

Effect of Aqueous Extract of *Solanum melongena* Fruits (Garden Eggs) on Some Male Reproductive Variables in Adult Wistar Rats

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Abstract: The effects of administration of aqueous extract of *Solanum melongena* on some reproductive variables of male Wistar rats were exploited in this work. 20 rats were randomized into four groups (Group A, B, C and D) of 5 rats each. Group A serve as the control and was administered 0.9% normal saline, group B, C and D serve as the extract group receiving 200mg/kg, 400mg/kg and 800mg/kg of the extract respectively. The extract was save with LD50 >5000mg/Kg. Sperm counts and motility were quantified; epididymal and general body weights were measured using a weighing balance. The histological studies of the testes and the anterior pituitary were also done. Results of the experiment revealed an increase in the sperm count and sperm percentage motility. There was decrease in body weight gain and epididymal weight across the groups receiving the extract, though not significant. This experiment thus revealed that aqueous extract of *Solanum melongena* possesses profertility properties which may be beneficial to those who consume it.

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1. Introduction

Medicinal plants have long been used by the peoples of the world for treating different types of illnesses, but some various substances found in them may be harmful to the organism. Many plants are known for their teratogenic and abortive properties (Norton, 1996; Mengue *et al.*, 2001) while others are known for their toxic effects on the male reproductive system (Soufir *et al.*, 1989).

There are several numbers of agents that can cause adverse effect on the male reproductive system. These occur by interfering with sexual maturation, production, and transport of spermatozoa, the spermatogenic cycle, sexual behavior and fertility (Kimmel *et al.*, 1995). These agents may also play an adverse or beneficial roles on the leydig cells, thereby affecting testosterone production (Mooradian *et al.*, 1987). Some studies have reported that the toxic effect of these agents in the epididymis affect fertility (Klinefelter *et al.*, 1990; Vieira-Filho *et al.*, 2002), by disturbing the sperm maturation process, and the functioning of the accessory sex glands (Zenick *et al.*, 1994).

About 80% of the population in developing countries use medicinal plants and plant products in handling some there primary medical problems, due to their accessibility, availability and affordability (Telefo *et al.*, 2002; Cherdshewasart *et al.*, 2007). In these countries, a variety of plants are claimed to have

fertility regulating properties and a few have been tested for such effects (Cherdshewasart *et al.*, 2007; Ganguly *et al.*, 2007).

Solanum melongena (garden egg), is a common and popular vegetable crop grown in the subtropics and tropics. It is called “brinjal” in India, “yalo” in the hausa tradition of northern nigerian and “aubergine” in Europe. Eggplant is a perennial plant but grown commercially as an annual crop (Veeraragavathatham *et al.*, 2006; Westerfield, 2008).

Solanum melongena is one of the most important vegetable crops grown on over 1.7 million hectares world wide. China, India, Bangladesh, Nepal and Srilanka accounts for about 75% of eggplant production. It has been established that *Solanum melongena* leaves has antipyretic and analgesic effect (Mutalik *et al.*, 2003), central nervous system depressant and it is also a multiallergenic vegetable (Vohora *et al.*, 1984). It fruits is also use for weight loss, long term control of asthma and as a prokinetic agent.

Some varieties of the plant are claimed to have fertility regulating properties and a few have been tested for such effect. There is dearth of information for *Solanum melongena* in that regards. But due to its chemical constituent that revealed the presence of dietary fiber, alkaloids, saponins, nasunin, ascorbic acid, steroids, tannins, flavonoids, proteins and carbohydrates in both the crown and the fruit (Hanson

et al., 2006; Noda *et al.*, 2000; Tiwari *et al.*, 2009), as well as its various medicinal claims and its high consumption in some part of the world especially in the Egbo tradition of the eastern part of Nigeria. We decided to search for its fertility effect. Therefore, the objective of the current study is to determine the fertility effect of aqueous extract of *Solanum melongena* fruit extract on some reproductive variables using adult male wistar rats. The variables studied in this research include; Sperm count, sperm motility, body weight change, epididymal weight and histology of testes and anterior pituitary glands.

2. Materials and Methods

2.1 Plant Material

Fresh fruits of *Solanum melongena* were bought from “Kasuwar Sabo” market in Zaria, Kaduna State of Nigeria, in the month of September. Botanical identification was performed at the Herbarium section of Biological Science Department of Ahmadu Bello University, Zaria, Kaduna State, Nigeria, and given a voucher number 1939.

