International Journal of Phytomedicine 6 (2014) 346-353
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Effect of *Salvadora persica* (*Miswak*) *leaves and stem aqueous* extracts on ovarian folliculogenesis and uterine histology in female albino rats

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Abstract

This work was conducted to evaluate the antifertility activities of *Salvadora persica* (*miswak*) aqueous leaves and stem extracts on female albino Wistar rats.

Control animals received 0.5 ml of distilled water (Group 1); experimental animals received 0.5 ml of aqueous solution (1:1 w/v) of *miswak* leaves (Group2) or stem extract (Group 3) for 14 consecutive days. At the end of experiment, animals were weighed and vaginal smears were obtained from control and treated groups. Control and experimental animals were anesthetized then scarified, ovaries and uterus were dissected out, weighed and the number of corpora lutea and surface ovarian follicles were counted. Ovaries and uterus were fixed then processed for paraffin sections and stained with Hematoxylin-eosin. Histological changes in ovaries and uterus were determined.

Results showed that administration of *miswak* leaves or stem aqueous extract is safe and have no side effects or mortalities, and it did not affect body weight of treated animals compared with control. However, administration of *miswak* leaves or stem aqueous extract significantly decrease ($P<0.05$) ovarian weight in treated groups. Uterine weight also significantly decreased ($P<0.05$) after administration of *miswak* leaves extract compared to control or stem extract groups. Moreover, number of surface ovarian follicles significantly decreased ($P<0.05$) after exposure to extract of *miswak* leaves or stem. Number of corpora lutea did not vary between groups. Histological examination revealed that administration of *miswak* leaves extract caused a significant decrease in the epithelial cell height, myometrial and stromal thickness of uterus compared to stem extract or control group.

The present study illustrated the antiovulatory and anti-uterotrophic effects of the aqueous extract of *miswak* leaves in female rats. This effect may be mediated through direct effect of the extract on the reproductive organs by disruption of ovarian folliculogenesis and inhibiting further development of the recruited ovarian follicles and/or by disruption of the hormonal balance in the hypothalamo-hypophysial ovarian and uterine axis. *Miswak* stem extract could affect follicular development but it did not affect the uterine structures.

Keywords: *Miswak*, Female rat, Antifertility, Endometrium, Ovarian follicles, Corpus luteum.

Introduction

Miswak is a product of the plant *Salvadora persica* which grows in different areas of the world including the middle east and Africa [1]. Miswak is deeply rooted in traditional Arabian medicine and have been used for centuries for tooth cleaning [2]. Nowadays, miswak is recommended by the World Health Organization (WHO) for mouth hygiene in areas where their use is customary. It contains a number of medically beneficial properties including abrasives, astringents and antiseptics. Its main constituents are trimethylamine, an alkaloid which may effectively be salvadorine, chlorides, sulphur, terpenes, vitamin C, glycosides, large amounts of fluoride and silica, small amounts of tannins, saponins and flavonoids [3-5]. Raj and Agarwal [6] isolated β-sitosterol, m-anisic acid, and salvadourea. Also, Miswak stem yielded octacosanol, 1-triacantanol, β-sitosterol, and β-sitosterol-3-O-β-D-glucopyranoside [7]. Miswak has potential medicinal and research activities, it has been claimed in traditional literature to be valuable against a wide variety of diseases [8]. Miswak was reported to exhibit antiulcer activity [9], anticonvulsant activity [10], analgesic activity [11], antibacterial activity [12, 13], hypoglycaemic [14], antimicrobial [15], anti-plasmodial [16], antirheumatic [17], antioxidant [18], as anticancer [19-21] Oral hygienic activity [22]. However, scanty literature was exists on the direct effect of miswak on reproductive function. Darmani et al. [23] showed that exposure to meswak extract did not have much effect on female mouse fertility, although it caused a significant decrease in the relative weights of the ovary and an increase in uterine weights. *Meswak* has adverse effects on male and female reproductive system and fertility.

Administration od aqueous extract of *Salvadora persica* to female mouse significantly decrease the number of breeding's, corpora lutei and fertility percentage of treated animals compared with

control. The concentrations of hormones (FSH, LH, estrogen) has decreased in experimental groups compared with control one, while the concentration of progesterone has increased in all groups compared with control group, and also the concentration of prolactin has increased in treated groups compared with control group [24]. Therefore, the present study was undertaken to evaluate the physiological changes in the reproductive system of female albino rats after oral administration of an aqueous extract of leaves or stem of miswak The results therefore will help in clarify its suitability as an anti-fertility agent.

