Partial Inhibitory Effect of Ethanol Extract of *Lepidagathis alopecuroides* (Vahl) on Spermatogenesis in Sprague-Dawley Rats

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**Abstract:** The aim of this investigation was to evaluate the inhibitory effect of ethanol extract of *Lepidagathis alopecuroides* on spermatogenesis in mammalian system. Thirty-six adult male Sprague-Dawley rats were used for this investigation and were divided into six groups of six rats each (A-F). Groups B-F were administered ethanol extracts of *L. alopecuroides* orally at a daily dose of 50-250 mg/kg body weight respectively, for 35 days. Testicular sections 5 μ thick were embedded with paraffin and stained according to standard Hematoxylin-eosin protocol. Computer-assisted histometric analysis was carried out on testicular seminiferous epithelium. Morphometric evaluation of the reproductive organs showed that there was significant (p<0.05) reduction in paired testes weights of the treated rats at higher concentrations, while the body weight, epididymal and *Tunica albuginea* weights were comparable (p>0.05) to those of the control rats. From analysis of the volumetric proportions of spermatogenic cells, extract interfered with the spermatogenic process in the treated rats inducing over 50% reduction in the number of preleptotene/leptotene primary spermatocytes, 57% decline in the number of advance primary spermatocytes at pachytene, about 60% reduction of secondary spermatocytes and 80% decrease in the expected number of spermatids against the number of spermatogonia. The progressive decline in the number of the various spermatogenic elements on critical examination of the seminiferous epithelium especially at concentrations 200-250 mg/kg body weight and the reduction of the spermatogenic efficiency to 20% shows that *Lepidagathis alopecuroides* possesses anti-spermatogenic properties in rats without the accompanying systemic toxicity reported in the catfish *Clarias gariepinus*.

**Keywords:** Cellular degeneration, *lepidagathis alopecuroides*, proliferation index, rat, spermatogenic cells, testis

**INTRODUCTION**

Herbal medicine has become the most accessible form of therapy available to the third world population for maintenance of health and treatment of various kinds of diseases (Gupta *et al.*, 2004). The World Health Organization (WHO) estimates that over 70% of the population in Africa makes use of herbal medicine (WHO, 2002).

Several plants commonly used for the treatment of various forms of diseases have not been assessed for possible toxicity on reproduction while a number of plants and plant products are reported to inhibit spermatogenesis in diverse classes of animals including rodents, domestic animals, birds and humans (D'Cruz *et al.*, 2010).

There is a steady and progressive accumulation of information on the phytochemical components of plant extracts and their active ingredients as well as compelling body of evidence of their effects on biological systems. A review of available literature indicates a wealth of plants with contraceptive effects on the males. Gossypol from *Gossypium herbaceum* administered at 30 mg/kg body weight acts directly on the testis and induced azoospermia or oligospermia in rats (Coutinho, 2002). *Ocimum sanctum* leaves administered to male rats at 250 mg/kg for 48 days decreased sperm count and motility of spermatozoa, reversible two weeks after withdrawal of treatment (Ahmed *et al.*, 2002).

Oral feeding of male rats with the ethanolic leaf extract of *Colebrookia oppositifolia* at dose levels of 100 and 200 mg/kg for 8-10 weeks, respectively did not cause body weight loss, while the weights of testes and epididymides were significantly decreased. Also reported was depression of spermatogenesis, decrease in the primary and secondary spermatocytes, sperm count, motility and fertility (Gupta *et al.*, 2001).

Chronic administration of *Mondia whitei* L. root bark extract (400 mg/kg/day) for 55 days caused testicular lesions resulting in the cessation of spermatogenesis, degenerative changes in the seminiferous tubules and...
epididymides. The wet weight of the seminal vesicle increased, whereas the weights of testes, epididymides and ventral prostate were unchanged (Watcho et al., 2001). Degeneration of germ cells and germinal epithelium, reduction in the number of Leydig cells and presence of vacuoles in the seminiferous tubules have been reported following oral administration of crude ripe seed extract of *Carica papaya* at 100 mg/kg to rats (Udoh and Kehinde, 1999).

