Academic Sciences

International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 5, Issue 4, 2013

Research Article

EFFECT OF AQUEOUS LEAF EXTRACT OF *Andrographis paniculata* IN THE REPRODUCTIVE ORGANS AND FERTILITY OF THE MALE WILD INDIAN HOUSE RAT (*Rattus rattus*)

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Received: 20 September 2013, Revised and Accepted: 30 September 2013

ABSTRACT

Effect of oral administration of aqueous leaf extract of Kalmegh (*Andrographis paniculata*) (250 mg/kg body weight/day) on the male reproductive organs of the wild Indian house rat (*Rattus rattus*) was investigated and its activity was noticed at 30th and 45th days of post treated animals. The treatment had no severe effect on body weight and the reproductive organs weight of rats. In treated rat, testes showed affected seminiferous tubules indicating mixing of the germ cell types in stages of spermatogenesis, atrophy of the spermatogenic elements, increases in number of Leydig cells, occurrence of giant cells and decreases of spermatozoa in the lumen of the seminiferous tubules as compared with control tubules. Seminiferous tubular area, sertoli cells nuclear diameter, tubular area, epithelial cell height and nuclear diameter of cauda epididymis were reduced with vaculation and decreased sperm. The histochemical distribution of different components within the testicular tissue of 30th and 45th days of post treated revealed that the sudanophilic lipid granules was found with higher intensities in the seminiferous tubules. The intensities of acid and alkaline phosphatases were decreased. But there was no remarkable change of Δ^5-3β HSDH and 17β-HSDH within the seminiferous tubules. The concentration of some biochemical components such as the cholesterol and ascorbic acid within the testicular tissues has been increased than the control value. We conclude that *Andrographis paniculata* leaf extract can suppress the process of spermatogenesis which can lead to infertility and may be included as a controlling agent in the control of fertility of the rodent pests.

Keywords: *Andrographis paniculata,* Testis, Spermatozoa, Fertility, Biochemical components, Histochemical components, Rodent pest.

INTRODUCTION

Among the vertebrate pests, rodents are the most destructive to the agricultural produce in India. These rodents cause damage to the standing crops, vegetables, fruits and household properties due to their burrowing, cutting and hoarding activities, to food in storage, in poultry farms and to other commodities [1-3]. Naturally on an emergency basis the rodent population should be controlled in a judicious way. Many synthetic chemicals compounds, physical agents, plant extracts have been used to control them. Use of aqueous leaf extract of Kalmegh (*Andrographis paniculata*) is one of the methods to control the rodent pest as an integrated pest management programme. Several plants and plant products are reported to impede various stages of testicular function in many animal species such as dogs, rats, monkeys and human [4-8]. There is growing interest in natural terpenoids because of their wide spectrum of activities against insect pests [9]. Andrographolide inhibits ovarian development, affecting the fertility and the reproductive potentiality of *Tribolium confusum* suggesting its use for the development of safe and specific anti-fertility agent for the control of the stored grain pest [10].

Andrographis paniculata (Family: Acanthaceae) is an important medicinal plant, occurring wild in India, and is both in Ayureveda and Unani systems of medicine [11]. The dried herb is a remedy for a number of ailments related to digestion, hepatoprotection, vermicidal, antiacne, analgesic, anti-inflammatory, antibacterial, antityphoid, antibiotic activities, hypoglycemic, besides immune enhancement [12-14]. Early reports of oral administration of powdered of stem of Andrographis *paniculata* indicated an antifertility effect in male Wistar mice, but no impact on fertility in female mice [15-16]. It has also been reported that administration of *Andrographis paniculata* resulted in abortion in pregnant rabbits [17-18] fed female mice sun-dried Andrographis powder at a dose of 2g/kg body weight/day for six weeks. When they were mated with untreated males of proven fertility, pregnancy was inhibited in 100 percent of the animals. Conversely, more than 95 percent of untreated female mice in the control group become pregnant when mated with males in a similar fashion. Akbarsha et al. [19] administered dry leaf powder to male albino rats (20 mg daily for 60 days). They reported inhibition of spermatogenesis, degenerative changes in the seminiferous tubules, regression of Leydig cells, and regressive and/or degenerative changes in the epididymis, seminal vesicle, ventral prostate and coagulating glands. The authors concluded that the observations suggested an antispermatogenic (sperm production blocking) or antiandrogenic (blocking effects of androgens) ability of the plant. Sperm count and sperm motility were decreased and sperm abnormalities were noted [20]. However, Burgos et al. [21] no testicular toxicity in male Sprague Dawley rats after treatment with a standardized dried extract in doses of up to 1000mg/kg daily for 60 days. Their analysis was based on testicular weight and histology, ultrastructural analysis of Leydig cells and testosterone levels. Some reports showed that the extract of *Andrographis paniculata*, at doses up to 1000mg/kg in male rats for 60 days and more [21-22] could not produce any significantly effect on plasma concentration of testosterone. Studies done in cultured human placental tissue showed that andrographolide sodium succinate (derived from *Andrographis paniculata*) was effective in inhibiting human progesterone production [23]. This hormone is necessary for pregnancy to be successful.

