Anti-androgenic Activity Of Aqueous Leaf Extract OF *Cnidoscolous aconitifolius*

***[1]Yakubu, M. T., [1]Lateef, I. A., [1]Ajiboye, T. O., [1]Akanji, M. A., [1]Oladiji, A. T. , [2]Ibrahim, O. O. K.**

[1] Phytomedicine, Toxicology, Reproductive and Developmental Biochemistry Research Laboratory, Department of Biochemistry, University of Ilorin, Ilorin, Nigeria
[2] Department of Pathology, University of Ilorin, Ilorin, Nigeria.

Abstract

Aim: Aqueous leaf extract of *Cnidoscolous aconitifolius* (a widely consumed vegetable) at the doses of 250, 500 and 1000 mg/kg body weight was investigated for anti-androgenic activity in the testes of Wistar rats.

Method: Twenty male rats (*Rattus norvegicus*) weighing 112.63 ± 15.47 g were completely randomized into four groups: A-D. Animals in Group A (control) were orally administered with 0.5 ml of distilled water once daily for sixty days while those in Groups B, C and D were treated like the control except they received 250, 500 and 1000 mg/kg body weight of the extract respectively.

Results: The extract significantly ($p<0.05$) decreased testes-body weight ratio as well as the levels of testicular sialic acid, glycogen, protein, cholesterol, testosterone, serum luteinizing and follicle stimulating hormones. There was also reduction in the activities of acid phosphatase and gamma glutamyl transferase in the testes of the animals. The extract also distorted the seminiferous tubules, reduced the amount of stroma as well as population of spermatogenic and supporting cells. The spermatocytes within the lumen became scanty with no evidence of spermatogenesis.

Conclusion: Overall, the study suggests that the extract of *C. aconitifolius* leaves possess anti-gonadotropic, anti-androgenic and anti-spermatogenic activities which impaired the normal secretory functioning of the testes and spermatogenesis. The aqueous leaf extract of *C. aconitifolius* may have consequential effects on the fertility of the animals.

Keywords: Anti-androgenic, Anti-gonadotropic, Anti-spermatogenic, *Cnidoscolous aconitifolius*, Euphorbiacae, Fertility

Correspondence to:
Yakubu, M. T. (Ph. D.),
Phytomedicine, Toxicology, Reproductive and Developmental Biochemistry Research Laboratory, Department of Biochemistry, University of Ilorin, PMB 1515, Ilorin, Nigeria. GSM: +2348037544437; Email: tomuyak@yahoo.com; tomuyak@gmail.com

Introduction

The incidence of infertility in humans is increasing empirically and is raising serious concerns. For example, community based data suggest that up to 30 per cent of couples in some parts of Nigeria may have proven difficulties in achieving a desired conception after two years of marriage without the use of contraceptives.[1] Male infertility with its serious negative impact on the social well being of the sufferer has myriad of etiological factors. Some of these include occupational hazards, some life styles (such as alcoholism), sexually transmitted diseases, side effects of drugs and contraceptives, male sexual dysfunction such as erectile dysfunction, premature ejaculation, disorders of the spermatogenetic pathways which presents as weak and watery sperm and anti-androgens. Anti-androgens exert their anti-fertility effect, by their action on hypothalamus-pituitary-gonadal axis or direct hormonal effect on reproductive organs, resulting in the inhibition of spermatogenesis.[2] Although, several botanicals such as *Ruta graveolens*, *Cordia multispicata, Chromolaena odoratum* leaves have been screened for their anti-spermatogenic, anti-androgenic and anti-fertility effects in rats[3-5], there is still the need to screen for more especially leafy vegetables with anti-gonadotropic, anti-androgenic and anti-sprematogenic activities which may eventually confer infertility in male.

