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# CERTAIN PHARMACOLOGICAL EFFECTS OF ARTEMISIA ALBA IN RATS

BY

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## SUMMARY

The effect of alcoholic extract of *Artemisia alba* in doses of 25.2 and 126 mg/kg. b.wt on male rat fertility were studied. The tested doses were given orally to male rats for 65 successive days. Sex organs weights, seminal characters and histopathological examination of the male genital organs were used to evaluate the reproductive efficiency of the treated rats.

A significant decrease in the weights of genital organs, sperm cell concentration and percentages of sperm motility and seminiferous tubules containing spermatozoa with an increase in the percentage of spermatozoal abnormalities of treated rats. Histopathological examination of genital organs revealed that alcoholic extract of *Artemisia alba* caused testicular lesions characterized by moderate to severe degenerative changes of spermatogonial cells and partial arrest of spermatogenesis.

## INTRODUCTION

*Artemisia alba* is a common plant growing in Syria in many regions, e.g. azzib valley, All-domir and All-hamed (sankari, 1971). It is a dwarf shrub, silver in colour with white leaves and aromatic smell & known by Shih. Sheep refuse to graze it during spring season. They eat the plant in autumn after dryness and evaporation of volatile oils (Al-Rabbat 1975).

*Artemisia alba* was used in folk medicine as anthelmintic due to its santonin content (Foulsham 1976), insecticide (abdallah et al., 1986), antibacterial in treatment of bronchial infections (Benouda and Benjilali, 1988), hypoglycemic (Cami and farjou, 1990) and in treatment of diarrhea, abdominal pain and other gastrointestinal disturbances (Al-waili, 1988). Yashphe et al. (1987) attributed most of the previous pharmacological effects to essential oil content of the plant.

The present work was design to accomplish some of important effects to elucidate other pharmacological effects.

## MATERIAL AND METHODS

### The plant:

*Artemisia alba* plant was obtained from Azzib valley of Syria.

### Preparation of extract:

Alcoholic extract was prepared by cold percolation of dried *Artemisia alba* plant with 95% ethanol till exhaustion then evaporated under reduced pressure till became 20%.

### Toxicological studies:

Acute toxicity of alcoholic extract of *Artemisia alba* plant was studied on 5 groups of mature albino mice (20 - 25 g b.wt. ) each. The tested extract was given orally in graded increased doses. Animals were kept under observation for 24 hr during which symptoms of toxicity and rate of mortality in each group were recorded.

$LD_{50}$ of alcoholic extract of *Artemisia alba* plant was determined as described by Kerber

(1941). For this purpose, 5 groups of 5 mice each were used in addition to a control group. The tested extract was administered orally in doses ranging from 800 to 1600 mg/kg b.wt. The number of dead animals and post-mortem findings in each group were recorded during 24 hours.

$LD_{50}$ = The biggest dose - $\frac{\sum (zxd)}{n}$

z = The mean of dead animals in 2 successive groups.
d = The constant factor between 2 successive doses.
n = Number of animals in each group.
$\sum$ = The sum of (zxd).

**Hormone-like effect:**

Oestrogen-like action of the tested extract was studied qualitatively on 3 groups of 5 ovariectomised rats using vaginal smear technique (Robson, 1947).

progesterone-like action was studied on 3 group of 5 immature female rabbits each as proceeded by Clauberg (1930).

The androgenic activity of the tested extract was performed on 3 groups of 5 castrated rats. Weights of prostates and seminal vesicles were recorded and compared with those of control group.

**Effect on male rat fertility:**

Male rats of proven fertility were divided into 3 groups of 10 animals each. The first group was kept as a control whhile the others were orally giren alcoholic extract of Artemisia alba plant in doses of 25.2 ($^1/_{50}$ $LD_{50}$) and 126 ($^1/_{10}$ $LD_{50}$) mg/kg b.wt, respectively. The alcoholic extract was administered daily for 65 successive days to cover a complete spermatogenic cycle (Hershberger et al., 1969). Rats were sacrificed by decapitation, their sexual organs were dissected out dried between two filter papers and weighed. Weights of testes and accessory glands of each rat were calculated in relation to its body weight. The epididymal content of each rat was obtained by cutting the tail of epididymis and squeezed it in a clean watch glass. It was diluted 10 times with 2.9% sodium citrate dihydrate solution and thoroughly mixed to estimate the progressive motility and concentration of sperms (Bearden and Flauquary, 1980). Eosin-nigrosin stained smears were made to determine the sperm abnormalities. Testes, epididymis, prostates and seminal vesicles of each rat were taken for histopathological examination using Harris Haematoxylin and Eosin method (Luna, 1968). The percentage of seminiferous tubules containing spermtozoa were also determined (Yunda and Kushniruk 1974). Each male in the control and treated groups was paired separately with 5 females of regular oestrus cycle for 2 days. The efficacy of fertility of male rats was calculated from the number of pregnancies resulted from their mating.

