



## Antifertility effects of crude different of *Adiantum lunulatum* Burm. on Reproductive Organs of male albino rats.

*D.K. Bhatia, A.K. Sharma\*, P.C. Pathania\*\* and Naveen Chandra Khanduri\*\*\**

*Deptt. of Zoology, Govt. Degree College Karanprayag, Chamoli  
\*ICFRE, Dehradun*

*\*\* Deptt. of Entomology, Punjab Agricultural University, Ludhiana*

*\*\*\*Deptt. of Zoology, Govt. PG College, Augustmuni Rudraprayag*

**Abstract :** Effects of crude extract, both alcoholic and decoction of whole plant of *Adiantum lunulatum* Burm. was observed on the reproductive structures of male albino rat after the oral administration of 100 mg/kg, 250 mg/kg; and 500mg/kg b.w. for 30, 60, 90 days respectively. A dose and duration dependent effects on testis, epididymis, vas deferens and accessory reproductive organs of the rats were observed. The treatment resulted in deformation in the germ cells of testis Leydig's cell were atrophied. No spermatozoa could be seen in the seminiferous tubules and were filled either with edematous fluid or degenerated cellular debris.

**Key works :** *Adiantum lunulatum*, alcoholic extract, decoction, albino rats.

### INTRODUCTION

The search for agents of plants origin capable of regulating the female and male fertility is as old as the human civilization and reference to them could be found in the ancient texts of Ayurveda and other Indian system of Medicine. The ever increasing population of the world at an alarming rate has led to intense the research efforts to discover novel compounds from the plant kingdom to control the fertility. The folklore medicine of primitive people frequently included a large number of plants said to be potent contraceptives and abortifacients. Some of these plants might have been included, among folklore. Medicines as a result of trial and error observations while for other there may be no reasonable basis. There is a need to verify these claims. A large number of plants have been tested throughout the world for their possible fertility regulating properties, very few plants however, have been studied for their possible male antifertility efficacy. Therefore, there exists a tremendous scope for carrying out research in the area of male fertility regulation to develop a cheaper and safer herbal oral contraceptive pill. On the basis of folklore and experimental data the following authors, Kirtikar and Basu (1935); de Laszlo and Henswas (1954), Bhatia et al. 2004; Joseph 2001; Murugan, 2002; Lohiya and Goyal, 1992; Ghosh, 2002 have reported many indigenous plants having fertility regulating properties. However, no work on fern species, otherwise occurring commonly appears to have been undertaken. In the present work *Adiantum lunulatum* have been selected for exploring their possible male antifertility activity using male albino rats.

### MATERIALS AND METHODS

**Plant Material:** *Adiantum lunulatum* Burm fern was collected from the Mussoorie hills and Siwalik hill ranges of Dehradun (U.K.). Their proper identification was done by

the taxonomists. Voucher specimens of the collections made, were preserved. The collected ferns were shade dried, powdered and stored in air-tight containers for further processing to obtain decoction and alcoholic extract for administration to the test animals.

Weighed amount of plant (*Adiantum lunulatum*) shade dried, powdered of whole plant were mixed with measured amount of water and boiled to reduce the volume to one fourth of original volume of water. This henceforth was designated as decoction (WHO Protocol, 1978, 1983) Link 1978, Hunter and Recht 1979 administered to test animal with the help of a metallic feeding needle called cannula (Khanna & Chaudhury, 1968).

**Animals:** The studies were conducted on Swiss albino male rats (*Rattus rattus nervegicus*) Fertility assessment studies were undertaken in Swiss albino female rats. They were statistically grouped by randomized design and assigned to 20 groups of 10 animals each. They were acclimatized to laboratory conditions for 15 days prior to the commencement of the treatment. The rats were kept in open air cages (60 × 45 × 45 cm) at room temperature. The rats were fed standard rodent pellet diet (Hindustan Lever Limited) and water was allowed ad libitum (Edmond, 1950)

**Decoction:** Weighed amounts of plant power were mixed with measured amount of water and boiled to reduce the volume to one fourth of original volume of water. This henceforth was designated as 'Decoction' and administered to test animals in graded doses for varying period of treatment.