Cnidoscolous aconitifolius Miller, (Family-Euphorbiacae) known as tree spinach (English), *efo iyana ipaja*, or *efo Jerusalem* (Yoruba) is commonly found growing in the tropic and sub tropical regions of the world, including Africa, South of Sahara, India, North and South America. The plant is an ornamental, evergreen, deciduous shrub between 3-5 m high.[6] The leaves are palmate lobed and large (32 cm long and 30 cm wide) in size. The leaves which are commonly eaten as vegetable in many localities in Nigeria, provide important nutritional sources of protein, vitamins A and C, calcium, iron, phosphorus, niacin, riboflavin and thiamine.[7] Phytoconstituents of the aqueous leaf extract include alkaloids (2.75%), saponins (2.60%), phenolics (1.86%), tannins (0.93%), flavonoids (0.30%), anthraquinones (0.072%), phlobatannins (0.065%) and

triterpenes (0.039%).[8] The shoots and leaves of *C. aconitifolius* are reportedly used as laxative, diuretic, diabetes, obesity, acne, kidney stone, eye problems, circulation and lactation stimulants.[9] Studies have revealed that the plant has nutritive values when compounded as leaf meal for broiler chicken.[10,11] Furthermore, Oladeinde et al[12] have also reported on the anti-diabetic properties of leaf extract of *C. aconitifolius* in in-bred type 2 diabetic mice. Recently, the ameliorative effects of *C. aconitifolius* on anaemia and osmotic fragility induced by protein energy malnutrition were reported by Oyagbemi et al[13]. In addition, the antimicrobial activity of the essential oil from the leaf of *C. aconitifolius* against *Escherichia coli* and *Salmonella typhi* as well as its potential as an endocrine disruptor that led to hormonal imbalance and consequently infertility in female rats have also been reported.[8-14] Our previous findings on the endocrine disruption potential of the aqueous leaf extract of *C. aconitifolius* in female rats coupled with the on-going screening of botanicals with anti-fertility activity in our laboratory have motivated the present research into the effect of the extract in male rats. Therefore, this study was aimed at evaluating the effect of aqueous leaf extract of *C. aconitifolius* on the secretory functional indices and histopathology of rat testes. This was with a view to determining the anti-gonadotropic, anti-androgenic and anti-spermatogenic and by extension, anti-fertility potentials of the plant extract in male rats.

Materials and methods

Plant materials and authentication

The plant sample, obtained from a single population within the premises of the main campus of University of Ilorin, Ilorin, Nigeria, was authenticated at the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan, Nigeria. A voucher specimen (FHI 10776) was deposited at the herbarium of the Institute.

Laboratory animals

Male albino rats (*Rattus norvegicus*) of Wistar strain weighing 112.63±15.47 g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were housed in clean plastic cages that provided free access to rat pellets (Bendel Feeds and Flour Mills limited, Ewu, Nigeria) and tap water that was free of contaminants. The cages were contained in a well ventilated standard housing conditions (temperature: 28-31ºC; photoperiod: 12 h natural light and 12 h dark; humidity: 50 – 55%).

Assay kits and enzyme substrates

The assay kits for testosterone, luteinizng and follicle stimulating hormones were products of Diagnostic Automation Inc., Calabasas CA, USA, while cholesterol assay kit was a product of Randox Laboratory, Co-Antrim, UK. 4-Nitrophenyl phosphate (disodium salt hexahydrate) and L-Glutamic acid 5-(4-nitroanilide) were products of Sigma-Aldrich CHEME GmbH, Steinheim, Germany. All other reagents used were of analytical grade and were prepared in glass distilled water.

Preparation of extract

A portion of the fresh leaves (358 g) of *C. aconitifolius* was oven dried at 40ºC until a constant weight of 58.28 g was obtained. The dried leaves were pulverized using USHA blender (model MG 2053, New Delhi, India) and the resulting powder stocked in a plastic container. A portion (30 g) of the powder was extracted in 1.3 L of cold distilled water for 48 hours at room temperature. The extract which was filtered with Whatman filter paper № 1 (Maidstone, UK) was thereafter concentrated over steam bath to give a residue of 5.63 g which is equivalent to a percentage yield of 24.39 ± 0.5 %. This was reconstituted in distilled water to give the required doses of 250, 500, and 1000 mg/kg body weight used in this study. The doses were as used previously by Yakubu et al[8].

