EFFECTS OF JATROPHA CURCAS EXTRACTS ON THE HORMONAL PROFILE OF MATURE FEMALE WISTAR RATS

[1]Ale, Ayotunde Oladunni.; [2]Odesanmi Selina Omotola; [1]Magbagbeola, Olubunmi Abiola and [1]Osalusi, Bamidele Sanya

[1] Department of Medicine, Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun state,

[2]Deparment of Biochemistry, College of Medicine, University of Lagos State

Correspondence: Ayoade2004@yahoo.com

ABSTRACT

Extracts from *Jatropha curcas*, a tropical shrub, serve as multi-purpose drugs in folk medicine. Previous studies have shown contraceptive and abortifacient potency of the extract of this plant. This study assessed the effect of oral administration of *J. curcas* root extracts on hormonal levels of follicle stimulating hormone (FSH), progesterone, estradiol (E2), luteinizing hormone (LH), and prolactin in Wistarrats. The root extracts of *J. curcas* in water (JCW) and 80% ethanol (JCE) were prepared using the Soxhlet extraction method. Four groups of mature female Wistar rats (Groups A-D) were administered varying doses of JCW or JCE once daily for 15 days. Another group (Group E) served as control with no extract. After 15 days of extract administration, the rats were fasted for 18 h and sacrificed. Venous blood samples were collected from each group for analysis of fasting glucose level and lipid profile using spectrophotometry. We observed that, except for prolactin, the levels of all other hormones decreased significantly in the rats treated with the root extracts, compared to the control(all $P < 0.05$). Among groups that were treated with varying doses of JCW, the levels of E2, progesterone, and LH increased with increase in the concentration of the JCW. Although, there was no dose-dependent increasing or decreasing trend in case of FSH and prolactin, the levels of these hormones varied significantly among the rats of the Groups A-C. Administration of the root extracts of *J. curcas* led to a decline in the serum FSH, LH progesterone, and E2, compared to the control group, indicating adverse effect of root extract on fertility of Wistar rats.

Keywords: *J. curcas*, Herbal contraceptive, Hormonal effects

Accepted date: 28 October 2021

Introduction

J. curcas (Greek: iatros – doctor, trophe – food) is a perennial shrub with poisonous activity, can reach up to 500cm in height. Belonging to *Euphorbiaceae* family, this plant is also known Barbaos nut, physic nut, Black vomit nut, or purging nut (Gadekar*et al*, 2006). It can grow in a wide range of temperature and soil of poor quality (Ishii *et al*, 1987; Munch and Kiefer, 1989). This plant originated in South and Central American countries and then spread to Asian and African countries by Portuguese seafarers (Johnson *et al*, 2014; Shukla *et al*, 2015). It has been shown to exhibit several medicinal activities in animals and humans, and its seed oil used as biodiesel (Achten *et al*, 2008). However, this plant contains several toxic compounds, such as phorbol esters, saponin, curcins, etc. because of which the plant and its parts, despite their benefits,

have been deemed unfit for direct consumption by the humans (Kumar *et al*, 2014).

In this study, we mainly focused on the effects on the *J. curcas* extracts on the serum levels of five hormones, namely estradiol (E2), progesterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), and prolactin. There have been several studies that focused on the effects on the *J. curcas* extracts on the male fertility and testosterone circulation (Katole *et al*, 2013). However, there have not been many researches on female sex hormones and fertility. Only few studies have shown abortifacient activity of *Jatropha* species; however, there is a scarcity of literature in this regard (Jain *et al*, 2013). Therefore, in this research we assessed the effects of J.*curcas* root extracts on the levels of abovementioned hormones, using female Wistar rats as the animal model.

Methods and Materials

This study was approved by the Department of Biochemistry, College of Medicine, University of Lagos, Nigeria.

J. curcasroot extracts

The dry roots of this plant were collected from different traditional Nigerian herbalists. The roots were further dried in a hot air oven at 60 °C for 24h, and then, grounded to powder form using the grinding machine at the Department of Pharmacology, College of Medicine, University of Lagos. The aqueous (JCW) and ethanolic (JCE) extracts of the dried roots were prepared by dissolving 40 g of the powder in 250 ml of either water or 80% ethanol solution in a Soxhlet apparatus for 72 h. The extract was then dried using a rotary evaporator at 45 °C and stored in a refrigerator at 4~8 °C until further use.

