Full Length Research Paper

Adverse effects of *Zataria multiflora* boiss on epididymal sperm quality, and testicular tissue following experimentally induced copper poisoning in mice

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Objective: The aim of the current study was to evaluate adverse effects of *Zataria multiflora* essential oil on epididymal sperm quality, and testicular tissue following experimentally induced copper poisoning in mice. Methods: The study comprised of four different groups of twelve mice as follows: Group N (Normal) and group Cu (Copper) received pure drinking water, while Groups Z (*Zataria multiflora*) and T (*Zataria multiflora* treated animals) received *Zataria multiflora* essential oil (800 ppm, per day) during experiment period in drinking water. Group Cu and T treated by gavage with copper sulfate at a dose of 200 mg/kg/day (0.2 ml) for 6 weeks, while group N and Z received the same volume of distilled water by gavage during experiment period. Results: The data obtained shows that sperm concentration, motility and viability in treatment group were significantly decreased (p < 0.05) in comparison with group Cu during experimental period. The testes of group T showed varying degrees of degenerative changes of seminiferous tubules and spermatozoa, and were lined by sertoli cells, spermatogonia, and primary spermatocytes. Conclusion: Results showed that *Zataria Multiflora* essential oil at a dose of 800 ppm has synergist toxic effect with copper sulfate at a dose of 200 mg/kg/day.

Keywords: *Zataria multiflora*, Copper, Mice, Sperm quality, Histopathology, Testes

INTRODUCTION

Copper is an essential trace element that is widely distributed in animal tissues. It is a component of a number of metalloenzymes such as catalase, peroxidases, and cytochrome oxidase, and is essential for the utilization of iron (Goyer et al., 1991; Stokinger, 1981). Copper sulfate is the most common copper salt; however, other important copper salts include carbonate, cyanide, oxide, and sulfide (Rosmarie, 1992). Copper can be absorbed into the systemic circulation from the gastrointestinal tract, the lungs, and skin (Rosmarie, 1992). The gastrointestinal absorption of copper is influenced by a number of factors, including its chemical form: soluble copper compounds (oxides, hydroxides, citrates and sulfate) are readily absorbed but water-insoluble compounds (sulfides) are poorly absorbed (Venugopal and Luckey, 1978). Absorbed copper binds to plasma albumin and amino acids in the portal blood and is transported to the liver where it is incorporated into ceruloplasmin and later released into the plasma (Rosmarie, 1992). Age, sex, amount of dietary copper, and overall health determine the amount of copper distributed to the various tissues (Rosmarie, 1992).

Exposure to environmental contaminants has been suggested to play a role in the pathophysiology of adverse reproductive health effects including decreased semen quality, sub-fertility, change in birth sex ratio, and an increase in the prevalence of developmental abnormalities of the male reproductive tract (Carlsen et al., 1992; Swan et al., 2000).

Many herbal concoctions are said to be effective in
chronic inflammatory conditions. Labiatae are generally known for their various effects such as analgesic and anti-inflammatory activities (Hernandez-Perez et al., 1995), antioxidant (Abdollahi et al., 2003; Cuppett and Hall, 1998; Giralut et al., 1998), hepatoprotective (Dawson et al., 1990; Salem et al., 2001; Wasser et al., 1998) and hypoglycemic actions (Hosseinzadeh et al., 1998).

Zataria multiflora is a valuable medicinal plant owned by the Labiatae family that is distributed only in Iran, Pakistan and Afghanistan (Ali et al., 2000). It is extensively used for medicinal and condimental purposes in these countries. This plant with the vernacular name of "Avishan Shirazi" in Iran has several traditional uses such as antiseptic, anti-fungal, antispasmodic, analgesic, antitussive, anti-inflammatory, antioxidant, antinociceptive, hypoglycemic and carminative properties (Hosseinzadeh et al., 2000; Mohagheghzadeh et al., 2004; Ramezani et al., 2004). There are also commercial pharmaceuticals with formulae based on Zataria multiflora essential oil. The chemical compositions of extracts have been extensively characterized in Iran (Javidnia et al., 1999; Mohagheghzadeh et al., 2004) and Pakistan (Saleem et al., 2004). The extract contains thymol, carvacrol zatrinal, oleanolic acid, betulic acid, rosmarinic acid (Mohagheghzadeh et al., 1999 and 2000; Saleem et al., 2004) and monoterpenoids, sesquiterpenoids, p-cymene and γ-terpinene (Mohagheghzadeh et al., 1999; Rosmarie, 1992). The aim of the current study was to evaluate adverse effects of Zataria multiflora on testular tissue following experimentally induced copper poisoning in mice.

**MATERIALS AND METHODS**

**Animals**

Forty eight 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 Faculty of Shahid Bahonar University of Kerman for 1 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 twelve 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, Iran) ad libitum. All ethical considerations using animals were considered carefully and the experimental protocol was approved by the Ethics Committee of KUMS.

**Experimental design**

The study comprised of four different groups of twelve mice as follows: Group N (Normal) and group Cu (Copper) received pure drinking water, while Groups Z (Zataria multiflora) and T (Zataria multiflora treated animals) received Zataria multiflora essential oil (800 ppm, per day) during experiment period in drinking water. Group Cu and T treated by gavage with copper sulfate at a dose of 200 mg/kg/day (0.2 ml) for 6 weeks, while group N and Z received the same volume of distilled water by gavage during experiment period.