Animal grouping and extract administration

A total of twenty, sexually experienced, male albino rats, after an initial acclimatization for two weeks were completely randomized into four groups (A-D). Animals in group A (control), received orally, 0.5 ml of distilled water while those in Groups B, C and D were orally administered with same volume of the extract containing 250, 500 and 1000 mg/kg body weight respectively. The administration was done, once daily, for 60 days (to cover one complete spermatogenic cycle made up of 53 days of spermatogenesis and 6-7 days of final transition through the epididymis), using oropharyngeal cannula. The animals were maintained on free access to rat pellets and tap water throughout the exposure period. The study was carried out after approval from the Departmental Ethical Committee on the Use and Care of Experimental Animals. The animals were handled humanely in accordance with the guidelines of European convention for the protection of vertebrate animals and other scientific purposes- ETS-123.[15]

Preparation of serum and testicular supernatants

The animals were sacrificed twenty-four hours after their last dose and the testes homogenates prepared using the method described by Yakubu et al[16]. Briefly, the rats were anaesthetized in diethyl ether fumes after being separately weighed. Under the anaesthesia, the jugular veins which were slightly displaced (to prevent contamination of the blood with interstitial fluid) were

quickly cut with sterile scapel blade and about 5 ml of the blood was collected into clean, dry centrifuge tubes. The blood was allowed to clot for 10 min at room temperature before being centrifuged at 224 g x 15 min using Uniscope Laboratory Centrifuge (Model SM800B, Surgifirend Medicals, Essex, England). The sera were aspirated into clean, dry, sample bottles with the aid of Pasteur pipette and used within 12 hours of preparation. The rats were thereafter quickly dissected and the testes excised. The testes were cleaned of superficial fatty layer, weighed again and then transferred into 0.25M sucrose solution. The organ was later blotted with tissue paper, cut very thinly with sterile scapel blade and homogenized in ice-cold 0.25M sucrose solution (1:5, w/v). The homogenates were further centrifuged at 1398 × *g* for 15 min to obtain the supernatant, which was then aspirated with Pasteur pipette into sample bottle, stored overnight at 4ºC before being used for the biochemical assays.

Determination of biochemical parameters

The testes-body weight ratio of the animals was computed using the expression described by Yakubu et al[16]. Other parameters were quantitatively determined in the testicular supernatant using standard procedures described for glycogen, protein, sialic acid, cholesterol, acid phosphatase (ACP) (EC 3.1.3.2) and Gamma glutamyl transferase (GGT) (EC 2.3.2.2).[17-22] The concentration of testosterone in the testes as well as serum luteinizng and follicle stimulating hormones were determined using the procedure outlined in the Manufacturer's Instruction Manual according to the principles described by Kleerekoper[23].

Histopathological examination

The left testes were fixed in 10% (v/v) formaldehyde, dehydrated through ascending grades of ethanol (70%, 90%, and 95%, v/v), cleaned in xylene, and embedded in paraffin wax (melting point 56 ºC).[24] Tissue sections were prepared according to the procedure described by Drury and Wallington[25] and stained with hematoxylin and eosin (H & E). The photomicrographs were captured at x 400 with software, Presto! Image Folio package.

Statistical analysis

Data were expressed as mean ± SD of five determinations. One way analysis of variance and Duncan Multiple Range Test complemented with Student's t-test were used to account for the differences among the means as well as the interaction between the variables. Differences were considered statistically significant at $p < 0.05$.

Results

Administration of aqueous leaf extract of *C. aconitifolius* at the doses of 250, 500 and 1000 mg/kg body weight for 60 days to male rats significantly ($p<0.05$) reduced all the testicular parameters investigated in this study. All the reductions were however not dose related and at varying degree. For instance, while the reduction in testicular protein, glycogen and testosterone were dose-related, those of sialic acid, GGT, ACP, cholesterol and testes-body weight were not dose dependent (Table I). Furthermore, the extract at 250, 500 and 1000 mg/kg body weight dose dependently decreased ($p<0.05$) testicular protein by 35.59, 47.19 and 76.30% respectively while that of glycogen was 16.90, 27.23 and 50.70% in the animals administered with the graded doses of the extract. In addition, the 250 mg/kg body weight of the extract reduced the testes-body weight ratio by about half the control value whereas the decrease produced by both the 500 and 1000 mg/kg body weight was one-seventh the control value. Although, the extract significantly ($p<0.05$) decreased the levels of sialic acid and cholesterol as well as the activities of GGT and ACP in the testes of the animals, these decreases which were not dose-related ranged from 26.54% to 66.14% of the control values (Table I).