Materials and Methods

Preparation and extraction of *Miswak*

Collection of *miswak* plant was done from the green tree cultivated in Kingdom of Saudi Arabia, during July 2009. The plant was authenticated at the Department of Botany, Faculty of Agriculture, University of Al-Azhar, Egypt. The plant was washed under running tape water, then dried in shade for 5 days. Leaves of the *miswak* plant were separated from its stem, then both were cut into small pieces.

Aqueous extracts was prepared by mixing 20.0 g of dry *miswak* leaves or stem with 250 ml of distilled water in two separate sterile, dry screw-capped bottles. The bottles were maintained at room temperature in a shaker at 400 rotations per min. The mixture was boiled for 30 min then the extracts were decanted and filtered using filter funnels fitted with Whatman No 1 filter papers. The extracts were pooled and concentrated using a Büchi rotary evaporator (Büchi Labortechnik, Flawil, Switzerland) set at 40–50 C to reach to the concentrated solution of 20.0 ml (1:1- w:v). The aqueous extracts of *miswak* leaves and stem were stored at 4 ^{o}C till animals administration.

Animals

Forty adult female albino Wistar rats weighing between 150 and 170 g were used as experimental model during this study. The animals were obtained from the Animal House, National Research Center, Egypt. The rats were kept at a controlled temperature of 23±2 ^{o}C, relative humidity of 60–70% and a light regime of 12 hr light : 12 hr dark (lights on at 6:00). Animals were quarantined and acclimatized for 7 days prior to the initiation of the study; animals were given well balanced pellet diet and tap water ad libitum. The use of animals in our study is conformed to the guidelines and bioethics of the Egyptian Scientific Research Academy that coincide well with the U.S. Department of Agriculture through the Animal Welfare Act (7USC 2131) 1985 and Animal Welfare Standards incorporated in 9 CFR Part 3, 1991.

Experimental design

Acute oral toxicity study

Ten albino Wistar rats (150-170g weight) were kept for overnight fasting period prior to extracts administration. Three groups of animals were used, which received a single oral dose 0.5 ml (1:1- w: v) of aqueous extracts of *miswak* leaves (n=3) or stem (n=3) or distilled water (DW) for control group (n=4). After extracts or DW administration, food was withheld for further 3–4 hours. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 hours (with special attention during the first 4 hours), and daily thereafter for a period of 2 weeks. Once daily, cage side observations including changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, heart rate, autonomic (salivation, lacrimation, piloerection, urinary incontinence, and defecation) and central nervous system (drowsiness, gait, tremors and convulsion) changes was performed. Mortality, if any, was determined over a period of 2 weeks.

Effect of *miswak* on female reproductive system

The albino Wistar rats were segregated into 3 groups of 10 animals/each. Group I, served as control and received 0.5 ml per day distilled water as a vehicle. In Group II and III, animals were treated with aqueous extracts (leaves or stem) of *miswak* at dose of 0.5 ml (1:1 w: v)/rat, respectively. The extracts or distilled water (control) were administered intragastrically using animal feeding intubation needles (Popper and Sons, New York) for 14 consecutive days. Twenty-four hours after the last day of animal treatment, animals were weighed and vaginal smear were obtained by the aspiration technique. The aspirated fluid was places on glass slide, and allow the smear to completely dry at room temperature. Once dry, the smears were immediately stained with crystal violet or stored and stained at a later date. Microscopic examination was done immediately after staining. The stages of the estrous cycle were determined based on presence or absence of leukocytes, cornified epithelial, and nucleated epithelial cells according to Felicio et. al. [25].

Control and experimental animals were scarified under alcohol : chloroform : ether (1 : 2 : 3) anaesthesia at fasting state. Ovaries and uterus were dissected out from the surrounding tissues then weighed and the number of surface ovarian follicles was recorded under stereomicroscope. Autopsy specimens were taken from the ovary and uterine horn of each rat directly after their killing. Specimens were immediately fixed in 10% formalin for at least 24 hours. Tissue specimens were dehydrated in a graded series of ethanol, cleared in xylene, embedded in paraffin, serially sectioned at 4 µm thickness and mounted on glass slides. Tissue sections were deparaffinized and stained by Hematoxylin and eosin (H&E) for histological structure. Every tenth section was photographed using Olympus microscope fitted with camera containing CCD camera.