**Alcoholic Extract:** Weighed amount of powdered plant material was soxhlated with measured volume of 50% ethanol distilled water solvent for such time till no more coloured solvent was obtained. The extract so obtained was reduced to a semi solid state under low temperature and

reduced pressure using a rotary apparatus (Khanna and Chaudhury 1968). This was henceforth designated as 'alcoholic extract'. Desired amount of this extract was weighed and mixed with 2% gum acacia to obtain an aqueous suspension for administration to test rats in graded dose for varying period of treatment.

**LD50 Studies:** The acute 24 hour lethality test was carried out according to WHO Protocol (1983); Link, 1978; Hunter, 1979; Overbeek, 1973. The extracts were dissolved in distilled water, and 0.5mg/100 body weight was administered orally using a metallic feeding cannula. The dose was increased until 50% mortality was obtained within 24 hours. Doses of 10 and 15mg/kg body weight were found to be associated with 1 to 2% mortality and 50% mortality was found at 20mg/kg body weight. Thus LD-50 IS 20mg/kg body weight, which according to WHO Standard reveals that the extract is non-toxic and were observed for any change in behaviour toxicity and mortality, if any up to 72 hours.

**Antifertility Studies :** The healthy male albino rats were divided into 13 groups of 10 animals each and treated with different extracts as follows :

Group 1 - Control, Group 2-4 - Decoction @ 100, 250, 500 mg/kg b.w. respectively for 30 days. Group 5-7 - Decoction @ 100, 250, 500 mg/kg b.w. respectively for 60 days. Group 8-10 - Alcoholic extract @ 100, 250, 500 mg/kg b.w. respectively for 30 days. Group 11-13 - Alcoholic extract @ 100, 250, 500 mg/kg b.w. respectively for 60 days. Thereafter, they were sacrificed by cervical dislocation and reproductive and non-reproductive organs were taken out for histological studies.

**Reversibility Studies:** The rats were divided into 7 groups of 10 rats each.

Group 1 - Control, Group 2-4 - Decoction @ 100, 250, 500 mg/kg b.w. respectively for 60 days. Group 5-7 - Alcoholic extract @ 100, 250, 500 mg/kg b.w. respectively for 60 days. Body weight of all the animal were recorded before and after the treatment. At the end of the treatment (days 60th) the treated male rats were co-caged with proven fertile female rats overnight. Vaginal smears were taken next morning to ascertain the success of mating. If the sperms were present in the smear it was designated as day 1 of pregnancy. Laprotomy of the pregnant females was done on day 10th to ascertain the number of implants, Corpora lutea they were then allowed to deliver full term and the litter size were recorded. Thirty days after cessation of treatment (90th day), the animals were sacrificed by cervical dislocation. Body weight of all the animals were recorded at the initiation of the experiment and continued at weekly intervals till the completion of the experiment. Reproductive tissues viz., testis, epididymis, vas deferens, seminal vesicle, prostate and non-reproductive organs viz., heart, lung, liver, spleen and kidney were removed. The fresh weight of tissues, organs was recorded. The tissues were taken processed for

histological studies (Overbeek et al., 1973; Coutinho, 2002).

**Statistical Analysis :** The data obtained was analysed using standard statistical techniques for the significance of differences by applying one way ANOVA followed by student's 't' test (Snedecor and Cochran, 1967).

## RESULTS AND DISCUSSION

**Acute Toxicity and LD50 Studies:** Varying doses of extracts of plants *Adiantum lunulatum* were given to different groups of rats (W.H.O. Protocol, 1983 and Link, 1978; Hunter, 1979) were given to different groups of rats. It was found that the toxicity level in all the three mentioned plants were safe upto 15 g/kg b.w.

**Effect of *Adiantum lunulatum* Linn. on the body and genital tissue weight in rats.**

**Decoction:** Administration 500 mg/kg of *Adiantum lunulatum* decoction for 30 and 60 days resulted in significant reduction of genital tissue wet weights, which were duration dependent that is 60 days treatment resulted in higher reduction compared to those of 30 days treatment duration. No change, however, was observed in the body weight. The results indicated that *Adiantum lunulatum* decoction produced a dose and duration dependent change in the genital organ wet weight in rats.