The extract dose dependently decreased the concentrations of both the luteinizing and follicle stimulating hormones in the serum of the animals. In addition, the 250, 500 and 1000 mg/kg body weight of the extract decreased the testicular testosterone concentration of the animals by 47.83, 69.57 and 84.78% respectively (Table II).

Histopathological examination of the control rat testes revealed well preserved seminiferous tubules, normal amount of stroma, normal population of spermatogenic and supporting cells with ample evidence of spermatogenesis (Figure I) whereas all these features were no longer intact in the testis of rats treated with various doses of the extract. For instance, the 250 mg/kg body weight of the extract distorted the seminiferous tubules and was accompanied with disorganised spermatogenic and supporting cells (Figure II). The same dose also reduced spermatogenesis as well as the number of spermatocytes in the lumen of the organ. Furthermore, the 500 and 1000 mg/kg body weight of the extract severely distorted the seminiferous tubules with scanty populations of spermatogenic and supporting cells (Figures III and IV). Evidence of spermatogenesis was also lacking and the number of spermatocytes was very few in the testes of animals administered with these doses.

Discussion

The administration of a chemical compound, including plant extract, may significantly alter the

Table I: Testicular parameters of male rats administered with aqueous leaf extract of *Cnidoscolous aconitifolius*

Animal Grouping	Testes-body weight ratio	Testicular protein (mg/ml)	Testicular glycogen (mg/100mg of tissue)	Testicular sialic acid (µg/mg protein)	Testicular GGT (nM/min/mg protein)	Testicular ACP (nM/min/mg protein)	Testicular cholesterol (mmol/L)	Testicular testosterone (ng/ml)
Control	0.0078 ± 0.002[a]	465.80 ± 72.45[a]	85.2 ± 2.68[a]	38.17 ± 3.49[a]	204.59 ± 34.45[a]	46.91 ± 0.07[a]	2.54 ± 0.33[a]	1.38 ± 0.16[a]
250 mg/kg body weight	0.0038 ± 0.003[b] (51.28)	300.00 ± 44.24[b] (35.59)	70.80 ± 6.5[b] (16.90)	16.58 ± 1.02[b] (56.56)	144.45 ± 24.46[b] (29.40)	24.46 ± 1.63[b] (47.86)	1.26 ± 0.22[b] (50.39)	0.72 ± 0.11[b] (47.83)
500 mg/kg body weight	0.0011 ± 0.001[c] (85.90%)	246.00 ± 16.43[c] (47.19)	62.00 ± 5.48[c] (27.23)	19.26 ± 1.14[c] (49.54)	150.30 ± 15.26[b] (26.54)	27.57 ± 1.08[c] (41.23)	1.54 ± 0.33[c] (39.37)	0.42 ± 0.11[c] (69.57)
1000 mg/kg body weight	0.0010 ± 0.02[c] (87.18)	110.40 ± 4.93[d] (76.30)	42.00 ± 2.08[c] (50.70)	19.65 ± 4.12[c] (48.52)	136.38 ± 7.22[c] (33.34)	29.71 ± 2.81[d] (36.67)	0.86 ± 0.06[d] (66.14)	0.21 ± 0.06[d] (84.78)

Values are means ± SD of 5 replicates
Values in bracket are computed percentage decrease
Statistical tools used were one way ANOVA and Duncan Multiple Range Test complemented with Student's t-test.
[a-d]Test values carrying superscript different from the control down the group for each parameter are significantly different (P <0.05).

