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Reproductive Effects of Hydromethanolic Leaf Extracts of *Cnidoscolus aconitifolius* (Euphorbiacea) in Streptozotocin Induced-diabetic Rats

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The potency of plants is largely due to the presence of phytochemicals contain in them. Plant-derived chemicals influence endocrine activities in animals and humans have received affluent attention due to their possible adverse effects or benefits, especially on sex hormones.
Aim: This study is aimed at evaluating the effect of hydromethanolic leaf extract of *Cnidoscolus aconitifolius* on sex hormones in streptozotocin induced-diabetic wistar rats.
Materials and Methods: Thirty (30) wistar rats with an average weight of 230 grams, were randomly assigned into five groups of 6 (six) animals each. Group 1: served as negative control (non-diabetic) and received normal animal chow and water *ad libitum*, group 2: served as positive control group and received 10 mg/kg bw of glibenclamide, groups 3, 4 and 5 served as

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experimental group and received 100 mg/kg bw, 150 mg/kg bw and 200 mg/kg body weight of C.A orally for 28 days after being induced with diabetics using streptozotocin.
Results: Phytochemical screening of extract revealed the presence of a highly abundant level of alkaloids and flavonoids, with moderate levels of tannins, phlobatannins, saponins, free anthraquinones, combined anthraquinones, terpenes, cardiac glycoside and cyanogenetic glycoside. Administration of the extract of C.A shows significant ($P<0.05$) decrease in LH, testosterone, estradiol, whereas that of serum prolactin concentration was significantly increased.
Conclusion: The biologically active phytochemicals in the hydromethamelic leaf extract of *Cnidoscolus aconitifolius* may disrupt the endocrine system, thus inducing hormonal imbalance or disorders in hormone deponent organ, infertility and contraception. *Cnidoscolus aconitifolius* might be a promising contraceptive agent and an excellent alternative natural remedy in reproductive and gynaecological studies.

Keywords: Cnidoscolus aconitifolius; phytochemicals; sex hormones; infertility.

1. INTRODUCTION

The potency of plants is largely due to the presence of phytochemicals contained in different parts of plants. These phytochemicals provides varied physiological effects and potential therapeutic properties of plants in the treatment of illnesses. Some of these plants are known to possess anti-fertility effect through their action on hypothalamic-pituitary-gonadal axis or direct effects on hormones resulting in inhibition of ovarian steroidogenesis [1]. Herbal plants are being increasingly utilized to treat a wide variety of clinical diseases [2], and some causing very great danger unknowingly to mankind. Many drugs commonly used today are of herbal origin, higher plants source of medicinal compound continuance to play a dominant role in the maintenance of human health since antiquities [3]. Many phytochemicals have been identified as components of food and more are still being discovered [4]. Some of the phytochemicals of greater importance are plant steroids, flavonoids, tannins, saponins, alkaloids and glucosides. Sex hormones are a group of steroids hormones that regulate the reproductive functions. In reproduction, however, the primary endocrine glands are the anterior pituitary gland which secretes the luteinizing hormones (LH) and the follicle stimulating hormones (FSH). The gonads (testes and ovary) which secrete testosterone, estrogen and progestin etc. [5]; the secretory functions of these glands are under the control of the arcuate and preoptic areas of the hypothalamus that secrete the gonadotropin-releasing hormones (GnRH) [5]. Diabetes is a metabolic disorder in which the body does not produce or properly utilize insulin. It causes a disturbance in carbohydrate, protein and lipid metabolism and some complications. Diabetes mellitus has become a common disease, very prevalent in the world affecting all ages in developing and developed nations [6]. The prevalence of diabetes mellitus in some countries has reached 2-3% of the total populations and in Africa, especially in Nigeria, it is on the increase [7].

