

EFFECT OF AQUEOUS AND ALCOHOLIC EXTRACT OF ANDROGRAPHIS PANICUALATA ON FERTILITY OF MALE ALBINO RAT (*RATTUS NORVEGICUS*).

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.

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I. 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) (Danial 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 (Danial 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.

II. 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* were taken. These parts will be washed and air dried. For extract preparation, 10g of dried plant parts will be dissolved in 100ml 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 was 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 groups 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).

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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.

III. RESULTS

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

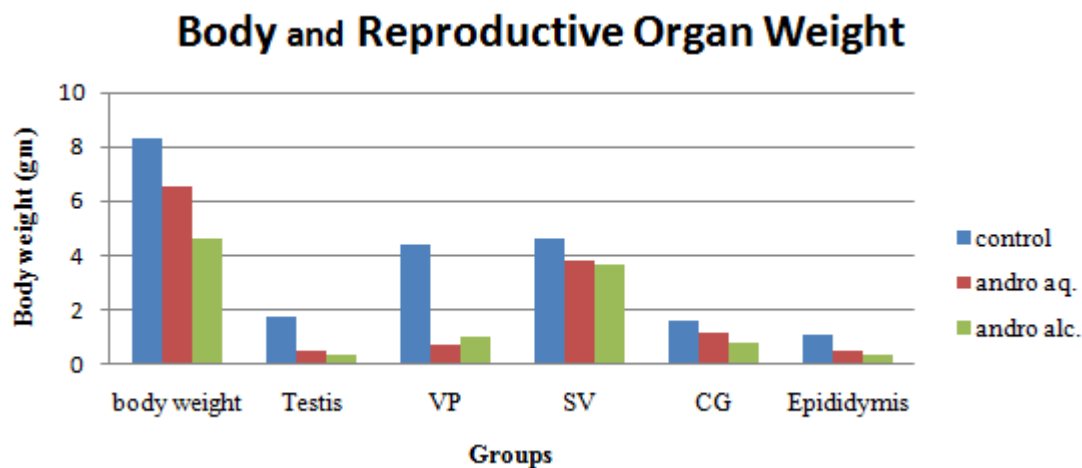
No. of Animals (6)	Change in body weight	Weight of Organs					Fructose in C.G (mg/100mg of tissue)	Protein in S.V (mg/100mg of tissue)	Acid phosphatase in V.P (mg/hr/100 mg of tissue)	Sperm count (million/ml)
		Testis	VP	SV	CG	Epid				
Control (6)	8.3±2.26	0.18±0.12	0.44±0.04	0.46±0.04	0.16±0.01	0.11±0.05	0.51±0.02	31.83±0.91	42.23±0.45	192X10 ⁴ ±4.21
<i>Andrographis paniculata</i> (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	79.6X10 ⁴ ±1.18
<i>Andrographis paniculata</i> (6) Alc.	4.6±2.48	0.04±0.06	0.1±0.06	0.37±0.01	0.08±0.07	0.04±0.13	0.14±0.001	12.4±1.12	15.58±1.37	70.8X10 ⁴ ±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.