Considering all aspects and especially to control their fertility the present investigation was undertaken to focus on antifertility efficacy of *Andrographis paniculata* aqueous leaf extract on male wild Indian house rat, *Rattus rattus*.

MATERIALS AND METHODS

Experimental animals

Adult male wild Indian house rat (*Rattus rattus*) weighing around 100-120gms were collected from the surrounding fields of the Kalyani University and were maintained in the usual laboratory conditions. A 12±1 hr light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions. Animals were fed on pellet and tap water *ad libitum*.

Plant collection and extract preparation

The leaves of Kalmegh (*Andrographis paniculata*) were collected from Kalyani University campus in January-March 2002. The leaves were washed to remove dirt and dried at room temperature and ground into course powder. 50gms of the powdered leaves was macerated with 200ml of distilled water for 48 hrs and filtered with filter paper. The filtrate was dried at room temperature and dried mass was stored at 4ºC. A weighed amount of the concentrated extract was then dissolved in distilled water to get the desired concentration for the experiments.

Experimental design

A total of eighteen animals were randomly divided into three groups, namely A, B and C. Group A served as the control and groups B and C as the treated groups. The A group (Control) received an equal volume of vehicle, i.e., distilled water. The B group received 250mg/kg body weight/day, for 30 days oral administration of aqueous leaf extract of *Andrographis paniculata* and the C group received 250mg/kg body weight/day, for 45 days oral administration of aqueous leaf extract of *Andrographis paniculata*. On the 31st and 46th day of post treatment the body weights of all rats were recorded and the animals were sacrificed by cervical dislocation. Testes, epididymes and seminal vesicle were quickly taken out, weighed on a torsion balance and processed for histological, histochemical and spermatokinetic studies.

Tissue preparation for histological observations

For histological work, testis and epididymis were fixed in Bouin's fluid and processed for routine microtomy. 6 µm thick paraffin sections were made, stained with Haematoxylin-Erythrosin and Fast Green sequence. From the well-stained sections of testes of three groups, observations were made and photomicrographs taken. For the quantitative estimation of the germ cell population, well stained tubular sections of the testis of six animals were counted to give the percentage of germ cell population at the magnification of 1000 x (oil immerssion objective 100x and ocular 10x). 200 different seminiferous tubules were randamly selected and counted from each rat. For the measurement of the seminiferous tubular area randomly sixty fields from each rat were traced with the camera lucida unit and their areas were measured with the help of an Allbritt disc planimeter with Zero setting device. To draw the areas of this component stained sections were magnified with the help of light microscope low power (10x10) magnification.

Assessment of sperm count

The cauda epididymis was removed within two minutes of autopsy and immediately placed in 5ml phosphate buffered saline at 37ºc. It was then finely minced with scissors and left for 20 min. to ensure an even distribution of sperm throughout the buffer solution. A drop of this suspension was placed in a Neubauer chamber of the haemocytometer and the number of sperm heads were counted [24-25].

Tissue preparation for biochemical estimations

For the quantitative estimation of total cholesterol [26], ascorbic acid [27], acid and alkaline phosphatases [28] concentration, testis of control and treated animals were weighed, homogenized and centrifuged in ice-cold solutions. The supernatants were taken and processed further for separate estimations. Spectronic 20 Genesys spectrophotometer was used for the measurement of the concentration of cholesterol (620 nm), ascorbic acid (520 nm), acid and alkaline phosphatases (405 nm). Estimations were made, the data were recorded and analysed.

