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Carbohydrate nature of lyophilized leaf powder of *Azadirachta indica* induced inhibition of the activities of accessory reproductive ducts in male rats

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Abstract
Reports have been shown that the chemical nature of leaf powder/extract was carbohydrate-rich in nature. The purpose investigation is designed to investigate the dose dependent effect of carbohydrate nature of lyophilized *A.indica* leaf powder on some of the androgen dependent biochemical parameters in the reproductive organs which include epididymis (divided in to caput, corpus and cauda), vas deferens, seminal vesicle and ventral prostate of treated rats given 25, 50 and 75 mg in suspension of 1 mL Propylene Glycol, respectively/kg body weight, once daily, orally, for 24 days. Results indicated no significant difference in the body weight of all treated animals. However, there was decreased ($P \leq 0.05$) in the weight of accessory reproductive organs. The biochemical analysis showed a dose dependent decrease ($P \leq 0.05$) in the total protein content and the activity of acid phosphatase (ACP) and an increase ($P \leq 0.05$) in the total free sugar, glycogen, cholesterol contents and the activities of alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in the reproductive organs of treated rats. Blood serum levels of testosterone, LH and FSH in treated groups were significantly ($P \leq 0.05$) reduced respectively when compared to controls. In the present study, the changes in biochemical composition of androgen dependent reproductive organs suggest a deficiency in the level of circulating androgen and decrease in the serum hormone levels in the lyophilized *A.indica* leaf powder treated animals clearly indicates the action of carbohydrate-rich leaf powder on the secretion of pituitary gonadotropins and inturn in the testosterone biosynthesis in the testis and reproductive organs.

Keywords: *A. indica* leaf powder, Reproductive organs, Biochemical, radioimmunoassay, Albino rats.

INTRODUCTION

Azadirachta indica A. Juss (Syn: *Melia Azadirachta* L, Meliaceae family) has been extensively used in the Ayurvedic system of medicine for a long time. Various parts of this plant are used for the treatment of various diseases. The medicinal utilities have been described especially for neem leaf [1] and its constituents are reported to exhibit immunomodulatory, anti-inflammatory, antihyperglycemic, antiulcer, antifungal, antibacterial, anticarcinogenic, nematicidal, antimalarial, insecticidal, and antioxidant properties [1, 2]. It has been reported that the crude oral administration of *A.indica* leaves cause decline in the fructose content of vas deferens [3]; ultrastructural changes in prostate gland, vas deferens and cauda epididymal epithelial cell types [4,5]; testis and cauda epididymal spermatozoa [6,7] and several such effects appear reversible [8].

Further, *in vitro* and *in vivo* experimental studies were shown that the lyophilized *A.indica* leaf powder is carbohydrate in nature and act as spermicidal in rat and human spermatozoa [9,10]; affecting the androgen dependent biochemical parameters of rat testis and epididymis [11]; and elicit depletion of antioxidant defense system by decreasing the activities of antioxidant enzymes and increasing the levels of lipid peroxidation and hydrogen peroxide generation in rat epididymal spermatozoa [12]. Though, various experimental studies reported by using this plant source on biochemical parameters, the purpose of the present investigation is to know the dose dependent effect of carbohydrate nature of lyophilized *A.indica* leaf powder on some of the androgen dependent biochemical parameters like, total protein, total free sugar, the enzymes like acid phosphatase (ACP), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in the reproductive organs which include epididymis (divided in to caput, corpus and cauda), vas deferens, seminal vesicle and ventral prostate of both control and dose dependent treated groups. In addition to these parameters, we further analyzed blood serum concentration of gonadotrophins (LH and FSH) and testosterone by radioimmunoassay (RIA) to confirm that whether this lyophilized *A.indica* leaf powder affects the process of spermatogenesis through the deprived androgen level mediated through the pituitary gonadotropins.

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MATERIALS AND METHODS

Preparation of neem lyophilized leaf powder

An aqueous extract was prepared from *A.indica* (neem) leaves. Neem leaves were ground in a mixer and filtered with 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 lyophilized leaf powder

The chemical nature of ingredients of lyophilized leaf powder was studied for the presence of lipid, protein and carbohydrate. Preparation of Fehling's solution for the carbohydrate estimation was performed as described elsewhere [10, 13]. Briefly, to the warm Fehling's solution, lyophilized leaf powder was added and the mixture was heated after each addition. The production of yellow or brownish-red cuprous oxide indicates that reduction has taken place. The differences in color of the cuprous oxide precipitates under different conditions are apparently due to difference in the size of the particles, the more finely divided precipitates having a yellow color, while the coarser ones are red.

Animals and treatment

Colony bred healthy adult male albino rats (Wistar strain) weighing 200 g were utilized for experiments. All animals were proven fertility 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 diet and water was given *ad libitum*. 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 powder suspended in 1 mL Propylene Glycol (25 mg/kg body weight);

Group III: Lyophilized *A.indica* leaf powder suspended in 1 mL Propylene Glycol (50 mg/kg body weight); and

Group IV: Lyophilized *A. indica* leaf powder suspended in 1 mL Propylene Glycol (75 mg/kg body weight).

The lyophilized *A. indica* leaf powder was then mixed with propylene glycol as required and administered orally (gavage) to the experimental animals [14]. The Propylene Glycol and the graded doses of lyophilized *A. indica* leaf powder were administered orally (gavage) on daily basis for 24 days. Twenty-four hours after the last dose, the control and treated animals were sacrificed by cervical dislocation. The reproductive organs viz., epididymis, vas deferens, seminal vesicle and ventral prostate were dissected out blotted free of mucus and used for biochemical parameters and blood samples were collected to analyze the serum concentrations of gonadotrophins (FSH and LH) and testosterone in both the control and experimental rats.

