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Combined Effect of Aqueous Extracts of *Artemisia monosperma* and *Mentha piperita* on the Reproductive Integrity of Male Albino Rats

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ABSTRACT

The aim of this study was to evaluate the effect of combinations of *Artemisia monospermia* with *Mentha piperita* on male fertility. Treated rats with different doses of *A. monosperma* or *M. piperita* alone induced severe testes histopathological lesions and histochemical alteration associated with a reduction in epididymis weight, abnormality in the sperm indices and a decline in serum testosterone levels which may have a bad impact on male infertility, on the other side, treatment the rats with the combined extract of different doses of *A. monosperma* or *M. piperita* attenuated the deleterious damage to the testes, the sperm index and the sex hormones. This study indicated that the traditional use of these two herbs as a mixture has no effects on male reproductive health.

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HEM, HNGE and RAE conceived the idea and performed the experiments. All authors discussed the results and contributed to the final manuscript.

Key words
***Artemesia monosperma*, Testis; *Mentha piperita*; Infertility; Tubuli recti**

INTRODUCTION

Herbal drug therapy is a common practice adopted in traditional and alternative medicine and has been used in the treatment of many diseases from ancient times (Pan *et al*., 2014; Gad-EL-Hak and Mobarak, 2020). *Artemisia monospermia* and *Mentha piperita* are two commen herbs.

A. monosperma is a common perennial shrub in the deserts (Huang *et al*., 2000) and one of the most common medicinial species of *Artemisia* used in folk medicine. *Artemisia* are aromatic fragrant plants that have a characteristic scent and taste (El-Massry *et al*., 2002). Some *Artemisia* species are used medically in repelling parasites (Goud and Swamy, 2015), in getting rid of intestinal gases and a gusher of menstruation (Nageeb *et al*., 2013), recommended for neurological disorders (Salah and Jäger, 2005), treatment of diabetes mellitus (Ribnicky *et al*., 2006), used as a cure against rheumatism (Adams *et al*., 2009) and to treat cold (Ballabh and Chaurasia, 2007). Some *Artemisia* species are used in traditional medicine as decoction for their antivenom (Nalbantsoy *et al*., 2013), anti-inflammatory (Choi *et al*., 2013) and antimicrobial (Ramezani *et al*., 2004) properties. It has been reported that different species of *Artemisia* grow wild in the uncultivated land have medicinal properties like anti-cholesterolemic (Riahi *et al*., 2013), antipyretic (Tan *et al*., 1998), antiseptic (Kordali *et al*., 2005) and used in the treatment of hepatitis (Mannan *et al*., 2010) and jaundice (Hayat *et al*., 2009). *Artemisia* should be taken carefully due to the santonin present, which has toxic effects if it is taken regularly (Hammond *et al*., 1997).

Mentha piperita (peppermint) is an important medicinal herb belongs to family Laminaceae, and commonly known as peppermint (Mimica-Dukic and Bozin, 2008). Peppermint is famous used as food additives in the form of spices (Kunnumakkara *et al*., 2009), flavoring enhancers (Kermode, 1972) and coloring agents (Oppenheimer *et al*., 1990) depend on common practice in human beings need. *M. piperita* act as preservatives (Sessou *et al*., 2012) and stomach carminatives (Girme *et al*., 2006). Peppermint has been consumed by many people in water by either as a juice or raw leaf addition in the tea and other herbs (Akdogan *et al*., 2004). *M. piperita* has a toxic effect on the testis (Akdogan *et al*., 2004) by causing a significant oligozoospermia (Ogbuewu *et al*., 2011) in experimental animals. Earliar studies indicated the structural alterations in testicular tissues, morphological deformities and inhibition of spermatogenesis of different mammalian species treated with peppermint (Sharma and Jacob, 1996; Sharma and Jocob, 2001).

The present study deals with impact of these two herbs administered in combination on the male fertility of albino rats.

MATERIALS AND METHODS

Plant collection and prepration of plant extract

A. monosperma and *M. piperita* aerial parts were collected from the northern Sinai desert, Arish of Egypt, in December 2018. It was authenticated by morphology

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and taxonomic elements in the Botany Department of Arish University, according to morphology and taxonomic aspects. Voucher specimens were deposited in the Herbarium of Faculty of Science, Arish University, North Sinai, Egypt with an accession number (2018001).

The aerial parts of *Artemisia monosperma* were air-dried under the shade and grinded into fine powder using electric blender. Approximately 50 g of *Artemisia* powder placed in a flask with 1000ml distilled water, then boiled for 15 min, then the mixture was filtered three times, The final concentration of prepared *Artemisia monosperma* was 5% as a total solids. The *A. monosperma* extract was preserved in a sterile dark bottle (500 ml) in a cool environment (4°C) until further use.

The leaves of *A. monosperma* were washed, wiped dry and cut the roots before immediately immerged into tap water. The concentration of the extract prepared was at 100 mg/ml which is corresponding to the daily recommended intake 200 ml for adult man (Barbalho *et al*., 2011). The water extract was then filtered into a bottle and kept for about one hour before use. Dose of 290 mg/kg b.wt. proven to be safe in a study of 14 days for all body organs (Johari *et al*., 2015).