2.2 Extraction of plant material

The fruits were shade-dried for 10 days and ground into powder. An aqueous extract was made from 1kg of *Solanum melongena* fruits which was soaked in distilled water (5L) and the mixture boiled for 15 minutes. The heated decoction was taken and allowed to cool at room temperature, filtered and oven-dried as described by Pierré *et al.*, (2009) to give oven-dried aqueous extract 60.2g (yield of extraction, 6.02%). The extraction was carried out in Department of Pharmacognosy Laboratory, Ahmadu Bello University, Zaria. The working solution was prepared at a final concentration of 150mg/ml in distilled water.

2.3 Animals

Male Wistar rats (110–200g) were procured from the Experimental Animal Unit of the Vector/Parasitology Unit of Nigerian Institute for Trypanosomiasis and Onchocerciasis Research (NITOR), Federal Ministry of Science and Technical, Kaduna State Nigeria and used throughout the study. They were housed in plastic boxes and acclimatized for two weeks in a controlled environment (temperature 25±2 °C and 12 h dark/light cycle) with standard laboratory diet and water *ad libitum*.

2.4 Acute Toxicity Studies

The acute toxicity studies (LD50) of *Solanum melongena* fruits was determined using the method of Lorke, 1983. The studies were done in two phases. In the first phase, 9 rats were used. The rats were randomly divided into three groups having 3 rats in each group. Group 1 received 10mg/Kg, group 2 received 100mg/Kg and group 3 received 1000mg/Kg via oral route respectively, and observed for signs of toxicity and death for 24 hours. In second phase, 4 rats

were used and consist of 4 groups with a rat in each group. Group 1 received 1000mg/Kg, group 2 received 1600mg/Kg, group 3 received 2900mg/Kg and group 4 received 5000mg/Kg. The median lethal dose (LD50) was determined at the end of the second phase.

2.5 Experimental protocol

Twenty male adult Wistar rats were randomly divided into four (4) groups; one (1) control and three (3) treatment groups. The rats in group A (Control) were administered with 0.9% physiological saline, while the treatment groups B, C, and D were administered with graded doses of the *Solanum melongena* fruits extract of 200, 400 and 800 mg/kg body weight respectively. The animals were dosed orally once daily for 28 days using rat oral gavage. This method was similar to that described by Oyeyemi, *et al.*, (2008). At end of the 28th day, the animals were sacrificed for investigation of some of these reproduction variables.

2.6 Semen Analysis

The rats were anaesthetized using chloroform soaked in cotton wool placed in a box. Orchidectomy was performed by open castration method. The testicle was exposed by incising the *tunica vaginalis* and the *cauda epididymis* were harvested. The *cauda epididymis* of rats in each of the experimental groups were removed and minced thoroughly in a specimen bottle containing normal saline for few minutes to allow the sperms to become motile and swim out from the *cauda epididymis* (Saalu *et al.*, 2008).

2.7 Sperm count and Motility studies

The semen was then taken with 1ml pipette and dropped on a clean slide, and covered with cover slips. The slides were examined under light microscope for sperm motility (Saalu *et al.*, 2008). And with the aid of the improved Neubauer hemocytometer (Deep1/10mm LABART, Germany) counting chamber as described by Pant and Srivastava (2003), the spermatozoa were counted under the light microscope. Counting was done in five thoma chambers.

2.8 Histological studies

The testes of all the rats were fixed in 10% formalin, while the pituitary glands were fixed using Bouin's fluid, and processed by the usual method for paraffin embedding at Anatomy Department in Ahmadu Bello University, Zaria, Nigeria. Section of 4-5 µm thickness by microtome was taken, stained with hematoxylin and eosin stain for histopathological examination through light microscope by the usual method described by Akpanatah *et al.*, (2003)

2.9 Statistical analysis

Student t-test and one way analysis of variance (ANOVA) were used to analyze the data. The results were expressed as mean ± standard error of the mean (SEM). The difference of the means was considered significant at p < 0.05.

3. Results

3.1 Acute toxicity studies

During the experimental procedure, no deaths, no locomotor activity alteration, no piloerection or any other clinical signs of toxicity were observed in any of the groups in both phases even at a dose of 5000mg/kg. Therefore, $LD_{50} > 5000 \text{ mg/Kg}$, indicate that the extract is safe and non-toxic.

