**EFFECT OF AQUEOUS AND ALCOHOLIC EXTRACT OF *Andrographis* *panicualata*  ON FERTILITY OF MALE ALBINO RAT (*Rattus norvegicus* ).**

**Richa Sharma\*, Varsha Anand\*, Atul Samiran\*, Ashok Kr Thakur\*\* & Mirza Md Ali\*\*\***

Research Scholar, University Department of Zoology, T.M.B.U., Bhagalpur\*

University Professor, University Department of Zoology, T.M.B.U., Bhagalpur\*\*

Asso. Professor, Dept of Botany, C.M. College , Bounsi, T.M.B.U., Bhagalpur\*\*\*

**Abstract:** Rising global human population, particularly in developing and underdeveloped countries, has a negative impact on the earth's life-sustaining systems. Plants have traditionally been used to heal a variety of diseases. It has been revealed that phytochemicals are becoming increasingly important in males. The effect of oral administration of *Andrographis paniculata* leaf extract (100 mg/kg body weight) on the male reproductive organs of male albino rats was examined in the current study. Numerous reproductive endpoints were evaluated, including sperm count, fructose content, total protein, and organ weight. Rats receiving treatment had degenerative alterations in their seminiferous tubules, as seen by testicular histology. The decreased sperm count, the weight of the reproductive organs, fructose in the coagulating gland (CG), protein content in the seminal vesicle (SV), as well as alterations in the spermatogenic components of the testis, show that test plant have antifertility properties.

**Keywords:** *Andrographis paniculata* , Biochemical parameters, Contraception , Reproductive organs, Sperm count.

**INTRODUCTION**

Population control is a global and public health issue. Many research on fertility have been conducted. One of the current societal concerns in global health is the stability of population increase (Choudhary et al; 1990). Many strategies have been created for women, although men have received insufficient attention in this arena. Men have not shared equally with women the duty for fertility regulation globally, and the absence of male involvement may partly reflect men's limited options (Gupta and Sharma, 2006).

The search for male antifertility factors in plants remains a potential area of investigation. However antispermatogenic activity has been reported in some plants (Pakrashi and Pakrashi, 1977). Only few plants are reported to possess male antifertility activity (Despande *et.al*; 1980 and Choudhary *et.al*; 1990).

Antifertility medicines, also known as oral contraceptives, are drugs that restrict fertility. (2014) (Daniyal and Akram). These medicines have an influence on the menstrual cycle and ovulation in females. In males, it impairs spermatogenesis, suppresses testosterone, or alters organ gonadotrophin or sperm mortality. Surprisingly, the contraceptive options available to men now do not change significantly from those available in the first century. Until recently, condoms and vasectomy were the primary methods of male contraception. (Ringheim,1993).

While researching alternative viable male contraceptive options, numerous hormonal approaches were discovered. Exploring the drugs having antifertility activity is the need of current time, and many plant extracts have been investigated for their antifertility effects (Chauhan and Prabha, 2019).

Medicinal herbs are a wonderful gift from nature that can be used to treat a wide range of human issues. It goes without stating that modern scientific research has demonstrated the medical potential of medicinal plants. (Gedia *et.al*;2011). Herbal remedies and their derivatives have been used in traditional medicine since the dawn of recorded history. However, it is only recently that the wider usage of medicinal plants has begun to gain recognition in the more expensive international sector. With numerous gaps in these approaches, it remains a challenge to develop chemicals that are universally acceptable, safe, cost-effective, and reversible (Daniyal and Akram, 2014).

The current study was undertaken to evaluate the effects of alcoholic and aqueous leaf extract of Andrographis *paniculata*  on the reproductive organs of male albino rat.

