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Effect of lyophilized *Azadirachta indica* leaf powder on biochemical parameters of testis and epididymis in albino rats

Ravindranath H. ALADAKATTI [1*], Mukhtar Ahmed G. GHODESAWAR [2], Mukhtar AHMED [3], Tulasigeri M.TOTIGER[1] and D. SANNADURGAPPA [4]

[1] *Central Animal Facility, Indian Institute of Science, Bangalore-560 012, India.*
[2] *Anjuman Arts, Sciences & Commerce College, Bijapur-586101, India.*
[3] *Research Centre, Electron Microscope Unit, # 5, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Kingdom of Saudi Arabia.*
[4] *Centre for Sustainable Technologies, Indian Institute of Science, Bangalore-560 012, India.*
[*] *Corresponding author,* ***E-mail address:*** *ravindranath@caf.iisc.ernet.in Tel: +91-080-22932457, Fax: +91-080-23606569*

ABTRACT

The aim of the present study was to investigate the effect of lyophilized *A. indica* leaf extract (125, 250 and 375 mg in suspension of 1 mL Propylene Glycol, respectively / kg body weight) on androgen-dependent biochemical parameters such as cholesterol and glycogen in the testis, total protein, total free sugar, enzymes like acid phosphatase (ACP), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in the testis and epididymis of both control and treated groups. Results indicated no significant difference in their body weight. However, testis and epididymis showed a significant decrease in their weights. The biochemical analysis showed a general decrease in the total protein content and the activity of ACP and, an increase in the total free sugar, glycogen, cholesterol contents and the activities of ALP and LDH in the dose-dependent treated rats. Since it is known that the accumulation of cholesterol and glycogen in the testis and epididymis are indicators of androgen deprivation. In this study such effects may have resulted from the deficiency in the level of circulating androgen, probably due to androgen deficiency resulting to the anti-androgenic property of the carbohydrate-rich nature of lyophilized *A. indica* leaf extract.

Keywords: Lyophilized *A. indica* leaf extract, Testis, Epididymis, Biochemical parameters, Albino rats.

INTRODUCTION

Azadirachta indica A. Juss (Syn: *Melia Azadirachta* L, Meliaceae family), commonly known as neem, is an important medicinal plant cultivated throughout India and Burma. This plant is extensively used as an astringent, antiperiodic, antispirochaetal, antiprotozoal; leprosy and bronchitis; for healing ulcers in urinary passages; for chronic fever and many other disorders (Bhakuni et al., 1990). It has been reported that the oral administration of crude extracts of *A.indica* leaves induces morphological changes in the head of rat spermatozoa (Aladakatti and

Nazeer Ahamed, 1999); sperm parameters (Aladakatti et al., 2001); fructose content in the vas deferens (Ghodesawar et al., 2003); and ultrastructural changes in testis, prostate gland, vas deferens and cauda epididymal epithelial cell types (Kasturi et al., 2002; Ghodesawar et al., 2004a, 2004b). Additionally, changes in spermatogenic pattern and ultrastructural changes in Sertoli cells, Leydig cells and cauda epididymal spermatozoa induced by *A. indica* leaves in albino rats (Aladakatti & Nazeer Ahamed, 2005a, 2005b; 2006). Several such effects appear reversible (Joshi et al., 1996). Further, study from Khillare and Shrivastv (2003) have been demonstrated and confirmed that the lyophilized *A. indica* leaves extract is carbohydrate in nature (nonisoprenoids, with proteins i.e. amino acids, sulphurous compounds, polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds, etc.).

Though, various experimental studies on biochemical parameters have been reported using this plant source, very little information about the influence of lyophilized *A. indica* leaf extract on the androgen-dependent biochemical parameters of the testis and caput and cauda of epididymis in albino rats. Hence, the purpose of the present investigation is to know the effect of lyophilized *A. indica* leaf extract on the androgen-dependent biochemical parameters of testis and the epididymis at a dose dependent level. The present work mainly deals with the assay of some of the androgen-dependent biochemical parameters such as cholesterol and glycogen in the testis, total protein, total free sugar, enzymes like acid phosphatase (ACP), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in the testis and the epididymis of both control and treated groups.

MATERIALS AND METHODS

Preparation of neem leaf extract

An aqueous extract was prepared from *A. indica* (neem) leaves. A voucher specimen (Zoo/herb/File No.52-Acc.No.17) was collected and deposited at Zoology Department, Karnatak University, Dharwad, India. Neem leaves were ground in a mixer and filtered with a gauze. The filtrate was washed with chloroform in 1:1 proportion, centrifuged at 3000 rpm for 20 min. The pellet was discarded and the supernatant frozen at -20 °C for lyophilization. The extracts were lyophilized separately.