3.2 Semen Analysis

3.2.1 Sperm count

There was a significant increase in the sperm count across the extract groups when compared with the control group, and the increase was dose-dependent (Table 1).

3.2.2 Sperm motility

Generally, there was an increase in the motility of sperm cells in all of the extract groups when compared with the control group receiving normal saline in a dose-dependent manner, but the increase was not significant. There was also an increased number of progressive motile sperm cells, while there was a decrease in the non-progressive motile and non-motile sperm cells in a dose-dependent manner. These observed changes were only statistically significant for the progressive and non-progressive motile sperm cells in the group receiving 200mg/kg of *Solanum melongena* extract (Table 1).

3.3 Change in body and organ weight

There was no significant decrease in the changes observed in the body and the epididymal weight of animals across the group receiving the aqueous extract of the *Solanum melongena* fruit (Table 1).

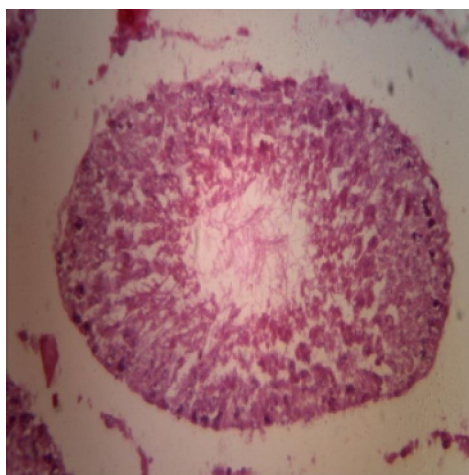
3.4 Histological studies

The histology of the testes in the group (A) receiving normal saline (figure 1: A1) appears normal, with abundant basal cells (primary spermatogonia cells) (Ps) and presence of sperm cells in the lumen (L) of the seminiferous tubule. While group (B, C and D) receiving 200mg/kg, 400mg/kg and 800mg/kg extract of *Solanum melongena* respectively, shows increased spermatogenic activities (arrows) toward the lumen (L) (figure 1: B1, C1 and D1). This is evidenced by the reduced number of primary spermatogonia cells (Ps) which is an indication that they might have differentiated to the next level of the spermatogenic cells. The histology of the anterior pituitary gland in the control group receiving normal saline appears normal, with abundant basophilic cells (b) and acidophilic cells (a) (figure 1: A2). There was no observed change in cytoarchitecture of the anterior pituitary gland in the group (B, C and D) receiving the extract (Figure 1: B2, C2, and D2). Thus, they all appear normal.

Table 1: Showing change in Body weight (g), epididymal weight (Wepi), Sperm count ($\times 10^6/\text{ml}$) and Sperm motility (%)

Groups	initial weight $W_i(\text{g})$	final weight $W_f(\text{g})$	weight gain $W_{\text{gain}}(\text{g})$	$W_{\text{epi}}(\text{g})$	Progressive motility (%)	non progressive motility (%)	Non motile cells (%)	Sperm count ($\times 10^6/\text{ml}$)
A(N/saline)	168.60 \pm 9.7	219.20 \pm 14.2	50.60 \pm 4.6	0.15 \pm 0.01	30.00 \pm 3.5	41.00 \pm 4.3	29.00 \pm 5.3	29.00 \pm 0.5
B(200mg/kg)	159.60 \pm 13.1	190.60 \pm 9.6	31.00 \pm 6.8	0.14 \pm 0.02	50.40 \pm 4.8*	26.20 \pm 4.3*	23.40 \pm 5.6	30.80 \pm 0.7
C(400mg/kg)	158.40 \pm 14.2	209.00 \pm 14.4	50.60 \pm 9.0	0.14 \pm 0.01	44.00 \pm 1.9	32.00 \pm 2.5	24.00 \pm 1.9	32.20 \pm 0.8*
D(800mg/kg)	165.00 \pm 4.3	189.00 \pm 14.6	24.00 \pm 11.5	0.13 \pm 0.01	51.60 \pm 1.4	29.80 \pm 1.3	18.60 \pm 1.0	32.60 \pm 0.4*