Tissue preparation for histochemical observations

For the cytochemical localization of lipids [29], Δ5-3β-hydroxysteroid dehydrogenase [30-31], 17β- hydroxysteroid dehydrogenase [32, 31], alkaline phosphatase [33] and acid phosphatase [34] within the testis of control and two experimental groups, different methods were followed. At the end of the cytochemical procedures, sections were mounted in glycerine jelly and observations were made under the light microscope. After thorough processing, the reactions within the testis of the control and experimental animals were carefully studied and their intensities were recorded properly. The necessary photomicrographs were taken from the well-stained sections.

Statistical analysis

All data were expressed as the mean ± S.E.M, and the level of significance was determined by student's t-test [35]).

RSEULTS

The treatment of adult male wild Indian house rat, *rattus rattus* with *Andrographis paniculata* aqueous leaf extract caused no significant effect on the body weight of the animals. In the 30th and 45th days of post treated groups, the weight of the testis and seminal vesicle were decreased but not significant statistically.

The section of the testis in control group (Gr. A) indicating normal spermatogenesis, the seminiferous tubules were lined with three or four layers of spermatogenic cells at different stages of maturation and mature spermatozoa were found (**Fig. 2A**). Testis showed effceted seminiferous tubules with decreased spermatozoa in the lumen of seminiferous tubules (**Fig. 2B**) in the 30th days of post treatment. The percentage of secondary spermatocytes and spermatozoa were decreased and spermatids were increased significantly in the 30th days of post treated male wild Indian house rat (**Fig. 1**). The damaged structures were observed in the seminiferous tubules i.e. appearance of foamy substance, occurrence of giant cells and tail debris of spermatozoa in the lumen of seminiferous tubules (**Fig. 2C and 2D**) of 45th days of post treated rats. The percentage of spermatogonia, primary spermatocytes and sertoli cells were greatly increased (**Fig. 1**). The spermatid and spermatozoa population has been decreased than the control value. In the 30th and 45th days of post treated rats, the seminiferous tubular area and sertoli cell nuclear diameter were decreased significantly (**Table-1**). Sperm number/mg cauda epididymis was decreased both in the 30th and 45th days of post treatment of *Andrographis paniculata* aqueous leaf extract. Gradual increase of the percentage of sperm abnormality was noticed both in the treatment group B and C (**Table-2**). More than 60% of the cases separated head and tail and abnormal shape of the head were observed.

Significant decrease in tubular area, nuclear size and epithelial cell height of the epididymis was recorded in the treatment groups (Group-B, C) compared to control. Maximum decreased were observed in the 45 days of post treated rats (**Table-3**).

The seminiferous tubules of control group contain less amount of sudanophilic lipids. The intensities of Δ^5-3β – hydroxysteroid dehydrogenase (HSDH), 17β- hydroxysteroid dehydrogenase (HSDH), alkaline phosphatase and acid phosphatase were quite higher in the control testis. Depletion of acid and alkaline phosphatase and intensities of Δ^5-3β – hydroxysteroid dehydrogenase (HSDH), 17β-hydroxysteroid dehydrogenase (HSDH) has been noticed within the testis of 30th and 45th days of post treated rats (**Table-4**). The sudanophilic lipid granules were found with higher intensities in the seminiferous tubules of the 45th days of treatment of *Andrographis paniculata* aqueous leaf extract.

Results of some biochemical components of the testis are presented in the **Table-5**. In comparison to control values, the concentration of acid and alkaline phosphatases were reduced both in 30th and 45th days of treatment of *Andrographis paniculata* aqueous leaf extract. On the other hand, ascorbic acid and cholesterol level were significantly increased both the treatment groups in comparison with control.

DISCUSSION

In the present study oral administration of aqueous leaf extract of Kalmegh (*Andrographis paniculata*) (250 mg/kg body weight/day) on the male reproductive organs of the wild Indian house rat (*Rattus rattus*) was investigated and its activity was noticed in 30 and 45 days of post treated animals. The treatment of adult male rat, *Rattus rattus* with *Andrographis paniculata* aqueous leaf extract caused no significant effect on the body weight of the animals. In the 30th and 45th days of post treated groups, the weight of the testis, epididymis and seminal vesicle were decreased but not significant statistically.

