Niger. J. Physiol. Sci. 30(2015) 095-101
www.njps.com.ng

Niger. J. Physiol. Sci.

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

***Oore-Oluwapo I. Daramola[1,2], Opeyemi O. Akindele[2], Adeyombo F. Bolarinwa[2] and Yinusa Raji[2]**

[1]Department of Physiology, College of Health Science, Igbinedion University, Okada, Nigeria. [2]Department of Physiology, College of Medicine University of Ibadan, Ibadan, Nigeria.

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 sub- divided into two. The sub-group-1 rats were mated with male breeders, the litter size and birth weight of their offsprings was 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.

**Address for correspondence:* ooore324@yahoo.com Tel: +2348038196338

Manuscript Accepted: December, 2015

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 *Curcumbitiacea.* It is found along the fringes of the closed forest in Africa and particularly cultivated in south-eastern part of Nigeria and Seiria 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 uwmenkhen. 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 poly unsaturated free fatty acids (Egbhekun *et al.*, 1998). It also has an acidic value that indicates that it is edible

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 45^0 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 60^0C to 80^0C. On completion of extraction, the petroleum ether in the extracted oil was completely evaporated at 40^0C (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 alcohol, cleared with two changes 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 Estrus Cycle

The length of estrous cycle before and during the treatment period was not significantly different. The length of estrus 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 (Figure 1 and figure 2). 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

Dosage	Body weight gain (g)		
	Week 1	Week 2	Week 3
Control	13 ± 1.53	12± 1.33	10 ± 1.50
400 mg/kg	11 ± 1.00	12 ± 1.33	9 ± 1.80
600 mg/kg	14 ± 1.67	15 ± 1.34	8 ± 1.00
800 mg/kg	11 ± 1.67	10 ± 2.00	7 ± 2.36

Values expressed as Mean ± SEM. n=5

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

Dosage	Relative ovary weight (%)	Relative uterus weight (%)
Control	0.02 ± 0.00	0.28 ± 0.08
400 mg/kg	0.02±0.00	0.21 ± 0.03
600 mg/kg	0.02± 0.00	0.19 ± 0.05
800 mg/kg	0.02± 0.00	0.28 ± 0.04

Values expressed as Mean ± SEM. n=5

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

Dosage	Estrous cycle length (days)	
	Before	After
Control	4.68 ± 0.19	4.67 ± 0.26
400 mg/kg	5.01±0.27	5.09 ± 0.30
600 mg/kg	5.21± 0.14	5.02 ± 0.14
800 mg/kg	4.90 ± 0.13	4.77 ± 0.15

Values expressed as Mean ± SEM. n=5

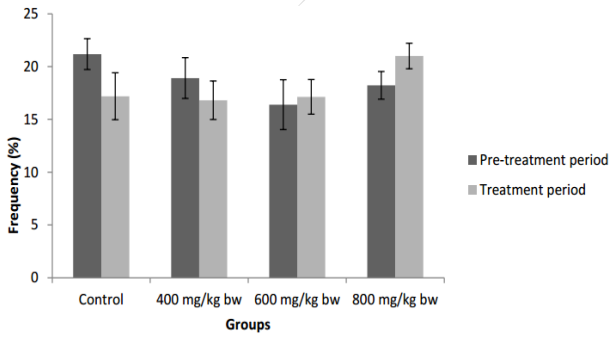

Figure 1: Effect of *T. occidentalis* seed oil on frequency of proestrus phase before and during treatment. n=5.

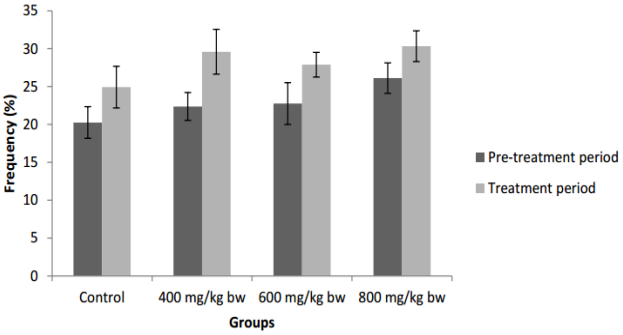

Figure 2: Effect of *T. occidentalis* seed oil on frequency of estrus phase before and during treatment. n=5.

