya.

Mokhtar I Yousef
Department of Environmental Studies, Institute of Graduate Studies and Research, University of Alexandria, Egypt.

Kamel I Kamel
Animal Production Research Institute, Dokki, Giza, Egypt.

Correspondence
Fayrouz A Khaled
Omar Al-Mukhtar University, Al Bayda, Libya.

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 spermatic 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 TPTCl. Also, testosterone levels, body weight (BW), relative weights of testes (RWT) and epididymis (RWE) were decreased. Thiobarbituric acid-reactive substances and lactate dehydrogenase were increased, while glutathione S-transferase, transaminases and phosphatases were decreased in seminal plasma of rabbits treated with TPTCl 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 TPTCl alleviates its toxic effects. Russo *et al.* (2006) [28] revealed that propolis protects sperm DNA from the oxidative damage caused by thiobarbituric 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 Bivd. 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 1 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 (Universalindikator pH 0-14 Merck, Merck 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.

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

^{abcd} 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].

Table 2: The overall means (±SE) of EV, pH, RT, PSV, SC and TSO during treatment of male rabbits with propolis, mono-sodium glutamine (MSG) and/or their combination.

Parameter	Groups			
	Control	propolis	MSG	propolis+MSG
EV (ml)	0.79 ± 0.140^{b}	0.94 ± 0.018^{a}	0.63 ± 0.012^{d}	0.74 ± 0.012^{c}
PH	7.7 ± 0.024^{b}	7.4 ± 0.033^{c}	7.9 ± 0.039^{a}	7.6 ± 0.018^{b}
RT (sec.)	9.7 ± 0.90^{c}	5.7 ± 0.51^{d}	19.3 ± 1.27^{a}	12.7 ± 1.10^{b}
PSV (%)	16.9 ± 0.20^{b}	20.4 ± 0.27^{a}	13.1 ± 0.30^{d}	15.1 ± 0.27^{c}
SC	209 ± 2.0^{b}	246 ± 4.0^{a}	157 ± 4.2^{d}	177 ± 2.3^{c}
TSO ($\times10^{6}$)	166 ± 3.7^{b}	231 ± 6.9^{a}	103 ± 4.3^{d}	133 ± 3.1^{c}

[abcd] Within row, means with different superscript letters differ significantly ($p < 0.05$).

Effect of propolis, MSG and their mixture on SM, TMS, DS, AbS, TFSF and IF

Data in Table (3) represent the mean values of rabbit sperm motility (SM), total motile sperm (TMS), dead sperm (DS), abnormal sperm (AbS), total function sperm fraction (TFSF) and initial fructose (IF). Treated male rabbits with MSG significantly decreased ($P < 0.05$) the SM, TMS, TFSF and IF values compared to control group. On the other hand, significant increase in DS and AbS parameters was observed in the MSG-treated rabbits compared with control group.

Table 3: The overall means (±SE) of rabbit SM, TMS, DS, AbS, TFSF and IF during treatment of male rabbits with propolis, mono-sodium glutamine (MSG) and/or their combination.

Parameter	Groups			
	Control	propolis	MSG	propolis+MSG
SM (%)	64.1 ± 0.7^{b}	76.4 ± 1.0^{a}	54.2 ± 1.2^{d}	59.8 ± 0.8^{c}
TMS ($\times10^{6}$)	130 ± 2.7^{b}	200 ± 6.9^{a}	75 ± 3.8^{d}	101 ± 2.3^{c}
DS (%)	34.4 ± 0.36^{b}	22.0 ± 0.63^{c}	40.7 ± 0.96^{a}	36.3 ± 0.42^{b}
AbS (%)	21.4 ± 0.2^{c}	14.0 ± 0.4^{d}	28.5 ± 0.5^{a}	23.4 ± 0.3^{b}
TFSF ($\times10^{6}$)	103 ± 7.2^{b}	172 ± 6.8^{a}	54 ± 3.7^{c}	77 ± 1.9^{c}
IF (mg/dl)	231 ± 2.3^{b}	250 ± 3.8^{a}	174 ± 4.4^{d}	196 ± 2.2^{c}

The mean value represents 96 values for each treatment.

[abcd] Within row, means with different superscript letters differ significantly ($p < 0.05$).

Nayatara *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 decreased 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].

