

## Effect of *Cnidoscoulous aconitifolius* (miller) i.m. Johnston leaf extract on sperm characteristics and reproductive hormones of male rats

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### Abstract

The increase in population growth rate has prompted urgent need to search for plants with antifertility potentials. The present study is concerned with the evaluation of the effect of *Cnidoscoulous aconitifolius* leaf ethanolic extract (LF) on sperm indices and reproductive hormones in male wistar rats.

Eighteen male albino rats were divided into three groups (A,B,C) of six animals each. Group A (control) received distilled water while groups B and C received 250 and 500 mgkg<sup>-1</sup> leaf extracts respectively on daily basis for fourteen days. Solvent partitioned fractions (n-hexane, dichloromethane, ethyl acetate and aqueous methanol) of the leaf crude ethanol extract were similarly evaluated using another twenty five male rats divided into groups of five. Cotton seed oil (5ml/kg) was used as reference standard

Results showed that administration of the extract produced significant decrease ( $p < 0.05$ ) in sperm count and weight of testis even though there was no significant difference in hormonal level between the treated animals and the control group

The reduction in sperm count by *C. aconitifolius* leaf is an indication of adverse effect on spermatogenesis and provides some justification for further exploitation of this plant as potential male contraceptive.

**Keywords:** *Cnidoscoulous aconitifolius*, sperm characteristics, testosterone, contraceptive.

### Introduction

*Cnidoscoulous aconitifolius* (Miller) I.M. Johnston, (Family – Euphorbiaceae) known as tree spinach (English), *efo iyana ipaja*, or *efo Jerusalem* (Yoruba) is commonly found growing in western part of Nigeria. It is an ornamental, evergreen, drought deciduous shrub with palmate lobed leaves alternately arranged. The leaves are widely consumed as vegetable within many localities in Nigeria [1, 2]. *Cnidoscoulous aconitifolius* (Chaya) is a large, fast growing leafy perennial shrub reaching a height of about 2-3 metres (6-9 feet). It resembles the hibiscus or the cassava plants. It is probably native to the Yucatan Peninsula of Mexico where it is known as Chaya [3, 4]. The genus *Cnidoscoulous* consists of forty or more species but only Chaya refers to the vegetables and can be harvested continuously unlike many leaf crops with short seasons. Chaya makes an excellent shrub in the yard or a hedgerow that can be continuously pruned for a large supply of vegetable greens from otherwise unproductive land. It can produce 8-10 tons of nutritious greens per hectare. It is very easy to grow needing little care and not much bothered by pests. It is relatively rich in protein, iron,

carotene and vitamins C [4, 5]. Most of the studies on the plant have been on the nutritive values of the leaf meal in broiler chicken [4, 6] and antimicrobial activity of the essential oil against *Escherichia coli* and *Salmonella typhi* [2].

Ethnomedicinally, the plant is used in managing an array of ailments and conditions including diabetes, obesity, acne, kidney stone and eye problems. The shoots and leaves of *C. aconitifolius* are also used as a laxative, diuretic, circulation and lactation stimulants [7, 8]. These usages are however without information on its effect on sperm indices and reproductive hormones [9, 10]. Several studies have shown that chemical compounds including plant extracts could alter the concentrations and functions of male and female reproductive parameters [9, 10 and 11]. This study was therefore designed to provide information on the effect of *C. aconitifolius* extracts on male reproductive hormones and sperm indices.

### Materials and Methods

#### Animals



Eighteen (18) mature male albino rats (*Rattus norvegicus*) of wistar strain weighing between 180-200 g were obtained from Oluwaseyi animal husbandry, Abadina, U I, and used for this study. They were divided into three treatment groups.

Group A to serve as control (not treated with the extract).

Group B received *C. aconitifolius* leaf extract (250 mg/kg body weight).

Group C received *C. aconitifolius* leaf extract (500 mg/kg body weight).

The rats were housed in standard cage under 12 hours day light and 12 hours darkness. They were fed with rat chow from Pfizer (Nig) ltd and given water *ad libitum* throughout the period. The average body weights at one week interval were taken before, during and after treatment using the Metler weighing balance and their cages and surroundings were kept clean daily. Animals were maintained according to the guidelines of institutional animal ethics committee.