Cnidoscolus aconitifolius is a perennial shrub of the family Euphorbiaceae commonly found in the tropics. It is commonly eaten as a vegetable in soup condiment in south western Nigeria, where it is called “iyana ipaja” and “efo Jerusalem”, in the Niger Delta region of Nigeria, it is referred to as “hospital too far” due to its multifaceted traditional uses. It has been demonstrated to contain phenols, saponins, cardiac glycosides, and phlobatannins [8]. Chaya consumption has become popular among the Hispanic population in southern Texas Florida. The nutritional value of chaya is very rich when compared with other common vegetables [9,10]. The plant which is also called spinach tree has great potential to alleviate deficits in a population of developing countries as it is rich in essential amino acid, vitamin and minerals [11,12] and [13]. High fibre content and antibacterial activities of *Cnidoscolus aconitifolius* have been reported [14]. Apart from the antibacterial activities, the ameliorative effect of the *Cnidoscolus aconitifolius* on anaemia and increased erythrocyte osmotic fragility induced by protein energy malnutrition (PEM) has also been reported [15]. While it’s antidiabetic property has also been elucidated.

Cnidoscolus acontifolus is a food plant; it has been used therapeutically for a number of ailments such as diabetes [16], arteriosclerosis, gallstone and high cholesterol. It is also believed that *Cnidoscolus aconitifolius* cleans the circulatory system, stimulates lactation, improved eyesight, strengthens nails, improves digestion

and is a diuretic and laxative agents [17], despite these array of uses of *Cnidoscolus aconitifolius* scanty literature is available on its effect on sex hormones in streptozotocin induced-diabetic wistar rats. Hence, this study, therefore, aims at scientifically evaluating the effect of hydromethanolic leaf extract of *Cnidoscolus aconitifolius* on sex hormones in streptozotocin induced-diabetic wistar rats.

2. MATERIALS AND METHODS

2.1 Plants Materials and Authentification

Fresh leaves of *Cnidoscolus aconitifolius* were obtained from a garden at Seventh Day Adventist Church, Choba, Port Harcourt, Rivers State and were correctly identified by Dr. N.E Edwin-Wosu of the Department of Plant Science and Biotechnology, College of Natural and Applied Sciences, University of Port Harcourt, Choba, Rivers State, Nigeria with reference number: UPH/PSB/015.

2.2 Extraction of Plant

The fresh leaves were air dried and pulverize with the electric grinding machine into minute pieces weighing 150 g. Hydromethanolic (1/4, v/v) extraction was carried out with Soxhlet extractor (Model no. 3567, Austria). The extract obtained was filtered using Whatmann no 1 filter paper. The filtrate was concentrated under reduced pressure in vacuum at 45°C using rotator evaporator (Gallenkamp, UK).The resulting residues were then transformed to a lot oven where they were dried to a constant weight at 45°C, the extract was stored at 4°C.

2.3 Experimental Design

Thirty (30) wistar female rats with an average weight of 230 gram were randomly assigned into five groups of six (6) animals each. Group 1: served as negative control (non-diabetic) and received normal animal chow and water *ad libitum*, group 2: served as positive control group and received 10 mg/kg bw of glibenclamide; group 3, 4 and 5 served as experimental groups and received 100 mg/kg bw, 150 mg/kg bw and 200 mg/kg bw of *Cnidoscolus acontifolius* leaf extract respectively orally for 28 days after been induced with diabetes using streptozotocin (STZ). Principles of laboratory animals care (NIH publication no. 85, revised 119, 1985), were followed as well as specific national laws were applicable [18]. At the end of extract administration, animals were anaesthetized using 25% urethane (Ethyl carbamate) at the dose of 0.6 ml/100 g bw intraperitoneally; blood samples collected for laboratory determination of serum sex hormones level.

2.4 Induction of Diabetes in Rats

After two weeks of acclimatization, diabetes was induced with a single intraperitonial injection of streptozotocin (STZ) at a dose of 60mg/kg body weight after 18 hours fast according to the method described by [19]. The STZ was freshly dissolved in citrate buffer (0.01M, P^H 4.5) [20]. The injection volume was prepared to contain 1.0 ml/kg [21]. After 12 hours blood glucose level w measured and animals with a concentration of more than 230 mg/dl were classified as diabetic and used for the experiment.

