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RESEARCH ARTICLE

Beneficial effects of *Anemone palmata* extracts on male rabbit fertility and reproduction

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Objective: The current study is intended to offer an insight on positive benefits of the herb Anemone palmate belonging to Ranunculaceae family, as an enhancing agent of male rabbit's fertility.
Methods: Twelve male rabbits, in the growth stage, were divided randomly into four equal groups: one serving as the control and three subjected to different treatments. Anemone palmata extracts (50, 100, and 200 mg/kg) were administered orally to the groups. Blood samples were collected to measure the serum levels of testosterone four weeks later. The weight of the testis and epididymis, sperm count, and sperm motility were determined histologically.
Results: The findings revealed a significant increase in the relative weights of the reproductive organs (testis and epididymis) and plasma testosterone levels in the groups that received the 100 and 200 mg/kg doses. Moreover a notable enhancement in the spermatozoa biology, including concentration, motility, and speed, was observed in the groups treated with 100 and 200 mg/kg doses compared to the control and the group treated with 50mg/kg. The histological study revealed some changes in the spermatogenesis and the structure of the organs, involving the presence of the spermatid phase. A significant increase in the thickness of seminiferous walls, a decrease in the interstitial space, and a reduction in the lumen and intra-tubular spaces were observed as well.
Conclusions: it is well indicated that Anemone palmata could improve fertility factors, and exhibit good effects on gonad and sperm parameters in rabbits.

Keywords: *Anemone palmata*, male rabbit, reproduction, sperm biology, histological study

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Introduction

The plant family Ranunculaceae, belonging to the order Ranunculales, encompasses approximately 60 genera and around 2200 species. Predominantly consisting of herbs, these plants range from small shrubs to woody vines. They are distributed worldwide, with a significant presence in temperate regions of the northern hemisphere. China has 42 genera and roughly 720 species, with a majority in the southwest hilly region [1]. At least 30 genera and roughly 220 species are used medicinally in China to treat a variety of ailments such as antibiosis, congestion, fever, cancer, arrhythmia, and malaria [2,3]. Ranunculaceae species are primarily found in the southwest of China [4] whereas there are 27 genera in Europe. The complex chemical composition of Ranunculaceae plants frequently has taxonomic repercussions [3]. Numerous studies have been accomplished on the pharmacology, phytochemistry, chemotaxonomy, and plant systematics of this family [5]. Numerous new chemical components, bioactivities, and therapeutic applications have been reported as a result of the advancement of chemical sciences and analytical technology, offering fresh chemotaxonomic evidence for therapeutic implications.

The genus *Anemone* of the Ranunculaceae family and endemic to temperate regions in both Northern and Southern hemispheres comprises more than 150 species of flowering plants [6]. Ethno-pharmacological survey of more than 50 Anemone species as well as pre-clinical and clinical investigations provided scientific support for some of the traditional claims made about these species [7]. Numerous chemicals have been linked to anemones, including triterpenoids, saponins, steroids, lactones, lipids and oils, sugars, and alkaloids [8]. Oleanolic acid triterpene saponin is a prevalent compound found in Anemone species. Additionally, Anemone contains ranunculin, anemonin, and protoanemonin, besides coumarins and flavonoids [9].

Anemone palmate was documented by Linnaeus in 1753 and was included in De Candolle's strict section in 1818 and 1824. Alongside *Anemone coronaria* L. and several other European-Mediterranean species, hold a noteworthy place in Anemonanthea DC [10]. *A. palmata* is a perennial plant (geophyte), with excellent actinomorphic flowers with many free stamens and carpels. *A. palmata* is classified as a western stenomediterranean species by Pignatti [11]

Generally, the plant typically grows in shrubby areas or at the borders of open woods, always in regions subject to some maritime influence [10]. It prefers calcareous and mildly humiferous soils rich in minerals but with low levels of decomposed organic matter. It enjoys partially shaded to sunny areas. Its flowering period typically occurs in spring and summer [12].

The aim of our study was to determine the effect of *Anemone palmata* leaf extracts on the testosterone profile

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and on the semen characteristics and morphology in male adult rabbits (*Oryctolagus cuniculus*). The work aims as well to the understanding of the properties of this plant and its impact on reproductive processes. To the best of our knowledge no investigation was reported on effects of *Anemone palmata* extract on reproductive health, particularly in male subjects. The results obtained are promising in the field of reproductive medicine and can potentially contribute to the development of therapies aimed at improving reproductive health and function.