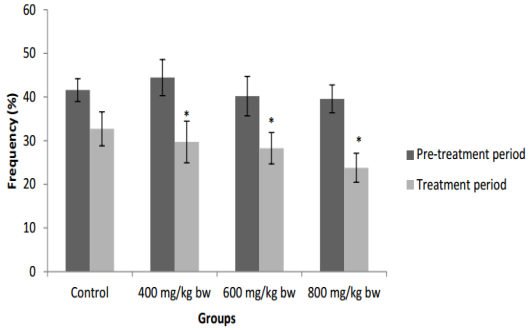

Figure 3: Effect of *T. occidentalis* seed oil on fequency of diestrus phase before and during treatment. n=5. *$p < 0.05$

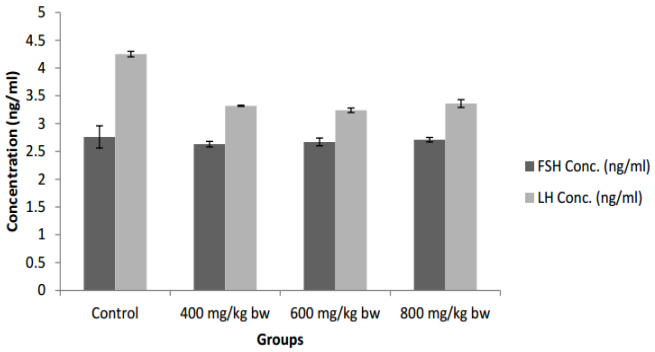

Figure 4: Effect of *T. occidentalis* seed oil on serum concentration of FSH and LH. n=5.

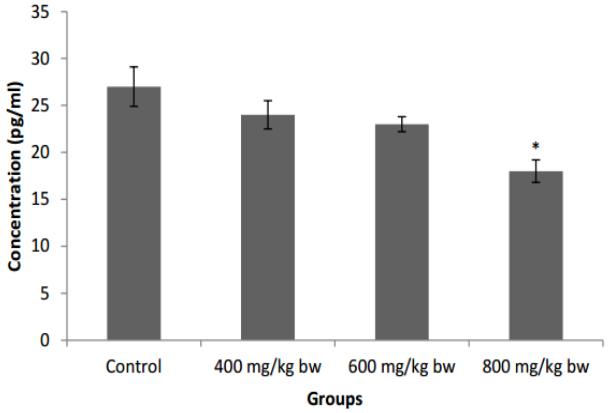

Figure 5. Effect of *T. occidentalis* seed oil on serum concentration of estrogen. n=5. *$p < 0.05$

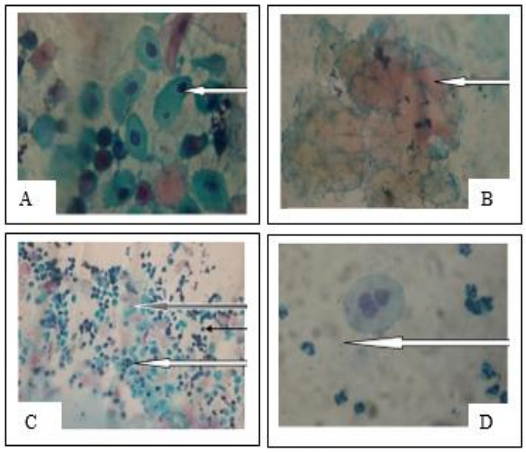

Figure. 6: **(A)** Nucleated epithelial cells in the proestrus phase of the estrous cycle (white arrow). **(B)** Estrus phase of the estrous cycle showing non-nucleated epithelial cells (white arrow). (**C)**Metestrus phase of the estrous cycle: the photomicrograph showing the presence of multinucleated 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.

Effect of T. occidentalis Seed Oil on Serum Levels of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Estrogen

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,

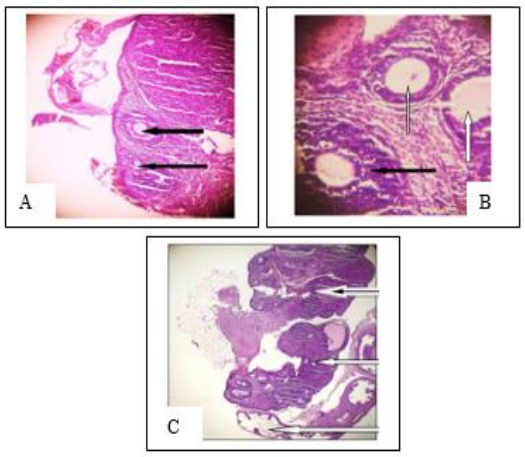

Figure 7:(A): Arrows (black) showing secondary follicles with normal cytoarchitecture, The cortex and inner medullar showed normal histology **(B)** Arrows showing secondary follicle with multilayered epithelium (black arrow) and primary follicle (white arrows) with normal component **(C)** Arrow (white)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