2.5 Phytochemical Screening

Preliminary phytochemical analyses of the hydromethanolic extracts were performed as described by [22], seeking to highlight the major group of secondary metabolites. It was evaluated, the presence of alkaloids [23] canthracyamines coumarins, anthracene derivatives, flavonoids [24], lignans, mono, and diterpenes, naphthoquinones [25] saponins [26] steroids [27] and terpenoids were revealed.

2.6 Hormonal Assay

Determination of follicle stimulating hormone (FSH): The FSH quantitative test is based on a solid phase (ELISA). A method described by Uotila M et al. [28].

2.7 Determination of Progesterone

The progesterone EIA is based on the principle of competitive binding between progesterone in the test tube and progesterone-HRP [29].

2.8 Determination of Prolactin

The prolactin quantitative test is based on a solid phase linked immunosorbent assay (ELISA) [29].

2.9 Determination of Estradiol

Extradiol is based on the principle of competitive binding between E2 in the test specimen and E2-HRP conjugate for a constant amount of rabbit antiestradiol [29].

2.10 Determination of Luteinizing Hormone (LH)

The luteinizing hormone quantitative test is based on a solid phase enzyme-linked immunosorbent assay (ELISA) [28].

2.11 Statistical Analysis

The result was expressed as the mean of 6 replicates ± standard error of the mean (SEM) and were analyzed, using the Statistical Package of Social Sciences (SPSS) version 20.0. One way analysis of variance (ANOVA) was performed to test the effect of each dose on the parameters under evaluation at 95% level of confidence. Values were considered statistically significant at ($p<0.05$) [30].

3. RESULTS

Phytochemical constituents of *Cnidoscolus aconitifolius* as shown in Table 1. The phytochemical screening of *Cnidoscolus aconitifolius* revealed highly abundant levels of alkaloids and flavonoids, with moderate levels of tannins, phlorotannins, saponins, anthraquinones, terpenes and cardiac glycoside.

Table 1. Results of phytochemical constituents of hydromethanolic leaf extract of *Cnidoscolus aconitifolius*

S/N	Phytochemicals	Inference
1	Alkaloids	+ +
2	Tannins	+
3	Phylobotannins	+
4	Saponins	+
5	Flavonoids	+ +
6	Free anthraquinones	+
7	Combined anthraquinones	+
8	Terpenes	+
9	Cardiac glycoside	+

+ Moderately abundant, ++ highly abundant

Table 2. Effects of hydromethanolic leaf extract of *Cnidoscolus aconitifolius* on some sex hormones

Groups	Sex hormones			
	LH(miu/ml)	FSH(miu/ml)	Estradiol(pg/ml)	Prolactin(ng/ml)
Non Diabetic Group (Negative control)	1.09±0.24	1.80±0.23	8.23±0.44	0.23±0.05
Glibendamide (Positive control)	1.23±0.03*	1.19±0.04	7.55±0.70*	0.26±0.03*
(100 mg/kg of CA)	1.83±0.23*	1.80±0.05	8.28±0.13	0.25±0.03*
(150 mg/kg of CA)	1.58±0.26	1.50±0.01	8.03±0.25	0.33±0.05*
(200 mg/kg of CA)	0.4±0.27	0.39±0.01	7.80±0.18*	0.35±0.05*

All values are expressed as mean ±S.E.M. n=6, P≤0.05 = statistically significant when compared to the negative control