Methods

Animals

The current study adhered to international and national standards for the ethical treatment of animals, following guidelines and complying with pertinent legislation. Twelve adult male rabbits, each weighting approximately 2.563 kilograms were placed in steel cages for a two-week adaptation period. During this time, they were exposed to controlled environmental conditions, including a temperature of 25 °C, humidity levels maintained between 40% and 50%, and a light cycle of twelve hours of daylight followed by twelve hours of obscurity. The rabbits were categorized into four groups, namely G1, G2, G3, and G4, and subjected to a thirty-day treatment period. Different graded doses of the extracts were allocated to each group as follows:

For groups G2, G3, and G4, the doses administered were 50mg/kg, 100mg/kg, and 200mg/kg respectively. Rabbits in group G1, designated as the control group, were given distilled water

Plant collection and preparation of extract

Plants samples were collected from the area of Chebket Sellaoua (South of Oum El Bouaghi). Taxonomic identification of leaves was confirmed by Prof. Zellagui at the department of Biological Sciences, Larbi Ben Mhidi University, Oum El bouaghi. The plant leaves were air dried under a shade for one week to prevent the loss of bioactive volatile compounds then finely sieved after being ground into powder using an electric grinder. The material was diluted in 70% ethanol, filtered twice, and subjected to extraction at 60°C using a rotary evaporator. Following the extraction process, it was dried for 15 days in a dark and well-ventilated environment.

Spermatozoa Characterization: spermogram

The epididymis was surgically removed, and slits were made in the epididymis to assess sperm characteristics, including movement, concentration, and speed. This assessment was conducted by placing 1µL drops of the milky white sperm into 50 µL of physiological fluid (NaCl = 0.9%) as stated by the World Health Organization guidelines [13]

Sperm Concentration

The sperm concentration was determined using a hemocytometer (Malassez slide) and an optical microscope by counting the spermatozoa in five squares of the slide (equivalent to 400x magnification). This was calculated using the following equation:

$$\text{Concentration (Spermatozoa} \times 106/\text{ml}) = (D \times V \times n)/N$$

Where:

D represents the dilution factor.

V is the volume of the hemocytometer

n is the number of spermatozoa counted in five squares of the hemocytometer.

N represents the total number of squares on the hemocytometer (usually 100 squares).

This calculation is in accordance with the guidelines provided by the World Health Organization [13]

Sperm motility

Sperm motility was evaluated by placing a semen sample on a standard microscope slide. In this assessment, five distinct fields of view were selected to determine the percentage of spermatozoa exhibiting active movement. The examination was performed under a microscope set at a magnification of 400x, adhering to the guidelines established by the World Health Organization in 1993.

Sperm Speed

The speed of spermatozoa was measured by placing a semen drop on the Nageotte hemocytometer and covering it with a cover slip. The time taken by the spermatozoa to traverse between two parallel lines was determined using a chronometer while observing them under an optical microscope at 400x magnification. This method aligns with the guidelines outlined by the World Health Organization [13].

Testosterone Analysis

The plasma testosterone level was measured using a commercial kit designed for in vitro chemiluminescence immunoassay. This quantitative determination of testosterone was performed with the MAGLUMI series fully automated chemiluminescence immunoassay analyzer. The testosterone concentration was expressed in nanograms per milliliter (ng/ml).

Histological Analysis

After removal, the testes and epididymis were promptly preserved in a formaldehyde solution. They underwent a series of processing steps involving graded ethanol to dehydrate the tissue, and ultimately, they were embedded in paraffin. The resulting samples were then subjected to histological analysis. Slices, each approximately 4-5 micrometers (µm) thick, were generated using a microtome. These 5µm thick paraffin slices were stained with hematoxylin and eosin, facilitating histological examination under a light microscope. The relevant portions of the tissue were examined and captured using a camera.

Statistical analysis

Statistical analysis of the data utilized Student's t-test to compare the means of two groups. The data analysis was conducted using the SPSS program in the year 2020. The statistical results were reported in terms of mean values along with their standard deviations (SD).In terms of interpreting the correlation, the following criteria were applied using probability (p):

- If $p > 0.05$, the result was considered not significant
- When $p = 0.05$, the result was deemed significant (*).
- A p-value of 0.01 was considered very significant (**).
- A p-value of 0.001 was regarded as extremely highly significant (***).

These guidelines helped assess the significance of correlations within the data

And the analysis of variance for classification criterion (ANOVA) was used to perform the multiple comparison of the means, and a value of $P<0.05$ was considered statistically significant.

Results

Evolution of body weight

The data in Table I display alterations in body weight observed over a four-week period, resulting from the daily administration of 50, 100, and 200 mg/kg/day of organic extract of *Anemone Plamata* to matured rabbits. These results effectively showcase the extract's influence on body weight. In both the treated and control groups, a noteworthy increase ($p < 0.05$) in final body weight was evident compared to their respective initial weights. Moreover, the application of the extract led to greater weight gain in comparison to the control group (T). Specifically, we observed a weight increase of 2.81% in G1, 4.92% in G2, and 4.71% in G3, while the control group showed an increase of 2.30%. These results indicate that the extract had a notable effect on enhancing weight gain in the rabbits when compared to the control group.

