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Original Article

The Therapeutic and Toxic Effects of *Artemisia herba alba* Extract on the Reproductive System and Fertility of Male Rabbits *Oryctolagus cuniculus*

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ABSTRACT

Background: Medicinal plants are generally considered beneficial to health because they contain secondary metabolites responsible for their therapeutic effects. However, some plants used in phytotherapy are toxic due to their high content of toxic components. **Objectives:** The purpose of this study is to assess the therapeutic and toxic action of *Artemisia herba alba* (AHA) by using increasing doses of the plant extract, on reproductive parameters, plasma testosterone levels, organs' relative weight, tissue structure, and immunolocalization of estrogen receptors ER in reproductive organs of adult male domestic rabbits. **Materials and Methods:** Thirty-six rabbits were divided into six groups (D0), (D1), (D2), (D3), (D4), and (D5), and received respectively NaCl (0.9%), 5, 15, 30, 60, and 120 mg/kg bw/day of AHA extract. The daily doses were administered orally for thirty days. **Results:** The results show progressive improvement in sperm quality in rabbits treated with 5, 15 and 30 mg/kg of (AHA) extract, an increase in sperm speed, concentration, motility, vitality, testosterone levels, and the organs' relative weight compared with the control. The marked improvement in sperm quality is observed in rabbits treated with 30 mg/kg of the AHA extract. However, from dose 60 mg/kg, sperm parameters are significantly reduced compared with the control, this reduction is severe in rabbits treated with 120 mg/kg. Histological sections of reproductive organs in rabbits treated with 5 and 15 mg/kg bw, do not reveal any remarkable morphological changes in tissue structure compared to the control, treatment with 30 mg/kg reveals an improvement of the structural integrity. However, at high doses, histological sections reveal more or less significant changes in tissue structure and morphology of reproductive organs. The immunochemical study shows a positive reaction in the reproductive organs of rabbits treated with low doses, the immunopositivity of the reaction decreases progressively with the increase of the extract doses. While an immunonegative reaction is observed in the organs of rabbits treated with a high dose of plant extract. **Conclusion:** The therapeutic effect of AHA extract on reproduction was manifested at low doses of extract; they had a positive influence on reproductive parameters. However, at the high doses, some reproductive parameters were slightly disturbed; these disturbances were accentuated and appeared significant at the highest dose of plant extract. The supplementation then of low quantities of AHA in rabbits' diets can improve reproductive performance, which in turn positively affects rabbit production.

Keywords: *Artemisia herba Alba*, Reproduction, Sperm Quality, Histology, Estrogen Receptor, Health.

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INTRODUCTION

Since Antiquity, medicinal plants have been commonly valued for their therapeutic virtues, where they were used for care, treatment, and cure of various diseases.[1] Despite current progress in the pharmaceutical and medicinal sciences, the use of natural plant resources has continued to grow for certain populations of the world, especially in developing countries,[2] but in some cases, plants with beneficial effects can become toxic when consumed in high concentrations or over a long period of time, because they contain toxic bioactive components, such as heterosides, alkaloids, anthocyanins, tannins, monoterpenes, and steroids, which can generate oxidative stress, and cause major cytotoxicity; this last poses a risk to the cardiovascular, respiratory, immune, nervous, renal, and reproductive systems. Furthermore, the presence of

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phytoestrogens; which are nonsteroidal polyphenolic compounds; in some plants, can affect reproductive functions leading to disruption of the reproductive system and loss of fertility, such as gamete production, sperm density, androgen secretion, sperm motility, secretion, and metabolism of reproductive hormones, and temporary infertility syndromes.[3-9]

Artemisia herba alba (AHA), known also as wormwood (known in Chinese as 苦艾 (Kǔ ài), Hindi as नागदौन (naagadaun), as ajenjo in Spanish, armoise blanche in French, and as shih (الشيح) in Arabic), is a popular medicinal plant in northern Africa, commonly used by the local population in human and veterinary medicine; inflammatory disorders[10] and infectious diseases are the main disorders treated by this plant.[11] The beneficial effect of (AHA) is due to their richness in bioactive components which provide the plant an anti-inflammatory, antioxidant, antibacterial, antidiabetic, and anti-cancer, properties.[12-25] The phytochemical studies revealed that the (AHA) contains herbalbin, sesquiterpene, monoterpenes, cis-chryanthenyl acetate, flavonoids,[25-28] mono- and di-cinnamoylquinic acids, and 5-caffeoylquinic acid which can be found in high concentrations, in addition to 3,5-dicaffeoylquinic acid and vicenin-2, coumarins, and acetylenic hydrocarbons.[20,29] Furthermore, the composition of (AHA) essential oil has also been investigated.[30,31] However, in high concentrations, (AHA) can cause structural and functional changes in organisms. Indeed, the toxic effect of high doses of Artemisia has been demonstrated by several studies.[14,32-34] Thujone is a monoterpene ketone, present in *Artemisia herba alba*, it is highly convulsive,[35] and causes feelings of disinhibition and hallucinations. It has two natural stereoisomeric forms, α and β thujone, α-thujone isomer is more toxic and is reported to be harmful to the kidney, brain,[35,36] and liver cells.[37]

Camphor is a component of (AHA), it is easily absorbed by the intestinal mucosa and causes damage to the kidney, liver, and brain,[38,39] it can also pass through the placental barrier and cause hemorrhagia in the placenta.[40] In addition, Camphor is also considered fetotoxic, since the fetus is unable to produce the enzymes required for its metabolism, leading to fetal abortion.[41]

Few studies have investigated the reprotoxicity induced by medicinal plants, especially if we take into account our limited knowledge of veterinary folk medicine, this effect could have repercussions on the economy because it affects animal reproductivity and productivity. On the other hand, limited studies have been carried out to assess the toxic influence of (AHA) on the reproductive performance of males, for those reasons, we carried out this study which aims to determine the therapeutic and toxic doses of (AHA) extract on reproductive parameters; sperm speed, concentration, motility, vitality, plasma testosterone levels, the testis and epididymis relative weight, reproductive organs' tissue structure, and immunolocalization of estrogen receptors.

MATERIALS AND METHODS

Plant extract process

The aerial part of the *Artemisia herba alba* (AHA) (Figure 1)[42] was obtained from the Oum El-Bouaghi region, Algeria, between October and January. The plant was dried in a dry, dark, and airy place, in order to preserve its therapeutic properties. After pulverizing the sample of the plant by using an electric grinder, the extraction process was carried out by diluting 1000 g of the powder in ethanol (70/30, v/v); 24 hr later, the solution was filtered 3 times and then placed in a rotary evaporator (BÜCHI Labortechnik AG, Switzerland) at 70°C to evaporate the solvent.

Animals and experiment

The experiments were performed on a total of thirty-six adult male rabbits of the local strain *Oryctolagus cuniculus*, weighing 2578±243.25 g. The animals were housed in conventional cages at room temperature in the animal house at the University of Oum El Bouaghi, Algeria,

They were fed with a pellet diet and water. After the adaptation period, the animals were divided into six groups:

The control group (D0) received NaCl (0.9%), the other groups (D1), (D2), (D3), (D4), and (D5) received respectively 5, 15, 30, 60, and 120 mg/kg bw/day of AHA extract. The daily doses were administered orally for thirty days.

Blood and organs sampling

After ending the treatment period, we weighed the rabbits and then killed them by decapitation. The blood was then collected in test tubes and centrifuged for 10 min at 4,000 rpm/min, in order to separate blood formed elements from the plasma. The latter was used to measure testosterone concentration. We also removed the testis and epididymis, weighed them, and stored them in a dilute formalin solution (10%), the samples were used for the histological and immunohistochemical studies.