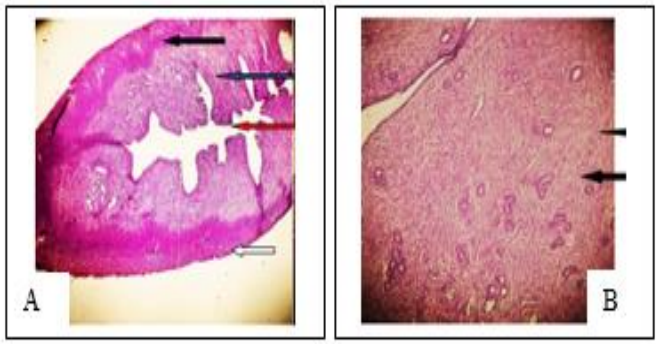

Figure 8: (**A**): Arrows showing myometrium (black arrow), endometrium (blue arrow), uterine cavity (red arrow), and perimetrium (white arrow) (X100). (**B**): Arrows showing normal endometrium and secretary tubular glands (black arrow) (X100).

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

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

Dosage	Birth Weight	Litter Size
Control	5.38±0.14	7.2±0.04
400 mg/kg	6.17±0.22	9.66±0.23
600 mg/kg	10.00±0.14*	8.0±0.20
800 mg/kg	6.66±0.23	10.25±0.10

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

Abdallah, S. and Daar Z. M. (2001). Infertility and social suffering: the case of ART in developing countries. Section 1, Infertility and assisted reproductive technologies in the developing world. Current Practices and Controversies in Assisted Reproduction: Report of a meeting on "Medical, Ethical and Social Aspects of Assisted Reproduction" WHO Headquarters, Geneva, Switzerland. 15-21, 17-21.

Agatemor, C. (2006). Studies of selected phytochemical properties of Fluted pumpkin *(Telfairia occidentalis* hook F.) seed oil and tropical Almond *(Terminalis catappia* I) seed oil. Pak. J. Nutr. 5: 306-307.

Ajayi, I. A., Oderinde, R. A., Taiwo, Y. O. and Agbedana, k O. (2004). Dietary effect of growth, plasma lipid and tissues of rats fed with non-conventional oil of *Telfairia occidentalis.* J. Sci. Food Agric. 18 (13): 1715-1712.

Akang, E., Oremosu, A. A., Dosumo, O. O., Noronhe, C. C and Okanlawon, A. O. (2010). The effect of Fluted pumpkin *(Telfairia occidentalis)* seed oil on testis and semen parameters. Agric. and Bio. J. North Am. 1 (4): 697-703.

Akubugwo, I. E. and Ugbogo, A. E. (2007). Physiochemical studies on oil of five selected Nigerian plant seed. Pak. J. Nutr. 6: 75-78.

Alada, A. R. A. (2000). The haematological effect of *Telfairia occidentalis* diet preparation. Afr. J. Biomed. Res. 3:185-186.

Bello, M. O., Akindele, T. L., Adeoye, D. O. and Oladimeji A. O. (2011). Physicochemical properties and fatty acids profile of seed oil of *Telfairia occidentalis.* Int. J. Basic Appl. Sci. 11 (6): 9-14.

Burkett, H. M. (1968). The results of plants of West Africa. Royal Botanical Garden, Kews, England. 1: 603-604.

Egbhekun, M. K., Nda-Suleiman, E. O. and Akinyeye, O. (1998). Utilization of Fluted pupkin *fruits (Telfairia occidentalis)* in marmalade manufacturing. Plant food Hum. Nutr. 52: 171-176.

Ehiagbonare, J. E. (2008). Conservation studies of *Telfairia occidentalis* Hook. F. An indigenous plant used in ethnomedicinal treatment of anaemia in Nigeria. Afr. J of Agric Res. 3(1): 074-077.

Giami, S. Y. and Barber, L. I. (2004). Utilization of protein concentrates from ungerminated and germinated fluted pumpkin *(Telfairia occidentalis* Hook F.) seeds in cookie formulations. *J. Sci. Food and Agric.* 84: 1901-1907.

Horsfall Jr., M. and Spif, I. A. (2005). Equilibrium sorption study of Al, CO3+, and Ag+ in aqueous solution of fluted pumpkin *(Telfairia occidentalis* Hook F) waste biomass. Acta. Chim. Slov.52:174-181.

Hunter, K. J. and Fletcher, J. M. (2002). The antioxidant activity and composition of fresh, frozen jarred and canned vegetable. Innov. food Sci. Emerg. Technol. 3: 399-406.

