Original Article

# Effects of aqueous leaf extract of Chaya (*Cnidoscolus aconitifolius*) on pituitary-gonadal axis hormones of male Wistar rats

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## Abstract

**Introduction:** Male fertility is controlled by a complex assortment of pituitary-gonadal hormones. This regulation is key to understanding problems with fertility. The level to which some plants consumed by man contribute to his fertility problems is yet to be fully explored. This study aimed at evaluating the effects of Chaya on pituitary gonadal hormone axis in male wistar rats. **Methodology:** The study was conducted using 24 wistar rats randomized into three control and three treatment groups of four rats each. The treatment rats received 1.5g/kg body weight of Chaya extract by gavage. Blood samples were collected at various time intervals for hormonal assay and statistical analysis performed. **Findings:** There was a statistically significant decrease ($P = 0.010$) in testosterone levels and elevated LH and FSH levels ($P = 0.432$ and $P = 0.939$ respectively) in the treatment rats. The testosterone / estrogen ratio was also elevated. These effects were duration of treatment dependent.

**Key words:** *Cnidoscolus aconitifolius*, pituitary-gonadal axis, testosterone

## INTRODUCTION

Hormonal problems may lead to testosterone deficiency from decreased testicular testosterone production or problems with the pituitary or hypothalamus, which controls testosterone production (Holdcraft and Braun, 2004). Excess prolactin production (hyperprolactinemia) may also reduce fertility (Ganong, 2005).

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In mammals, the action of a complex assortment of peptide and steroid hormones plays an important role in spermatogenesis by regulating the normal functioning of the seminiferous epithelium. These hormonal messengers are critical not only for regulation of male germ cell development but also for the proliferation and function of the somatic cell type required for proper development of the testes (Sharpe, 1994; McLachlan *et al*.; 2002). These include; the interstitial Leydig cells (Mendis-Handagama, 1997), the myoid cells (Maekawa *et al*., 1996) and sertoli cells (Griswold, 1998).

Follicle stimulating hormone (FSH) and luteinizing hormone (LH) secreted by the anterior pituitary act directly on the testes to stimulate somatic cell function in support of spermatogenesis (Pierce and Parson, 1981).

Testosterone and its metabolite dihydrotestosterone and estradiol are collectively referred to as the sex hormones. The primary function of estrogen in the male reproductive tract is the regulation of luminal fluid reabsorption in the rete testes and efferent ducts linking

the testes and epididymis (Hess *et al*.; 2000; Lee *et al*.; 2000). In contrast, androgen and androgen receptor function are essential for proper sexual differentiation and the maintenance of normal spermatogenesis (Lindzey *et al*.; 1994). Progesterone is required in the pathway for the synthesis of testosterone and estrogen (Thomas and Gerald, 2006).

The use of medicinal plants in disease treatment has aroused the interest of man for millennia. Especially, since prehistory man has tried to lessen pain or treat disease using plants with medicinal properties (Yamada, 1998).

It has been reported that about 70% of the human population is dependent wholly or partially on plant-based medicine (Raven *et al*.; 2006). This plant-based traditional medicine system plays an essential role in health care with about 80% of the world's population relying on it due to its availability and cheap source (Owolabi *et al*., 2007). In Nigeria and most developing countries of the world, rural and urban dwellers, literate and illiterate rely heavily on herbal preparations for the treatment of various diseases despite availability of orthodox medicine (Salahdeen and Yemitan, 2006). Many of these medicinal plants are used by males and females of reproductive age and for treating reproductive problems such as infertility (Sakpa and Uche-Nwachi, 2014).

Many plant extracts have been tested for their fertility or antifertility effects on human and animal models, e.g. the crude extract of *Allium sativum* (Garlic) caused a decrease in serum testosterone levels with effects being evoked at a very low dose (Hammami *et al*.; 2008; Hammami *et al*.; 2009). *Gossypol herbaceum* linn invoked antifertility effects in rats at 30 mg/kg body weight with lesser doses causing infertility in humans (Rahim *et al*.; 2010). Other studies using plant extracts, include the antifertility effects of *Bulbine natalensis* (Yakubu and Afolayan, 2009), *Junipeus phoenica* (Shukukani *et al*.; 2007), *Aegele marmelos* (Chauhan and Agarwal, 2008), *Dendrophyte falcate* (Gupta and Kacchawa, 2007), all of which reduced testosterone levels. Plants such as *Capparis aphylla* had antisteroidogenic effect while *Garcina cambogia* caused degeneration of leydig cells (Sarathchandran *et al*.; 2007).