The ovaries and uteri of control animals were evaluated first, while ovaries and uteri in the experimental groups were evaluated blind to treatment. Histological changes, epithelial cell height, stromal thickness, and myometrial thickness were recorded.

Statistical Analysis

The results were expressed as mean values ± S.E.M. (standard error of mean) for the three groups. Statistical comparison was carried out by analysis of variance (ANOVA). The difference between the means of treated groups and the non-treated control group was evaluated by Duncan's test. Statistical analyses were performed using SPSS (version 15.0 for Windows; SPSS Inc, Chicago, IL, U.S.A., 2008). The results were considered statistically significant when $P < 0.05$.

Results and Discussion

There is quest for the oral contraceptive agent to control human fertility. Although, a variety of synthetic contraceptive agents are available, but these cannot be used continuously because of their side effects. So, natural plant substances could offer an effective non-conventional source of contraception with less deleterious side effects.

In the present work, acute toxicity study of oral administration of *miswak* leaves or stem aqueous extract revealed the absence of any signs and symptoms of toxicity in the extract treated animals, suggests that the extract was non-toxic and safe to the female rats at the dose employed.

In the present work, administration of aqueous extract of *miswak* leaves or stem significantly extended the period of diestrus phase in treated female rats. In control group, vaginal smears examination indicated that 5 out of 10 animals were in estrus phase (50%), whereas the rest of animals were in proestrus or metestrus phase (40% and 10%, respectively). The smear entirely consists of cornified superficial cells that forming large flacks (Table 1). In *miswak* treated groups, microscopic examination of vaginal smear revealed that 90% and 70% of female rats were in diestrus phase for leaves and stem extracts, respectively, and the rest of animals were in metestrus phase. The vaginal smears consistently lack cornified cells whereas leukocytes were very plentiful (Table 1). It is clearly noticeable that the administration of *miswak* extracts induced progesterone like effect, and consequently it extended the diestrus phase of the estrus cycle. The influence of progesterone on ovariectomized rats, resulting in the presence of intermediate cells, and polymorphs and mucus forming a noticeable background to the smear [26].

Table 1: Assessment of predominant cell typology in vaginal smears after *miswak* leaves and stem extracts administration in Wister rats.

Group %		Nucleated epithelial cells	Cornified squamous epitheli cells	Leukocytes	Over all **estrous stage**
Control	**40**	**+++**	-	-	Proestrus
	50		**+++**		Estrus
	10	+	-	+++	Metestrus
Leaves	**90**	+	+	+++	Diestrus
	10	-	++	+++	Metestrus
Stem	**70**	+	+	+++	Diestrus
	30	-	++	+++	Metestrus

%= Means percent of rats showing this type of cells

Cell Number: +++= High, ++= Moderate, += Low, **-** = Nil

Table 2: Effect of aqueous extracts of *miswak* on body, ovarian and uterine weights in female Wistar rats.

Treatment	Body weight on day of sacri (g)	Ovarian weight (mg)	Uterine weight (mg)
Control	186.60 ± 13.49 ^{a}	128.30 ± 9.18^{b}	476.80± 16.20^{b}
Leaves extract	179.20± 14.66 ^{a}	99.80 ± 11.73^{a}	354.30 ± 18.05 ^{a}
Stem extract	172.89 ±12.11^{a}	105.10 ± 8.86^{a}	442.10 ± 14.10^{b}

Mean ± SEM (n=10)

$^{a,b,..}$ Means having different superscripts are significantly different at $P<0.05$.

In addition, in the present work no significant change in the final body weight was observed after 14 days of administration of *miswak* leaves or stem extract. However, a significant reduction in genital organ weight was noticed after administration of *miswak* extracts. Since body weight gain did not altered in the extract treated rats in comparison with the controls, the morphological and histological changes observed on the female rat reproductive system following treatment may be attributed to the effect of the extract itself on reproductive system. In the current work, administration of either *miswak* leaves or stem aqueous extract significantly decreased ($P<0.05$) ovarian weight of treated rats compared to control group. Furthermore, aqueous extract of *miswak* leaves or stem significantly decreased ($P<0.05$) the number of surface ovarian follicles compared with the control group (Figure. 10. While, the number of corpora lutea per ovary did not vary between control and treated animals (Figure. 1). After