**MATERIALS AND METHODS**

**Plant material**

One of the most well-known medicinal plants, *Andrographis paniculata* (family *Acanthaceae*), has been used for centuries in Asia, America, and Africa to treat a variety of illnesses, including cancer, diabetes, high blood pressure, ulcers, leprosy, bronchitis, skin diseases, flatulence, colic, influenza, dysentery, dyspepsia, and malaria. It contains a number of photochemical components with distinct and intriguing biological characteristics. In order to fill the knowledge vacuum that will necessitate future research possibilities, this review discusses the past and present status of research on *Andrographis paniculata* with respect to medicinal usage, phytochemistry, pharmacological activities, toxicity profile, and therapeutic usage. This evaluation is based on a review of the literature, which included books and scientific journals obtained in libraries and online. The plant's extract and pure compounds have been shown to have anti-microbial, cytotoxic, anti-protozoan, anti-inflammatory, anti-oxidant, immunostimulant, anti-diabetic, anti-infective, anti-angiogenic, hepato-renal protective, sex hormone/sexual function modulation, liver enzymes modulation, insecticidal, and toxicity properties. Numerous tests to determine how poisonous extracts and metabolites from this plant were on experimental animals failed to detect any appreciable acute toxicity. Future research must include a detailed and all-encompassing toxicity profile on mammalian tissues and organs.

**Plant extract preparation**

**Aqueous extract**

Fresh mature plant parts of *Andrographis paniculata* was taken. These parts will be washed and air dried. For extract preparation, 10g of dried plant parts will be dissolved in100ml of distilled water and left for 12hrs ,filtered and diluted with distilled water for required dose. After 12 hrs the solution will be filtered. The filtrate is now ready for dilution and required dose preparation(0.1ml/rat/day).(Shubhangi *et.al*,2018 and Choudhary *et.al*;1990).

**Alcoholic extract**

The plant extract was prepared by the method adopted by ( agokei *et.al*,2010). Fresh mature leaves of *Andrographis paniculata* were taken for investigation. The parts of the test plant were washed and dried at room temperature. The dried plant parts were powdered by using a mixer grinder. 20gm of plant powder of each test plant was poured into a conical flask containing 150ml of 50% of ethanol. The mixture was stirred, allowed to settle, and kept covered. At the end of the second day the extract was filtered with no.1 Whatman filter paper. The filtrate was taken on a petri dish and evaporated at room temperature. The residue that remained in the petri dish was ready for the experiment.

**Experimental animal**

Male Sprague Dawley rats (180-200) gm of body weight of proven fertility were selected for the experiment. Three separate groups (one for control and two for experimental) of male rats were selected. Each group containing 6 animals. The experimental group of rats were administered orally with suspension of test plant extracts(both aqueous and alcoholic) at a dose of 100mg /kg body weight for 21 days. The control group was fed with distilled water for the same period of treatment (choudhary *et.al*,1990). A separate group of rats taken as control received distilled water only for the same period of treatment.

**Fertility performance test**

The fertility performance of individual rats was done from day 16 to 21st of treatment. Each male rat was caged separately with 2 coveal females of proven fertility. The presence of vaginal smear indicated that the females had mated to the particular male and the day of mating was considered to be the day 1st of pregnancy. Laparotomy was done on 8th day of pregnancy to examine the record of Corpora lutea and Implantation sites. Litters were examined and litter size was recorded at term(Choudhary et.al,1990 & Choudhary &sharma,2023).

**Biochemical parameters**

Male rats were sacrificed on the 22nd day and different tissues were collected and weighed on Torsion balance. Fructose estimation in the coagulating gland(CG) was evaluated by colourimetry ( Mann, 1981; Choudhary *et.al*,1990). Acid phosphatase activity in ventral prostate (VP) was evaluated by the method adopted by Sigma Technical Bulletin no. 104. Protein estimation in the seminal fluid was estimated by colourimetry ( Lowry *et.al*,1951). Spermatozoa collected from Caput, Corpus and Cauda epididymis and vas were examined under the compound microscope and their number and morphology were recorded. (Singh *et.al*, 1969 ; Choudhary *et.al*, 1990; Subhangi *et.al*,2018). The data were analysed statistically using student t-tests.