Another twenty five sets of mature male albino rats of wistar strain weighing between 180-200 g were also used to evaluate the solvent partitioned *C. aconitifolius* leaf extract and were divided into groups of five.

Group I to serve as negative control (not treated with the extract).

Group II received *C. aconitifolius* combined (DCM/EtOAc) leaf fraction (50 mg/kg body weight).

Group III received *C. aconitifolius* combined (DCM/EtOAc) leaf fraction 100 mg/kg body weight).

Group IV received *C. aconitifolius* aqueous methanolic leaf fraction (50 mg/kg body weight).

Group V received *C. aconitifolius* aqueous methanolic leaf fraction (100 mg/kg body weight).

### Plant Materials and Extraction Procedure

The plant sample was collected from a single population at Major Salawu area, Agbowo, Ibadan, Oyo State, Nigeria between the months of January to February 2011. The plant material was identified at the Department of Botany Herbarium, University of Ibadan and authenticated at the Forest Herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan where a voucher specimen (FHI 108884) is deposited. The leaf sample was air dried under shade to a constant weight. The sample was grinded to coarse powdery form and kept in an air-tight plastic container until the time of usage. One kilogram of dried powdered leaf was macerated in 70% ethanol for 72 hours. The sample was filtered to remove the marc. The resulting filtrate was concentrated on a rotary evaporator and dried to give 152.8g of the leaf extract. The yield was 15.28% w/w.

One hundred grams (100g) of the dried leaf extract was dissolved in 80ml of aqueous methanol (3:1) and solvent partitioned into n-hexane, dichloromethane, ethyl acetate and methanol. The resulting fractions were separately concentrated and dried for further study.

### Semen Collection

At the end of the treatment period, the animals were sacrificed by cervical dislocation. The abdomen was opened to harvest the right epididymis and the caput lacerated on a glass slide using a warm (27°C) sterile lancet to release the semen sample [12].

### Sperm Motility Study

Some drops of sodium citrate were added to the sperm sample on the slide to effect full motility of the spermatozoa (Turner and Giles, 1982). The average gross motility was scored under the microscope x 40 objectives according to [12, 13]. The testis were also harvested, weighed, and stored in normal saline.

### Sperm Morphology Study

Two drops of eosin-nigrosin stain were added to mount the semen on the slide. It was latter drawn into a film using a cover slip held at angle 45° to dry. The film was mounted under a cover slip using Canada (Depex) mounting fluid and examined under light microscope. Live and dead spermatozoa were revealed and expressed as ratio in percentages [12, 14]. The slide was scored for such abnormalities as tailless head, headless tail, rudimentary tail, curved tail, curved mid piece, bent mid piece and looped tail. The scoring was done under light microscope.

### Sperm Count

Sperm concentration was determined using the left epididymis, immersed in a 5ml of normal saline. The displaced volume was taken as the volume of the epididymis. The epididymis above was matched into suspension and examined on improved Neubauer Haemocytometer. Counting was done in 5 large Thomas square and adjustment was made for the volume of the normal saline added. The count was calculated from counts/ml= No of sperm cells in 5 large Thomas square \*32000 \*dilution [12, 15].

### Hormonal Assay

Blood was collected through orbital sinus of the animals and used for hormonal assay. Blood serum was used for this experiment. Blood sample was collected in E/U sample tubes, centrifuged, and the serum separated [13, 16]

This enzymeimmunoassay (EIA) system was developed for the measurement of total Testosterone, FSH and LH in human serum or plasma without extraction using EIA assay kits (England) and assayed at Chemical Pathology Department, University College Hospital, Ibadan.

## Data Analysis

All data were analysed using the student T test and ANOVA, the results presented as Mean  $\pm$  S.D. The level of significant was placed at  $p < 0.05$ .

## Result and Discussion

This study investigates the effect of oral administration of *C. aconitifolius* leaf ethanol extract and ethanolic fractions on sperm indices and reproductive hormones of male wistar rats. The phytochemical screening of the *Cnidioscolous aconitifolius* powdered leaf shows the presence of alkaloids, saponins, phenolics, tannins, glycosides and anthraquinones. This also agrees with the work of Awoyinka *et al.*, 2007 on the phytochemical screening of *C. aconitifolius* (Euphorbiaceae) [17, 18].

