

Effects of Aqueous Root Extracts of *Anacyclus pyrethrum* on Gonadotropins and Testosterone Serum in Adult Male Rats

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ABSTRACT

In this study, we investigated effects of *Anacyclus pyrethrum* L. aqueous root extracts on gonadotropins (LH, FSH) and testosterone serum in adult male rats. A total number of 60 male adult rats were divided randomly into five groups (n=12) including one control group (A), sham control (B) and three test groups (C, D and E). Test groups (C, D and E) received 50, 100, 150 mg/kg does of *A. pyrethrum* aqueous root extracts intraperitoneally for a period of 28 days. At the end of the treatment period, the reproduction variables such as gonadotropins (LH, FSH) and serum testosterone levels were determined. The results depicted that gonadotropins and serum testosterone levels, showed a significant difference between the treatments and control groups ($P < 0.05$). The results of this study showed that aqueous root extract of *A. pyrethrum* increased gonadotropins (LH, FSH), as well as serum testosterone levels. Experimental reports was showed that these effects may be caused due to the presence of chemical compounds in the plant. *A. pyrethrum* root decomposition was repeatedly shown that the extract was a resin compound called pyrethrin or pellitorin. It was reported that the alkyl amide of *A. pyrethrum*, pellitorin have positive effects on reproduction.

Keywords: Pellitorin, Spermatogenesis, Serum testosterone, Gonadotropins.

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INTRODUCTION

Medicinal plants play a significant role in provides food for humans¹⁻³. Notwithstanding plants are extremely exploited in traditional healing systems, only in some cases their therapeutic potential in human has been substantiated^{4,5}.

Infertility is a disease that has affected over 80 million people worldwide. Unfortunately, the largest global share willing to treat infertility has nil access to medical treatment. Even in developed and economical countries, there are differences to access the medical diagnosis and treatment of infertility. It is one of the main problems of modern life, and almost 30% of infertility relates to males⁶. Non-reproductive causes are diverse and one of the prime causes of this disorder is combination of sperm and ovocit, which causes the non-formation of a zygote and gestational. In the present circumstances, only 40% of infertility cases are diagnosed, and 60% of cases are diagnosed pathological. Many research studies shown that a variety of factors, including toxins, pesticides, radiation, influence infertility. Smoking can also be associated with male infertility⁷.

Use of medicinal plants has been considered by human beings and their integration in new drug formation has become an individual necessity. World Health Organization (WHO) in its meeting has emphasized the use of medicinal plants for developed countries essentially due to its efficacy, safety and standards⁸. Experimental studies have shown that substances found in herbs, viz. flavonoids have androgenic effects⁹. Androgens play an important role in fertility and they are an optimal option to treat sexual dysfunction in both males and females¹⁰.

A.pyrethum root is rather the size of the little finger, with sometimes leaf - remains at the top, and beset with few or no

hair-like rootlets; externally brownish, deeply fissured longitudinally. It breaks with a few force, showing a rather thick bark adhering closely to the pale brown wood, from which it is separated by a narrow cambium line. This woody column is transverse by broad, distinct medullary rays, and contains as does also the bark, large scattered resin ducts. The stems are numerous, procumbent, somewhat branched, and pubescent. The radical leaves are spreading, petiolated, smoothish, and pinnately divided; the segments much cleft into linear, subulate lobes; and the cauline leaves sessile, Branches 1-headed. The root is harvested in the autumn and dried for stored for later use¹¹.

Anacyclus pyrethrum root commonly known as pellitory and Akarkara in Hindi local language is perfectly recognized in traditional and herbal medicine and has a positive effect on regulating the immune system¹². This plant is used as a medicine to stimulate the salivary glands; and treat chronic catarrh. Moreover, people use it as a remedy to improve irritated skin. Surprisingly, chewing leaves of this plant are helpful for toothache, muscle breakdown and Trachea apnea. *A.pyrethrum* root have been analysed frequently and its activity proved the presence an acrid matter, known as pyrethrin. However, this so-called pyrethrin, is in fact, a mixed substance; consisting of a brown acrid resin. The root also contains a little volatile oil, gum and traces of tannic acid. Phytoconstituents mainly reported from the plant are N-isoButyldienedynamide and polysaccharides. Also the roots of this plant contain anacyclin that it used traditionally as a tonic to the nervous system. In addition, the antibacterial and anti-inflammatory activities of the *A.pyrethrum* root was reported¹¹. Though Pellitory is a native plant in Asia and Africa, but it is primarily found