Animals and treatments

Forty healthy adult male Spargue-Dawely rats were obtained from the animal house of the Zoology Department, Faculty of Science, Suez Canal University Ismailia, Egypt. They were housed in groups five per cage, maintained under controlled conditions of temperature (22 ± 2°C) and light (12 dark: 12 light) in well ventilated room and provided with rodent food and water *ad libitium*. All animal experiments were carried out in accordance and approved with the guidelines of the Institutional Animals Ethics Committee of Faculty of Science, Suez Canal University Ismailia, Egypt. The animal experiments were performed according to the principles of the care and use of laboratory animals established by the National Institutes of Health (NIH Publication, No. 85-23, revised 1985) (Care *et al*., 1985). The animals were weighted and their behavioral changes were recorded before the start and at the end of the experiment with animal weighting balance. Animals were divided into eight groups of five rats each into: control group: rats were orally given distilled water. AL group: rats were orally given 100 mg/kg aqueous extract of *A. monosperma* daily for 2 weeks. AM group: rats were orally given 300 mg/kg aqueous extract of *A. monosperma* daily for 2 weeks. AH group: Rats were orally given 600 mg/kg aqueous extract of *A. monosperma* daily for 2 weeks. M group: Rats were orally given *M. piperita* at a dose level of 290 mg/kg daily for 2 weeks. ALM group: Rats were given the same dose of *A. monosperma* given to animals of group AL followed by 290 mg/kg *M. piperita* daily for 2 weeks. AMM group: Rats were given the same dose of the same dose of *A. monosperma* given to animals of group AM followed by 290 mg/kg *M. piperita* daily for 2 weeks. AHM group: Rats were given the same dose of *A. monospermia* given to animals of group AH and 290 mg/kg *M. piperita* daily after 2weeks he selected doses of A. monosperma according to the studied LD50 of *A. monosperma* to the rats which is more than 900 mg/kg. Animals were anaesthetized by intraperitoneal injection of Ketamine (50 mg/kg) (Struck *et al*., 2011). Blood samples were collected in the morning by cardiac puncture into a set of plain sample bottles, and allowed to clot. The clotted blood samples were centrifuged to obtain serum. The separated serum samples were stored in the refrigerator until required for the hormonal assay. All assays were done within 24 h of the sample collection. The serum samples were assayed for FSH, LH and testosterone using enzymes immunoassay methods. Calculations of the concentrations of hormones were made according to the method given in the immunoassay kit's instruction.

Sperm indices (sperm kinetics and morphology)

Epididymides were dissected out, weighed, immediately minced in 5 ml of physiological saline and then incubated at 37 °C for 30 min to allow spermatozoa to leave the epididymal tubules. The sperm indices were evaluated using computer-assisted SpermVision™ CASA System (CASA) (MiniTüb, Tiefenbach, Germany) with Olympus BX 51 phase contrast microscope (Olympus, Tokyo, Japan). Sperm kinetics parameters were determined which included the percentage of total motile spermatozoa (MT), the percentage of progressive motility (PR) is spermatozoa moving actively either linearly or in large circle regardless of speed, velocity curved line (VCL, µm/s), velocity straight line (VSL, µm/s), velocity average path (VAP, µm/s), distance curved line (DCL, µm), distance straight line (DSL, µm), distance average path (DAP, µm), mean angular degree (MAD, ^{0}), amplitude of lateral head displacement (ALH, µm), beat cross frequency (BCF, Hz), linearity (LIN, %), wobble (WOB, %) and straightness (STR=VSL/VAP %).

Spermatozoa from each rat were examined morphology and individually scored normal or abnormal, according to the strict sperm morphology criteria. The morphological abnormalities were divided into head, midpiece and tail defects. The percentages of normal and abnormal shaped sperms, the multiple abnormalities per abnormal spermatozoa (MDI), the total number of defects divided by the number of sperm counted (TZI), the total number of defects divided by the number of sperm counted (SDI) was calculated.

Histological examination of tests

The testes and the epidydimis of dissected rats were removed, cleaned of accessory tissues and weighed. The gonadosomatic index was calculated by dividing the testis and epidydimis weight by body weight of each animal getting the relative weight of testes and epididymis. The testes and the rete tubules were processed for histological examination according to Gad El-Hak and Mobarak (2020) and were stained with haematoxylin and eosin for the histopathological studies. For the histochemical investigations of testes, the deparaffinised sections were stained with blue Feulgen technique for DNA using Teomics kits according to the kits instruction and periodic acid Schiff's (PAS) for demonstration of polysaccharides (El-Hak, 2017). While For the histochemical investigations of the rete testes, the deparaffinised section were stained with silver nitrate stain for reticular fibre (Elder and Hsu, 1981).