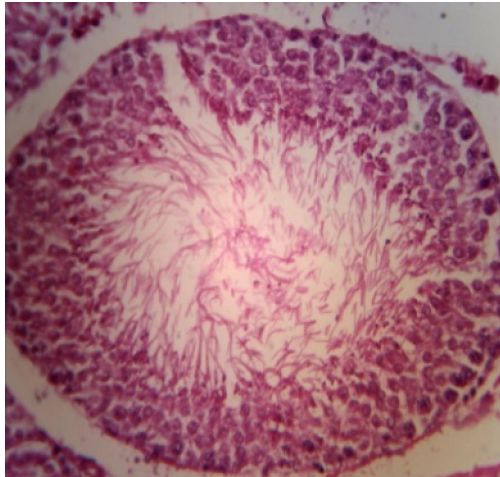
*P Value < 0.05 significant



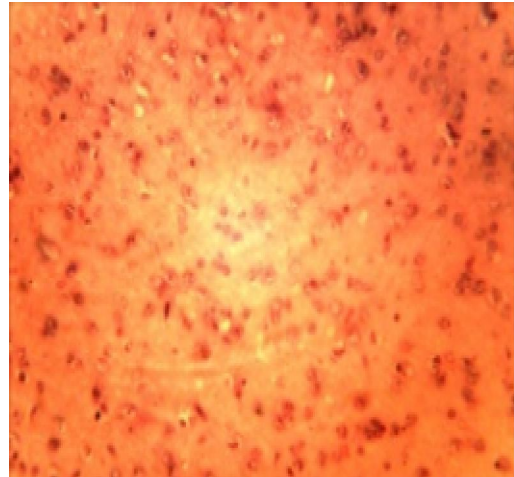
A1



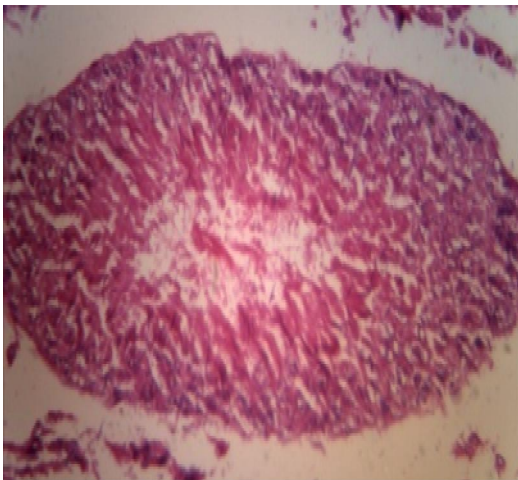
B1



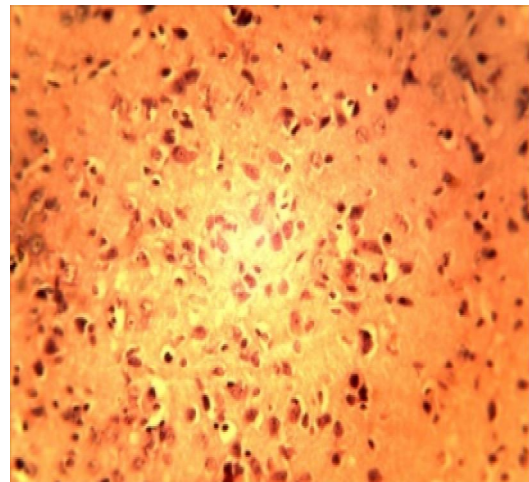
C1



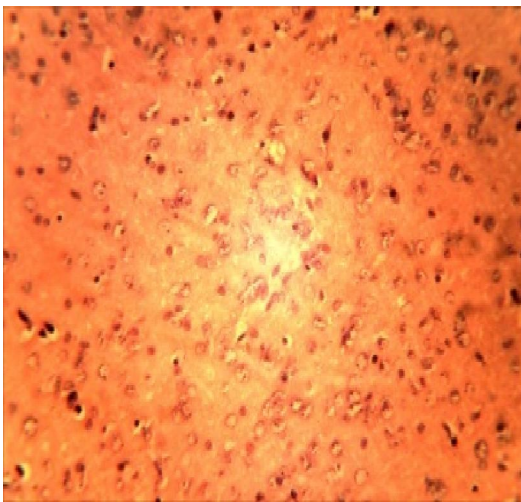
B2



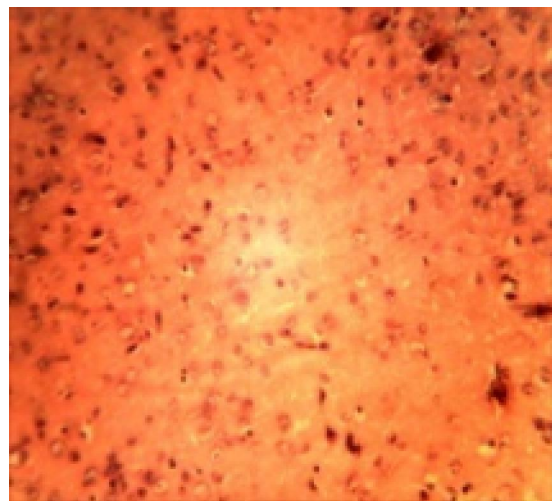
D1



C2



A2



D2

Figure 1: Histology of Testes and Anterior Pituitary glands (A1, B1, C1 and D1 are testes histology, while A2, B2, C2 and D2 are the anterior pituitary gland histology) H&E x 250

4. Discussion

The present study showed a significant increase in epididymal sperm concentration in the treated group which was in a dose dependent manner. The observed increase could be ascribed to the importance of *Solanum melongena* as a potent antioxidant and free radical scavenger (Tiwari *et al.*, 2009). Thus, support the findings of Saalu *et al.*, (2007a) that potent antioxidant ameliorate the increased free radicals generated by the natural and experimental stress, thereby increasing the spermatogenic activity by increasing the synthesis of testosterone from the interstitial cells of Leydig (Saalu *et al.*, 2007a).

Generally, there was increase in motility of sperm cells in all the extract treated groups as compared to the control group in a dose dependent manner, but the increase was not statistically significant. There was increase in the number of progressive motile sperm cells in a dose dependent manner, but the increase was only statistically significant in group receiving 200mg/kg of *Solanum melongena* extract. The non progressive motile sperm cells number decrease non significantly in all the test groups receiving *Solanum melongena* extract when compared to the control group in a dose dependent manner. Except for the group receiving 200mg/kg extract that the decrease was significant. The frequency of the non motile sperm cells decrease in a dose dependent manner when compared to the control group, though, the decrease was not statistically significant. This result indicates that *Solanum melongena* fruits extract has an effect on the mitochondria found in the body of the spermatozoon where energy is been synthesis in the form of adenosine triphosphate, that increases the sperm motility (Duke, 1997).

The basophilic cells of the anterior pituitary gland is responsible for the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which act on the testes to enhances the process of spermatogenesis (Moenter *et al.