Fetouh, and Azab (2014) [1] found that the daily sperm production decreased markedly due to gentamicin treatment with as compared to control group ($p<0.05$), and with co-administration of propolis, the daily sperm production improved significantly as compared to gentamicin treated group.

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 steroidogenesis of testicular Leydig cells (Mclachlan *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

1. Abd El-Aziz GS, El-Fark MO, Hassan SM, Badawoud MH. Effects of prolonged oral intake of monosodium glutamate (MSG) on body weight and its correlation to stomach histopathological changes in male rats Thai J Vet Med. 2014; 44(2):201-208.
2. Abu-Aita NA, Hashesh MA, Mohamed AH. Clinicopathological and cytogenetic studies on the ameliorative effect of propolis against profenofos toxicity in rats. Global Veterinaria; 2012; 9(6):669-682.
3. Adeyemo GO, Longe OG, Adejumo DO. The reproductive performance of breeder cocks fed cottonseed

cake-based diets. International Journal of Poultry Science. 2007; 6(2):140-144.
4. Adrienne S. The Toxicity-Safety of MSG. Acct. Res 1999; 6(4):259-310.
5. Aitken RJ. Free radicals, lipid peroxidation and sperm function. Reprod. Fertil. Dev 1995; 7:659-668.
6. Andrew OE. Histological studies of the effects of monosodium glutamate on the kidney of adult Wistar rats. The Internet Journal of Health. 2007, 6(2).
7. Banskota AH, Tezuka Y, Adnyana IK, Midorikawa K, Matsushige K, Message D *et al.* Cytotoxic, hepatoprotective and free radical scavenging effects of propolis from Brazil, Peru, the Netherlands and China. J Ethnopharmacol. 2000; 72:239-246.
8. Beyreuther K, Biesalski HK, Fernstrom JD, Grimm P, Hammes WP, Heinemann U *et al.* Consensus meeting: monosodium glutamate - an update. Eur J Clin Nutr. 2007; 61(3):304-13.
9. Blom E. A one-minute live-dead sperm stain by means of eosin-nigrosin. J Fertil Steril. 1950; 1:176-177.
10. Burde RM, Schainker B, Kayes J. Acute effect of oral and subcutaneous administration of monosodium glutamate on the arcuate nucleus of the hypothalamus in mice and rats. Nature 1971; 233:58-60.
11. Correa JR, Zavos PM. Preparation and recovery of frozen-thawed bovine spermatozoa via various sperm selection techniques employed in assisted reproductive technologies. Theriogenology, 1996; 46:1225-12232.
12. El-Masoudy RH, Attia AA, El-Shenawy NS. Protective role of propolis against reproductive toxicity of chlorpyrifos in male rats. Pestic Biochem Physiol, 2011; 101:175-181.
13. Fetouh FA, Azab AE. Ameliorating effects of curcumin and propolis against the reproductive toxicity of gentamicin in adult male guinea pigs: Quantitative analysis and morphological study. American Journal of Life Sciences. 2014; 2(3):138-149.
14. Hinshelwood MM. Steroidogenesis, overview. In: Neill JD, Knobil E (Eds.): Encyclopedia of Reproduction. Academic Press, New York, 1998; 4:644-653.
15. IFIC (Institute of Food Technologists'). Review of Monosodium Glutamate, Examining the Myths. IFIC, 1994; 41(5):143-145.
16. Igwebuike UM, Nwankwo IA, Ochiogu IS. Effects of oral administration of monosodium glutamate (MSG) on serum testosterone levels and muscle mass development in male rats. Animal Research International, 2011; 7:1212-1217.
17. Jasprica D, Mornar A, Debeljak Z, Smolcic-Bubalo A, Medic-Saric M, Mayer L *et al. In vivo* study of propolis supplementation effects on antioxidative status and red blood cells. J Ethnopharmacol. 2007; 110:548-554.
18. Khadiga A, Ati A, Mohammed S, Saad AM, Mohamed HE. Response of broiler chicks to dietary monosodium glutamate. Pakistan Vet. J. 2009; 29(4):165-168.
19. Khalil ML. Biological activity of bee propolis in health and disease. Asian Pac. J Cancer Prev. 2006; 7:22-31.