The biological study of semen

Sperm parameters were estimated according to the method of OMS.[43] The sperm was collected by microsurgery using one or more epididymal micro-incisions; the sample was diluted in a physiological solution of 0.9%, which has been used to evaluate sperm speed (expressed as mm/s), concentration (expressed as million/mL), motility (expressed as a percentage), and vitality (expressed as a percentage).

Testosterone measurement

The plasma testosterone concentrations were determined by the Enzyme-Linked Immuno-Sorbent Assays (ELISA) method by using of TOSOH AIA System Analyzers (3-8-2 Shiba, Minatoku, Tokyo, Japan) apparatus, and the commercial ELISA kits for plasma testosterone (ST AIA-Pack Tosoh Bioscience.

ST AIA-PACK™ Test Cups: Testosterone, Tokyo, Japan). This method is based on the visualization of labelled antigen-antibody reactions by adding TMB (tetramethyl benzidine) substrate. The color intensity is inversely proportional to the quantity of antigen present in the sample.

Histological study

Histological procedures were performed according to the method of Martoja and Martoja,[44] with moderate modifications in the method. The objective of these procedures is to produce thin, transparent sections of tissues that can be observed under a light microscope.

Organ samples preserved in diluted formalin (10%), were dehydrated in ascending concentrations of ethyl alcohol, substituted in xylene, and impregnated in paraffin. The sections of 4 µm were confectioned by using a manual microtome (SLEE CUT 4062, TECH Inter Lab, Thoiry, France). Deparaffinization and rehydration; are two procedures that were performed before staining with Hematoxylin-eosin. The samples were then protected against drying out by using the mounting medium (Eukitt® Classic, ORSAtec GmbH, Bobingen, Germany).

Immunohistochemical study

The procedure was carried out by placing the paraffin-embedded sections of epididymis and testis at 70°C, in order to adhere the sample to the slide firmly. Immunostaining was performed following the avidin-biotin method. After deparaffinization and hydratation, samples were incubated in 3% H_2O_2 to inhibit endogenous peroxidase, followed by heating in the microwave for 20 min after immersion of samples in an antigen retrieval solution (0.01 M Sodium citrate buffer, pH 6). For blocking nonspecific protein binding, normal goat serum (10%) was used for 30 min. Incubation with primary antibody was carried out by using mouse antihuman monoclonal IgG ER (Santa Cruz Biotechnology, CA) overnight at 4°C. After washing with PBS solution, samples were incubated with secondary antibody by using biotinylated goat anti-mouse IgG (Santa Cruz Biotechnology, CA) for 1 hr, washed with PBS, and incubated in streptavidin peroxidase for 20 min. Samples were then incubated in DAB substrate solution until the desired stain intensity developed, dark brown precipitate was produced. Samples were counterstained in hematoxylin, stabilized in mounting medium, and covered with a glass coverslip.

Statistical analysis

All data are shown as mean±SD. To compare two means, the t-student test was used, while the one-way Analysis of Variance (ANOVA) (GraphPad Prism 9.5.1 software, LLC, USA) was employed to compare different means. The statistical test was considered significant at $p < 0.05$ level ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). [a] test ANOVA, [b, c, d, e, f] test *t*-student. [b] D0 vs D1. [c] D0 vs D2. [d] D0 vs D3. [e]D0 vs D4. [f]D0 vs D5.

RESULTS

Effect of *Artemisia herba alba* extract on sperm quality

Biological analysis of sperm parameters; speed, motility, concentration and vitality of sperm, reveals a progressive improvement in sperm quality in rabbits treated with 5, 15 and 30 mg/kg of (AHA) extract. Treatment with 5 mg/kg shows moderate changes in sperm speed (Figure 2) and vitality (Figure 3), while concentration (Figure 4) and motility (Figure 5) show a significant increase compared to the control. While, the treatment with 15 mg/kg results in a significant increase in sperm parameters, except for sperm speed, which shows non significant difference. The marked improvement in sperm quality is noted in rabbits treated with 30 mg/kg of the AHA extract. However, from 60 mg/kg, sperm parameters are significantly reduced compared with the control, this reduction is severe in rabbits treated with 120 mg/kg.

Effect of *Artemisia herba alba* extract on testosterone concentration

Hormone measurements show a significant elevation in plasma testosterone concentrations in rabbits treated with 15 and 30

Figure 1: Photograph of the aerial part of *Artemisia herba alba.*[42]

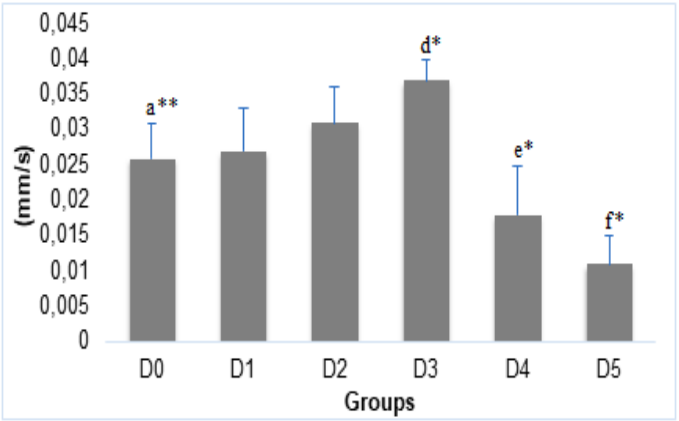

Figure 2: Variations in sperm speed in rabbits received increasing doses of AHA extract.

mg/kg of (AHA) compared to the control, while the lower dose produces non significant difference. In addition, treatment with 60 and 120 mg/kg of plant extract produces a marked decrease, which seems to be more pronounced in rabbits treated with the higher dose (Figure 6).

Effect of *Artemisia herba alba* extract on organs' relative weight

A non significant increase in the testis relative weight is recorded in rabbits treated with 5 and 15 mg/kg. Indeed, rabbits treated with 30 mg/kg show a remarkable increase in the testis relative weight compared with the control. In contrast, from 5 to 60 mg/kg, there is non significant change in the epididymis relative weight. Whereas at the highest dose, 120 mg/kg, there are significant changes in the organs' relative weight, reflecting a marked decrease in their relative weights (Figures 7 and 8).

Effect of *Artemisia herba alba* extract on organs' structure

Histological sections performed on the testis show that treatment with 5 and 15 mg/kg bw of AHA extract (Figures 9D1 and D2) does not lead to any remarkable morphological modifications in testicular tissue structure compared with the control (Figure 9D1). There is a similarity between them. Furthermore, treatment with 30 mg/kg (Figure 9D3) reveals an increase in interstitial cell clusters or Leydig cells, and the structural integrity of the seminiferous tubule walls, which, in fact, represent the stages of the spermatogenesis process. Histological sections of rabbit testis treated with 60 and 120 mg/kg (Figures 9D4 and D5) show more or less significant alterations, with dilatation of the tubular lumen due to narrowing of the walls, which in turn results in the absence of certain stages in the spermatogenesis process. In addition, parts of the rabbit testis treated with 120 mg/kg show severe changes, with vacuolization in the interstitial tissues and walls of the seminiferous tubules, and degeneration of the sperm cell membranes.

Histological sections of the epididymis of rabbits treated with 5 mg/kg (Figure 10D1) show a similar tissue structure compared to the control (Figure 10D0). Moreover, rabbits treated with 15 and 30 mg/kg (Figure 10D2 and D3) show an increase in the epithelium cell thickness, which covers the epididymal duct, and the accumulation of mature spermatozoa in the epididymal duct lumen. Regarding rabbits treated with 60 and 120 mg/kg (Figure 10D4 and D5), histological sections show some disturbances, represented by enlargement of the stromal space, degeneration, and narrowing of the epithelium cells in certain areas of the epididymis, leading to dilatation of its lumen and absence of mature spermatozoa in some parts of the duct.