*, 2003). No change was observed in the cytoarchitecture of the anterior pituitary glands. The basophilic and the acidophilic cells in the anterior pituitary gland were intact in all the extract groups when compared with those in the control group. Thus, these results revealed to us that *Solanum melongena* (garden egg) fruits extract does not affect the functionality of anterior pituitary gland. Thus, support the finding of Vohora *et al.*, (1984). There is no any observable lesion in the histology of the testes in the entire extract groups when compared with the control. This is in line with the work of Cody *et al.*, Harbone and Williams, Who stated that plants containing flavonoids are effective in prevention of lesion, mainly because of their antioxidant properties (Cody *et al.*, 1986; Harborne and Williams, 2000). However, in all the test groups, there was observed

increase in spermatogenic activities towards the lumen of the seminiferous tubule. This increase cellular activities was from the basement membrane up to the lumen of the seminiferous tubules of the testes. This was evidenced by the reduced number of primary spermatogonia cells. This is an indication that they might have differentiated to next level of spermatogenic cells. This was mainly due to the presence of potent antioxidant like favonoids that sacavage free radicals and increase testosterone formation by the interstitial cells of Leydig (Saalu *et al.*, 2007a).

There was no any disturbance in the cytoarchitecture of pituitary gonadotrophic cells which secrete stimulating hormones responsible for reproductive endocrine functions. These also oppose the finding of Elbetieha *et al.*, (2001), who stated that an increase or decrease in the weight of reproductive organs is under hormonal control and could suggest a disturbance of the reproductive endocrine functions.

The effect of aqueous extract of *Solanum melongena* in this study shown that *Solanum melongena* fruits has a potential to decrease mean body weights of rats, although the changes in the mean body weight was not statistically significant, when compared to the control group. This is agreement with the work of Edijala *et al.*, (2005), who also recorded that garden egg plant reduce weight gain by reducing the serum total cholesterol, triglyceride and increase serum HDL-cholesterol in his comparative study of the effect of *Solanum melongena* (garden egg) fruit, oat and apple on serum lipid profile. There was slight decrease in weight of epididymis in the test groups in a dose dependent manner when compared with the control group, though it was not statistically significant despite the increase in cellular activity in the testes. This oppose the findings of Shittu *et al.*, (2007) that says increased cellular activities are key factor to be considered in the evaluation of organ weights.