Stastistical analysis

The body weights and organ weight were expressed as the mean ± standard error. The parametric data analyzed by *One Way ANOVA* subsequent Multiple comparison by Duncan test. The nonparametric data analyzed by Kruskal-Wallis test and subsequent individual comparison by the Mann-Whitney *U-test* using SPSS statistical version 16 software package (SPSS 4 Inc., USA). *A p≤0. 05* was considered to be statistically significant.

RESULTS

Body and organ weights

Table I shows effect of *A. monosperma* and *Mentha piperita* administered alone or in combition on body and organ weight. There was non-significant increase ($p \geq 0.05$) in the initial and final body weight, relative testicular weight of rat in all the treated group when compared with the control group.

Serum sex hormones

Table II shows the effects of *A. monospermia* and *M. piperita* alone or combined on serum sex hormones of male rats. The results showed that there was no significant difference ($p \leq 0.05$) in FSH and LH levels in all treated groups compared to control group, while testosterone level was significantly dropped ($p<0.05$) in the *A. monospermia* groups (AL , AM and AH) and mint group (M) compared to control group.

Sperm indices

Sperm of treated and control rats were evaluated through the sperm class analyzer for progressive kinetics and for sperm morphology Tables III and IV. The sperm kinetics analysis showed significant changes in all motion parameters of treated rats compared to control group. There was a significant decrease in MT and PR ($P<0.05$) in M group. Also, there were increased distance parameters, including DAP ($P<0.05$) in ALM, AM and AH groups, decrease in DCL ($P<0.05$) in AMM, AHM and M groups, and increased DSL ($P<0.005$) in ALM group. While there were significant decrease in STR ($P<0.005$), LIN ($P<0.005$), ALH ($P<0.05$) and BCF ($P<0.005$) in M, AL, A, AH, AMM and AHM groups.

Table I. Effct of *Artemesia monosperma* and *Mentha piperita* extracts administered alone or in combination for 14 days on initial and final body weight, the relative testicular and epidydimal weight in rats of different groups after 14 days treatment.

Groups	Initial body weight (g)	Final body weight (g)	Relative testicular weight (%)	Relative epidydimal weight (%)
Control	268±12.7	269±16.6	1.93±0.2	0.414±0.1
M	279±16.9	301±36.9	1.63±0.3	0.390±0.04
AL	274±19.1	267±20.7	2.17±0.1	0.365±0.01
AM	249±17.42	286±28.1	2.07±0.1	0.450±0.04
AH	278±23.8	318±68.4	1.85±0.2	0.566±0.04
ALM	276±15.1	295±25.4	2.13±0.1	0.482±0.1
AMM	258±32.6	275±75.2	1.85±0.2	0.566±0.05
AHM	268±11.8	271±34.1	1.84±0.2	0.482±0.01

M, *M. piperita* extract with dose 290 mg/kg/day for 14 days; AL, *A. monospermia* extract with dose 100 mg/kg/day for 14 days; AM, *A. monospermia* extract with dose 300 mg/kg/day for 14 days; AH, *A. monospermia* extract with dose 600 mg/kg/day for 14 days; ALM, *A. monospermia* extract with dose 100 mg/kg/day + *M. piperita* extract with dose 290 mg/kg/day for 14 days; AMM, *A. monospermia* extract with dose 300 mg/kg/day + *M. piperita* extract with dose 290 mg/kg/day for 14 days; AHM, *A. monospermia* extract with dose 600 mg/kg/day + *M. piperita* extract with dose 290 mg/kg/day for 14 days.

Table II. Effects of *Artemesia monosperma* or *Mentha piperita* extracts administered alone or in combined for 14 days on serum sex hormones of male albino rats.

Groups	FSH (mIU/ml)	LH (mIU/ml)	Testosterone (ng/dl)
Control	0.005 ±0.002	0.003±0.0001	0.522±0.06 [c, d]
M	0.003±0.001	0.004±0.0001	0.250±0.14 [a]
AL	0.004±0.001	0.003±0.0004	0.137±0.03[a]
AM	0.0035±0.003	0.005±0.0006	0.272±0.22 [a, b]
AH	0.005±0.004	0.003±0.0001	0.334±0.21 [a, b, c]
ALM	0.006±0.001	0.004±0.0001	0.546±0.09 [d]
AMM	0.005±0.001	0.033±0.029	0.454±0.154 [b, c, d]
AHM	0.006±0.004	0.004±0.0007	0.518±0.041 [c, d]

Data is represented ad mean±SE. different letters represent significant ($P < 0.005$) compared to the control group. For details of groups, see Table I.

The sperm of rats from the AL and ALM showed nonsignificant increases ($P<0.05$) in VAP and VCL, consistently with ALH ($P<0.005$) while, BCF values were significantly decreased ($P<0.05$). There was no significant difference in MOT and PR parameters in the groups M, AHM, AM and AL compared to control. Furthermore, ALH parameter was significantly decreased ($P<0.005$) in M, AL, AM, AH, AMM and AHM groups. Conversely, significant decreases were detected in progression parameters VSL ($P<0.05$) in M, AM, AH, AMM and AHM groups, decreased LIN ($P<0.005$) and vigor parameters WOB ($P<0.005$) in M, AM and AL groups and decreased BCF ($P<0.005$) in M, AL, AM, AH, ALM, AMM and AHM groups compared to control.