Evolution of organs weight

Evolution of organ weight refers to the changes or alterations in the weight of organs within a biological organism over a specific period or under particular conditions. The results obtained, as presented in **Table II**, reveal that the oral administration of the organic extract over a 4-week period leads to a notably significant increase in the relative weight of both the right and left testis in rabbits treated with doses of 100 and 200 mg/kg/day ($p < 0.05$). This increase in testis weight is observed when compared to the control groups. In contrast, rabbits treated with a dose of 50 mg/kg/day did not exhibit a significant increase in testis weight ($p > 0.05$) when compared to the control group. These findings indicate that the organic extract had a pronounced effect on increasing testis weight at higher doses (100 and 200 mg/kg/day) but did not significantly impact testis weight at the lower dose of 50 mg/kg/day when compared to the control group. However, we observed a notably significant rise ($p < 0.01$) in the relative weight of epididymis rabbits treated with the two doses 100 and 200 mg/kg/day, and no significant increase was noted in rabbits treated by dose 50 mg/kg/day ($p > 0.05$) in comparison with the control one.

Testosterone level

The results depicted in Figure 1 illustrate that the oral administration of the organic extract leads to an extremely significant increase ($p < 0.001$) in the plasma testosterone concentration among rabbits treated with doses of 100 and 200mg/kg/day in G3 and G4, respectively, when compared to the control group. In contrast, it is noteworthy that there was no significant increase ($p > 0.05$) in the

Table I. Fluctuations in body weight among both the control and treated batches

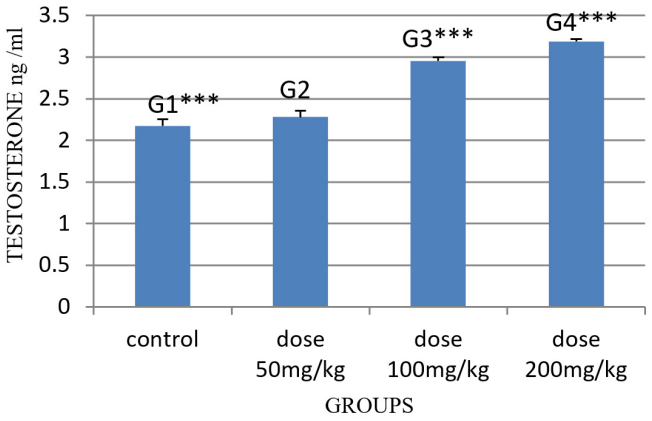
Parameters	Experimental batches			
	Control (T) Mean ± S.D.	G1(50 mg/kg) Mean ± S.D.	G2(100 mg/kg) Mean ± S.D.	G3(200 mg/kg) Mean ± S.D.
Initial body weight (g)	2460.00±52.91 -	2606.66±40.41 (5.96%)	2573.33±66.58 (4.60%)	2616.66±56.86 (6.36%)
Final body weight (g)	2516.66±75.71 -	2680.00±26.45 (4.11%)	2700.00±50.00 (7.28%)	2740.00±79.37 (8.87%)
Weight gain (g)	56.66 ±22.8 -	73.34 ±13.96 (29.43%)	126.67 ±16.58 (123.56%)	123.34 ±22.51 (117.68%)
increase Rate	2.30%	2.81%	4.92%	4.71%

(%) represents the percentage of variation of weights between treated groups and control group

Table II. Changes in Relative Weight of Testis and Epididymis (Mean ± Standard Deviation) in Male Rabbits Following 4 Weeks of Treatment

Parameters	Experimental batches			
	Control G1 (T) Mean ± S.D.	G2 (50 mg/kg) Mean ± S.D.	G3 (100 mg/kg) Mean ± S.D.	G4 (200 mg/kg) Mean ± S.D.
Right testis	0.105 ±0.008 -	0.104 ±0.009 (0.95%)	0.140** ±0.003 (33.33%)	0.145*** ±0.004 (38.09%)
Left testis	0.104 ±0.007 -	0.107 ±0.007 (2.88%)	0.137*** ±0.002 (31.73%)	0.148*** ±0.003 (42.30%)
Epididymis	0.022 ±0.003 -	0.025 ±0.002 (13.63%)	0.037** ±0.003 (68.18%)	0.044*** ±0.004 (100%)

If $p > 0.05$, the result was considered not significant; $P < 0.05$, the result was deemed significant (*); $P < 0.01$: very significant (**); $P < 0.001$: extremely highly significant (***)