Biochemical estimation of reproductive organs

A small quantity of each reproductive 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 the biochemical estimation of total protein, total free sugar contents and the activities of ACP, ALP and LDH using Hitachi.U.V. Vis. Spectrophotometer.

Estimation of total protein

The total protein content of each reproductive tissue was estimated by the method of Lowry et al. [15] 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 each reproductive tissue was estimated following the method of Folin and Wu [16]. The O.D. was recorded at 420 nm and expressed as mg sugar 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 [17]. 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 LDH

LDH levels in each reproductive tissue were determined by the method of King [18].The intensity of the colour was measured at 440 nm and expressed as µMole/gm/hr.

Radioimmunoassay (RIA) of gonadotrophins and testosterone

For the determination of serum levels of testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), on completion of the treatment, blood samples from control and treated groups were collected by cardiac puncture under light ether anesthesia and allowed to clot at room temperature for 30 min. The serum was then collected, centrifuged at 2000×g for 10 min and the clear supernatant was used for hormone assays [19]. The assays were performed using the commercial enzyme immunoassay kits as per manufacturer's instructions (Omega Diagnostics, UK). The sensitivity of the assays was 0.5 mg/l for LH, 0.65 mg/l for FSH and 10 Pg for testosterone. Hormone concentrations in the each sample were calculated from standard curves and expressed as ng/ml.

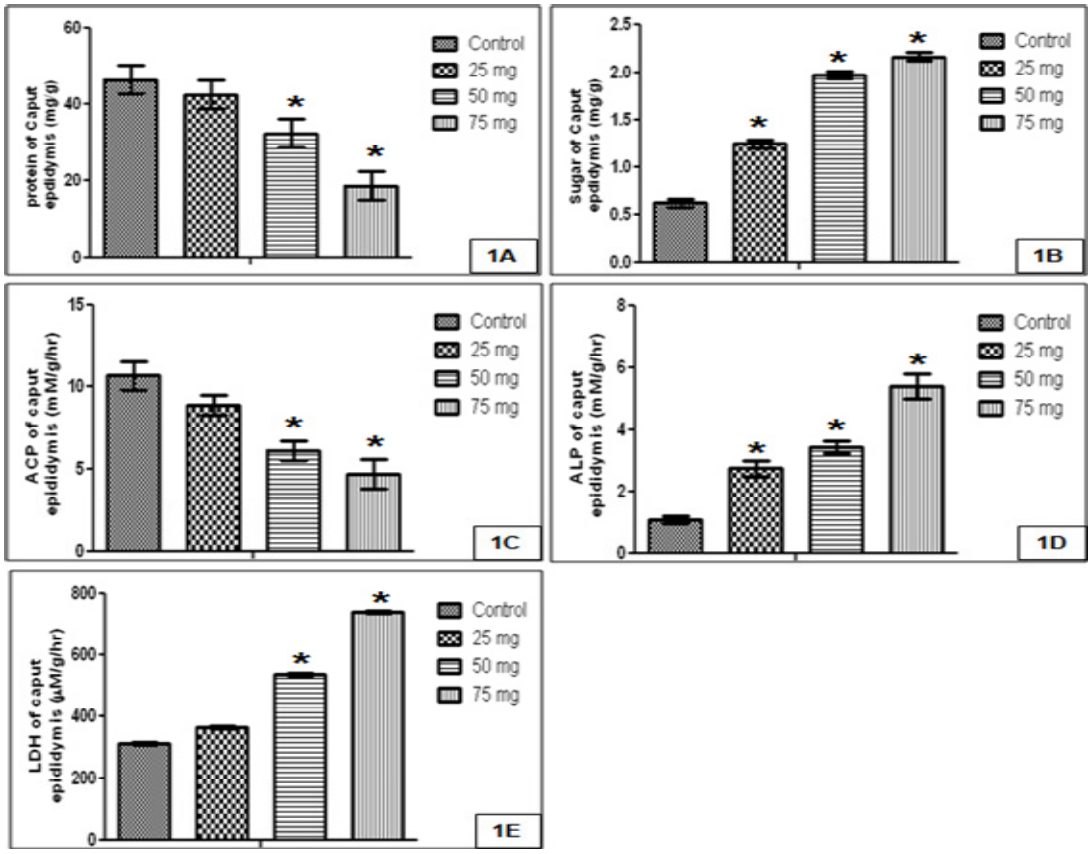
Statistical analyses

Data were analyzed using one way analysis of variance (ANOVA) using the Graph Pad Prism software method, followed by Dunnet test by comparing all treated groups against controls. Values represented are mean ± SEM (n=3). $P \leq 0.05$ is considered to indicate a significant difference between experimental and controls.

