Hormonal and Histomorphologic Effects of *Azadirachta indica* leaf Extract on the Pars Anterior of Wistar Rats

Efectos Hormonales e Histomorfológicos del Extracto de la Hoja de *Azadirachta indica* en la Pars Anterior de Ratas Wistar

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**SUMMARY:** *Azadirachta indica* is a tree whose medicinal value is unquantifiable. Any part of the tree can be used in the treatment of malarial infection. Reports have indicated its antifertility effects, and this necessitated this study on the effects of the methanol leaf extract on serum luteinizing (LH) and follicle stimulating hormones (FSH) levels and the histomorphology of the pars anterior of Wistar rats. Thirty adult male Wistar rats were equally divided into 3 groups of A, B and C. Group A was the control and the animals received distilled water orally, while groups B and C were treated with 200mg/kg and 400mg/kg respectively of the leaf extract by oral gavage for fourteen days. On day fifteen, the animals were sacrificed by chloroform anaesthesia. Blood was obtained from their hearts, while the skull was opened to assess the hypophysis. Hormonal assay showed that luteinizing (LH) and follicle stimulating (FSH) hormone levels in the serum were lower in groups B and C treated with 200mg/kg and 400mg/kg respectively of the leaf extract, while that of LH were significant (P<0.001). Histomorphologic sections of the pars anterior revealed reduced acidophil and basophil populations, with prominent degranulated chromophobes which were larger in the group treated with 400mg/kg of *A. indica* leaf extract. This group also presented hypertrophy of the basophils compared to the control. In conclusion, methanol leaf extract of *A. indica* decreases serum LH and FSH and caused histomorphologic changes in the pars anterior of adult male Wistar rats.

**KEY WORDS:** *Azadirachta indica*; Pars anterior; Luteinizing hormone; Follicle stimulating hormone; Wistar rat.

**INTRODUCTION**

The plant, *Azadirachta indica* commonly called neem has been used widely due to its medicinal values (Subaprinya & Nagini, 2005). Its chemical components includes various alkaloids where some are; nimbitin, azadirachtin and salanin (Koul et al., 1990). These alkaloids contribute to the general properties of the plant.

Different parts of this plant have been used as insecticides and pesticides (Koul et al.; dos Santos et al., 2004; Siddiqii et al., 2004; Jin et al., 1995), and have been reported to have inhibitory effects on several ranges of micro-organisms (Talwar et al., 1997; Garq et al., 1998; Baswa et al., 2001; Bocke et al., 2004).

*A. indica* regulates metabolism, and functions as anti-diabetic, anti-hyperglycaemic and anti-carcinogenic agents. Immuno-modulatory and anti-inflammatory effects have been reported as well (Subaprinya & Nagini; Chattophyay, 1998; Khosla et al., 2000).

Extracts of *A. indica* have been reported to have several anti-fertility effects (Awasthy, 2001; Raji et al., 2003). These include; reduction in serum testosterone and luteinizing hormone levels, inhibition of folliculogenesis and immunocontraceptive activity (Awasthy; Raji et al.; Roop et al., 2005; Akpantah et al., 2010).

The serum level reduction of the hypophysis hormones as well as hypophysis induced processes as seen in previous works raised our concern to carry out this study on the effect of the leaf extract of *A. indica* on the luteinizing and follicle stimulating hormones levels and histomorphology of the pars anterior of adult male Wistar rats.
MATERIAL AND METHOD

Thirty adult male Wistar rats were assigned equally into 3 groups (A, B and C). The animals weighed 110-150g and the experiment commenced after acclimatizing for two weeks in the Animal House of the Department of Anatomy, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria. They were cared for in compliance with the international guidelines for animal research study, and ethical approval was obtained from the institution. Fresh leaves of A. indica were washed and oven-dried at 60ºC for twenty-four hours. The leaves were blended into powder by an electric blender. Soxhlet extractor using methanol was employed in extracting 220g of the powder. A stock solution of 40g was arrived at. Distilled water was used to dilute the stock solution to concentrations of 200mg/kg and 400mg/kg.

Group A animals served as the control and the animals received distilled water, while groups B and C were the experimental and were treated with 200mg/kg and 400mg/kg of the leaf extracts of A. indica respectively for fourteen days by oral gavage.

