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The effects of *Mentha crispa* on the spermatogenesis of wild rats (*Rattus norvegicus*) from Sudan

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Abstract

The health benefits and inadequacies of the *Mentha* species have already been demonstrated, but spearmint quality and composition vary in different species, places and still has to be studied. In Sudan, peppermint (*Mentha crispa*) is widely consumed for different purposes, but has not been used as a male infertility contraceptive agent. The objective of this study was to examine the effects of this plant on the body and vital organ weights of wild rats (*Rattus norvegicus*; n= 30), as well as sperm parameters and spermatogenesis, based on the administration of an aqueous extract of *M. crispa* for 60 days.

The results of the present study showed that the average body weight decreased significantly in the experimental groups, for animals that received 50 and 100 mg/kg/rat of *M. crispa* extract, as compared with the control group that received only distilled water. There was no significant difference (p>0.05) in the kidney, spleen, heart and liver weight of rats, as well as in the reproductive organs (testis, epididymis, prostate and seminal vesicle) in the experimental groups. However, there was a significant difference (p> 0.05) in sperm count, sperm viability and sperm motility (%) in the experimental groups.

These findings suggest that the aqueous extract of *M. crispa* has inhibitory effects on male spermatogenesis (sperm phenotype and quality) which could be useful in the development of male infertility contraceptive agents and free rodenticides, in the sustainable management of rodents.

Keywords: *mentha crispa, rattus norvegicus* male, spermatogenesis, contraceptive agent, rodent management

1. Introduction

Mentha is a common name for mint plant, and is also known as peppermint, lamb mint or brandy mint. The plant is perennial, grows to a height between50 to90 cm, and is a genus of the family Lamiaceae [6]. It is estimated that 13 to 18 species exist but the exact distinction between species is still unclear, as hybridization occurs naturally between some species [12]. The different species (such as *Mentha cervina*, *M. pulegium*, *M. longifolia*, *M. spicata*, *M. crispa*, *M. viridis* and *M. Piperita*) have great importance in folk medicine, and are used worldwide from traditional medicinal plant stores to local markets [9, 10]. The genus has worldwide distribution across Europe, Africa, Asia, Australia and North America. The plant's main active components are essential oil, phenolic acids and flavonoids [6]. Its essential oil contains the principal active ingredients of Mentha namely menthol, menthone and menthyl acetate. Menthyl acetate is responsible for peppermint's minty aroma and flavor, while menthol provides the cool sensation of the herb. The menthol content of peppermint oil determines the quality of its essential oil which varies between species. These variations depend on the climate, habitat, and where the peppermint is endemic. Peppermint also contains vitamins A and C, magnesium, potassium, inositol, niacin, copper, iodine, silicon, iron and sulfur. Another constituent known as rosmarinic acid (RA) plays a role in modulating inflammatory diseases including allergies, asthma and atherosclerosis [3, 2, 15]. However, Mentha plants are commonly used in the treatment of appetite loss [17]. vomiting [3]. And spasmodic responses [10].

Many studies have investigated the health benefits of the *Mentha* species and various volatile components have been analyzed and isolated from them. However, evidences show that despite its beneficial effects, spearmint quality and composition vary in different species and may result in several shortcomings [2]. Showed that spearmint herbal tea has adverse effects on testicular tissue and testosterone level, and alters the level of follicle stimulating hormone (FSH) and luteinizing hormone (LH). Other studies confirmed that the volatile extracts of *M. arvensis* contain menthol, muurolol, eugenol, thymol, ketones (methyl acetate and jasmonate) and hydrocarbons (dihydroactinidiolide, germacrene D and calamenene). In *M. piperita* leaves, menthol and myrcene have been identified as key volatile components. The above mentioned phenolic components are responsible, at least in part, for the antioxidant and antiperoxidant effects. Previous studies have demonstrated the toxic and adverse effects of *Mentha*. In addition, the severe biochemical and histopathological effects of *M. piperita* and *M. spicata* on the kidney, liver and uterine tissues of mice have been documented by previous authors.

In Sudan, the Family Labiatae; genus Mentha includes several species (*M. piperita*, *M. crispa*, and *M. viridis*), and *M. crispa* is number one among the fifty-one most widely used plants in the country [17].

This study assessed and evaluated the potential effects of Mentha (*M. crispa*, family: Lamiaceae) and showed its effects on the body organ weights of rat (e.g total body weight, liver, kidney, spleen and heart weights) and spermatogenesis. This study involved the testing of mint products from Sudan which may help in improving rural employment, supplying local industries and generating export revenues in the region.