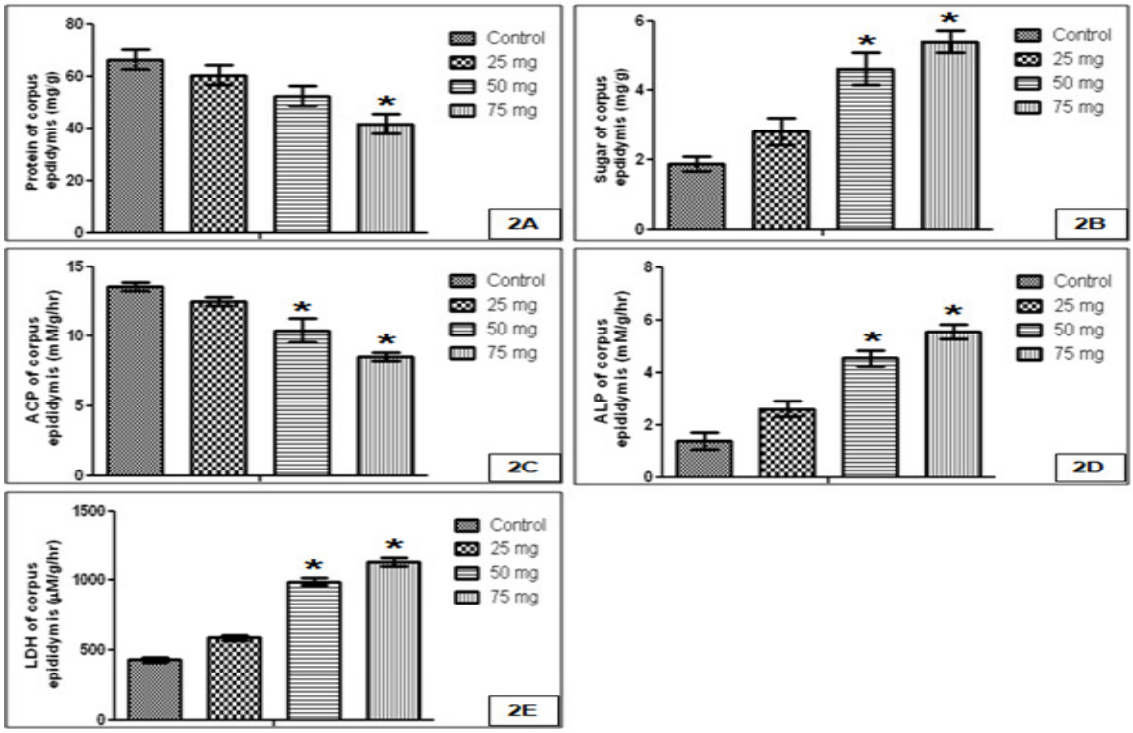
RESULTS

Androgen dependent biochemical parameters in lyophilized *A. indica* leaf powder treated rats

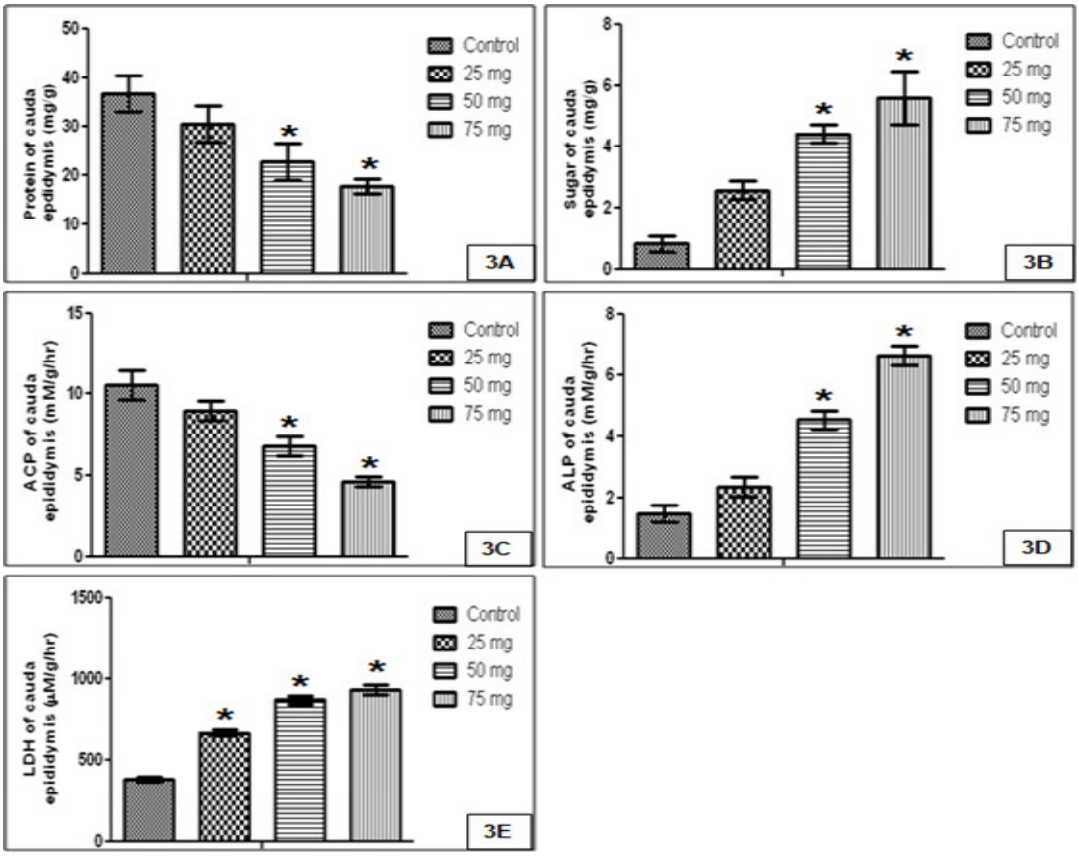
Biochemical composition of protein, free sugar content, ACP, ALP and LDH in the epididymis (caput, corpus and cauda), vas deferens, seminal vesicle and ventral prostate were observed in the control and dose dependent concentration of lyophilized *A. indica* leaf powder treated rats (Graphs.1-6).



