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The Effects of Aqueous Extract of Anacyclus Pyrethrum on Sperm Count and Reproductive Organs in Adult Male Rats

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Abstract

***Background*:** More than 80 million individuals suffer from infertility globally. Various factors such as some drugs and toxins have harmful effects on fertility. *Anacyclus pyrethrum* plant in Indian traditional medicine is used for treatment of many diseases including infertility.

***Materials and Methods*:** In this experimental study 48 male adult rats were divided randomly into four groups (N=12) including one control group (A) and three test groups (B, C and D). Test groups (B, C and D) received root aqueous extract of *A. pyrethrum* intraperitoneally with doses of 50, 100 and 150 mg/kg for 28 days, respectively. At the end of the treatment period, the reproduction variables such as weight of body and sex organs, the sperm count in epididymis and right and left vas deferens and percent of abnormal spermatozoids were determined. The test groups were compared to the controls using analysis of variance following Tukey.

***Results*:** Data analysis of body and sex organs' weight, sperm count of epididymis and right and left vas deferens and percent of abnormal spermatozoids showed a significant difference between the tests and control groups (p=0.02, p=0.0001); however, no significant difference was found between two groups regarding vas deferens weight.

***Conclusion*:** The results of the present study showed that root aqueous extract of *A. pyrethrum* increased the weights of body and sex organs, increase of sperm count of epididymis and right and left vas deferens, and reduction of percent of abnormal spermatozoids in treated rats.

Introduction

Infertility is a complex disorder affecting more than 80 million people worldwide [1]. Approximately 30 % of infertilities are due to male factors [2]. In only 40% of all cases the infertility is detectable, and in the remaining 60% it is not pathologically detectable [3]. Many factors including drug treatment, chemotherapy, toxins, air pollutions and insufficient vitamins intake have destructive effects on a normal spermatogenesis [4]. Some toxic compounds such as 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (TCDD) can increase the production of abnormal sperms [5]. Besides, study of Saba et al. [6] showed that extract of *Lagenaria breviflora* augments the production of abnormal sperms [7].

Although androgens are primarily used in the treatment of sexual disorders, in both men and women [8], the use of medicinal herbs have always been under attention [9]. The World Health Organization (WHO) in its 31th summit in developed countries has recommended administration of medicinal herbs, because of their standards, advantages and healthiness [10]. Experimental reports suggest that compounds with androgenic effects could increase the weight of reproductive organs and glands [11].

Various studies have shown that compounds such as flavonoids in medicinal herbs have androgenic effects and stimulates the spermatogenesis [12]. Anacyclus pyrethrum is a medical herb which derives from the root Pellitory and in Indian language is called Akarkara. In Indian traditional medicine this plant has been shown to strengthen the immune system of human [13].

Medicinally, *A. pyrethrum* root has a pungent efficacy in promoting a free flow of saliva, in relieving toothache, in alleviating chronic catarrh and acne [14]. Pellitory root in Indian traditional medicine has been widely used as an aphrodisiac and as a medicine for rejuvenation and vitality [15].

It has been reported that oral administration of the root powder has aphrodisiac actions [16], so that in male rats the administration of alkylamide-rich extracts of *A. pyrethrum* improved their sexual behaviors [17]. Therefore, the present study was aimed to evaluate the effects of intraperitoneal injection of aqueous extract of *A. pyrethrum root* on weight of reproductive organs and glands and on sperm count in epididymis and vas deferens of male adult rats.

Materials and Methods

In this experimental study was performed on 48 Wistar strain male albino rats, aging 5-7 month, weighting 225±50 g, which were kept at the Animal laboratory of Zahedan University of Medical Sciences. In this experimental study, the animals were housed at room temperature (20±20°C), and light was set at 12 h light–dark cycle. They were maintained in plastic cages separately and had free access to the food and water. The study protocol was approved by the Institutional Animal Ethics Committee. After weighting of animals with a digital balance (EK-b10, Japan) the rats were randomly divided into four groups each with 12 animals; group A were given only standard dry rat pellet diet and water ad libitum. The test groups, (B, C and D) received doses of 50, 100 and 150 mg/kg of aqueous extract of A. pyrethrum root for 28 days by intraperitoneal route, respectively [18]. In the present study, a Sham group also was used for comparison, but since the results for the Sham and control group were similar, we eliminated the Sham group from the study to facilitate analyzes and interpretation of our results. *A. pyrethrum* roots purchased from a local market in Zahedan, and were approved at the Herbarium center of Biology department of University of Sistan and Balouchestan.

For the preparation of aqueous extract of *A. pyrethrum*, the plant roots were dried, powdered and then subjected to soxhelet apparatus for extraction with distilled water for 24 h. The extract obtained was filtered through a 30×100 mm filter paper and then dried at temperature of 37ºC to get the powder form. At the time of experiments, the dried powder was dissolved in physiologic serum in determined doses and then was injected intraperitoneally to the experimental groups [19]. At the end of the treatment period, the animals were anaesthetized with ether, killed by cervical dislocation and subjected for the various analyses. Body, testes, vas deferens and right and left epididymides were weighed and removed for sperm analysis after an inguinal dissection.