Table II: Testicular testosterone and serum luteinizing and follicle stimulating hormones of male rats administered with aqueous leaf extract of *Cnidoscolous aconitifolius*

Animal Grouping	Testosterone (ng/mL, Testes)	Luteinizing Hormone (ng/mL, serum)	Follicle Stimulating Hormone (ng/mL, serum)
Control	1.38 ± 0.16[a]	1.88 ± 0.06[a]	9.24 ± 0.40[a]
250 mg/kg body weight	0.72 ± 0.11[b] (47.83)	1.02 ± 0.07[b] (45.74)	7.21 ± 0.14[b] (21.97)
500 mg/kg body weight	0.42 ± 0.11[c] (69.57)	0.72 ± 0.01[c] (61.70%)	5.16 ± 0.08[c] (44.16)
1000 mg/kg body weight	0.21 ± 0.06[d] (84.78)	0.46 ± 0.06[d] (75.53)	3.14 ± 0.11[d] (66.02)

Values are mean ± SD of 5 replicates
Values in bracket are computed percentage decrease
Statistical tools used were one way ANOVA and Duncan Multiple Range Test complemented with Student's t-test
[a-d]Test values carrying superscript different from the control down the group for each parameter are significantly different (P <0.05)

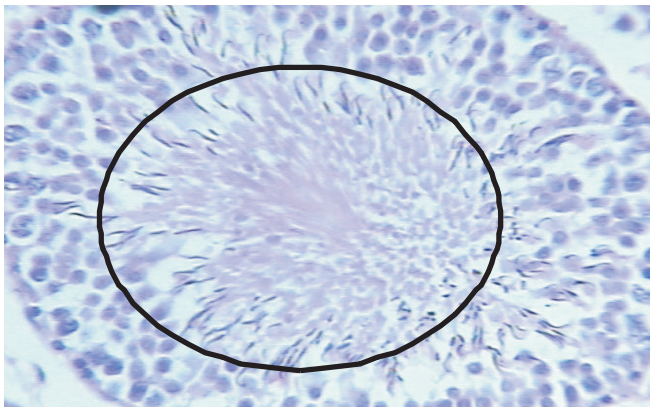

Figure I: Photomicrograph of the cross section of male rat testis (magnification x400) orally administered with distilled water for 60 days (Control). The circled spot shows well preserved seminiferous tubules with normal amount of stroma, normal population of spermatogenic and supporting cells. The spermatocytes within the lumen are also intact with evidence of spermatogenesis (H & E)

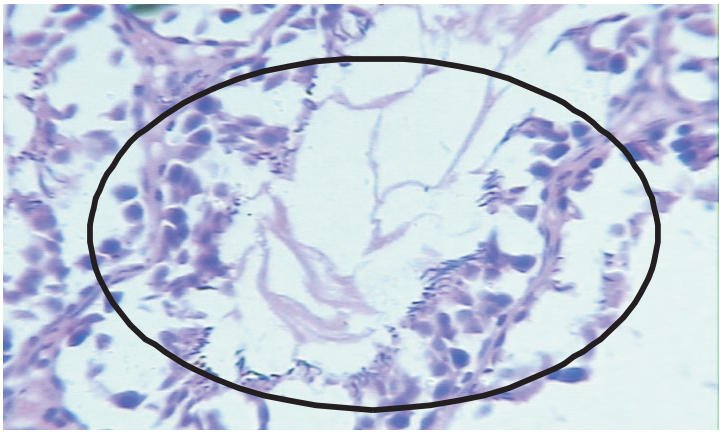

Figure II: Photomicrograph of the cross section of male rat testis (magnification x400) orally administered with 250 mg/kg body weight of the extract. The circled spot shows distorted seminiferous tubules with disorganised population of spermatogenic and supporting cells. The spermatocytes within the lumen are very few with evidence of reduced spermatogenesis (H & E)