**Alcoholic Extract:** The lower dose does not show any significant changes however, administration of 500 mg/kg dose of *Adiantum lunulatum* decoction for 30 days produced marked reduction in the size of the tubules. The seminiferous tubules presented significant degenerative changes. The changes invoked by the treatment consisted of the damage of the germinal epithelium and degeneration of spermatozoa, spermatogonia, spermatocytes and spermatids. The interstitium was highly reduced and packed with atrophied Leydig's cells. Thus, higher dose produced marked reduction in genital weight of testes, caput and cauda epididymis, vas deferens, seminal vesicle and prostate glands.

**Effect of *Adiantum lunulatum* 50% alcoholic and decoction extract on histology of testis:** Administration of 50% alcoholic and decoction extract of *Adiantum lunulatum* at 500 mg/kg for 30 days resulted in the arrest of spermatogenesis at secondary spermatocytes stage. The tiered arrangement of the germ cells was disturbed and most of them were seen to have migrated into the lumen. Sloughing of the dead cells occurred into the lumen of the tubules. However upon treatment with same dose (500 mg/kg) for 60 days, the seminiferous tubules showed degenerative changes. The lumen of the seminiferous tubules was filled with oedematous fluid and cellular debris. The higher alcoholic dose cause severe disintegration of spermatocytes, resulting spermatogenic arrest with severe tubular necrosis (Figure 1 and 2).

**Histology of normal caput and cauda epididymis:** The transverse section of epididymis presented a normal histological picture. The epithelial cells of the caput were

tall, columnar with nuclei arranged in a row near the thin basement membrane. The epithelium of the cauda consisted of low cuboidal cells. The lumen of the ductules was larger in the cauda and smaller in the caput segments of stereocilia were more profuse in the caput region than in the cauda. Both the portions of the epididymis were full of spermatozoa. Intertubular connective tissue and vascularity was observed to be normal in both caput and cauda epididymis. The higher alcoholic dose cause severe disintegration of spermatocytes, resulting in spermatogenic arrest with severe tubular necrosis.

**Effect of Adiantum lunulatum 50% alcoholic and decoction extract on histology of caput and cauda epididymis:** When 50% alcoholic and decoction at dose level of 500 mg/kg dose of Adiantum lunulatum was administered for 30 days, the histological alterations were more visible in caput than cauda. The inter tubular spaces were wider in caput and filled with some unknown material. The stereocilia were lacking in caput. In cauda, the epithelial cells were distorted and nuclear pyknosis appeared among the epithelial cells. Administration of same dose for 60 days induced marked histological changes. The lumen of ducts contained cellular debris. The interstitial spaces were filled with loose connective tissue. No sperms were visible. In cauda there were very little spermatozoa which had clumped. The interstitial spaces were filled with little loose connective tissue, which has clumped. The interstitial spaces were filled with loose connective tissue.

Administration of 500 mg/kg dose of 50% alcoholic and decoction for 30 days was observed to have caused mild alterations in the histology of epididymis. The histological changes were more pronounced in caput than in cauda region. In caput, the lumen of tubules contained little or no spermatozoa. The epithelial nuclei were observed to have migrated into the lumen of the tubules. In cauda, the intertubular spaces were widened and were observed to be filled with unidentifiable fibrous material. Administration of same dose for 60 days, showed more degenerative changes in cauda than in caput epididymis.

**Histology of normal vas deferens:** The transverse section of the vas deferens of control rats showed normal histological picture with three distinct muscular patterns, namely, the outer longitudinal, middle circular and inner longitudinal layers. The lamina propria was present between the inner longitudinal muscle layer and the pseudo-stratified epithelium which contained stereocilia.

**Effect of Adiantum lunulatum 50% alcoholic and decoction extract on histology of vas deferens:** Administration of 500 mg/kg dose of 50% alcoholic and decoction for 30 and 60 days caused reduction in the thickness of lamina propria and resulted in the absence of spermatozoa. The height of luminal folds was affected. The epithelial layer was observed to have broken at several places. Similarly, administration of 250 mg/kg dose for 30 and 60 days caused distortion in the muscular arrangement

at several places and vacuoles were also seen in these layers. The luminal folds, which formed villi present a distorted appearance and was broken at some place. The stereocilia and spermatozoa were completely absent. When 500mg/kg dose of this decoction was administered for 30 and 60 days the luminal epithelium showed nuclear pyknosis especially in the region of the folds. The lumen was devoid of spermatozoa clumped and muscular arrangement was disturbed.