5. Conclusion

This work reveals that oral administration of aqueous extract of *Solanum melongena* fruits possesses profertility properties which may be beneficial to those who consume it. These profertility properties can be exploited in male fertility therapy.

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References

1. Akpanatah, A.O., Oremosu, A.A., Ajala, M, O., Noronha, C.C. and Okanlawon, A.O. (2003). The effect of Grade extract of *Garemia Kola* seed on histology of hormonal Milieu of male Wister rats reproductive organs. *Nigerian Journal of Health and Biomedical Science*, 2 (1): 40-46.
2. Cherdshewasart, W., Kitsamai, Y. and Malaivijitnond, S. (2007). Evaluation of the estrogenic activity of the Wild *Pueraria mirifica* by vaginal cornification assay. *Journal of Reproduction and Development*, 53: 385-393.
3. Cody, V., Middleton, E. and Harbone, J.B. (1986). Plant Flavonoids in Biology and Medicine (eds.): *Biochemical, Pharmacological and Structural-Activity Relationships*, Alan Liss, New York, pp. 22-25.
4. Duke, J. (1997). The ultimate compendium of Natural remedies from the worlds foremost Authority on healing and herbs. The Green Pharmacy Rodale press, 248; 334; 463; 481.
5. Edijala, J.K., Asagba, Samuel, O., Eriyamremu, G.E. and Atomatofa, U. (2005). Comparative Effect of Garden Egg Fruit, Oat and Apple on Serum Lipid Profile in Rats Fed a High Cholesterol Diet. *Pakistan Journal of Nutrition*, 4 (4): 245-249.
6. Elbetieha, A., Bataineh, H., Darmani, H., Al-Hamood, M.H. (2001). Effects of ling-term exposure to manganese chloride on fertility of male and female mice. *Toxicology Letters*, 119: 193-201.
7. Ganguly, M., Borthakur, M., Devi, N. and Mahantam, R. (2007). Antifertility activity of the methanolic leaf extract of *Cissampelos pareira* in female albino mice. *Journal of Ethnopharmacology*, 111: 688-691.
8. Hanson, P.M., Yanga, R.Y., Tsoua, S.C.S., Ledesmaa, D., Englea, L. and Lee, T.C. (2006). Diversity in eggplant (*Solanum melongena*) for superoxide scavenging activity, total phenolics, and ascorbic acid. *Journal of Food Composition and Analysis*, 219: 594-600.
9. Harborne, J. B. and Williams, C. A. (2000). Advances in flavonoids research since 1992. *Phytochemistry*, 55(6): 481-504.
10. Kimmel, G.L., Clegg, E.D. and Crisp, T.M. (1995). Reproductive toxicity testing: A risk assessment perspective. In: Witorsch R.J. (Ed). *Reproductive Toxicology*. 2nd (ed.) New York: Raven Press, 75-98.
11. Klinefelter, G.R., Laskey, J.W., Roberts, N.R., Slott, V. and Suarez, J.D. (1990). Multiple effects of ethane dimethanesulfonate on the epididymis of adult rats. *Toxicology and Applied Pharmacology*, 105: 271-287.
12. Lorke, D. (1983). A new Approach to Acute toxicity Testing. *Archive of Toxicology*, 54:275-287.
13. Mengue, S.S., Mentz, L.A. and Schenkel, E.P. (2001). Uso de plantas medicinais na gravidez. *Revisita Brasil Farmacognosia*, 11: 21-35.
14. Moenter, S.M., Defazio, R.A, Straume, M., Nunemaker, C.S. (2003). Steroid regulation of GnRH neurons. *Annals of the New York Academy of Sciences*, 1007:143.
15. Mooradian, A.D., Morley, J.E. and Korenman, S.G. (1987). Biological actions of androgens. *Endocrine*, 8: 1-27.
16. Mutalik, S., Paridhavi, K., Mallikarjuna, R.C. and Udupa, N. (2003). Atipyretic and analgesic effect of leaves of *solanum melongena* linn. in rodents. *Indian journal of pharmacology*, 35: 312-315.
17. Noda, Y., Kneyuki, T., Igarashi, K. and Packer, M.L. (2000). Antioxidant activity of nasunin, an anthocyanin in eggplant Peels. *Toxicology*, 148: 119-123.
18. Norton, S. (1996). Toxic effects of plants. In: Klaassen CD (Ed) Casarett and Doull's Toxicology. *The Basic Science of Poisons*, New York, Mcgraw-Hill, 841-854.
19. Oyeyemi, M.O., Oluwatoyin, O., Ajala, Leigh, O.O. and Adesiji, A.F. (2008). The Spermioiogram of male wister rats treated with