Sperm morphology

The evaluations of normal and abnormal spermatozoa are shown in Table IV. Morphological analysis of semen samples revealed a significantly lower percentage of spermatozoa with abnormal morphology in all experimental groups ($P<0.005$). The significantly increased incidence of sperm with abnormalities in M group were detected. Treatment with Artemesia alone or with Mint did not produce any variation in the frequency of the sperm head, tail and midpiece abnormalities.

Histological and histochemical examination of testis

Histological examination of testicular section of control rats showed the normal histological testicular architecture with normally arrangement different series of spermatogenic layers, spermatozoa and the interstitial tissues with Leydig cells (Fig. 1A). Testis of rats treated with *Artemisia* (Fig. 1C, 1D and 1E) or mint (Fig. 1B) showed testicular degeneration of germ cells within the seminiferous tubules which were greatly depleted of germ cells. Some treated rats showed disturbance in the arrangement of germ cell rows of spermatogenic cells which appears as areas lacking spermatogenic activity with exfoliation of cells in many of the tubules. While testicular examination of rats which received a combined dose of Artemisia sp. and Mint showed normal seminiferous tubules with normally arrangement different series of spermatogenic layers (Fig. 1F, 1G and 1H).

Figure 2 shows Quantitative evaluation of Polysaccharides PAS-positive materials in the testes of control rat and in those given the combination of *Artemisia* sp. and peppermint aqueous extract appeared increase in the basement membrane of the tubules (the tunica albuginea) as well as in the intertubular connective tissue of the testes. Testes of rats treated with peppermint aqueous extract only and doses of *Artemisia* revealed a mild decrease of PAS-positive materials.

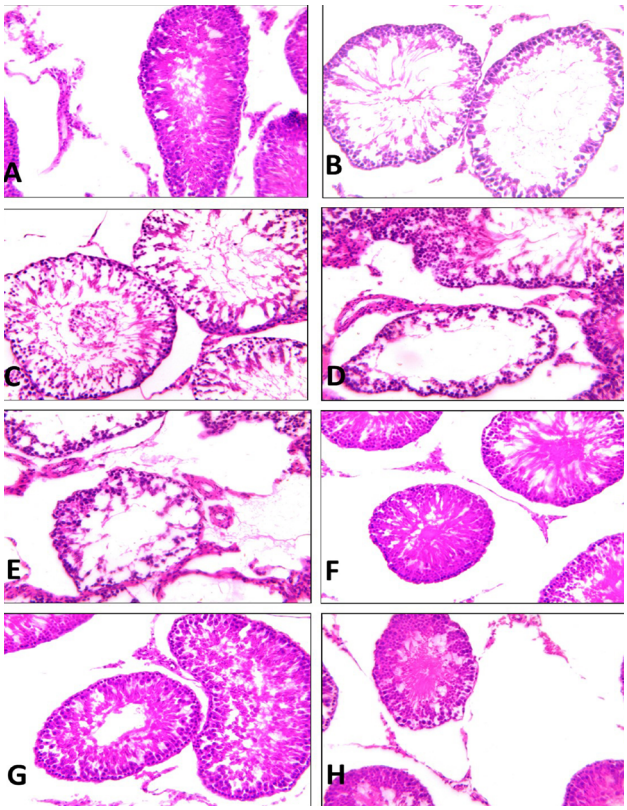

Fig. 1. (A) Testis of control rats showing the normal histological structure of seminiferous tubules with different series of active spermatogenic layers, spermatozoa and the interstitial tissues. (B) Testis of mint treated group showing alternations of some tubules in the form of reduced the number of layers of the germinal epithelium, spermatozoa with vacuolated spermatogenic cells. (C, D and E) Testis of AL, AM and AH group showing tubular shrinkage with extensive degeneration of the germinal epithelium. The shrinkage tubules contained degenerated Sertoli cells with few germ cells. (F, G and H) Testis of ALM, AMM and AHM group showing no prominent histological changes. Most of the seminiferous tubules appeared to increase of spermatogenic cells and an increase in the number of sperm bundles was seen. (Stain HX and magnification x200).

DNA deoxyribose sugar was histochemically demonstrated using the blue Feulgen reaction technique (Fig. 3). The DNA present in the nucleus of spermatogonia and spermatocytes. The mean DNA content of spermatogonia and spermatocyte in all treated groups was the same as the control group.

Figure 4 shows the histological examination of the tubuli recti in the control and the treated rats with the combination of *Artemisia* sp. Peppermint groups did not show any histological damage. The histological examination on the tubuli recti in M, AL, AM and AH groups reduced

sperm reserves relative to the control and vacuolation appeared in the cuboidal epithelium lining the tubules.