**Histology of normal seminal vesicle:** The transverse section (T.S.) of seminal vesicle of control rats showed mucosal folds extending into the lumen. The lumen was filled with secretions produced by glandular epithelium. The epithelial lining of mucosa consisted of a single layer of tall columnar cells with basal oval nuclei.

**Effect of Adiantum lunulatum 50% alcoholic and decoction extract on histology of seminal vesicle:** The lower dose does not show any alteration in the histo-architecture of the tissue. However, administration of 500 mg/kg dose of 50% alcoholic extract of Adiantum lunulatum for 30 and 60 days caused reduction in lumen size due to creeping in of mucosal folds and lowering of secretion. The patterns of mucosal folds were highly distorted. The muscle layers were thin. The lamina propria and connective tissue were in poor condition. The treatment with 500 mg/kg dose decoction of Adiantum lunulatum for 30 and 60 days also caused inhibition of the secretions in the lumen. The epithelial cells and changed their shape. The lamina propria and muscle layers were disturbed and reduced in height. The mucosal crypts were no more discernible.

**Histology of normal prostate glands:** The histological structure of the prostate gland in T.S. of control animals showed a number of alveoli lined by the low columnar epithelium with basal nuclei. The follicular lumen was full of secretions. There was an intervening fibre muscular stroma. The epithelium had proliferated into the crypts having invaded the lumen. Folding of the mucosal lining was observed in smaller tubules but distended tubules had no mucosal folds.

**Effect of Adiantum lunulatum 50% alcoholic and decoction extract on histology of prostate glands :** Administration of 50% alcoholic and decoction at dose level of 500 mg/kg for 30 and 60 days produced significant alterations in the histological structure of prostate glands of the treated animals. The various alveoli were in the process of disintegration. The lumen had less secretory material in some follicles and the mucosal folds were damaged. The epithelium had degenerated cellular elements.

**Reversibility.** Withdrawal of treatment results in the restoration of the changes induced by the treatment irrespective of the dose and type of formulation (decoction or alcoholic) the values were observed to be comparable to the control groups. No effect of Adiantum lunulatum on non reproductive tissue weight was observed. It shows that administration of Adiantum lunulatum decoction and

alcoholic extract at various dose levels did not result in any change in the wet weight of various non reproductive organs of the treated rats compared to that of the controls.

Treatment of 100 mg/kg dose of alcoholic extract of *Adiantum lunulatum* did not impair the fertility rat. However, 250 and 500 mg/kg doses induced complete infertility in the treated males. The above loss of fertility rat was observed to be reversed significantly upon the withdrawal of treatment followed by 30 days of rest period. Similarly, administration of *Adiantum lunulatum* decoction a 250 and 500 mg/kg doses induced 100% infertility in treated animals. However the cessation of treatment and rest period for 30 days was observed to reverse the above infertility rat significantly among the treated animal. Coutinho, 2002