histological examination, higher number of surface ovarian follicles showed signs of atresia in rats treated with *miswak* leaves extracts compared with stem extract or control group (Figure 2A, B, C). Similarly, administration of ethanolic extract of miswak to female mice was accompanied with significant reduction in ovarian weight [23]. Numerous studies have reported the effects on fertility of female animals treated with extract of various plant species. Oral administration of M. charantia (27), S. trifoliatus (28), H. rosasinensis flowers (29), and M. azedarach extract (30) was reported to prolong the diestrus phase, decreased the weight of ovaries, decreased the number of ovarian follicles and increased the number of atretic follicles in female rats. Also Solomon et al. [31] reported that administration of Rumex steudelii to female rats decreased ovarian weight. The effects of plant extracts at the ovarian level may be indirect due to their primary effects occurring at the hypothalamic or hypophysial level, the result of which is suppression of normal gonadotropin release (24, 28). The alteration of gonadotropin secretion leads to inhibition of normal ovarian steroidogenesis (estrogen and progesterone) and feedback within the hypothalamus-pituitary-gonadal axis, consequently estrous cycle was disrupted and reduced the follicular development and ovulation (32, 33).

The effect of aqueous extract of *miswak* leaves or stem on histological structure of rats uterus is presented in Figure 3. In the current work, uterine weight significantly ($P<0.01$) decreased in rats given an aqueous extract of *miswak* leaves compared with stem extract and control groups. In control group, the uterine lumen was lined by very large tall secretory columnar epithelium with small nucleus present at the base of the cell (Figure 3A), and also there was a high incidence of vacuolar degeneration. Large number of neutrophils infiltrated into the lamina propria and endometrial gland (Figure 3B). Administration of miswak leaves extract to female rat caused atrophic effect on the uterine tissue as revealed by a significant reduction in the epithelial cell height, stromal thickness and myometrial thickness. Uterine epithelium was composed of small cuboidal cells, the uterine glandular epithelium also lined with small columnar epithelial cells and dark staining nuclei situated mainly at apical and middle aspects of the cells (Figure 3C,D). High incidence of mitotic figures and low number of neutrophils remain within the lamina propria (Figure 3D). For stem extract group, the luminal epithelium and glandular epithelium were low columnar cells and the number of neutrophil infiltration markedly increased as in control group (Figure 3E,F). The decrease in the uterine weight is indicative of the antiestrogenic nature of the plant since antiestrogenic substances are known to decrease the weight of the rodents uterus [23]. The extract either directly act on the uterine tissue as estrogen antagonist or indirectly by disruption of the hormonal balance on the hypothalamo-hypophysial-ovarian-uterine axis. It is known that estrogen induces a rapid increase in microvascular permeability in may be attributed to the absence or reduced availability of ovarian hormones and gonadotropins., leading to stromal edema and marked increase in uterine weight [34]. The results observed in the present study on the histology of the uterus is in complete agreement with previous studies in which treatment of female rats with antifertility plants like Rumex steudelii seeds [31] or Sesbania sesban seeds [35] caused great reduction in endometrial height, atrophy of uterine glands, compact stroma and poor vascularity. Contrary, Darmani et al. [23] recorded that administration of *meswak* ethanolic extract to female mice increased uterine weight. This contradiction may be due to the method of *miswak* extraction, source of plant or the dose used, or species difference.