Chaya is a plant of ancient origin, with a long history of human use, propagation and domestication. The name chaya comes from the Yucatan Maya word "Chay," the generic and most commonly used name for the plant. Many of the names, especially in Spanish (Ortiga, Pica, Mala Mujer, Tread-softly, Spurge nettle, and others) are due to the urticating hairs of the plant. Other names such as tree spinach, or cabbage, etc., refer to Chaya's association with other leafy green vegetables. In South Western Nigeria, it is known as "Iyana ipaja" (Oyagbemi *et al*.; 2008), whereas in South Eastern Nigeria, it is called "Hospital too far" (Iwalewe *et al*., 2005) because of its blood boosting effects.

It is currently a widespread cultivar of increasing popularity, and both historic and ethnogeographic evidence suggests that it has been a plant of importance as food and medicine (Ross-Ibarra and Molina-Cruz, 2002). Its high nutritious value, ease of propagation, productivity, tolerance of poor growth conditions and resistance to pests and diseases make Chaya a valuable potential crop that could benefits peoples of many different regions (Ross-Ibarra and Molina-Cruz, 2002).

A wide variety of claims have been made as to the medical efficacy of Chaya as a treatment for numerous ailments, ranging from the ability to strengthen fingernails and darken graying hair, to its use as a cure for alcoholism, insomnia, venereal disease, gout, scorpion stings, and as an improvement of brain function and memory. A wild relative of Chaya, is even attributed with anticontraceptive properties (Ross-Ibara and Molina-Cruz, 2002).

In South Western Nigeria, the leaves and young shoots are often squeezed with water and drank alone or with milk and tomato paste added. The local folks believe that it has a blood-boosting effect, and so is commonly taken by pregnant women and young children who are anemic (Iwalewe *et al*.; 2005). Studies have shown that *Cnidoscolus aconitifolius* has ameliorative effects on anemia and osmotic fragility induced by protein-energy malnutrition in male Wistar rats (Oyagbemi *et al*.; 2008). It has been reported that the ethanol leaf extract of *C. aconitifolius* at LC50 of 10 μg/ml showed evidence of cytotoxicity with brine shrimp larvae (Senjobi *et al*.; 2011).

Previous work done had also showed that Chaya caused histological changes in testicular and epididymal tissue. However, the mechanism of the effects on the testes and epididymis could not be elucidated (Sakpa and Uche-Nwachi, 2014), hence the need for the present hormonal study.

## MATERIALS AND METHODS

Fresh leaves of *C. aconitifolius* registered as FHI 109528 in the herbarium at Forestry Research Institute, Ibadan were collected from the premises of the Department of Biochemistry, University of Benin, Benin City. The aqueous leaf extract was prepared according to the method by Salahdeen and Yemitan. A dose of 1500 mg/kg body weight which was less than the calculated $LD_{50}$ value of 7,348 g/kg body weight (Adebiyi *et al*.; 2012) for *C. aconitifolius* was administered to the rats.

Twenty-four male Wistar rats weighing between 250 and 350 g were used for the study. The rats were randomized into three control groups KA, KB, and KC and three treatment groups A, B, and C consisting of four Wistar rats each. The rats were fed with standard rat chow (Bendel Livestock Feed, Edo state, Nigeria) and had free access to water throughout the entire study period of 8 weeks. All control groups- KA, KB, KC; representing 2, 4 and 8 weeks of study respectively, had 1 ml of sterile water daily by gavage, while treatment groups A, B, and C representing 2, 4 and 8 weeks study periods respectively received 1.5 g/kg bodyweight (1.5 g/kg bodyweight) of *C. aconitifolius* extract daily by gavage. All animals were weighed at the commencement and end of each study period, and the weights were recorded as initial and final weights.