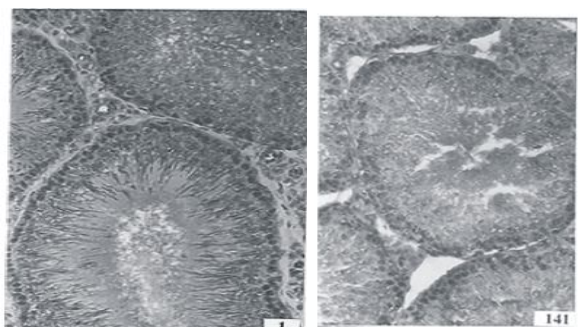
Treatment of rats with *Adiantum lunulatum* whole plant decoction at higher doses of 250 and 500mg/kg b.w for longer period (60 days) of treatment caused various degrees of damage in all the reproductive tissues. The seminiferous tubules were disfigured spermatogenesis was arrested at spermatogonial stage (Verma 2002). The Leydig's cell was mostly atrophied. The interstitial spaces were filled with oedematous fluid. The vas deferens shows marked changes in histological features. The stellate form of lumen was distorted. The luminal epithelium showed nuclear pyknosis. No spermatozoa were observed in the lumen. The seminal vesicle showed mild alteration in its histology. The administration of same doses for same period showed alteration in the histology of prostate. The lumen had less secretion. The epithelium had pyknotic nuclei especially in the mucosal fold. Dose and duration dependent effects on the testis, epididymis, vas deferens and accessory reproductive organs of the rats treated with 50% ethanolic extract of *Adiantum lunulatum* was also observed. The doses of 250 and 500mg/kg b.w. for 30 and 60 days caused deleterious effects on various reproductive organs. The treatment resulted in vacuole formation in the germ cells of the testis. Leydig's cells atrophied totally. No spermatozoa could be seen in the seminiferous tubules and they were filled either with oedematous fluid or degenerated cellular debris. Caput and cauda epididymis showed marked histological alterations in the epithelium of the ductules (Verma and Chinoy 2002). The seminal vesicles were affected with little secretions, damaged epithelial cells and poor vascularity in lamina propria. The histology of prostate was also affected by these plant extract treatments. A significant reduction in reproductive and accessory sex organs weights were also observed. The results of the present study revealed arrest of spermatogenesis at the spermatogonial stage. It may be because of the effect of the treatment (*Adiantum lunulatum*) on the circulating levels of LH and FSH. (Liu et al 2004, Gupta et al, 2004) Further, the atrophy of Leydig's cells under the influence of the present treatments may have resulted in the lowering of testosterone levels indicating antiandrogenic activity. It seems that the extracts exert their effect on spermatogenesis through the alteration

of the hypothalamus-pituitary-gonadal axis function and regulation. The structural alterations induced by the treatment of the rats with extracts of *Adiantum lunulatum* suggest that the antifertility effect manifested by these agents is essentially as a result of antiandrogenic properties present in the plant species studied. Further, the treatment might have altered the bio-chemical and physiological milieu of the epididymis contributing to the non-maturation of the sperm which might explain the lower fertility rate observed amongst the treated rats. A comparative study of various histophysiological features of proximal and distal vas deferens reveals that it is not a mere connection for sperm transport, but is an important organ contributing actively to the maintenance of sperm structure, maturation, survival and viability (Ghose, 2002; Chinoy et al., 1997; Sur, 2002; Bai Jun Ping, 2002; Tamboura et al., (2004).

The treatment with *Adiantum lunulatum* in the present study revealed lowering of secretory activity of both seminal vesicle and prostate gland besides alterations in their histo-architecture. These point out specific antiandrogenic effects of these agents on these accessory sex organs, resulting in inhibition of the contributing factors for sperm activation and vitality. This could be the cause of reduced fertility rates as observed in the present study. No work on male antifertility activity of *Adiantum lunulatum* has been reported so far. However, the histological and physiological actions manifested by the treatment with these agents in the present study are in conformity with similar changes in the reproductive organs reported for many other plant species (Farook et al., 1989; Purohit, 1990; Lohiya and Goyal, 1992; Chaturvedi et al., 1995; Manonayagi et al., 1989; Parveen et al, 1993; Kasturi et al., 1995; Bhargava, 1989; Malini et al., 1999 and Hiremath et al., 1997.

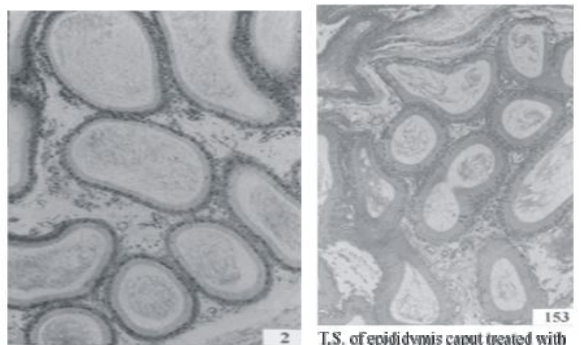
There were varying degree of spermatogenesis and the prominent feature was nuclear degeneration in varying germinal cell types. Most of the tubules were devoid of spermatids and spermatozoa. The absence of secretion indicated impairment in their normal function. Absence of sperms in caput epididymis suggests an acute effect of the extract on the process of spermatogenesis. The absence of secretion in seminal vesicle and ventral prostate appears to indicate a change in function of Leydig's cell or inhibition of androgenic effect of hormones on the target organs by plant extracts. The weight of the reproductive and accessory sex organs was reduced considerably. Drastic reduction in the weight of testes indicates severe damage of the testicular material. These effects suggest strong antiandrogenic properties vested in the plant species studied. The results of the present study point out that *Adiantum lunulatum* possibly act as an antiandrogenic agent and alter the physiology and metabolism of the reproductive organs viz. testis, epididymis, vas deferens and accessory sex organs like seminal vesicle and prostate. The restoration of histological features back to normal and reversal of functional fertility upon the cessation of treatment reveals