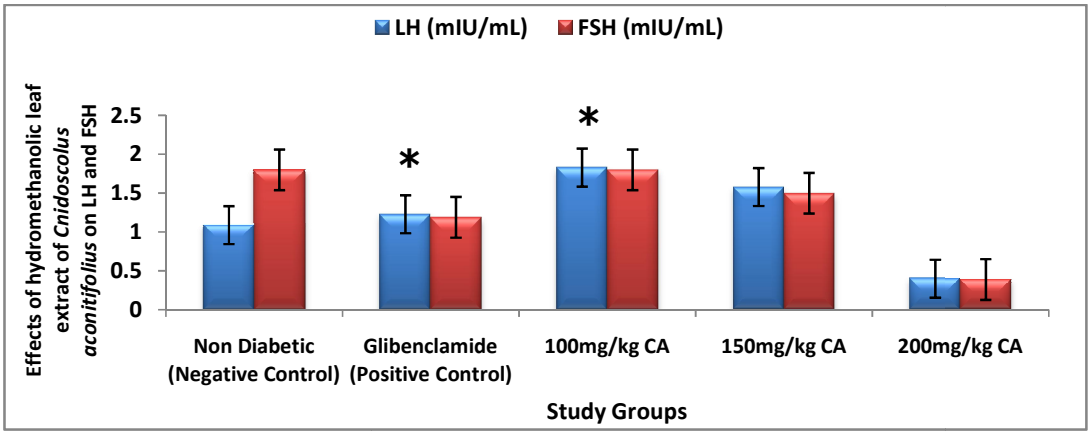

Fig. 1. Effects of hydromethanolic leaf extract of *Cnidoscolus aconitifolius* on serum LH and FSH levels

*N/B: * = Significant compared to Negative control at P<0.05*

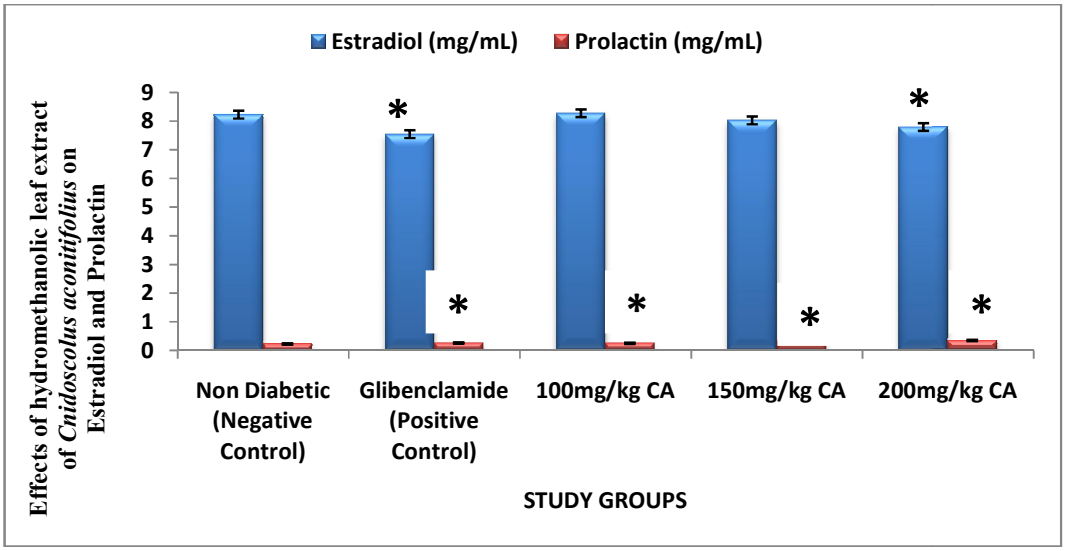

Fig. 2. Effects of hydromethanolic leaf extract of *Cnidoscolus aconitifolius* on Testosterone level

*N/B: * = Significant compared to Negative control at P<0.05*

A statistically significant (P<0.05) increase was recorded in the serum LH concentration level of group 2 and group 4 animals for which there is no statistical significance in FSH serum level in all groups. Estradiol recorded a statistically significant (P<0.05) decrease in group 2 (glibenclamide and group 5 (200 mg/kg bw of C.A).

Paradoxically, a statistically significant (P<0.05) increase was recorded in the serum prolactin level of group 2, 3, 4 and 5 respectively in a dose-dependent manner.