in northern India¹³. The root of *Anacyclus pyrethrum* (AP) due to its rejuvenating qualities is very popular in Hindi traditional medicine¹⁴. This plant has a positive effect on men's sexual and reproductive capacity. Hypothalamus-pituitary-testicle axis is a central regulator of testicular function. Drugs that alter the correct operation of hypothalamus-pituitary-testicle axis are influenced both directly and indirectly through the release of gonadotropin hormones *via* other regulators¹⁵. Thus, in this investigation, we evaluated the intraperitoneal administration effect of *Anacyclus pyrethrum* L. aqueous root extracts on serum testosterone levels and gonadotropins.

MATERIAL AND METHOD

Plant preparation

The *Anacyclus pyrethrum* L. roots (Fig. 1) were purchased from a local market in Zahedan, Sistan and Baluchestan, Iran, and approved at the Herbarium Center of Biology Department of University of Sistan and Baluchestan.

Plant aqueous extraction

For the preparation of aqueous extract of *A. pyrethrum*, the plant roots were dried, powdered and subjected to soxhelt apparatus for extraction with distilled water for 24 h. The extract obtained was filtered through a 30×100 mm filter paper and dried at temperature of 25°C to get the powder form. During the experiment, the root dried powder was dissolved in the physiologic serum in determined doses and intraperitoneally injected to the experimental groups¹⁶.

Experimental assays

The present study was performed on 60 male albino rats (Wistar strain), aging 5-7 month, weighing 225±50 g, and kept at the Animal laboratory of Zahedan University of Medical Sciences. In this experimental study,

the animals were housed at room temperature (25°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. They were randomly divided into five groups each with 12 animals (N=12). This included control group (A), sham control (B) and three test groups (C, D and E). The test groups (C, D and E) received 50, 100 and 150 mg/kg of *A. pyrethrum* L. aqueous root extracts intraperitoneally for a period of 28 days¹⁷.

At the end of the treatment period, the animals were anaesthetized with ether (Merck, Germany), and killed by cervical dislocation¹⁶. The blood of rats was centrifuged due to obtain serum. The assay kits used for the analysis for LH and FSH were supplied by Life science Inc.wuhan430056, PR China Houston, TX77082, USA, whereas the ELISA kit (using a double antibody) for testosterone was supplied by Eiagen Testosterone kit manufactured by Adaltis Italia, Italy¹⁷.

Statistical analysis

Results are expressed as Mean±SE. The test groups were compared to the controls using analysis of variance following Tukey test. All the statistical analyses were carried out using the SPSS version of 19 software. A *p*-value below 0.05 was considered statistically significant.

RESULT

The reproductive activities of *A. pyrethrum* root extracts were assayed by intraperitoneally injecting four groups of rats. Tukey test data analysis revealed a significant difference of gonadotropins and testosterone serum levels between *A. pyrethrum* treated rats and the control groups. In all treated groups, a dose-dependent increase in serum testosterone, FSH, and LH concentrations was observed ($P < 0.0001$). Mean values of each

control and test group is shown in Table 1. The maximum concentration for serum testosterone was measured in the E treated group (150 mg/kg), (4.15 ± 0.49 ng/mL (Mean \pm SE). Similarly, as compared with the control group, serum levels of FSH and LH were found significantly higher (25.55 ± 0.53 ng/mL and 4.32 ± 0.13 pg/mL, respectively) in E treated group (150 mg/kg).