Wistar rats Treatment.

Twenty female Wistar rats were acquired from the University of Ibadan and allowed to acclimatize for 15 days. The rats were kept in ample light and ventilation and given rat chow and water *ad libitum*. They were then divided into five groups containing four rats each (Groups A-E). All the rats were weighted on the first day of the experiment, and then, at 3-day intervals until the end of the experiment. Groups A, B, and C received JCW at doses of 5, 10, and 15 mg/200 mg body weight (b.wt.) of the rats. The rats in Group D received JCE at the concentration of 10 mg/200 mg b.wt. of the rats. The rats in Group E did not receive either JCW or JCE (control group). All the extracts were administered orally via stomach tubes. After 15 days, the rats were fasted for 18 h and sacrificed after being anesthetized using an intraperitoneal injection of 25% urethane chloralose. The venous blood samples were collected in plain sample bottles for hormone profiling. The serum was isolated via centrifugation at 3000 revolution per minute for 20 minutes. The serum samples were stored at 4 °C before analyses.

Determination of hormonal levels

We utilized the hormone-specific ELISA kits provided by the Randox Laboratories Pvt. Ltd., UK.

E2 Assay – For this assay, the serum samples were incubated along with a reaction mixture in the wells of the microtiter plates coated with goat anti rabbit IgG for 90 min at room temperature. Then, the wells were washed to remove the unbound E2 peroxidase conjugate. Next, the wells were filled with TMB reagent and incubated for 20 min at room temperature. Addition of TMB reagent causes formation of blue color, the intensity of which was assessed at 450 nm. Intensity of the color correlates with the levels of E2 in the serum samples.

LH Assay – The serum samples were added to the microtiter plate wells precoated with an antibody specific to a unique epitope on LH hormone. After that, the wells were filled with assay diluents and the plates were incubated. Then, the plates were washed to remove unbound LH molecules. Then, the wells were filled with another horseradish peroxidase-conjugated antibody, which is specific for another unique epitope on the LH molecule. After secondary incubation, the plates were washed again, and the wells were filled with a substrate solution containing a chromogen, which leads to the formation of a colored product that could be assessed using a spectrophotometer at 450 nm.

Intensity of the color correlates with the LH levels in the serum samples.

FSH Assay – This assay was performed in a similar manner as the LH Assay.

Progesterone Assay – In this assay, the serum sample was added along with the progesterone antibody in the microtiter plate wells, which were already precoated with antibody specific antiprogesterone antibody. Then, the plates were incubated. Next, the wells were filled with a progesterone enzyme conjugate, which competes with progesterone present in the serum samples for a limited number of binding sites. After incubation, the wells were washed and filled with a chromogen, resulting in the formation of a colored product. The color intensity was assessed at 450 nm. The color intensity correlates with the levels of progesterone in the serum samples.

Prolactin Assay – The serum samples were added to the wells precoated with prolactin-specific antibody. After that, the wells were filled with assay diluents and the plates were incubated. Then, the plates were washed. The wells were then filled with substrate solution, which leads to the formation of a colored product that could be assessed using a spectrophotometer at 450 nm. Intensity of the color correlates with the prolactin levels in the serum samples.

Data Analysis

All the statistical calculations were presented as mean ± SD. Three or more groups comparison was carried out using one-way ANOVA, whereas comparison between two groups were done using the Student's test. Where variances were found not to be homogenous, non-parametric Kruska Wallis test was used. Statistical analysis was performed using SPSS version 21. Differences with P value < 0.05 regarded as statistical significance.

Results

Effect of varied doses of *J. curcas* root extract on the hormonal profile of mature female rats *J. curcas root extract on serum E2 and progesterone.* The mean serum E2 and progesterone were significantly lower in the rats that were treated with the root extracts of *J. curcas* than control group(P = 0.008 and 0.04, respectively) (Table 1). Furthermore, among the Group A-D, the rats of Group A exhibited the lowest E2 and progesterone levels (Table 2).

J. curcas root extract on serum LH

Similar to E2 and progesterone, in comparison to the control group, the levels of LH were significantly lower in the rats that were treated with the root extracts of *J. curcas* (all P = 0.01) (Table 1). However, among the Groups A-D, the rats of Group D exhibited the lowest levels of LH (P < 0.05) (Table 2). Furthermore, the levels of LH between the rats of Groups A and B were not significantly different (P = 0.15).