## RESULTS

**Toxicological studies:**

The results obtained showed that minimal lethal dose (MLD) and $LD_{50}$ of the alcoholic extract of Artemisia alba plant are 1000 and 1260 mg/kg b.wt. respecively (Table 1). The symptoms of toxicity were characterized by increased respiration and gasping.

**Hormone-like effect:**

Subcutaneous injection of alcoholic extract of *Artemisia alba* in doses of 25.2 and 126 mg/kg b.wt to ovariectomised and castrated rats had no oestrogen and androgen-like actions, respectively.

**Table (1): $LD_{50}$ of alcoholic extract of *Artemisia alba* plant (n = 5 mice).**

| Dose (mg/kg b.wt.) | No. of dead animals | z | d | ∑(z x d) |
|---|---|---|---|---|
| 800 | 0 | 0.5 | 200 | 100 |
| 1000 | 1 | 1.5 | 200 | 300 |
| 1200 | 2 | 2.5 | 200 | 500 |
| 1400 | 3 | 4.0 | 200 | 800 |
| 1600 | 5 | | | 1700 |

$LD_{50} = 1600 - \frac{1700}{5}$

= 1260 mg/kg b.wt.

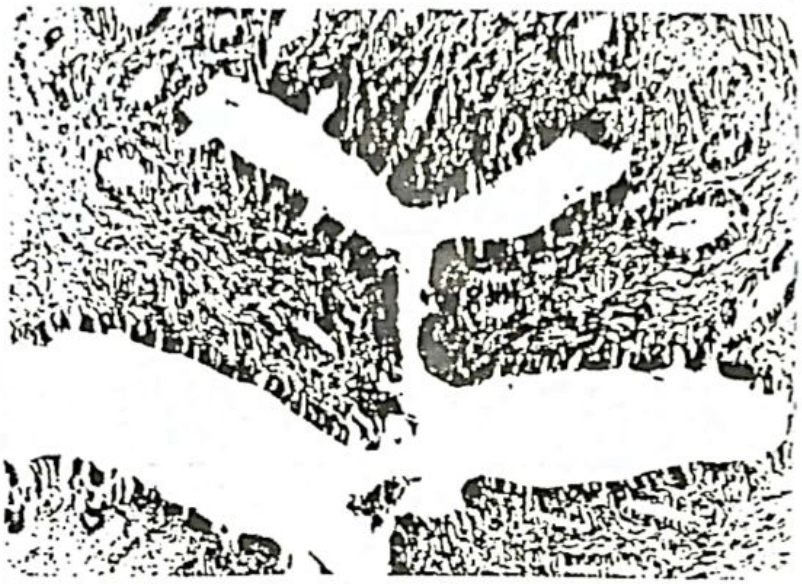

Fig. 1: Showing progestational proliferation in uterus of an immature female rabbit given alcoholic extract of *Artemisia alba* plant in a dose of 126 mg/kg b.wt. (H & E, x 400)

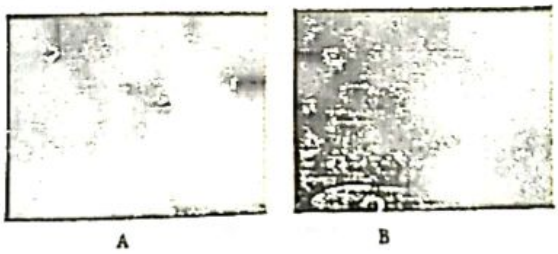


Fig. 2: Showing headless (A) and bent tail (B) sperms obtained from rats given alcoholic extract of *Artemisia alba* in a dose of 126 mg/kg b.wt. for 65 successive days (E & N, x 100)

Table (2): Effect of prolonged oral administration of alcoholic extract of Artemisia alba for 65 days on weights of sexual organs and semen picture (n = 5 rats).