DISCUSSION

The results of this study showed that the aqueous root extracts of *Anacyclus pyrethrum* significantly increased serum gonadotropin hormone (LH, FSH) and testosterone serum in treated groups as compared to control groups. Several researchers have conducted studies on the administration of the oral alcoholic extract of *A. pyrethrum*, and reported that this extract increases gonadotropins and testosterone serum in the treated groups in relation to the control groups¹⁶. Physiologically, LH and FSH hormones are secreted in the anterior pituitary *via* gonadotropin releasing hormone secreted by the hypothalamus (GnRH), and circulate throughout the blood in the body to arrive and connect the specific receptors in Leydig cells and Sertoli in testicular tissue through increased production of secondary messenger (cAMP) stimulating cell activity. It was reported that gonadotropins (LH, FSH) and testosterone act on testis leading it to secrete testosterone. LH stimulates the Leydig cells to secrete testosterone, whereas FSH levels largely induce sertoli cells to regulate spermatogenesis. FSH has a stimulatory effect on testicular tissues and is essential for normal reproduction. Thus, increased serum levels of LH may lead to increase in testosterone secretion of Leydig cells. Besides, increased serum levels of LH and FSH is suggested to be due to the plant extract directly inducing hypothalamic - pituitary - testicular axis, which in turn influences the gonads¹⁷. In addition, Pellitory root

decomposition has repeatedly shown that the extract has a resin compound called pyrethrin with brown spicy taste, and the root of the plant contains volatile oil, gum and tannic acid¹². Additionally botanists have reported that the plant has N-isobutyldienedynamide and hot water soluble polysaccharides^{19,20}. Another phytochemical study has shown that this plant has alkyl amide and polymeric polysaccharides exhibiting strong androgenic effects. It was reported that the alkyl amide of *A. pyrethrum*, Pellitorin has positive effects on reproduction^{18,21}. Similarly, Zheng *et al.*²² reported that the extract of *Lepidium meyenii* plant containing alkyl amide not only acts like testosterone, but also increases the body weight and genital organs.

CONCLUSION

Based on our observation, we therefore conclude that, the aqueous extract root of *A. pyrethrum* has compounds that could directly or indirectly promote testosterone to increase reproductive activity.

REFERENCES

1. Sharifi Rad J, Hoseini Alfatemi SM, Sharifi Rad M, Sen DJ. Phytochemical and Antimicrobial Evaluation of the Essential Oils and Antioxidant Activity of Aqueous Extracts from Flower and Stem of *Sinapis arvensis* L. *Am J Advan Drug Deliv.* 2013; 1 (1), 001-010.
2. Abdolshahi A, Majd MH, Sharifi Rad J, Taheri M, Shabani A, JAT da Silva. Choice of solvent extraction technique affects fatty acid composition of pistachio (*Pistacia vera* L.) oil. *J Food Sci Technol.* 2013; 1-6.
3. Hoseini Alfatemi SM, Sharifi Rad J, Sharifi Rad M, Mohsenzadeh S, JAT da Silva. Chemical composition, antioxidant activity and *In vitro* antibacterial activity of *Achillea wilhelmsii* C. Koch essential oil on methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* spp. *3 Biotech.* 2014; 1-6.