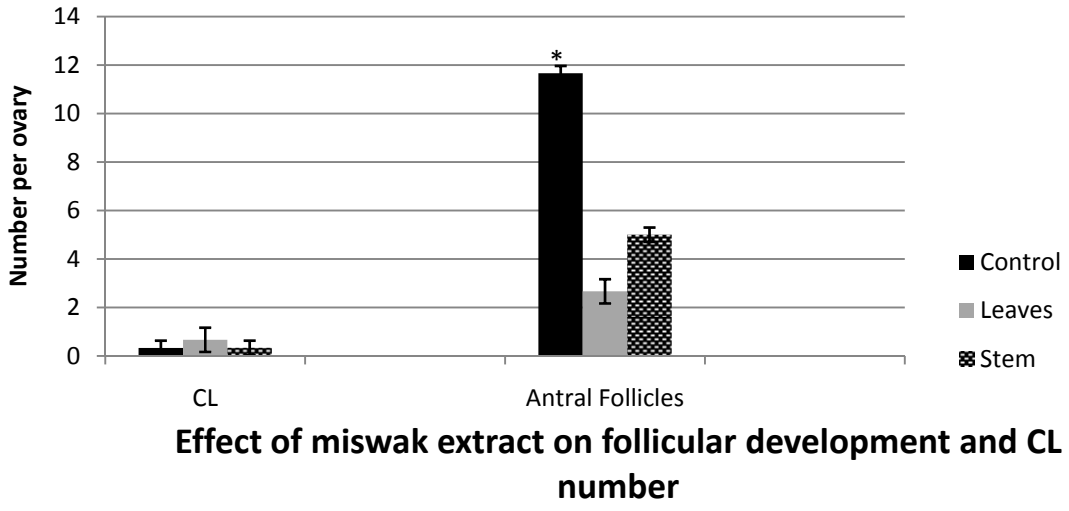

Figure. 1: Effect of *miswak* leaves or stem extracts administration on the development of antral follicles and corpus luteum in rat ovaries.

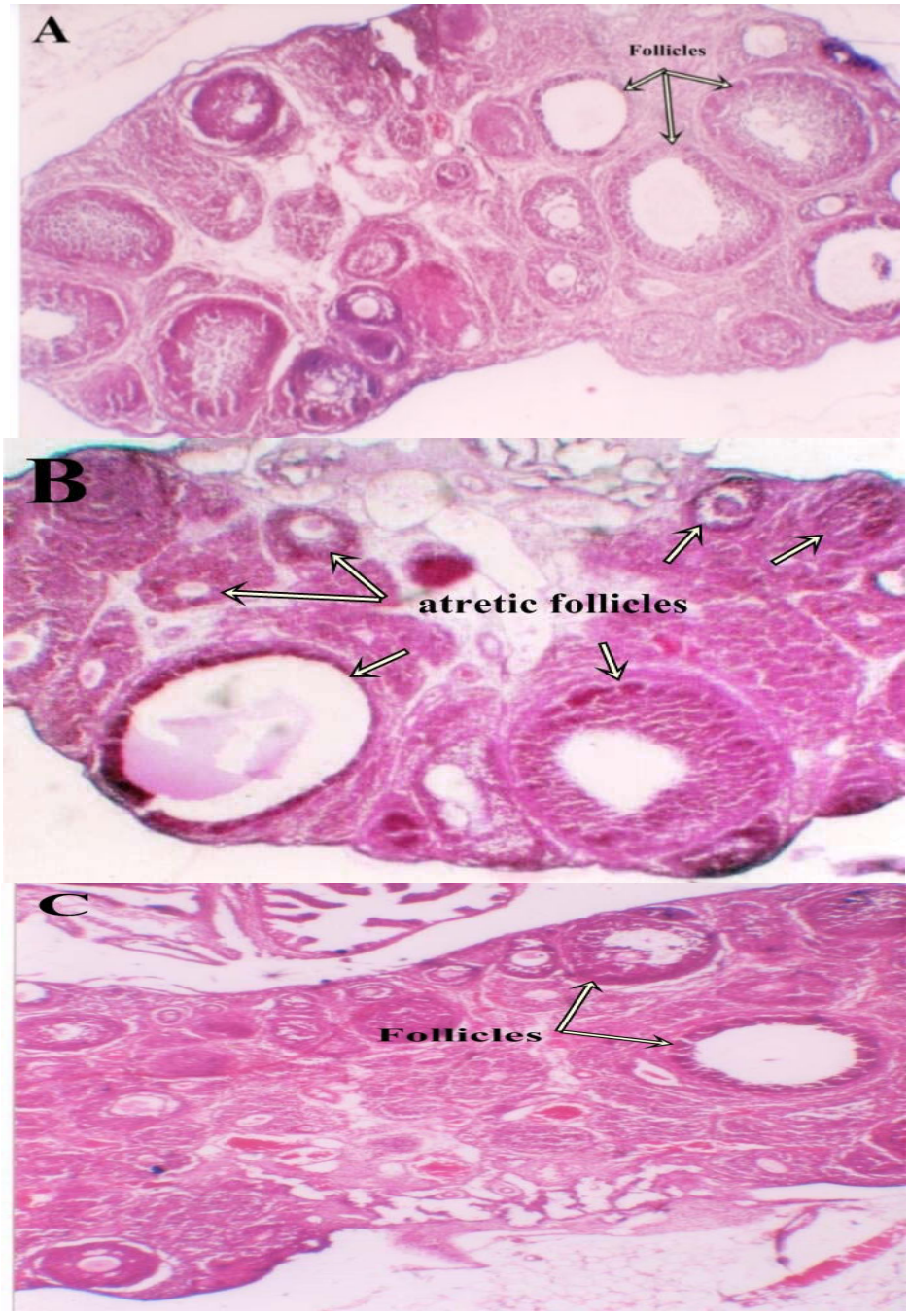

Figure. 2: Photomicrographs of cross-sections from equivalent regions of the ovary of control rats (A) and rats treated with aqueous extract of *miswak* leaves (B) or stem (C). (H&E 40X).