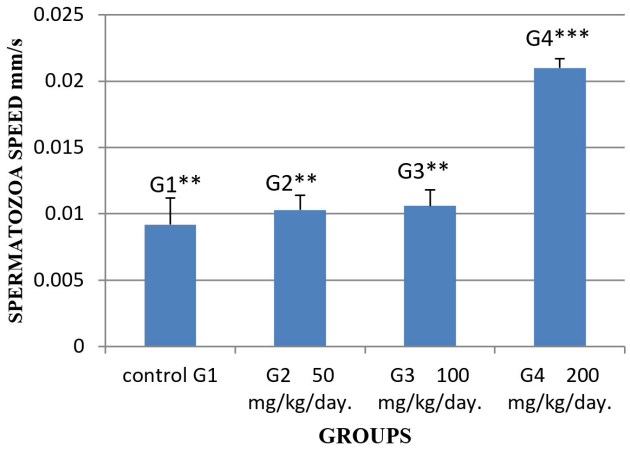
no significant difference between G2 and G1; ***: extremely highly significant difference between G3 and G1; ***: extremely highly significant difference between G4 and G1 (t-student test); G1***: extremely highly significant difference between the groups (ANOVA test).

Fig. 1. The testosterone levels (X±SD) in rabbits following four treatment weeks of Treatment.

plasma testosterone concentration observed in G2, where rabbits were treated with a dose of 50 mg/kg/day

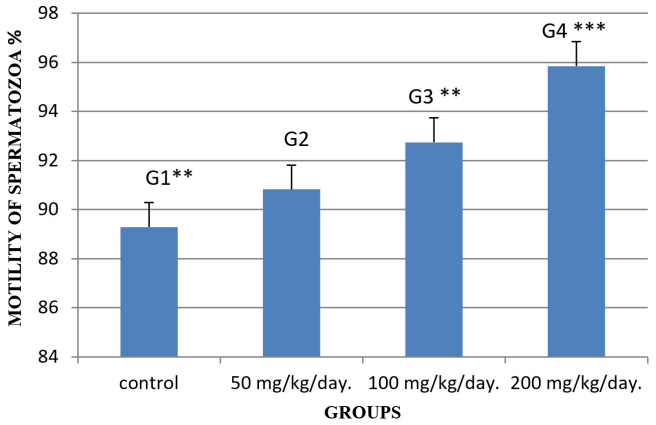
Sperm parameters

The findings of the epididymal sperm assay demonstrate a noteworthy increase in all sperm parameters, including sperm speed, concentration, and motility, in the group of rabbits that received doses of 100 and 200 mg/kg/day. However, the group subjected to 50 mg/kg/day shows no significant elevation compared with the other groups (Figures 2, 3, and 4)



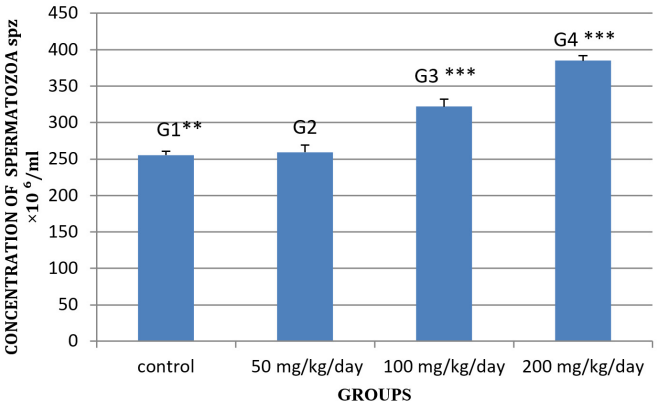
: high difference significant difference between G2 and G1; **: high difference significant difference between G3 and G1; *: extremely highly significant difference between G4 and G1 (t-student test); G1**: high difference significant between the five groups (ANOVA test).

Fig. 2. Variations in Sperm Speed (Mean ± Standard Deviation) in Male Rabbits After 4 Weeks of Treatment.



No significant difference between G2 and G1; **: high difference significant difference between G3 and G1; ***: extremely highly significant difference between G4 and G1 (t-student test); G1**: high difference significant between the five groups (ANOVA test).

Fig. 3. Variations in Sperm Motility (Mean ± Standard Deviation) in Male Rabbits After 4 Weeks of Treatment.



no significant difference between G2 and G1; ***: extremely highly significant difference between G3 and G1; ***: extremely highly significant difference between G4 and G1 (t-student test); G1**: high difference significant between the five groups (ANOVA test).

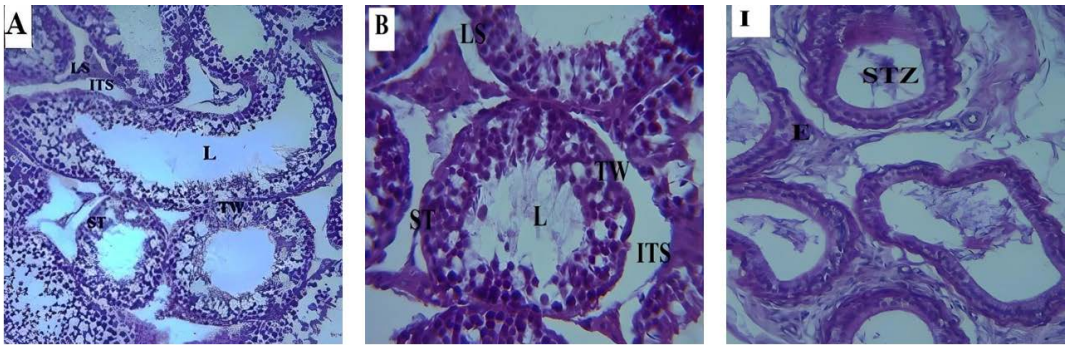
Fig. 4. Variation in Sperm Concentration (Mean ± Standard Deviation) in Rabbits throughout 4 Weeks of Treatment.