In this study histopathological observations showed that *Andrographis paniculata* affected the process of spermatogenesis and disrupted the production of sperm. Sperm number/mg cauda epididymis was decreased both in the 30th and 45th days of post treatment. Gradual increase of the percentage of sperm abnormality was noticed both in the treatment group B and C. Significant decrease in tubular area, nuclear size and epithelial cell height of the epididymis was recorded in the treatment groups compared to control. There is a close relationship between spermatid losses and the ultimate decline in sperm numbers [36]. The reduction in sperm motility in cauda epididymis is of importance with regard to

fertilization [37]. Inadequate concentration and immortality of the spermatozoa means they cannot penetrate the cervical mucus and thus fail to fertilize the ova [38]**.** Neem leaf (*Azadirachta indica*) extract also reduced sperm counts and motility and increased the number of abnormal sperm (26). He also reported reductions in weight, epithelial cell height and nuclear diameter of the ventral prostate and seminal vesicles as indirect evidence of the anti-androgenic action of neem extract. The effects of andrographolide on sperm have been reported earlier either negative [19-20], positive [21, 39] or no effect [22].

Testis showed effceted seminiferous tubules with decreased spermatozoa in the lumen of seminiferous tubules in the 30th days of post treatment. The percentage of secondary spermatocytes and spermatozoa were decreased and spermatids were increased significantly in the 30th days of post treated male wild Indian house rat without a significant changed in testis, epididymis and seminal vesicle weight. Several reports have shown degenerative changes in seminiferous tubules without a significant change in organ weight [40].

Male reproductive toxicity of the plant *Andrographis paniculata* was reported earlier by Akbarsha and Murugaian, 2000. *Andrographis paniculata* found to obsessed anti-fertility and pregnancy-terminating effects [41] and stopped spermatogenesis in male rats. A male reproductive toxic effect of a therapeutic use of andrographolide and confirmed the possible prospective use of andrographolide as a male contraceptive was observed by Akbarsha and Murugaian [20] .

In comparison to control values, the concentration of acid and alkaline phosphatases were reduced both in 30th and 45th days of post treatment of *Andrographis paniculata* aqueous leaf extract (both biochemical and histochemical). The alkaline phosphatase is said to be a histochemical marker for primordial germ cells of various species, including rat [42] and mouse [43]. It is known that this enzyme is required for the synthesis of glycogen, which in turn apparently participates in the metabolic process of spermatogenesis [44]. Seminal and prostatic acid phosphatase has been associated with the nutrition of spermatozoa [45-46] and with their fertilizing ability [47]. It is also known from the present investigation that ascorbic acid from the testicular tissues has been increased due to oral administration of leaf extract of Kalmegh (*Andrographis paniculata*). Role of ascorbic acid in the process of steroidogenesis is well known [48]. Again as ascorbic acid is a known catalyst for both lipid peroxidation and alteration of unsaturated fatty acid composition [49], so the involvement of ascorbic acid in the process of steroidogenesis in the testis of the rats of control and treated groups may be taken into consideration. Adverse effects of leaf extract of kalmegh on the level of fructose in the seminal vesicle and sialic acid in the duct was noticed in mice by Mishra and Singh [50].Cholesterol is involved in steroidogenesis in the testes, an increased level of cholesterol is attributed to decreased androgen concentration, resulting in impaired spermatogenesis [51].

The anti-fertility effect of *Andrographis paniculata* has been evaluated in the screening programme done by Zoha et al. [18] with both male and female mice and confirmed the antifertility effect [52, 18]**.** It has been suggested that with exposure to *Andrographis paniculata* ovulation may be inhibited because hormonal level of LH, FSH, estrogen and progesterone were decreased in female rats [53].

Depletion of the intensities of Δ^5-3β – hydroxysteroid dehydrogenase (HSDH), 17β- hydroxysteroid dehydrogenase (HSDH) has been noticed within the testis of 30th and 45th days of post treated rats. The sudanophilic lipid granules were found with higher intensities in the seminiferous tubules of the 45th days of treatment of *Andrographis paniculata* aqueous leaf extra. High amount of lipid droplets within the Sertoli cells of mammals seem to be due to the phagocytosed lipid materials of the degenerating germ cells, which is also pointed out by Lacy [54]. It is also known that the lipid inclusion in the Sertoli cell may be in the control of FSH secretion from the pituitary [55]. Serum FSH have been shown to be related to the germinal cell component, particularly spermatogonial numbers [56]. Both the lipids and steroid dehydrogenases are usually known to show inverse relationship between them. Higher content of lipids and lower activity levels of the dehydrogenases are characteristic of steroidogenically inactive gonads. It has been suggested [57] that 17 β-ol-dehydrogenase (oxidase) is located within both the tubules and the interstitial elements and is diminished slightly during the oral administration of neem leaf (*Azadirachta indica*) extract. This phenomenon is directly related to the destructive changes associated with the depopulation of the seminiferous tubules. Several animal studies showed that *Andrographis paniculata* may have contraceptive or anti-fertility effects following long-term treatment at high doses (20mg/rat) [19].