The total sperm count, motile sperm count and normal morphological features has been reported as indices of fertility in males [7, 12]. As shown in Table 2, administration of 500 mg/kg of ethanol leaf extract of *C. aconitifolius* for 14 days significantly ( $p < 0.05$ ) decreased the sperm number from  $104 \pm 14.82$  in the control group to  $72.0 \pm 9.92 \times 10^6$  in the 500 mg/kg-1 treated male rats. The results revealed that *C. aconitifolius* leaf extract adversely affects the male reproductive functions. The cause of decreases in testicular weight (Table 4, Fig.2) may possibly be due to the depletion in the number of spermatogenic elements and spermatozoa [19, 20]. Statistically significant reduction in the weight of accessory sex organs has been proved to reflect interference on testosterone output and anti androgenic nature of plant extract [21].

The sperm motility and sperm morphology were not significantly affected and there was also no significant difference in the volume of the caudal epididymis (Table 1 and 2). This means the extract is not responsible for any significant abnormalities in the sperm characteristics of the animals. This observation is similar to an earlier work of Etuk and Muhammed [22].

The administration of *C. aconitifolius* revealed its nutritive value and high proteinous content as was demonstrated by Sarmiento – Franco *et al.*, 2002 [6]. The leaf extract shows significant increases

in body weight of the animals compared to untreated groups and is dose dependent (Table 3, Fig.1).

The anti-fertility activity demonstrated in the crude extract led to the solvent-partitioned fractionations of the ethanol extract. The effects of the partitioned fractions on different parameters studied are shown on the (Table 6, Fig, 3, 4 and 5). The extracts do not have significant effect on the reproductive hormones of the male rats (Table 7, Fig 6). It shows that the extracts may not have direct effect on the hormones of the male animals but on the spermatogenesis, though *C. aconitifolius* leaf extract has been reported to disrupt hormonal balance in female rats by Yakubu *et al.*, 2008 where the leaf extract adversely affect the oestrogen level [8].

The aqueous methanol fraction was more active than the combined (Dichloromethane and Ethylacetate) fractions (CF) at a dose of 50 mg/kg body weight. The sperm number reduced from  $96.25 \pm 10.04$  in the negative control group to  $77.67 \pm 4.93$  in the group treated with 50 mg/kg body weight of aqueous methanol fraction just as the positive control group (5ml Cottonseed oil/kg body weight) significantly lower the sperm count to  $64.5 \pm 6.46$  (Table 6). The extract also significantly reduced the testis weight from  $2.32 \pm 0.22$  grams (negative control group) to  $1.96 \pm 0.61$  grams in the group treated with 100 mg/kg body weight of aqueous methanolic fraction and  $1.88 \pm 0.69$ g in the group treated with 5 ml/kg body weight of cotton seed oil (Fig 5 )  $p < 0.05$ . There was increment in the body weight of all the animals, this may be attributed to the expected growth in the animals (Figure 3, Table 3).

Ever since the call by WHO for the discovery of new medicinal agents from natural sources, increasing number of African traditional medicinal plants are being screened for anti-fertility activity. African medicinal Plants are highly untapped and present great opportunity for science to discover active compounds and develop new anti-fertility agent. Traditionally, herbs are often used in simple or compound recipes. This is advantageous because medications may then possess several anti-fertility mechanisms and limit complications while lowering fertility. The remarkable efficacy, rich resources, low incidence of adverse effects are factors that will make traditional herbs more acceptable as scientist acquire more precise and detailed knowledge about them [23].