Effect of *Artemisia herba alba* extract on localization of estrogen receptor

The immunochemical study shows a positive reaction in the testis of rabbits treated with 0, 5, 15, and 30 mg/kg bw (Figures

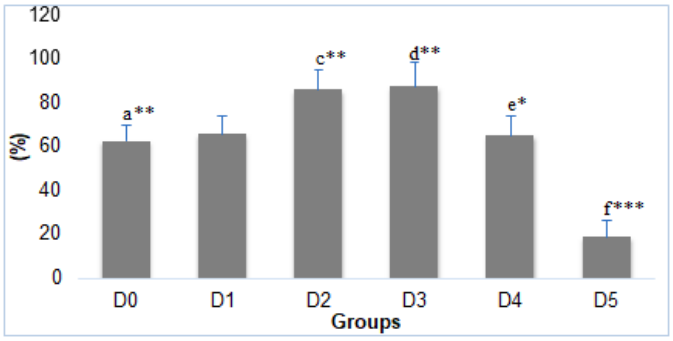

Figure 3: Variations in sperm vitality in rabbits received increasing doses of AHA extract.

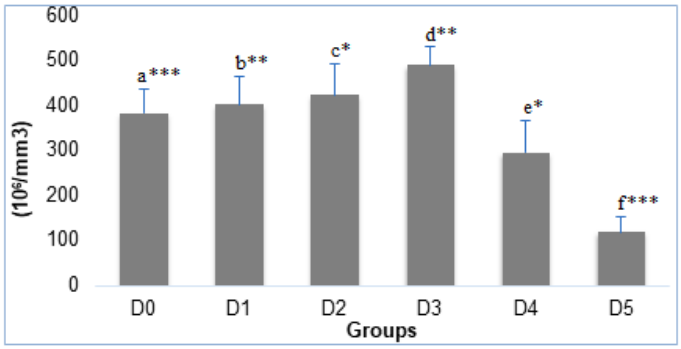

Figure 4: Variations in sperm concentration in rabbits received increasing doses of AHA extract extract.

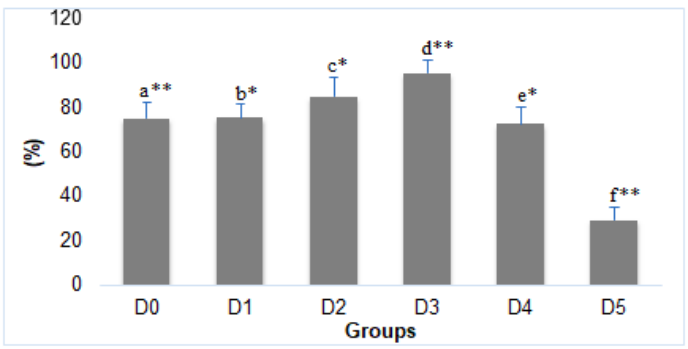

Figure 5: Variations in sperm motility in rabbits received increasing doses of AHA extract.

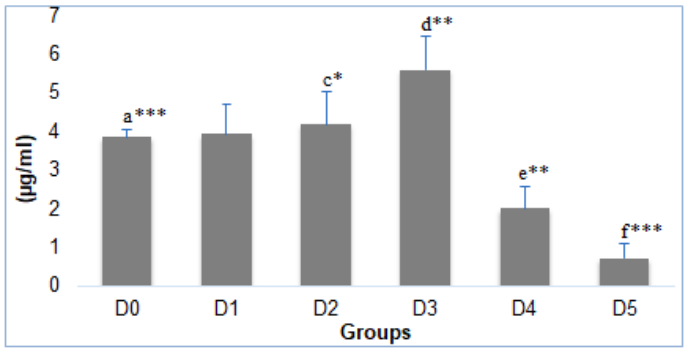

Figure 6: Variations in plasma testosterone levels in rabbits received increasing doses of AHA extract.

11D0, D1, D2 and D3), receptors are localized in Leydig cells in interstitial tissue clusters, and in germ cells; spermatogonia, a weak staining is also observed in spermatocytes, corresponding to a low number of estrogen receptors. While, an immunonegative reaction is observed in the testis of rabbits treated with high doses (Figures 11D4 and D5), due to the degeneration of a large number of interstitial and germ cells.

for sections performed on the epididymis, an immunopositive reaction appears in stromal cells, and epithelial cells of the epididymal duct in rabbits treated with 0 to 30 mg/kg of plant extract (Figures 12D0, D1, D2 and D3), The immunopositivity of the reaction decreases progressively with the increase of the extract doses, weak staining is observed at 60 mg/kg bw (Figure 12D4). Whereas, an immunonegative reaction is observed in rabbits treated with 120 mg/kg bw (Figure 12D5), due to a narrowing of the epithelium cells of the epididymal duct, and degeneration of epithelial and stromal cells.

DISCUSSION

Artemisia herba alba (AHA) extract causes variable effects related closely to the administered doses. Based on the results of this study, AHA induces positive effects on sperm quality when administered at low doses. Indeed, the plant extract improves sperm speed, concentration, motility, and vitality in rabbits treated with low doses of AHA. According to Abid *et al.*[45]

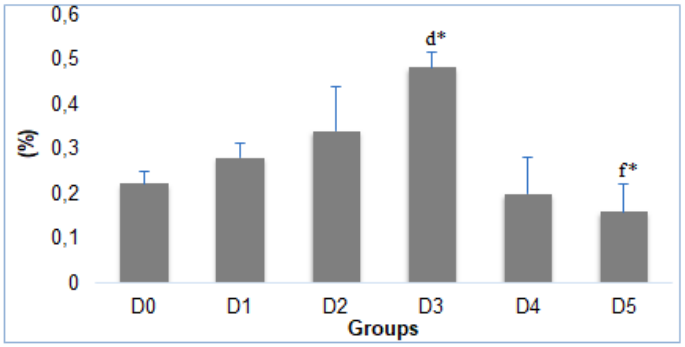

Figure 7: Variations in the testis relative weight in rabbits treated with increasing doses of AHA extract.

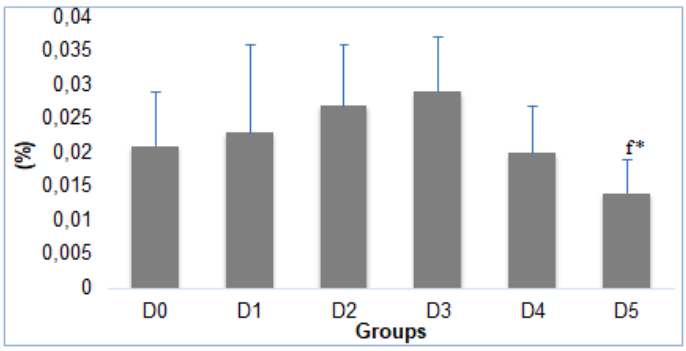

Figure 8: Variations in the epididymis relative weight in rabbits treated with increasing doses of AHA extract.