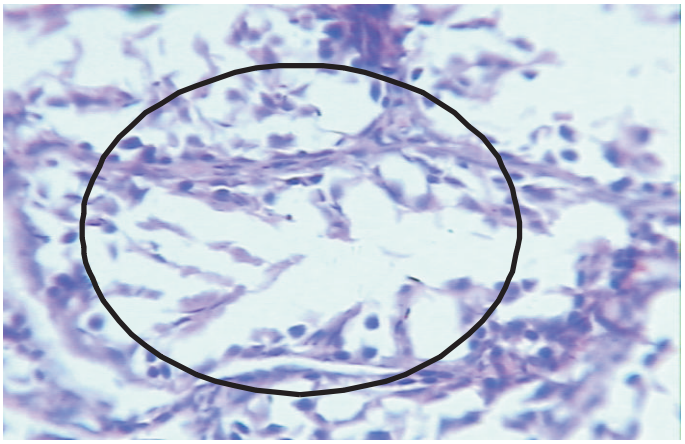

Figure III: Photomicrograph of the cross section of male rat testis (magnification x400) orally administered with 500 mg/kg body weight of the extract. The circled spot shows distorted seminiferous tubules with scanty population of spermatogenic and supporting cells. The spermatocytes within the lumen are very few with no evidence of spermatogenesis (H & E)

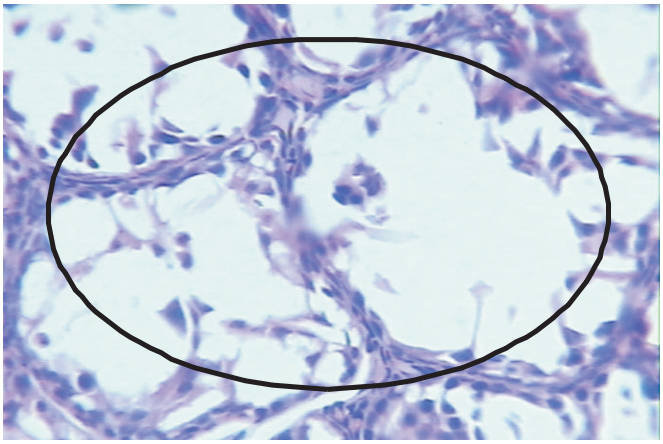

Figure IV: Photomicrograph of the cross section of male rat testis (magnification x400) orally administered with 1000 mg/kg body weight of the extract. The circled spot shows distorted seminiferous tubules with scanty germ cells. The spermatocytes within the lumen are very few with evidence of no spermatogenesis (H & E)

concentration and metabolic transformation of biomolecules as well as the histoarchitecture of several organs of the animal which may lead to structural and functional dysfunction. These effects which could be mild or severe may be beneficial or deleterious at the sub-cellular, cellular and or organ levels. In the present instance, aqueous extract of *C. aconitifolius* leaves adversely affected the normal functioning of the rat testes as evidenced by the biochemical and histological changes in this study.

Testes-body weight ratio and indices of secretory function of the testes (protein, glycogen, sialic acid, cholesterol, testosterone, ACP and GGT activities) as well as the normal histology of the testes are closely regulated by androgen.[26] Since androgens, notably testosterone, are produced by the testes, the evaluation of these biomolecules as well as the histopathological examination of the testes could serve as useful indices of organ damage, androgenicity and indirect assessment of fertility in male rats.

Computed organ-body weight ratio can be used to indicate organ swelling, atrophy, or hypertophy. Therefore, the reduction in the testes-body weight by the extract in this study may suggest atrophy, reduced tubule size, spermatogenic arrest and/or inhibition of steroid biosynthesis in the Leydig cells. Since the histopathological examination of the testes did not reveal any atrophy, the impairment in the organ to synthesize or secrete some biomolecules such as protein, testosterone, alkaline phosphatase as reported in the present study may account for the reduction in the testes-body weight ratio. It is also an indication of impairment at testicular, pituitary, or hypothalamic level and will adversely affect the normal functioning of the testes.

Testicular protein which is dependent on testosterone action also plays a role in the maturation of spermatozoa.[27,28] Furthermore, study has shown that the administration of anti-androgens could reduce among other, protein content in both the testes and epididymes of animals.[29] The testicular fluid also contains both stimulatory factors as well as inhibitory factors that selectivity alters the protein secretions.[30] Therefore, the decrease in testicular protein by the extract of *C. aconitifolius* leaves may be associated with anti-androgenic activity, more so, when the level of testosterone was also reduced by the extract in the present study. Such reduction in testicular protein content of the animals may be responsible, at least in part, for the reduced spermatocytes and spermatogenesis revealed by the histopathological analysis in this study.