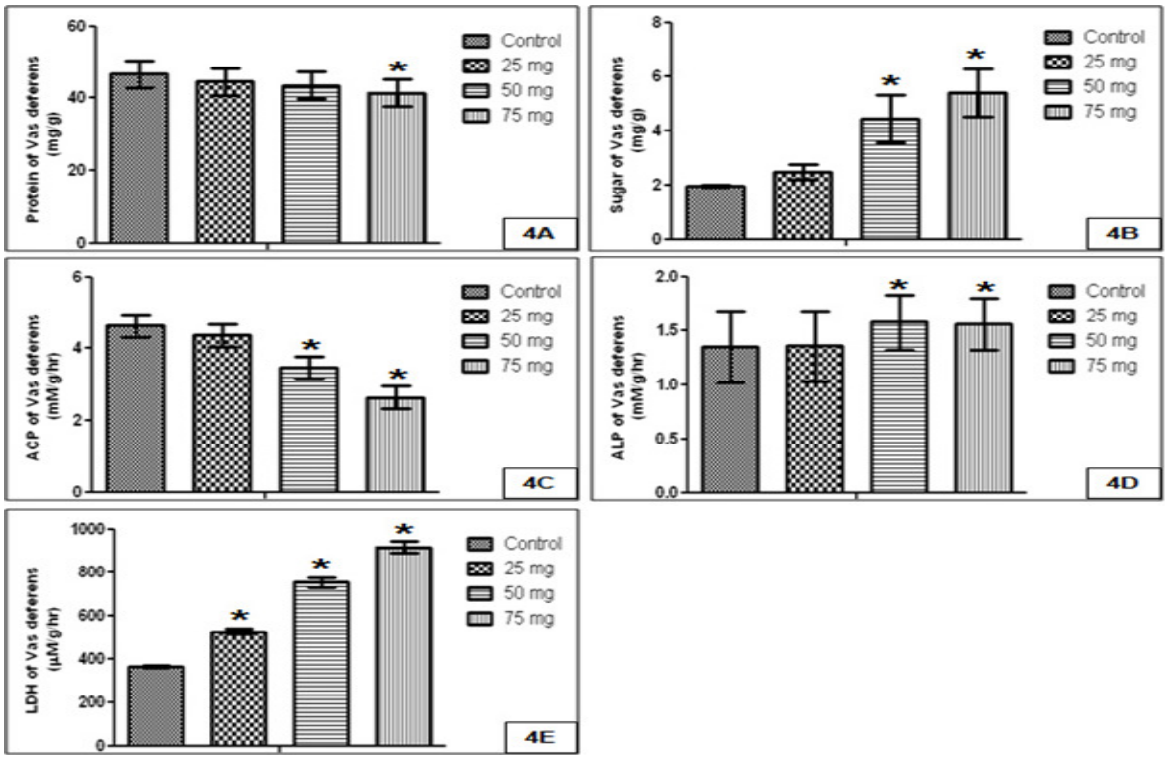
Graphs.1A-E. Dose dependent effect of lyophilized *A.indica* leaf powder suspended in 1 mL Propylene Glycol/kg body weight on caput epididymal biochemical parameters of protein 1A, sugar 1B, ACP 1C, ALP 1D and LDH 1E respectively for period of 24 days. Values are mean ± SEM n = 10 and * indicates significant P ≤ 0.05 compared to control.



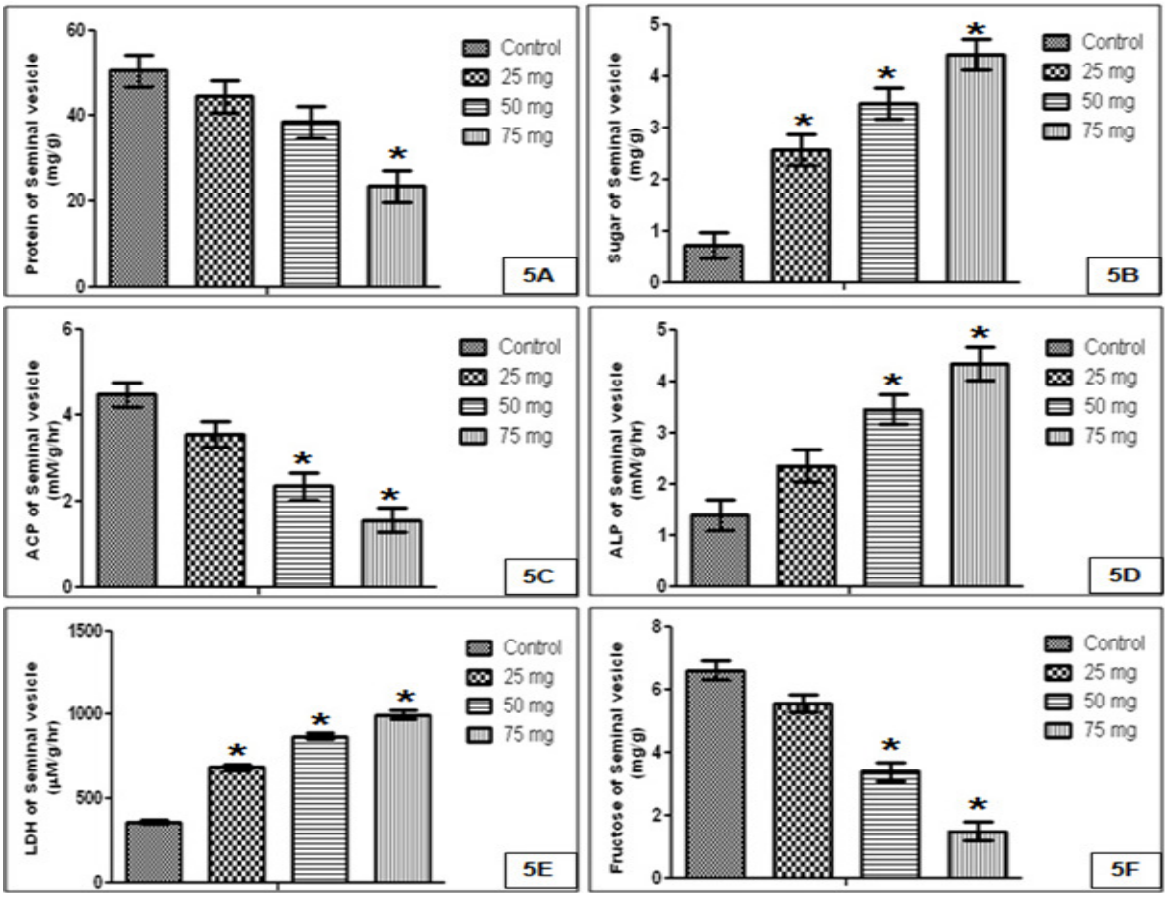
Graphs.2A-E. Dose dependent effect of lyophilized *A.indica* leaf powder suspended in 1 mL Propylene Glycol/kg body weight on corpus epididymal biochemical parameters of protein 2A, sugar 2B, ACP 2C, ALP 2D and LDH 2E respectively for period of 24 days. Values are mean ± SEM n = 10 and * indicates significant P ≤ 0.05 compared to control.