On the fifteenth day the animals were sacrificed by chloroform anaesthesia. Blood was collected from the heart and stored in heparinized test tubes for hormonal assay. The hypophysis was dissected out after excision of the sphenoid bone, the pars anterior separated and preserved in Bouins fluid. Routine haematoxylin and eosin method was used in the histological processing. Luteinizing (LH) and follicle stimulating (FSH) hormones in the serum were determined by the Enzyme-Linked Immunoabsorbent Assay (ELISA) method using Microwell’s kits.

One way analysis of variance was employed in determining the relationship of the hormonal levels between the control and experimental groups, and differences at p<0.05 were regarded as significant.

RESULTS

Hormonal Assay. The luteinizing hormone (LH) level of groups B and C treated with 200mg/kg and 400mg/kg respectively of A. indica leaf extract were significantly (P<0.001) lower than the control.

The follicle stimulating hormone (FSH) level of groups B and C treated with 200mg/kg and 400mg/kg respectively of A. indica leaf extract were not significantly (P<0.05) lower than the control. These are as presented in Table I below.

Histomorphology of the pars anterior. The control pars anterior section showed normal histomorphology. It presented two cell types: chromophils and chromophobes irregularly distributed. The chromophils were further grouped into the acidophils (A) and the basophils (B). (Fig.1).

Fig. 1. The control pars anterior section showed normal histology. It presented two cell types: chromophils and chromophobes (C) irregularly distributed. The chromophils were further grouped into the acidophils (A) and the basophils (B). H & E. Mag. x400.

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>200mg/kg of A. indica</td>
<td>400mg/kg of A. indica</td>
</tr>
<tr>
<td>Luteinizing Hormone (LH)</td>
<td>1.24±0.09</td>
<td>0.62±0.18***</td>
<td>0.34±0.24***</td>
</tr>
<tr>
<td>Follicle Stimulating Hormone (FSH)</td>
<td>0.34±0.11</td>
<td>0.24±0.003 NS</td>
<td>0.26±0.04S</td>
</tr>
</tbody>
</table>

Results are presented as Mean ± SEM. **Significantly different from control at P<0.001 NS Not significantly different from the control at p<0.05 A. indica-Azadirachta indica.
Group B pars anterior section treated with 200mg/kg of A. indica leaf extract for fourteen days presented numerous degranulated chromophobes and reduced acidophils and basophils compared to the control (Fig.2).

![Fig. 2. Group B pars anterior section treated with 200mg/kg of A. indica leaf extract for fourteen days presented numerous degranulated chromophobes (DC) and reduced acidophils (A) and basophils (B) compared to the control. H & E. Mag. x400.](image)

Group C pars anterior section treated with 400mg/kg of A. indica leaf extract presented reduced acidophil distributions, less but hypertrophied basophils and large degranulated chromophobes compared to the control (Fig. 3).

![Fig. 3. Group C pars anterior section treated with 400mg/kg of A. indica leaf extract presented reduced acidophil (A) distributions, less but hypertrophied basophils (B) and large degranulated chromophobes (DC) compared to the control. H & E. Mag. x400.](image)

**DISCUSSION**

The hypophysis serves as a master gland by dictating to other glands in the body. It does this by secreting different types of trophic and neuro-hormones (Murray et al., 2000). It has two parts; the pars posterior, and the pars anterior. The pars anterior is further divided into the pars distalis, tuberalis and intermedia (Bowen, 2006). The different cells of the pars anterior are divided into chromophils and chromophobes using haematoxylin and eosin staining technique (Bancroft & Steven, 1992; Young & Heath, 2004; Bowen). The chromophils are further divided into the acidophils and the basophils based on their colour staining to acidic and basic dyes respectively. The acidophils are made up of cells called the somatotrophs and the mammotrophs; these secrete the polypeptide hormones. The basophils are made up of cells called the corticotrophs, thyrotrophs and the gonadotrophs; these secrete the glycoprotein hormones (Bowen).

The gonadotrophs are the gonadal cells that secrete the glycoprotein hormones called the LH and FSH (Young & Heath). Assay of the serum levels of these hormones revealed lower levels in the two treatment groups, with the LH being significantly \( P<0.001 \) reduced.

FSH indirectly stimulates gametogenesis in both sexes and directly stimulates estrogen synthesis and follicular development. It also maintains the structure of the gonads in conjunction with LH (Guyton & Hall, 2001). On the other hand, LH is critical to luteinization of the ovarian follicles and post-ovulatory follicular function (Murray et al.). Reduced levels of FSH may result in the inability of follicular cells to reach maturation, and hence ovulation and complete spermatogenesis in females and males respectively may be hindered. This situation may inhibit the synthesis of the LH whose maximum effect is felt after ovulation. The limitation to the normal physiologic process may be exploited in contraception, hence this extract may be useful as a contraceptive.