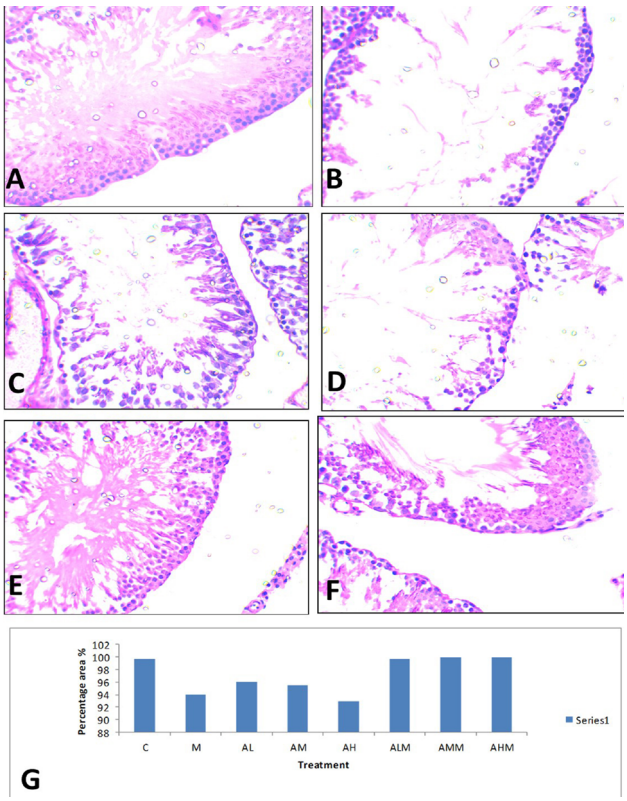

Fig. 2. Cross sections from testis, (A) control group with positive PAS increase in the basement membrane of the tubules (the tunica albuginea) as well as in the intertubular connective tissue of the testes. (B, C, D and E) Testis of mint, AL, AM and AH treated group showing decrease PAS reaction. (F, G and H) Testis of ALM, AMM and AHM group showing normal positive PAS. (PAS staining technique, 400X). (I) Percentage of PAS stained area in the treated group.

Figure 5 shows silver stains of the tubuli recti showed strong stain which revealed the presence of extensive and dense network of reticular fibers around the peritubular walls of the control and the treated rats with the combination of *Artemisia* sp. and peppermint groups. The tubuli recti in M, AL, AM and AH groups showed fragmented and degenerated reticular fibers with weal stained area with silver nitrate stained area.

DISCUSSION

Various tissue and organ systems of an individual differ in their response to herbal treatment, especially organs characterized by high cell proliferation. Testis considered one of the most sensitive organ due to its rapidly dividing cell renewal system.

In this study, the reproductive toxicity was conducted prior to assessment of aqueous extract of peppermint, different doses of *A. monosperma* and combination the different dose of *A. monosperma* with peppermint. The results showed that there was no sign of toxicity, no mortality within the entire period of 14 days of herbs administration. The extract did not trigger any changes in behavior, breathing, cutaneous effect, urination and defecation and did not suppress their appetite or retard their development. Neither body weight nor relative weights of testes and epidydimis of treated rats were significantly changed relative to the control group as the relative weight is a measure of the health status of the organ (EL-Hak *et al*., 2019).

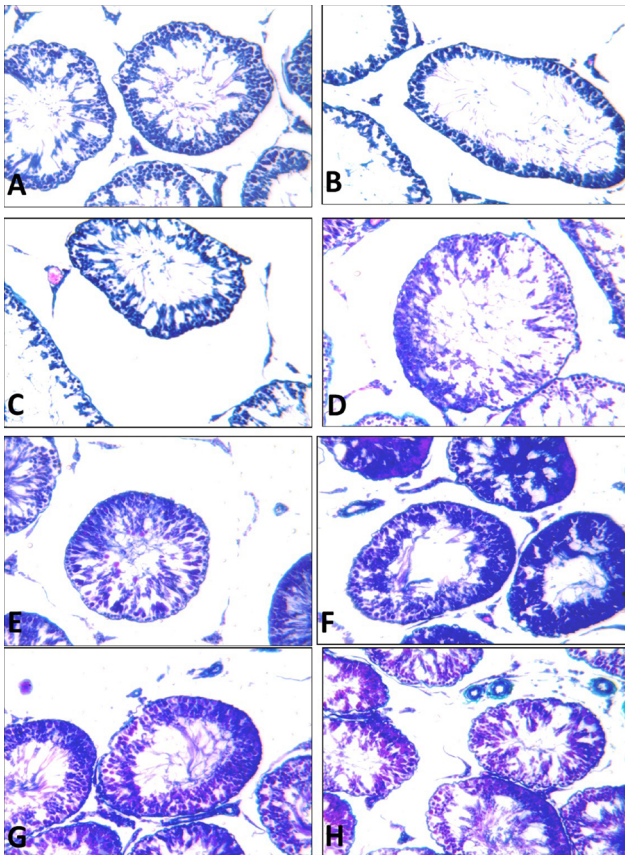

Fig. 3. Cross sections from testis, (A) control group with positive Feulgen reaction in the nucleus of spermatogonia and spermatocytes. (B, C, D and E) Testis of mint, AL, AM and AH treated group showing a decrease in The intensity of DNA blue color. (F, G and H) Testis of ALM, AMM and AHM group showing normal positive Feulgen reaction. (Feulgen staining technique, 200X).