Several other plant extracts which exhibit anti-spermatogenic activity and reduced sperm motility and concentration have been reported *Aegle marmelos* in rat (Chauhan and Agarwal, 2008), *Albizia lebbeck* (Gupta et al., 2006), *Syzygium aromaticum* (Mishra and Singh, 2009) *Eurycoma longifolia* (Wahab et al., 2010), *Leptadenia hastata* (Bayala et al., 2011), *Physalis alkekengi* (Naser et al., 2008), *Ruta graveolens* (Bazrafkan et al., 2010)

*Lepidagathis alopecuroides* (Vahl) is a herbal piscicide (Obomanu et al., 2007) that grows naturally in the Deltaic region of southern Nigeria. The aqueous leaf crude extract is used locally for the treatment of persistent sores and administered orally in the local gin for serious stomach upset. Sublethal concentrations of the aqueous leaf extract has been reported to induce degenerative changes in the germinal epithelium of fresh water catfish *Clarias gariepinus* during spermatogenesis (Orlu and Gabbriel, 2011a). Post testicular effect vis-à-vis significant reduction in sperm quality, motility and hatchability (Orlu and Ogbalu, 2011) and the effect on some Liver biomarkers and biochemistry have also been reported (Orlu and Gabbriel, 2011b).

However, there is no report in literature on the effect of this plant extract in any mammalian system, despite its traditional use as an herbal medicine. The aim of this investigation was the assessment of the effect of the ethanol extract of *Lepidagathis alopecuroides* on the reproductive tract and spermatogenesis in Sprague-Dawley rats.

**MATERIALS AND METHODS**

**Experimental location:** The investigation was carried out in the Postgraduate Laboratory of the Department of Applied and environmental Biology, Rivers State University of Science Technology, Port Harcourt (Coordinates: 4°48′14″N 6°59′12″E). The experiment lasted from October-December 2011.

**Experimental animals and management:** Thirty-Six adult male Sprague-Dawley rats weighing 245±18.71 g (mean GSI 0.01±0.001) used in this study were purchased from the animal house of the Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria. Animals were housed individually in plastic cages at room temperature and had access to commercial standard rodent Pellets and cool clean water *ad libitum*. The animals were acclimated for 14 days, before the commencement of the experiments. The experiments were conducted according to the institutional animal care protocols at the Rivers State University of Science and Technology Nigeria and followed approved guidelines and ethics for the treatment of Laboratory animals.

**Preparation of plant extract:** The leaves of *Lepidagathis alopecuroides* (Vahl) were air-dried and ground into fine powder with Moulinex blender and extracted in 70% Ethanol and the final concentrations adjusted to the following: Control (0.00 mg/kg), 50, 100, 150, 200 and 250 mg/kg body weight, respectively.

**Experimental setup:** Experimental animals were divided into six groups of six rats each. Group A (control 0.00 mg/kg.) was given water, Groups B-F were given 70% Ethanolic extract of *Lepidagathis alopecuroides* leaves orally at dose levels of 50, 100, 150, 200 and 250 mg/kg/day, respectively for 35 days. Histological and morphometric evaluation were carried out on the testes.

**Histology of the testis:** At the end of the experimental period, the animals were weighed and euthanized by cervical dislocation, dissected and the testes and epididymides were removed, freed of adhering connective tissues and immediately weighed. Known weights of the testes were fixed in Bouins’ liquid for histological analysis using the paraffin embedding and Periodic-Acid-Schiff technique and counter-stained with haematoxylin-eosin (Orlu and Egbonike, 2009).

Morphometric analysis of the paired testes and epididymides weights were obtained and the data used for the calculation of the means and standard deviations of each organ at the various concentrations.

**Testicular histometry:** The histometric and cytometric observations of the seminiferous epithelium were made at x100 immersion oil lens using Digital Microscope Model DB1-180M (China) connected to a computer through a CCD Camera system with a USB 1.0 output. The photomicrographs were captured and processed using the Software DN-2 Microscopy Image Processing System.

**Testicular cytometry:** The volumetric proportions of the various germ cell types were determined using 400-point graticule according to Berndtson (1977). Based on the cellular proportions the following indices of spermatogenesis intrinsic yield were derived.

**Spermatogonial mitoses efficiency:** This was determined as the coefficient of early primary spermatocytes (Preleptotene/Leptotene) to spermatogonia type A.
**Meiotic index:** Ratio of round spermatids to Pachytene primary spermatocytes.

**General spermatogenesis efficiency:** The number of round spermatids generated by one spermatogonium A

**Estimation of cellular losses:** The ratio of Preleptotene and Leptotene to Pachytene primary spermatocytes.

**Statistical analysis:** Data were subjected to analysis of variance and the Students t-test using the software XLSTAT 2011. All data in figures and tables are presented as the mean±SD and the significance level was set at p<0.05.