Idrisa, A. (2001). Infertility in: Kwawukume E.Y. and Emuveyan E. E. (eds.) (2005). Comprehensive gynaecology in tropics. Accra graphic packaging, 333-343. Trop J Obstet Gynaecol. 18:87-90.

Inhorn, M. C. (2003). Global infertility and the globalization of new reproductive technologies: illustrations from Egypt. Soc. Sci. Med. 56:1937-1951

Kage, D. N., Malashetty, V. B., Seetharam, Y. N., Suresh, P. and Patil S. B. (2009). Effect of ethanol extract of whole plant of *Trichosanthes cucumerina* var. Cucumerina L. on gonadotropins, ovarian follicular kinetics and oestrous cycle for screening of antifertility activity in albino rats. Intern. J. Morphol., 27(1): 173 - 182.

Kowalska, D. (2008). Effect of essential unsaturated fatty acid in fish oil on litters and composition of rabbit milk in does. Nutr. and Digestive Physiol. 705-709.

Long, J. A. and Evans, H. M. (1922). The Estrous cycle in the rat and its associated phenomena. Memories of University of Carlifonia. 6: 1- 148.

Longe, O. G., Rarinu, G. O. and Fetnoa, B. L. (1983). Nutritious value of fluted pumpkin *(Telfairia occidentalis).* J. Agric. Food Chem. 31: 982-992.

Lovejoy, J. C. (2002). The influence of dietary fats in insulin resistance. Current diabetes reports. 2 (5): 430-440.

Marcondes, F. K., Bianchi F. J. and Tanno A. P. (2002). Determination of estrous cycle phases of rats: Some helpful considerations. Braz. J. Biol. 62: 609-614.

Mascarenhas, M. N., Flaxman S. R., Boerma T., Vanderpoel S., and Stevens G. A. (2012). Global prevalence of infertility, infecundity and childlessness. National, regional and global trends in prevalence since 1990: A systematic analysis of 277 Health surveys. World Health J. P. Med. 9(12): 1-12.

Nworgu, F. C., Ogungbenro, S. A. and Solasi, K. S. (2007). Performance and some blood chemistry indices of broiler chicken served fluted pumpkin leaves extract supplement. *Am. Eurasian J. Agric. Environ. Sci.* 2: 90- 98.

Okigbo, B. N. (1977). Neglected plants of horticultural and nutritional importance in traditional farming system of tropical Africa. Acta. Hortic. 55: 131-149.

Okoli, E. B. and Magbeogu, C. M. (1983). Fluted pumpkin *(Telfairia occidentalis).* West Africa vegetable. Crop. Eco. Botany. 37(2): 145-147.

Okoli, R. I., Aigbe, O., Ohaju-obodo, J. O. and Mensah, K. (2006). Medicinal herbs used for managing some common ailments among Esan people of Edo state. Pak. J. Nutr. 6(5): 490-496.

Qui, R. Z. (2001). Sociocultural dimensions of infertility and assisted reproduction in the far East. Current practices and controversies in assisted reproduction: Report of a meeting on "medical, ethical and social aspects of assisted reproduction" WHO Headquaters, Geneva, Switzerland. 75-80.

Reinhold, B. O. (1992). Evaluation of the chemical properties of plant extracts in Africa. Journal of Egypt Society of Horticulture. 16(14): 497-498.

Salman, T. M., Olayaki L. A and Oyeyemi, W. A. (2008). Aqueous extract of *Telfairia occidentalis* leaves reduces blood sugar and increases haematological and reproductive indices in male rats. Afr. J. Biotechnol., 7: 2299-2303.

Satue, K. and Gardon, J. (2013). A review of the estrous cycle and the neuroendocrine mechanisms in the Mare. Steroids Horm. Sci. 4 (2): 1-8

Shaikh, A. A. (1971). Estrone and estradiol levels in the ovarian venous blood from rats during estrous cycle and pregnancy. Biol. Reprod. 5: 297-307.

Tindal, H. D. (1968). Commercial vegetable grown. Oxford press, London. Pg. 69.

Whigham, L. D., Watras, A. C. and Schoeller, D. A. (2007). Efficacy of conjugated linoleic acid for reducing fat mass: a meta-analysis in humans. Am. J. Clin. Nutri. 85 (5): 1203-1211.

Yoshinaga, K., Hawkins, A. A. and Stocker, J. F. (1969). Estrogen secretion by the rat ovary *in vivo* during the estous cycle and pregnancy. Endocrinol. 85: 103-112.