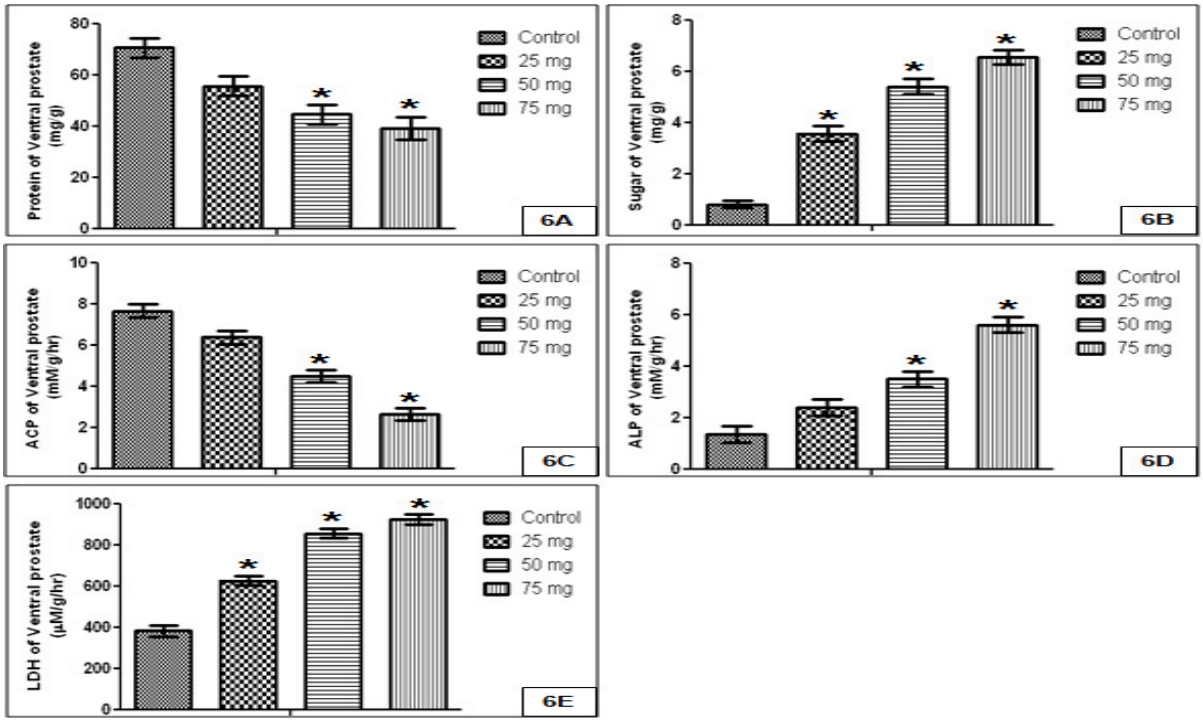
Graphs.3A-G. Dose dependent effect of lyophilized *A.indica* leaf powder suspended in 1 mL Propylene Glycol/kg body weight on cauda epididymal biochemical parameters of protein 3A, sugar 3B, ACP 3C, ALP 3D and LDH 3E respectively for period of 24 days. Values are mean ± SEM n = 10 and * indicates significant P ≤ 0.05 compared to control.



