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Original Research Article

Improved Reproductive Functions of Male Wistar Rats Administered with Leaf Extract of *Cnidoscolus aconitifolius*

Ijeoma Ezebuiro[1], Chibuike Obiandu[2*], Abiye Tamuno-Opubo[2], Bright .I. Owhorji[2] and Adesua .C.Obiandu[3]

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Universityof Port Harcourt, Nigeria
[2]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria
[3]Senior Secondary Schools Board, Port Harcourt, Rivers State, Nigeria

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***Corresponding author:** Chibuike Obiandu
Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria

Abstract

This study investigated the effects of hydromethanol leaf extract of *Cnidoscolus aconitifolius* on some male reproductive functions in Cadmium chloride ($Cdcl_2$) toxicity-induced male Wistar rats. Twenty four adult male Wistar rats were used for this study. The animals were divided into four groups (6 rats each) as follows: Group 1 (negative control), Group 2 (positive control) which received 5mg/kg $CdCl_2$, Groups 3 and 4 received 200 and 400 mg/kg BW of extract and 5mg/kg $CdCl_2$ respectively. Duration of extract administration was 30 days, while males were co-habited with females in the last 10 days of the study to determine fertility index. Subsequently, the rats were sacrificed following light chloroform anaesthesia, while samples were collected for analysis. Phytochemical screening of the leaf extract revealed the presence of alkaloids, flavonoids, phlobatannins, saponins, triterpenoids and cardenolide; and the analysis of data was by SPSS version 23. The analysed result indicated that the extract caused a significant ($p<0.05$) increase in FSH and testosterone levels. The percentages of viable sperm cells, sperm cells with normal morphology and actively motile sperm cells as well as sperm count were significantly increased. In addition, the fertility index in the rats that received higher dose (400mg/kg) of the extract was significantly increased. Conclusively, extract of *Cnidoscolus aconitifolius* mitigated against $Cdcl_2$ induced toxicity in some reproductive parameters of male Wistar rats.

Keywords: Reproductive Functions, Male Wistar, Cnidoscolus aconitifolius.

INTRODUCTION

Plants are recognized to have tremendous therapeutic potentials, and have been exploited by traditionalists to find techniques of treating disease in single form or in mixes since antiquity (Umeaku *et.al.*, 2018). This has been linked to the abundance of pharmacologically active chemicals embedded in the plants, which have been shown to be effective against a variety of diseases and medical problems, as well as their potential to protect humans against a variety of ailments (Oyewole *et.al.*, 2012). *Cnidoscolus aconitifolius* belongs to the *Euphorbiaceae family* and is a huge, fast-growing leafy perennial shrub (Ebeye *et.al.*, 2015). It is commonly referred to as *tree spinach* and is known by a variety of names by people from various geographical regions, including *"Iyana-Ipaja*" or *"lapalapa*" by Yorubas, "*Ncheobo*" or "*Ugu-oyibo*" by Igbos, "*Bindazugu"* by Hausa, and "*Ochiga*" by Ogirra, all in Nigeria (Oyewole *et al.*, 2012; Ebe & Chukwuebuka, 2019).This plant, which reaches a height of about 6 meters, has big, chartaceous or sometimes succulent alternate palmate lobed leaves, as well as succulent stalks packed with sap and little white flowers (Ross-Ibara & Molina-cruz, 2002; Ebeye *et al.*, 2015). The existence of several nutritional components imbedded in the plant, such as protein, beta-carotene, vitamins, ascorbic acid, calcium, potassium, and iron, have been discovered in several research (Jimenez-Arellanes *et al.*, 2014). It also contains antioxidants like Vitamin C and E, as well as flavonoids and other phytochemicals like saponins, cardiac glycosides, flavonoids, alkaloids, antraquinones, terpenoids, and tannins (Ehimwenma & Osagie, 2007; Barlett & Eperjesi, 2008; Ilondu & Enwa, 2013).