Histological study

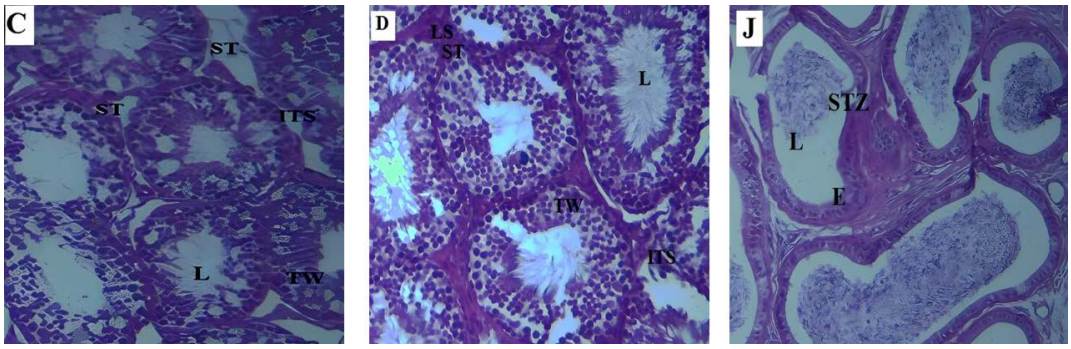
In rabbits given various doses, microscopic analysis of the testis and epididymis reveals certain architectural modifications to the testicular tissue. X100 magnification provides a broad overview of the tissue structure and shows the presence of Leydig cells and a reduction in the lumen and intertubular spaces in rabbits treated with two doses of 100 and 200 mg/kg/day.

Contrary, the control group and the group with low dose indicate an increase of lumen and intertubular spaces. Under a magnification of x40, the seminiferous tubules exhibited enhanced clarity and detail in the groups receiving the 100 and 200 mg/kg/day doses. These tubules had thicker walls and consisted of approximately seven layers of cells during differentiation and maturation. In addition, histological sections of rab-

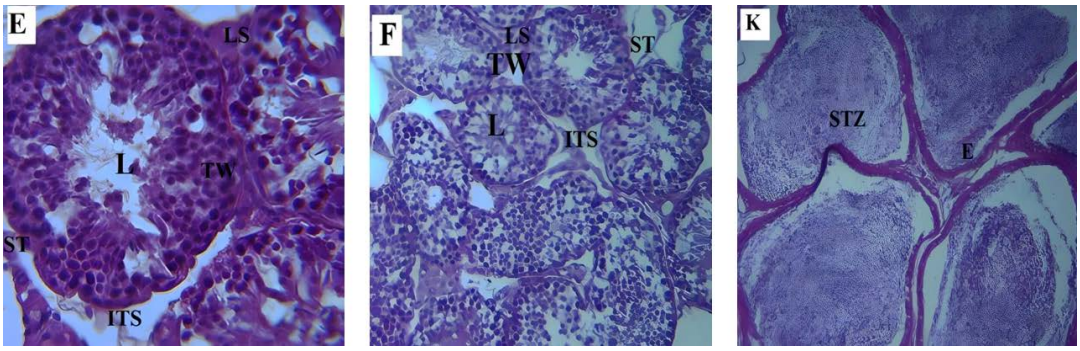
bits administered higher doses of the extract displayed a more uniform and regular arrangement of spermatozoa. Furthermore, the lumens of the seminiferous tubules appeared to be regular and filled with mature spermatozoa. Specifically, in the high-dose histological section of a rabbit's epididymis, the tubular structure appeared normal and regular, and the lumen was populated with mature spermatozoa. This observation stands in contrast to both the control group and the group treated with 50 mg/kg/day (Figure 5).



