Effects of *Telfairia Occidentalis* Seed Oil on Female Reproductive Functions in Wistar Rats

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**Summary:** The effects of *T. occidentalis* seed oil on some female reproductive indices were investigated in Wistar rats. The study was divided into two phases: (estrous cycle and pregnancy). Animals were grouped into four: group A received distilled water (control), groups B, C and D received 400, 600 and 800 mg/kg bw of *T. occidentalis* seed oil respectively. The pattern of estrous cycle was determined for three weeks before and during the treatment. Thereafter, each group was subdivided into two. The sub-group-1 rats were mated with male breeders, the litter size and birth weight of their offsprings were determined. Sub-group-2 rats were sacrificed and histology of organs and serum levels of LH, FSH and estrogen were assayed. There was no significant difference between the pre-treatment and post-treatment estrous cycle length. However, there was a significant decrease in the frequency of diestrus phase during treatment in all the experimental groups when compared with pre-treatment period (p < 0.05) but there was no significant difference in the diestrus phase when compared with the control group. Serum estrogen concentration was significantly reduced (p < 0.05) in the group that was treated with 800 mg/kg bw of *T. occidentalis* seed oil. Histology of the ovary and uterus in the experimental groups were similar to that of the control group. Birth weight of pups was significantly increased in the group treated with 600 mg/kg bw of *T. occidentalis* seed oil when compared with the control group (p < 0.05). The results of this study suggest that *T. occidentalis* seed oil does not alter estrous cycle in Wistar rats.

**Keywords:** *T. occidentalis*, Wistar rats, Estrous cycle, diestrus, Estrogen.

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**INTRODUCTION**

Infertility is a problem of global proportions. The World Health Organization estimates that 1 in every 4 couples in developing countries is infertile (Mascarenhas, 2012). It is also estimated that one in three couples is affected in countries within Central and West Africa (Inhorn, 2003). In sub-sahara African countries, report showed that 20 - 46 % of couples are infertile (Idrisa, 2001). The consequences of infertility in these countries range from economic hardship to social isolation, violence and denial of proper death rites (Abdallah and Daar, 2001).

In oriental cultures reproduction is one of the highest valued factors (Qui, 2001). The way in which people try to solve the problem of infertility is at least partly affected by the values and socio-cultural norms of the community in which they live (Inhorn, 2003). Herbal plants are used in the cure of infertility in Africa. Many of these plants are common vegetables and have been proven scientifically, to actually have fertility effects as well as other health benefit rooted in their physiological effect as a result of their phytochemical and nutritional constituents (Hunter and Fletcher, 2002).

*T. occidentalis* is commonly known as fluted pumpkin. It belongs to the family of *Curcumbitaacea*. It is found along the fringes of the closed forest in Africa and particularly cultivated in south-eastern part of Nigeria and Sierra Leone (Burkett, 1968). It is a creeping vegetable shrub that creeps low across the ground with large leaves and long twisting tendrils (Horsfall and Spiff, 2005). The leaves and the young shoot of the plant are frequently eaten as potherb (Tindal, 1968; Okigbo, 1977; Okoli and Mgbeogun, 1983). In Ibo, it is called ugu, in Yoruba, the seed is called egusi iroko, and in Benin, it is called uwenkhen. There is hardly any home in Nigeria where ugu is not consumed in daily meal due to its health restoration (Ehiagbonare, 2008).

The seed of *T. occidentalis* is located in its fruits that may weigh up to 13kg. It is dark red in color. It is used as soup condiment in certain communities in rural region of south-eastern Nigeria (Agatemor, 2006). It is also cooked and taken as a meal. Physiochemical analysis of fluted pumpkin seed oil showed that it is rich in iodine, linoleic acid, tannins and polyunsaturated free fatty acids (Egbeheku *et al*., 1998). It also has an acidic value that indicates that it is edible.
Effect of T. Occidentalis seed oil on female reproductive functions

and can be used in marmalade production (Akubugwo et al., 2007).

Experiments have shown that *T. occidentalis* seed oil has beneficial effects on male fertility in rats (Akang et al., 2010). It has also been shown that its addition to rat feed led to varying degree of pathology in the heart, liver, kidney and skin (Ajayi et al., 2004). The aqueous extract of *T. occidentalis* has a haematinic effect (Alada, 2000). Research carried out by Salman et al., (2008) suggested that the aqueous extract of the *T. occidentalis* leaf improved male reproductive functions by increasing sperm count, sperm motility and sperm viability. However, there is no scientific report on its effects on the female reproductive functions. The present study was therefore designed to investigate the effects of *T. occidentalis* seed oil on female reproductive functions in Wistar rats.

