Time and dose-dependent adverse effects of four medicinal plants on male reproductive system

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Original Article

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ABSTRACT

Introduction: the point of the present survey was to evaluate time and dose-dependent adverse effects of four restorative herbs on male reproductive system.

Methods: the study involved of seventeen distinctive gatherings of ten mice as takes after: group C1 to C4 (Cuminum cyminum), which received 0.1 ml Cuminum cyminum essential oil at dosage of 0.1, 1, 10 and 100 mg/kg/day, respectively. Group R1 to R4 (Rosa damascene), which received 0.1 ml Rosa damascene essential oil at dosage of 0.1, 1, 10 and 100 mg/kg/day, respectively. Group Z1 to Z4 (Zataria multiflora), which received 0.1 ml Zataria multiflora ethanolic extract at dosage of 100, 200, 400 and 800 mg/kg/day, respectively. Group O1 to O4 (Origanum vulgare), which received 0.1 ml Origanum vulgare ethanolic extract at dose of 100, 200, 400 and 800 mg/kg/day, respectively and finally group Normal which got similar amount of normal saline.

Results: the results demonstrated that sperm density, mobility and viability in groups R4, O4, Z2, Z3, Z4, C2, C3 and C4 after 4 weeks were altogether diminished in comparison with normal group.

Discussion: according to the results, mentioned medicinal plants should not be used frequently for prolonged duration.

Key Words: Sperm quality, Medicinal plant, Adverse effects, Mice.

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INTRODUCTION

Cuminum cyminum is a fragrant yearly plant belonging to the Apiaceae family which is generally developed in Iran; specifically, in dry and semi-dry locals. Cuminum cyminum is used to flavor foods, for pharmaceutical and medical preparations, food industries and added to fragrances[1]. In Iranian folk medicine, the fruits of this plant have been used to treat diarrhea, toothache and epilepsy[2]. The main contents occurring in Cumnum cyminum are cuminaldehyde, 1, 8-cineole, o and p-cymene, α and β-pinene, α and γ-terpinene, limonene, safranal and linalool[3].

Rosa damascene belongs to the family Rosaceae and genus Rosa and is a fragrant, light pink plant with connotation from economical and research perspective[4]. Rosa damascene commonly known as Damask rose[5] is known as Gole Mohammadi in Iran[6]. This plant is developed initially in focal part of Iran in Kashan Province for get ready rose water and perfume[4]. The Damask rose is originally in the Middle East and some evidences demonstrate that the starting point of rose water is Iran, however the source of its fragrant oil and extracts is Greece[2]. Rose oil is an exceptionally prized item utilized as a part of perfumery, beautifying agents, food industry and drug store[7]. Rosa damascene was offered as a rich wellspring of polyphenols, especially flavonols, which have been exhibited to apply antioxidant and free-radical scavenger's properties[8, 9]. This plant inclusive carboxylic acid[10], myrcene[11], vitamin C[12], kaempferol and quercetin[13]. Flowers likewise inclusive a bitter principle, tanning matter, fatty oil and organic acids[14].

Zataria multiflora is a valuable medicinal plant in the Labiatae family that is appropriated just in Iran, Pakistan

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and Afghanistan[15]. It is widely utilized for therapeutic and flavouring purposes in these nations. This plant, is known to "Avishan Shirazi" in Iran, has various customary uses, for example, anti-inflammatory, antiseptic, antioxidant, antinociceptive, anti-fungal, analgesic, antitussive and hypoglycemic properties[16]. There are additionally business medicine with formulae in view of Zataria multiflora essential oil[15]. The extract contains zatrinal, thymol, carvacrol, oleanolic acid, betulic acid, sesquiterpenoids, rosmarinic acid and also monoterpenoids, γ-terpinene and p-cymene[17].

The genus Origanum (Labiatae) is a yearly, enduring and shrubby herb that is local to the Mediterranean, Euro-Siberian and Irano-Siberian regions[18]. A total 38 Origanum species are known in the World. Origanum species grow abundantly on stony slopes and in rocky mountain areas at a wide range of altitudes (0–4000 m)[19]. Due to the variability in chemical and aroma characteristics, Origanum plants belonging to different species and ecotypes (biotypes) are generally utilized as a part of agribusiness and the medicine and cosmetic industries as a culinary herb, seasoning materials of food products, alcoholic drinks and perfumery for their spicy aroma[18, 20- 22]. It has additionally been utilized as a conventional treatment to regard different illnesses, for example, an antimicrobial, carminative, expectorant and aromatic for whooping, convulsive coughs, digestive disorders and menstrual problems[18, 23- 28].

PATIENTS AND METHODS

Animals:

A total of one hundred and seventy sexually mature male albino mice of strain NMRI aged 2 months and average weight 28 ± 3 g were used. The mice were randomly classified into seventeen groups of ten animals and kept in stainless steel cages at 22 ± 2 °C in dark-light cycles of 12:12 h and fed under standard lab diet.