J. curcas root extract on serum FSH

Again, all the rats administered with the extracts of *J. curcas*root showed significantly lower levels of FSH than the control group (all P < 0.05) (Table 1). The FSH levels were significantly different among the rats of Groups A-D (all P < 0.05). Furthermore, the rats of Group B exhibited the lowest levels of FSH (Table 1).

J. curcas root extract on serum prolactin

Similar to the findings related to the other hormones, the prolactin levels in the rats of Groups A-D differed significantly than those of the rats of control group (all P < 0.05). However, unlike other hormones, we observed that the rats that were administered with the root extract at the dosage of 10 mg/200 g b.wt. exhibited the maximum levels of prolactin among all the groups (including control group). However, the rats of the rest of the treated groups exhibited significantly less prolactin levels than the control group rats (all P < 0.05) (Tables 1 and 2).

Table 1: Effect of varied doses of *J. curcas* root extract on the hormonal profile of mature female rats

Parameter	Group A	Group B	Group C	Group D	Group E	Over all p values	A vs. B	A vs. C	B v s. C
Estradiol (E2) (pg/mL)	12.23 ± 0.21	32.67±1.16	56.13±0.12	41.00±1.73	71.00±1.00	0.008	<0.0001	<0.0001	<0.0001
Progesterone (nmol/L)	5.07 ± 0.12	10.23±0.25	17.17±0.12	16.47±13.93	32.27±0.25	0.04	<0.0001	<0.0001	<0.0001
Luteinizing hormone	4.72 ± 2.80	7.03±0.06	9.57±0.15	4.07±0.12	10.7±0.10	0.01	0.15	0.01	<0.0001
Follicle stimulating hormone (nmol/L)	2.07 ± 0.06	0.83±0.06	1.77±0.06	1.67±2.02	4.03±1.19	0.008	<0.0001	0.0004	<0.0001
Prolactin (ng/ml)	9.15 ± 0.35	32.67±1.16	12.33±0.31	16.03±0.15	19.67±0.58	0.01	<0.0001	<0.0001	<0.0001

All values were presented as mean ± SD. Comparisons among three or more groups were done using one-way ANOVA and overall p value was calculated to find the significance of the difference of means among the groups. Two groups comparison was done by Students t test. Level of significance (p level) was set at< 0.05.

Table 2: Effect of varied doses of *J. curcas* root extract on the hormonal profile of mature female rats: Comparison between aqueous extract and ethanolic extract

Parameter	Group A	Group B	Group C	Group D	Over all p value	A vs. D	B vs. D	C v s. D
Estradiol (E2) (pg/mL)	12.23 ± 0.21	32.67±1.16	56.13±0.12	41.00±1.73	<0.0001	<0.000	<0.000	<0.0001
Progesterone (nmol/L)	5.07 ± 0.12	10.23±0.25	17.17±0.12	16.47±13.93	<0.0001	<0.0001	<0.0001	<0.0001
Luteinizing hormone(nmol/L)	4.72 ± 2.80	7.03±0.06	9.57±0.15	4.07±0.12	<0.0001	<0.0001	<0.0001	<0.0001
Follicle stimulating hormone (nmol/L)	2.07 ± 0.06	0.83±0.06	1.77±0.06	1.67±2.02	<0.0001	<0.0001	<0.0001	<0.0001
Prolactin (ng/ml)	9.15 ± 0.35	32.67±1.16	12.33±0.31	16.03±0.15	<0.0001	<0.0001	<0.0001	<0.0001

All values were presented as mean ± SD. Comparisons among three or more groups were done using one-way ANOVA and overall p value was calculated to find the significance of the difference of means among the groups. Two groups comparison was done by Student's t test. Level of significance (p level) was set at< 0.05.