Chemical nature of extract

The chemical nature of ingredients of leaf extracts was studied for the presence of lipid, protein and carbohydrate according to the method of Khillare and Shrivastv (2003). It has been observed that the ingredient was rich in carbohydrate. The details of carbohydrate estimation by Fehling's test are given below.

Preparation of Fehling's solution

Fehling's solution was prepared by mixing copper sulfate solution and alkaline tartrate solution. Copper sulfate solution was prepared by dissolving 34.65 g copper sulfate in distilled water and made up to 500 ml. Alkaline tartrate solution was prepared by dissolving 125 g of potassium hydroxide and 173 g Rochelle salt in distilled water and made up to 500 ml. To prevent deterioration, these solutions were preserved separately in a rubber-stoppered bottle and equal volumes were mixed before using.

Procedure

To the warm Fehling's solution, an extract was added and the mixture was heated after each addition. The production of yellow or brownish – red cuprous oxide indicated that reduction had taken place. The differences in colour of the cuprous oxide precipitates under different conditions were apparently due to difference in the size of the particles, the more finely divided precipitates having a yellow colour, while the coarser ones are red.

Animals

Colony bred healthy adult male albino rats (Wistar strain) weighing 190-200 g were utilized for experiments. All animals

were proven fertile and obtained from the rat colony maintained in the department. They were housed at a temperature of 26 ± 2 °C and exposed to 13-14 h of daylight and maintained on a standard rat pellet diet (Gold Mohar, Hindustan Level Ltd., Hyderabad) and water was given *ad libitum*. The animals were acclimatized to the laboratory conditions before conducting experiments. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and the care of the laboratory animals was taken as per the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) regulations.

Study protocol

The animals were equally divided into 4 groups containing 10 rats each and treated as follows: Group I, oral administration of 1 mL propylene glycol/rat/ day and served as control; Group II, lyophilized *A. indica* leaf extract suspended in 1 mL Propylene Glycol (125 mg/kg body weight) Group III; Lyophilized *A.indica* leaf extract suspended in 1 mL Propylene Glycol (250 mg/kg body weight); and Group IV. Lyophilized *A. indica* leaf extract suspended in 1 mL Propylene Glycol (375 mg/kg body weight). The lyophilized *A. indica* leaf extract was then mixed with propylene glycol as required and administered orally (gavage) to the experimental animals (WHO, 1983). The Propylene Glycol and the graded doses of lyophilized *A. indica* leaf extract were administered orally (gavage) on daily basis for 24 days. Five animals from each group were used for fertility test. Twenty-four hours after the last dose, the control and treated animals were sacrificed by cervical dislocation. The testis and caput and cauda of epididymis were dissected out, blotted free of mucus and weighed to the nearest milligram.

Biochemical analysis

50-100 mg of each tissue was quantitatively homogenized in 1 ml of 0.1 M Tris-HCl buffer (pH 7.2), 0.1 M phosphate buffer (pH 7.2) or distilled water and then centrifuged at 8000 g for 15 minutes at 4 °C. The supernatants were collected and used for various biochemical analyses using Hitachi.U.V. Vis. Spectrophotometer.

Estimation of total protein

The total protein level in the testis was estimated by the method of Lowry et al. (1951) using Bovine Serum Albumin as standard. The OD of the resultant colour was read at 660 nm and expressed as mg protein per gm wet tissue.

Estimation of total free sugar

The total free sugar content of the tissue was estimated following the method of Folin and Wu (Oser, 1965). The O.D. was recorded at 420 nm and expressed as mg sugar per gm wet tissue.

Estimation of glycogen

The glycogen content of the testis was estimated according to the method of Caroll et al. (1956). The intensity of the colour developed was read at 620 nm and expressed as mg glycogen per gm wet tissue.

Estimation of cholesterol

The cholesterol of the testis was estimated according to the method of Zarrow et al. (1964) and O.D. was taken at 650nm and expressed as mg cholesterol per gm wet tissue.

Estimation of acid phosphatase (ACP) and alkaline phosphatase (ALP)

The enzyme assays were carried out according to the method described by Andersch and Szezypinski (1947). The O.D. of the resultant colour was read on a colorimeter at 400 nm and both phosphatase activities were expressed in terms of mMoles of P-nitrophenol formed per hour per gram protein.