Table III. Sperm Kinetics parameters for control rats and those treated with *Artemesia monosperma* or *Mentha piperita* extracts alone or in combination.

Parameter	Control	M	AL	AM	AH	ALM	AMM	AHM
MT (%)	45.01±0.5	17.7±5.4*	28.3±3.2*	24.6±4.0*	23.2±3.8*	29.7±10.6*	24.7±1.8*	31.7±3.2*
PR (%)	45.37±0.4	23.09±5.8*	34.3±3.3	26.4±5.7*	30.2±5.2*	35.2±4.5	23.6±2.9*	29.1±2.1*
VCL (µm/s)	23.03±0.5	9.45±3.0*	13.35±1.4*	12.9±1.6*	11.2±2.5*	17.26±3.2	11.56±2.8*	16.8±2.5
VSL (µm/s)	2.76±0.2	1.030±0.1*	2.27±0.37	1.58±0.41*	1.48±0.28*	2.141±0.45	1.25±0.12*	1.24±0.07*
VAP (µm/s)	4.05±0.1	2.25±0.5*	3.49±0.4	2.53±0.5*	2.63±0.4*	2.79±0.4	1.84±0.24*	2.44±0.1*
DCL (µm)	26.54±1.4	12.98±3.1	22.1±2.6	15.85±4.1	15.77±2.3	18.45±1.8	12.39±1.2	13.76±1.08
DSL (µm)	0.577±0.09	1.60±0.6	1.60±0.4	2.71±0.2*	3.34±0.8*	3.69±0.7*	2.14±0.45	1.59±0.8
DAP (µm)	0.265±0.08	0.50±0.2	0.65±0.2	1.15±0.1*	1.29±0.2*	1.13±0.2*	0.58±0.08	0.80±0.4
MAD (^{0})	1.22±0.4	2.96±2.7	3.52±2.6	5.57±1.7	7.20±3.7*	7.31±4.2*	4.21±1.6*	3.38±3.2
ALH (µm)	4.76±0.3	2.21±0.4*	3.15±0.4*	2.03±0.4*	2.49±0.5*	3.45±0.6	2.7±0.4*	2.5±0.4*
BCF (Hz)	2.22±0.1	0.75±0.1*	1.11±0.1*	0.88±0.1*	0.92±0.1*	1.54±0.3*	1.07±0.2*	1.25±0.1*
LIN (%)	10.78±1.06	4.06±0.5*	6.88±1.05*	4.45±0.94*	4.71±1.01*	6.78±1.07*	5.61±0.91*	5.40±1.8*
WOB (%)	4.58±0.8	0.8±0.4	0.67±0.1	1.38±0.6	2.02±0.7*	4.39±1.5*	2.56±1.06*	2.06±0.5*
STR (%)	32.4±2.63	16.4±3.4*	15.8±1.44*	17.5±3.5*	16.1±2.98*	28.3±4.4	19.5±3.4*	17.7±3.2*

Data is represented ad mean±SE. (*) significant ($P<0.005$) compared to the control group. For details of groups, see Table I.
MT, motile spermatozoa; PR, progressive motility; VCL, velocity curved line; DCL, distance curved line; DSL, distance straight line; DAP, distance average path; MAD, mean angular degree; ALH, amplitude of lateral head; BCF, beat cross frequency; LIN, linearity; WOB, wobble; STR, straightness.

Table IV. Effects of *Artemesia monosperma* or *Mentha piperita* extracts administered alone or in combintion on sperm morphology of male albino rats.

Parameter	Group							
	Control	M	AL	AM	AH	ALM	AMM	AHM
Head defect	1.176±0.04	1.10±0.00	1.19±0.05	1.11±0.01	1.21±0.11	1.30±0.2	1.10±0.00	1.61±0.02
Midpiece defect	0.594±0.3	0.00±0	3.22±3.1	0.82±0.8	1.02±1.02	1.02±1.02	1.02±1.02	1.02±1.02
Tail defect	2.20±2	5.80±5.8	2.20±2.2	21.82±7.2	20.4±9.07	17.8±9.1	21.8±6.3	7.6±3.1
MDI	34.5±5.1*	39.8±9.2	29.1±4.4	3.75±2.7*	8.28±6.4*	9.89±4.77*	1.02±1.02*	8.02±4.1*
TZI	22.11±7.5	47.06±7.4	32.64±3.0	25.4±5.7	28.8±10.9	27.5±7.1	23.5±5.01	19.1±4.4
SDI	67.08±1.03	52.9±7.4	67.3±3.00	74.5±5.7*	71.1±10.9	72.8±7.4	76.4±5.01*	80.8±4.4*

Data is represented ad mean±SE. (*) significant ($P<0.005$) compared to the control group. For details of groups, see Table I.

