

See discussions, stats, and author profiles for this publication at: <http://www.researchgate.net/publication/228839079>

# Sperm Immobilization Properties of Aqueous Ethanolic Extract of Hymenocardia Acida Stem Bark

ARTICLE · SEPTEMBER 2011

DOI: 10.3889/MJMS.1857-5773.2011.0185

---

CITATIONS

2

---

READS

69

3 AUTHORS, INCLUDING:



Adakole Abu

4 PUBLICATIONS 11 CITATIONS

SEE PROFILE



Chukwuka Uchendu

University of Nigeria

64 PUBLICATIONS 148 CITATIONS

SEE PROFILE

## Sperm Immobilization Properties of Aqueous Ethanolic Extract of *Hymenocardia Acida* Stem Bark

Adakole Abu<sup>1</sup>, Chukwuka Uchendu<sup>2</sup>, Raphael Ofukwu<sup>3</sup>

<sup>1</sup>Department of Veterinary Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Agriculture, P.M.B, 2373, Makurdi, Nigeria; <sup>2</sup>Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria; <sup>3</sup>Department of Veterinary Public Health and Preventive Medicine, College of Veterinary Medicine, University of Agriculture, P.M.B, 2373, Makurdi, Nigeria

### Abstract

**Citation:** Abu A, Uchendu C, Ofukwu R. Sperm Immobilization Properties of Aqueous Ethanolic Extract of *Hymenocardia Acida* Stem Bark. Maced J Med Sci. 2011 Sep 30; 4(3):261-264. http://dx.doi.org/10.3889/MJMS.1957-5773.2011.0185.

**Key words:** albino rats; *Hymenocardia acida*; immobilization; spermicidal.

**Correspondence:** Adakole Abu. Department of Veterinary Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Agriculture, P.M.B, 2373, Makurdi, Nigeria. Tel.: +234(0)8060396898. E-mail: adakoleabu@yahoo.co.uk

Received: 05-Mar-2011; Revised: 24-Jun-2011; Accepted: 28-Jun-2011; Online first: 16-Sep-2011

**Copyright:** ©2011 Abu A. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Competing Interests:** The authors have declared that no competing interests exist.

*Hymenocardia acida* (Large red-heart) is widely used in Nigeria and many African countries, and has many therapeutic benefits. This study evaluated the sperm immobilization property of aqueous ethanolic extract of *Hymenocardia acida* stem bark. It was found that the extract of *Hymenocardia acida* reduced sperm motility in concentration-dependent manner. At 10 % concentration, the spermatozoa immediately became immotile. The results showed that *Hymenocardia acida* stem bark extract possesses sperm immobilization property.

### Introduction

In several countries and all through the ages, medicinal plants have been widely used to enhance or regulate fertility. In Nigeria, the folkloric uses of plant preparations for reproduction related purposes are well documented [1]. Elsewhere, Gupta *et al.*, 2006 reported that herbal contraceptives are used because of affordability, ease of availability from local sources and less side effects [2]. Large numbers of plants used as abortifacient, contraceptive or sterility agents prevent implantation [3], suppress spermatogenesis [4] or are

spermicidal [5]. In vitro screening of plant extracts for their spermicidal properties involve loss of functionality or motility as an endpoint. Several reports have shown sperm immobilization properties of some plants like *Achyranthes aspera* and *Stephania hernandifolia* [5], *Ruta graveolens* [6], *Cestrum parqui* [7], *Allium sativum* [8] and *Carica papaya* [9].

*Hymenocardia acida* (Tul.) is a small browse tree or shrub with palatable foliage, widely distributed within the savannah region of Nigeria. All parts of the plant are useful as remedies for many ailments. The

decoction of powdered root is used for fever, diarrhoea and dysentery. The root ash is used to treat mouth infections. The powdered roots are also used as depurative and for treating colds, muscular pains, headaches, jaundice, hypotension, enteralgia, chest pains and nephritis [10]. Among the Idoma and Igede people of North Central Nigeria, the decoction of root and stem bark is used in the treatment of diabetes [11].