AHA contributes to improving the status of zinc, copper and antioxidants, the activity of glutathione peroxidase. It also prevents various disturbances, in particular oxidative stress,[45] leading to positive effects on reproduction. The flavonoids, sesquiterpene lactones, phenolics, and waxes in AHA are bioactive compounds with powerful antioxidant properties,[42] other studies on rams have demonstrated that the supplementation of AHA to diet can lead to an impact on different growth parameters, both muscular and skeletal, as well as on energy metabolisms, certain endocrine pathways, sexual performance, and reproductive function of rams.[46,47] In addition, the fresh leaves of AHA can enhance reproductive performance by improving semen parameters and increasing sperm concentration and motility.[48] AHA essential oil also enhances plasma testosterone levels and testis spermatid count in male rats.[49] However, administration of a high dose (300 mg/kg bw) of the extract induces a reduction of fertility in female rats,[32] an impairment in sexual activity in pregnant mice, a significant decrease in litter size, and pups in litter.[50] According to Khataibeh and Daradka,[51] AHA decreases spermatogenesis and sperm motility. Another study showed that the administration of an aqueous extract of AHA resulted in an increase in sperm speed, concentration, and motility in rabbits treated with 20 mg/

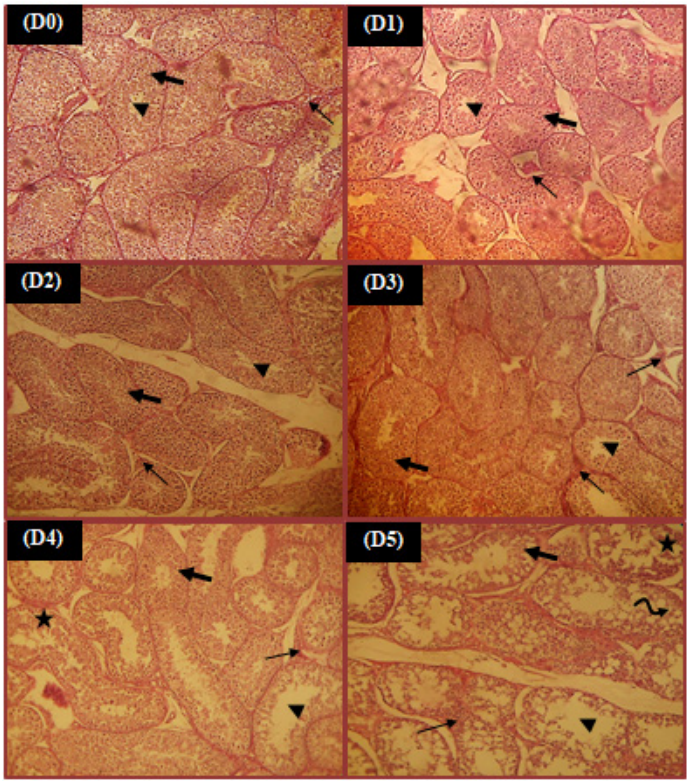

Figure 9: Histological sections of the testis in rabbits received increasing doses of AHA extract. (D0) control group, (D1) the group received 5 mg/kg bw of plant extract, (D2) the group received 15 mg/kg bw of plant extract, (D3) the group received 30 mg/kg bw of AHA extract, (D4) the group received 60 mg/kg bw of AHA extract, and (D5) the group received 120 mg/kg bw of AHA extract (H&E staining, magnification 100x, zoom 1.9x). Thin arrow: interstitial cell clusters, Thick arrow: seminiferous tubule wall. Arrowhead: tubular lumen. Curved arrow: vacuolization. Asterisk: degeneration of cell membranes.

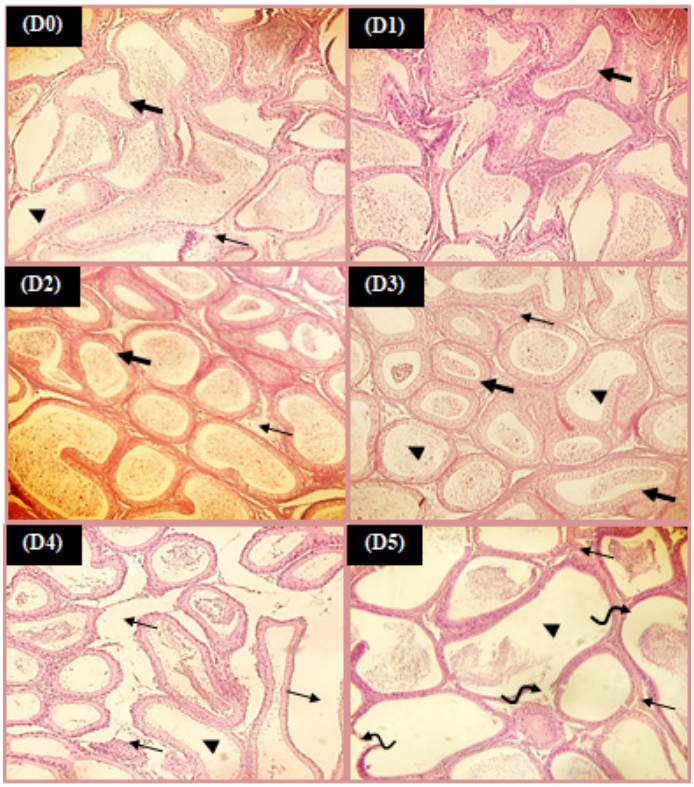

Figure 10: Histological sections of the epididymis in rabbits received increasing doses of AHA extract. (D0) control group, (D1) the group received 5 mg/kg bw of AHA extract, (D2) the group received 15 mg/kg bw of AHA extract, (D3) the group received 30 mg/kg bw of AHA extract, (D4) the group received 60 mg/kg bw of AHA extract, and (D5) the group received 120 mg/kg bw of AHA extract (H&E staining, magnification 100x, zoom 1.9x). Thin arrow: stromal tissue. Thick arrow: epithelium cell. Arrowhead: epididymal duct lumen. Curved arrow: narrowing and cell degeneration.

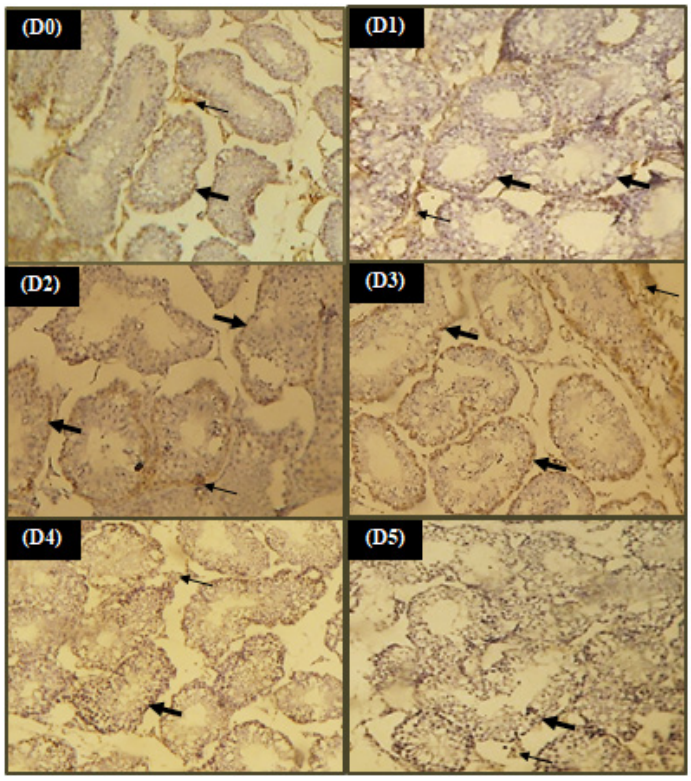

Figure 11: Immunohistochemical staining of estrogen receptor in testis in rabbits received increasing doses of AHA extract. (D0) control group, (D1) the group received 5 mg/kg bw of AHA extract, (D2) the group received 15 mg/kg bw of AHA extract, (D3) the group received 30 mg/kg bw of AHA extract, (D4) the group received 60 mg/kg bw of AHA extract, and (D5) the group received 120 mg/kg bw of AHA extract (sections were stained with Anti-ER immunostain, magnification 100x, zoom 2.4x). Thin arrows: Leydig cells, thick arrow: germinal cells.

kg; but all reproductive parameters were reduced at 30 mg/kg bw.[52]

