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Asian Pacific Journal of Reproduction
journal homepage: www.apjr.net

Original research http://dx.doi.org/10.1016/j.apjr.2015.12.008

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

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ARTICLE INFO

Article history:
Received 2 Jun 2015
Received in revised form 12 Oct 2015
Accepted 8 Nov 2015
Available online 18 Dec 2015

Keywords:
Rosa damascene
Copper
Sperm quality
Mice

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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Peer review under responsibility of Hainan Medical College.

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.

Table 1

Mean ± SE of the sperm quality parameters (sperm motility, concentration and vitality) following administration of normal saline (C), copper sulfate (Cu), essential oil of *R. damascene* (R) and combination of copper sulfate and essential oil of *Rosa damascene* (T) for 6 weeks in mice.

Groups	Sperm count ($\times 10^6$/mL)	Sperm motility (%)	Sperm viability (%)
Group C	73.60 ± 2.30^{a}	77.00 ± 1.83^{a}	82.30 ± 1.31^{a}
Group R	78.25 ± 3.11^{b}	76.33 ± 1.96^{b}	80.00 ± 2.58^{b}
Group Cu	38.10 ± 0.90^{a,b}	43.60 ± 2.59^{a,b,c}	47.10 ± 2.37^{a,b,c}
Group T	65.00 ± 1.70^{c}	66.40 ± 1.94^{c}	69.00 ± 3.67^{c}

^{a,b,c}Same superscript alphabets in each column show significant difference between the groups ($P < 0.05$).

*A minimum of five microscopic fields were assessed to evaluate sperm motility and vitality on at least 200 sperm for each animal.

However, a complete recovery was observed in *R. damascene* essential oil administered following copper poisoning in comparison with copper group.

Figures 1–4 illustrate histology of testes of animals in different groups. In the control group, seminiferous tubules had organized epithelium including different stages of germ cells (Figure 1). In the testicular sections of Cu group, degenerative changes of seminiferous tubules were observed. Most seminiferous tubules showed disorganization and vacuolation of seminiferous epithelium. Germ cells of seminiferous epithelium had been markedly decreased (Figure 2). In the treatment group, the most seminiferous tubules had normal structure and only few tubules showed mild degenerative changes (Figure 3). In the Rose group, normal histology of seminiferous epithelium was observed (Figure 4).

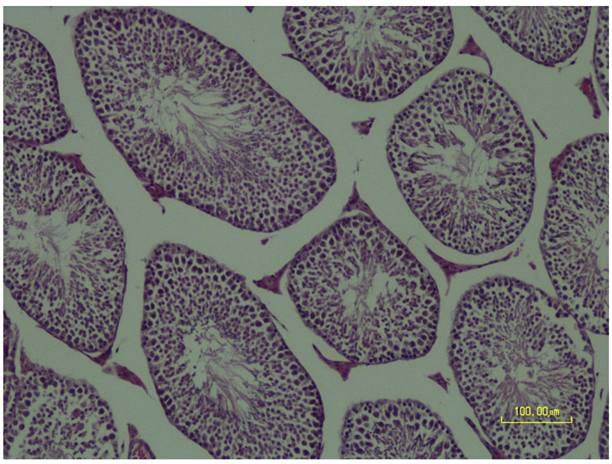

Figure 1. Control group. Normal histology of seminiferous tubules. H&E. Bar = 100 μm.

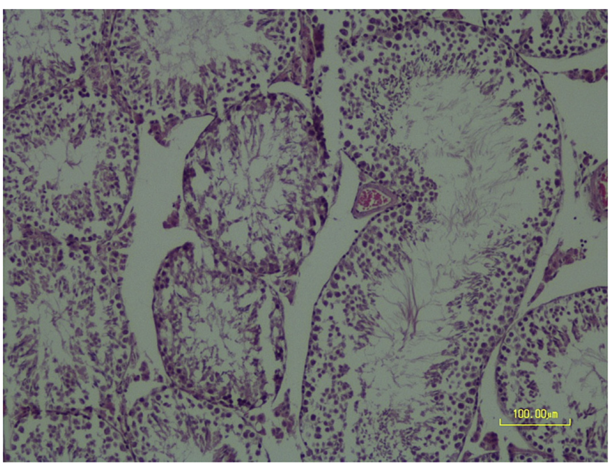

Figure 2. Cu group. Disorganization and vacuolation of seminiferous epithelium. Bar = 100 μm.