The rats were anesthetized with chloroform and cardiac puncture done to obtain blood for male fertility hormone profile. The blood samples were stored in a plain bottle (i.e. without anticoagulant) for the hormone studies. The blood samples were then assayed for FSH, LH, and Prolactin (Uotila *et al*., 1981), Progesterone (Tietz, 1995), Oestrogen (Hall, 1988; Ratcliffe *et al*., 1988) and Testosterone (Tietz, 1986) using enzyme-linked immunosorbent assay (ELISA) methods.

Ethical approval for this study was obtained from the Research and Ethics Committee of the College of Medical Sciences, University of Benin, Benin City, Edo State.

Data were collated and analyzed using International Business Machines Statistical Product and Service Solutions (IBM-SPSS) Version 20.0 (International Business Machines Corporation, 2011). Means were calculated, and then compared using one-way analysis of variance. Correlation charts were drawn using Microsoft Excel 2013. The level of statistical significance was set at a $P < 0.05$ for all statistical test conducted.

## RESULTS

The mean LH level reduced from 2.30 ± 0.86 μ/L at 2 weeks to 1.82 ± 0.15 μ/L at 8 weeks in control rats [Table 1]. The mean LH levels of rats in the treatment group elevated gradually from 2.26 ± 1.16 μ/L at 2 weeks to 3.55 ± 0.68 μ/L at 8 weeks [Table 2].

Follicle stimulating hormone levels fluctuated in the control rats peaking at 4 weeks (6.12 ± 3.43 μ/L), while treatment rats' FSH levels numerically elevated gradually from 2 weeks peaking at 8 weeks (7.58 ± 2.62 μ/L) on administration of Chaya extract [Tables 1 and 2].

Mean testosterone levels of treatment rats declined gradually from 2.85 ± 1.55 to 0.18 ± 0.10 pg/ml at the end of the study period [Table 2] while mean testosterone levels for control rats remained relatively constant within the 8 week study period (5.50 ± 1.29; 5.44 ± 1.30; 5.35 ± 1.25 at 2, 4 and 8 weeks respectively).

**Table 1: Comparison of mean hormonal values of rats in control groups KA, KB, and KC**

| Hormone | Control group | | | *P* |
|---|---|---|---|---|
| | KA | KB | KC | |
| FSH (mean±SD) | 5.56±2.29 | 6.12±3.43 | 5.32±3.85 | 0.939 |
| LH (mean±SD) | 2.30±0.86 | 2.00±0.05 | 1.82±0.15 | 0.432 |
| Prolactin (mean±SD) | 5.58±2.37 | 4.92±2.54 | 4.83±3.42 | 0.920 |
| Progesterone (mean±SD) | 3.46±2.44 | 3.02±1.88 | 3.33±1.36 | 0.948 |
| Oestrogen (mean±SD) | 13.44±5.05 | 10.86±5.24 | 14.52±8.08 | 0.708 |
| Testosterone (mean±SD) | 5.50±1.29 | 5.44±1.30 | 5.35±1.25 | 0.986 |
| Testosterone/estrogen ratio | 1:2.44 | 1:2.00 | 1:2.71 | - |

SD - Standard deviation, FSH - Follicle stimulating hormone, LH - Luteinizing hormone, KA - Control group A, KB - Control group B, KC - Control group C

**Table 2: Comparison of mean hormonal values of rats in treatment groups A, B and C**

| Hormone | Treatment groups | | | *P* |
|---|---|---|---|---|
| | A | B | C | |
| FSH (mean±SD) | 4.58±3.23 | 5.42±3.65 | 7.58±2.62 | 0.426 |
| LH (mean±SD) | 2.26±1.16 | 3.40±1.91 | 3.55±0.68 | 0.755 |
| Prolactin (mean±SD) | 6.48±1.49 | 6.47±2.73 | 6.23±2.11 | 0.983 |
| Progesterone (mean±SD) | 5.06±1.62 | 2.52±1.40 | 4.43±1.32 | 0.083 |
| Oestrogen (mean±SD) | 11.50±3.41 | 12.98±6.02 | 10.73±2.41 | 0.753 |
| Testosterone (mean±SD) | 2.84±1.55 | 0.87±0.63 | 0.18±0.10 | 0.010 |
| Testosterone/oestrogen ratio | 1:4.00 | 1:14.92 | 1:59.61 | - |

SD - Standard deviation, FSH - Follicle stimulating hormone, LH - Luteinizing hormone

Estrogen levels fluctuated in control (between 10.86 ± 5.24 and 14.52 ± 8.08) and treatment groups (between 10.73 ± 2.41 and 12.98 ± 6.02) and the results were not statistically significant.