Plasma testosterone levels reflect fertility status and the activity of the reproductive system.[53-55] The results of our study show an increase in plasma testosterone levels in rabbits that received low doses of AHA. Indeed, the findings of Khnissi *et al.*[48] revealed that the addition of the fresh leaves of AHA to the diet of rams resulted in an increase in plasma testosterone concentration.[52] However, high doses of AHA extract can disrupt testosterone synthesis, due to impairments in the endocrine function of the hypothalamus-pituitary-testis pathway, and in the sites of androgen production. The study of Khataibeh and Daradka,[51] showed a decrease in sperm count, hypothalamus-pituitary-testis pathway function, reproductive function, plasma levels of testosterone and FSH in AHA-treated rats, leading to difficulty in gestation and a decrease in the implantation sites and fetus number in female rats coupled with AHA-treated males. The findings of Domiaty,[56] demonstrated that the high dose of AHA extract (500 mg/kg bw) may induce any variation in serum testosterone levels, relative weight, and morphology of testicular tissue. On the other hand, testosterone secretion is controlled by estrogen hormone, a decrease in estrogen synthesis leads to the release of gonadotropins, which stimulate testosterone synthesis.[57] However, the absence of ER estrogen receptors, which clearly manifested as an immunonegative reaction in rabbits treated with high doses of AHA extract, causes a negative effect on testosterone production and spermatogenesis. However, at low doses, AHA extract induces an immunopositive reaction in Leydig cells and germ cells; the estrogen receptor presence indicates the major roles of these female hormones in testicular function, development, and spermatogenesis regulation.[58] Androgens can regulate the reproductive organs' weight.[35] Indeed, the effect of AHA extract on the testis and epididymis relative weight was observed when it was administered in high doses, due to their positive or negative action on the hypothalamus-pituitary-testis pathway, this last can in turn affect the production of androgen hormones. According to Olsen,[35] the weight of reproductive organs can be changed by androgen hormone levels. However, a decrease in plasma androgen levels leads to a decrease in the reproductive organs' weight, and vice versa.[32] The decline in the weight of the gonads causes a reduction in spermatogenesis, sperm quantity and quality.[59,60] Disruptions in the tissue structure and morphology of the testis and epididymis can also affect reproductive performance and sperm quality. Indeed, administration of low doses of AHA

Table 1: The effect of different doses of *Artemisia herba alba* on reproduction

Dose and nature of the AHA extract	Period of exposure	Animal species	Effects on reproduction
300 mg/kg bw of AHA extract	4 weeks and 12 weeks	Female Sprague-Dawley rats	Adverse effects on the reproductive system and fertility result in a decrease in plasma androgen levels, a reduction in the percentage of pregnancies and the number of implantation sites, and a decrease in the viable fetus's number.[32]
Unlimited consumption of AHA decoction in the diet	9 weeks	Male Wistar rats	Positive effect on reproduction, through the prevention of oxidative stress and the improvement of antioxidant status, glutathione peroxidase, Zn and copper levels.[45]
400 mg/kg bw of essential oils	95 days	Barbarine lambs	Positive impact on the antioxidant status of animal tissue leading to a positive effect on reproduction.[46]
20g/day of fresh foliage	70 days	Rams	Impact on endocrine pathway and reproductive performance.[47]
20g/day of fresh leaves	2 months	Rams	Enhancement of reproductive performance, semen parameters, sperm concentration, and motility. An increase in plasma testosterone level.[48]
50 mg/kg bw of essential oils	21 days	Male Wistar rats	Enhancement of antioxidant enzyme activity (GPx, SOD, CAT) in testis, epididymis and spermatozoa, increase in testis spermatid count and testosterone, reduction in epididymis sperm count.[49]
80 and 150 mg/kg/day of the methanol extract	the entire period of gestation	Female pregnant mice	An impairment in sexual activity in pregnant mice, a significant decrease in litter size, and pups in litter.[50]
100 mg/kg bw aqueous extracts	60 days	Male Albino rats	Decrease in the weight of reproductive organs, sperm motility and density in cauda epididymis and testicular ducts, spermatogenesis activity in the somniferous tubule, number of spermatocytes and spermatids, testosterone and FSH levels, number of female rats impregnated by males receiving treatment, a decrease in the implantation sites and viable fetuses number.[51]
20 and 30 mg/kg of aqueous extract	30 days	Male rabbits	An increase in sperm speed, concentration, motility, and plasma testosterone levels at 20 mg/kg; all reproductive parameters were reduced at 30 mg/kg bw.[52]
500 mg/kg bw of aqueous extract,	Alternate days for 3 weeks	Male Wistar rats	There is no impact on plasma testosterone levels, the relative weight of the testis, and the morphology of testicular tissue.[56]

extract improved the tissue morphology of the reproductive organs, due to the beneficial components of the plant, as antioxidants, which preserve the cellular integrity and tissue morphology of the organs. However, the high doses cause severe reprotoxicity resulting in the inhibition of spermatogenesis and degeneration of cell membranes, our findings are in agreement with those of Khataibeh and Daradka,[51] who demonstrated that AHA causes a disorder of spermatogenesis and an absence of their final stages, leading to a decrease in spermatocyte and spermatid numbers. Several studies have attributed the plant's toxicity to its components, such as flavonoids, phytoestrogens, and thujone.[50] Flavonoid toxicity is a field of research to be explored, as the mechanisms of their toxicity are complex and not yet completely clear. Indeed, flavonoids act on several targets at different doses.[61] Several investigations *in vitro* and on animals, as well as humans, have demonstrated that consumption of flavonoids with doses exceeding the threshold leads to harmful effects on health.[61] Phytoestrogens are components of AHA, they have estrogenic activity in the reproductive system, and on the hypothalamus-pituitary pathway.[62] According to Cederroth *et al.*[63] and Hamilton-Reeves *et al.*[64] the endocrine function can be disrupted by increased consumption of phytoestrogens because they have the ability to bind to estrogen receptors ER due to their similarity to the estrogen hormone[65] leading to a reduction of estrogen response. According to Ramírez *et al.*[66] phytoestrogens have a potential effect on the reproductive parameters of domestic animals, acting as agonists or antagonists of estrogen receptors. In addition, laboratory animals that were exposed to very high doses of estrogenic chemicals such as phytoestrogens, showed a decrease in gonad volume, quality, and sperm count.[67,68] However, low doses (physiological doses) and moderately high doses (pharmacological doses) that have a stimulating or inhibiting

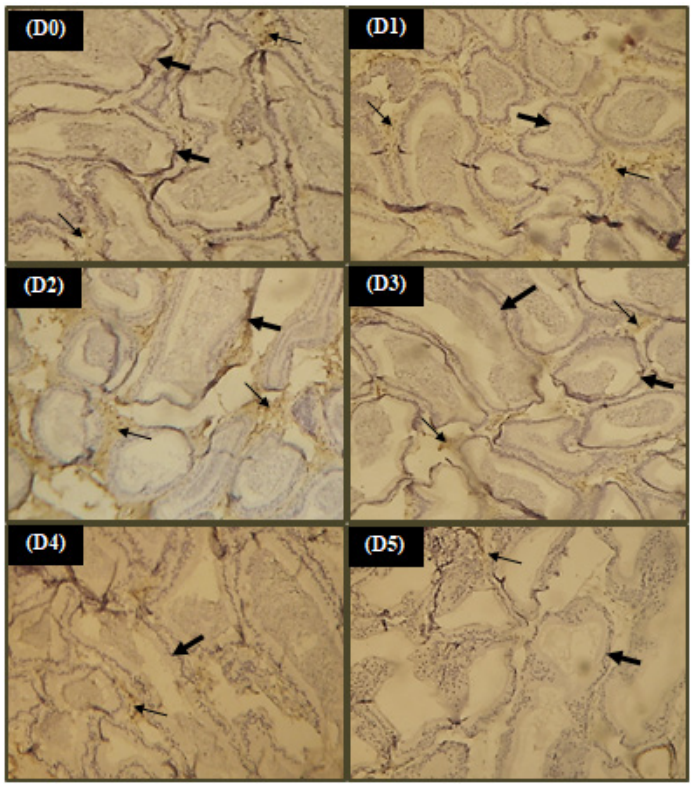

Figure 12: Immunohistochemical staining of estrogen receptor in epididymis of rabbits received increasing doses of AHA extract. (D0) control group, (D1) the group received 5 mg/kg bw of AHA extract, (D2) the group received 15 mg/kg bw of AHA extract, (D3) the group received 30 mg/kg bw of AHA extract, (D4) the group received 60 mg/kg bw of AHA extract, and (D5) the group received 120 mg/kg bw of AHA extract (sections were stained with Anti-ER immunostain, magnification 100x, zoom 2.4x). Thin arrows: stromal cells, thick arrow: epithelium cells.