Discussion

E2 is the major female sex hormone responsible for regulation of the menstrual cycles in the females. During embryonic development, this hormone is involved in the formation of the primary and the secondary sex organs, including vagina, uterus, fallopian tubes, mammary glands, etc. It is also critical in secondary sexual characteristics of the females, including development of breasts. This hormone is produced using the cholesterol (Weinbauer GF and Nieschlag E, 1996). Progesterone is a female steroid hormone synthesized and secreted by the corpus luteum after ovulation. It aids in the pregnancy of the animal. In addition, during the pregnancy period, this hormone inhibits further ovulation. It stimulates the development of the breast tissue of the females and prevents lactation during the pregnancy (Smith *et al*,1994). FSH is a gonadotrophic hormone. It is primarily involved in the puberty development of the females and in the development of male testes and female ovaries. In males, this hormone stimulated the Sertoli cells of the testes to release sperm (spermatogenesis). In the females, this hormone stimulates the ovarian follicles in the ovaries to release an egg (ovulation). This hormone works in conjugation with another gonadotrophic hormone, LH(Hiller-Sturmhofel and Bartke, 1998). LH is synthesized by the anterior pituitary gland. Primarily, LH is involved in the release of testosterone in males and estradiol in females. In female mammals, LH also stimulates the corpus luteum to release progesterone during the pregnancy (Niswender *et al*, 2000). Prolactin, also known as luteotropin, is primarily involved in the production of milk in the breasts of the female mammals. However, it also participates in the regulation of the immune system. It is also primarily produced by the anterior part of the pituitary gland (Hiller-Sturmhofel and Bartke, 1998).

Since ancient times, *J. curcas* has been extensively used for several medicinal purposes. It has been used as a purgative, laxative, abortifacient, vulnerary, styptic agent, etc. (List and Horhammer, 1969; Duke and Wain, 1981). Different parts of this plant exhibit varying pharmacological uses, such as antioxidant activity (Diwani *et al*, 2009), hepatoprotective activity (Balaji *et al*, 2009), wound healing activity (Shetty *et al*, 2006), antimetastatic and antiproliferative activity (Balaji *et al*, 2006), antimicrobial activity (Igbinosa *et al*, 2009), antidiabetic activity (Mishra *et al*, 2010), anti-inflammatory activity (Mujumdar *et al*, 2004), pregnancy terminating effect (Goonasekera *et al*, 1995), antiulcer activity (Kannappan *et al*, 2008), antihelminthic activity (Ahirrao *et al*, 2008), antifungal activity (Donlaporn *et al*, 2010). Previous studies have reported that *J. curcas* can be used to treat several kinds of ailments, such as malaria, dermatitis, STDs, cough, jaundice etc. (Prasad *et al*., 2012; Morton and Thomas, 1981; Sofowora, 1993). Apart from medicinal values, *this plant is also an excellent source* of biodiesel fuel (Singh and Mehta, 2005). Oil of its seeds serves as fungicide, insecticide, and pesticide (Agaceta *et al*., 1981; Thomas, 1989). Its bark is used for development of fish poisons (Watt and Breyer-Brandwijk, 1962) and its sap is used to make histological stains (Mitchell and Rook, 1979).

In their review, Pathak *et al* (2005) reported that the fruit of *J. curcas* exhibits abortifacient activity. Jain *et al* assessed the estrogenic activity of *Jatropha gossypifolia* extracts in rats. They reported that both the aqueous and ethanolic extracts of the plant increased the uterine weight of the treated rats in a dose-dependent manner. They also reported that the per cent antifertility activity of the extracts increased with increase in its dose (Jain *et al*, 2013).The aqueous seed extracts of this plant have been used as a contraceptive (Goonasakera *et al*, 1995).

In this study, we observed that the levels of E2, FSH, progesterone, and LH were significantly reduced in the rats treated with either JCE or JCW (Groups A-D) than control group rats (all $P < 0.05$). However, in case of prolactin, we observed the highest levels of this hormone in the serum of rats administered with JCW at concentration of 15 mg/200 g b.wt. of rat (Group B). On comparison between the rats who were administered with JCW (Groups A-C) and those administered with JCE (Group D), we observed that the levels of E2, progesterone, FSH, and prolactin in the rats of Group D were intermediate of the corresponding values of rats of Groups B and C. Among the rats

treated with the *J. curcas* extracts, Groups A, A, D, B, and A exhibited the lowest levels of E2, progesterone, LH, FSH, and prolactin respectively.

Our study had a few limitations. First, the root extracts were administered orally via stomach tubes. Brander *et al* (1991) has shown that the mode of administration of a drug/biological compound significantly affects its bioavailability. Oral administration of a biological agent often reduces the quantity of the agent that reaches the host blood, which, in turn, reduces its effects (Brander *et al*, 1991). Second, compared to previous studies, we administered very low dosages of the root extracts in this study. It might be possible that the use of low dosages might have led to generation of mild changes in the hormonal levels of the rats. Larger concentrations of the extracts might have led to more prominent changes.