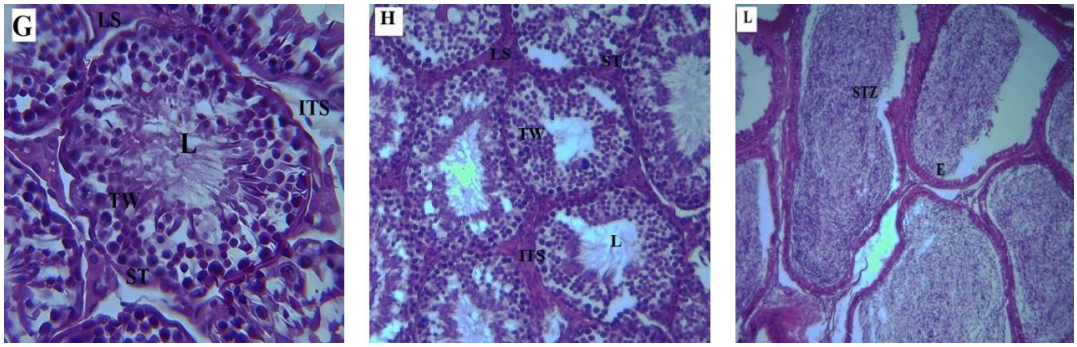
Group G1: Histological sections of the control group testis (A magnified x 40; B magnified x 100) (I) Histological sections of the control group epididymal tail



Group G2: Histological sections of the treated group (dose 50 mg) testis (C magnified x 40; D magnified x 100) (J) Histological sections of the group 2 epididymal tail



Group G3: Histological sections of the treated group (dose 100 mg) testis (E magnified x 40; F magnified x 100) (K) Histological sections of the group 3 epididymal tail



Group G4: Histological sections of the treated group (dose 200 mg) testis (G magnified x 40; H magnified x 100) (L) Histological sections of the group 4 epididymal tail

Fig. 5. Histological sections of the testis and the epididymal tail of the control and treated groups, LS: Leydig cells, L: Lumen, TW: Tubular wall, ITS: Intertubular spaces; ST: Seminiferous tubules, STZ: spermatozoide

Discussions

As far as our understanding extends, this study represents the primary investigation dealing with the assessment of *Anemone palmata*'s whole impact on the testes and testosterone outline in rabbits. The results are consequently generalized and compared to other species.

Due to their ease of handling and upkeep, rabbits were chosen as model animals. They grow in any climate and are economical from a scientific perspective. Rabbits also engage in sexual activity throughout the entire year. Furthermore, the semen evaluation averred an important tool for clarifying the effect of high doses of *Anemone palmata* extracts on male reproduction. The rabbits were categorized into four groups: Control G1, G2, G3, and G4, corresponding to doses of 0, 50, 100, and 200 mg/kg/day, respectively. These treatments were administered over a 30-day period.

Comparing the last body weight of mature rabbits to their preliminary mass, a statistically significant increase was observed: 4.92% for G3 and 4.71% for G4. In contrast, for G2 and the control group G1, The increase in body weight was less notable at 2.81% for G2 and 2.30% for the control group G1. A similar observation was previously reported [14].

The study investigated the impact of daily oral administration of Nigella sativa seed oil for duration of 60 days in twenty adult male rabbits and demonstrated that rabbits treated with 5ml/kg body weight/day had the greatest weight and the gain ratio was significantly increased. The relative weight of the reproductive organs both testis and epididymis showed a notable increase when rabbits were given the highest doses compared with the control. these findings are in accordance with the previous results which reported that *Nigella sativa* belong to the same family of Ranunculaceae [15,16].

The results from this study revealed a greatly momentous increase in the relative weight of both the testis and epididymis for G4 and G3 ($p < 0.01$) related to the control groups. These results align with the findings reported byUmar *et al* [14], who showed that treatment with the extract of *Nigella sativa* led to an increase in the weight of reproductive organs. Moreover, our results align with those stated by in Mohammad *et al.* [17]. These similarities in results suggest that the administration of the organic extract has a positive effect on the relative weight of the testis and epididymis. Likewise previous research dealing with *Nigella sativa* extract, found that treatments of rats using doses of 300 mg per kg for 60 days induced a significant increase in the fresh weight of the testis and epididymis ($p <0.01$). Additionally, *A.palmata* alkaloids possess estrogenic properties[18], possibly due to the fact that the plant estrogen is included in the extract, which binds to the testicular receptors and promotes feeding by raising the weight of the reproductive organs[19]. The highly significant Increase ($p < 0.001$) of plasma testosterone concentration by *Anemonepalmata* can be inferred by the presence of saponins and their action on the hypothalamic-pituitary axis ,Testes' Leydig cells are induced by saponins to produce more testosterone[20] .

The saponins found in plant extracts may have helped to stimulate an increase in the body's endogenous testosterone production. Because they increase androgen, plant saponins are thought to enhance aphrodisiac effects, according to studies. The pituitary glands secretion of LH, which raises the testosterone dose, and aids in the maintenance of testosterone levels [21]. The observed increase in testosterone levels may suggest a potential positive impact on the process of spermatogenesis, as indicated by the obtained results. In actuality, estrogens are crucial for controlling male reproductive activity [22].