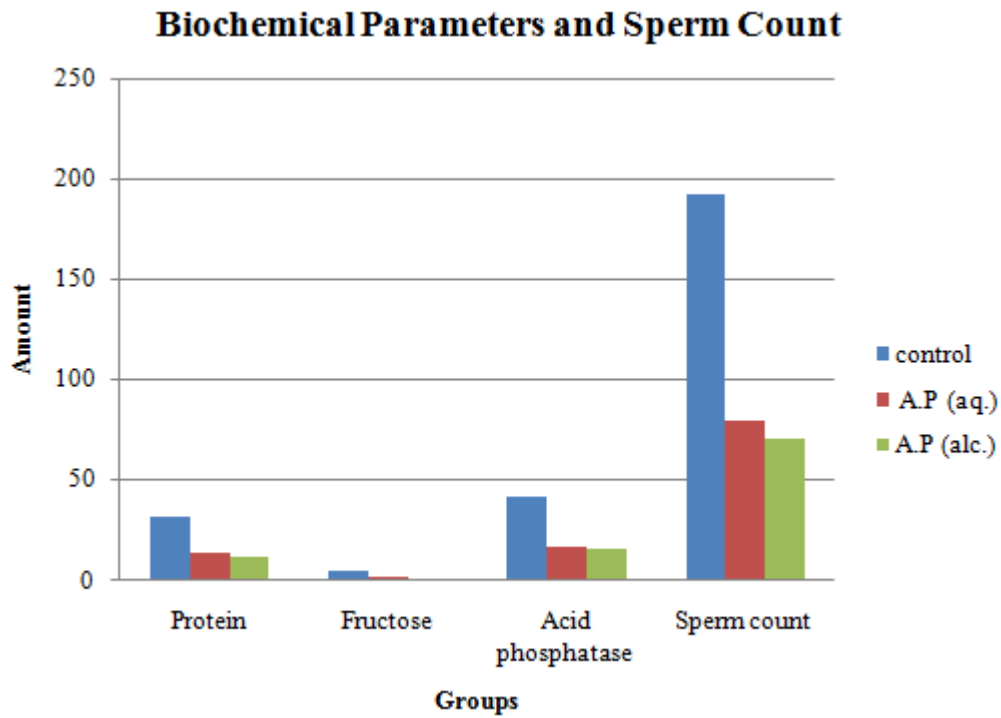
No. of animals(6)	Number of breeder males	Number of successful male	Number of mated females	CL Sites	Implantation sites	Litters
Control(6)	6	6	7	4.3±0.28	2.42±0.29	4.17±0.48
<i>Andrographis paniculata</i> (6) Aq.	6	4	4	3.12±0.55	2.0±0.42	4±0.82
<i>Andrographis paniculata</i> (6) Alc.	6	5	5	2.4±0.452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

CL Sites: Corpus Leutum sites

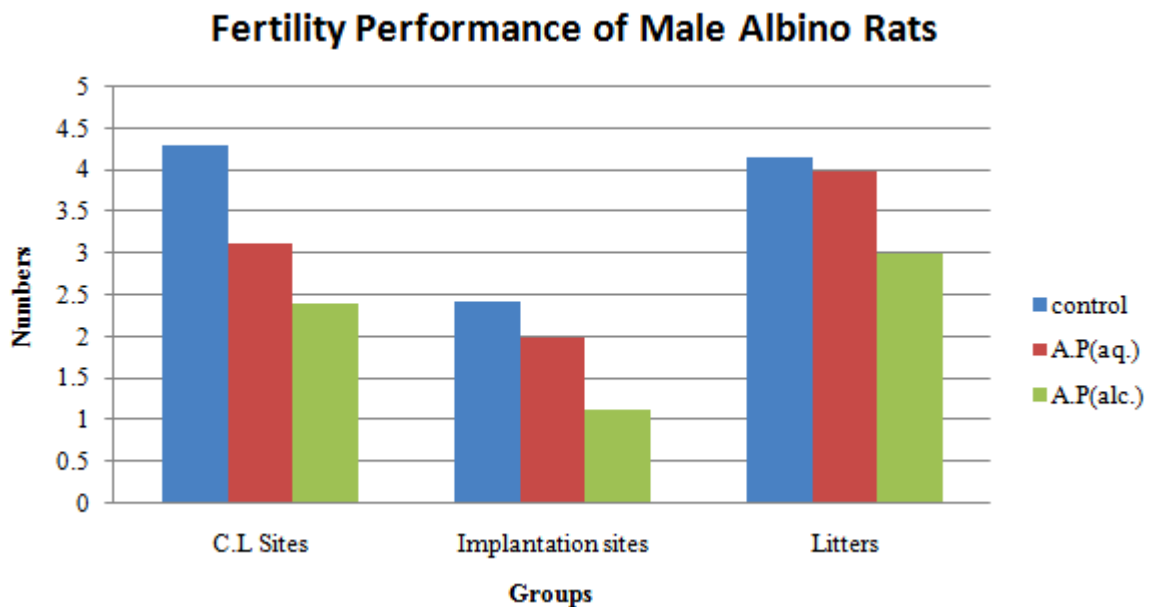


Graph 1: Represent Change in body weight and reproductive organ weight in male albino rats and plant extract fed.

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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.

IV. 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.al 2002 & Smith et.al, 2014).

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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 antioviulatory 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)

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