**MATERIALS AND METHODS**

**Plant Material and Oil Preparation**

Healthy fruits of *T. occidentalis* were obtained from Ojo market, Ibadan, Oyo state. The plant and its seed were identified at Forestry Research institute of Nigeria (FRIN), Ibadan, Oyo state, with the identification number, 108846. The fruits were broken and the seeds were collected, decocted and the naked seeds were air-dried for 2 days. The seeds were thereafter oven-dried at 45°C for 1 week during which consistent dry weight was obtained. The seeds were blended into powdery form using super master blender and preserved for oil extraction.

The oil extraction was done at the National Institute of Science and Technology (NIST), Samonda, Ibadan, Oyo state, Nigeria. The oil extract was obtained using petroleum ether in continuous extraction with a Soxhlet reflux apparatus as described in earlier works (Reinhold, 1992; Ojaiko and Nwajo, 2006; Akang et al., 2010) at a temperature of 60°C to 80°C. On completion of extraction, the petroleum ether in the extracted oil was completely evaporated at 40°C (Reinhold, 1992). The oil left after the evaporation of petroleum ether was the desired sample.

The desired oil sample had a yellow color and an agreeable smell, though different from that of other known oil. It was liquid at room temperature.

**EXPERIMENTAL ANIMALS**

Forty female Wistar rats (120-150g) and ten male Wistar rats (150-180g) were purchased from the Central animal house, University of Ibadan, Ibadan, Nigeria. They were maintained under standard laboratory condition and fed with rat feed and they had access to drinking water *ad libitum*. All animals were acclimatized for two weeks.

The female animals were randomly divided into four groups of ten animals each. Group A was the control group and was administered distilled water. Group B was administered a dose of 400 mg/kg bw of *T. occidentalis* seed oil. Group C was administered 600 mg/kg bw of *T. occidentalis* seed oil and group D was administered 800 mg/kg bw of *T. occidentalis* seed oil. The dosage regime was adopted from Akang et al., (2010). The treatment was done orally for a period of 21 days. The duration of administration was based on the fact that the mean estrous cycle length of rat is 4 days (Long and Evans, 1922). This provided the opportunity to study the effect of *T. occidentalis* seed oil on rat in 5 consecutive estrous cycles. The weight of the animals was recorded every week during treatment.

**EXPERIMENTAL PROCEDURE**

**Estrous Cycle**

The estrous cycle of each rat was established for three weeks without treatment. Thereafter, each experimental group was treated with its designated dosage while the control was treated with distilled water for another three weeks and estrous cycle was also carried out throughout the period. The outcome of both period of estrous cycle was later compared.

Determination of estrous cycle was done using the technique described by Marcondes et al., (2002). This was done between the hours of 7:00- 8:00am every morning. The slides used for preparing vaginal smear for each rat were thereafter stained using Papanicolaou’s technique. The morphology of the cells was observed under the microscope using x40 magnification lens and photograph was taken.

**Mating of the Animals**

At the end of estrous cycle determination, each group was sub-divided into two. Sub-group 1 was cohabited with male breeders during proestrus phase at the ratio of 2:1 (female: male). The day spermatozoa were seen in the vagina smear of rats was taken as day one of pregnancy. Birth weight of offspring and litter size in each group were determined on the day of parturition.

**Hormone Assay and Histology**

Animals in sub-group 2 were used for this purpose. During proestrus, blood sample was collected through the orbital sinus of each rat into sterile plain bottles using heparinized capillary tube. This was used to determine serum concentration of luteinizing hormone, follicle stimulating hormone and estrogen using the Enzyme-linked immunosorbent assay kits (Inteco®, UK). The animals were then immediately sacrificed by cervical dislocation. Each animal was opened through the linea alba. The uterus and the
Ovaries of each animal were harvested, freed of any adherent tissues and weighed immediately. All organs were then fixed in 10% formalin for at least 5 hours. Samples were dehydrated using ascending grades of xylene, embedded in paraffin wax, trimmed, nicked and sectioned using a microtome and stained with haematoxylin and eosin (H&E) for the purpose of determining the general morphology.

Statistical Analysis
The data from each group were analyzed using Student’s paired T-test and one-way analysis of variance (ANOVA) followed by Waller-Duncan’s post hoc test. The results were expressed as mean ± standard error of the mean (Mean ± SEM) and p-value < 0.05 was considered significant.

RESULTS
Effect of T. occidentalis Seed oil on Body Weight gain and Relative Organ Weight
There was no significant difference in the body weight gain (Table 1) and relative organ weight (Table 2) of the experimental groups when compared with control.