The present sperm count results indicate that *Anemone palmata* leaf extracts with increasing doses increased the epididymal and motility and moreover augmented the speed of sperms. Such results are in agreement with the findings of Bashandy [23], who reported that giving healthy rats 0.5 ml/day of Nigella sativa oil orally for two months had a number of beneficial effects. These included increased plasma testosterone levels, enhanced sperm motility and count, decreased sperm abnormalities, increased weight of seminal vesicles, and improved sperm motility and count. The inclusion of necessary components, especially antioxidants like vitamin C and vitamin E, as well as flavonoids like quercetin and anthocyanins, may be responsible for this beneficial effect. These antioxidants can mitigate the negative effects of free radicals produced as a result of oxidative stress [24].

Controlling oxidative stress is a critical factor for cells to maintain their viability. Mitochondria, found in all species, utilize oxygen molecules (O_2) in a process known as oxidative phosphorylation to generate energy. This process yields adenosine triphosphate (ATP), as well as water and carbon dioxide. As part of regular oxygen metabolism, there is a continuous, low-level production of reactive oxygen species (ROS) or free radicals. However, when there is an excessive generation of reactive oxygen species or free radicals, it can become toxic to cells, necessitating their metabolic neutralization or removal to prevent harm to the cells.

Substances identified as antioxidants possess the capability to inhibit or delay the formation of reactive oxygen species (ROS) intermediates. Cells can obtain antioxidants through ingestion or internal synthesis. Nigella sativa, a plant from the Ranunculaceae family also known as *anemone palmata*, has been shown to inhibit the production of hydroxyl, superoxide (O2), and hydrogen peroxide [17].

Indeed, reactive oxygen species (ROS) or their precursor molecules can be neutralized by antioxidants. Antioxidants can be either exogenous (derived from external sources) or endogenous (produced within the body).These substances also have the ability to bind to metal ions that are necessary for catalyzing the synthesis of ROS. By scavenging ROS and inhibiting their formation, antioxidants play a crucial role in maintaining cellular health and mitigating oxidative stress-related damage [25]. Also, the flavonoids have

the ability to scavenge reactive oxygen species such the superoxide anion and hydroxyl radical, which can cause oxidative damage. In addition, Quercetin and catechin are members of the flavonoid family. the presence of quercetin in AP which is a powerful antioxidant is also considered a free radical scavenger therefore it can protect spermatozoa against damage caused by (ERO) by inhibiting lipid peroxidation and modifying the antioxidant defense pathway *in vivo* and *in vitro* [26] Interestingly, Numerous studies have validated that severe oxidative stress can result in infertility due to its adverse effects on critical events like acrosome reaction and sperm-oocyte fusion [27]. Elevated levels of reactive oxygen species (ROS) can potentially harm spermatozoa's ability to fertilize and their genetic integrity [28]. Compounds such as steroids, flavonoids, saponins, and lipids have been identified to stimulate sexual behavior and enhance sperm quality, which could explain the observed improvement in the potency of *A. palmata* extracts.

The effect of the highest doses of 100 and 200 mg/kg/day of *A.palmata* extract on sperm motility in cauda epididymis was high significantly increased (P< 0.001) observed in this study. Also, Mohammad *et al.* [17] found that the treated group with Nigella sativa at a dose of 300. Mg/kg body weight in adult male albino rats induces a significant increase in the sperm motility.

The ability of the sperm to ascend the female reproductive canal to the site of fertilization and the requirement for fertilization both depend on motility [29] As a result, superior-quality sperm should be active and numerous, and their glycolytic or fructolytic rates should be higher than those of inferior-quality sperm Caused to Testosterone secretion from the testes is necessary for the accessory glands to produce and secrete fructose. This might be caused by *A.palmata*'s impact on the oxidative phosphorylation enzymes [30]. Reports are indicated that *A.palmata* contains flavonoid components[31].That have positive effect on the sperm Quality[32]and contain anthocyanins too which have roles as antioxidants, phytoalexins, or antibacterial agents revealed the presence of Anthocyanins in *A.palmata* which serve a variety of physiological purposes. They prevent diseases by defending living cells from oxidative damage[33].

The sperm parameters in animals treated with *A.palmata* extract have recorded elevations greater than those in the control condition, which may be attributed to its components, which include thymoquinone, saponins, and flavonoids; Oleanolic acid, triterpene, coumarins, Benzenoids, fatty acid derivatives is abundant in Anemone species [41). this finding was in good agreement with that of Tousson *et al* [34]. Moreover, El-Tohamy*et al.* [24] showed that *Nigella sativa*, which belongs to the same family as *Anemone palmata*, can significantly increase both sperm motility and sperm count in rabbits when compared to control groups. This finding suggests that *Nigella sativa* may have a positive impact on reproductive parameters in rabbits, potentially enhancing fertility or overall reproductive health. Additionally, Mehraban *et al.* [35] explained this increase by the components of *A.palmata* extracts activating estrogen hormones resulting in increased sperm production, and according to Hao *et al.* [36], alkaloids were also detected in AP extracts that improve sexual performance by maintaining male erection by increasing blood flow in the sexual organs due to vasodilatation.