20. Kumar P, Bhandari U. Protective effect of Trigonella foenum-graecum Linn. On monosodium glutamate-induced dyslipidemia and oxidative stress in rats. Indian J Pharmacol. 2013; 45:136-40.
21. Mann T. Fructose content and fructolysis in semen: Practical application in the evaluation of semen quality. J Agric Sci. (Camb.) 1948; 38:323-331.
22. Mclachlan RI, Wreford NG, O'Donnell L, De Kretser DM, Robertson DM. The endocrine regulation of spermatogenesis: independent roles for testosterone and FSH. J Endocrinol. 1996; 148:1-9.
23. Moore KL. Congenital Malformations Due to Environment In Developing Humans. 2nd ed. Philadelphia, W.B. Saunders co. Ltd, 2003, 173-183.
24. Nayatara AK, Vinodini NA, Damodar G, Ahmed B, Ramaswamy CR, Shabarienth S. Role of ascorbic acid in monosodium glutamate mediated effect on testicular weight, sperm morphology and sperm count in rat testis. Journal of Chinese clinical Medicine. 2008; 3(1):1-5.
25. Nosseir NS, Ali MHM, Ebaid HM. A histological and morphometric study of monosodium glutamate toxic effect on testicular structure and potentiality of recovery in adult albino rats. Research Journal of Biology. 2012; 02:66-78.
26. Ochiogu IS, Ogwu D, Uchendu CN, Okoye CN, Ihedioha JI, Mbegbu EC. Serum luteinising hormone, testosterone and total cholesterol levels, libido and testicular histomorphology of male West African Dwarf goats orally or subcutaneously treated with monosodium l-glutamate. Veterinarni Medicina, 2015; 60(5):253-260.
27. Onyema OO, Aisil CS, Ihetuge AP. Monosodium Glutamate Induces Oxidative Stress and Affects Glucose Metabolism in the Kidney of Rats. International Journal of Biochemistry Research & Review. 2012; 2(1):1-11.
28. Russo A, Troncoso N, Sanchez F, Garbarino JA, Vanella A. Propolis protects human spermatozoa from DNA damage caused by benzo[a]pyrene and exogenous reactive oxygen species. Life Sci. 2006; 78:1401-1406.
29. Sakr SA, Badawy GM. Protective effect of curcumin on monosodium glutamate-induced reproductive toxicity in male rats. Global Journal of Pharmacology. 2013; 7(4):416-422.
30. Samuels A. The Toxicity/Safety of MSG, A study in suppression of information. Accountability in Research 1999; 6(4):259-310.
31. Seo HJ, Ham HD, Jin HY, Lee WH, Hwang HS, Park SA *et al.* Chronic administration of monosodium glutamate under chronic variable stress impaired hypothalamic-pituitary-adrenal axis function in rats. Korean J Physiol Pharmacol. 2010; 14:213-221.
32. Sharma A, Prasongwattana V, Cha'on U, Selmi C, Hipkaeo W. Monosodium Glutamate (MSG) Consumption Is Associated with Urolithiasis and Urinary Tract Obstruction in Rats. PLoS ONE 2013; 8(9):e75546. doi:10.1371/journal.pone.0075546.
33. Sobocanec S, Sverko V, Balog T, Saric A, Rusak G, Likic S *et al.* Oxidant/antioxidant properties of Croatian native propolis. J Agric Food Chem. 2006; 54:8018-8026.
34. Statistical Analysis System (SAS), SAS user's guide: statistics, version, 6th edition. SAS Institute Inc., Cary, NC, USA, 1996.
35. Tikhonov AI, Mamontova INS. Production and study of a lyophilized phenolic polysaccharide preparation from propolis. Farmatsevtichnii Zhurnal. 1987; 3:67-68.
36. Vinodini NA, Nayanatara AK, Damodaragawda KM, Ahamad B, Shabarinath S. Effect of monosodium glutamate-induced oxidative damage on rat testis. J of Chinese Clinical Medicine. 2008; 3(7):370-373.
37. Walker R, Lupien JR. The safety evaluation of monosodium glutamate. J Nutr. 2000; 130(4S Suppl):1049S-1052S.

38. Yousef MI, Kamel IK, Hassan MS, El-Morsy AM. Protective role of propolis against reproductive toxicity of triphenyltin in male rabbits. Food and Chemical Toxicology. 2010; 48:1846-1852.
39. Yousef MI, Salama AF. Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. Food Chem. Toxic, 2009; 47:1168-1175.