**Sperm quality analysis**

Sperm samples were obtained from each group at the end of 6th week. Samples of mature sperm were collected from the cauda region of epididymis by mincing it finely in PBS at 37°C. Sperm quality was determined by three parameters: Sperm concentration, motility and viability.

Sperm concentration was analyzed using the haemocytometer method. Sperm suspensions from the caudal epididymis were diluted 1:200 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 haemocytometer 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 sec, 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.

**Histopathological assays**

After necropsy, the testes of each group were preserved in 10% neutral buffered formalin solution for histological examination at the end of 6th week. Formalin-fixed samples were processed by the standard paraffin wax technique, and sections of 5 m thickness were cut and stained with hematoxylin and eosin (H&E).
Table 1. Adverse effects of *Zataria multiflora* on epididymal sperm concentration, motility, and viability following copper poisoning in mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Concentration (× 10⁶ / ml)</th>
<th>Motility (%)</th>
<th>Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>44 ± 3.23</td>
<td>26 ± 2.51</td>
<td>31 ± 1.44</td>
</tr>
<tr>
<td>Z Group</td>
<td>88 ± 2.19</td>
<td>86 ± 3</td>
<td>87.3 ± 2</td>
</tr>
<tr>
<td>Cu Group</td>
<td>62.25 ± 2.48</td>
<td>74 ± 1.01</td>
<td>80 ± 2.67</td>
</tr>
<tr>
<td>Normal Group</td>
<td>90 ± 2.64</td>
<td>87 ± 3.1</td>
<td>92 ± 2.17</td>
</tr>
</tbody>
</table>

1Data are expressed as means ± SE; the same symbols (a, b and c) for each parameter show statistical significance between the groups.

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.

RESULTS

Results of evaluation of sperm quality analysis are presented in Table 1. The data obtained shows that sperm concentration, motility and viability in Treatment group were significantly decreased (p < 0.05) in comparison with groups Cu and N during experimental period, and there is no significant difference between Z and N groups.

Figures illustrate histology of testes of animals in different groups. The testes of group C and Z showed normal seminiferous tubules with all stages of germ cells from spermatogonia to spermatozoa (Figure 1). The testes of group T showed varying degrees of degenerative changes of seminiferous tubules. Some tubules were shrunken with sloughed cell and vacuolation of epithelium (Figure 2). Some tubules lacked spermatids and spermatozoa, and were lined by sertoli cells, spermatogonia, and primary spermatocytes (Figure 3).
DISCUSSION

Results of current study show a significant decrease in sperm concentration, motility and viability indicate the possibility of Adverse effects of Zataria multiflora Boiss on epididymal spermatozoa of mice, following experimentally induced copper poisoning.

The pathological manifestations observed during a toxicological study are indicative of copper toxicosis (Stalker and Hayes, 2007), similar results have been described in naturally occurring cases in young animals (Devoy, 2002; Hamar et al., 1997; Steffen et al., 1997; Sullivan et al., 1991).

The data obtained show that copper sulfate at a dose of 200 mg/kg/day plus Zataria multiflora essential oil at a dose of 800 ppm, after 6 weeks caused frank testicular atrophy correspondingly reduced sperm density in epididymal lumen, lowered motility, and induced structural abnormalities in sperm. The deterioration of seminiferous germinal epithelium or a spermicidal effect of copper sulfate may be responsible for such effects.

A more recent study in rats found that tetrathiomolybdenum caused a reduction in epididymal weights, sperm concentration, motility, and normal morphology at high dose levels (Lyubimov et al., 2004). Interestingly, dietary copper supplementation prevented the adverse effects on sperm at the same high molybdenum dose levels in the study [(Lyubimov et al., 2004)]. Meeker et al. (2008) found evidence of an inverse association between high copper levels and semen quality, which is consistent with a number of animal and human studies (Massanyi et al., 2004; Meeker et al., 2008; Yuyan et al., 2007).

Flavonoids as the main constituent of Zataria multiflora extract are a class of plant phenolics with significant antioxidant and chelating properties. Their positive effects come from their ability to inhibit lipid peroxidation, chelate redox-active metals and attenuating other procedure involving reactive oxygen species [(Le Marchand, 2002)]. There is evidence that flavonoids have anti-phosphodiesterase activity and thus could elevate intracellular levels of cyclic nucleotides (Abdollahi et al., 2003). Recent studies well indicate that both cAMP and cGMP can diminish oxidative stress in many biological systems and diseases (Yuyan et al., 2007). Sharififar and colleagues showed that oxidation of linoleic acid was effectively inhibited by Zataria multiflora extract. It seems that this activity is mostly related to the presence of the phenolic compounds such as flavonoids and phenolic acids in this extract (Sharififar et al., 2007).

Sakhaee et al., showed that Zataria multiflora essential oil at a dose of 800 ppm has adverse effect on hepatocytes in spite of its antioxidant effects (unpublished data). It seems that mentioned over dose may invert beneficial effects of Zataria Multiflora. Results of present study show that Zataria Multiflora essential oil has synergist toxic effect with copper sulfate on testes.

CONCLUSION

In conclusion, we have determined that Zataria Multiflora essential oil at a dose of 800 ppm has synergist toxic effect with copper sulfate at a dose of 200 mg/kg/day and may increase effects on testes.

ACKNOWLEDGEMENTS

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REFERENCES

Abdollahi M, Chan TS, Subrahmanyam V, O’Brien PJ (2003). Effects of
phosphodiesterase 3,4,5 inhibitors on hepatocyte cAMP levels, glycosylation, gluconeogenesis and susceptibility to a mitochondrial toxin. Mol Cell Biochem. 252: 205-211.