Through the presence of the required compounds in *A.palmata* extracts, they protect the sperm plasma against the loss of membrane fluidity and integrity caused by peroxidative damage, and sperm are longer capable of taking part in the membrane fusion processes necessary for fertilization [37].

Androgen is necessary for the function of the spermatogenesis process and the accessory reproductive organs. In the present study, the histological sections of rabbit testis supplemented with high doses of *Anemone palmata* extracts exhibit distinctive characteristics. These sections display tubules with thicker walls, which are densely populated with spermatozoa. Moreover, there is a notably higher number of spermatozoa in these sections compared to the control group. These observations suggest that the administration of high doses of *Anemone palmata* extracts may have a positive impact on testicular histology and sperm production. This outcome is consistent with research from Toussone*t al.* [34] who showed a development in the testes structure and contained numerous Leydig cells. Also, the number of sperms was significantly ($p<0.05$) higher compared with the control group in rabbits' group fed a diet supplemented with *N. Sativa* and also compared with other plants species used for treating infertility like *Pheonix dactylifera*L.

Selmani*et al.* [38] showed that feeding rats with Pollen suspension at two different doses (120 mg/kg and 160 mg/kg) displayed an increased male reproductive system. In another study by Bawazir [39], it was reported that the boosted amount of testosterone in the blood, correlated favorably with improved spermatogenesis and a notable rise in the quantity of mature sperm.

The significant increase ($p < 0.05$) in the epididymis and testes-body weight ratio, particularly at doses of 100 mg/kg and 200 mg/kg following the administration of *A. palmata* extracts, may be attributed to the heightened secretory activity of the testes. This is supported by the observed rise in testosterone concentrations in the present study. This outcome could be a result of the plant's abundance and the presence of necessary bioactive components within *A. palmata* extracts. These findings, which are consistent with previous reports [40], confirmed the effect of increasing testosterone levels necessary on the testis and epididymis. The present results indicate that *A.palmata* with the increasing dose increased the epididymal sperm count and moreover augmented the percentage number of normal sperms in the histological examination of the epididymis lumen. Furthermore, rabbits treated with *A. palmata* at doses of 100 and 200 mg/kg exhibited an increase in serum testosterone levels. This dose-dependent effect is likely due

to the positive impact on Leydig and spermatogonia cells. It's important to note that estrogen is synthesized in the male reproductive system by various cell types, including Sertoli, Leydig, and germ cells. Adimoelja [41] obtained a positive effect on the histological analysis of rabbit groups that revealed the normal testicular structure and contained numerous Leydig cells and found the development and thickness of seminiferous tubules increased. Therefore, it appears that the *A palmata* extract can help protect the reproductive system in part as this plant contains a number of antioxidants, including anthocyanins, flavonoids.

Conclusion

In conclusion, the study investigated the effects of administering *Anemone palmata* extract to mature male rabbits over a 30-day period. The results revealed promising outcomes regarding reproductive parameters. Administration of higher doses (100 and 200 mg/kg/day) of the extract significantly increased the relative weight of the testis and epididymis, indicating a potential positive influence on these reproductive organs. Moreover, a substantial elevation in plasma testosterone levels was observed in rabbits receiving these higher doses, highlighting a potential hormonal regulatory effect.

Importantly, the extract exhibited significant enhancements in sperm parameters—speed, concentration, and motility—in the rabbits receiving the 100 and 200 mg/kg/day doses, emphasizing potential benefits for fertility. Histological analysis further supported these findings, showcasing notable changes in testicular tubules that could contribute to increased sperm production. Additionally, the extract demonstrated antioxidant properties by inhibiting the production of reactive oxygen species. These findings collectively suggest that *Anemone palmata* extract may hold promise as a natural supplement for improving male reproductive health. However, further research is essential to unravel the underlying mechanisms and ensure long-term safety and effectiveness of this extract.

Looking ahead, this study opens up the opportunity for more research. The precise processes underlying the reported effects should be clarified in further studies, along with the extract's long-term safety and effectiveness, and dose optimization should also be taken into account. In order to confirm these results and determine whether *Anemone palmata* extract may be used to address issues with male reproductive health, clinical research involving human participants are also necessary. *Anemone palmata* extract is one of the natural therapies being thoroughly investigated for its potential to advance reproductive medicine and improve general health.

Authors' contribution

AM(Conceptualization; Investigation; Data curation; Methodology; Writing – original draft), HH (Methodology; Project administration; Formal analysis), NA (Investigation; Formal analysis; Validation) SB (Methodology; Supervision; Validation), YM (Formal analysis; Validation; Visualization), YB (Validation; Visualization; Supervision), AS (Formal analysis; Validation; Visualization), NG(Conceptualization; Supervision; Writing – review & editing Resources)

Conflict of interest

None to declare.

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