

Antifertility activity of ethanol extract of *Dioscorea esculenta* (L.) Schott on male albino rats.

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Abstract: Antifertility effects of ethanol extract of *Dioscorea esculenta* tuber were observed in male albino rats. The relative weight of testes and epididymis were decreased significantly ($p < 0.01$). No significant changes were observed in the vas deferens, seminal vesicle and prostate. The epididymal sperm count, motility and sperm abnormality were reduced significantly in treated rats. No significant changes were noted in the serum biochemical and liver marker enzymes (SGOT, SGPT and ALP) in the drug treated rats. The results of the hormonal assay showed that increased serum levels of FSH and estrogen but decrease in the serum levels of LH and testosterone compared to control. The results of the present study concluded that, ethanol extract of *Dioscorea esculenta* treatment inhibited sperm concentration, motility and testosterone which might result in a male fertility.

Key words: *Dioscorea esculenta*, testes, sperm concentration, testosterone.

Introduction

The options available to men for fertility control are much more limited compared to those of women. The male reproductive system, particularly the process of spermatogenesis, sperm maturation and transport and also the sperm-egg interaction are so complex that it has not so far been possible to find an effective intervention that can be converted into a product. Continued efforts

over the past three decades to develop additional methods of male contraception have made some significant contributions in the field. However, there is still no method available in the field of male contraception that satisfies the essential criteria of safety, efficacy, economy and complete reversibility¹.

Plant preparations play an important role in fertility regulation, a fact that has been reported in the

ancient literature of indigenous systems of medicine. A number of plant species have been tested for fertility regulation beginning about 50 years ago and were subsequently fortified by national and international agencies^{2, 3}. Ethnobotanical knowledge provides very useful basic clues not only in the problem relating to nomenclature identification of crude drug extracts but also in the discovery and the use of medicinal plants⁴.

Dioscorea esculenta (Dioscoreaceae) is an edible tuber found in Nigeria, China, Mexico, India and some other parts of the world. Externally, the tuber paste has been applied to ulcers, boils and abscesses. It contains allantoin, a cell-proliferant that speeds up the healing processes. It has been used traditionally as a contraceptive in the treatment of menopausal symptoms and various disorders of the genital organs. It has been suggested for ethnomedicinal uses as an antifatigue, anti-inflammatory, anti-stress, anti-spasmodic and immune deficiency remedies in various ethnomedicines. The main constituent of *Dioscorea* species is the well known saponin, dioscin. The aglycone, diosgenin, is the major starting material used in the industrial production of steroidal hormones. Diosgenin has also been utilized for hundreds of years to treat rheumatism and arthritis like ailments^{5, 6}. In view of the above said medicinal properties, the present study was designed to investigate the antifertility activity of ethanol extract of *Dioscorea esculenta* tuber.

Materials and Methods

Plant material

The tuber of *Dioscorea esculenta* (L.) Scott was collected from Pechiparai, Kanyakumari District, Tamil Nadu and identified by the Botanical Survey of India, Coimbatore. A voucher specimen was retained in Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin for further reference.

Preparation of plant extract for phytochemical Screening and Hepatoprotective Studies

The tuber of the plant was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder, which was then subjected to successive extraction in a Soxhlet apparatus using ethanol. The extract was subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures^{7, 8, 9}. The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for antifertility activity.

Animals

Normal healthy male Wistar albino rats (180-240g) were used for the present investigation. Animals were housed under standard environmental conditions at temperature (25±2°C) and light and dark (12:12h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum*.

Acute Toxicity Studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study¹⁰. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 100 and 2000 mg/kg body weight.

Experimental Design

The male rats were divided into four groups consisting of 5 animals.

Group I: Rats received normal saline daily for 14 days (control).

Group II: Rats received ethanol extract of *Dioscorea esculenta* tuber in a dose of 100 mg/kg body weight daily for 14 days (low dose).

Group III: Rats received ethanol extract of *Dioscorea esculenta* tuber in a dose of 300 mg/kg body weight daily for 14 days (moderate dose).