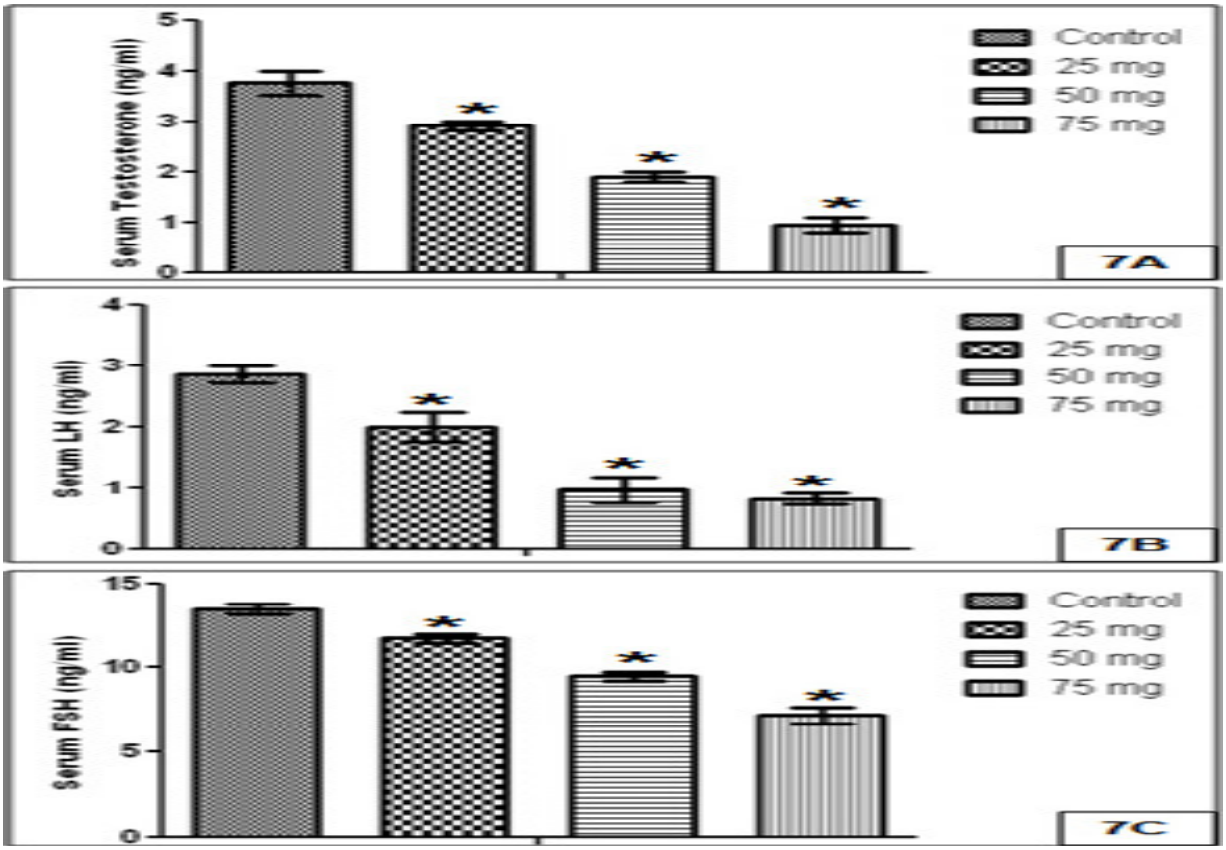
Graphs.4A-E. Dose dependent effect of lyophilized *A.indica* leaf powder suspended in 1 mL Propylene Glycol/kg body weight on vas deferens biochemical parameters of protein 4A, sugar 4B, ACP 4C, ALP 4D and LDH 4E respectively for period of 24 days. Values are mean ± SEM n = 10 and * indicates significant P ≤ 0.05 compared to control.



Graphs.5A-E. Dose dependent effect of lyophilized *A.indica* leaf powder suspended in 1 mL Propylene Glycol/kg body weight on seminal vesicle biochemical parameters of protein 5A, sugar 5B, ACP 5C, ALP 5D, LDH 5E and fructose content 5F respectively for period of 24 days. Values are mean ± SEM n = 10 and * indicates significant P ≤ 0.05 compared to control.



Graphs.6A-E. Dose dependent effect of lyophilized *A.indica* leaf powder suspended in 1 mL Propylene Glycol/kg body weight on ventral prostate biochemical parameters of protein 6A, sugar 6B, ACP 6C, ALP 6D and LDH 6E respectively for period of 24 days. Values are mean ± SEM n=10 and * indicates significant P ≤ 0.05 compared to control.



Graphs.7A-C. Dose dependent effect of lyophilized *A.indica* leaf powder suspended in 1 mL Propylene Glycol/kg body weight on blood serum levels of testosterone 7A, LH 7B and FSH 7C respectively for period of 24 days. Values are mean ± SEM n = 10 and * indicates significant P ≤ 0.05 compared to control.

Change in protein content

The protein content of control rat's caput, corpus and cauda epididymis (Group I) was 46.50 ± 3.75 (Graph.1A), 66.39 ± 3.48 (Graph.2A) and 36.60 ± 3.70 (Graph.3A) mg/gm respectively. In 25 mg/kg body weight of lyophilized *A. indica* leaf powder treated rats (Group II), the content of caput, corpus and cauda epididymis was reduced and difference was insignificant against the control. Where as in the rats belonging to the 50 mg and 75 mg treated animals (Groups III and IV), the content was significantly (P ≤ 0.05) decreased. However, 50 mg/kg body weight leaf powder treated rats exhibited no significant difference in the content of corpus epididymis when compared to controls (Graph.2A). In vas deference and seminal vesicle of Group IV animals, the content was decreased significantly (P ≤ 0.05) and there was insignificant difference in Groups II and III when compare to controls [(Groups I, 46.63 ± 3.70 mg/gm (Graph.4A) and 50.53 ± 3.54 mg/gm (Graph.5A)]. However, the content in the ventral prostate belonging to the 50 mg and 75 mg treated animals (Groups III and IV), was decreased significantly (Graph.6A, P ≤ 0.05) and no difference in the 25 mg/kg body weight of lyophilized *A. indica* leaf powder treated animals against the control (70.58 ± 3.57 mg/gm).

Change in free sugar content

The free sugar content of control rat's caput, corpus and cauda epididymis (Group I) was 0.62 ± 0.04 (Graph.1B), 1.88 ± 0.22, (Graph.2B) and 0.81 ± 0.26 (Graph.3B) mg/gm respectively. The content of caput was significantly (Graph.1B, P ≤ 0.05) increased in all graded doses of 25, 50 and 75 mg/kg body weight of lyophilized *A. indica* leaf powder treated animals (Groups II, III and IV). However, in corpus and cauda epididymis in rats (Groups III and IV), there was significant increased (P ≤ 0.05) in the content and in insignificant in the Group I when compared to control (Graphs. 2B and 3B). In vas deference, the content was increased significantly (P ≤ 0.05) in of Groups III and IV animals (Graph.4B) and there was insignificant difference in Groups II when compare to controls (Groups I, 1.95 ± 0.05 mg/gm). Whereas, the content of seminal vesicle (0.71 ± 0.25 mg/gm) and ventral prostate (0.79 ± 0.14 mg/gm) was significantly (P ≤ 0.05) increased in all graded doses of 25, 50 and 75 mg/kg body weight of lyophilized *A. indica* leaf powder treated animals (Groups II, III and IV) when compared to control (Graphs.5B and 6B).

