

ORIGINAL RESEARCH ARTICLE

Antifertility Activity of Aqueous Leaf Extract of *Andrographis paniculata* in Male Albino Rats

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ABSTRACT

The present study was to evaluate the effect of antifertility in aqueous leaf extract of *Andrographis paniculata* has been studied in Albino Rat. The study was divided into three groups of six animals for each. The first group (I) received distilled water serve as control. The second and third groups (II and III) of animals were administered the aqueous leaf extract daily on 100mg/kg body wt., and 200mg/kg body wt., respectively for a period of 45days significant decreases in the weights of testis, epididymes and seminal vesicle were observed. A dose related reduction in the testicular sperm count, epididymal sperm count and motility and abnormal sperm count were observed. The results suggest that the aqueous extract of the leaves of *Andrographis paniculata* has spermicidal activity.

Key words: Sperm, Testis, Medicinal plants, *Andrographis paniculata*, Antifertility.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources [1]. The World Health Organization suggested that effective, locally available plants be used as substitutes for drugs. Since the population explosion is a leading cause of poverty and pollution in developing countries, they created a population control programmer, which includes studies of traditional medical practices. Fertility Control is an issue of global and national public health concern. Current methods of contraceptive result in an unacceptable rate of unintended pregnancies. Approximately 50% of all pregnancies are unintended at conception; 50% of those occur in the 94% of sexually active couples who report using some method of contraception [2]. The only male-specific contraceptive methods currently available are withdrawal, condoms, and vasectomy. As concerns regarding side effects and inconvenience of these existing methods prevent their universal acceptance [3,4], the development of additional male methods of fertility control can

provide tremendous social and public health benefits.

Andrographis paniculata (Family: *Acanthaceae*) is an important medicinal plant, occurring wild in India, and is used both in Ayurveda and Unani systems of medicine [5]. The dried herb is a remedy for a number of ailments related to digestion, hepatoprotection, vermifugal, antiacne, analgesic, anti-inflammatory, antibacterial, antityphoid, antibiotic activities, hypoglycemic, besides immune enhancement [6,7,8]. This plant is well documented and several *in vitro* and *in vivo* studies describe its anti-cold, anti-urothelial and anti-hepatotoxic properties [9,10]. Some studies have also reported its antimalarial properties [11,12]. In spite of considerable development in contraceptive technology, searching for male antifertility agents continues to be a potential area of investigation. Hence the present investigation was undertaken to focus on antifertility efficacy of *Andrographis paniculata* aqueous leaf extract on male albino rat.

MATERIALS AND METHODS

Collection of Plant material

Andrographis paniculata leaves were freshly collected in January-February 2011 in and around Kathiyavadi village (Vellore Dt, Tamilnadu, India) and were cleaned with distilled water and shade dried at room temperature. The plant material was authenticated by Dr.B.Annadurai, Department of Botany, C. Abdul Hakeem College, Melvisharam, Vellore Dt, Tamil Nadu., and voucher specimens were kept at Department of Botany, C.Abdul Hakeem College, Melvisharam, Vellore District, Tamilnadu.

Preparation of plant extract

100gms of powdered plant materials were taken and mixed with 500ml of distilled water and were magnetically stirred in separate containers of overnight at room temperature. The residue was removed by filtration and the aqueous extracts were concentrated under vacuum to get solid yield of 10% extract. The plant extracts was kept in refrigerator for further experimental works.

Animals

Adult male *albino* rat weighing around 150-180gms were procured from Tamilnadu Veterinary and Animal Sciences University, Chennai. The animals were kept in polypropylene cages (four in each cage) at an ambient temperature of $25\pm 2^{\circ}\text{C}$ and 55-65% relative humidity. A 12 ± 1 hr light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions, and were fed with commercially available rat chow (Hindustan Lever Ltd., Bangalore. India) and had free access to water. General health condition and body weight of the animals were monitored throughout the experimental period. Animals were maintained according to the Guidelines of Institutional Animal Ethics Committee.

Experimental design

The daily dose of the plant extract was freshly dissolved in 0.5 mL of distilled water and orally administered to each experimental animal every morning for 45 days.

GROUP I: Control rat received 0.5 mL/day of the vehicle, that is, distilled water.

GROUP II: Rat treated with *Andrographis paniculata* extract at 100 mg/ kg /body weight

GROUP III: Rat treated with *Andrographis paniculata* extract at 200 mg/ kg / body weight.

Estimation of sperm motility and count

The spermatozoa were obtained by making small cuts in caudae epididymis and vasdeferens placed in 1ml of modified Krebs Ringer-bicarbonate buffer (pH 7.4). The sperm suspension was

evaluated for sperm count, percent motility. The percent of motility was determined by the progressive and non-progressive movements of sperms observed under a compound microscope. The sperm count was determined under a Neubauer haemocytometer^[13,14]. To evaluate the spermatozoa abnormalities, the sperm suspension was stained with eosin; smears were made on slides, air dried and made permanent.