**Results**

Table 1: Showing the effects of ethanolic and aqueous leaf extract of *Andrgraphis paniculata* on Body weight, Reproductive organ weight, Sperm count and Biochemical constituents.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No. of Animals  (6) | Change in body weight | Weight of Organs | | | | | Fructose in C.G(mg/100mg of tissue) | Protein in S.V (mg/100mg of tissue) | Acid phosphatase  in V.P (mg/hr/100mg of tissue) | Sperm count(millions/ml) |
| Testis | VP | SV | CG | Epid |
| Control(6) | 8.3±2.26 | 0.18±0.12 | 0.44±0.04 | 0.46±0.04 | 0.16±0.01 | 0.11±0.05 | 0.51±0.02 | 31.83±0.91 | 42.23±0.45 | 192X104±4.21 |
| *Andrographis paniculata (6)*  *Aq.* | 6.52±3.47 | 0.05±0.01 | 0.07±0.02 | 0.38±0.06 | 0.12±0.01 | 0.05±0.03 | 0.16±0.04 | 14±2.92 | 16.56±2.27 | 79.6X104±1.18 |
| *Andrographis paniculata (6)*  *Alc*. | 4.6±2.48 | 0.04±0.006 | 0.1±0.006 | 0.37±0.01 | 0.08±0.007 | 0.04±0.013 | 0.14±0.001 | 12.4±1.12 | 15.58±1.37 | 70.8X104±2.05 |

VP- Ventral Prostate , SV- Seminal Vesicle, CG- Coagulating Gland Epid.- Epididymis.

Effect of plant extracts on fertility of male rats. (The number of breeder males used is 6)

Table 2: Showing the effects of ethanolic and aqueous leaf extract of *Andrographis paniculata* on Fertility performance of male rats.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| No. of animals(6) | Number of breeder males | Number of successful male | Number of mated females | CL Sites | Implantation sites | Litters |
| Control(6) | 6 | 6 | 7 | 4.3±0.28 | 2.42±0.29 | 4.17±0.48 |
| *Andrographis paniculata (6) Aq.* | 6 | 4 | 4 | 3.12±0.55 | 2.0±0.42 | 4±0.82 |
| *Andrographis paniculata (6) Alc.* | 6 | 5 | 5 | 2.4±0.452 | 1.12±0.359 | 2.4±0.74 |

CL Sites : Corpus leutum sites

Graph 1:Represent Change in body weight and reproductive organ weight in male albino rats and plant extract fed.

Graph 2: Represent Changes in Sperm count and biochemical parameters ( Fructose, Protein content and acid phosphatase activity) in reproductive organ tissue in male albino rats and plant extract fed.

Graph 3: Represent fertility performance of male albino rats and plant extract fed.

**Results and discussion:**

The testis, which serves as the major reproductive organ in the male reproductive system, is joined by several accessory structures and is primarily responsible for producing sperm. The amount and quality of spermatozoa, which are influenced by a variety of reproductive variables, will be affected by substances that modify testicular function. In this study, the effects of *Andrographis paniculata* leaf extract on the reproductive characteristics of male albino rats were examined.

**Body weight**:

Significant reduction(P<0.001) in body and reproductive organ weight was observed in male rat after oral administration of both the aqueous and alcoholic leaf extract of *Andrographis paniculata* compared with the control group( Table 1 &Graph 1). The decreased body weight may be due to suppression or less secretion of growth hormone(GH) from pituitary gland (Choudhary and Sharma 2023).

**Reproductive Organ weight :**

Result showed that the weight of various reproductive organs such as Testis, Seminal vesicle(SV), Coagulating gland(CG), Epididymis, Ventral prostate(VP) of male albino rats treated with both alcoholic and aqueous extract of leaf of *Andrographis paniculata* were found significantly decreased(P<0.001) with compared to control group( Table 1 and graph 1). Significantly reduced gonadotrophic activity or decreased androgen levels that interfere with the development and maturation of spermatozoa may be the cause of the significant weight loss of several reproductive organs in treated male rats. Because circulating androgen is necessary for the structural and functional integrity of reproductive tissues, even a slight variation in testosterone level may cause the weight of the reproductive organs to decrease(Hammami et.al,2008 ; Das et.al,2017 & Choudhary &Sharma 2023).

**Biochemical parameters :**

There was a significant reduction(P<0.001) in fructose content in coagulating gland (CG), protein content in seminal vesicle (SV) and acid phosphatase activity in Ventral prostate (VP) observed in male rats treated with alcoholic and aqueous extract of leaf of *Andrographis paniculata* compared with control (Table 1 Graph 2). Reduced release of endogenous androgen may have contributed to the decline in acid phosphatase activity and fructose content in the ventral prostate and regulating gland, respectively. When compared to the control group, the treated rats' SV had less protein because of toxic manifestations that caused protein to break down and impeded ATP generation, which is a source of energy(Kumar et.al,2012).