Change in acid phosphatase activity (ACP)

In control rat's ACP activity (Group I) was in caput, corpus and cauda epididymis [10.67 ± 0.88 (Graph.1C), 13.55 ± 0.30 (Graph.2C); 10.55 ± 0.87(Graph.3C) mM/g/hr respectively], vas deference (Graph.4C, 4.63 ± 0.32 mM/g/hr), seminal vesicle (Graph.5C, 4.48 ± 0.28 mM/g/hr) and ventral prostate (Graph.6C, 7.65 ± 0.32 mM/g/hr). However, rats belonging to the 50 and 75 mg/kg body weight of lyophilized *A. indica* leaf powder treated rats (Groups III and IV), there was a significant decrease in the activity of all organs (P ≤ 0.05). Where as in 25 mg treated rats (Group II), the activity of organs was reduced but the difference was insignificant against the control.

Change in alkaline phosphatase activity (ALP)

The ALP activity of control rats' caput, corpus and cauda epididymis (Group I) was 1.07 ± 0.12 (Graph.1D), 1.38 ± 0.31 (Graph.2D) and 1.48 ± 0.28 (Graph.3D) mM/g/hr respectively. In 50 and 75 mg/kg body weight of lyophilized *A. indica* leaf powder treated rats (Groups III and IV), the activity was significantly ($P \leq 0.05$) increased. However, the activity of caput epididymis was increased significantly ($P \leq 0.05$) in 25 mg/kg body weight treated rats (Group II) and rest of corpus and cauda epididymis exhibited no significant difference in corpus epididymis when compared to controls (Graph.1D). In Groups III and IV, vas deferens (Graph.4D), seminal vesicle (Graph.5D) and ventral prostate (Graph.6D) exhibited a significant ($P \leq 0.05$) increase in the activity. Whereas, there was absolutely no difference or insignificant difference in the activity of these organs (Groups II) when compare to controls (Groups I, 1.34 ± 0.33, 1.39 ± 0.31 and 1.35 ± 0.32 mM/g/hr, respectively).

Change in lactate dehydrogenase activity (LDH)

In control rats (Group I), the LDH activity was in caput, corpus and cauda epididymis [315.2 ± 14.3 (Graph.1E), 428.8 ± 16.1 (Graph.2E); 379.6 ± 15.8 (Graph.3E) µM/g/hr respectively], vas deference (Graph.4E, 382.1 ± 26.4 µM/g/hr), seminal vesicle (Graph.5E, 359.4 ± 11.6 µM/g/hr) and ventral prostate (Graph.6E, 7382.1 ± 26.4 µM/g/hr). All organs exhibited the significant increased activity ($P \leq 0.05$) in all graded doses of 25, 50 and 75 mg/kg body weight of lyophilized *A. indica* leaf powder treated animals (Groups II, III and IV). However, in caput and corpus epididymis (Group II), exhibited no significant difference in the activity when compared to controls (Group I).

Change in fructose content

In the seminal vesicle of the control rats (Group I) the fructose content was 6.59 ± 0.30 mg/g. In 25 mg/kg body weight of lyophilized *A. indica* leaf powder treated animals (Group II), the content was reduced to 5.54 ± 0.29 mg/g. The difference was insignificant against the control. Where as in the rats belonging to the 50 mg and 75 mg treated animals (Groups III and IV) groups, the content (3.36 ± 0.32 and 1.48 ± 0.28 mg/g respectively) was significantly ($P \leq 0.05$) decreased when compared to controls (Graph. 5F).

Hormone levels in blood serum

The serum level of testosterone (Graph.7A), LH (Graph.7B) and FSH (Graph.7C) in all graded doses of 25, 50 and 75 mg/kg body weight of lyophilized *A. indica* leaf powder treated animals, were significantly ($P \leq 0.05$) reduced in a dose dependent manner when compared to controls (Group I, 3.76 ± 0.24 ng/ml, 2.85± 0.14 ng/ml and 13.46 ± 0.29 ng/ml respectively).

DISCUSSION

The accessory system of male ducts and glands are morphologically and physiologically dependent upon the production of androgens [20]. The reproductive system in male is governed by a system of hormones which stimulate the spermatogenic, androgenic functions of the testis and maintain the structural and functional properties of sex accessory glands [21]. Testosterone being an important role for the maintenance of accessory sex organs. In turn the synthesis and release of androgens depends on the availability of pituitary gonadotrophins like FSH and LH/ICHS [22]. Most of medicinal plant extracts have been reported as spermicidal/ antiandrogenic nature by changing in the testicular and other accessory organs and altered biochemical and hormone levels in rats and mice [23]. In the present study, a decrease in the concentrations of protein and ACP activity and increase in total free sugar, ALP and LDH activities of the reproductive organs like epididymis, vas deferens, seminal vesicle and ventral prostate on treatment with lyophilized *A.indica* leaf extract reveals the antiandrogenic property as above mentioned biochemical parameters are androgen sensitive. These observations are similar to those found in studies which reported that the structural and functional integrity of the reproductive organs depends on the circulating level of the androgen, and any small change in the androgen level results in the reduction in the biochemical parameters of organs leading to reduction of fertility [11, 24-31].