Serum testosterone

Serum levels of testosterone were assayed in duplicate using specific RIA method^[15]. Serum samples were separated by standard procedure and stored at -20°C for subsequent analysis.

Statistical analysis

The results were expressed in mean \pm standard deviation. Statistical analysis was carried out by using one way ANOVA as in standard statistical software package of social science (SPSS) version 12.

RESULTS

In the period the treatment with *Andrographis paniculata* leaf extract, no significant clinical and behavioural changes were observed in both group II and group III animals. The treatment of rats with *Andrographis paniculata* aqueous leaf extract caused no effect on the body weight of the animals; weight gain was normal in all the experimental groups. The treatment with *A. paniculata* aqueous leaf extract treated rats caused a highly significant decrease in the accessory sex organ weights, namely testis, epididymis and seminal vesicle in all treated groups. The group III animals the sex organ weights were highly significant when compared to that of Group-II animals. A highly significant decline in serum testosterone was observed in both groups when compared to that of group-I animals (Table-2). The sperm of the control rats had normal counts, motility, and morphology (Table: 1). In *Andrographis paniculata* aqueous leaf extract treated rats the cauda epididymidal sperm parameters showed evidence of dose dependent toxicity. The sperm counts were significantly decreased in group II and group III animals when compared to normal animals (Table 1). In group III animals the sperm count were very much reduced when compared to that of group II as well as group-I. The sperm motility was very much inhibited in both group II and group III animals (Table 2). More than 50% of the sperm had abnormal morphologies of various kinds, which included globose head, coiling of tails, fusion of tails of two or more sperm etc., were observed.

The plant extract intoxication exerted a significant decrease epididymal sperm concentration and sperm progress motility. The live/dead sperm count was increased in both group II and group III

animals. The reduction of sperm count and sperm motility were significantly ($P < 0.001$) higher in Group III treated animals when compared to that of Group-II.

Table 1: Antifertility effect of *Andrographis paniculata* aqueous leaf extract on male albino rats

Treatment	x				
	Initial	Final	Testis	Epididymis	Seminal Vesicle
Group-I	182.23 ± 1.75	202±2.02	998±14.2	465±8.2	547±5.73
Group-II	185±3.74	205±5.05	871.2±4.35*	413±5.24*	446±3.51*
Group-III	192±2.6	201±3.56	623±5.4*	318±6.2*	388±4.23*

Data is expressed as Mean ±SD of 6 individual observations. * Significant ($P < 0.001$). Group II and Group III were compared to Group I (Control).

Table 2: Antifertility effect of *Andrographis paniculata* aqueous leaf extract on male albino rats: Activity levels of Serum testosterone, total sperm count, % of motile and % of abnormal counts

Treatments	Serum testosterone	Total sperm	Motile	Abnormal	
				% HD	% Tail
Group-I	4.67±0.27	7.8±2.62	94±1.93	1±0.01	1±0.01
Group-II	1.98±0.24*	4.7±4.41*	59±4.10*	16±2.02*	15±2.12*
Group-III	0.78±0.14*	2.9±4.62*	32±2.11*	30±3.32*	27±4.7*

Data is expressed as Mean ±SD of 6 individual observations. * Significant ($P < 0.001$). Group II and Group III were compared to Group I (Control).

DISCUSSION

The present study was undertaken to evaluate the effects of aqueous extract of *Andrographis paniculata* leaves on antifertility, seminology and general physiology, if any, in the male following oral administration. Presence of phytochemicals flavonoids [16] and diterpenoid lactones [17], in *Andrographis paniculata* have been reported. Sperm motility and density in the cauda epididymis was adversely affected after the treatment. The reduction in sperm motility in cauda epididymis is of importance with regard to fertilization [18]. Inadequate concentration and immotility of the spermatozoa means they cannot penetrate the cervical mucus and thus fail to fertilize the ova [19]. Reduced testicular and epididymal protein content could be correlated with the absence of spermatozoa in the lumen [20], since the luminal fluid of epididymis contains a number of proteins [21], some of which remain bound to spermatozoa. Cholesterol is involved in steroid genesis in the testes; an increased level of cholesterol is attributed to decreased androgen concentration, resulting in impaired spermatogenesis [22]. A significant decrease in glycogen content after the treatment with *Aegle marmelos* possibly could be explained by an inhibition of glycolysis during spermatogenesis [23].

In conclusion, our results in revealed that *Andrographis paniculata* leaf extract treatment and durations employed in the present study causes marked alterations in the male reproductive organs and that the alterations are reversible after cessation of treatment. Treatment also had a reversible effect on suppression of fertility in males. Further, did not show any toxic effects in treated rats.

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