There was a fluctuation in progesterone levels of control animals (lowest at 4 weeks – 3.02 ± 1.88; and highest at 2 weeks – 3.46 ± 2.44) with a similar fluctuation in progesterone levels in treatment animals (lowest level recorded was 2.52 ± 1.40 at 4 weeks while levels were highest at 2 weeks 5.06 ± 1.62). There was no statistically significant difference in comparison of the mean progesterone levels at 2, 4 and 8 weeks in control ($P = 0.948$) and treatment ($P = 0.083$) groups, respectively.

There was a statistically significant difference ($P = 0.010$) in comparison of mean testosterone levels of rats in the various treatment groups. The testosterone/estrogen ratio at 2 weeks was 1:4.00 in the treatment group declining to 1:14.92 at 4 weeks and lowest at 8 weeks (1:59.61). In comparison, the testosterone/estrogen ratio in control rats were 1:2.44; 1: 2.00; and 1:2.71 at 2, 4 and 8 weeks, respectively.

**Correlations**

In the control group, a negative significant correlation was found between LH and testosterone ($r = -0.625$; $P = 0.030$) [Figure 1], while the correlation between LH and testosterone in the treatment group was nonsignificant ($r = -0.036$; $P = 0.913$) [Figure 2]. A negative nonsignificant correlation was found between testosterone and FSH in control ($r = -0.267$; $P = 0.402$) and treatment ($r = -0.360$; $P = 0.250$) groups, respectively [Figures 3 and 4].

## DISCUSSION

Previous work done showed that *C. aconitifolius* caused histological changes in the rat testes and epididymis. In the testes, it caused disruption in the seminiferous epithelium with spermatogenic arrest, failure of spermiogenesis and release of immature spermatogenic cells into the lumen of the seminiferous tubules at early stages and at late stages depletion of these cells within the lumina. In the epididymis, there was presence of clumps of immature germ cells within the tubule during early stages of treatment and at later stages, depletion of spermatogenic cells in the lumen (Sakpa and Uche-Nwachi, 2014).

The present study showed that *C. acontitifolius* gradually elevated the LH levels in the treated rats when compared with the decrease recorded in the control rats. In contrast, FSH levels fluctuated in control rats peaking at 4 weeks (6.12 ± 3.43 μ/L) while the levels in the treated rats progressively rose from the 2nd week of treatment and peaked at 8 weeks (7.58 ± 2.62 μ/L). However, these changes were not statistically significant.

Luteinizing hormone through specific receptors found on the surface of leydig cells controls the production and secretion of testosterone (deKrester *et al.*; 1971). In males, FSH receptor expression (FSH-R) is limited to the testicular sertoli cells (Ranniki *et al.*, 1995). Genetic and pharmacological studies in rodents indicate that the primary role of FSH in spermatogenesis is stimulation of sertoli cell proliferation during prepubertal development (Heckert and Griswold, 2002) and sertoli cell number largely determines the number of germ cells (Sharpe, 1994) and hence male fertility. Estrogen and testosterone have a negative feedback on the production of these hormones in the pituitary hence a decrease in serum testosterone level will cause a concomitant increase in the levels of LH and FSH. The findings in this study support this assertion because the gradual decline in testosterone levels resulted in elevation of serum LH and FSH levels in treated rats [Table 2]. This is also in keeping with the negative correlations recorded between FSH and testosterone as well as between LH and testosterone in both the control and treatment groups [Figures 1-4]. Prolactin secretion is stimulated by prolactin releasing factor in the hypothalamus. Consequently, high levels of prolactin (hyperprolactinemia) inhibits LH and FSH