- aqueous leaf extract of *Venercinae ntygdalina. Foliav eterinarias*, 22: 98-101.
20. Pant and Srivastava (2003). Testicular and spermatotoxic effects of quinalphos in rats. *J Appl. Toxicol.* 2003; Jul-Aug;23(4):271-4. Available: <http://www.ncbi.nlm.nih.gov/pubmed/12884411>.
 21. Pierre, W., Esther, N., Nkeng-Efouet, P., Alango, N.T. and Benoît, A.K. (2009). Reproductive effects of *Ficus asperifolia* (Moraceae) in female rats *African Health Sciences*, 9 (1): 49-53.
 22. Saalu, L. C., Udeh, R., Oluyemi, K. A., Jewo, P. I. and Fadeyibi, L. O. (2008). The Ameriorating Effects of Grapefruit Seed Extract on Testicular Morphology and Function of Varicocele Rats, *Int. J. Morphol.* 26(4):1059-1064. Available: <http://dx.doi.org/10.4067/S0717-95022008000400042>.
 23. Saalu, L.C, Oluyemi, K.A. and Omotuyi, I.O. (2007a) a-Tocopherol (vitamin E) attenuates the testicular toxicity associated with experimental cryptorchidism in rats. *African Journal of Biotechnology*, 6 (12): 1373-1377.
 24. Shittu, L. A. J., Bankole, M. A., Oguntola, J. A., Ajala, O., Shittu R. K., Ogundipe, O. A., Bankole M. N., Ahmed, T. and Ashiru, O. A. (2007). Sesame leaves intake improve and increase epididymal spermatocytes reserve in adult male Sprague Dawley rat. *Scientific Research and Essay*, 2 (8): 319-324.
 25. Soufir, J.C., Radique, C., Dantec, M.C., Garner, D. and Jegou, B. (1989). Gossypol-induced modifications in the microenvironment of rat epididymal spermatozoa. *Journal of Reproduction and Fertility*, 86: 427-434.
 26. Telefo, P.B., Moundipa, P.F. and Tchouanguép, F.M. (2002). Oestrogenicity and effect on hepatic metabolism of the aqueous extract of the leaf mixture of *Aloe buettneri*, *Dicliptera verticillata*, *Hibiscus macranthus* and *Justicia insularis*. *Fitoterapia*, 2 (73): 472-478.
 27. Tiwari, A., Jadon, R. S., Tiwari, P. and Nayak, S. (2009). Phytochemical Investigations of Crown of *Solanum melongena* fruit. *International Journal of Phytomedicine*, 1: 9-11.
 28. Veeraragavathatham, T., Jawaharlal, M. and Seemanthini R. (2006). *SUSVEG- Asia Bringal Manual (TNAU)*, Tamil Nada Agricultural University, Combatore.
 29. Vieira-Filho, S.A, Duarte, L.P., Silva, G.D.F., Mazaro, R. and Stasi, L.C.D. (2002). Constituintes químicos e atividade antiespermatogênica em folhas de *Austroplenckia populnea* (Celastraceae). *Revisita Brasil Farmacognosia*, 12 (1): 123-124.
 30. Vohora, S.B., kumar, I. and Khan, M.S.Y. (1984) Effect of alkaloids of *solanum melongena* on the central nervous system. *Journal of ethnopharmacology*, 11: 331-336.
 31. Westerfield, Robert (2008). "Pollination of Vegetable Crops" (pdf). <http://pubs.caes.uga.edu/caespubs/pubs/PDF/C934.pdf>. Retrieved 2009-07-01.
 32. Zenick, H., Clegg, E.D., Perreault, S.D., Klinefelter, G.R. and Earl, Gray, L. (1994). Assessment of male reproductive toxicity: a risk assessment approach: In: Hayes AW, (Ed.) *Principles and Methods of Toxicology*, New York: Raven Press, 937-988.