Estimation of lactate dehydrogenase (LDH)

LDH levels in the testis were determined by the method of King (1965) .The intensity of the colour was measured at 440 nm and expressed as μMole/gm/hr.

Statistical analysis

Results are expressed as the mean value ± standard error of mean (S.E.M.). All

percentage data were subjected either to Student's *t*-test or one-way analysis of variance (ANOVA) followed by multiple t-tests for analysis.

RESULTS

Chemical nature

Using Fehling's test, the leaf extract was found to be carbohydrate in nature. Carbohydrates possessing a free or potentially free Aldehyde or ketone group have the property of readily reducing the ions of certain metals such as copper, bismuth, mercury, iron and silver. The most widely used tests for sugar are based on this property. For example, when blue cupric hydroxide suspended in an alkaline medium is heated, it is converted into insoluble black cupric oxide. However, in the presence of reducing agents such as certain sugars, the cupric hydroxide is reduced to insoluble yellow or red cuprous oxide. Based on the above property, the chemical nature of leaf extract was studied using Fehling's test. The production of yellow or brownish-red cuprous oxide indicated that reduction has taken place. The difference in color of cuprous oxide precipitated under different conditions was apparently due to differences in the size of the particles, the more finely divided precipitates having yellow color while the coarser ones were red. This test was found to be positive, indicating that the leaf extract is carbohydrate in nature.

Body and organ weights

The body weight of the rats did not differ significantly due to the dose dependent treatment of lyophilized *A.indica* leaf extract, whereas in rats 250 and 375 mg/kg body weight, exhibited significant ($P \leq 0.05$) decrease in the weights of testis and epididymis when compared to the control rats (Table 1).

Biochemical estimations in testis

Biochemical composition of protein, glycogen, cholesterol, ACP, ALP and LDH in the testis was observed in the control and graded doses of lyophilized *A. indica* leaf extract treated rats (Table 2).

Change in protein content

In the testis of the control rats, the protein content was 37.10 ± 2.25 mg/gm. No significant change was observed in 125 mg/kg body weight treated rats**.** Where as in 250 mg and in 375 mg/kg body weight treated rats, a gradual ($P \leq 0.05$) and significant decrease ($P \leq 0.001$) in protein content was noticed when compared to the controls (Table 2).

Change in free sugar content

In the testis of the control rats, the free sugar content was 0.60 ± 0.05 mg/gm. In 125 mg/kg body weight treated rats, the free sugar content was higher ($P \leq 0.05$). However, in 250 mg and in 375 mg/kg body weight treated rats, this level increased significantly ($P \leq 0.001$) when compared to the controls (Table 2).

Change in glycogen content

In the testis of the control rats, the glycogen content was 4.66 ± 1.33 mg/gm. In 125 mg/kg body weight treated rats, the glycogen content increased ($P \leq 0.05$). However, in 250 mg and in 375 mg/kg body weight treated rats, the level increased significantly ($P \leq 0.001$) when compared to the controls (Table 2).

Change in cholesterol content

In the testis of the control rats, the cholesterol content was 3.15 ± 0.12 mg/gm. In 125 mg/kg body weight treated rats, the cholesterol content was increased ($P \leq 0.05$). However, in 250 mg and in 375 mg/kg body weight treated rats, this content level was increased significant ($P \leq 0.001$) in both when compare to the controls (Table 2).

Change in acid phosphatase activity (ACP)

In the testis of the control rats, the ACP activity was 2.96 ± 0.67 mM/gm/hr. No significant change was observed in 125 mg/kg body weight treated rats**.** However, in 250 mg and in 375 mg/kg body weight treated rats, this activity was significantly decreased ($P \leq 0.001$) in both when compare to the controls (Table 2).

Change in alkaline phosphatase activity (ALP)

In the testis of the control rats, the ALP activity was 1.22 ± 0.72 mM/gm/hr. No significant change was observed in 125 mg/kg body weight treated rats**.** However, in 250 mg and in 375 mg/kg body weight treated rats, this activity was increased significant ($P \leq 0.001$) in both when compare to the controls (Table 2).

Change in lactate dehydrogenase activity (LDH)

In the testis of the control rats, the LDH activity was 268.02 ± 5.78 µM/gm/hr. No significant change was observed in 125 mg/kg body weight treated rats**.** However, in 250 mg and in 375 mg/kg body weight treated rats, this activity was increased significant ($P \leq 0.001$) in both when compare to the controls (Table 2).