Experimental design

The animals divided into seventeen experiment groups of ten as follows: group C1 to C4 (Cuminum cyminum), which received 0.1 ml Cuminum cyminum essential oil at dose of 0.1, 1, 10 and 100 mg/kg/day, respectively. Group R1 to R4 (Rosa damascene), which received 0.1 ml Rosa damascene essential oil at dose of 0.1, 1, 10 and 100 mg/kg/day, respectively. Group Z1 to Z4 (Zataria multiflora), which received 0.1 ml Zataria multiflora ethanolic extract at dose of 100, 200, 400 and 800 mg/kg/day, respectively. Group O1 to O4 (Origanum vulgare), which received 0.1 ml Origanum vulgare ethanolic extract at dose of 100, 200, 400 and 800 mg/kg/day, respectively and finally group normal which received the same volume of normal saline. The mice were gavaged every day by supplements, during experiment. Five mice in each group were sacrificed at week 4 and the other five at week 6.

Sperm quality analysis

For epididymal sperm preparation, semen samples were taken from each group after 30 and 45 days. Caudal region of epididymis of each mouse was minced finely in phosphate buffer solution at 37°C. Sperm quality was determined by three parameters: Sperm concentration, motility and vitality.

In order to analyze sperm concentration using a Neubauer slide, sperm suspensions were diluted 1:200 in phosphate buffer solution. The mixture was put into the counting chamber and counted using a haemocytometer slide under a light microscope. The sperm concentration was represented as ×106.ml-1. To evaluate sperm motility, the suspension was placed on a preheated slide and the motile and non-motile spermatozoa observed under a light microscope. In order to evaluate sperm viability, 10 µL of sperm suspensions was mixed 20 µL of 1% Eosin Y – nigrosin. After one min of incubation at room tempreture, slides were observed under light microscope. Dead sperms were stained pink but the live ones took no color. The vitality of sperm was represented as the percent of viable spermatozoa.

Statistical analysis

Results were shown as mean ± standard error mean for each group and analyzed by SPSS (Version 16; SPSS Inc., Chicago, USA), using one-way analysis of variance (ANOVA), followed by Tukey HSD test as post hoc. All data were assessed at $p < 0.05$ and considered statistically significant.

RESULTS

Results of assessment of sperm analysis have been presented in Table 1 to 4. The data show that sperm vitality, motility and count in group C3 and C4 after 30 days and group C2 after 45 days were significantly decreased ($p < 0.05$) compared to the normal group. Mentioned factors in group O4 and R4 after 45 days were significantly ($p < 0.05$) decreased, and finally, the results (Table 4) revealed that sperm vitality, motility and count were decreased significantly ($p < 0.05$) after administration of Zataria multiflora ethanolic extract at dose of more than 200 mg/kg/day at 4th week compared to the normal group.

Table 1: Time and dose-dependent adverse effects of Cuminum cyminum essential oil on epididymal sperm concentration, motility, and viability in mice.

	Week 4			Week 6		
Groups	Count (×106/ml)1	Motility (%)1	Vitality (%)1	Count (×106/ml)1	Motility (%)1	Vitality (%)1
Normal	84±8.7	81.6±1.43	75.2±2.37	86.4±5.6	77.6±1.12	79.0±2.88
C1	81.3± 1.14	80±3.1	75.6± 2	83.5±0.9	71±3.3	69.4± 1.1
C2	59.2±7.7*	62.0±7.18*	52.0±3.71*	51.6±12.5*	63.0±6.63*	61.4±3.31*
C3	48.7±4.4*	56±6.1*	50.4±2.5*	43.9±7.6*	60.6± 3.7*	54.1± 4.5*
C4	42.8±5.2*	52.3±1.6*	49.3±6.9*	37.4±2.5*	53.6±3.7*	49±4.4*

1: Data were expressed as means ± SE
*: Statically significant difference from respective control, p <0.05.

Table 2: Time and dose-dependent adverse effects of Rosa damascene essential oil on epididymal sperm concentration, motility, and viability in mice.

	Week 4			Week 6		
Groups	Count (×106/ml)1	Motility (%)1	Vitality (%)1	Count (×106/ml)1	Motility (%)1	Vitality (%)1
Normal	84±8.7	81.6±1.43	75.2±2.37	86.4±5.6	77.6±1.12	79.0±2.88
R1	79.1±1	81.1±2.8	77.3±4.2	83.9±6.5	72±4.11	77.8±5.9
R2	85.4±2.9	80.3±7.3	76.9±1.5	82.7±4.9	73.5±4	81.64±4.3
R3	80.11±4.7	76±3.3	69.5±3.9	77.3±5.1	75.2±2	73.32±7.2
R4	63.7±4.8*	71±3.61*	65±1.54*	41.4±8.7*	58.6±3.9*	53.2±7.39*

1: Data were expressed as means ± SE
*: Statically significant difference from respective control, p <0.05.

Table 3: Time and dose-dependent adverse effects of Origanum vulgare ethanolic extract on epididymal sperm concentration, motility, and viability in mice.