Further studies could help explore the effects of *J. curcas* extracts on other hormones. *J. curcas* could be used as a medicinal plant, food source, and protein supplement to the animal. However, its extracts have been previously reported to be toxic after consumption and not fit for human use.

Conclusion

In this study, our findings showed that oral administration of*J. curcas* root extract induced decline in LH, FSH, prolactin and E2 levels in female Wistar rats, indicating the adverse effect of the extract on the fertility of the rats. Future studies need to focus on the effects of this plant on other hormones in humans and animals and mechanism of action of the active components of this plant that modulate the hormone profiles.

References

Abd-Elhamid HF (2004). Investigation of induced biochemical and histopathological parameters of acetonitrile extract of Jatropha curcas in albino rats. Journal of Egyptian Society of Parasitology, 34: 397-406.

Achten WMJ, Verchot L, Franken YJ, Mathijs E,Leuven KU (2008). Jatropha biodiesel production and use (a review). Biomass and Bioenergy, 32(2): 1063-1084.

Agaceta LM ***Dumag PU, BatolosJA*** (1981). Studies on the control of snail vectors of fascioliasis; molluscicidal activity of some indigenous plants. NSDB Technology Journal, 6: 30-34.

Ahirrao RA Pawari SP, Borse LB, Borse SL, Desai SG (2008). Anthelmintic activity of leaves of Jatropha curcaslinn and Vitexnegundolinn. Pharmacology Online News Letters, 279-293.

Balaji R, Suba V, Rekha N, Deecaraman M (2009). Hepatoprotective activity of methanolic fraction of Jatropha curcas on Aflatoxin B1 induced Hepatic Carcinoma. International Journal of Pharmaceutical Sciences,1: 287-296.

Brander GC, ***Pugh DM, Bywater RJ***(1991). Veterinary Applied Pharmacology and Therapeutics. 5th Ed., W.B. Saunders Co., Philadelphia, ISBN-13: 978-0702013669, Pages: 636.

Diwani G, Rafie SE, Hawash S (2009). Antioxidant activity of extracts obtained from residues of nodes leaves stem and root of Egyptian Jatropha curcas. African Journal of Pharmacy and Pharmacology, 3: 521-530.

Donlaporn S and Suntornsuk W (2010). Antifungal activities of ethanolic extract from Jatropha curcas seed cake. Journal of Microbiology and Biotechnology, 20: 319-324.

Duke JA and Wain KK(1981). Medicinal plants of the world. Computer Index with More than 85,000 entries, Volume 3, Plants Genetics and Germplasm Institute, Agriculture Research Service, Beltsville, Maryland.

Gadekar KP (2006). Vegetative propagation of Jatropha curcas, Karanji and Mahua by stem cuttings, Grafting, Budding and Air-layering, M.Sc. Forestry Thesis, Department of Forestry, Indra Gandhi Agricultural University, Raipur.

Goonasekera MM and Gunawardana VK (1995). Pregnancy terminating effect of Jatropha curcas in rats. Journal of Ethnopharmacology, 47: 117-123.

Hiller-Sturmhofel S and Bartke A(1998). The

endocrine system. Alcohol Health and Research World, 22(3): 153-164.

Igbinosa OO, Igbinosa EO, Aiyegoro OA(2009). Antimicrobial activity and phytochemical screening of stem bark extracts from Jatropha curcas (Linn). African Journal of Pharmacy and Pharmacology, 3: 58-62.

Ishii Y, Takeuchi R, Tokida K (1987). Trans esterifiedcurcas oil as a farm diesel engine fuel. In: Proceedings of the international symposium on agriculture mechanization and international cooperation in the high technology era. 239. University of Tokyo.

Jain S,Choudhary GP,Jain DK (2013). Pharmacological Evaluation and Antifertility Activity of Jatropha gossypifolia in Rats. Biomed Research International, 2013; 2013: 125980.

Johnson M, Olufunmilayo LA, Adegboyega CC, Adetayo OM. (2014). Evaluation of Antidiabetic and the Effect of Methanolic Leaf Extract of Jatropha curcas on Some Biochemical Parameters in Alloxan-induced Diabetic Male Albino Rats. European Journal of Medicinal Plants, 4(12): 1501-1512.

