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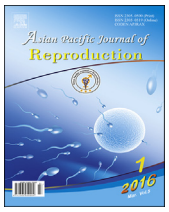

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Histopathological evaluation of supportive effects of *Rosa damascene* on mice testes, following long term administration of copper sulfate

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ABSTRACT

Objective: To evaluate the supportive effects of *Rosa damascene* (*R. damascene*) essential oil on epididymal sperm quality and histopathology of testes following long term administration of copper sulfate in mice.

Materials and methods: The study comprised of four different groups of six mice as follows: group Cu, which received 0.1 mL copper sulfate solution at dose of 100 mg/kg, group R which received 0.1 mL *R. damascene* essential oil at dose of 1 mg/kg, treatment group (T) which received copper sulfate solution (100 mg/kg) and treated by *R. damascene* essential oil (1 mg/kg), and control group (C) which received the same volume of normal saline. The supplements were gavaged in all animals every other day, during experimental period. All animals of each experimental group were sacrificed 42 days after the beginning of experiment.

Results: Results showed that sperm concentration, motility and viability in group Cu were significantly decreased after 6 weeks, and severe degenerative changes were observed in testicular tissues in comparison with the control group. In treatment group, significant improve in the sperm count, motility and viability, and normal architecture in most seminiferous tubules with organized epithelium was observed compared to the group Cu.

Conclusions: Administration of the essential oil of *R. damascene* owning to its anti-oxidant properties is able to protect the testis and epididymal sperm from the adverse effects of copper poisoning in mice.

1. Introduction

The genus *Rosa* comprises over 100 species, found in Europe, Asia, the Middle East and North America [1,2]. *Rosa damascene* (*R. damascene)* belongs to the Family Rosaceae and genus *Rosa* and is an aromatic, light pink plant with signification from economical and research point of view [3]. They grow both in the lowlands and in mountainous regions, where some are found above the tree line and among mountain pine thickets [4]. Generally hardy, these plants flourish in natural environments without needing as Damask rose [5] is known as “Gole Mohammadi” in Iran [6]. This plant is cultivated originally in Kashan city, Esfahan Province (central part of Iran) for preparing rose water and attar [3]. The origin of Damask rose is the Middle East and some evidences indicate that the origin of rose water is Iran, but the origin of its fragrant oil and extracts is Greece [7]. Rose oil is a highly prized product used in perfumery, cosmetics, food industry and pharmacy [8,9]. Rose species have long been used for food and medicinal purposes in many cultures. Rose hips are used in many foodstuffs and drinks including teas, jellies, jams, and alcoholic beverages [4,10]. As an herbal remedy, rose hips are used in skin care as well as for the treatment of various ailments including colds, flu, inflammations, chronic pain and ulcers [10–12]. In French folk medicine, the rose flower is used as a cure for scurvy and hemorrhoids, as an anthelmintic and fortifying agent. In Bulgaria, the rose flower is still used to cure diseases of the gastrointestinal tract, while in Russia it is recommended for the treatment of lung diseases and infections of the upper respiratory tract [13]. Plant-derived foods contain a broad spectrum of secondary plant metabolites such as

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polyphenols that inhibit human low density lipoprotein oxidation, thus are made responsible for the beneficial effects on human health [14]. *R. damascene* was suggested as a rich source of polyphenols, particularly flavonols, which have been demonstrated to exert antioxidant and free-radical scavengers properties [15–17]. Several components were isolated from flowers, petals and hips (seed-pot) of *R. damascene* including terpenes, glycosides, flavonoids, and anthocyanins [15,18–20]. This plant contains carboxylic acid [21], myrcene [22], kaempferol and quercetin [23]. Flowers also contain a bitter principle, tanning matter, fatty oil and organic acids [24].