4. DISCUSSION

The result of the present study indicates that the hydromethanolic leaf extract of *Cnidoscolus aconitifolius* possesses possible strong effect on the regulation of the oestrous cycle, conception and reproduction [31]. However, a study is on going to microscopically assess vaginal smear and confirm the regulatory potency of the extract on the estrous cycle. Alkaloids and flavonoids have been shown to reduced serum concentrations of luteinizing hormone, estradiol and follicle stimulating hormones [29]. Therefore, the presence of these phytochemicals may account for the alteration in the levels of circulating sex hormones in a diabetic state observed in this study. Luteinizing hormones stimulates secretion of sex steroids from the gonads, therefore, the reduction in the serum LH levels may be explained by an inhibitory effect of the extract on the release of LH which may trigger disruption of ovulation [28]. This may result in impairment of oestrous cell; hamper conception and normal reproduction [31].

Follicle stimulating hormone is the focal hormone of mammalian reproduction, important for gonadal development and maturation at puberty as well as gamete production during fertile life [32]. It acts on the receptors located on the granulosa cells to stimulate growth and maturation of ovaries follicle. The non - statistically significant reduction in follicle stimulating hormone by the hydromethanolic leaf extract of *Cnidoscolus aconitifolius* in streptozotocin induced diabetic wistar rats may disturb folliculogenesis and delay maturation of the follicle in the preovulatory phase [33]. The secretion of FSH is regulated by the gonadotropic releasing hormone secreted by the hypothalamus, the reduction could be that the extract may have exerted its effect on the hypothalamic-pituitary axis. Reduction in this hormone may negatively affect conception in female animals, this corresponds with the work of Beme et al. [34] where administration of Afromosia laxiflora, plerocarpus erinaceous and cola nitida stem bark deceased the release of the gonadotropisms. The possible mechanism of action could be the effect of flavonoids on the sertole cells of the somniferous tubules in males and in the granulosa cells of the ovarian follicles

in the females to secrete inhibin that specifically inhibits the anterior pituitary's secretion of FSH without affecting the secretion of LH [35].

Estradiol stimulates the growth of the uterine lining, causing it to thicken during the preovulating phase of the cycle [35]. It is well established that estradiol is directly responsible for the growth and development of reproductive organs [36]. In synergy with FSH, estradiol stimulates granular cell proliferation during follicular development [28]. The fall in estradiol secretion in women can be useful in the treatment of endometrioses. In this condition, ectopic endometrial tissue from the uterus (dependent on estradiol for growth) is found growing outside the uterus. The significant decrease in estradiol in this study is an indication that *Cnidoscolus aconitifolius* may ameliorate uterine fibroids (leiomyomas) non-malignant growth [36] Prolactin helps to initiate breast development by inducing lobuloalveolar growth of the mammary gland. It stimulates lactogenesis. The enhanced level of prolactin observed in this study may be attributed to the effect of the extract probably acting as a dopamine antagonist.

High prolactin levels tend to suppress the ovulating cycle, by inhibiting the secretion of both follicle stimulating and gonadotropic-releasing hormone (GnRH). The increased level of prolactin in this study justifies the folkloric use of *Cnidoscolus aconitifolius* in stimulating lactations.

5. CONCLUSION

The biologically active phytochemicals in the hydromethamelic leaf extract of *Cnidoscolus aconitifolius* may disrupt the endocrine system, thus inducing hormonal imbalance or disorders in hormone deponent organ, infertility and contraception. It also shows the possible potency of ameliorating endometriosis and uterine fibroid, owing to it's thus, hydromethanolic leaf extract of *Cnidoscolus aconitifolius* might be a promising contraceptive agent. Further collaborative studies are ongoing with the Department of Obstetrics and Gynaecology, College of Health Sciences, University of Port Harcourt, Rivers State, Nigeria on human subjects to compare with the findings of this study.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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