**Table 1: Effect of *C. aconitifolius* leaf ethanol extract (LF) on morphology of semen in male rats.**

Group/Dose	TH	HT	RT	BT	CT	BMP	CMP	LT	TOTAL SPERM CELLS
Control	4.6± 1.02	4.6± 1.02	2.2± 0.75	11.8± 0.98	10.8± 0.98	12.2± 0.75	11.2± 1.17	1.8± 0.75	405± 4.47
250 mg/kg	5.0± 0.82	5.0± 1.41	2.25± 0.96	8.75± 1.2	8.25± 0.96	9.25± 0.96	8.5±1.29	1.75±0.96	402.5± 5.0
500 mg/kg	4.33± 1.11	4.5± 1.26	2.17± 0.90	11.50± 1.38	10.0± 0.00	10.17± 0.90	10.67± 1.11	2.0± 0.82	403.33± 3.73

N= 6 / group,  $\bar{x}$  = mean, SD = standard deviation of the mean,  $P < 0.05$   
 HT= Headless tail; TH= Tailless head; RT= Rudiment tail; BT=Bent tail; CT Curved tail;  
 BMP Bent mid piece; CMP= Curved midpiece; CT= Looped tail;

**Table 2: Effect of *C. aconitifolius* leaf ethanol extract (LF) on motility, live/dead ratio, caudal epididymis volume and sperm count in male rats**

Group/Dose	Motility (%)±SD	Live/dead (%)±SD	Caudal epididymis volume (ml) ± SD	Sperm count (x106)±SD
Control	70.0±8.94	92.2±6.76	0.16±0.05	104±14.82
250 mg/kg	67.5±6.29	89.25±1.5	0.15±0.06	77.75±5.56*
500 mg/kg	68.333±22.67	94.0±6.40	0.18±0.04	72.0±9.92*

N= 6 / group,  $\bar{x}$  = mean, SD = standard deviation of the mean \* =  $P < 0.05$ ,

**Table 3: Effect of *C.aconitifolius* leaf ethanol extract (LF) on body weight of male rats**

Group/Dose	Weight of animals in gram ± S.D		
	Week 0	Week 1	Week 2
Control	185.74 ±5.76	190.05±0.52	193.05±0.54
250 mg/kg	184.52 ±13.3	199.68 ±17.5	203.95±17.4*
500 mg/kg	188.01 ±4.05	199.73 ±4.62	207.46±2.99*

N= 6 / group,  $\bar{x}$  = mean, \* =  $p < 0.05$ , SD = standard deviation of the mean

**Table 4: Effect of *C. aconitifolius* leaf ethanol extract (LF) on testicular weight of male rats**

Group/Dose	Weight of testis in grams ± S.D
Control	1.09±0.07
250 mg/kg	1.02±0.03*
500 mg/kg	0.92±0.05*

N = 6 / group,  $\bar{x}$  = mean, SD = standard deviation of the mean

**Table 5: Effect of *C. aconitifolius* leaf solvent partitioned fractions on morphology of semen of male rats**

Groups +Tween 80	TH	HT	RT	BT	CT	BMP	CMP	LT	TOTALSPERM CELLS
Postive control (5ml/kg of Cottonseed oil)	4.5 ± 1.29	3.5 ±1.0	1.75 ± 0.96	5.25 ± 3.59	7.0 ± 0.82	7.25 ± 0.96	6.75 ±0.5	1.75 ± 0.96	406.25 ±7.5
Negative control (Distilled water)	5.0 ± 0.82	4.5 ± 1.29	2.25 ± 0.96	8.0 ± 1.15	7.75 ± 1.5	9.25 ±1.5	8.25 ± 0.96	2.25 ± 0.96	403.75 ±4.79
50 mg/kg DCM&Ethyl acetate fraction	4.75 ± 1.26	4.5 ± 1.29	2.25 ± 0.96	9.5 ± 2.38	9.25 ± 1.26	9.75 ±1.5	9.25 ± 0.96	2.25 ± 0.96	405.0 ±4.08
100 mg/kg DCM & Ethyl acetate fraction	4.75 ± 0.96	2.25 ± 0.96	2.25 ± 0.96	8.75± 1.26	9.25 ± 1.5	9.25 ± 0.96	8.75± 1.71	2.0± 1.15	403.75±4.79
50 mg/kg aq/methanol fraction	4.25± 0.96	4.5±1.29	2.0±1.15	8.25 ± 1.29	8.25 ± 1.29	7.75± 1.26	8.25 ± 0.96	2.25 ± 0.96	405±4.08
100 mg/kg aq/methanol fraction	4.5±1.29	3.75± 0.96	2.25 ± 0.96	10.25± 2.22	9.25± 1.71	9.5± 2.08	9.5± 1.91	2.0± 0.82	403.75± 4.79

N= 5 / group,  $\bar{x}$  = mean, SD = standard deviation of the mean = P<0.05 HT= Headless tail; TH = Tailless head; RT= Rudiment tail; BT= Bent tail; CT= Curved tail; BMP= Bent mid piece; CMP= Curved midpiece; LT= Looped tail.