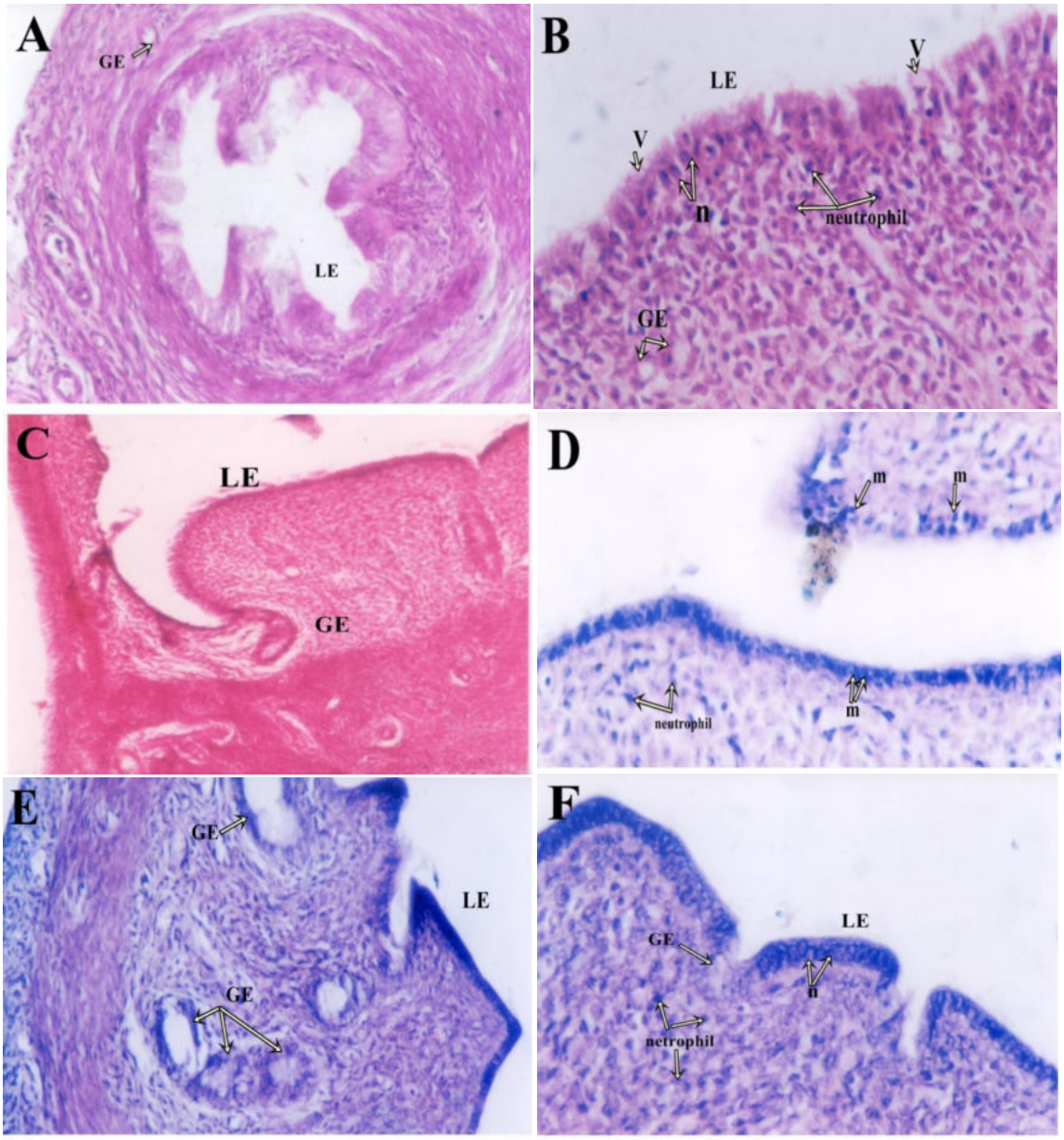

Figure. 3: Photomicrographs of cross-sections from equivalent regions of the uterine horn of control rats (A,B,) and rats treated with aqueous extracts of *miswak* leaves (C, D) or stem (E,F). H&E stain, A,C, E magnification at 40X, and B, D, F magnification at 200X.