that the effect of these agents is essentially reversible. The treatment of rats with these agents does not cause any permanent genetical loss that is one of the main and key areas of concern for any contraceptive to be safe.



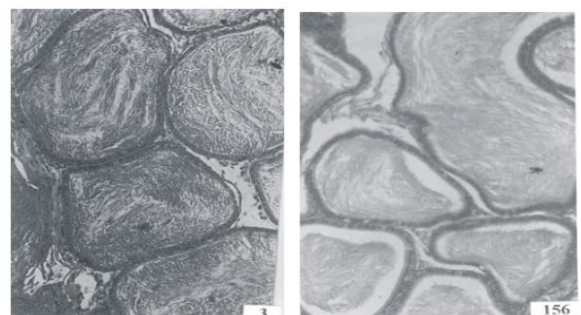
T.S. of testes (Control albino rats)

T.S. of testes treated with *A. lunulatum* alcoholic extract.



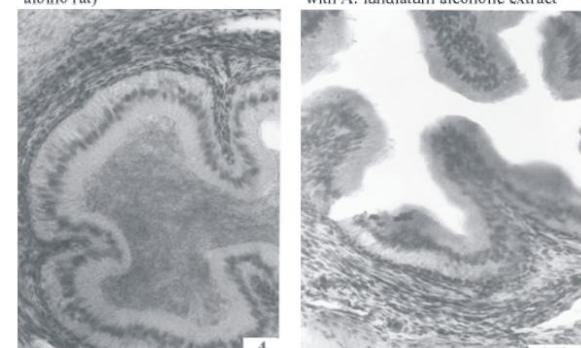
T.S. of caput epididymis (Control albino rat)

T.S. of epididymis caput treated with *A. lunulatum* alcoholic extract



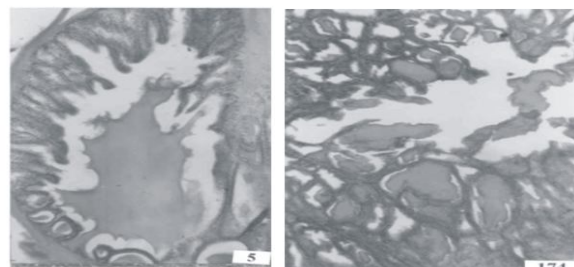
T.S. of epididymis cauda (control albino rat)

T.S. of epididymis cauda treated with *A. lunulatum* alcoholic extract



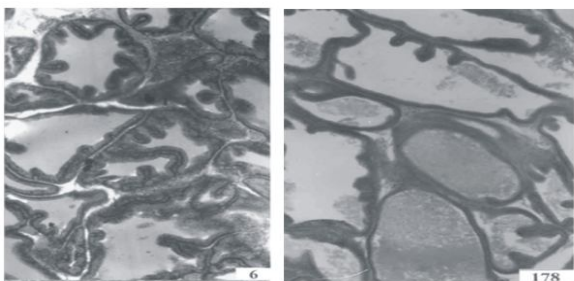
T.S. of vas deferens (control albino rats)

T.S. of vas deferens treated with *A. lunulatum* alcoholic extract



T.S. of seminal vesicle (control albino rats)

T.S. of seminal vesicle treated with *A. lunulatum* alcoholic extract.



T.S. of prostate gland (control albino rat)

T.S. of prostate gland treated with *A. lunulatum* alcoholic extract.

Thus, the present study reveals that *Adiantum lunulatum* could be possibly exploited pharmacologically to develop a safer, effective and reversible male antifertility agent(s). Detailed studies are however, required to be undertaken before these plants could be developed as a simple, safe, effective and reversible male contraceptive.

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