**Sperm Count**:

The result showed that the sperm counts had significantly reduced (P<0.001) in treated male rats with both the aqueous and alcoholic extract but sperm count is lesser in rats treated with alcoholic extract of leaves of *Andrographis paniculata* as compared to the control one. The decreased sperm count seen in treated male rats may result from decreased testosterone synthesis and leydig cell and seminiferous tubule degradation. It is well recognised that testosterone influences the epididymal environment and is crucial for spermatogenesis. Thus, it must have had an impact on spermatogenesis. Thus, it was evident that *Andrographis paniculata* leaf extracts that were both alcoholic and aqueous have antiandrogenic properties that might prevent male rats from being fertile(Kumar et.al,2012; Mali et.al2002 & Smith et.al,2014).

**Fertility test**:

Females mated by males treated with leaf extract of *Andrographis paniculata* showed a significant reduction(P<0.001) in Corpora lutea sites and Implantation sites whereas significant reduction(P<0.005) in litter size was observed in females mated by males treated with leaf of *Andrographis paniculata* . Out of 6 males treated with aqueous extract of leaves of *Andrographis paniculata* only 4 males could mate with 4 females and only 5 male could mate with 5 females with oral administration of alcoholic extract of leaves of *Andrographis paniculata* (Table 2 and graph 3). Evidently, changes in the endocrine activity of lutea structures were the cause of the decrease in implantation sites. There have been numerous reports of antiovulatory and anti-implantational traits in plants. The extracts did, however, have abortifacient effects as evidenced by the diminution of the viable litter size. These abortifacient effects may be caused by hazardous substances entering the female genital canal with semen, decreased implantation, greater foetus resorption, alterations in the maternal estrogen/progesterone ratio, or any of these factors. Additionally, the lower sperm count and motility may both be major factors in the decreased number of implantations (Shreedhar et.al,2001; Ghosh et.al,2017 & Choudhary &Sharma 2023)