Exposure to xenobiotics such as heavy metals has been linked to lower sex organ weight, reproductive performance, fertilization, and implantation, as well as mitotic suppression and chromosome aberration (Mateo, 2009). (Ait *et al*., 2009; Cinar *et al*., 2015;;Deutcheu *et al*., 2019). Cadmium toxicity research has established it as a common environmental human carcinogen (Nakamura *et al.*, 2002) and one of the most well-known reproductive toxins in a wide range of animals (Jahan *et al.*, 2014). Chronic exposure to environmentally relevant cadmium causes elevated cadmium levels in people, particularly in sterile men (Bernoff *et al.*, 2009). According to Al-Snafi (2016), improving healthy sexuality is feasible through the use of natural plants and nutrients that have multiple therapeutic mechanisms due to the presence of numerous chemical elements (Drewes *et al.*, 2003; Cao *et al.*, 2008). The aim of this research is to investigate the effects of the hydromethanol leaf extract of *Cnidoscolus aconitifolius* on some male reproductive functions of male Wistar rats exposed to $CdCl_2$ toxicity.

MATERIALS AND METHODS

Animal Models

A total of twenty four (24) male Wistar rats were procured from the animal house of the Department of Human Physiology, University of Port Harcourt and used as the experimental model. The animals, which weighed an average of 120-130g, were kept in conventional laboratory settings and supplied standard meals and water in compliance with national and institutional norms for animal use in research. The animals were also given a 14-day acclimatization period before the experiments began.

Plant Extraction

The *Cnidoscolus aconitifolius* leaves utilized in this study were obtained from the University of Port Harcourt, Nigeria, Botanical Garden and later identified by a taxonomist at the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. The herbarium received a voucher specimen of the plant specimen. The leaves were air dried for two weeks before being pulverized into powder and extracted at the University of Port Harcourt's Pharmacognosy Department with a Soxhlet extractor and hydromethanol (80% methanol) as the solvent. The solution was filtered through Whatmann filter paper, and the filtrate was concentrated in vacuum at 45°C under reduced pressure.The extract yield was placed in a hot oven and dried to a consistent weight at 45°C before being kept at 4°C. Before administration, the extract was resuspended in distilled water.

Experimental Design/Procedure

A total of 24 male Wistar rats were used in this investigation, which were divided into four groups of six rats each. Group 1 served as the negative control group and received distilled water, while Group 2 served as the Positive Control group, receiving 5mg/kg BW Cadmium Chloride ($CdCl_2$). Groups 3 and 4 received 200 and 400 mg/kg BW of *Cnidoscolus aconitifolius* hydromethanol leaf extract, respectively, followed by 5 mg/kg BW of $CdCl_2$.

The entire administration was done orally once daily for 58 days, to accommodate the spermatogenic cycle in Wistar rats as well as the sperm transit time through the epididymis. The extract dose was based on the findings of Iyke *et al.*, (2018), as well as a toxicity (LD50) study conducted by Adebiyi *et al.*, (2012), which found that the plant leaves are largely non-toxic with a lethal dose exceeding 5000mg/kg BW (7, 348mg/kg BW).

At the end of the administration, the rats were sacrificed under light chloroform anaesthesia. The testes and epididymis were harvested through abdominal incision, weighed and used for sperm analysis (count, morphology, viability and motility). Blood samples were collected via cardiac puncture into the appropriate bottles for hormonal profile assay. The sera samples were separated and then assayed for FSH, LH and Testosterone, using Enzyme-linked Immunisorbent Assay (ELISA).

STATISTICAL ANALYSIS

Results are expressed as mean ± standard error of mean (SEM). Significant differences were determined by one-way analysis of variance (ANOVA) using SPSS version 23 (SPSS incorporated, Chicago, Illinois, USA). The differences in values were considered to be statistically significant at $p<0.05$.

RESULTS

The result for the study is presented in tables 1-5

Table-1: Qualitative Phytochemical Analysis of The Hydromethanolic Leaf Extract of Cnidoscolus aconitifolius

Phytochemicals	Presence
Alkaloid	+
Flavonoid	+
Phlobatannins	+
Free anthraquinone	-
Combined anthraquinone	-
Triterpenoid	+
Cyanogenic glucosides	-
Fixed oils	-
Cardenolide	+
Saponins	+

Key: + = present, **-** = absent

Table-2: Effect of Extract of Cnidoscolus aconitifolius on Organ Weight of Wistar rats

GROUPS	Testis (g)	Epididymis (g)
I	1.29 ± 0.18	0.26 ± 0.02
II	1.22 ± 0.25	0.24 ± 0.02
III	1.37 ± 0.75	0.26 ± 0.02
IV	1.42 ± 0.76	0.25 ± 0.02

Values are expressed as mean ± SEM, n=6.

Table-3: Effect of extract of Cnidoscolus aconitifolius on Some Reproductive Hormones of Wistar rats.