Effect of T. occidentalis Seed Oil on Estrous Cycle
The length of estrous cycle before and during the treatment period was not significantly different. The length of estrous cycle was also not significantly different in all the experimental groups when compared with the control group (Table 3). There was no significant change in the frequency of proestrus phase and estrus phase before and during the treatment in all the experimental groups. There was also no significant difference in the proestrus phase and the estrus phase of the experimental groups when compared with the control group (Table 3). However, there was a significant decrease in the frequency of diestrus phase in all experimental groups during the treatment period when compared with the pre-treatment period (p < 0.05) but there was no significant difference in the diestrus phase when compared with the control group (Figure 3).

Table 1: Effects of T. occidentalis seed oil on Body Weight gain

<table>
<thead>
<tr>
<th>Dosage</th>
<th>Body weight gain (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
</tr>
<tr>
<td>Control</td>
<td>13 ± 1.53</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>11 ± 1.00</td>
</tr>
<tr>
<td>600 mg/kg</td>
<td>14 ± 1.67</td>
</tr>
<tr>
<td>800 mg/kg</td>
<td>11 ± 1.67</td>
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</tbody>
</table>

Values expressed as Mean ± SEM. n=5

Table 2: Effect T. occidentalis seed oil on Relative Organ Weight

<table>
<thead>
<tr>
<th>Dosage</th>
<th>Relative ovary weight (%)</th>
<th>Relative uterus weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.02 ± 0.00</td>
<td>0.28 ± 0.08</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>0.02 ± 0.00</td>
<td>0.21 ± 0.03</td>
</tr>
<tr>
<td>600 mg/kg</td>
<td>0.02 ± 0.00</td>
<td>0.19 ± 0.05</td>
</tr>
<tr>
<td>800 mg/kg</td>
<td>0.02 ± 0.00</td>
<td>0.28 ± 0.04</td>
</tr>
</tbody>
</table>

Values expressed as Mean ± SEM. n=5

Table 3: Effect of T. occidentalis seed oil on estrous cycle length

<table>
<thead>
<tr>
<th>Dosage</th>
<th>Estrous cycle length (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>Control</td>
<td>4.68 ± 0.19</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>5.01 ± 0.27</td>
</tr>
<tr>
<td>600 mg/kg</td>
<td>5.21 ± 0.14</td>
</tr>
<tr>
<td>800 mg/kg</td>
<td>4.90 ± 0.13</td>
</tr>
</tbody>
</table>

Values expressed as Mean ± SEM. n=5

Figure 1: Effect of T. occidentalis seed oil on frequency of proestrus phase before and during treatment. n=5.

Figure 2: Effect of T. occidentalis seed oil on frequency of estrus phase before and during treatment. n=5.

Figure 3: Effect of T. occidentalis seed oil on frequency of diestrus phase before and during treatment. n=5. *p < 0.05
Effect of T. occidentalis seed oil on female reproductive functions

There was no significant difference in the serum levels of FSH and LH in all the experimental groups when compared with the control group (Figure 4). However, the serum concentration of estrogen in the group treated with 800 mg/kg bw decreased significantly when compared with the control group (p < 0.05) (Figure 5).

**Effect of T. occidentalis Seed Oil on Ovarian and Uterine Histology**

The histological section of the ovaries in the experimental groups showed no visible lesion with intact cortex and inner medullar. The surface of the germinal epithelium and tunica albuginia showed normal histological appearance with normal follicular growth. The germinal discs seen in some sections appeared normal. The animals in all the experimental groups showed normal features of the uterus. The uterine cavity, perimetrium, myometrium and endometrium all appeared normal. The endometrium

**Figure 4:** Effect of *T. occidentalis* seed oil on serum concentration of FSH and LH. n=5.

**Figure 5:** Effect of *T. occidentalis* seed oil on serum concentration of estrogen. n=5. *p < 0.05

**Figure 6:** (A) Nucleated epithelial cells in the proestrus phase of the estrous cycle (white arrow). (B) Estrus phase of the estrous cycle showing non-nucleated epithelial cells (white arrow). (C) Metestrus phase of the estrous cycle: the photomicrograph showing the presence of multinucleated cells (white arrow) single nucleated cells (slender arrow) and non-nucleated epithelial cells (black arrow) (D) Diestrus phase of the estrous cycle showing predominant leucocytes cells (white arrow). H&E X400.

**Figure 7:** (A): Arrows (black) showing secondary follicles with normal cytoarchitecture. The cortex and inner medullar showed normal histology (B) Arrows showing secondary follicle with multilayered epithelium (black arrow) and primary follicle (white arrows) with normal component (C) Arrow (white) showing several follicle with normal cytoarchitecture at different stages of development. The cortex and inner medullar (black) shows normal histology.

**Figure 8:** (A): Arrows showing myometrium (black arrow), endometrium (blue arrow), uterine cavity (red arrow), and perimetrium (white arrow) (X100). (B): Arrows showing normal endometrium and secretary tubular glands (black arrow) (X100).
laminae propria mucosa and secretory tubular glands are normal in all the groups.