Experimental studies have confirmed anti-HIV and anti-inflammatory [12], and antiplasmodial [13] as well as *in vitro* trypanocidal activities [14, 15] of this plant. Recently, Abu and Uchendu, 2010 [16] reported antispermatogenic activity of aqueous ethanolic extract of *Hymenocardia acida* stem bark in Wistar rats. To our knowledge, there is yet no report on sperm immobilization or spermicidal property of *Hymenocardia acida*. This study evaluates the sperm immobilization property of aqueous ethanolic extract of *H. acida* stem bark.

## Materials and Methods

The stem bark of *Hymenocardia acida* was collected within the premises of University of Agriculture, Makurdi and authenticated at the College of Forestry. Voucher specimen (No. 209) was deposited at the College herbarium. The stem bark was washed, air dried at room temperature for one week, pulverized and stored in air-tight container until required. One hundred gram of powdered material was soaked in 500 ml of 70% ethanol and stirred intermittently for 48 hours at room temperature. The material was filtered using sterile cotton wool and Whatman (No. 1) filter paper; the residue was resuspended in the same amount of solvent and then filtered three more times. The filtrates obtained were dried at room temperature under the electric fan, to obtain a crude extract with a yield of 18 %. The extracts were stored in air-tight containers at 4°C until needed.

### Phytochemical screening

Analysis of the major phytoconstituents was carried out qualitatively using standard procedures [17].

### Animals

Five white albino rats weighing 150 g – 200 g were obtained from the College of Health Sciences, Benue State University, Makurdi, Nigeria. The animals were kept in polypropylene cages under room temperature, with 12-hour light and 12-hour dark cycle and were allowed to acclimatize for two weeks. The animals were provided commercial feed (Grand Cereals

and Oil Mills Ltd, Bukuru, Jos, Nigeria) and clean water *ad libitum*. Protocols for this experiment was in accordance with the guidelines on the care and well being of research animals [18] and was approved by the Departmental Ethics Committee.

### Experimental design

The rats were anaesthetized with diethyl ether. A scrotal incision was made to exteriorize the testis and epididymides. The epididymides were carefully dissected out of the testes and blotted free of blood. To prepare sperm suspension, epididymal sperm were obtained by teasing the cauda epididymides placed in prewarmed beaker containing 2 ml of physiological saline (maintained at 37°C). Sperm suspension obtained from each rat was used for the *in vitro* immobilization activity.

*In vitro* sperm immobilization activity: Ten micro litres of the plant extract dissolved in physiological saline solution at varying concentrations (1%, 2%, 4%, 8% and 10 %) were mixed with epididymal sperm suspension (1:1 v/v) and tested for their effect on sperm motility. A drop of the evenly mixed sample was immediately placed on a clean and dry glass slide covered with cover slip and mounted on a prewarmed stage. This slide was then examined under the binocular microscope (Olympus, Japan) at magnifications of x10, x40. At least five fields were rapidly examined and 100 spermatozoa were counted. For the control, 10 µl of physiological saline was used instead of plant extract. The motility of spermatozoa was observed at various time intervals (15, 30 60, 90, 120 and 180 seconds).

### Statistical analysis

The results were expressed as mean ± S.E.M. using Graph Pad Prism Version 3.0 for Windows (Graph Pad Software, San Diego, California).

**Table 1: Phytochemical screening of hydroethanolic extract of *H. acida* for alkaloids, glycosides, flavonoids, saponins, tannins, terpenoids, anthraquinones and Phlobatannins.**

Alkaloids	+++
Anthraquinones	--
Glycosides	++
Flavonoids	+++
Saponins	+++
Tannins	+++
Phlobatannins	--
Terpenoids	+

+++ = highly present; ++ = moderately present; + = lightly present; - = absent.

## Results

Phytochemical screening of aqueous ethanolic extract of *H. acida* stem bark revealed the presence of alkaloids, glycosides, flavonoids, saponins, tannins and terpenoids (Table 1). Table 2 shows the *in vitro* effect of aqueous ethanolic extract of *H. acida* stem bark on sperm motility at different times (duration in seconds). The extract caused significant decreases ( $P < 0.05$ ) in spermatozoa motility in a concentration-dependent manner. No motility was observed at 120, 90, and 60 seconds when 2 %, 4% and 8 % concentrations were applied respectively. At 10 % concentration, the spermatozoa immediately became immotile.