Changes in the biochemical parameters in rats, such as significant decreases in protein content, but marked increases in cholesterol, acid phosphatase and alkaline phosphatase levels with appearance of fructose in the reproductive system, recommended anti-fertility effects of the andrographolide [58]**.**

In this study with wild rats, it can be concluded that aqueous crude leaf extract of Kalmegh (*Andrographis paniculata*) has effective contraceptive activity for the control of agricultural rodent pests and reasonable safety at anti-fertility doses used. Further study on the possible mechanism of action should be investigated.

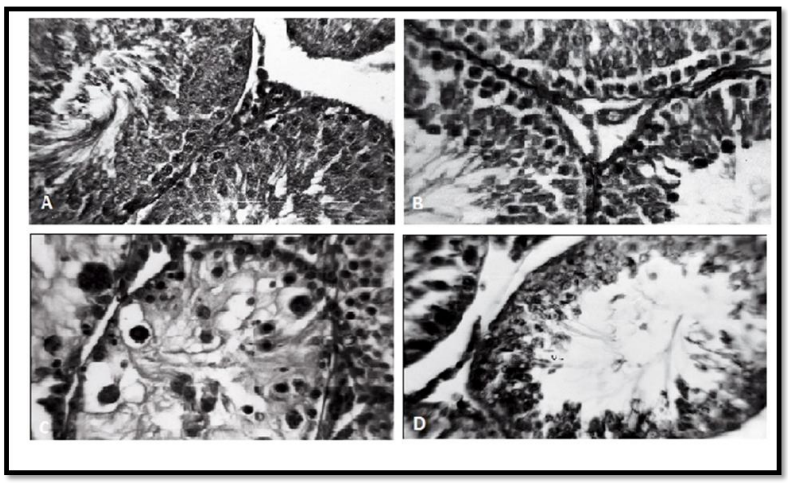

Fig. 2A: Section of the testis of the control male wild Indian house rat, *Rattus rattus* showing normal seminiferous tubules and spermatogenesis (X450); 2B. Section of the testis of 30 days leaf extract of *Andrographis paniculata* post treated rat showing affected seminiferous tubules, atrophy of spermatogenic elements, and degenerated appearance of germ cells and decreased of spermatozoa in the lumen of seminiferous tubules (X450); 2C. Section of the testis of 45 days leaf extract of *Andrographis paniculata* post treated rat showing necrotic seminiferous tubules, absence of spermatozoa, foamy substance and occurrence of giant cells in the seminiferous tubules (X450); 2D. Section of the testis of 45 days leaf extract of *Andrographis paniculata* post treated rat showing tail debris of spermatozoa in the lumen of seminiferous tubules (X450).

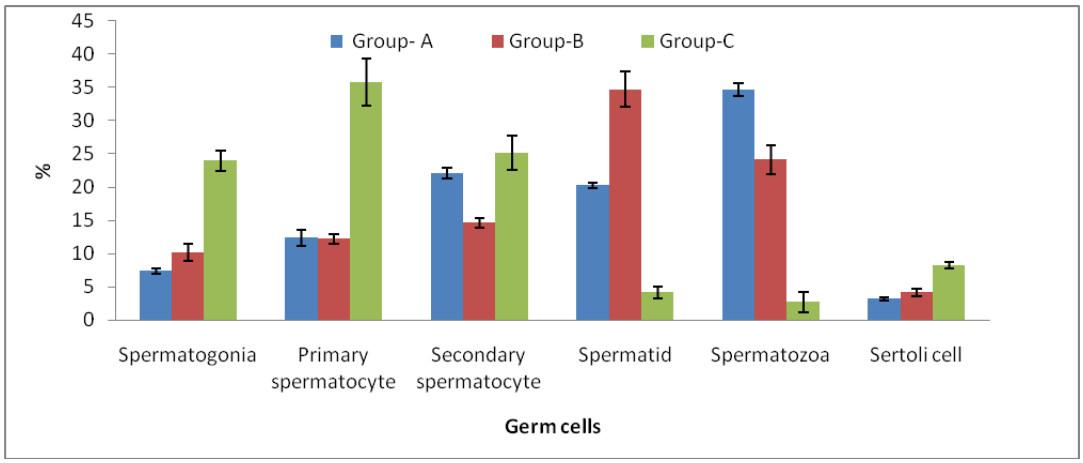

Fig. 1: Percentage of different germ cell types due to administration of the leaf extract of *Andrographis paniculata* in the adult male wild Indian house rat, *Rattus rattus*