**RESULTS**

The gross morphology of the reproductive system of the experimental rats is presented (Fig. 1)

**Morphometric data:** The morphometric analysis of the testes, epididymides and Tunica albuginea of the rats treated with ethanolic extracts of *Lepidagathis alopecuroides* showed a concentration-dependent reduction in the testicular weights of treated rat (Table 1). From the mean weights of the organs the decline in the paired testes weights was significant (p<0.01) while paired epididymal and paired Tunica albuginea weights were not significant (p>0.05).

**Table 1:** Testicular and epididymal weights of control and *pidagitasis alopecuroides*-treated rats (Mean±SD)

<table>
<thead>
<tr>
<th>Parameters weight (mg)</th>
<th>Concentration of <em>Lepidagathis alopecuroides</em> (mg/kg bodyweight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>Paired testes (mg)</td>
<td>288±15</td>
</tr>
<tr>
<td>Left testis (mg)</td>
<td>148±8.0</td>
</tr>
<tr>
<td>Right testis (mg)</td>
<td>140±7.0</td>
</tr>
<tr>
<td>Paired epididymis (mg)</td>
<td>81±6.0</td>
</tr>
<tr>
<td>Left epididymis</td>
<td>42±2.7</td>
</tr>
<tr>
<td>Right epididymis</td>
<td>39±4.1</td>
</tr>
<tr>
<td>Paired tunica albuginea</td>
<td>11±0.54</td>
</tr>
<tr>
<td>Left tunica albuginea</td>
<td>6.0±0.00</td>
</tr>
<tr>
<td>Right tunica albuginea</td>
<td>5.0±0.05</td>
</tr>
</tbody>
</table>

*: Mean±SD significant (p 0.05); **: Mean±SD significant (p 0.01)

**Germ cell proliferation:** Mitotic proliferation index of the primordial spermatogonia was not affected (p>0.05) by any concentration of the plant extract used (Table 2). There was no significant (p>0.05) reduction in the number of type A spermatogonia at all concentrations. For early primary spermatocytes at preleptotene and leptotene stages there was significant (p<0.01) dose-dependent decline of over 50% in volume observed at 250 mg/kg body weight. The volumetric proportion of the late stage Pachytene/Diplotene also showed similar pattern of significant (p<0.01) cellular reduction (57% at 250 mg/kg). The number of spermatids observed also showed significant (p<0.01) concentration-dependent decrease of up to 80%. The mitotic index, meiotic index, efficiency of spermatogenesis decreased and cellular degeneration increased in a concentration-dependent manner.

**Table 2:** Germ cell counts and cellular degeneration in rats treated with ethanol extracts of *Lepidagathis alopecuroides*

<table>
<thead>
<tr>
<th>Spermatogenic elements</th>
<th>0.00</th>
<th>50</th>
<th>100</th>
<th>150</th>
<th>200</th>
<th>250</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spermatogonia A (SpgA)</td>
<td>3.80±0.5</td>
<td>3.80±0.41</td>
<td>3.80±0.54</td>
<td>3.80±0.33</td>
<td>3.80±0.64</td>
<td>3.80±0.45</td>
</tr>
<tr>
<td>Prelep/Lep</td>
<td>12.9±1.10</td>
<td>12.8±1.01</td>
<td>11.9±1.05</td>
<td>10.67±1.21</td>
<td>8.85±0.96**</td>
<td>6.55±0.99**</td>
</tr>
<tr>
<td>Pachytene</td>
<td>28.8±1.8</td>
<td>28.60±1.22</td>
<td>22.86±1.35</td>
<td>22.65±1.09*</td>
<td>18.82±1.75*</td>
<td>12.41±1.53*</td>
</tr>
<tr>
<td>20SPC</td>
<td>25.6±1.5</td>
<td>25.25±1.98</td>
<td>22.96±1.79</td>
<td>20.21±1.64</td>
<td>12.25±1.58**</td>
<td>10.20±1.87**</td>
</tr>
<tr>
<td>Stds</td>
<td>89.82±2.43</td>
<td>84.52±2.76</td>
<td>68.45±2.98</td>
<td>66.95±2.54*</td>
<td>35.06±2.62**</td>
<td>15.64±2.85**</td>
</tr>
<tr>
<td>Mitotic index2</td>
<td>3.39±0.21</td>
<td>3.37±0.42</td>
<td>3.14±0.50</td>
<td>2.81±0.63*</td>
<td>2.32±0.66**</td>
<td>1.72±0.74**</td>
</tr>
<tr>
<td>Meiotic index2</td>
<td>3.11±0.16</td>
<td>3.01±0.18</td>
<td>2.99±0.42</td>
<td>2.96±0.21*</td>
<td>1.86±0.15**</td>
<td>1.26±0.12**</td>
</tr>
<tr>
<td>Efficiency2</td>
<td>23.64±2.53</td>
<td>22.77±2.11</td>
<td>18.01±2.16</td>
<td>17.62±2.13*</td>
<td>9.22±1.14**</td>
<td>4.12±0.58**</td>
</tr>
<tr>
<td>Germ cell loss3</td>
<td>22.9±2.18</td>
<td>24.53±2.74</td>
<td>25.43±2.24</td>
<td>27.21±2.16*</td>
<td>62.48±2.89**</td>
<td>83.85±3.25**</td>
</tr>
</tbody>
</table>