Copper (Cu) sulfate is a potent emetic and powerful oxidizing agent causing corrosive damage to mucous membranes [25]. The oxidizing properties of copper sulfate may cause a hemolytic anemia and/or methemoglobinemia. Coma and convulsions ensue in the most severely poisoned patients and fatalities have occurred [26,27]. Clinical manifestations associated with copper poisoning and its pathological features specially in organs such as liver, kidney, spleen, lung and intestine have been well demonstrated in animals [28]. Recently, the adverse effect of copper poisoning on sperm quality has been reported [29]. Attempt has been made to prevent the occurrence of the disease by dietary supplementation with molybdenum and sulfate but despite of the success of these reports, there has been fear of inducing a copper deficiency state [29,30]. According to the reported effects for this plant and regarding to the above-mentioned points about copper poisoning, the aim of the present study was to evaluate supportive effects of *R. damascene* essential oil on epididymal sperm quality and histopathology of testes, following long term administration of copper sulfate in mice.

2. Materials and methods

2.1. Animals

Twenty four sexually mature male NMRI mice were purchased from the animal laboratory of Kerman University of Medical Sciences (KUMS), Kerman, Iran and kept in the center for laboratory animal care at the Veterinary Medicine School of Shahid Bahonar University of Kerman for one week before treatment. The mice weighed 25–30 g and were the same age (1.5–2 months old). The experimental animals were randomly divided into four groups of six animals and were housed in standard polypropylene cages with wire mesh top, at 21 °C in a 12 h/12 h dark–light cycle. During the study, the animals received water and pellet food (Javaneh Khorasan Co, Mashhad, Iran) *ad libitum*. All ethical considerations using animals were considered carefully and the experimental protocol was approved by the ethics committee of KUMS.

2.2. Experiment design

The study comprised of four different groups of six mice as follows: group Cu, which received 0.1 mL copper sulfate (Merck, Germany) solution (in distilled water) at dose of 100 mg/kg [29]. Group R which received 0.1 mL *R. damascene* essential oil (diluted with distilled water) (Barij Essence, Kashan, Iran) at dose of 1 mg/kg [31]. Treatment group (T) which received copper sulfate solution (100 mg/kg) and treated by *R. damascene* essential oil (1 mg/kg), and control group (C) which received the same volume of normal saline. The supplements were gavaged in all animals every other day, during experimental period. All Animals of each experimental group were sacrificed upon diethyl ether anesthesia by cervical dislocation 42 days after the beginning of experiment.

2.3. Sperm quality analysis

Sperm samples were obtained from each group at the end of 6th week. The testes and epididymis were gently excised and weighed and the cauda epididymis were isolated and placed in a Falcon tube containing 2 mL of D-PBS (pH = 7.4, mosm = 295). The tissue of cauda epididymis was minced by using sharp scissors to release spermatozoa. The spermatozoa were allowed to swim out and then incubated for 15 min in an atmosphere of 5% CO_2 at 37 °C, prior to determining sperm quality. Sperm quality was determined by three parameters: sperm concentration, motility, and viability. Sperm concentration was analyzed using the hemocytometer method (World Health Organization 1999) [32]. Sperm suspensions from the caudal epididymis were diluted 1:100 with PBS and transferred into microcentrifuge tubes. The diluted samples were put into the counting chamber and the number of sperm was counted using a hemocytometer with improved doubles Neubauer ruling under a light microscope. The sperm concentration was expressed as $\times 10^6$/mL. Sperm motility was analyzed and averaged by counting the motile and non-motile spermatozoa and expressed as the percent motility. Sperm viability was performed by the Eosin–Nigroson staining. One drop of sperm suspensions was mixed with two drops of 1% Eosin Y. After 30 s, three drops of 10% Nigrosin were added and mixed well. A smear was made by placing a drop of mixture on a clean glass slide and allowed to air dry. The prepared slide was examined. Pink-stained dead sperm and unstained live sperm were counted under the light microscope. The viability of sperm was expressed as the percent of viable spermatozoa.

2.4. Histopathological assays

After necropsy, the testis samples from all the animals of each group were preserved in 10% neutral buffered formalin (Merck, Germany) solution for histological examination. Formalin-fixed samples were processed by the standard paraffin wax technique, and sections of 5 μm thickness were cut and stained with hematoxylin and eosin (H&E).

2.5. Statistical analysis

All data were expressed as mean ± standard error of the mean. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Post hoc Tukey HSD test. A value of ($P < 0.05$) was considered statistically significant.

3. Results

Results of evaluation of sperm quality analysis are presented in Table 1. The data obtained shows that sperm concentration, motility and viability in Cu was significantly decreased ($P < 0.05$) in comparison with control group C during experimental period.