Biochemical estimations in caput and cauda of epididymis

Change in protein content

In the caput and cauda of epididymis of the control rats, the protein content was 37.19 ± 2.20 mg/gm and 37.19 ± 1.54 mg/gm respectively. No significant change was observed in 125 mg/kg body weight treated rats**.** However, in 250 mg and in 375 mg/kg body weight treated rats, a gradual ($P \leq 0.05$) and significant decrease ($P \leq 0.001$) in protein content of both when compare to the controls (Tables 3 & 4).

Change in free sugar content

In the caput and cauda of epididymis of the control rats, the free sugar content was 0.59 ± 0.12 mg/gm and 0.68 ± 0.26 mg/gm respectively. In 125 mg/kg body weight treated rats, the free sugar content was increased ($P \leq 0.05$). However, in 250 mg and in 375 mg/kg body weight treated rats, this content level was increased significant ($P \leq 0.001$) in both when compare to the controls (Tables 3 & 4).

Change in acid phosphatase activity (ACP)

In the caput and cauda of epididymis of the control rats, the ACP activity was 11.15 ± 0.12 mM/gm/hr and 7.95 ± 0.23 mM/gm/hr respectively. No significant change was observed in 125 mg/kg body weight treated rats. Where as in 250 mg and in 375 mg/kg body weight treated rats, a gradual ($P \leq 0.05$) and significant decrease ($P \leq 0.001$) in ACP activity of both when compare to the controls (Tables 3 & 4).

Change in alkaline phosphatase activity (ALP)

In the caput and cauda of epididymis of the control rats, the ALP activity was 1.23 ± 0.14 mM/gm/hr and 1.36 ± 0.17 mM/gm/hr respectively. In 125 mg/kg body weight treated rats, the activity was increased ($P \leq 0.05$). Where as in 250 mg and in 375 mg / kg body weight treated rats, this ALP activity was increased significant ($P \leq 0.001$) in both when compare to the controls (Tables 3 & 4).

Change in lactate dehydrogenase activity (LDH)

In the caput and cauda of epididymis of the control rats, the LDH activity was 307.65 ± 1.48 µM/gm/hr and 365.19 ± 2.20 µM/gm/hr respectively. In 125 mg/kg body weight treated rats, the activity was increased ($P \leq 0.05$). Where as in 250 mg and in 375 mg/kg body weight treated rats, this LDH activity was increased significant ($P \leq 0.001$) in both when compare to the controls (Tables 3 & 4).

DISCUSSION

After treatment with lyophilized *A.indica* leaf extract at 375 mg/kg body weight for 24 days, the body weight of rats was not significantly altered. It is known that monitoring body weight provides information on the general health level of animals, which can be important to the interpretation of reproductive effects (US EPA, 1996). The present data show that the administration of lyophilized *A.indica* leaf extract brought about a decrease in the mean testis and epididymis weight may be due to the decrease in serum testosterone concentration. As testosterone plays a major if not sole role in the maintenance of structural integrity and functional activities of the accessory sex

Table 1: Effect of the treatment of lyophilized *A. indica* leaf extract on the body weight (g), testes and epididymis (caput and cauda) weights (mg/100 g body weight).

Group & Treatment	Body weight	Testis	Epididymis	Caput	Cauda
I (1 mL Propylene Glycol/rat)	185.00 ± 1.48	658.20 ± 0.57	274.17 ± 0.30	124.16±1.02	105.10 ± 1.84
II 125 mg in 1 mL Propylene Glycol / kg body weight	183.61 ± 1.25	655.18 ± 0.73	272.65 ± 1.10	122.86 ± 0.68	103.71 ± 1.60
III 250 mg in 1 mL Propylene Glycol / kg body weight	174.00 ± 2.45	643.27 ± 0.82*	265.19 ± 1.13*	116.20 ± 0.71*	99.91 ± 1.30*
IV 375 mg in 1 mL Propylene Glycol / kg body weight	175.20 ± 2.15	640.10 ± 1.12*	263.80 ± 2.05*	112.15 ± 0.55*	97.19 ± 1.24*

Data are mean ± S.E.M. of 10 replicates.

* Significant difference at $p < 0.05$ level, when compared with control group.

Table 2: Effect of the treatment of lyophilized *A. indica* leaf extract on various biochemical analysis of the testis.