effect, induce stimulation of spermatogonia proliferation *in vitro*,[69] and restoration of spermatogenesis in germ cell-depleted testis of hypogonadal mice.[70] Estrogen concentrations that are below the threshold are therefore essential for maintaining the function and the structural integrity of the testis, and their receptors are necessary for maintaining fertility.[71]

CONCLUSION

Artemisia herba alba is commonly used in human and veterinary medicine, and despite its many benefits, its excessive and/or long-term use can induce an adverse effect on reproduction and animal production, which in turn has an impact on the economy. In fact, the effect of this plant, whether positive or negative, is dependent on the dose, the period of exposure, and the nature of the plant extract (Table 1). Based on the findings of this study, the limit between beneficial and toxic doses on reproductive quality was noted, the low doses can improve fertility and reproductive parameters, resulting in an increase in animal productivity. In contrast, the high doses cause reprotoxicity leading to a decrease in fertility and reproductive performance, which in turn has a negative impact on animal production and the economy overall.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AHA: *Artemisia herba alba*; **ER:** Estrogen receptors; **NaCl:** Physiological solution; **ELISA:** Enzyme-Linked Immuno-Sorbent Assays; **TMB:** Tetramethyl benzidine; **H_2O_2:** Hydrogen peroxide; **IgG:** Immunoglobulin G; **PBS:** Phosphate-buffered saline; **DAB:** 3,3'-diaminobenzidine.

SUMMARY

This study aimed to determine the therapeutic and toxic doses of *Artemisia herba alba* (AHA) extract on the reproductive capacity of adult male rabbits *Oryctolagus cuniculus*.

Increasing doses of AHA extract were administered by gavage for thirty days. The results showed that low doses of AHA (5, 15, and 30 mg/kg) are considered therapeutic doses, they positively affect the reproductive system by enhancing fertility and improving reproductive performance in male rabbits due to its content of beneficial bioactive components.

However, the high doses of AHA extract (60 and 120 mg/kg) which are considered toxic doses, can cause severe reprotoxicity, resulting in morphological changes and disruption of reproductive markers. These changes are due to the presence of toxic components such as camphor and thujone. In addition, other bioactive components which usually have a positive impact on health, become toxic when administered in high doses and/or over long periods.

On the other hand, our findings were different from those obtained in previous studies conducted to determine the beneficial and harmful doses of the plant. These differences may be due to the nature of the plant extract, the period of exposure, the animal species, and the experimental conditions. The following Table 1 recapitulates the results of previous investigations on the positive and negative effects of AHA on reproduction and fertility.

REFERENCES

1. Maldonado-Miranda JJ. Medicinal plants and their traditional uses in different locations. Phytomedicine. 2021:207-23. doi: 10.1016/B978-0-12-824109-7.00014-5.
2. Tabuti JR, Lye KA, Dhillion SS. Traditional herbal drugs of Bulamogi, Uganda: plants, use and administration. J Ethnopharmacol. 2003;88(1):19-44. doi:10.1016/s0378-8741(03)00161-2, PMID 12902048.
3. Whitten PL, Patisaul HB. Cross-species and interassay comparisons of phytoestrogen action. Environ Health Perspect. 2001;109(1):5-20. doi:10.1289/ehp.01109s15, PMID 11250801.
4. Al-Majed AA, Al-Yahya AA, Al-Bekairi AM, Al-Shabanah OA, Qureshi S. Reproductive, cytological, and biochemical toxicity of Yohimbein male swiss albino mice. Asian J Androl. 2006;8(4):469-76. doi:10.1111/j.1745-7262.2006.00174.x, PMID 16763724.