The M, AL, AM and AH treated groups showed significant decreases in serum testosterone level which may have resulted from the direct effect of *M. piperita* and *A. monosperma* on Leydig cells according to Dym and Madhwa Raj (1977). These reduction in testosterone level may cause a decrease in libido, reproductive functions and hence resulting in impotence according to Blute *et al.* (2009).

In the present study, treatment with *M. piperita* and *A. monosperma* alone or in combination did not affect the level of LH and FSH. That may be according to previous results of Pradhan *et al.* (2013) that found *Artemesia* sp. suppresses feedback secretion of the pituitary which suppressed the regulatory changes in LH and FSH secretion.

In the present study, results indicate significant decline in sperm motility and morphology of rats treated with *Artemesia* sp., or peppermint which may be from the androgen deprivation effect and the decrease of testosterone (Handelsman *et al.*, 1996). As the epididymal spermatozoa are highly dependent on testosterone for their final maturation and development of progressive motility and fertilizing capacity (Dyson and Orgebin-Crist, 1973). These findings are similar to those reported by D'cruz *et*

al. (2010; Smith (2010); Ogbuewu *et al*. (2011) reported decrease in sperm concentration and motility, increase in dead and abnormal sperm of rats and impairment of spermatogenesis in animals treated with mint or *Artemesia* sp.

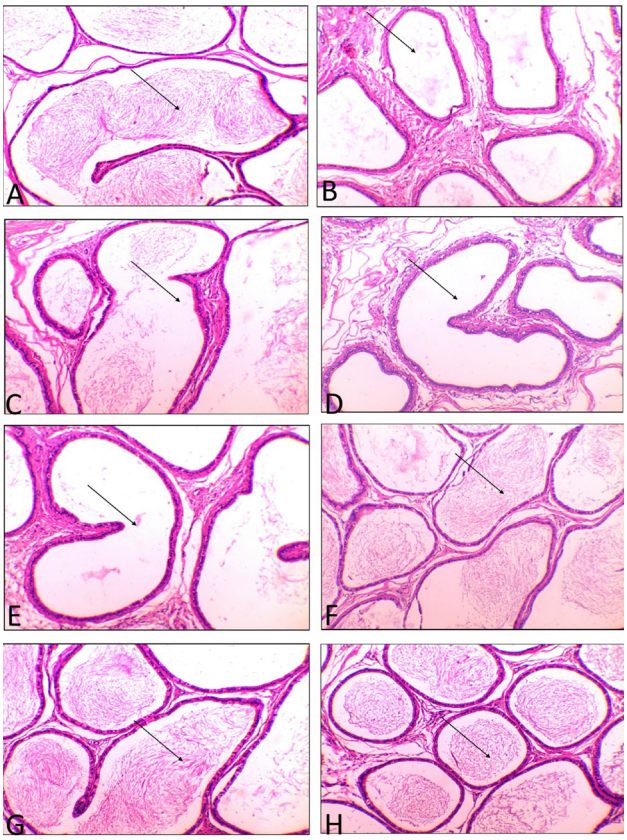

Fig. 4. (A) Tubuli recti of the control group showing the normal histological structure lining with cuboidal epithelium and present of sperm in the lumen (←). (B) Tubuli recti of mint treated group showing alternations of some tubules in the form of reduced sperm in the lumen (←). (C, D and E) Tubuli recti of AL, AM and AH group showing vacuolation of the cuboidal epithelium with extensive absent of the sperm in the lumen (←). (F, G and H) Tubuli recti of ALM, AMM and AHM group showing no prominent histological changes with increase of sperm in the lumen (←). (H and E, x200).

The alteration in sperm parameters could be attributed to direct effect of these plants on testicular tissue which leads to reproductive dysfunction such as reduced sperm motility and increase sperm abnormailities in the morphology (Wong *et al*., 2000). The present study found that the combination of *A. monospermia* extract and *M. piperita* extract play a specific role in maintaining the structural and functional integrity of the sperm cell. Treatment with combined *Artemesia* sp. and peppermint displayed a higher incidence of hyperactivated like motility than caused by *Artemesia* or peppermint alone. Interestingly, simultaneous treatment with *Artemesia* sp. or peppermint produces an adverse effect on the sperm morphology. The possibility that *Artemesia* sp. or peppermint may cross the epididymal epithelium based on its lipophilic properties and reach the stored spermatozoa would explain its damaging effects on sperm structure and function according to Adamkovicova *et al*. (2016). The fertilization capabilities of sperm were significantly reduced because of spermatozoa movement dysfunction and reduction of testosterone hormone (Fallah *et al*., 2018).

