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Antiandrogenic Activity of *Artemisia herba-alba* in Male Albino Rats, with Emphasis on Biochemical Parameters

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The aqueous extracts of *Artemisia herba alba*. solution was fed orally to male albino rats at a dose of 100 mg/kg body weight for 60 d. A total of 20 rats were involved in this study and were divided into two groups. Group (A) a vehicle-treated control and group (B) a treated group with *Artemisia herba-alba*. The dose induces a significant decrease in the weight of reproductive organs ($p < 0.01$) when compared to controls. The sperm motility and density in cauda epidydimides and testicular ducts were significantly decerased ($p < 0.01$). A significant decreased ($p < 0.001$) in spermato-gensis activity is observed in somniferous tubule. Our results demonstrated that administration of *Artemisia herba-alba* in a dose of 100 mg/kg of body weight for 60 d induces a very significant decrease in glucose level. A significant ($p \leq 0.01$) increase in total serum cholesterol level, triglycerides, phospholipids, serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT). Treated rats testicular cell population showed a decrease in number of spermatocytes and spermatids ($p < 0.001$) when compared to controls. Serum hormonal assay indicated a decrease in testoterone and follicular stimulating hormone (FSH) levels in treated rats. A decreased in number female rats impregnated by males receiving treatment was observed and demonstrated by a decrease in the implantation sites and viable fetuses number ($p < 0.01$).

Key Words: *Artemisia herba-alba*, Fertility, Spermatogenesis, Male and female albino rats.

INTRODUCTION

Plant preparations play an important role in fertility regulation, a fact that has been reported in the ancient literature of indigenous systems of medicine. A number of plant species have been tested for fertility regulation beginning about 50 years ago and were subsequently fortified by national and international agencies[1,2].

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Artemisia herba alba (Composate) is commonly known by the Arabic name "sheh" and is a popular folk remedy for the treatment of diabetes mellitus in Jordan. For this purpose, the native use a hot water decoction made from the fresh leaves and branch lets. *A. herba alba* used by local population of some middle east countries as an antidiabetic activity[3,4], *A. herba alba* also used as an antihelminitic[5]. A literature survey revealed that certain other species of Artemisia also shown antimalarial[6], antibacterial[7] and insecticidal[8].

Phytochemical investigation of *A. herba alba* have shown that it contains santonin[5], sequiterpene lactones[9] and flavonoids[10]. Components of the essential oil have also been investigated[11]. The aim of the present study is to present the presence or absence of antifertility activity for this plant using male albino rats.

EXPERIMENTAL

Adult male and female albino rats of Sprague Dawley strain, weighing about 300 g were raised in the Animal House Unit in Faculty of Medicine, Jordan University of Science and Technology and under controlled temperature of 21 ± 1°C and 12 h light: 12 h darkness schedule (lights on 06.00 am - 18.00 pm). Food and Water were available *ad labitum*.

Sample of *Artemisia herba-alba* plant were collected from mafraq area, between September to December, the plant were identified in our laboratory, then the plant was dried and grinded with a grinder into powder perparation for extraction.

The *Artemisia herba-alba* powder was extracted by water-ethanol mixtuer (70/30,v/v) for 6 h, this step was repeated three times. The filtrate was pooled and concentrated under vacuum (not exceeding 50°C) and dissolved in normal saline (freshly prepared) to a final of 250 mg/kg for further use. The extract administered orally to rats using animal feeding intubations needles (Popper and Sons, New York) in concentration of 250 mg/kg.

Male rats were divided into following groups:

G-1 Intact (Control) rats of this group received vehicle (distilled water) treatment for 60 d).

G-2 Rats of this group received an aqueous extracts of *Artemisia herba-alba* in a dose of 100-mg/kg body weight for 60 d representing the fully reproductive cycle.

After 24 h of the last dose, animals were weighed and autopsied under light ether anesthesia. The blood was collected through cardiac puncture using a sterile syringe for serum analysis.

Fertility test: Fertility was estimated in adult male rats treated with aqueous extracts of *A. herba-alba* and in the control male counterparts.