4. Sharifi Rad J, Hoseini Alfatemi SM, Sharifi Rad M, Iriti M. Free Radical Scavenging and Antioxidant Activities of Different Parts of *Nitraria schoberi* L. *TBAP*, 2014;4 (1): 44 – 51.
5. Sharifi Rad J, Hoseini Alfatemi SM, Sharifi Rad M. *In vitro* assessment of antibacterial activity of *Salicornia herbacea* L. seed extracts against multidrug resistant gram-positive and gram-negative bacteria. *Int J Biosci.* 2014;4 (6): 217-222.
6. K Purvis, E Christiansen. Infection in the male reproductive tract Impact, diagnosis and treatment in relation to male infertility. *Int J Androl.* 1993; 16: 1–13.
7. Yang WM, Kim HY, Park SY, Kim HM, Chang MS, Park SK. *Cynomorium songaricum* induces spermatogenesis with glial cell-derived neurotrophic factor (GDNF) enhancement in rat testes. *J Ethnopharmacol.* 2010; 128: 693-696.
8. World Health Organization Resolution: drug Policies and management: Medicinal plant. Geneva, Switzerland: World Health Organisation.1978; 31-33.
9. Sharma V, Thakur M, Chauhan NS, Dixit VK. Evaluation of the anabolic, aphrodisiac and reproductive activity of *Anacyclus pyrethrum* DC in male rats. *Sci Pharm.* 2009; 77: 97-110.
10. EU Etuk, AA Muhammad. Fertility enhancing effects of aqueous stem bark extract of *Lophira lanceolata* in male Spargue dawley rats. *Int J Plant Physiol Biochem.* 2009; 1 (1): 1-4.
11. Annalakshmi R , Uma R , Subash chandran G , Muneeswaran A. A treasure of medical herb – *Anacyclus pyrethrum* A review. *Indian J. Drugs Dis* 2012; 1 (3): 59-67.
12. Sharma V, Thakur M, Chauhan NS, Dixit VK. Immunomodulatory activity of petroleum ether extract of *Anacyclus pyrethrum*. *Pharm Biol.* 2010; 48: 1247-1254.
13. Bentley R, Trimen H. Medicinal Plants. 2004. Third volum, Asiatic Publishing House. Delhi.
14. The Ayurvedic pharmacopoeia of India. Government of India, Ministry of health and family welfare, Department of Indian system of medicine and Homeopathy, Part1, Vol 11, 1st edition, New Delhi;2004.
15. Sharma V, M Thakur, NS Chauhan, Dixit VK. Effect of petroleum ether extract of *Anacyclus pyrethrum* DC on sexual behaviour in male rats. *Chin J Integr Med.* 2010; 8:767–773.
16. Parandian R, Yousofvand N, Gorbani R. The enhancing effects of alcoholic extract of *Nigella sativa* seed on fertility potential, plasma gonadotropins and testosterone in male rats. *Iran J Reprod Med.* 2012; 10(4): 355-62.
17. Sharma V, Boonen J, Spiegeleer BD, Dixit VK. Androgenic and spermatogenic activity of alkylamide-rich ethanol solution extract of *Anacyclus pyrethrum* DC. *Phytother Res.* 2013; 27(1): 99-106.
18. Sharma V, Boonen J, Chauhan NS, Thakur M, De Spiegeleer B, Dixit VK. *Spilanthes acmella* ethanolic flower extract: LC–MS alkylamide profiling and its effects on sexual behavior in male rats. *Phytomedicine.* 2011; 18: 1161-1169.
19. Crombie L. Isolation and structure of an N-iso Butyldienedynamide from Pellitory (*Anacyclus pyrethrum* DC). *Nature.* 1954; 174:832-833.
20. Bendjeddou D, Lalaoui K, Satta D. Immunostimulating activity of the water-soluble polysaccharide extracts of *Anacyclus pyrethrum*, *Alpinia galangal* and *Citrullus colocynthis*. *J Ethnopharmacol.* 2003; 88: 155-160.
21. Cicero AF, Bandieri E, Arletti R. *Lepidium meyenii* walp. Improves sexual behavior in male rats independently from its action spontaneous locomotor activity. *J Ethnopharmacol.* 2001; 4: 225-229.
22. Zhenga BL, Hea K, Kima CH, Rogersa L, Shaoa Y, Huangb ZY, Lub Y, Yanc SJ, Qiend LC, Zhenga QY. Effect of a lipidic extract from *Lepidium meyenii* on sexual behavior in mice and rats. *Urology.* 2000; 55(4):598-602.

Table 1. Effects of *Anacyclus pyrethrum* root extract on gonadotropins and testosterone serum levels (n=12)

TH (ng/mL)	FSH (ng/mL)	LH (pg/mL)	Parameters Groups
1.93±0.22	18.38±0.22	1.23±0.24	A
1.85±0.17	1.85±0.17	1.44±0.51	B
0.9	0.9	0.8	P-value
2.41±0.26	21.20±0.93	2.76±0.2	C
0.008*	< 0.0001*	< 0.0001*	P-value
3.33±0.43	23.71±0.47	3.66±0.17	D
<0.0001	< 0.0001*	< 0.0001*	P-value
4.15±0.49	25.55±0.53	4.32±0.13	E
<0.0001*	< 0.0001*	< 0.0001*	p-value

*Comparisons with the Control groups.