Conclusion

Oral administration of aqueous extract of *miswak* to female albino rat prolonged the diestrus phase of estrous cycle, reduced ovarian weight and disrupt folliculaogenesis and produced anti-uterotrophic effects. This effect may be mediated through the direct effect of the extract on the reproductive organs by suppressing follicular growth and increased incidence of follicular atresia in the ovary and/ or by disruption of the hormonal balance in the hypothalamo-hypophysial ovarian and uterine axis. *Miswak* stem extract has less antifertility effect on female rats.

Competing Interest

Authors declare that they have no competing interests

Authors contribution

Abdoon A.S., Abdel-Rahman H.A. and Kandil O.M. design the work, performed all experiments, Mohamed A.A. and Al-Saghier O.

available miswak and performed the plant authenticate, Abdoon write the manuscript and all the authors read and approved the final manuscript.

Acknowledgement

This paper was funded by the National Research Center (NRC) of Egypt (in-house project 9040207). The authors, therefore, acknowledge with thanks the technical and financial support for NRC.

References

[1]. Eid MA, Al-Shammery AR, Selim HA. The relationship between chewing stick (*miswak*) and periodontal health. Relationship to plaque, gingivitis pocket depth and attachment loss. Quintessence Int. 1990; 21:1019-1022.

[2]. Sadhan AL, Almas K., 1999. *Miswak* (chewing stick) - A cultural and scientific heritage. Saudi Dent J. 1999;11:81.

[3]. Asolkar LV, Kakkar KK, Chakre OJ, 1992. Second Supplement to Glossary of India Medicinal Plants with Active Constituents, India, NISCAIR, 1992. pp. 1965-1985.

[4]. Bungorn S, Varima W, Pisamai L, Jamsai S, Durit J. Diuretic effects of selected Thai indegenous medicinal plants in rats. J Ethnopharmacol. 2001;75:185-190.

[5]. Chatterjee A. Treatise of Indian Medicinal Plants. Council for Scientific and Industrial Research, India, 1990, 327.

[6]. Raj KP, Agarwal YK. Heavy metal contents of the leaf-gall. Sci. Cult. 1979;45:35.

[7]. Jain M, Saxena VK. Chemical constituents of the Stem of Salvadora persica. Acta Ciencia Indica 1984;10:127-128.

[8]. Khare CP, 2007. Indian Medicinal Plants: An Illustrated Dictionary, Springer Publishers, 2007. pp. 574.

[9]. Monforte MT, Miceli N, Mondello MR, Sanogo R, Rossitto A, Galati EM. Antiulcer activity of *Salvadora persica* on experimental ASA-induced ulcer in rats: ultrastructural modifications. Ind J Expl Biol. 2001;39:289-292.

[10]. Mohamed SA, Jalaluddin A, Khan JA. Antioxidant capacity of chewing stick miswak Salvadora persica. BMC Complement Altern Med. 2013;13: 40. doi: 10.1186/1472-6882-13-40.

[11]. Sukkarwalla A, Ali SM, Lundberg P, Tanwir F: Efficacy of *Miswak* on oral pathogens. Dent Res J (Isfahan). 2013;10:314-320.

[12]. Monforte MT, Trayoto A, Foresteieri AM, Aquino DA, Rossitto A, Galati EM. Anticonvulsant and sedative effects of *Salvadora persica L.* stem extract. Phyto Res. 2002;16:395-397.

[13]. Talea H, Mansoor A, Mohammad S, Asif R. Analgesic activity of *Salvadora persica* in mice. Med Channel. 2011;4:22-24.

[14]. Almas K, Skaug N, Ahmad I. In vitro antimicrobial comparison of *miswak* extract with commercially available non-alcohol mouthrinses. Int J Dent Hyg. 2005;3:18-24.

[15]. Sofrata AH, Claesson RL, Lingstram PK, Gustafsson AK. Strong antibacterial effect of miswak against oral microorganisms associated with periodontitis and caries. J Periodontol. 2008;79:1474-9.