**Table 2: Effect of *Hymenocardia acida* extract on spermatozoa motility (expressed as percentage). Data are expressed as mean  $\pm$  SEM.**

Concentrations	0 seconds	15 seconds	30 seconds	60 seconds	90 seconds	120 seconds	150 seconds	180 seconds
0 %	85.80 $\pm$ 2.96	85.80 $\pm$ 2.37	79.00 $\pm$ 3.32	77.00 $\pm$ 2.00	76.00 $\pm$ 2.45	76.00 $\pm$ 2.24	72.00 $\pm$ 2.00	69.00 $\pm$ 2.45
1 %	76.00 $\pm$ 2.45	60.00 $\pm$ 2.37	53.33 $\pm$ 3.34	43.33 $\pm$ 3.30	35.50 $\pm$ 4.01	33.75 $\pm$ 3.75	27.60 $\pm$ 2.01	18.20 $\pm$ 2.30
2 %	28.00 $\pm$ 3.74	24.00 $\pm$ 2.45	20.00 $\pm$ 2.45	16.00 $\pm$ 4.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
4 %	32.00 $\pm$ 2.00	22.00 $\pm$ 2.00	6.00 $\pm$ 2.45	4.40 $\pm$ 1.86	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
8 %	15.40 $\pm$ 3.50	14.80 $\pm$ 2.15	9.80 $\pm$ 3.01	0.00 $\pm$ 0.00				
10 %	0.00 $\pm$ 0.00							

## Discussion

The present study evaluated sperm immobilization properties of aqueous ethanolic extract of *Hymenocardia acida* stem bark and revealed a concentration-dependent reduction ( $P < 0.05$ ) in the motility of sperm cells. The extract of *Hymenocardia acida* caused instant immobilization of the rat epididymal spermatozoa at 10% concentration. An impaired motility as an index of spermicidal activity of aqueous extract of *Azadirchta indica* leaves [19], *Achyranthes aspera* and *Stephania hernandifolia* [5] have been reported. As was observed with *Cestrum parqui* [7], aqueous ethanolic extract of *Hymenocardia acida* stem bark possesses *in vitro* sperm immobilizing effect on rat sperm in concentration- and time- dependent manner.

A large number of plants screened for their spermicidal or sperm immobilization property were reported to contain saponins, flavonoids and phenol compounds [20]. Recently, most plant derived spermicides which caused sperm immobilization in animals and humans have been confirmed to contain saponins [21, 22]. Phytochemical screening of *H. acida*

stem bark extract revealed the presence of saponins and other phytoconstituents. The activity shown by the extract might be due to the presence of these metabolites.

Although the mechanism of action was not determined in the present study, other studies have attributed sperm immobilization properties of plants to cell death [9], disruption of the cell membrane [8] and ATP depletion or chromatin damage [23]. Lohiya and co-investigators showed that partially purified compounds of ethyl acetate sub fractions of *Carica papaya* seeds when administered at 2% concentration reduced motility of spermatozoa [9].

The results of the present study show that the aqueous ethanolic extract of *Hymenocardia acida* has an effect on the motility of the rat spermatozoa. The efficacy of active components of *H. acida* as vaginal spermicidal agents should be further evaluated.