**Figure 1:** *Cnidoscolus aconitifolius* (Chaya)

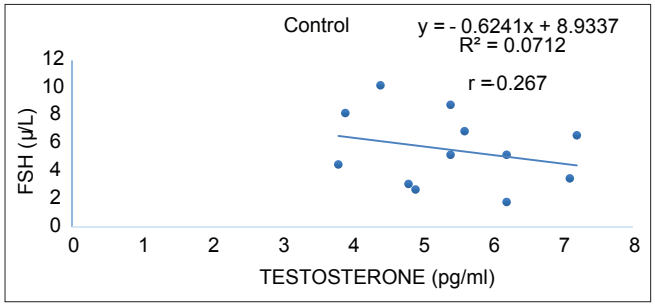


**Figure 3:** Relationship between follicle stimulating hormone and testosterone in control grou

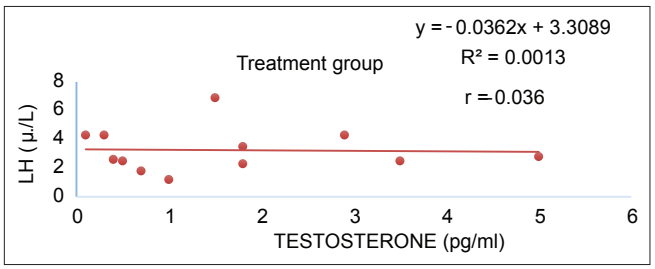


**Figure 2:** Relationship between luteinizing hormone and testosterone in treatment group

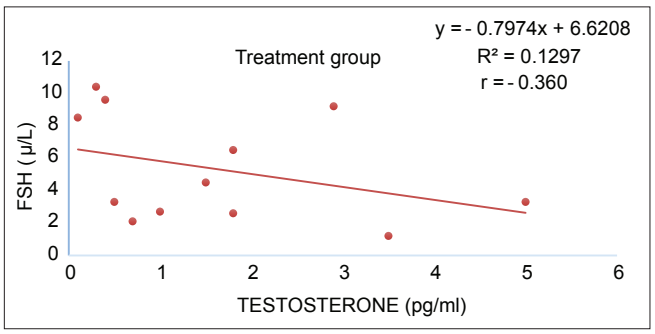


**Figure 4:** Relationship between follicle stimulating hormone and testosterone in treatment group

pulsatile secretion, lowers testosterone levels and inhibit spermatogenesis (Thomas and Gerald, 2006). Prolactin levels gradually dropped in both control and treatment groups in this study which showed that *C. aconitifolius* did not affect prolactin secretion.

Progesterone and estrogen did not reveal any regular pattern in their serum levels in control and treatment groups in this study. While progesterone is necessary for the production of testosterone, estrogen is a product from the final conversion of testosterone. This study did not show any form of responsiveness to these hormones during treatment with *C. aconitifolius*. These effects may have resulted from extragonadal production of these hormones.

In contrast, there was a slight decline in the levels of testosterone in the control group but this was not statistically significant ($P = 0.986$) when compared with the treatment groups in which testosterone levels steeply declined to a statistically significant level ($P = 0.010$) from week 2 to week 8. This also reflected in the testosterone/oestrogen ratio, which also steeply declined in the treatment groups. A decline in testosterone levels may have resulted from Leydig cell damage or inhibition of the enzymes in the pathway for testosterone production.

Put together, the findings of high levels of gonadotropins and the decrease in testosterone levels recorded in the treated rats suggests a likely cause for the observed spermatogenic arrest and failure of spermiogenesis in the histology of rat testes and epididymis treated with Chaya extract in an earlier study. The findings corroborate the assertion that the testosterone is critical for the completion of meiosis, entry into and progress through spermiogenesis in rodents (McLachlan *et al*.; 2002; Haywood *et al*.; 2003).

These antifertility effects of Chaya are likely due to its alteration in pituitary-gonadal axis hormones, and since the changes are more marked in the testosterone levels, the probable site of action is most likely in the testes.

## CONCLUSION

*Cnidoscolus aconitifolius* (Chaya) produced a decline in testosterone level with a consequent elevation of LH level. Since testosterone was the only hormone that showed significant statistical difference among all hormones assayed, it is likely that the action of *C. aconitifolius* is at the level of Leydig cells by inhibiting testosterone production. The mechanism of this inhibition needs further evaluation with histochemical studies.

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