[16]. Yadav JP, Saini S, Kalia AN, Dangi AS. Hypoglycemic and hypolipidemic activity of ethanolic extract of *Salvadora oleoides* in normal and alloxan-induced diabetic rats. Indian J Pharmacol. 2008;40:23-27.

[17]. Al-Bagieh NH, Almas K. In vitro antimicrobial effects of aqueous and alcohol extracts of *miswak*. Cairo Dental Journal 1997;13:221-224.

[18]. Ali H, Konig GM, Khalid SA, Wright AD, Kaminsky R. Evaluation of selected Sudanese medicinal plants for their in vitro against hemoflagellates, selected bacteria, HIV-1-RT and tyrosine kinase inhibitory and for toxicity. Journal of Ethnopharmacology 2002;83: 219-228.

[19]. Khatak M, Khatak S, Siddqui AA, Vasudeva N, Aggarwal A, Aggarwal P. *Salvadora persica*. Pharmacogn Rev. 2010;4:209–214.

[20]. Harlev E, Nevo E, Lansky EP, Lanskyc S, Bishaye A. Anticancer attributes of desert plants: a review. Anti-Cancer Drugs. 2012;23:255–271.

[21]. Ibrahim AY, El-Gengaihi SE, Hemaia M, Motawe HM. Phytochemical and cytotoxicity investigation of *Salvadora persica* bark extracts. JASMR 2011;6:127-133.

[22]. Iyer D, Patil UK. Efficacy of Stigmast-5-en-3β-ol Isolated from *Salvadora persica L.* as antihyperlipidemic and anti-tumor agent: Evidence from animal studies. Asian Pacific Journal of Tropical Disease 2012:S849-S855.

[23]. Darmani H, Al-Hiyasat AS, E1betieha AM, Alkofahi A. The effect of an extract of Salvadora persica (Meswak chewing stick) on fertility of male and female mice. PhytoMedicine 2003;10:63–65.

[24]. Al-Humesh MJM, Mahmood AY, Lateef RH. Effect of watery extract of the *Salvadora persica* on the concentration of the sexual hormones and number of fertility parameters of white mice females. Tikrit Journal Agricultural Sciences University 2012;12: 62-68.

[25]. Felicio LS, Nelson JF, Finch CE. Longitudinal studies of estrous cyclicity in aging C57BL/6J mice: II. Cessation of cyclicity and the duration of persistent vaginal cornification. Biol Reprod. 1984;31:446–453.

[26]. Montes GS, Luque EH: Effects of ovarian steroids on vaginal smears in the rat. Acta Anat (Basel); 1988, 133: 192-199.

[27]. Sharanabasappa A, Vijayakumar B, Patil SB. Effect of *Momordica charantia* seed extracts on ovarian and uterine activities in albino rats. Pharm. Biol. 2002;40:501-507.

[28]. Duragkar NJ, Gore S, Katolkar PP. Contraceptive effects of saponins from *Sapindus trifoliatus* DC, 2008. Patent IN200700001-I3.

[29]. Kholkute SD, Chatterjee S, Udupa KN. Effect of *Hibiscus rosasinensis* Linn on estrous-cycle and reproductive organs in rats. *Indian* Journal of Experimental Biology 1976; 14:703-704.

[30]. Roop JK, Dhaliwal PK, Guraya SS. Extracts of *Azadirachta indica* and *Melia azedarach* seeds inhibit folliculogenesis in albino rats. Brazilian Journal of Medical and Biological Research 2005;38:943-947.

[31]. Solomon T, Largesse Z, Mekbeb A, Eyasu M, Asfaw D. Effect of *Rumex steudelii* methanolic root extract on ovarian folliculogenesis and uterine histology in female albino rats. African Health Sciences. 2010;10: 353 – 361.

[32]. Farnsworth NR, Bingel AS, Cordell GA, Crane FA, Fong HFS. Potential value of plants as sources of new antifertility agents I. Journal of Pharmaceutical Sciences 1975;64:535-598.

[33]. Farnsworth NR, Bingel AS, Cordell GA, Crane FA, Fong HFS. Potential value of plants as sources of new antifertility agents II. Journal of Pharmaceutical Sciences 1975; 64:717-754.

[34]. Carroll WR. Variation in the water content of the rat uterus during continuous estrogenic treatment. Endocrinology. 1945;36: 266-271.

[35]. Pal SS. Fertility control of female through *Sesbania Sesban* seeds. Journal of Research and Education in Indian Medicine 1990;4: 27 -32.