Our report is in line with Raji et al. and Akpantah et al. who reported a reduction in serum LH level after treatment with extracts of A. indica. Gbotolorun et al. (2004) reported partial blockage of ovulation and alteration in the estrous cycle of Sprague-Dawley rats treated with seed extract of A. indica. This may have contributed to the levels of the LH and FSH seen in this study.
Histomorphologically, Groups B and C treated with 200mg/kg and 400mg/kg respectively of the extract showed reduced acidophil and basophil populations, with prominent degranulated chromophobes which were larger in group C. Group C also presented hypertrophy of the basophils compared to the control.

The reduced presence of acidophils and basophils in the sections of the groups treated with 200mg/kg of A. indica leaf extract may indicate a destructive effect of the leaf extract of A. indica to the somatotrophs, lactotrophs, corticotrophs, thyrotrophs and the gonadotrophs. This may have caused the degranulated chromophobes seen in this study. The reduced basophil population may have resulted in reduced gonadotrophs which secretes FSH and LH, and this may be a reason for the reduced hormonal levels in this study.

The hypertrophy of the basophils in the 400mg/kg extract group may be due to the increased dose of the extract resulting in their blotted size in order to cope with the dose. By so doing, they occupy greater spaces and may cluster together as seen in this study.

The group treated with 200mg/kg and 400mg/kg of the extract showed degranulated chromophobes which increased in population in the higher dose group. Chromophobes have been speculated to probably represent resting or degranulated chromophil cells (Young & Heath). Their increase may be due to the loss of the active neuromolecules of the tissue, since these are responsible for the tissue’s staining ability. Yajurvedi et al. (1985) earlier reported the same effect on pars distalis after treatment of frogs with gonadotropin releasing hormone.

A. indica has been reported to have antifertility effects (Awasthy; Raji et al.; Roop et al.), though the degree of effect by different parts of the plants cannot be ascertained. The decreased basophils of the pars distalis and the decreased LH and FSH may be a pointer to already reported antifertility effects of this plant with other extracts. These results indicate that the leaf extract’s antifertility effect of the plant may be a result of hypophysis changes that ultimately cause the reduction of LH levels.

In conclusion, methanol leaf extract of A. indica decreases serum LH and FSH levels and causes histomorphologic changes in the pars anterior of adult male Wistar rats. Hence the plant may be exploited in contraception, though with caution.

**RESUMEN:** Azadirachta indica es un árbol cuyo valor medicinal es invaluable. Cualquier parte del árbol se puede utilizar en el tratamiento de la infección por malaria. Reportes han indicado su efecto antifertilidad, lo que requirió estudiar los efectos del extracto metanólico de la hoja sobre los niveles séricos de las hormonas luteinizante (LH) y folículo estimulante (FSH) y la histomorfología de la pars anterior de ratas Wistar. Treinta ratas Wistar adultas fueron divididas en tres grupos. El grupo A fue utilizado como control y los animales recibieron agua destilada por vía oral, mientras que los grupos B y C fueron tratados con 200 mg/kg y 400 mg/kg respectivamente, con extracto de hoja mediante una sonda nasogástrica durante catorce días. A los quince días, los animales fueron sacrificados por anestesia con cloroformo. Se obtuvo sangre desde sus corazones, mientras que el cráneo fue abierto para evaluar la hipófisis. Los ensayos hormonales mostraron que los niveles en suero de la LH y FSH se redujeron en los grupos B y C, tratados con 200 mg/kg y 400 mg/kg respectivamente, siendo la reducción de LH significativa (p<0,001). Secciones histomorfológicas de la pars anterior revelaron una reducción de las poblaciones acidófilas y basófilas, con prominentes cromófobos degranulados que fueron mayores en el grupo tratado con 400 mg/kg del extracto de A. indica. Este grupo también presentó hipertrofia de los basófilos en comparación con el control. En conclusión, el extracto alcohólico de la hoja de A. indica disminuye el nivel sérico de LH y FSH y provoca cambios histomorfológicos en la pars anterior de ratas Wistar adultas.

**PALABRAS CLAVE:** Azadirachta indica; Pars anterior; Hormona luteinizante; Hormona folículo estimulante; Rata Wistar.