Each male was placed in an individual cage with two virgin untreated females of the same strain they were left together for 10 d during which two estrous cycles had elapsed[12]. One week after the removal of the exposed males, pregnant females rats were sacrificed by cervical dislocation under light ether anesthesia and the number of implantation sites, the number of viable fetuses and the number of resorption sites were recorded.

Body and organ weights: The initial and final body weights of the animal were recorded. The reproductive tract was taken out trimmed free of fat and each organ was weighed sparately on electronic balance. The reproductive organs taken into account for study in male include testes, epididymides, ventral prostrate, seminal vesicle, and vas deferens. Some vital organs such as the liver and heart were also obtained, weighed and kept in 90 % formaldehyde for further analysis. Reproductive organs along with a small piece of the obtained liver, heart and kidney were fixed in Bouin's fixative for histological studies.

Sperm motility and count: To determine the sperm count and motility, a 100 mg of cauda epididymides was minced in 2 mL of physiological saline and one drop of the evenly mixed sample was applied to a Neubauer's counting chamber under cover slip. Quantitative motility expressed as percentage was determined by counting both motile and immotile spermatozoa per unit area. Cauda epididymal and testicular sperm counts were made by routine procedure and expressed as million/mL of suspension[13].

Histological analysis: The Bouin's fixed reproductive organs (testes, epididymides, seminal vesicle, ventral prostate, and vas deferens) along with liver, kidney and heart were cut into small pieces and processed for histological slides. After dehydration using different concentration of alcohol, specimens were embedded in parafin blocks and sectioned at 5 µm, placed on a clean histological glass slide and stained using haematoxyline and eosin.

Histometry: With the help of Camera Lucida, one hundred of circular appearing somniferous tubles were traced at 80x magnification and the diameter of each tubule was measured separately. The measurement was expressed in terms of mean of all the traced tubules. Similarly, Leyding cell and their nuclei were traced at 800x. In addition, epithelial cell height of cauda epididymides, caput epididymides and seminal vesicle were also traced at X-360 and recorded.

Testicular cell population counting: Spermtogenic elements namely spermatogonia, spermatocytes and spermatids were counted in 5 µm thick cross section is of 10 somniferous tubules obtained from 10 animals of each group. All raw counts were transformed to true counts by an adaptation of Abercrombie formula[14] from germ cell diameter measurement. Interstitial cell types (such as fibroblast, immature and matuer Leydig cells

and degenerating cells) were estimated, applying a differential count over 200 cells population and statistically verified by the binomial distribution[15].

Serum biochemistry: Total glucose, cholesterol, triglycerides, phospholipids, serum aspartate aminotransferase (AST) and serum alanine amino -transferase (ALT) were determined in blood serum using commercial kits (Cis BIO International Gif sur Yvette, France)

Hormonal assays: Blood plasma FSH and testosterone concentrations was measured by radioimmunoassay using two commercial kits (Cis BIO International Gif sur Yvette, France).

Statistical analysis: All values of body/organ weight biochemical estimation and histometric analysis were experssed in terms of mean value ± S.D. The different treatment groups were compared[16] with control group using chi-square test and Student's "t" test.

RESULTS AND DISCUSSION

Effect of *Artemisia herba-alba* on body and organ weight: Tabel-1 shows the effect of intra-gastric administration of *Artemisia herba-alba* caused an increased in body weight in treated animals, when initial and final body weight were compared with controls. The weight of the tests, epididymides, seminal vesicle, ventral prostate and vas deferens however, were found to be significantly decreased in treated male rats when compared with the weights of the same organs obtained from control rats.

TABLE-1
BODY AND ORGAN WEIGHTS OF MALE ALBINO RATS TREATED WITH *ARTEMISIA HERBA* FOR 60 D

Condition	Body weight (g)		Test	Epidid-ymides	Seminal Vesicle	Ventral Prostate	Vas Deferens
	Initial	Final	(mg/100 g body weight)				
Control Group	304 ± 2.80	319 ± 2.65	895 ± 25.21	367 ± 21.61	376 ± 14.38	226 ± 4.1	113 ± 4.36
Treatment Group	287 ± 3.11	302 ± 4.55	809** ± 17.19	343** ± 22.87	354** ± 17.98	197** ± 3.67	86* ± 2.56

Results are expressed as mean ± S.D.
Ten rats were included per group.
*p < 0.05, **p < 0.01 significantly different from control group (Student's "t" test).