The concentration of glycogen which has also been found to be directly proportional to the steroid hormones in the cells of animals is also a useful index of energy storage.[31] In addition, the glycogen contained in the Sertoli cell and spermatogonia provide reserve of carbohydrate for the cells of the seminiferous tubules. Therefore, the decrease in testicular glycogen by the aqueous leaf extract of *C. aconotifolius* suggest among others, inhibition of the activation of the phosphorylase or depletion of certain other enzymes which could block the synthesis of androgen. Such reduction in the glycogen content will adversely affect protein synthesis, since, the biosynthetic pathway in spermatogenic cells, is dependent on energy obtained from glucose.[32] This result agrees with the finding of Gupta *et al*[33], who also observed reduced glycogen and protein concentration in the testes of rats administered with extract of *Barleria prionitis* root.

Sialic acid, known also as acetylated neuraminic acid, has its synthesis dependent on androgen. There is also a direct relationship between the levels of androgen and the concentration of sialic acid in the testes of animals. Sialic acid reduces friction among spermatozoa and in the process facilitates their upward movement within the lumen of the testes as well as during their transit through the epididymis and the vagina.[34] Acetylated neuraminic acid is also involved in the maturation of spermatozoa and in the maintenance of structural integrity of the membranes of spermatozoa. Therefore, the reduction in the level of sialic acid by the extract of *C. aconitifolius* leaves in the present study may account for the observed scanty germ cells. This trend of reduction in sialic acid may consequentially affect the structural integrity of acrosomal membrane, metabolism, motility, and fertilizing capacity of spermatozoa.[35] Thus, it is possible that the extract might contain some chemical compound(s) which might have negatively interfered with the synthesizing machinery of the testes that is responsible for producing and secreting sialic acid. It is also possible that the extract promoted the catabolism of sialic acid in the testes of the animals higher than its biosynthesis.

Cholesterol is the major precursor responsible for the anabolic effect of testosterone in males[36], and a constant supply of the lipid is required for the synthesis of steroid hormones.[37] The synthesis of testosterone via steroidogenesis is dependent on the testicular cholesterol. Therefore, the reduced level of testicular cholesterol by the extract of *C. aconitifolius* leaves may not only account for the corresponding decrease in testosterone obtained in the present study, but also suggest impaired steroidogenesis.

ACP is widely distributed in the testes of animals where it enhances spermatogenic activity as well as the exchange of materials between the germinal cells and the Sertoli cells. The testicular acid phosphatase gene is up-regulated by androgens and is down-regulated by estrogens.[38] Furthermore, activities of phosphatases have been shown to rise when testicular steroidogenesis is increased.[39] Thus, the reduction in the specific activity of

the testicular enzyme by the aqueous leaf extract of *C. aconitifolius* in the present study may reflect decreased testicular steroidogenesis in the animals and this may be correlated with reduced secretion of gonadotropins in this study.[40] Such reduction in the enzyme activity may adversely affect the normal functioning of the testes by hindering the activity of the sperm cells and the adequate exchange of materials between the germinal cells and the Sertoli cells.

GGT, a membrane bound enzyme, is considered as a 'marker' enzyme required for the normal functioning of the Sertoli cells.[41] The Sertoli cells function by enhancing sperm maturation. Thus, the reduction in the testicular enzyme following the administration of aqueous leaf extract C. aconitifolius may hamper the normal functioning of the Sertoli cells. The alterations in the testicular enzymes in this study suggest anti-androgenic activity for the plant extract.