| Group | Dose (mg/kg b.wt.) | Weight of sexual organs (g/100 g b.wt) | | | Semen picture | | | Seminiferous tubules containing sperms (%) |
|---|---|---|---|---|---|---|---|---|
| | | Testes | Seminal vesicles | Prostate glands | Concentration ($10^6$/ml) | Motility (%) | Abnormality (%) | |
| Control | 0.0 | 1.74 ± 0.08 | 0.38 ± 0.02 | 0.29 ± 0.01 | 586.2 ± 13.43 | 88.3 ± 4.05 | 3.4 ± 0.16 | 95 ± 5.26 |
| Alcoholic extract of Artemisia alba | 25.2 | 1.50* ± 0.06 | 0.34 ± 0.03 | 0.26 ± 0.02 | 374.8*** ± 9.85 | 53.6*** ± 3.21 | 14.2*** ± 0.40 | 54*** ± 3.11 |
| | 126 | 1.41* ± 0.09 | 0.28** ± 0.02 | 0.20** ± 0.02 | 0.90.7*** ± 7.86 | 34.0*** ± 3.36 | 26.8*** ± 0.62 | 29*** ± 2.08 |

Significant at: * P < 0.05 ** P < 0.01 *** P < 0.001

Table (3): Effect of prolonged oral administration of alcoholic extract of Artemisia alba to male rats on the percentage of pregnancies after mating trials with normal females after 65 days (n = 5 )

| Group | Dose (mg/kg b.wt) | Percentage of pergnancies |
|---|---|---|
| Control | 0.0 | 100 |
| Alcoholic extract of Artemisia alba | 25.2 | 30 |
| | 126 | 22 |

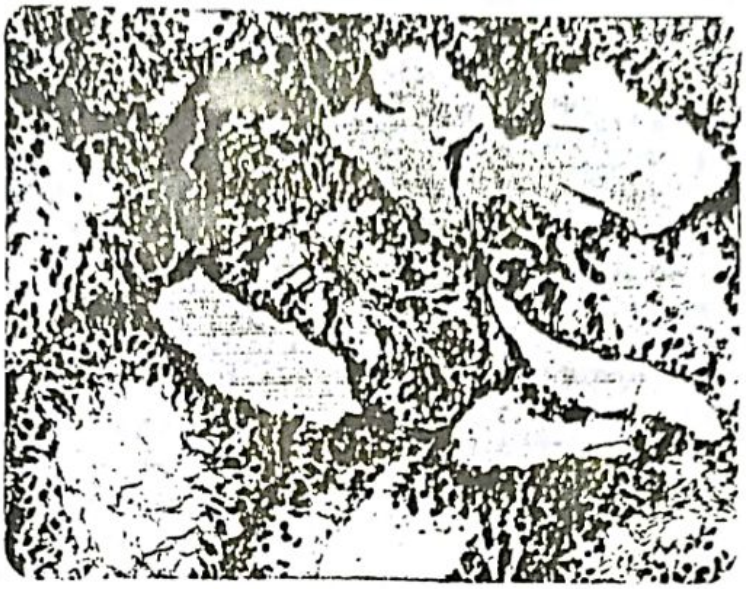

Fig. 3: Showing irregular arrangement of spermatogenic cells in testis of a rat given alcoholic extract of *Artemisia alba* in a dose of 126 mg/kg b.wt. for 65 successive days (H & E, x 200)

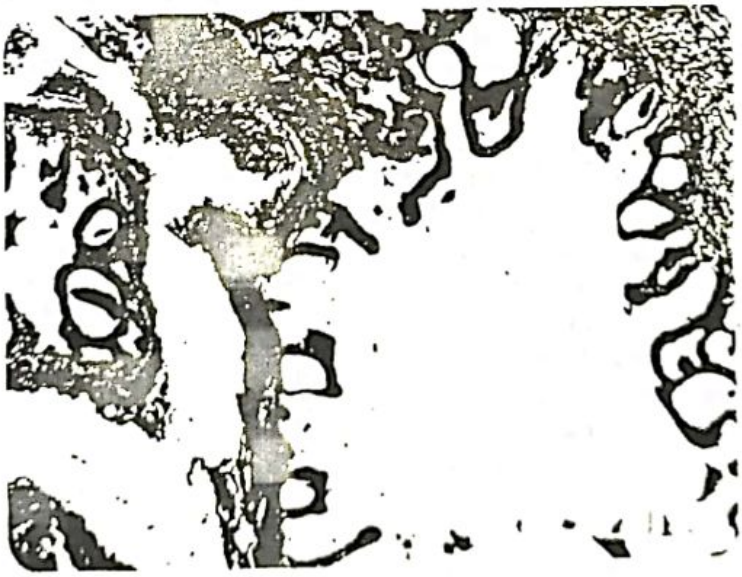

Fig. 4: Showing degenerative changes and scanty secretions in the alveoli of prostate gland of a rat given alcoholic extract of *Artemisia alba* in a dose of 126 mg/kg b.wt. for 65 successive days (H & E, x 400).