Effect of *Artemisia herba-alba* on sperm dynamics and histometrical parameters: Table-2 shows that the sperm motility in cauda epididymis was decreased to a significant levels ($p < 0.001$) in treated animals with *Artemisia herba-alba L* when compared with the controls. A significant decrease in sperm density, somniferous tubule diamter and leydig cell nuclear diameter was also observed in treated rats ($p < 0.01$). Epithelial cell height in epididymides (cauda and caput) and seminal vesicle were found to be significantly decreased as well in treated group ($p < 0.01$).

TABLE-2
HISTOMETERICAL PARAMETERS AND SPERM DYNAMICS FINDINGS IN MALE ALBINO RATS TREATED WITH *ARTEMISIA HERBA* FOR 60 D COMPARED TO CONTROLS

Condition	Sperm Density (million/mL)		Sperm motility (%)	Semi-niferous tubule	Leydig cell nuclear	Epithelial cell height		
	Testes	Cauda	Cauda	Diameter	Diameter	Caput	Cauda	Seminal Vesicle
Control group	4.75 ± 0.47	56.0 ± 1.94	74.1 ± 1.94	290.6 ± 3.2	6.45 ± 0.96	38.8 ± 0.4	26.08 ± 0.32	17.32 ± 0.17
Treatment group	3.67** ± 0.14	35.67** ± 1.08	52.33† ± 1.08	246.87** ± 2.35	4.63** ± 0.762	33.47** ± 2.66	22.98** ± 2.68	14.5** ± 0.27

Results are expressed as mean ± S.D.
Ten rats were included per group.
*p < 0.05, **p < 0.01, †p < 0.001 significantly different from control group (Student's "t" test).

Effect of *A. herba-alba* on testicular cell population dynamics: Table-3 shows that the administration of *A. herba-alba* extract causes a significant decreased (p < 0.001) in rats germinal cell population namely spermatocytes (Primary and secondary) and spetmatids. Similarly, the immature and mature Leydig cells number were also decreased to a significant levels, whereas the degenerating cell numbers were observed to be significantly increased (p < 0.001). Fibroblast and spermatogonia numbers were not altered in treated rats.

TABLE 3
TESTICULAR CELL POPULATION DYNAMICS FINDINGS IN MALE ALBINO RATS TREATED WITH *ARTEMISIA HERBA* FOR 60 D COMPARED TO CONTROLS

Condition	Germinal cell types				Interstitial cell type			
	Spermatogonia	Spermatocyte (primary)	Spermatocyte (secondary)	Spermatids	Fibroblast	Immature Leydig Cell	Mature Leydig Cell	Degenerating Cell
Control Group	23.99 ± 0.93	18.85 ± 0.80	64.126 ± 3.51	147.71 ± 4.87	63.83 ± 1.64	65.195 ± 3.47	70.64 ± 1.03	18.34 ± 1.67
Treatment Group	18.66 ± 4.44	13.45† ± 2.41	23.45† ± 3.73	36.7† ± 6.82	44.56 ± 1.33	43.11† ± 1.65	49.33† ± 0.78	34.98† ± 0.76

Results are expressed as mean ± S.D.
Ten rats were included per group.
†p < 0.001 significantly different from control group (Student's "t" test).

Effect of *Artemisia herba-alba* on biochemical changes analysis: The results presented in Table-4 shows that blood glucose level was decreased to a significant values (p < 0.001), while total cholesterol, triglycerides, phospholipids, ALT and AST were increased (p < 0.01) in treated groups when compared to controls. Levels of plasma FSH and testosterone were found to be significantly decreased (p < 0.001) in treated group when compared with controls.