Sperm counting was performed according to the method described by Sharma et al. [20]. In brief, a hemocytometer with improved Neubauer ruling was employed for counting the spermatozoa. A 20-fold dilution was made by mixing the sperm suspension with normal saline (0.9% NaCl). The preparation was then thoroughly mixed, and one drop was added to both sides of the hemocytometer. Spermatozoa on both sides of the hemocytometer were counted, and the average number was recorded. The average number of spermatozoa counted was multiplied to volume, surface and dilution coefficients.

Since a number of sperms are damaged during mincing of epididymis, only intact spermatozoa were counted. To evaluate the sperm abnormalities, one drop of sperm suspension was stained with 1% eosin (alcoholic) smeared on slides, fixed and made permanent slides. The slides were examined under the microscope (Olympus) using ×400 objectives and abnormal sperm cells were counted and the percentage was calculated [21-22]. Results are expressed as mean±SEM. The test groups were compared to the controls using analysis of variance following tukey tests. All the statistical analyses were carried out using SPSS-19 software. A *p*-value below 0.05 was considered statistically significant.

Results

Analysis of data by the Tukey test revealed no significant difference of vas deferens weight in *A. pyrethrum* treated rats and the control. However, a significant difference was observed between the groups in the weights of body, testes and epididymis (Table 1 and 2). The effects of *A. pyrethrum* extract on weight of body, testes, right and left epididymis, right and left vas deferens and sperm count in left and right epididymis have been fully described in the table 1 and 2.

Table 1. Effects of A. pyrethrum extract on weight of body, testes and right and left epididymis (N=12)

Variables Groups	Body weight (g)	Right testis (g)	Left testis (g)	Right epididymis (g)	Left epididymis (g)
A (mean±SEM)	239.50±10.41	0.880±0.006	0.882±0.008	0.485±0.066	0.490±0.067
B (mean±SEM)	256.16±18.31	0.910±0.004	0.911±0.007	0.485±0.066	0.490±0.067
p-Value	0.02	<0.0001	0.001	0.001	0.004
C (mean±SEM)	291.58±11.82	0.947±0.003	0.951±0.007	0.675±0.010	0.667±0.025
p-Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
D (mean±SEM)	296.00±12.03	1.012±0.031	1.005±0.010	0.698±0.011	0.699±0.011
p-Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Table 2. Effect of A. pyrethrum extract on weight of right and left vas deferens and sperm count in left and right epididymis (N=12)

Variables Groups	Right vas deferensi (g)	Left vas deferens (g)	Sperm count in right epididymis (10^6/mL)	Sperm count in left epididymis (10^6/mL)
A (mean±SEM)	0.105±0.010	0.100±0.010	44.27±30.89	44.44±26.12
B (mean±SEM)	0.099±0.027	0.097±0.027	51.28±26.74	51.41±27.45
p-Value	0.9	0.8	<0.0001	<0.0001
C (mean±SEM)	0.110±0.017	0.0108±0.017	60.90±23.49	61.25±23.83
p-Value	0.8	0.9	<0.0001	<0.0001
D (mean±SEM)	0.106±0.013	0.103±0.013	64.42±24.39	64.24±23.72
p-Value	0.9	0.9	<0.0001	<0.0001

Table3. Effect of A. pyrethrum extract on sperm count in right and left vas deferens and percentage of abnormal spermatozoa (N=12)

Variables Groups	Sperm count in right deferens (10^6/mL)	Sperm count in left deferens (10^6/mL)	Abnormal Spermatozoa (%)
A (mean±SEM)	5.18±2.95	5.24±2.82	1.91±0.66
B (mean±SEM)	5.88±1.84	5.73±2.25	1.75±0.62
p-Value	<0.0001	<0.0001	0.004
C (mean±SEM)	6.57±1.85	6.63±1.22	1.66±0.65
p-Value	<0.0001	<0.0001	0.002
D (mean±SEM)	7.31±3.76	7.28±2.38	0.75±0.62
p-Value	<0.0001	<0.0001	<0.0001

*p<0.05 Comparisons with the Control group (A)

Moreover, analysis of data using the Tukey showed that there is a significant difference between the test and control groups in sperm count in epididymis and vas deferens and also in percentage of spermatozoa with abnormal morphology (Tables 2, 3). The effects of *A. pyrethrum* extract on sperm count in right and left vas deferens and percentage of abnormal spermatozoa is presented in table 3.