Values are Mean±SD; *: Values significant (p<0.05); **: Values significant (p<0.01)

[1] SpgA: Spermatogonia A; Stds: Round spermatids; 20SPC: Secondary spermatocytes; Prelep: Preleptotene; Lep: Leptotene; Mitotic Index was the estimate of the ratio of Preleptotene/Leptotene to Spermatogonia A; Meiotic Index was the ratio of Round spermatids to primary spermatocytes at Pachytene; Efficiency of spermatogenesis was estimated as the number of round spermatids produced by one spermatonium type A; Germ cell loss was the ratio of early primary spermatocytes at preleptotene/leptotene to pachytene.

Fig. 1: Abdominal section of Sprague-Dawley rat showing Left Testis (LT) Right Testis (RT). Note the Caput (Cp), Corpus (Cr) and Cauda (Cd) epididymis
Fig. 2A: Intact seminiferous epithelium of control rat showing normal germ cell composition. Primary spermatocytes (1°SPc) at Pachytene, proliferating interstitial cell of Leydig (LC) and Lumen (L) filled with spermatozoa.

Fig. 2B: SE at first and second meiotic divisions (M1 and MII) with secondary Spermatocytes (2°SPC), Early stage Spermatids (Estds).

Fig. 2C, D: SE of rat treated with 50 mg/kg of *L. alopecuroides*. Epithelium retains its architectural integrity. Proliferating spermatogonia, primary spermatocytes (1°SPC) and Elongating Spermatids (Est).

Fig. 2E, F: SE of rats treated with 100 mg/kg of the extract showing Spermatogonia (Spg), Pachytene primary spermatocytes (1°SPc, P), Leydig Cells (LC) in the Interstitial Space (I-S).

Fig. 2G, H: Showing SE at 150 mg/kg with Spermatogonia (Spg), Elongating Spermatids (Est) and primary spermatocytes at Pachytene (P).

Spermatogenesis and the amount of germ cell loss were significantly (p<0.01) altered with increase in the concentration of *Lepidagathis alopecuroides* extract (Table 2). The spermatogenic efficiency declined to about 20%.

Histological evaluation of the seminiferous epithelium of the control rats revealed an intact epithelium with normal germ cell complements during late stage...
(I, J): Pycnotic cells (+) and very sparsely populated seminiferous epithelium

(K, L): At 250 mg/kg concentration Germ cell degeneration and vacuolation of the seminiferous epithelium, marked decrease in the number of primary spermatocytes and germcell loss. Absence of elongating spermatids and partial arrest of spermatogenesis

Fig. 2: Seminiferous Epithelium (SE) of control and rats treated with increasing concentrations (50-250 mg/kg) with ethanol extracts of Lepidaga thisalopecuroides (Vahl)

spermatogenesis (Fig. 2A) with interstitial Leydig cells and maturing spermatozoa, the lumen filled with spermatids following spermiation. Figure 2B shows an epithelium undergoing second meiotic division producing secondary spermatocytes. Some secondary spermatocytes are also undergoing mitotic division to produce round spermatids (arrow head) while some of spermatids are beginning to elongate (Estds). Interstitial cells of Leydig were observed and in appreciable number.