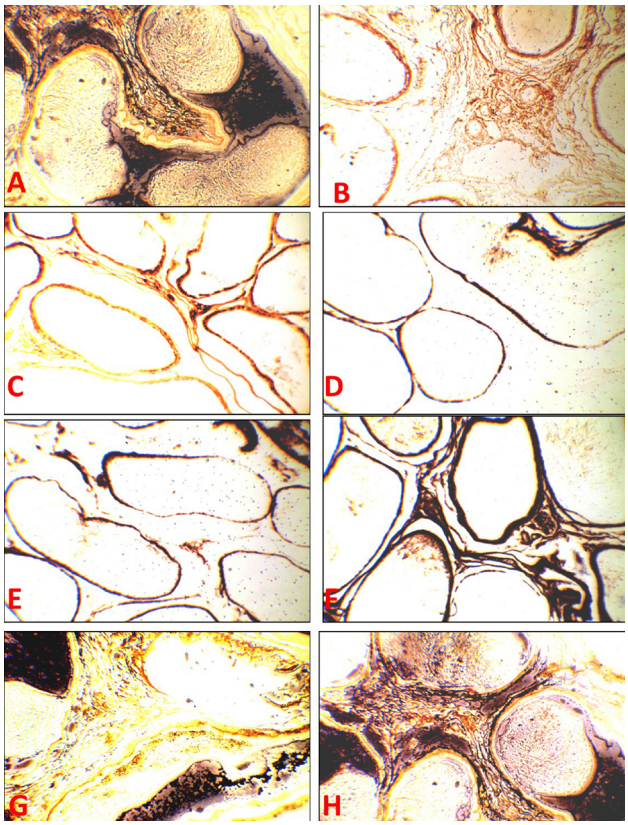

Fig. 5. (A) Tubuli recti of the control group showing normal reticular fibers. (B, C, D and E) Tubuli recti of M, AL, AM and AH group showing degeneration and fragmentation of reticular fibers (F, G and H) Tubuli recti of ALM, AMM and AHM group showing the normal appearance of reticular fibers. (Silver stains x200).

In the present study using histological evaluation of testis, we observed that oral administration of peppermint or different doses of *A. monosperma* could exert adverse effects on spermatogenesis process and cause some histological changes. Control groups showed a compact

and regular arrangement of spermatogenic cells in the seminiferous tubules whereas in peppermint or different doses of *A. monosperma* treatment groups, deceased cell layers and degeneration were observed. Histopathological examination showed a slight increase in the number of tubules with histologically disruption in the AH group. The explanation for this finding may be due to the indirect effect of decrease in the serum testosterone level in M, AL, AM and AH group which has been reported in the present study. Furthermore, reduced number of interstitial cells in M, AL, AM and AH treated groups which are testosterone secreting cells in the male reproductive system could be considered supporting evidence for the aforementioned finding. The histological examination of the different spermatogenic stages revealed that no stage was specifically susceptible to treatment with the extract. Exfoliation of germ cells was observed in the results of this study in the M, AL, AM and AH group and can be explained according to Li *et al.* (2016) who postulated that it may be due to disruption of cell junctions leading to loss of adhesion or it may be due to disruption of Sertoli cell cytoskeletal fibers leading to sloughing of apical Sertoli cell cytoplasm and attached germ cells. The mechanism by which *A. monosperma* or *M. piperita extract* produces lesions in the testes remains unclear but several factors may be considered. Decreased testosterone production has been proposed as the probable cause of damage to the seminiferous tubules of rats (Rich *et al*., 1979). In addition, data from the present study has indicated that serum testosterone levels were altered in rats. Rats treated with the combination of *Artemisia* sp. and peppermint aqueous extract showed normal seminiferous tubules. Our data point to the need to further analyze the exact effect of the biomolecules present in *A. monosperma* or *M. piperita* extract or combination of both extracts on the the male reproductive integrity individually or collectively. The rats treated with Mint or Artemesia showed diminution in percentage area of PAS +ve materials which can be explained according to Gupta *et al*. (2002) by impairment of spermatogenesis resulted from the decreased androgen concentration. *Artemesia* sp. with Mint gave a normal +ve result that can be explained by thickening of the basement membrane of seminiferous tubules. Histochemically, the rats treated with mint or *Artemesia* or both exhibit no difference in DNA content in testis as also reported by Cheville *et al*. (1998) and Tseng *et al*. (2006).

In the present study and decrease of sperm in the tubuli recti, degeneration of reticular fibres between the tubules were observed in M, AL, AM and AH groups. Tubuli recti, form the junction between the seminiferous tubules and the rete (Dym, 1974). The rete testis serves as a collecting reservoir for sperm within the testis (Free *et al*., 1980). The Low Tubuli recti sperm density and degeneration of the reticular fibers may be due to alteration in the androgen metabolism (Scarano *et al*., 2006). In the other hand, ALM, AMM and AHM groups showed the presence of sperm and normal apperarnce of reticular fibres. Reticular fibers is important in the Tubuli recti to transmit the sperm in the male reproductive organ (Pathak *et al*., 2014).

CONCLUSION

The study revealed that administration of *A. monospermia* or *M. piperita* alone to male rats resulted in damage induced in rat testes and sperm quality that in the future may lead to reproductive integrity and infertility. In the other hand, the administration the mixture of both *A. monospermia* and Peppermint extract has the potential to prevent and antidote that damage.

Statement of conflict of interest

The authors have declared no conflict of interest.

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