The same extract showed progesterone-like action in immature female rabbits (Fig. 1)

**Effect on male fertility:**

Oral administration of alcoholic extract of *Artemisia alba* plant in doses of 25.2 and 126 mg/kg b.wt for 65 successive days, significantly decreased the weight of testes. A significant decrease in the weights of accessory glands was induced only by the large dose. Both doses of the alcoholic extract significantly decreased sperm cell concentration and percentages of sperm motility and seminiferous tubules containing spermatozoa but increased the abnormalities of sperm (Table 2).These changes in semen picture were more prominent in rats given the higher dose.

Morphologic abnormalities seen in spermatozoa of rats given alcoholic extract of *Artemisia alba* plant in either doses were in the form of detatched heads and bent tails (Fig.2. ) . Oral administration of the tested extract in doses of 25.2 and 126 mg/kg b.wt. to male rats for 65 successive days then mated with normal oestrus females, significantly decreased the percentage of pregnancies as compared with the control group (Table 3 ).

Histopathological examinations of the male genitalorgans of rats given alcoholic extract of *Artemisia alba* plant ( 25.2 and 126 mg/kg b.wt) for 65 successive days showed pathological alterations as compared to control group. The higher dose of the to sted extract caused degenerative changes in the testes more than that of smaller one.Most of the seminiferous tubules showed irregular arrangement of spermatogenic cells with reduction in the number of spermatids and spermtozoa (Fig. 3). Some tubules were completely free from spermatozoa. The interstitial tissue was greatly atrophied. Epididymis of these animals showed marked reduction in the number of spermatozoa. Sections from seminal vesicles and prostate glands of treated rats showed a degenerative changes associated with the presence of scanty secretions in their alveoli (Fig. 4).

## DISCUSSION

The toxicity of commonly used plants and their effects on the efficiency of reproductive organs were highly indicated to be studied. In this respect, the effect of alcoholic extract of *Artemisia alba* plant on male reproductive organs was investigated. The present study revealed that the minimal lethal dose (MLD) and $LD_{50}$ of the alcoholic extract of *Artemisia alba* plant are 1000 and 1260 mg/kg b.wt, respectively. These values indicated that the tested extracts are non toxic in mice since Buck et al., (1976) stated that substances possessing $LD_{50}$ bigger than 50 mg/kg b.wt, are considered non toxic.

Prolonged oral administration of the tested extract in doses of 25.2 and 126 mg/kg b.wt. to male rats showed serious reproductive changes and decreased their fertilizing capacity (Nemethalla 1976). Both doses significantly decreased weights of sexual organs, sperm cell count and percentages of progressive motility and seminiferous tubules containing spermatozoa. Moreover, they increased the abnormalities of sperms.

It was proved that the studied extract had no oestrogen and androgenic effect.

No available literature could be obtained on the effect of studied extract on reproductive organs and fertility of males. The present decrease in epididymal sperm characters could be explained by degenerative changes seen in testicular and accessory gland structures (Murdui 1978). These effects could impair or even stop the process of spermatogenesis resulting in infertility of male rats. The observed disturbances in epididymal sperm characters are in accordance with those obtained by Hollinger and Davis (1960) who demonstrated that prolonged treatment with *Artemisia alba* plant resulted in inhibition of spermatogenesis and testicular function.

Histopathological examination of sexual organs is characterized by degenerative changes in the testes and accessory glands of treated rats with reduction in the number of spermatids and spermatozoa. These pathological changes explain the decrease in the weight of sexual organs, sperm cell count and percentages of progressive motility and seminiferous tubules containing spermatozoa.

Conclusively, *Artermisia alba* plant impaired the

ilizing capacity of males, therefore it is advisa- to avoid its administration to them especially se used in natural mating or kept for artificial emination.

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