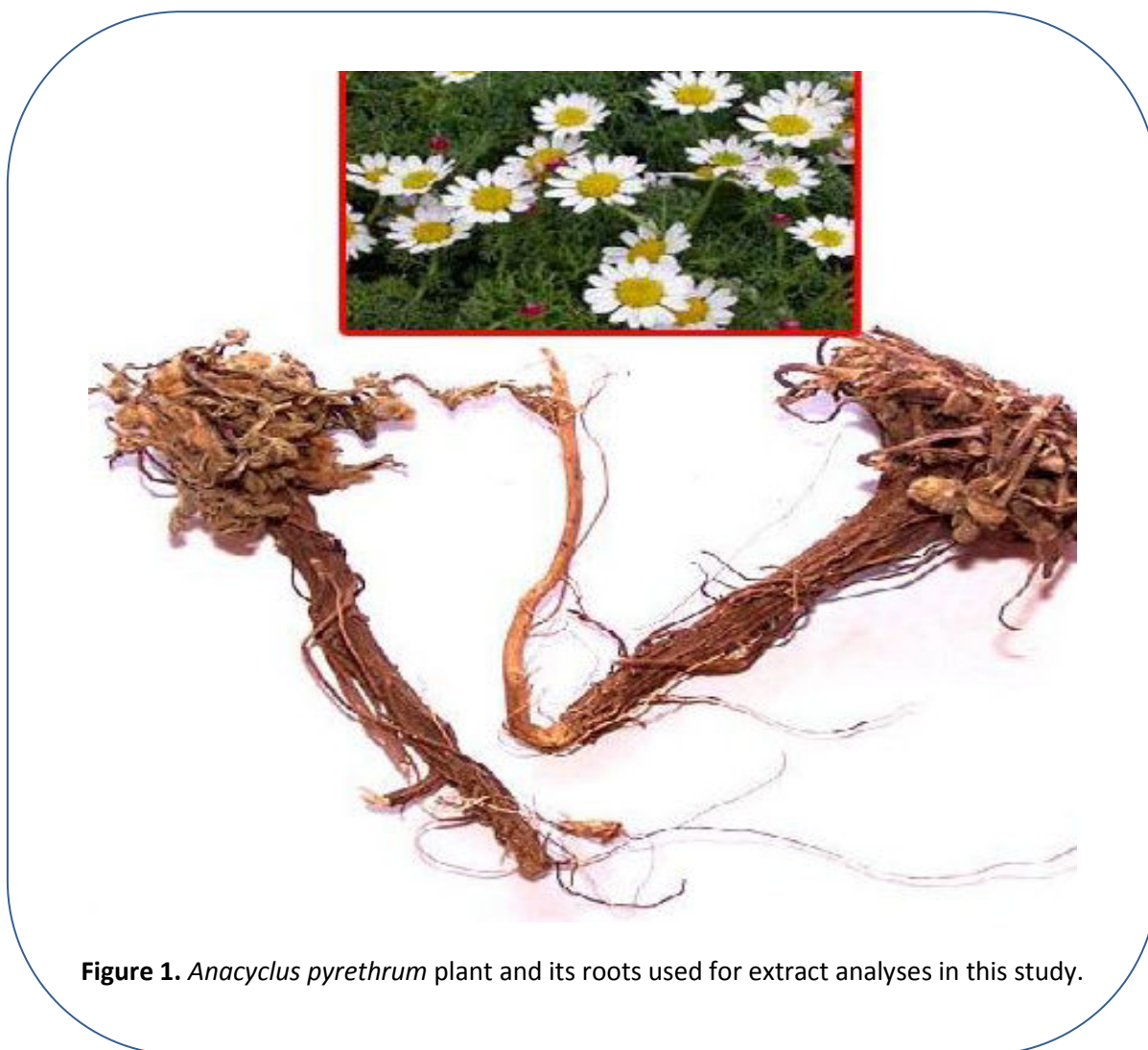


Figure 1. *Anacyclus pyrethrum* plant and its roots used for extract analyses in this study.