**Table 6: Effect of *C. aconitifolius* leaf solvent partitioned fractions on motility, live/dead ratio, caudal epididymis volume and sperm count in male rats.**

Group + Tween 80	Motility (%) ± SD	Live/dead (%) ± SD	Caudal epididymis volume (ml)	Sperm count (x106) ±SD
Postive control (5ml/kg b.wt of Cottonseed oil)	75±10.0	95.25±3.77	0.18±0.05	64.5±6.46*
Negative control (Distilled water)	88.75±6.29	96.0±4.0	0.18±0.05	96.25±10.04
50 mg/kg b.wt DCM &Ethyl Acetate fraction	82.5±5.0	95.25±3.77	0.2±0.0	87.0±14.31
100 mg/kg b.wt DCM &Ethyl Acetate fraction	91.25±2.5	79.25±1.5	0.13±0.05	85.5±9.47
50 mg/kg b.wt aqueous methanol fraction	76.25±4.79	96.0±2.45	0.175±0.75	77.67±4.93*
100 mg/kg b.wt aqueous methanol fraction	86.25±4.79	95.25±3.77	0.15±0.06	89.25±8.22

N= 5 / group,  $\bar{x}$  = mean, SD = standard deviation of the mean \* = P<0.05

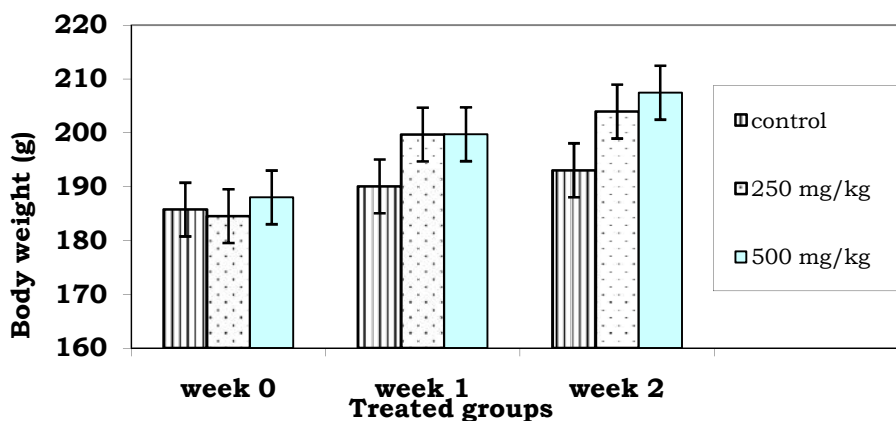


**Table 7:** Effect of *Cnidoscolous aconitifolius* leaf ethanol extract (LF) on LH, FSH & Testosterone of male rats.

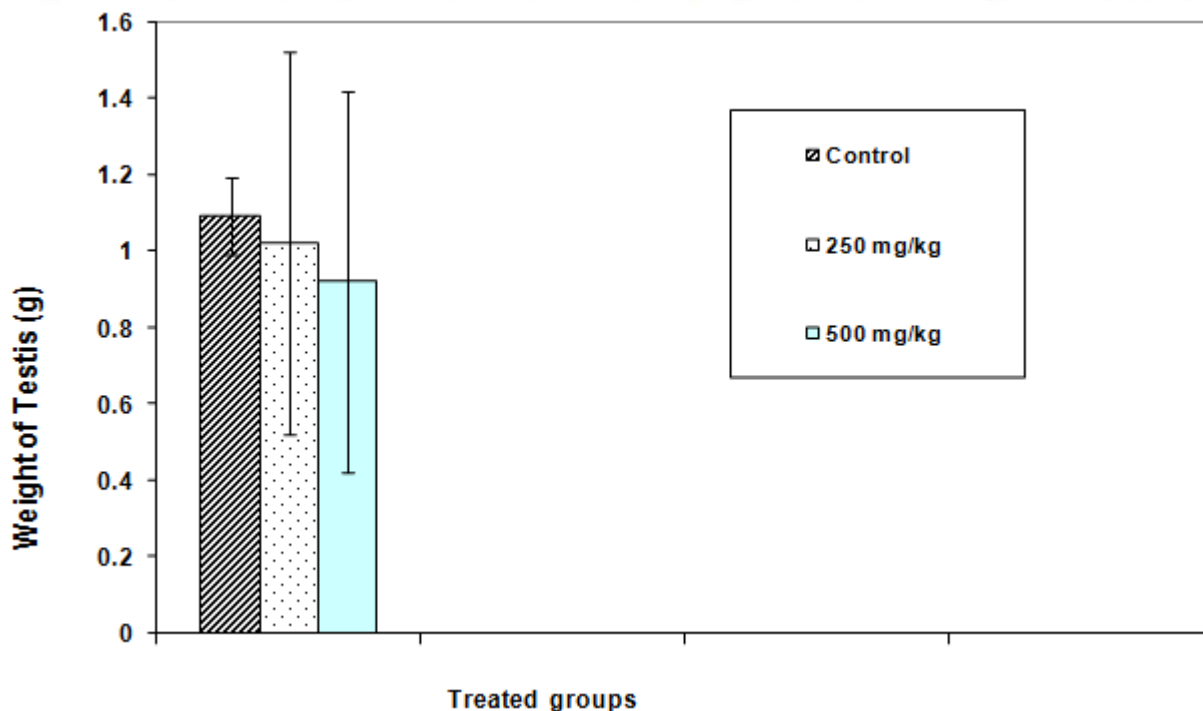
Group/Dose	Hormone concentration		
	LH(IU/L)	FSH(IU/L)	TESTOSTERONE(nmol/L)
250 mg	0.106±0.189	0.348±0.325	52.1±9.61
500 mg	0.015±0.008	0.54±0.105	58±0.00
Control	0.038±0.0359	0.403 ±0.23	58±0.00

LH= luteinizing hormone, FSH=follicle stimulating hormone, IU/L= International unit per litre

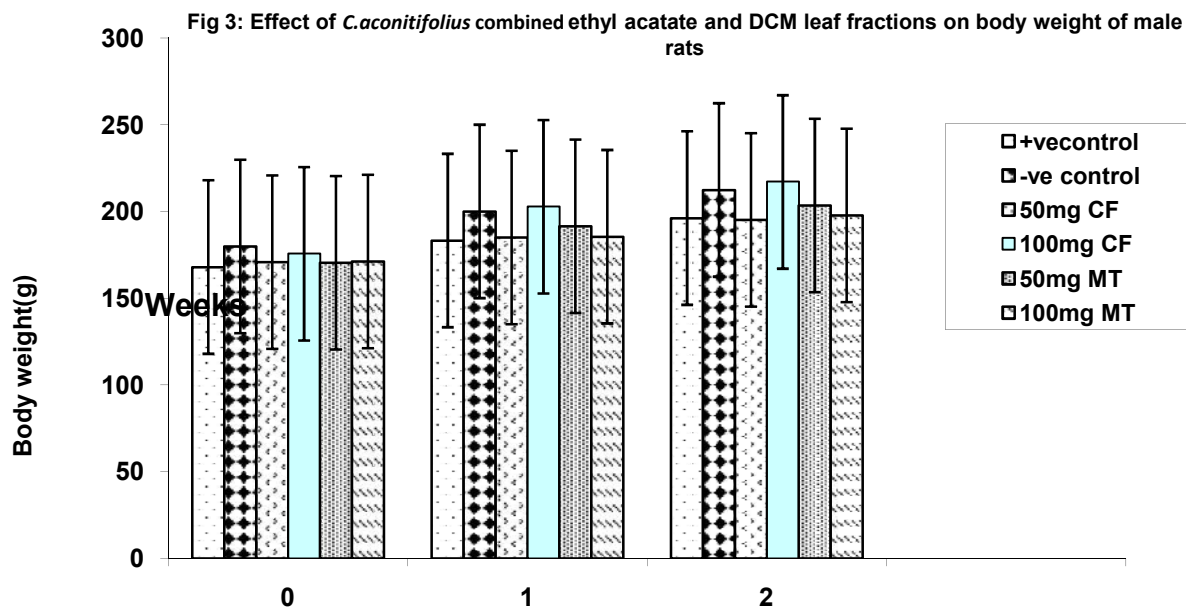
**Fig 1:** Effect of *Cnidoscolous aconitifolius* leaf ethanol extract (LF) on the body weight of male rats