**References:**

1. Choudhary, D. N., Singh, J. N., Verma, S. K., & Singh, B. P. (1990). Antifertility effects of leaf extracts of some plants in male rats. Indian journal of experimental biology, 28(8), 714-716.
2. Choudhary, D. N., Singh, J. N., Verma, S. K., & Singh, B. P. (1990).Abortifacient and teratogenic effects of PlumeriaacutifoliaPior.on rats. Indian J. Applied&PureBiol, 5(2), 79-80.
3. Das, P., Kumar, J., Sunhangi, S., Verma, A., Singh, V.N. (2017). Antifertility Effects of Aqueous Suspension of Allium sativum on seminal profile of swiss albino mice. International Journal of Science and Research, 7(5), 1807-1809.
4. Ogbuewu, I. P., Unamba-Oparah, I. C., Odoemenam, V. U., Etuk, I. F., &Okoli, I. C. (2011). The potentiality of medicinal plants as the source of new contraceptive principles in males. North American journal of medical sciences, 3(6), 255.
5. Ghosh, A., Jana, K., Pakhira, B. P., Tripathy, A., &Ghosh, D. (2015). Anti-fertility effect of aqueous-ethanolic (1: 1) extract of the fruit of Terminalia chebula: Rising approach towards herbal contraception. Asian Pacific Journal of Reproduction, 4(3), 201-207.
6. Kumar, D., Kumar, A., &Prakash, O. (2012). Potential antifertility agents from plants: A comprehensive review. Journal of Ethnopharmacology, 140(1), 1-32.
7. Chauhan, A., &Agarwal, M. (2010). Evaluating the antifertility potential of an aqueous extract from Cassia fistula seeds in male rats. Fertility and sterility, 93(5), 1706-1710.
8. Sathiyaraj, K., Sivaraj, A., Thirumalai, T., Baskaran, N., Vinothrasu, K., Inbasekar, P., & Kumar, B. S. (2011). Antifertility activity of aqueous leaf extract of Andrographispaniculata in male albino rats. Int J Pharm Biol Arch, 2(4), 1179-82.
9. Kasture, V. S., Chopde, C. T., &Deshmukh, V. K. (2000). Anticonvulsive activity of Albizzialebbeck, Hibiscus rosasinesis and Buteamonosperma in experimental animals. Journal of Ethnopharmacology, 71(1-2), 65-75.
10. Anitha, P., & Indira, M. (2006). Impact of feeding ethanolic extract of root bark of Canangaodorata (Lam) on reproductive functions in male rats.
11. Revathi, P., Vani, B., Sarathchandiran, I., Kadalmani, B., Shyam, K. P., &Palnivel, K. (2010).Reproductive toxicity of Capparisaphylla (Roth.) in male albino rats. Int J Pharm Biomed Res, 1(3), 102-112.
12. Mishra, R. K., & Singh, S. K. (2009). Antispermatogenic and antifertility effects of fruits of Piper nigrum L. in mice.
13. Mandal, R., &Dhaliwal, P. K. (2007). Antifertility effect of Meliaazedarach Linn.(dharek) seed extract in female albino rats.
14. Joshi, S. C., Sharma, A., &Chaturvedi, M. (2011). Antifertility potential of some medicinal plants in males: An overview. Int J Pharm PharmSci, 3(5), 204-217.
15. Pare, S., Zade, V., &Dabhadkar, D. (2013). Evaluation of potential antifertility activity of plant Trianthemaportulacastrum in female albino rat. Int. JA PS. BMS, 2(1), 007-011.
16. RAHMAN, K., SULTANA, A., & RAHMANA, S. (2012). GossypiumHerbaceum: An Ethnopharmacological Review.
17. Agokei, O. E., &Adebisi, A. A. (2010). Tobacco as an anesthetic for fish handling procedures. Journal of Medicinal Plants Research, 4(14), 1396-1399.
18. Choudhary, D.N., Singh, J.N.,and Singh B.P.(1991). Effect of some medicinal plants on fertility of Albino rats.Indian Journal of Pharmacology, 23:253-57.
19. Choudhary, D.N., Sahay,G.R.,and Singh, J.N.(1994).Antifertility and Canniblistic properties of some Myotoxins in Albino rats.J.Food Sci. Technol.31(6), 497-499.
20. Singh, A., & Singh, S. K. (2008). Reversible antifertility effect of aqueous leaf extract of Allamandacathartica L. in male laboratory mice. Andrologia, 40(6), 337-345.
21. Mann T. (1964) The Biochemistry of semen and of the male reproductive tract. Willey, NY Co. GLTD London.
22. Sigma Technical Bulletin.Sigma chemical company, Dekalb St Louis 18, Mo 1963.No.104 pp 170.
23. Lowary ,O.H.,N.J.Rosenberg, A.L Fan and R.J.Randel(1951).Protein measured with the Folin-Ciocalteu reagent.J.Biol.Chem.,193:265-267.
24. Singh JN, SettyBS,Kar AB( 1969). Effect of estrogen on survival of spermatozoa in the genitalntract of castrated male rats. Indian J Exp Biol;7:174-8.