## References

1. Akaneme FI. Identification and preliminary phytochemical analysis of herbs that can arrest threatened miscarriage in Orba and Nsukka towns of Enugu state. *Afri J Biotechnol.* 2008; 7(1):6-11.
2. Gupta RS, Kachawa JB, Chaudhury R. Antifertility effects of methanolic pod extract of *Abizzia lebbeck*. *Asian J Androl.* 2004;6:155-159.
3. Vasudeva N, Sharma SK. Estrogenic and pregnancy interceptory effects of *Achyranthes aspera* Linn. root. *Afr J Trad. Compl Altern Med.* 2007;4(1):7-11.
4. Raji Y, Akinsomisoye OS, Salman TM. Antispermatogenic activity of *Morinda lucida* extract in male rats. *Asian J Androl.* 2005;7(4):405-410.
5. Paul D, Bera S, Jana D, Maiti R, Ghosh D. *In vitro* determination of the contraceptive activity of a composite extract of *Achyranthes aspera* and *Stephania hernandifolia* on human sperm. *Contraception.* 2006;73(3):284-288.
6. Harat ZN, Sadeghi MR, Sadeghipour HR, Kamalinejad M, Eshraghian MR. Immobilization of *Ruta graveolens* on human sperm: A new hope for male contraception. *J Ethnopharmacol.* 2008;115:36-41.
7. Souad K, Ali S, Mounir A, Mounir TM. Spermicidal activity of extract from *Cestrum parqui*. *Contraception.* 2007;75(2):152-156.
8. Chakrabarti K, Sulagna Pal S, Bhattacharyya AK. Sperm immobilization activity of *Allium sativum* L. and other plant extracts. *Asian J Androl.* 2003;5:131-135.
9. Lohiya NK, Kothari LK, Manivannan B, et al. Human sperm

- immobilization effect of *Carica papaya* seed extracts: an *in vitro* study. *Asian J Androl.* 2000;2:103-109.
10. Irvine FR. Woody plants of Ghana. Oxford University Press, London, 1961:pp878.
11. Igoli SO, Ogeji OG, Tor-Anyiin TA, Igoli NP. Traditional medical practice amongst the Igede people of Nigeria. Part II. *Afr J Trad Compl Altern Med.* 2005;2(2):134-152.
12. Muanza DN, Euler KL, Williams L, Newman DS. Screening for antitumor and anti-HIV activities of nine medicinal plants from Zaire. *Intern J Pharmacol.* 1995;33(2):98-106.
13. Vonthron-Senecheau C, Weniger B, Quattara M, et al. *In vitro* antiplasmodial activity and cytotoxicity of ethno botanically selected Ivorian plants. *J Ethnopharmacol.* 2003;87:221-225.
14. Hoet S, Opperdoes F, Brun R, Adjakidje, V, Quetinleclercq J. *In vitro* antitrypanosomal activity of ethno pharmacologically selected Beninese plants. *J Ethnopharmacol.* 2004;91:37-42.
15. Abu AH, Uchendu CN, Ofukwu RA. *In vitro* antitrypanosomal activity of some selected Nigerian medicinal plants. *J Appl Biosci.* 2009;3(3):468-472.
16. Abu AH, Uchendu CN. Antispermatogetic effects of aqueous ethanolic extract of *Hymenocardia acida* stem bark in Wistar rats. *J Med Plants Res.* 2010;4(23):2495-2502.
17. Odebiyi OO, Sofowora EA. Phytochemical screening of Nigerian medicinal plants. *Lloydia.* 1978;41:234-235.
18. National Institute of Health (N.I.H). Guide for the care and use of laboratory animals. DHEW Publication. Office of Science and Health Reports, Bethesda, U.S.A., 1985.
19. Khillare B, Shrivastava TG. Spermicidal activity of *Azadirchta indica* (neem) leaf extract. *Contraception.* 2003;68:225-229.
20. Farnsworth NR, Waller DP. Current status of plant products reported to inhibit sperm. In: Zatzuchni GI, editor. *Research frontiers in fertility regulation.* 1982;2:1-16.
21. Saha P, Majumdar S, Pal D, Pal BC, Kabir SN. Evaluation of spermicidal activity of Misaponin A. *Contraception.* 2007;75(2):152-156.
22. Dubey R, Dubey K, Sridhar C, Jayaveera KN. Sperm immobilization activity of aqueous methanolic and saponins extract of bark of *Ziziphus Mauritania*. *Der Pharmacia Sinica.* 2011;2(2):11-16.
23. Hikim AP, Lue YH, Wang C, Reutrakul V, Sangsuwan R, Swerdloff RS. Posttesticular antifertility action of triptolide in the male rat: evidence for severe impairment of cauda epididymal sperm ultrastructure. *J Androl.* 2000;21(3):431-7.