2. Materials and methods

Experimental animals

Young wild rats, *Rattus norvegicus* (n=30) were used in the present study. The captured rodents were examined for rodent species identification by external morphological measurements, and physical appearances. Only young adult males of *R. norvegicus* were transported to the animal facility of the Sudan Natural History Museum of Khartoum University, Sudan for further study. The captured male adult rats with average weight of 150-200 g were kept under standard condition (temperature 25°C, humidity 40-70% and 12 h/12 h light/dark cycle). They were divided into three experimental groups, each with 10 rats as follows: control group (Group I) and experimental groups (Groups II and III). They had free access to food (standard rat diets) but the daily intake of water was monitored for at least one week before commencing treatments to determine the amount of water needed per experimental animal. Thereafter, the control group received 8 ml of distilled water daily. However, the experimental groups: Groups II and III, received 50 and 100 mg/kg/rat of aqueous solution of *M. crispa*, respectively, for 60 consecutive days.

Sample collection and numerical measurements

The rats were sacrificed after anaesthesia by using diethyl ether. The right and left testes were quickly removed. Then, the epididymis were carefully separated from the testes and put in labeled tubes. Thereafter, the weight of the testes and epididymis were measured together and separately according to [13]. By using a digital Vernier caliper and a digital balance (Milton ® UK balances), respectively. The sperm suspension was drawn into a white blood cell pipette and diluted to 1:100 with proteolytic enzyme solutions, followed by transfer into 2 ml of a medium containing 0.5% bovine serum albumin and incubated at 37°C (with 5% CO_2), the cauda epididymis sperm concentration was determined using the standard hemocytometric method. Also, the sperm morphology, viability and motility were analyzed with a microscope (Olympus IX70) at 10 field and reported as the mean of motile sperm, according to Wells and Awa (1970) and WHO method [19].

Statistical analysis

Statistical analyses were conducted using the ANOVA test for comparison of data in the control and experimental groups. The difference in means were considered significant at $p<0.05$.

3. Results

Body and organ weights of rats

The results obtained for the body and organ weights of rats after 60 days treatment with aqueous *M. crispa* leaves are illustrated in Table 1. The average body weight decreased significantly ($p<005$) in the experimental Group I treated with 50 mg/kg/rat, and there was no significant increase in Group II rats treated with 100 mg/kg/rat as compared with the control group. However, there were no significant differences ($p>0.05$) between the experimental groups, in terms of the kidney, spleen, heart and liver weights of rats.

Table 1: Body and organ weights after 60 days treatment with aqueous *Mentha piperita* leaves extract in male rats, *Rattus norvegicus*. Fertility of male mice in the control (Group I with distilled water) and experimental groups (Groups II and III with 50 and 100 mg/kg b.wt/day, respectively).

Weight	Group I (8 ml dH_2O/day)	Group II (LD 50 mg/Kg/day)	Group III (HD 100 mg/Kg/day)
Body weight	151.00±1.69	136.00±0.93*	142.00±0.84
Kidney weight	0.94±0.10	0.93±0.21	1.00±0.34
Spleen weight	0.90±0.12	0.92±0.14	0.96±0.11
Heart weight	0.81±0.09	0.78±0.07	0.82±0.81
Liver weight	3.20±0.17	3.35±0.18	3.55±0.12

The effects of *Mentha piperita* aqueous extracts on the reproductive organs of rats

Table 2 shows the effects of *M. crispa* aqueous extracts on the reproductive organ weights of male rats. The results obtained show no significant difference ($p>0.05$) in the testes, epididymis, prostate, and seminal vesicle weights ($p>0.05$) for both experimental groups, as compared with the control group.

Table 2: The reproductive organ weights in grams after 60 days treatment with aqueous *Mentha piperita* leaves extract in male rats, *Rattus norvegicus*. The fertility of male mice in the control (Group I with distilled water) and experimental groups (Groups II and III with 50 and 100 mg/kg/day, respectively).

Parameter	Group I (8 ml dH_2O/day)	Group II (LD 50 mg/kg/day)	Group III (HD 100 mg/kg/day)
Testis weight	0.67±0.12	0.67±0.38	0.63±0.60
Epididymis weight	0.62±0.09	0.60±0.01	0.61±0.09
Prostate weight	0.61±0.09	0.70±0.07	0.70±0.04
Seminal volume	0.06±0.10	0.06±0.02	0.059±0.01

LD and HD represent low and high doses (50 and 100 mg//kg/day, respectively). * Significantly different at $p < 0.05$ level.