25. Subhangi, S.,Verma, A., Das, P.K., Singh, V.N. (2018). Contraceptive effect of Momordicacharantia seeds on seminal profile of mice. International Journal of Scientific Research,7(4)577-578.
26. Gupta, R. S., & Sharma, A. (2003). Antifertility effect of Tinosporacordifolia (Willd.) s tem extract in male rat s.
27. Mali, P. C., Ansari, A. S., &Chaturvedi, M. (2002). Antifertility effect of chronically administered Martyniaannua root extract on male rats. Journal of ethnopharmacology, 82(2-3), 61-67.
28. Hammami, I., Nahdi, A., Mauduit, C., Benahmed, M., Amri, M., Amar, A. B., & May, M. V. E. (2008). The inhibitory effects on adult male reproductive functions of crude garlic (Allium sativum) feeding. Asian journal of andrology, 10(4), 593-601.
29. Das, P., Kumar, J., Sunhangi, S., Verma, A., Singh, V.N. (2017). Antifertility Effects of Aqueous Suspension of Allium sativum on seminal profile of swiss albino mice. International Journal of Science and Research, 7(5), 1807-1809.
30. Sherines R.J., Howards S.S. In: Harrison JH, Gittes RF, Perimutter AD, Stamey TA, Walsh PC, Eds. Campbell’s Urology. 4th ed. Philadelphia, Pa: W.B. Saunders Co. 1978; pp. 715
31. Zeherea, M .N., Reddy, S. P., Reddy, S. P., Ravindra., and Saraswati, B. Patil (1998) Antispermatogenic and androgenic activities of MomordicaCharantia (Karela) in albino rats. J. Ethnopharmacol. 61, 9- 16.
32. Chung J.Y., Kim Y.J., Kim J.Y. et al. Benzo [a] pyrene reduces testosterone production in rat Leydig cells via a direct disturbance of testicular steroidogenic machinery. Environ. Health Persp.2001; 119: 1569-1574.
33. Singh K, Gupta R.S. Antifertility Activity Of β-Sitosterole Isolated from BarleriaPrionitis(L.) Roots in Male Albino Rats.Int. J. Pharm. Pharm. Sci. 2016; 8(5): 88-96.
34. Sharma, R., Lakhne, R., & Gupta, R. S. Antispermatogenic Activity of MomordicaDioicaMethanolic Root Extract.
35. Chinoy, N. J., Sheth, K. M., Seethalakshmi, L., Parmar, P. Y., Sanjeevan, A. G., Rao, M. V., &Trivedi, D. G. (1982). Studies on reproductive physiology of animals with special reference to fertility control. Comparative physiology and ecology.
36. Thejashwini, M. S., & Krishna Ram, H. (2012). Reversible antifertility effect of cyamposispsoralioides in male swiss albino mice. International Journal of advanced biological research, 2(4), 657-665.
37. Kamble A, Reddy C, Patil S. Testicular Activity of Mice Treated WithMeOH Extract of AchyranthesasperaLeaves. Jour. of Adv. Med. Sci. and App. Tech. 2017; 3(2); 93-100.
38. Obianime, A. W., Aprioku, J. S., &Esomonu, C. T. (2010). Antifertility effects of aqueous crude extract of Ocimumgratissimum L. leaves in male mice. Journal of Medicinal Plants Research, 4(9), 809-816.
39. Smith, L. B., & Walker, W. H. (2014, June). The regulation of spermatogenesis by androgens.In Seminars in cell & developmental biology (Vol. 30, pp. 2-13).Academic Press.
40. Chauhan, A., &Prabha, V. (2019). Evaluation of sperm impairing factor from Serratiamarcescens as male contraceptive in mouse model. BioMed Research International, 2019.
41. Raji Y, Bolarinwa A.F. Antifertility activity of Quassiaamara in male rats in vivo study. Life Sciences. 1997; 61(11):1067–74.
42. Shreedhara C.S., Pai K.S.R., Vaidya V.P. Postcoital antifertility activity of the root of Momordicadioicaroxb. Ind. Jour. of Pharma. Sci. 2001;63(6):528–531.
43. Ghosh, A., Pakhira, B. P., Tripathy, A., &Ghosh, D. (2017). Male contraceptive efficacy of poly herbal formulation, contracept-TM, composed of aqueous extracts of Terminalia chebula fruit and Musa balbisiana seed in rat. Pharmaceutical biology, 55(1), 2035-2042.
44. Pusuloori, R., Radhika, P., &Vangoori, Y. (2017). Evaluation of effect of momordicadioica extract on reproductive system of male and female rats. Biomedical and Pharmacology Journal, 10(3), 1419-1425.
45. Keel, A. B., and Abney, T (1980) Influence of bilateral cryptorchidism in the mature rat; Alteration in testicular function and serum hormonal level. Endocrinology; 107: 1226-1233.
46. Sharma,R.& Choudhary,D.N(2023). Effect of ethanolic leaf extract of Calotropis procera and Terminalia chebula on reproductive organs of male albino rats ( Rattus norvegicus ).International Journal of Pharmaceutical Research and Application,8(4),2141-2149.