Figure 2C, D show the epithelium of rats treated with 50 mg/kg body weight of the extract. The integrity of the seminiferous epithelium architecture was still intact. Germ cells at the pre-meiotic spermatogonia stage were seen as well as primary spermatocytes at pachytene. Elongated and maturing spermatids were observed ready for spermiation. From the concentration of 100 to 250 mg/kg degenerative histological changes were observed in the seminiferous epithelium of the treated rats and the effect was concentration-dependent. There was progressive reduction in the number of spermatogonia committed to meiosis and reduction in the number of primary spermatocytes at early meiotic prophase (Fig. 2E, F 100 mg/kg), reduction in the number of secondary spermatocytes, spermatids at early stage of elongation and maturation (Fig. 2G-J 150-200 mg/kg), vacuolation of the seminiferous epithelium and absence of elongating spermatids and spermatozoa (Fig. 2K-L 250 mg/kg) The interstitial space progressively lost the Leydig cells.

DISCUSSION

The result of this investigation revealed that oral administration of ethanol extract of Lepidagathis alopecuroides did not cause significant reduction in the body weight of the treated rats indicating non-systemic toxicity of the extract in rats. This is in contrast to reduction in bodyweight in Clarias gariepinus (Burchell, 1822) treated with sublethal concentration of this extract (Orlu and Gabriel, 2011a; Orlu and Ogbalu, 2011). This result, however, agrees with Venma et al. (2002) who reported a lack of systemic side effects on male albino rats administered 70% methanol extract of Sarcostemma acidum and other similar results by Gupta et al. (2006), Watcho et al. (2001) and Bayala et al. (2011). The significant (p<0.01) reduction in testicular weight observed in this investigation, is indicative of reproductive toxicity since there is a linear and positive correlation between testis weight and sperm concentration (Orlu and Egbunike, 2009; Orlu and Egbunike, 2010ab; Orlu and Ogbalu, 2011; Gupta et al., 2004).

The reduction in Meiotic and Mitotic Indices observed in this study has also been reported in the fresh water catfish Clarias gariepinus (Burchell, 1822) exposed to sublethal concentration (0.75-1.0 ppm) of Lepidagathis alopecuroides Vahl (Orlu and Gabbriel, 2011a). This result is in agreement with (Gupta et al., 2006; Bazrafkan et al., 2010) who reported similar observations in rats treated with methanol extract of Albizia lebbeck and Ruta graveolens, respectively. Reduction in mitotic and meiotic
indices occurred due to degenerative changes in the seminiferous pithelium. Failure of spermatogonia to become committed to meiosis due to inhibition of the last mitotic division reduces the number of preleptotene/leptotene primary spermatocytes. The conversion of spermatogonia (mitotic germ cells) into primary spermatocytes (meiotic cells) is powered by a surge of testosterone produced by interstitial Leydig cells. Inhibition of and reduction in the population of Leydig cells would result in reduction of testosterone levels resulting in germ cell degeneration (Yang et al., 2004). This observation agrees with other reports on plants with anti-spermatogenic activities, including (Udoh and Kehinde, 1999) who reported degeneration of germinal epithelium, germ cells, reduced Leydig cell and presence of vacuoles in rats fed crude extracts of ripe Carica papaya seeds. Reports on decrease in the number of primary spermatocyte, interference and arrest of spermatogenesis, reduction in male fertility by extract of Tinospora cordifolia (Gupta and Sharma, 2003), Eurycoma longifolia (Wahab et al., 2010) Leptadenia hastata (Bayala et al., 2011), Ruta graveolens (Bazrafkan et al., 2010), Mondia whitei (Watcho et al., 2001) have been documented.

Analysis of the indicators of the efficiency of spermatogenesis in this investigation shows that ethanol extract of Lepidagathis alopecuroides interfered with the spermatogenic process in the treated rats by inducing over 50% reduction in the number of stage preleptotene/leptotene primary spermatocytes, 57% decline in the number of advance primary spermatocytes at Pachytene, about 60% reduction of secondary spermatocytes and 80% decrease in the expected number of spermatids against the number of spermatogonia type-A (Table 2, Fig. 2E-L). The progressive decline in the number of the various spermatogenic elements, presence of vacuoles in the seminiferous epithelium especially at higher concentrations imply that Lepidagathis alopecuroides possesses anti-spermatogenic properties in rats without the accompanying systemic toxicity reported (Orlu and Gabriel, 2011a) in the catfish Clarias gariepinus.

REFERENCES