	Week 4			Week 6		
Groups	Count (×106/ml)1	Motility (%)1	Vitality (%)1	Count (×106/ml)1	Motility (%)1	Vitality (%)1
Normal	84±8.7	81.6±1.43	75.2±2.37	86.4±5.6	77.6±1.12	79.0±2.88
O1	83.74±0.34	82.7±4	76.1±3.8	82.22±3.1	74.8±6.14	71.4±3.3
O2	79.5±3.3	82±5.44	73.9±1.1	85.6±4.9	77.7±4.1	70±1.9
O3	88.31±5.5	83.7±5.33	75±4	86.1±9.4	75.7±3.2	81.4±6.1
O4	71.44±1.9*	79.3±3.8	66±6.57*	64.5±0.88*	59.4± 5.99*	57±4.43*

1: Data were expressed as means ± SE
*: Statically significant difference from respective control, p <0.05.

Table 4: Time and dose-dependent adverse effects of Zataria multiflora ethanolic extract on epididymal sperm concentration, motility, and viability in mice.

	Week 4			Week 6		
Groups	Count (×106/ml)1	Motility (%)1	Vitality (%)1	Count (×106/ml)1	Motility (%)1	Vitality (%)1
Normal	84±8.7	81.6±1.43	75.2±2.37	86.4±5.6	77.6±1.12	79.0±2.88
Z1	82.4±3	79.6±7.2	77±3.13	85.3±3.3	74.11±5.1	73±9.7
Z2	73.7±9*	63±2.2*	71.4±4.5	69.8±3.9*	61±8*	70.5±6.6*
Z3	52.45±3*	68.8±3.1*	60.7±7.6*	58.5±4.9*	62.1±5.3*	55.7±3.2*
Z4	46.9±12*	51.5±11.4*	53.4±9.54*	39.3±14*	49.1±4.8*	48.3±9.7*

1: Data were expressed as means ± SE
*: Statically significant difference from respective control, p <0.05.

DISCUSSION

Cuminum cyminum, Rosa damascene, Zataria multiflora and Origanum vulgare are generally utilized as an herbal remedy. Potential unfavorable impacts of herbal drugs have received intense attention in recent years. Although most body organs can be affected by drugs and toxins, liver and kidney are of greatest importance in this regard[29 -32].

To the best of the authors' knowledge, there are not many documented reports about toxic effects of O. vulgare. Many researchers evaluated antimicrobial effects of the herb. However, essential oils and aqueous and alcoholic extracts of mentioned plant are used for food preservation or medicinal purposes[33]. Benavides, *et al*. (2010) showed O.vulgare extract have no toxic effects on development and morphology of pre-implantation mouse embryo, but dose enhancement reveal a significant delay in the formation of blastocysts [34]. However, our study confirmed male reproduction toxicity of extraordinary dose of the extract after 6 weeks administration.

Results of previous researches have been shown that Zataria multiflora Boiss essential oil at dosage of 800 ppm has unfavorable impacts on hepotocytes despite of its antioxidant impacts[35]. Our study confirms the adverse effect of this medicinal plant and our results demonstrated a meaningful diminishing in sperm density, mobility and viability indicate the possibility of toxic effects of Zataria multiflora on male reproductive function.

Ali Esmail Al –Snafi in his review stated that the therapeutic dose of extract of Cuminum cyminum in mice is safe and there are no documented reports about adverse effects of it in mentioned dose in rat. While, the consequences of another study showed ill thriftiness, hepato-encephalopathy and anemia due to great dose of mentioned oil administration in rat[36]. Our results confirm that unfavorable impacts of mentioned oil at a dose of more than 10 mg/kg on function of male reproductive organ in mice after four weeks.

A preliminarily study on toxic effect of Rosa damascene extract on dogs was carried out by Akbari, *et al.* Consequences of the study indicated nephrotoxicity and hepatotoxicity at extraordinary high doses. Present research stated that Rosa damascene extract at a dose of higher than 100 mg/kg/day after 42 days reduce sperm density, mobility and viability in mice.

Flavonoids as the primary constituent of all of the plants in question are a class of plant phenolics with noteworthy antioxidant and chelating attributes. It seems that extraordinary doses of these medicinal plants may reverse useful impacts of them, because of limitation of detoxifying activity of hepatocytes.

Results of previous studies show that spermatozoa are especially helpless to peroxidative harm since them inclusive high concentrations of polyunsaturated fatty acids furthermore have a critical capacity to produce reactive oxygen species, predominantly superoxide anion and hydrogen peroxide. Oxidative stress created by gathered respective oxygen species required in an assortment of pathological processes. Germ cells are as defenseless as different cells to the potential unfavorable impacts of reactive oxygen species[37].

According to the consequences of the current research, it seems that overdose administration of Cuminum cyminum, Rosa damascene, Zataria multiflora and Origanum vulgare have poisonous impacts on epididymal sperm quality in mice due to their oxidative effects.

CONCLUSION

We deduced that all previously mentioned plants caused oxidative damages, therefore theses medicinal plants should not be used frequently for prolonged duration. In order to elucidate possible safe dose low concentration of these remedies should be tested.

ETHICAL APPROVAL

All ethical considerations utilizing animals were considered conscientiously, furthermore, the trial convention was affirmed by the Ethics Committee of KUMS, Kerman, Iran.

CONFLICT OF INTEREST

There are no conflicts of interest.

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