Table 1: Effect of the leaf extract of *Andrographis paniculata* on the area of the Seminiferous tubules and nuclear diameter of the Sertoli cells of the adult male wild Indian house rat, *Rattus rattus*

Groups	Seminiferous tubular area (Cm^2)	Sertoli cells Nuclear diameter (um)
A (6)*	20.32 ± 0.44**	2.77 ± 0.13
B	12.87 ± 0.69 [a]	2.23 ± 0.14 [c]
C	11.51 ± 0.56 [a]	2.14 ± 0.07 [b]

* Number of animals in each group; ** Mean ± Standard error

[a] p<0.001; [b] P<0.01; [c] p<0.05

Table 2: Effect of the leaf extract of *Andrographis paniculata* on the Sperm count and percent of Sperm abnormality of the adult male wild Indian house rat, *Rattus rattus*

Groups	Group-A(6)*	Group-B	Group-C
Sperm count/mg Cauda epididymis X 10^4	32.06 ± 2.73**	22.24 ± 1.45 [b]	22.01 ± 0.44 [c]
% of Sperm abnormality	3.75 ±0 .62	9.11 ± 2.15 [b]	9.57 ± 0.39 [a]

*Number of animals used; **Mean ± Standard error

[a] p<0.001; [b] P<0.01; [c] p<0.05

Table 3: Effect of the leaf extract of *Andrographis paniculata* on the epithelial cell height, nuclear size and tubular area of the epididymis of the adult male wild Indian house rat, *Rattus rattus*

Groups	Epithelial cell height of the epididymis (µm)	Nuclear size of the epididymis (µm)	Epididymal tubular area (cm^2)
A (6)*	22.56 ± 0.40**	7.26 ± 0.23	37.71 ± 0.13
B	20.33 ± 0.15 [a]	6.48 ± 0.02 [b]	35.02 ± 0.14 [a]
C	19.15 ± 0.18 [a]	6.32 ± 0.01 [b]	24.86 ± 0.21 [a]

*Number of animals used; **Mean ± Standard error

[a] p<0.001; [b] P<0.01

Table 4: Histochemical reactions showing the intensity within the testis of the adult male wild Indian house rat *(Rattus rattus)* due to the administration of the leaf extract of *Andrographis paniculata*

Methods	Regions	Group-A	Group-B	Group-C
Sudan	i) Basement membrane	++	++	++
III & IV	ii) Seminiferous tubule	+	+	++
	iii) Interstitium	++	++	++
Acid	i) Basement membrane	++	+	±
phosphatase	ii) Seminiferous tubule	±	+	±
	iii) Interstitium	++	+	+
Alkaline	i) Basement membrane	++	+	+
phosphatase	ii) Seminiferous tubule	++	+	±
	iii) Interstitium	++	++	+
17β-hydroxy steroid	i) Basement membrane	+	+	+
dehydrogenase	ii) Seminiferous tubule	+	+	±
	iii) Interstitium	+	+	+
Δ^5-3β- hydroxy steroid	i) Basement membrane	++	++	+
dehydrogenase	ii) Seminiferous tubule	+	+	+
	iii) Interstitium	+++	++	+

Intensities of reactions : + : Positive ; - : Negative ; ++ : Moderate ; +++ : Highly positive or intensed ; ± : Not significant

Table 5: Effect of leaf extract of *Andrographis paniculata* on the biochemical components of the testes of the adult male wild Indian house rat, *Rattus rattus*

Groups	(mM/100mg fresh testicular tissue)		(mg/100mg fresh testicular tissue)	
	Acid phosphatase	Alkaline phosphatase	Ascorbic acid	Cholesterol
A (6)*	0.597 ± 0.029**	0.276 ± 0.019	0.019 ± 0.003	0.538 ± 0.04
B	0.558 ± 0.031	0.144 ± 0.016 [a]	0.021 ± 0.001 [a]	0.756 ± 0.04 [a]
C	0.461 ± 0.064 [d]	0.137 ± 0.026 [b]	0.022 ± 0.0007 [a]	0.811 ± 0.16 [a]

* Number of animals in each group; **Mean ± Standard error

[a] p<0.001; [b] P<0.01; [d] p<0.10

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