KatoleS,SahaSK, DasA, SastryVRB, LadeMH, Prakash B (2013). Nutrient intake, digestibility, and blood metabolites of goats fed diets containing processed jatropha meal. Tropical Animal Health and Production, 45: 1563-1569.

List PH and Horhammer L. Harger'sHandbuck Der Pharmaceutischen. Springer-Verlag, Berlin, Germany, 1969.

Kannappan N (2008). Antiulcer activity of methanolic extract of *Jatropha curcas*Linn on aspirin induced gastric lesions in Wistar rats. Pharmacology Online Newsletters, 1: 279-293.

Kumar V, Singh SP, Shukla A, Chaudhary JK (2014). Evaluation of subacute toxicity of *Jatropha curcas* seeds and seed oil toxicity in rats. Indian Journal of Veterinary Pathology, 38(2): 88-93.

Mishra SB, Vijayakumar M, Ojha SK, Verma A (2010). Antidiabetic effect of Jatropha curcas L. leaves extract in normal and alloxan-induced diabetic rats. International Journal of Pharmaceutical Sciences, 2: 482-487.

22. Mitchell J and Rook A (1979), Botanical Dermatology. Green Glass Ltd., Vancouver, Canada, ISBN-13: 9780889780477, Pages: 787.

23. Morton JF and Thomas HQ (1981). Anti-malarial activity of Jatropha curcas leaf extract. India Herbal Pharmacopoeia Public Regional Research Laboratory, 20: 53-57.

24. Mujumdar AM, *Misar AV, Salaskar MV, Anuradha U* (2001). Antidiarrhoeal effect of an isolated fraction (JC) of Jatropha curcas roots in mice. *Journal of Natural Remedies,* 1: 89-93.

25. Mujumdar AM and MisarAV (2004). Anti-inflammatory activity of Jatropha curcas roots in mice and rats. *Journal of Ethanopharmacology,*90: 11-15.

26. Munch E and Kiefer J (1989). Purging nut (J. curcas L) Multi-use plant as a source of fuel in the future. Schriftenreihe der GTZ, 209: 1-32.

27. Niswender GD,Juengel JL, Silva PJ, Rollyson MK, McIntush EW.(2000). Mechanisms controlling the function and life span of the corpus luteum. Physiological Reviews, 80(1): 1-29.

28. Pathak AK, Mallurwar VR, Kondalkar AK, Soni S(2005), A Review of Plants with Anti-Fertility Activity. *Nigerian Journal of Natural Products and Medicine,* 9: 4-10.

29. Prasad DMR,IzamA, Md. Maksudur Rahman Khan MMR (2012). Jatropha curcas: Plant of medical benefits. *Journal of Medicinal Plants Research,* 6: 2691-2699.

30. Shetty S., Udupa SL, Udupa AL, Vollala VR (2006). Wound healing activity of bark extract of Jatropha curcas L. in *albino rats. Saudi Medical Journal,* 27: 1473-1476.

31. Shukla A, Singh S,Tiwari S (2015). Transformation of toxic potential of Jatropha curcas (Ratanjyot) into protein source: A mini-review. *Journal of Advanced Veterinary and Animal Research,* 2(2): 89-

94.
32. Singh S and Mehta RS (2005). Environment-Jatropha biodiesel. *Autocar India,* 2005; 7: 169-175.
33. Smith MF, McIntush EW, Smith GW (1994). Mechanisms associated with corpus luteum development. *Journal of Animal Science,* 72(7): 1857-1872.
34. Sofowora A. (1993). Medicinal Plants and Traditional Medicine in Africa. *2nd Edn., Spectrum Books Ltd., Ibadan*, Nigeria, ISBN-13: 9782462195, Pages: 289.
35. Thomas O.O. (1989). Re-examination of the antimicrobial activities of Xylopiaaethiopica, Carica papaya, Ocimumgratissimum, and Jatropha curcas. Fitoterapia, 60(2): 147-155.
36. Watt JM and Breyer-Brandwijk MG (1962). The Medicinal and Poisonous Plants of Southern and Eastern Africa. 2nd ed., E and S Liningstone Ltd., London, UK., Pages: 1457.
37. Weinbauer GF and Nieschlag E (1996). Hormonal Regulation of Reproductive Organs. In: Greger R., Windhorst U. (eds) Comprehensive Human Physiology. Springer, Berlin, Heidelberg.