5. Raji Y, Oloyo AK, Morakinyo AO. Effect of methanol extract of *Ricinus communis* seed on reproduction of male rats. Asian J Androl. 2006;8(1):115-21. doi:10.1111/j.1745-7262.2006.00055.x, PMID 16372130.
6. Pérez-Rivero JJ, Martínez-Maya JJ, Pérez-Martínez M, Aguilar-Setién Á, Serrano H. Efecto del coumestrol sobre la producción espermática y la conducta de exploración olfatoria de perros estimulados con moco vaginal estral. Veterinaria México; 2009;40(1):9-16.
7. Peña-Corona S, León P, Mendieta E, Villanueva M, Salame A, Vargas D, *et al.* Effect of a single application of coumestrol and/or dimethyl sulfoxide, on sex hormone levels and vaginal cytology of anestrus bitches. Vet Méx OA. 2019;6(1):1-15. doi: 10.22201/fmvz.24486760e.2019.1.656.
8. Gunes S, Sengupta P, Henkel R, Alguraigari A, Sinigaglia MM, Kayal M, *et al.* Microtubular dysfunction and male infertility. World J Mens Health. 2020;38(1):9-23. doi: 10.5534/wjmh.180066, PMID 30350487.
9. Wyse J, Latif S, Gurusinghe S, McCormick J, Weston LA, Stephen CP. Phytoestrogens: a review of their impacts on reproductive physiology and other effects upon grazing livestock. Animals (Basel). 2022;12(19):2709. doi: 10.3390/ani12192709, PMID 36230450.
10. Bouchara N, Senejoux F, Fraisse D, Felgines C, Caldéfie-Chezet F, Vasson MP, *et al.* Anti-inflammatory and prolonged protective effects of*Artemisia herba-alba* extracts via glutathione metabolism reinforcement. S Afr J Bot. 2021;142:206-15. doi: 10.1016/j.sajb.2021.06.018.
11. Abu-Darwish MS, Cabral C, Gonçalves MJ, Cavaleiro C, Cruz MT, Efferth T, et al. *Artemisia herba-alba* essential oil from Buseirah (South Jordan): chemical characterization and assessment of safe antifungal and anti-inflammatory doses. J Ethnopharmacol. 2015;174:153-60. doi: 10.1016/j.jep.2015.08.005, PMID 26277492.
12. Al-Shamaony L, al-Khazraji SM, Twaij HA. Hypoglycaemic effect of*Artemisia herba alba*. II. Effect of a valuable extract on some blood parameters in diabetic animals. J Ethnopharmacol. 1994;43(3):167-71. doi:10.1016/0378-8741(94)90038-8, PMID 7990489.
13. Kordali S, Kotan R, Mavi A, Cakir A, Ala A, Yildirim A. Determination of the chemical composition and antioxidant activity of the essential oil of*Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish*Artemisia absinthium*,*A. dracunculus*,*Artemisia santonicum* and*Artemisia spicigera* essential oils. J Agric Food Chem. 2005;53(24):9452-8. doi:10.1021/jf0516538, PMID 16302761.
14. Iriadam M, Musa D, Gümüflhan H, Baba F. Effects of two Turkish medicinal plants *Artemisia herba-alba* and *Teucrium polium* on blood glucose levels and other biochemical parameters in rabbits. J Cell Mol Biol. 2006;5:19-24.
15. Ben Abid ZB, Feki M, Hédhili A, Hamdaoui MH. *Artemisia herba-alba* Asso (Asteraceae) has equivalent effects to green and black tea decoctions on antioxidant processes and some metabolic parameters in rats. Ann Nutr Metab. 2007;51(3):216-22. doi: 10.1159/000104140, PMID 17587792.
16. Khennouf S, Iratni N, Baghiani A, Harzellah D, Arrar L. Antioxidant and antibacterial activities of extracts from *Artemisia herba alba Asso*, leaves and some phenolic compounds. J Med Plants Res. 2010;4(13):1273-80. doi: 10.5897/JMPR09.379.
17. Adel K, Zied Z, Zarai Z, Neacute ji G, Mohamed D, Radhouane G, *et al.* Chemical constituents and antioxidant activity of the essential oil from aerial parts of *Artemisia herba alba* grown in Tunisian semi-arid region. Afr J Biotechnol. 2011;10(15):2923-9. doi: 10.5897/AJB10.2491.
18. Awad NE, Seida AA, Shaffie ZE, Abdelaziz AM. Hypoglycemic activity of*Artemisia herba-alba* (*Asso*) used in Egyptian traditional medicine as Hypoglycemic remedy. J Appl Pharm Sci. 2012;2:30-9. doi: 10.7324/JAPS.2012.2306.
19. Khlifi D, Sghaier RM, Amouri S, Laouini D, Hamdi M, Bouajila J. Composition and antioxidant, anticancer and anti-inflammatory activities of*Artemisia herba-alba*,*Ruta chalpensis* L. and*Peganum harmala* L. Food Chem Toxicol. 2013;55:202-8. doi:10.1016/j.fct.2013.01.004, PMID 23333573.
20. Boudjelal A, Siracusa L, Henchiri C, Sarri M, Abderrahim B, Baali F, *et al.* Antidiabetic effects of aqueous infusions of *Artemisia herba-alba* and *Ajuga iva* in alloxan-Induced diabetic rats. Planta Med. 2015;81(9):696-704. doi: 10.1055/s-0035-1546006, PMID 26018915.
21. Dif MM, Benali Toumi F, Boukaaza H, Mokaddem F, Benyahia M, Bouazza S. Phenolic content and antioxidant activity of*Artemisa herba alba*, a medicinal plant from Algerian arid zone. Phytothérapie. 2018;16(2):91-5. doi: 10.3166/s10298-016-1077-9.
22. Yun C, Jung Y, Chun W, Yang B, Ryu J, Lim C, *et al.* Anti-inflammatory effects of Artemisia leaf extract in mice with contact dermatitis *in vitro* and *in vivo*. Mediators Inflamm. 2016; 2016:8027537. doi:10.1155/2016/8027537, PMID 27647952.
23. Sekiou O, Boumendjel M, Taibi F, Boumendjel A, Messarah M. Mitigating effects of antioxidant properties of *Artemisia herba alba* aqueous extract on hyperlipidemia and oxidative damage in alloxan-induced diabetic rats. Arch Physiol Biochem. 2019;125(2):163-73. doi: 10.1080/13813455.2018.1443470, PMID 29482369.
24. Mohammed MJ, Anand U, Altemimi AB, Tripathi V, Guo Y, Pratap-Singh A. Phenolic composition, antioxidant capacity and antibacterial activity of white wormwood (*Artemisia herba-alba*). Plants (Basel). 2021;10(1):164. doi: 10.3390/plants10010164, PMID 33467047.
25. Boukhennoufa A, Benmaghnia S, Maizi Y, Touil A, Meddah B. Antifungal and antioxidant activities of *Artemisia Herba-Alba Asso*. Eur J Biol Res. 2021;11:493-500. doi:10.5281/zenodo.5552721.
26. Moufid A, Eddouks M. *Artemisia herba alba*: a popular plant with potential medicinal properties. Pak J Biol Sci. 2012;15(24):1152-9. doi: 10.3923/pjbs.2012.1152.1159, PMID 23755405.
27. Salah SM, Jäger AK. Two flavonoids from *Artemisia herba alba Asso* with *in vitro* GABAA-benzodiazepine receptor activity. J Ethnopharmacol. 2005;99(1):145-6. doi:10.1016/j.jep.2005.01.031, PMID 15848034.
28. Stermitz FR, Scriven LN, Tegos G, Lewis K. Two flavonols from*Artemisa annua* which potentiate the activity of berberine and norfloxacin against a resistant strain of*Staphylococcus aureus*. Planta Med. 2002;68(12):1140-1. doi:10.1055/s-2002-36347, PMID 12494348.
29. Da Silva JA. Mining the essential oils of the Anthemideae. Afr J Biotechnol. 2004;3(12):706-20.
30. Hudaib MM, Aburjai TA. Composition of the essential oil from *Artemisia herba-alba* growing in Jordan. J Essent Oil Res. 2006;18(3):301-4. doi: 10.1080/10412905.2006.9699096.
31. Saleh MA, Belal MH, El-Baroty G. Fungicidal activity of*Artemisia herba alba Asso* (Asteraceae). J Environ Sci Health B. 2006;41(3):237-44. doi:10.1080/03601230500354774, PMID 16484084.
32. Almasad MM, Qazan WS, Daradka H. Reproductive toxic effects of *Artemisia herba alba* ingestion in female Sprague-Dawley rats. Pak J Biol Sci. 2007;10(18):3158-61. doi:10.3923/pjbs.2007.3158.3161, PMID 19090117.
33. Aloui S, Skhiri H, Ltaief A, Elmay M. An exceptional case of acute renal failure: is there a renal toxicity of *Artemisia herba-alba*? Ren Fail. 2010;32(8):1009-11. doi: 10.3109/0886022X.2010.501932, PMID 20722571.
34. Abderrahman SM, Jamal Shbailat SJ. Genotoxic and cytotoxic effects of *Artemisia herba-alba* on mammalian cells. Caryologia. 2014;67(4):265-72. doi: 10.1080/00144235X.2014.974355.
35. Olsen RW. Absinthe and γ-aminobutyric acid receptors. Proc Natl Acad Sci U S A. 2000;97(9):4417-8. doi: 10.1073/pnas.97.9.4417, PMID 10781032.
36. Naser B, Bodinet C, Tegtmeier M, Lindequist U. *Thuja occidentalis* (arbor vitae): a review of its pharmaceutical, pharmacological and clinical properties. Evid Based Complement Alternat Med. 2005;2(1):69-78. doi: 10.1093/ecam/neh065, PMCID1062158, PMID15841280.
37. Höld KM, Sirisoma NS, Ikeda T, Narahashi T, Casida JE. Casida. α-thujone (the active component of absinthe): γ-aminobutyric acid type A receptor modulation and metabolic detoxification. Proc Natl Acad Sci U S A. 2000;97(8):3826-31. doi:10.1073/pnas.070042397, PMID 10725394.
38. Siegel E, Wason S. Camphor toxicity. Pediatr Clin North Am. 1986;33(2):375-9. doi: 10.1016/s0031-3955(16)35008-8, PMID 3515302.
39. Smith AG, Margolis G. Camphor poisoning; anatomical and pharmacologic study; report of a fatal case; experimental investigation of protective action of barbiturate. Am J Pathol. 1954;30(5):857-69. PMID 13197534. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1942492/.
40. Phelan WJ. Camphor poisoning: over-the-counter dangers. Pediatrics. 1976;57(3):428-31. doi: 10.1542/peds.57.3.428, PMID 1256960.
41. Rabl W, Katzgraber F, Steinlechner M. Camphor ingestion for abortion (case report). Forensic Sci Int. 1997;89(1-2):137-40. doi: 10.1016/S0379-0738(97)00099-6, PMID 9306672.
42. Mohamed AE, El-Sayed M, Hegazy ME, Helaly SE, Esmail AM, Mohamed NS. Chemical constituents and biological activities of*Artemisia herba alba*. Rec Nat Prod. 2010;4(1):1-25. doi: 10.13140/RG.2.1.2544.8806.
43. World Health Organization. Who. Analyze human sperm and interaction with sperm cervical mucus. INSERM; 1993. p. 55-6.
44. Martoja R, Martoja M. Initiation aux techniques de l'histologie animale. Ed Masson et Cie. Paris; 1967.
45. Abid ZB, Feki M, Hédhili A, Hamdaoui MH. *Artemisia herba-alba* Asso (Asteraceae) has equivalent effects to green and black tea decoctions on antioxidant processes and some metabolic parameters in rats. Ann Nutr Metab. 2007;51(3):216-22. doi:10.1159/000104140, PMID 17587792.
46. Aouadi D, Luciano G, Vasta V, Nasri S, Brogna DM, Abidi S, *et al.* The antioxidant status and oxidative stability of muscle from lambs receiving oral administration of*Artemisia herba alba* and Rosmarinus officinalis essential oils. Meat Sci. 2014;97(2):237-43. doi: 10.1016/j.meatsci.2014.02.005, PMID 24583334.
47. Baazaoui I, Soumri N, Chouikh Z, Khnissi S, Lassoued N, Bedhief-Romdhani S. Estimation *in vivo* of some carcass quality parameters in Barbarine breed rams receiving diets supplemented with *Artemesia herba alba* or*Rosmarinus officinalis* foliage. Ann INRAT. 2017;90:190-201. doi: 10.12816/0040328.
48. Khnissi S, Bomboi G, Khémiri I, Salem IB, Dattena M, Sai S, *et al.* Incorporation of fresh leaves of wormwood (*Artemisia herba alba*) and/or rosemary (*Rosmarinus officinalis*) in the diet of rams: effect on testicular function, sexual behavior, and blood parameters. Food Sci Nutr. 2023;11(6):3121-30. doi:10.1002/fsn3.3293, PMID 37324835.
49. Selmi S, Rtibi K, Grami D, Hajri A, Hosni K, Marzouki L, *et al.* Antioxidant properties of*Artemisia herba-alba* and*Eucalyptus camaldulensis* essential oils on malathion-induced reproductive damage in rat. RSC Adv. 2016;6(112):110661-73. doi: 10.1039/C6RA18268B.
50. Laadraoui J, Aboufatima R, El Gabbas Z, Ferehan H, Bezza K, Ait Laaradia M, *et al.* Effect of*Artemisia herba-alba* consumption during pregnancy on fertility, morphological and behaviors of mice offspring. J Ethnopharmacol. 2018;226:105-10. doi:10.1016/j.jep.2018.08.017, PMID 30118834.