Table 4: Effect of *T. occidentalis* seed oil on Birth Weight of pups and Litter Size

<table>
<thead>
<tr>
<th>Dosage</th>
<th>Birth Weight</th>
<th>Litter Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.38±0.14</td>
<td>7.2±0.04</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>6.17±0.22</td>
<td>9.66±0.23</td>
</tr>
<tr>
<td>600 mg/kg</td>
<td>10.00±0.14*</td>
<td>8.0±0.20</td>
</tr>
<tr>
<td>800 mg/kg</td>
<td>6.66±0.23</td>
<td>10.25±0.10</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM. n=5 *p < 0.05

Effect of *T. occidentalis* Seed Oil on the Vaginal Epithelial Cells

The vagina epithelial cells that characterized each phase of estrous cycle in all groups appeared normal as was observed in the Papanicolaou’s staining (Figure 6).

Effects of *T. occidentalis* Seed Oil on Birth Weight and Litter Size of Pups.

There was a significant increase in birth weight of pups in the group treated with 600 mg/kg bw of *T. occidentalis* seed oil when compared with the control group (Table 4). There was no significant increase in litter size in all the experimental groups when compared with the control group (Table 4).

**DISCUSSION**

This study was conducted to determine the effects of *T. occidentalis* seed oil on body weight gain, relative organ weight, estrous cycle, serum concentration of luteinizing hormone (LH), follicle stimulating hormone (FSH) and estrogen, histology of the ovary, birth weight and litter size of pups.

It has been reported that *T. occidentalis* seed oil is used for cooking and in the production of cookies (Giami and Barber, 2004). The body weight gain, relative ovary weight and relative uterus weight of groups treated with *T. occidentalis* seed oil was not disrupted. This showed that the oil has no adverse effect on the body and organs. It also showed that the oil is well tolerated by the animals and is indicative of its safety for consumption.

Furthermore, the length of estrous cycle was not affected. However, the occurrence of the diestrus phase reduced in a dose dependent manner. This reduction in frequency of diestrus caused a shift in estrous cycle to favor estrus phase more than it did for proestrus. Although, the reduction of diestrus phase in this study is similar to the report of Kage *et al.*, (2009) in which the estrogenic nature of *Trichosanthes cucumerina* was responsible for the reduction of diestrus phase. However, in this study, the reduction in diestrus phase of the estrous cycle in all the experimental groups is suggestive of the fact that *T. occidentalis* seed oil may be beneficial to the development and maturation of follicles because prominent among the events of diestrus phase are rising estrogen level (Shaikh, 1971; Yoshinaga *et al.*, 1969) and low FSH level, which at some point causes growth of preovulatory follicle (Satue and Gardon, 2013). This eventually leads to the next phase (proestrus) during which follicles enlarges and estrogen increases thereby preparing the dominant follicle for ovulation.

The significant reduction in estrogen concentration caused by the administration of 800 mg/kg bw of *T. occidentalis* seed oil is similar to the report of Akang *et al.*, (2010) in which 800 mg/kg bw of the *T. occidentalis* seed oil caused a decrease in serum testosterone concentration. Estrogen production by the ovary or corpus luteum occurs as a result of interplay of different endocrine glands and enzymes. It is possible that *T. occidentalis* seed oil was able to reduce estrogen concentration by interfering with one of the steps that leads to its production.

The histology of ovaries in the experimental groups was similar to that of the control group. Lovejoy, (2002) suggested that linoleic acid and oleic acid both of which are present in *T. occidentalis* seed oil increased membrane fluidity, allows for osmosis, intracellular and extracellular gaseous exchange and caused an increase of lipid storage in lipid droplet and thus, an improvement of oocyte developmental competence respectively.

The increased birth weight in the group treated with 600 mg/kg bw of *T. occidentalis* seed oil is similar to the study of Kowalska, (2008) in which fish oil constituting 3% of the feed fed to rabbits resulted in increased birth weight. Like fish oil, *T. occidentalis* oil contains poly unsaturated fatty acid which is made up of linoleic acid and oleic acid (Nworgu *et al.*, 2007; Bello *et al.*, 2011). It has been reported that polyunsaturated fatty acid maintains the development of mammalian body before and after birth (Kowalska, 2008).

The result of this study showed that oral administration of *T. occidentalis* seed oil did not alter body weight, organ weight and estrus cycle. It also did not have any adverse effect on ovarian and uterine histology. Birth weight however increased in the 600 mg/kg bw. It caused a decrease in frequency of diestrus in all the experimental groups which is of course beneficial. The decreased estrogen concentration that was observed in the 800 mg/kg bw group did not have any effect on the other parameters measured. The results of this study suggest that *T. occidentalis* seed oil does not alter estrous cycle in Wistar rats.
REFERENCES