Testosterone, secreted by the Leydig cells under the influence of luteinizing hormone, is the main secretory constituent of the testes as well as the major hormonal marker of androgenicity.[42] A minimum level of blood androgen is thus required, among others for the maintenance of body shape, nocturnal penile tumescence, normal secretory function and sexual desire, maintenance of the normal histology of the testes, metabolism, motility and fertilizing capacity of the spermatozoa. The reduction in testosterone by all the doses of the extract in the present study suggests hindrance/impairment in the synthesis of the hormone or its secretion by the Leydig cells of the testes. Such decrease in the hormone might be a consequence of the reduced level of cholesterol, the starting material for the synthesis of androgens. Therefore, the decrease in testosterone by the extract, an indication of anti-androgenicity will hamper sexual drive, fertility and reproductive activities in the male rats. Furthermore, the decrease in the male hormone may also account for the reduced secretory function of the testes in the present study, and thus suggest that the aqueous leaf extract of C. aconitifolius is anti-androgenic in the male rats. The various findings in this study are similar to the reports of Sharma et al[43] and Gupta et al[44].

Normal male reproductive function depends on the secretion of luteinizing and follicle stimulating hormones by the pituitary gland and this is under the influence of hypothalamic gonadotropin-stimulating hormone (GnRH). LH in males stimulates the testicular Leydig cells to secrete testosterone while FSH induces spermatogenesis in the seminiferous tubules.[45,46] Therefore, the reduction in the levels of the gonadotropins in this study may be attributed to inhibitory effect of the extract on the hypothalamic-pituitary-gonadal axis and this may suggest anti-gonadotropic effect. This may account for the reduction in the testicular testosterone and the scanty/reduced spermatogenic cells observed from the histopathological examination in the present study.

Histopathological analysis of tissues of animals following administration of chemical compounds can be used to corroborate biochemical changes in the same tissue of interest. Studies have shown that the preservation of normal histo-architecture as well as growth and function of the testes is dependent on a minimum level of testosterone in the animals.[26,43] Therefore, the distortions in the seminiferous tubules, disorganised and scanty population of the spermatogenic and supporting cells are consequences of reduced testosterone levels by the extract of C. aconitifolius leaves. The disorganised and scanty population of spermatogenic and supporting cells as well as the number of spermatocytes (primary and secondary) within the lumen could possibly imply that the morphologic characteristics and the abundance of spermatozoa were adversely affected by the extract. All these features may also arise from impaired spermatogenesis. The anti-testosteronic effect of the extract and alterations in the testicular microenvironment could lead to impaired structural and functional activity of the organ and may adversely affect the fertility of the animals. The findings are similar to the report on the antispermatogenic activity of extract of Allium sativum pod in rats.[47]

Several studies have implicated neoflavones, flavonoids, prenyl flavones, triterpenes, steroidal alkaloids and saponins as anti-androgenic and anti-spermatogenic agents in rats.[3,48,49] It is possible that the anti-androgenic and anti-spermatogenic activities of the aqueous leaf extract of C. aconitifolius may be attributed to any of alkaloids, saponins, flavonoids and triterpenes or their combination that was earlier reported to be present in the extract.[8] Although, the present study did not address the mechanism of action of the extract of C. aconitifolius leaves as anti-androgenic and anti-spermatogenic agents, it is possible to propose that the extract may act by (a) affecting the hypothalamus-pituitary axis to prevent the release of signal required for the synthesis and secretion of testosterone (b) inhibit or regulate the activity of one or more enzymes in the biosynthetic pathway of testosterone (c) inhibit or impair the synthesis of cholesterol (d) block the receptor site of testosterone and thus prevent the hormone from eliciting its effect on the testes (e) destroying the Leydig cells of the testes. All these propositions will await our further studies.

Overall, the results of the present study has shown that aqueous leaf extract of Cnidoscolous aconitifolius at the dose range of 250-1000 mg/kg body weight exhibited toxicological and histopathological effects on the testes of the animals. These may be the consequence of anti-gonadotrpic, anti-androgenic and anti-spermatogenic

properties of the extract. These alterations may impair normal secretory functioning of the testes, spermatogenesis and fertility in male rats. Work is in progress on the isolation and characterization of the anti-androgenic and anti-spermatogenic principle(s) in the plant extract.

Conflict of interest
The authors declare no conflict of interest. The authors alone are responsible for the content of the paper

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