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

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

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

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

Abstract

*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 hemandifolia* [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 antiplasmoidal [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>
<thead>
<tr>
<th>Phytochemical screening</th>
<th>Alkaloids</th>
<th>Anthraquinones</th>
<th>Glycosides</th>
<th>Flavonoids</th>
<th>Saponins</th>
<th>Tannins</th>
<th>Phlobatannins</th>
<th>Terpenoids</th>
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<td>+ + +</td>
<td>-</td>
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<td>+</td>
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</table>

+ + + = 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 ± SEM.

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>0 seconds</th>
<th>15 seconds</th>
<th>30 seconds</th>
<th>60 seconds</th>
<th>90 seconds</th>
<th>120 seconds</th>
<th>150 seconds</th>
<th>180 seconds</th>
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<tr>
<td>0%</td>
<td>85.0 ± 2</td>
<td>80.0 ± 2</td>
<td>75.0 ± 2</td>
<td>70.0 ± 2</td>
<td>65.0 ± 2</td>
<td>60.0 ± 2</td>
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<tr>
<td>1%</td>
<td>2.45</td>
<td>2.37</td>
<td>2.34</td>
<td>2.30</td>
<td>2.26</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
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<tr>
<td>2%</td>
<td>1.74</td>
<td>1.45</td>
<td>1.45</td>
<td>1.40</td>
<td>1.00</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td>4%</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<td>0.00</td>
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<td>8%</td>
<td>0.00</td>
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<tr>
<td>10%</td>
<td>0.00</td>
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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 hernandoifolia* [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

9. Lohiya NK, Kothari HK, Manivannan B, al. Human sperm...