GROUPS	FSH (mIU/L)	LH (mIU/L)	T (mIU/L)
I	0.93 ± 0.16	1.75 ± 0.10	1.39 ± 0.14
II	0.91 ± 0.48	1.03 ± 0.13	1.52 ± 0.18
III	0.62 ± 0.77	1.24 ± 0.94	2.67± 0.62^{a,b}
IV	1.49 ± 0.19^{a,b}	2.03 ± 0.48	3.41 ± 0.41^{a,b}

Values are expressed as mean ± SEM, n=6. a- significantly different from negative control group (P<0.05) b-significantly different from positive control group (P<0.05)

Table-4: Effect of Extract of Cnidoscolus aconitifolius on Sperm Parameters of Wistar rats

GROUPS	Sperm Volume (ml)	Viable Sperm Cells (%)	Normal Morphology (%)	Actively Motile (%)	Sperm Count ($x10^6$ml)
I	0.24 ± 0.07	81.00 ± 4.00	79.00 ± 4.00	78.00 ± 3.37	142.00 ± 17.43
II	O.09 ± 0.03^a	67.00 ± 3.00^a	60.00 ± 4.18^a	35.00 ± 5.00^a	44.00 ± 11.22^a
III	0.18 ± 0.04	79.00 ± 1.87^b	75.00 ± 2.24^b	70.00 ± 2.74^b	90.00 ± 10.95^b
IV	0.21 ± 0.02	81.00 ± 3.32^b	75.00 ± 1.78^b	77.00 ± 3.39^b	116.00 ± 11.66^b

Values are expressed as mean ± SEM, n=6. b- Significantly different from positive control group (P<0.05

Table-5: Effect of Extract of Cnidoscolus aconitifolius on Fertility Index

GROUPS	Mated M/F (n)	Pregnant Females (n)	Foetus (n)	Fertility Index (%)
I	6	3	8	50
II	6	0	0	0
III	6	0	0	0
IV	6	4^b	6^b	66.67^b

Values are expressed as mean ± SEM, n=6. b- Significantly different from positive control group (P<0.05)

DISCUSSION

According to Orji *et al.*, (2016), phytochemical constituents are responsible for plant species' medicinal activity, and the phytochemical screening results in this study are similar to those of Lennox *et al.*, (2018), who found the presence of alkaloid, glycosides, saponins, flavonoids, reducing compounds, and polyphenols in the extracts, but Phlobatanins and anthraquinones were absent. Tannins, on the other hand, were only found in the aqueous extract. In a similar study, Iyke *et al.* (2018b) discovered moderate quantities of tannins, saponins, free anthraquinones, mixed anthraquinones, terpenes, cardiac glycoside, and cyanogenetic glycoside in *Cnidoscolus aconitifolius* hydromethanolic leaf extract.The presence of alkaloids, phenols, tannins, phlobatannins, saponins, cyanogenic glycoside, flavonoids, oxalates, and anthraquinone steroids has also been recorded routinely in the ethanolic extract (Jimenez-Aguilar & Grusak, 2015; Sanchez-Hernandez *et al.*, 2017. In addition, the phytochemical screening of extracts of fresh leaves of *Cnidoscolus aconitifolius* reported by Chikezie *et al.*, (2016) revealed the presence of saponin, flavonoids, alkaloids, phlobotannins, steroids, anthraquinones, and phenols in the ethanolic extract and the presence of cyanogenic glycosides, tannins, and oxalate in the water extract.

In a study, Adeniran *et al.*, (2013) found that the leaf extract included steroids, tannins, alkaloids, cardiac glycosides, terpenoids, and saponins, but phlobatannins and flavonoids were lacking. Various scholars have assessed the significance of these phytochemicals in health and disease. Because of the phenol concentration of the extract, it can be utilized to treat a variety of diseases and could even be employed as a cancer therapy. Saponins, on the other hand, are thought to be responsible for wound healing as well as protective effects (against hyperglycemia, hypercholesterolemia, and hypertension), as well as antibiotic and anti-inflammatory properties, while terpenoids are thought to have antibacterial and anti-diarrheal properties (Orji *et al.*, 2016). Tannins are also responsible for healing and anti-inflammatory properties, according to Araujo *et al.*, (2008).