TABLE-4
SERUM BIOCHEMISTRY FINDINGS IN MALE ALBINO RATS TREATED WITH *ARTEMISIA HERBA* FOR 60 D COMPARED TO CONTROLS

Condition	Glucose	Choles-terol	Trigly-cerides	Phospho-lipid	ALT	AST	Testost-erone	FSH
	(mmol)			(mg/100 mL)	(µL)		(mmol/L)	(µL/L)
Control group	7.3 ± 0.212	1.04 ± 0.147	0.8 ± 0.07	217 ± 3.14	36.7 ± 6.98	77.7 ± 7.14	14.4 ± 1.33	23.25 ± 1.44
Treatment group	4.4† ± 1.03	0.16** ± 0.07	0.07** ± 0.04	193** ± 2.04	78.8** ± 1.44	120** ± 8.3	9.24** ± 1.69	16.55** ± 0.68

Results are expressed as mean ±S.D.
Ten rats were included per group.
**p < 0.01, †p < 0.001 significantly different from control group (Student's "t" test).

Effect of *Artemisia herba-alba* on male rat fertility: The results presented in Table-5 shows that intra-gastric administration of *Artemisia herba-alba* diet at dose (100 mg/kg body weight) for 60 d to male rats causes a significant decreased ($p < 0.01$) in the number of females impregnated by male treated rats. The number of implantations and the number of viable fetuses calculated after cesarean suctions were significantly decreased ($p < 0.01$) in female rats impregnated by treated males when compared with females impregnated with untreated rats. On other hand the number of resorptions sites were found to be increased to a significant values ($p < 0.05$) in females impregnated by treated male rats when compared to controls.

TABLE-5
EFFECT OF *ARTEMISIA HERBA L* FED TO INTACT ON MALE ALBINO RATS ON FERTILITY

Condition	Male No.	Female No.	Pregnant Females No.	Implantation Sites No.	Viable Fetuses No.	Resorption Sites No.	Resorption No./total Implantation No.
Control group	10	20	18/20 (85 %)	9.62 ± 2.66	9.37 ± 1.16	8	8/173 (5 %)
Treatment group	10	20	15/20 (90 %)	8.3 ± 3.31	6.63 ± 1.54	19	19/125 (15.4 %)

In this experiment, the animal model has been used previously by several workers to assess the adverse effects of this extract obtained from medicinal plants on reproductive functions in male[17,18].

In rats the whole spermatogenic cycle process requires 53 d out which spermatozoa spends the last 6 to 7 d in its final transition though epididymides[19]. Treatment presented as an extract obtained from *Artemisia herba-alba* was administrated for one complete spermatogenic cycle. The present investigation clearly shows that oral administration of *Artemisia herba-alba* promoted a decreased male albino rats fertility. This is further illustrated from the data obtained regarding the weight of reproductive

organs were a markedly decreased in the organs weight was observed (Table-1). It is well known that the weight, size and the secretory function of tests, epididymes, seminal vesicles, ventral prostate, and vasa differentia are closely regulated by androgens hormones[20,21]. Therefoer, it could be that the treatment may act directly or indirectly on the pituitary gland secretory function leading to an increase in the main hormones controlling spermatogenesis process. It has ben demonstrated that the process of spermatogenesis and the accessory reproductive organs functions are androgen dependent. Therefore, a change in the androgen production would reflect and explain the decrease in number of mature Leydig cells and their functional status. In this present study, the findings indicated that the number of degenerating Leydig cells were significantly decreased that lead to a decrease in the serum androgen level observed in our results. In these findings, a decrease in number of spermatocytes (primary and secondary) and spermatids observed together with this perversely mentioned observations go hand in hand with and further confirm our hypotehsis leading to conclude that these stages are completely androgen dependent[22].

Histometric analysis of reproductive organs, on the other hand, further confirmed the androgenic effect. This is illustrated by the finding that a significant decrease in the sperm motility was observed in the cauda epididymis in treatment group. The results presented also indicated that ingestion of *Artemisia herba-alba* byadult male rats reduces the number of females' impregnation as shown in Table-5. In addition, the number of implantations and the number of viable fetuses were decreased, this decreased could be a reflect and may be due to the decerase in sperm motility and sperm density observed in this study. This may be due to the activity effects of *Artemisia herba-alba* on the enzymes involved in the oxidative phosphorylation proces. In conclusion, *Artemisia herba-alba* ingestion diet possesses strong compound or principles that decreased fertility mainly by affecting pituitary gland cells, Further studies are in progress to isolate and identify the active principle(s) with precise mode of its action.

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