The effects of *M. crispa* aqueous extracts on sperm, motility, viability and counts

Animals treated with 50 and 100 mg/kg/day aqueous extract of *M. crispa* leaves, for 60 consecutive days, showed significant ($p< 0.05$) decrease in sperm count, sperm viability and sperm motility in the experimental groups (Groups II and III) as compared with the control group. However, the sperm count, viability and motility in the control group were significantly higher in comparison with the experimental groups (Table 3).

Table 3: Fertility of male rats, *Rattus norvegicus* after 60 days of treatment with aqueous extract of *Mentha piperita* leaves in the control (Group I with distilled water) and the experimental groups (Groups II and III with 50 and 100 mg/kg/day, respectively).

Parameter	Group I (8 ml dH2O/day)	Group II (LD 50 mg/Kg/day)	Group III (HD 100 mg/Kg/day)
Sperm count (x10^6)	91.00±12.36	77.00±9.51*	46.00±3.95*
Sperm viability (%)	60.40±5.02	38.00±2.23*	28.50±2.22*
Sperm motility (%)	84.00±9.85	49.00± 8.35*	31.00±5.33*

LD and HD represent low and high doses (50 and 100 mg//kg/day, respectively).* significantly different at $p < 0.05$ level.

4. Discussion and Conclusion

The result of the present study showed that there were significant differences in the body weight but there were no significant difference in the vital and reproductive organ weights. Our results are in total agreement with the study that demonstrated the effects of *M. piperita* juice and tea on the plasma lipids (decrease in triacylglycerols and cholesterol values) of Wistar rats due to substantial reduction in food intake, and percentage of weight gain [5]. It is interesting to note that the average body weight decreased in Group II, and slightly increased in Group III, when compared with the control group. Furthermore in another study, the menthol in *M. piperita* caused hepatocellular changes in rats [3]. And the daily consumption of four cups of spearmint tea reduced libido in men and recorded strong antimutagenic effects on sperm abnormalities [4, 15].

Furthermore, sperm concentration, motility and viability in the cauda epididymis also decreased [16]. In another study, when an aqueous solution of the extract (10 mg per day per mouse) was administered orally to male mice with proven fertility for 20, 40 and 60 days, it caused inhibition of fertility while maintaining their normal sexual behavior [4]. However, by increasing the treatment duration, a corresponding decrease in the mean weight of the testes and accessory reproductive organs was evident.

Also there was a significant difference ($p>0.05$) in the sperm count, viability and motility in the experimental groups (Groups II and III) at the high dose of the extract and timing close to the period of spermatogenesis in rats (50 days), were used for ensuring the effects of the extract on sperm quality and quantity. The data in this study corroborates the results of previous studies [16, 4, 15]. The findings suggest that the administration of an aqueous extract of *M. crispa* in a period of 60 days, at 50 and/or 100 mg/kg/rat, will produce suppressive effects on male spermatogenesis (sperm quality and quantity). This could be useful in the development of a male infertility contraceptive agent and free rodenticides in the sustainable management of rodents.

However, there are several medicinal plants such as Chirmi seed (*Abrus precatorius*), bael leaf (*Aegle marmelos*), Siris pods (*Albizzia lebbeck*), golden trumpet leaf (*Allamanda cathartica*), Aloe vera leaf (*Aloe Barbadensis*), soya seeds (*Anethum graveolens*), neem seeds (*Azadirachta indica*), papaya seeds (*Carica papaya*), whites ginger root bark (*Mondia whitei*), pan leaf-stalk (*Piper betle*), long pepper fruit (*Piper nigrum*), Surinam wood bark (*Quassia amara*), Harad fruit (*Terminalia bellirica*) and neem giloy stem (*Tinospora cordifolia*) that have antifertility properties [8]. Although, very few herbal contraceptives have been developed from plant extracts, their potential is not accurately determined, and their mode of action has been beyond our knowledge until now. This is because there are many problems in assessing plant extracts, including specific variety and dose quantity, beside the lack of a definite active portion of the extract [14, 1]. Therefore, quality control and assessment of the doses of natural products, has to be promoted with regard to the ongoing research and development in this field for the tremendous male-based shift paradigm for contraception with social and public health benefits [7, 11, 8, 15].

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