When compared to both controls, administration of the extract had no significant effect on the organ weight of the animals. The weight of the testis and epididymis of the experimental groups, on the other hand, grew independently of the extract effects. According to Thakur & Dixit (2007), this could be regarded as a biological indicator for the effectiveness of the plant extract in improving the genesis of steroidal hormone (testosterone) levels in blood (Amini & Kamkar, 2005), while Adeleke *et al.*, (2016) found that giving the ethanol leaf extract for 14 days caused testicular weight to decrease.

LH stimulates gonad secretion of sex steroids (Testosterone), whereas FSH, the focus hormone of mammalian reproduction, is known to play important roles in gonadal development, steroidogenesis, and maturation throughout puberty, as well as gamete formation (spermatogenesis) during fertile life (Iyke *et al.*, 2018). The extract was more successful in boosting the studied reproductive hormones when supplied at a greater dose, as seen by the dose dependent rise reported in this study. Iyke *et al.*, (2018) found that hydromethanolic leaf extracts of *Cnidoscolus aconitifolius* significantly ($p<0.05$) enhanced serum LH concentration levels in Streptozocin-induced diabetic rats. Moreover, whereas Lucky and Festus (2014) found a significant ($p=0.010$) drop in testosterone levels in rats treated with the aqueous leaf extract of *Cnidoscolus aconitifolius*, LH and FSH levels were significantly ($p=0.010$) rose. The study by Adeleke *et al.*, (2016), on the other hand, found that ethanol leaf extracts had no significant influence on the reproductive hormones of male rats. Because the gonadotropin releasing hormone (GnRH) generated by the hypothalamus regulates LH and FSH secretion, the significant ($p<0.05$) rise in FSH and Testosterone in this study could be due to a putative stimulatory effect the extract had on the hypothalamo-pituitary-gonadal axis, which increased their secretion.

Male fertility indices have been reported to include total sperm count, motile sperm count, and normal morphological traits (Oyeyemi *et al.*, 2000). The results of this study clearly show that administering hydromethanolic leaf extracts of *Cnidoscolus aconitifolius* had a beneficial effect on spermatogenesis in rats, which is dose dependent, and it also mitigated the effect of administering $CdCl_2$ in the same way. Flavonoids, which have been identified as antioxidants with ameliorative effects against toxicity-induced testicular deficits in animal tissues and the ability to stimulate testicular androgenesis, could be responsible (Salem *et al.*, 2001; Kujo, 2004).This contradicts recent findings from Ebeye *et al.*, (2015), who found that male rats given the aqueous leaf extract of *Cnidoscolus aconitifolius* saw a severe decline in sperm count and spermatogenesis was stopped. Adeleke *et al.*, (2016) found that injection of ethanol leaf extract dramatically reduced sperm count while having no effect on the volume and morphology of the animals' sperm cells. The variation in the effect of the extract on sperm parameters could be attributed to the use of hydromethanol as the extraction solvent in this investigation, which is a significant change from the nature of the solvents employed in the previous studies. The number of pregnant females in a group divided by the number of mated females in the group multiplied by one hundred is the fertility index. The fertility rate of female rats was determined after mating with males in this study, which represents the number of offspring born per mating pair, individual, or population. When given in higher doses, the extract improved fertility and mitigated the harmful effects of $CdCl_2$ on the male reproductive system, according to the findings. According to Lebas *et al.*, (1997), the average liter size ranges from 3 to 12, with limitations ranging from 1 to 20, and the results of this investigation revealed that the extract-treated group, as well as the 400mg/kg BW extract and $CdCl_2$ coadministration group, all fell within this range. According to Boutalbi (2014), this improvement can be ascribed to the components' androgenic characteristics (phenols and flavonoids), as demonstrated by our phytochemical studies.

CONCLUSION

This study found that administering hydromethanol leaf extract of *Cnidoscolus aconitifolius* to adult male Wistar rats with $CdCl_2$ toxicity caused a significant ($P<0.05$) increase in reproductive hormones (FSH and T) and sperm parameters (sperm cell volume, viability, normal morphology, motility, and count), improved the fertility index, and reversed the negative effect of $CdCl_2$ on the reproductive indices. In this study, the extract may have increased hormone secretion by acting on the hypothalamo-pituitary-gonadal axis. As a result, the extract may have a hormonal and sperm-boosting effect, as well as a pro-fertility effect. Additional research can be done to discover and isolate the specific bioactive chemicals in the plant that brought about these effects.

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