Discussion

The results of present study showed that the root aqueous extract of *A. pyrethrum* increased the body weight in the treated group compared to the control group. Several experiments including the Sharma et al. [19] study have demonstrated that aqueous or alcoholic extracts of this plant can enhance body weights of the test group in comparison with the control group. Additionally, the present study showed that testes and epididymis average weight were elevated significantly in the defined doses, which is in accordance with previous studies on other types of herbs [19, 23, 24]; however, vas deferens average weight was not affected. Our study showed that the plant extract leads to considerable elevation of average sperm count in epididymis and vas deferens, which fully supports the results of Sharma et al. [19, 23, 24]. To the best of our knowledge, our study for the first time revealed that the aqueous extract of *A. pyrethrum* reduces the percentage of spermatozoa with abnormal morphology.

Gaining weight is usually associated with steroid production, and is a biological indicator for effectiveness of medicinal herbs in production of steroid hormones [15]. Production of testosterone can result from gonadotropin activity or from increase of testosterone steroid precursors [25]. It is also presumed that steroidogenic components in the plant extract may improve the function of gonadotropin [26].

With respect to structure and performance, a male reproductive organ is dependant to testosterone and other androgens, two male sex hormones which are involved in growth and secretory action of reproductive glands [27-29]. Androgens are essential for development, growth and normal activity of male testes and sex glands, and previous studies have shown that there is a strong correlation between testosterone levels in blood and weight of testes, epididymis, seminal vesicle and prostate [30]. The mechanism of the testosterone activity in increasing the weight of sex organs is through stimulation of protein expression in target cells. After a short time which testosterone is converted to dihydro testosterone (DHT) by α-5 reductase enzyme the DHT binds to cytoplasmic protein receptors and enters the nucleus to stimulate the transcription of DNA to RNA which through translation of RNA to proteins, eventually lead to increase of the weight of body and reproductive organs [31, 32]. Administration of this plant orally to male rats has been reported to increase of serum levels of testosterone compared to the control group [18]. Since androgenic effects are associated with the blood testosterone levels, probably *A. pyrethrum* extract has a role in testosterone secretion from gonads [33]. These effects may be correlated to neurotransmitters level or their activity in the cell [34, 35].

Spermatogenesis is a complex process among structural epithelial elements of testes and hormonal system [36]. FSH is a stimulator of spermatogenesis and LH accelerates the release of testosterone. Testosterone augments the blood flow, improves the growth of target tissues, and directly motivates spermatogenesis [37]. Accordingly, our results possibly are associated with chemical constitutes of *A. pyrethrum*. The root of this plant has been analyzed and it was determined that the root contains a brown spicy resin substance named Pyrethrin. Additionally, the root of this plant involves vaporizing oil, resin and various Tannic acids [14]. Botanists have reported that this plant contains N-isobutyldienediynamide and warm water soluble polysaccharides [38, 39]. Phytochemical studies on this plant have demonstrated the presence of alkylamide and polymeric polysaccharides both of which has potent androgenic effects and also stimulates secretion of the testosterone [40]. Additionally, Sharma et al. in their previous studies have shown that isolated alkylamides from extract of *A. pyrethrum* root have positive effects on reproduction variables such as weight of body and sex organs as well as the sperm count in epididymis [39].

Similarly, Cicero et al. reported that alkyl amide from the *A. pyrethrum* has positive effects on reproduction [38]. Tail domain of spermatozoa is prone to oxidation due to the presence of unsaturated fatty acids in the plasma membrane. Thakur et al. showed that date seed oil

with antioxidant properties suppresses lipids peroxidation, hence reduces the number of abnormal permatozoids [22]. In the present study, antioxidant effects of *A. pyrethrum* plant have not been examined, but Kalim et al. in their study have evaluated the antioxidant effects of the *A. pyrethrum* plant [42]. Therefore, it is probable that *A. pyrethrum* plant contributes to the reduction of abnormal spermatozoids through a similar mechanism.

Therefore, with respect to the noted evidences, the significant increase of some examined variables could be explained through the fact that increasing the weights of reproductive organs is associated with androgenic and anabolic factors. Androgens can stimulate the growth of reproductive organs and then increase their weight [9]. Thus it is probable that natural components have androgenic properties [11]. It is presumed that the existence of Plitorin, an alkyl amide in the *A. pyrethrum* extract, might be related to the observed effects. The main reason for this hypothesis is fortification of sex characteristics by alkyl amides that are extracted from Lepidium meyenii root [40]. It seems that the alkyl amid has a testosterone-like activity or stimulates the testosterone secretion [41]. In conclusion, this study indicated that intraperitoneal injection of aqueous extract of *A. pyrethrum* root in male adult rats increased the weight of body, epididymides and vas deferens and augment of sperm count in these organs; however it reduces the number of abnormal spermatozoids, and in this way improves the quality of spermatozoids. Therefore, the positive effects of *A. pyrethrum* plant on different reproductive parameters might improve the production of forthcoming drugs and dietetic components for prognosis or treatment of infertility

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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