Group & Treatment	Protein (mg/g)	Sugar (mg/g)	Glycogen (mg/g)	Cholesterol (mg/g)	ACP (mM/g/hr)	ALP (mM/g/hr)	LDH (µM/g/hr)
I (1 mL Propylene Glycol/rat)	37.10 ± 2.25	0.60 ± 0.05	4.66 ± 1.33	3.15 ± 0.12	2.96 ± 0.67	1.22 ± 0.72	268.02 ± 5.78
II 125 mg in 1 mL Propylene Glycol / kg body weight	29.63 ± 1.16	1.89 ± 0.10*	7.18 ± 1.80*	7.65 ± 0.60*	1.73 ± 0.04	2.69 ± 0.68	382.40 ± 3.29
III 250 mg in 1 mL Propylene Glycol / kg body weight	20.48 ± 1.47*	3.18 ± 0.14**	10.87 ± 1.54**	10.46 ± 1.38**	0.87 ± 0.08**	4.20 ± 1.21**	674.15 ± 6.59*
IV 375 mg in 1 mL Propylene Glycol / kg body weight	16.23 ± 1.65**	4.08 ± 0.12**	12.10 ± 1.20**	11.50 ±1.71**	0.65 ±0.12**	5.93 ± 0.32**	786.0 ±3.60**

Data are mean ± S.E.M. of 10 replicates.

* Significant difference at $p < 0.05$ level, when compared with control group.

** Significant difference at $p < 0.001$ level, when compared with control group.

Table 3: Effect of the treatment of lyophilized *A. indica* leaf extract on various biochemical analysis of the caput of epididymis.

Group & Treatment	Protein (mg/g)	Sugar (mg/g)	ACP (mM/g/hr)	ALP (mM/g/hr)	LDH (µM/g/hr)
I (1 mL Propylene Glycol/rat)	**37.19 ± 2.20**	**0.59 ± 0.12**	**11.15 ± 0.12**	**1.23 ± 0.14**	**307.65 ± 1.48**
II 125 mg in 1 mL Propylene Glycol / kg body weight	36.10 ± 1.71	1.56 ± 0.16*	8.64 ± 0.65	3.80 ± 0.24*	406.10 ± 2.10*
III 250 mg in 1 mL Propylene Glycol / kg body weight	20.12 ± 0.46*	2.10 ± 0.25**	5.95 ± 0.16*	5.60 ± 0.19**	720.24 ± 2.33**
IV 375 mg in 1 mL Propylene Glycol / kg body weight	16.90 ± 1.80**	2.90 ± 0.15**	5.05 ± 0.15**	6.65 ± 0.15**	789.14 ± 1.71**

Data are mean ± S.E.M. of 10 replicates.

* Significant difference at $p < 0.05$ level, when compared with control group.

** Significant difference at $p < 0.001$ level, when compared with control group.

Table 4: Effect of the treatment of lyophilized *A. indica* leaf extract on various biochemical analysis of the cauda of epididymis.

Group & Treatment	Protein (mg/g)	Sugar (mg/g)	ACP (mM/g/hr)	ALP (mM/g/hr)	LDH (µM/g/hr)
I (1 mL Propylene Glycol/rat)	37.19 ± 1.54	0.68 ± 0.26	7.95 ± 0.23	1.36 ± 0.17	365.19 ± 2.20
II 125 mg in 1 mL Propylene Glycol / kg body weight	30.42 ± 0.81	3.70 ± 0.20*	7.10 ± 0.20	3.06 ± 0.03*	760.35 ± 2.16*
III 250 mg in 1 mL Propylene Glycol / kg body weight	22.64 ± 1.23*	5.22 ± 0.18**	6.42 ± 0.18*	5.59 ± 0.13**	863.28 ± 2.57**
IV 375 mg in 1 mL Propylene Glycol / kg body weight	17.83 ± 1.72**	5.76 ± 0.18**	4.90 ± 0.08**	6.86 ± 0.04**	921.23 ± 1.17**

Data are mean ± S.E.M. of 10 replicates.

* Significant difference at $p < 0.05$ level, when compared with control group.

** Significant difference at $p < 0.001$ level, when compared with control group.

organs and reduction in accessory sex organ weight is a reflection of decreased testosterone level in the blood (Chandra et al., 2007, Gupta et al., 2007). Treatment of male reproductive organs with lyophilized *A. indica* leaf extract reveals accumulation of glycogen and cholesterol, a decrease in the protein and ACP activity and an increase in the activities of ALP and LDH in the testis and epididymis suggesting that the carbohydrate nature of this lyophilized leaf extract execute antiandrogenic quality as the above said biochemical parameters are androgen sensitive. It has been shown that a significant reduction in the total protein content in the testes of male albino rats occurs when treated with methanolic *Dendrophthoe falcata* stem extract (Gupta et al., 2007). Since, the most pronounced general metabolic action of the androgen is the promotion of protein anabolism (Steinberger, 1971), the reduction in protein content observed in the present study would have resulted from a general disturbance in the protein anabolism in the testis and reproductive organs.