**Fig 2:** Effect of *C aconitifolius* leaf ethanol extract (LF) on testicular weight of male rats







CF=Combined fraction (dichloromethane and ethyl acetate), MT= aqueous methanolic fraction

**Fig 4: Effect of *C.aconitifolius* leaf ethanol extract (LF) on % change in body weight of male rats.**

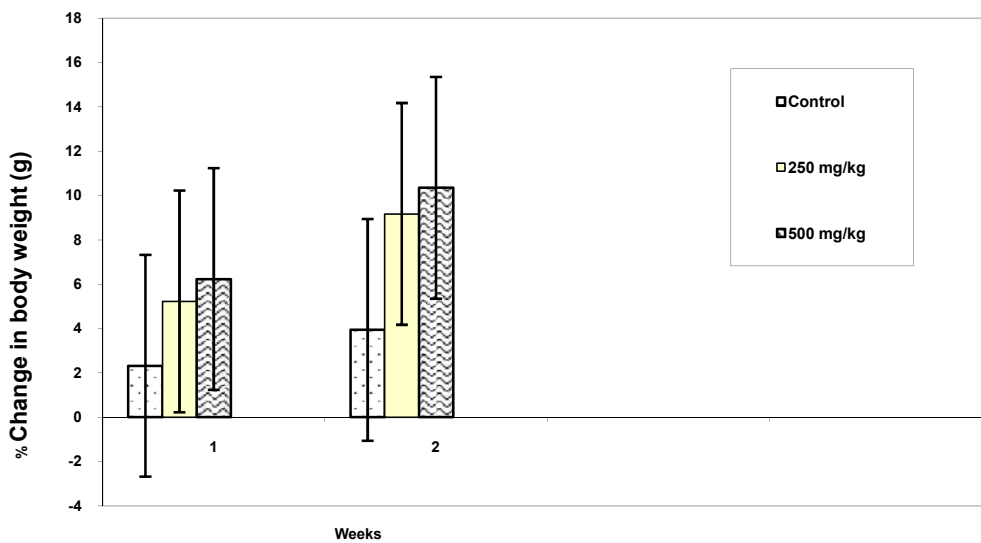
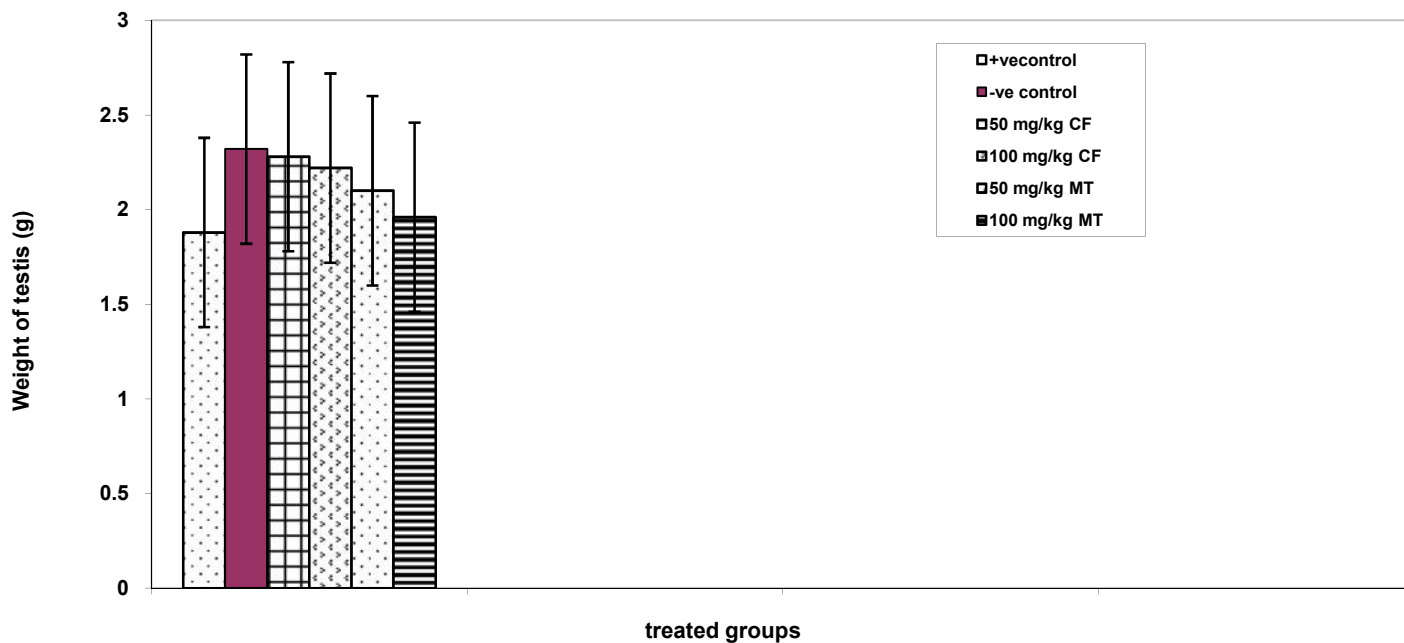
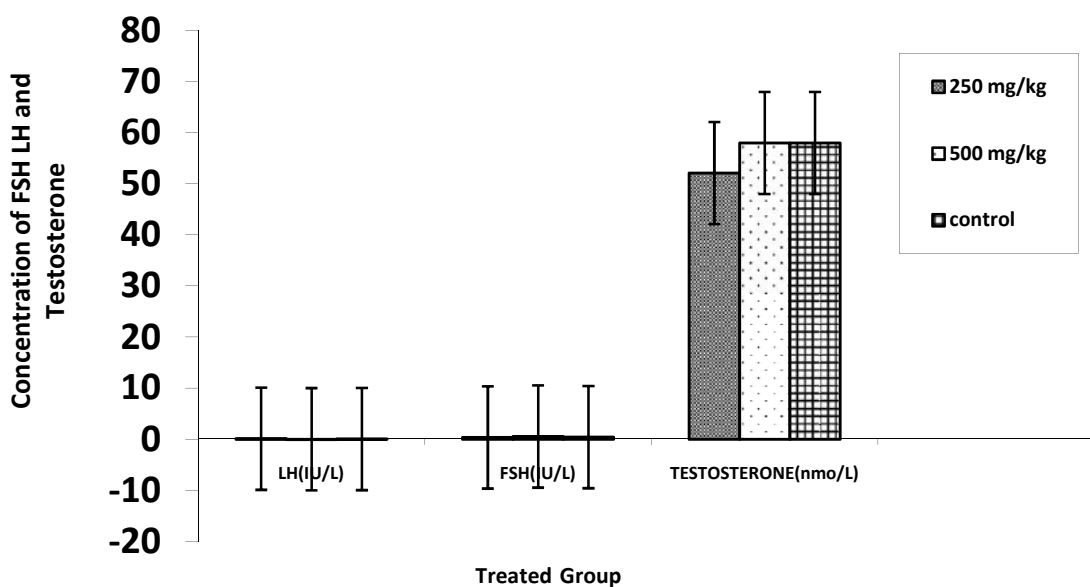


Fig 5: Effect of *C. aconitifolius* leaf solvent partitioned fractions on testicular weight of male rats after 14 - days of administration



CF = Combined fraction (dichloromethane and ethyl acetate). MT = aqueous-methanolic fraction

Fig 6: Effect of *Cnidioscolus aconitifolius* leaf ethanol extract (LF) on LH, FSH and Testosterone





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