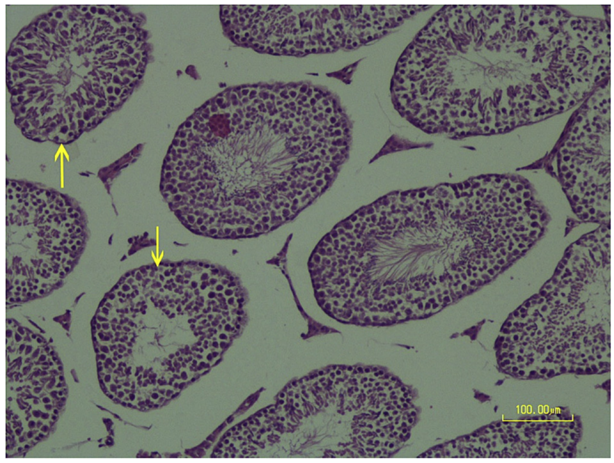

Figure 3. Treatment group. Seminiferous tubules have normal structure and only few tubules show mild degenerative changes (arrows). Bar = 100 μm.

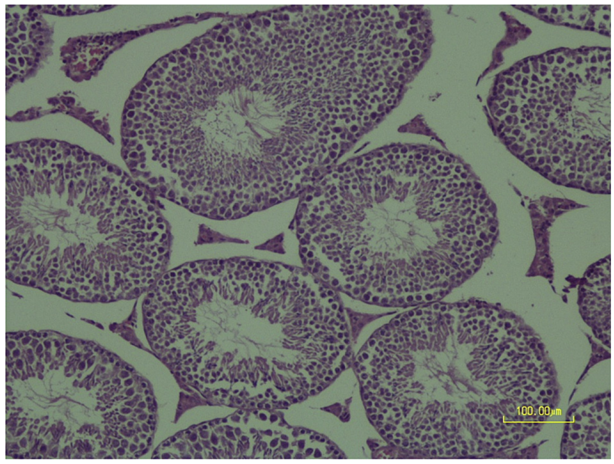

Figure 4. Rose group. Seminiferous tubules show normal structure. Bar = 100 μm.

4. Discussion

Sperm morphology and motility analysis, testis and epididymis weight determination were performed in rats as part of a subchronic dietary study [33]. Aydemir *et al.* [34] showed that copper levels in serum and seminal plasma in the subfertile male group were significantly higher than those in the fertile male group. Results of recent studies have shown a significant decrease in sperm concentration, motility, and viability, also degenerative changes in testicular tissue that indicating the possibility of adverse effect of copper poisoning on the testes and epididymal spermatozoa [32,35]. Histopathological observations in Kheirandish *et al.*, [36] study showed that the long term administration of Cu caused the spermatogenic damage accompanied by reduction in mean diameter of Sertoli cell nuclei in the mice. Spermatozoa are particularly susceptible to peroxidative damage because they contain high concentrations of polyunsaturated fatty acids and also possess a significant ability to generate reactive oxygen species (ROS), mainly superoxide anion and hydrogen peroxide. Superoxide dismutase protects spermatozoa from this peroxidative damage. Oxidative stress caused by accumulated ROS is closely involved in a variety of pathological processes [37]. Results of previous studies show that germ cells are as vulnerable as other cells to the potential detrimental effects of ROS and may thus require antioxidant protection at sites of gamete production, maturation and storage and embryo implantation [37]. Copper might be mediator of the effect of oxidative damage and plays an essential role in spermatogenesis and male infertility.

In the present study, we administered *R. damascene* essential oil as a valuable natural antioxidant to prevent the adverse effect of chronic copper toxicosis on male reproductive organ in mice. Results of previous studies have shown that *R. damascene* essential oil has high antioxidant activities. Sakhaee *et al.*, [31] showed beneficial effects of *R. damascene* essential oil on mice ovarian tissue, following experimentally induced copper poisoning. They observed ovarian damages in different types of follicles and various ovary components in copper group and showed that *R. damascene* essential oil treatment group had less toxic ovarian damage and few atretic follicles with degenerative changes and apoptotic cells.

Sakhaee *et al.*, [35] evaluated supportive effects of vitamin C as an antioxidant on testes following long term administration of copper sulfate in mice. They confirmed shrinkage and collapse of tubules, decreased number of germinal cells and decrease in epithelial height in testes of Cu group, while, vitamin C as an antioxidant group showed normal morphology with presence of spermatozoa in their lumen.

Results of present study show that treatment by *R. damascene* essential oil in mice, involved in cooper toxicity; significantly repaired epithelium of seminiferous tubules. Furthermore, results of sperm quality analysis revealed that sperm concentration and motility and sperm vitality improved compared to the group Cu. It seems that three flavonol glycosides including quercetin-3-O-glucoside, kaempferol-3-O-rhamnoside and kaempferol-3-O-arabinoside induced protective effects.

According to the results of the present study, we conclude that administration of the essential oil of *R. damascene* owning to its antioxidant properties is able to protect the testis and epididymal sperm from the adverse effects of copper poisoning in mice. Considering the nontoxic and safe nature of *R. damascene* essential oil, it can be one of the suitable choices for preventive therapeutic effects and/or improvement in testicular tissue after Cu toxicosis.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This research was financially supported by the research council of Shahid Bahonar University of Kerman, Iran (grant No. 94/142).

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