51. Khataibeh MH, Daradka H. Antiandrogenic activity of *Artemisia herba-alba* in male albino rats, with emphasis on biochemical parameters. Asian J Chem. 2007;19(4):2595-602.
52. Al Marghani FA, Alostaomar SM. The physiological effect of *Artemisia herba alba* extracts on sperm activity in male rabbits. Egypt Acad J Biol Sci (B Zool). 2021;13(1):217-23. doi: 10.21608/EAJBSZ.2021.174152.
53. Apfelbeck B, Flinks H, Goymann W. Variation in circulating testosterone during mating predicts reproductive success in a wild songbird. Front Ecol Evol. 2016;4:107. doi: 10.3389/FEVO.2016.00107.
54. Preston BT, Stevenson IR, Lincoln GA, Monfort SL, Pilkington JG, Wilson K. Testes size, testosterone production and reproductive behavior in a natural mammalian mating system. J Anim Ecol. 2012;81(1):296-305. doi: 10.1111/j.1365-2656.2011.01907.x, PMID 21958300.
55. George N, Nassar SW, Leslie. Physiology, testosterone. StatPearls Publishing LLC; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK538429/.
56. Domiaty DM. Artemisa herba-alba ameliorates CCl 4 -induced spermatozoa toxicity through the downregulation of ERCC1 expression. Pharmacophore. 2022;13(3):91-7. doi: 10.51847/ZU0qiYb8sn.
57. Al-Tikriti AA, Al-Khateeb E, Abbas MA. *Teucrium polium* hexane extract downregulated androgen receptor in testis and decreased fertility index in rats. Hum Exp Toxicol. 2017;36(12):1248-55. doi: 10.1177/0960327116688069, PMID 28071240.
58. Rochira V, Fabbi M, Valassi E, Madeo B, Carani C. Estrogens and male reproduction. Androl. 2003;13(1):57-61. doi: 10.1007/BF03034410.
59. Guvvala PR, Ravindra JP, Rajani CV, Sivaram M, Selvaraju S. Protective role of epigallocatechin-3-gallate on arsenic induced testicular toxicity in Swiss albino mice. Biomed Pharmacother. 2017;96:685-94. doi: 10.1016/J.BIOPHA.2017.09.151, PMID 29040955.
60. Uygur R, Aktas C, Caglar V, Uygur E, Erdogan H, Ozen OA. Protective effects of melatonin against arsenic-induced apoptosis and oxidative stress in rat testes. Toxicol Ind Health. 2016;32(5):848-59. doi: 10.1177/0748233713512891, PMID 24318767.
61. Tang Z, Zhang Q. The potential toxic side effects of flavonoids. Biocell. 2022;46(2):357-66. doi: 10.32604/biocell.2022.015958.
62. Mostrom M, Evans TJ. Phytoestrogens. reproductive and developmental toxicology; 2011;707-22. doi: 10.1016/B978-0-12-382032-7.10052-9.
63. Cederroth CR, Auger J, Zimmermann C, Eustache F, Nef S. Soy, phyto-oestrogens, and male reproductive function: a review. Int J Androl. 2010;33(2):304-16. doi: 10.1111/J.1365-2605.2009.01011.X, PMID 19919579.
64. Hamilton-Reeves JM, Vazquez G, Duval SJ, Phipps WR, Kurzer MS, Messina MJ. Clinical studies show no effects of soy protein or isoflavones on reproductive hormones in men: results of a meta-analysis. Fertil Steril. 2010;94(3):997-1007. doi: 10.1016/j.fertnstert.2009.04.038, PMID 19524224.
65. Smith-Brown P, Morrison M, Krause L, Davies PS. Dairy and plant based food intakes are associated with altered fecal microbiota in 2 to 3 years old Australian children. Sci Rep. 2016;6:32385. doi: 10.1038/srep32385, PMID 27694811.
66. Ramírez MM, Estrada DV, Rodríguez IJ, Pérez-Rivero JJ, Reséndiz AS, González HF, *et al.* Effects of phytoestrogens on the reproductive physiology of productive species [review]. Rev Mex Cienc Pecuarias. 2022;13(3):803-29. doi: 10.22319/rmcp.v13i3.5878.
67. Witorsch RJ. Low-dose *in utero* effects of xenoestrogens in mice and their relevance to humans: an analytical review of the literature. Food Chem Toxicol. 2002;40(7):905-12. doi:10.1016/S0278-6915(02)00069-8, PMID 12065211.
68. Akingbemi BT, Hardy MP. Oestrogenic and antiandrogenic chemicals in the environment: effects on male reproductive health. Ann Med. 2001;33(6):391-403. doi:10.3109/07853890108995952, PMID 11585100.
69. Pentikäinen V, Erkkilä K, Suomalainen L, Parvinen M, Dunkel L. Estradiol acts as a germ cell survival factor in the human testis *in vitro*. J Clin Endocrinol Metab. 2000;85(5):2057-67. doi:10.1210/jcem.85.5.6600, PMID 10843196.
70. Ebling FJ, Brooks AN, Cronin AS, Ford H, Kerr JB. Estrogenic induction of spermatogenesis in the hypogonadal mouse. Endocrinology. 2000;141(8):2861-9. doi:10.1210/endo.141.8.7596, PMID 10919273.
71. Rochira V, Granata AR, Madeo B, Zirilli L, Rossi G, Carani C. Estrogens in males: what have we learned in the last 10 years? Asian J Androl. 2005;7(1):3-20. doi:10.1111/j.1745-7262.2005.00018.x, PMID 15685347.

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