It has been reported that protein level is directly correlated with the secretory activity of the testis and accessory glands, which in turn depends on the androgen levels [32]. The most pronounced general metabolic action of the androgen is the promotion of protein anabolism [33]. Protein decrease primarily from the peripheral cells of epididymal epithelium and it may be one of the constituents that ensure the maturation of sperms. Androgen regulates the synthesis of epididymal proteins in rats and castration reduces the protein composition of the epididymal fluid and causes lysis of epididymal sperms. The vas deferens of mammals, with emphasis on laboratory rodents, plays other roles through synthesis and secretion of proteins [34].The reduction in protein content observed in the present study may be attributed to the reduction in secretory activity of the testis and other organs because of the androgen deprivation effect which indirectly indicates the anti-androgenic property of lyophilized *A.indica* leaf powder.

The sugar is an important source of energy and optimum concentration of sugar is required for the proper functioning of spermatozoa and when the concentration of sugar is increase, the metabolism of spermatozoa will be affected and hypertonic environment will be created which affects the sperm motility adversely [35]. It is generally known that any interference in the normal reproductive physiology would result in decrease of carbohydrate metabolizing enzymes [36]. Any such decrease in the levels of such enzymes would result in under utilization of sugar and hence its accumulation in the target organs [11, 37]. Taking into consideration the above reports, it can be presumed that an increase in the total free sugar content of reproductive organs of lyophilized *A.indica* leaf extract treated rats may be due to a decrease in the carbohydrate metabolizing enzymes, resulting in accumulation of sugar in the target organs.

Both ACP and ALP are sensitive functional indicators of the reproductive animal status and thus responsible for the secretory concentration of target organs. The effects of androgen on the target organs are mediated through ACP and ALP [38, 39]. Altering in these enzymes might have caused an unfavorable environment for the morphology of sperm and their survival [40. In the present findings of decreased activity of ACP in the reproductive organs of lyophilized *A.indica* leaf extract treated rats is reflecting decreased androgen output. The increased activity of ALP may be suggested as due to increased transport of organic and inorganic materials across

the cell membranes due to degeneration and lysis of sperms. The tissues levels of ACP and ALP in the reproductive organs indicates that the secretory function of these organs was impaired and decline in the ACP and increase in ALP activity possibly due to the decrease in serum testosterone and as well as degeneration of testicular germ cells [39]. Taking all the above findings into consideration ACP and ALP are 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 reproductive organs. This agrees with the observations of pomegranate peel extracts [29]; aqueous extract of *Chromolaena odoratum* [29]; lyophilized *A.indica* leaf powder in rats [11] and suggested the suppression of androgen biosynthesis is due to treatment of extract.

It is well known as the increase in LDH level in the reproductive organs is elevation of the substrate lactate level as LDH is one of the key enzymes in Embden-Mayeroff pathway of carbohydrate metabolism and the low activity of the enzyme has been used as a marker for active spermatogenesis. Increased LDH activity indicates increased anaerobic glycolysis. It is suggested that increased activity of LDH is a shift in the tissue respiration from anaerobic to aerobic type which would be adverse to the metabolism of organs [25, 41]. In the present study, a considerable elevation in the reproductive organs LDH activity of treated rats indicate the production of substrate lactate, suggesting a switch over from aerobic to anaerobic type of respiration or altered physiological/metabolic activity which may have a definite influence on androgen-regulated glycolytic enzyme activities in the male accessory organs, thereby indirectly affecting the secretory activities of these tissues.

The fructose content of the seminal vesicle content is a better 'marker' for the functioning of seminal vesicle [42]. There is a close interdependence between the chemical composition of seminal plasma and the quantity of androgen present in the semen [38]. It has been shown that the reduction in the fructose content of the seminal fluid may be an indication that the secretory ability of the seminal vesicle was hindered by the extract [43], and this will adversely affect its nutritive potentials for the semen which will in turn affect sperm motility. Such pattern of alterations agrees with studies of administration of *Semecarpus anacardium* fruits [24]; ethanolic extract of root bark of *Cananga odorata* [25]; aqueous extracts of *Fadogia agrestis* stem [27] in reproductive function of male rats. In the present study, the decreased fructose content at the higher doses of 50 and 75 mg lyophilized *A.indica* leaf possibly gives a clue that the changes in biochemical composition suggest a deficiency in the level of circulating androgen; the results indirectly reflect the antiandrogenic property of the lyophilized *A.indica* leaf powder.

It has also been shown that the testosterone level in serum and plasma correlate with sperm concentration and sperm motility [44]. Two possible hypotheses may be proposed to explain the antigonadal activities of the herbal agent. One hypothesis is that the active ingredient(s) of the extract may alter the pituitary gonadotropins hormones i.e. LH and FSH hormone [45]. Low levels of these hormones decrease endogenous testosterone secretion from the testis depriving developing sperm of the signal required for normal maturation and also it suppress testicular steroidogenesis and spermatogenesis [45] since the pituitary-testicular axis is a central regulatory conduit for testicular function that culminates in the production of spermatozoa [46]. Studies carried out on serum hormone profile by using various plant sources and suggested that all these effects were due to the extract's effect on hypothalamic-gonadal axis and inturn significant decrease in the level of assayed gonadotrophins which are essential for the gonadal development and steroidogenesis in male rats [28-31]. It has been demonstrated that histochemical localization of activities of testicular $\Delta^5$3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase declined in a dose-dependent manner on lyophilized *A.indica* leaf powder treatment which is again consistent with the low production of testosterone by Leydig cells since both the enzymes play an important role in steroid hormone synthesis [47]. In the present study, the decrease in the serum concentrations of FSH, LH and testosterone in the lyophilized *A.indica* leaf powder treated animals clearly indicates the action of leaf powder on the secretion of pituitary gonadotropins and inturn in the testosterone biosynthesis in the testis and reproductive organs. Perhaps the dose dependent decrease in serum LH, FSH and testosterone levels observed in our study might have been caused by one of these hypothesized mechanisms, but this is the subject of future research.

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