It is generally known that any interference in the normal reproductive physiology results in decreased carbohydrate metabolizing enzymes like pyruvate kinase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (Aruldhas et al., 1982a, 1982b). Any such decrease in the levels of such enzymes of the pentose phosphate pathway would result in decreased sugar utilization (Verma et al., 1980). In the present study, the increased level of glycogen in the testis reflects accumulation of non-utilizable sugars in the Sertoli cells due to an arrested spermatogenesis. Apparently, a significant increase in the total free sugar content of epididymis treated rats may be due to a decrease in the carbohydrate metabolizing enzymes of pentose phosphate pathway, resulting in accumulation of sugar in the target organs.

Cholesterol is involved in testicular steroidogenesis and is the most important precursor in the synthesis of steroid hormones. In this study, the high cholesterol content observed in the testis and epididymis also indicates the reduction in the androgen production due to lowered conversion of cholesterol to androgens which depends on the pituitary gonadotrophins (Vijaykumar et al., 2004, Yakubu et al., 2007). The accumulation of cholesterol indicates reduced steroidogenesis by testis and forms a direct evidence for the antiandrogenic action (Hammami et al., 2008).

Both ACP and ALP are sensitive functional indicators of the reproductive status of animal. Experimental studies showed that ACP is widely distributed in lysosomes of Sertoli cells, spermatogonia and late spermatids (Chemes, 1986) and activities of free lysosomal enzymes have been shown to rise when testicular steroidogenesis is increased (Mathur and Chattopadhyay, 1982). In the present study, the decrease in ACP activity might reflect decreased testicular function in the treated rats and might be associated with the reduced secretion of testosterone. Indeed; decline activity of ACP in the epididymis is due to decreased activity of spermatozoa, reflecting decreased androgen output. Changes in reproductive organs of male rats exposure to hypoxia showed that there was an increase in ALP in the epididymis. The increase in these enzymes may be concerned with resorption and lysosomal action for quick disposal of enormous numbers of abnormal or dead spermatozoa found in the lumen of the epididymis (Riar et al., 1979). Increase in the ALP of testis and epididymis might have caused an unfavorable environment for the morphology of sperm and their survival (Akbarsha et al., 1990, Yakubu et al., 2007). Taking all the above findings into consideration ACP and ALP biochemically provide indirect evidence that the treatment of lyophilized *A. indica* leaf extract exerts a decreasing effect on ACP and an increase in ALP in the testis and epididymis.

The LDH is a very important marker of testicular function and cytotoxicity (Jagetia et al., 2000) and it is present in pachytene spermatocytes, spermatids and mature spermatozoa, as well as in the matrix of sperm mitochondria (Burgos et al., 1995). In this study, a considerable elevation in the LDH activity in testis and epididymis is due to *A. indica* leaf powder-induced damage of seminiferous epithelium and several sperm abnormalities with complete absence of plasma membrane and disorganization of the mitochondrial sheath in the epididymis, leading to reduced fertilizing ability of the sperm as seen in our previous experimental conditions (Aladakatti & Nazeer Ahamed, 2005b, 2006).

In conclusion, the oral administration of lyophilized *A.indica* leaf extract to male rats produced dose-related effects on biochemical parameters of testis and epididymis. The functional state of male accessory reproductive organs can be accurately evaluated by determining the chemical composition (Turner and Bagnara, 1976). It can be suggested that a change in the chemical composition of the testis and the epididymis in the present study is probably due to a deficiency in the level of circulating androgen; the results indirectly reflect the antiandrogenic property of the lyophilized *A. indica* leaf extract. Based on the present findings, it can be concluded that the lyophilized *A. indica* leaf extract possess one or more constituents which probably affect the androgen synthesis and thus exhibit antiandrogenic effects on androgen sensitive target glands like testis and epididymis. Effects such as decrease in the weight of testis and epididymis and changes in androgen are sensitive biochemical parameters that clearly indicate some dwindling in the androgenic status of the treated rats. Though, these conclusions are based on a preface study where the rats were forced fed with the lyophilized leaf extract of *A. indica*, additional, advanced and sophisticated studies involving separation procedures, classification of active principles and more critical study on their effects on androgen dependent sites of the albino rat are relevant.

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