Group IV: Rats received ethanol extract of *Dioscorea esculenta* tuber in a dose of 600 mg/kg body weight daily for 14 days (High dose).

After 24 hours of last treatment, the final weight was recorded and the animals were sacrificed by decapitation. Blood was collected; sera were separated by centrifugation at 3000g for 10 minutes and stored at -20° C until used for various biochemical assays. Then testes, epididymis, vasdeferens, seminal vesicle and ventral prostate were dissected out, trimmed off extraneous and weighed accurately on a torsion balance. The organ weights were expressed in terms of mg/100gm body weight.

Sperm Count

Epididymal fluid (for sperm count) was collected from caput and cauda segments separately and diluted with Sorenson's buffer (pH 7.2). The separated fluid was taken for sperm count. Sperm count was carried out by using Neubauer's haemocytometer as described by Zaneveld and Polakoski¹¹.

Sperm motility and abnormality

After anaesthetizing the rats, the caudal epididymis was then dissected. An incision (about 1mm) was made in the caudal epididymis and drops of sperm fluid were squeezed onto the microscope slide and 2 drops of normal saline were added to mobilize the sperm cells. Epididymal sperm motility was then assessed by calculating motile spermatozoa per unit area.

Morphology (abnormality) was evaluated on sperm from the cauda epididymis. The total morphological abnormalities were observed as described by Linde *et al*¹².

Serum biochemical analysis

Serum protein¹³ and serum albumins were determined by quantitative colorimetrically method by using bromocresol green. The total protein minus albumin gives the globulin, urea¹⁴, creatinine¹⁵, serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by using the method of Reitman and Frankel¹⁶. Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong¹⁷.

Hormonal Assay

Blood removed from the rats by intracardiac method. Blood was centrifuged at 3000rpm to separate the serum for the measurement of testosterone, luteinizing hormone (LH), estrogen and follicle stimulating hormone (FSH). The quantitative determination of hormones was done by using Enzyme Immunoassay Method (EIA). The EIA kit was obtained from Immunometrics (London, UK).

Statistical Analysis

Data were expressed as Mean \pm SEM. Student's t test was used for statistical comparison.

Results

Body and Reproductive organ weight

The ethanol extract of *Dioscorea esculenta* tuber at different concentration were treated on male Wistar albino rats for antifertility activity.

The administration of ethanol extract of *Dioscorea esculenta* tuber to rats did not cause any significant change in the body weight (Table 1) and on the libido of treated rats whereas weights of testes and other accessory sex organs were decreased significantly ($p < 0.01$) (Table. 1). Among the accessory sex organs, a significant weight reduction was seen in the caput and caudal epididymal segment. The weight reduction was dose dependent i.e. high dose (600 mg / kg body weight) treated groups (Group IV) drastically reduced followed by moderate (300 mg / kg body weight) (Group III) and less in low dose groups (Group II) (100 mg / kg body weight). No change was observed in vasdeferens, seminal vesicle and prostate.

Sperm count and Sperm motility

Sperm motility and sperm density in caudal epididymis, significantly decreased and the reduction was severe in higher dose treated group (Group IV) followed by moderate and low dose groups (Group III and II respectively) (Table 2) and the same trend was seen in the caput epididymal sperm density when compared to control (Group I).

Sperm abnormality

Sperm abnormality in caput and caudal region was drastically affected by ethanol extract of *Dioscorea esculenta* tuber ($p < 0.05$). Among the three dose treatment groups, high dose group have shown significant and drastic abnormality in the sperm morphology, further tail region of the sperm in all the treated groups much affected than the head region.

Serum biochemical profile

Serum protein, albumin, globulin, urea and creatinine and the activity of liver marker enzymes (SGPT, SGPT and ALP) levels of control and treated rats were depicted in the Table 3. No significant changes were noted in the serum biochemical and liver marker enzymes in the entire drug treated groups when compared to control group.

Table - 1 Effect of ethanol extract of *Dioscorea esculenta* on the reproductive organ weight of adult male albino rats.

Treatment Groups	Body wt(gm)		Testis (gm)	Epididymis (mg)		VD (mg)	SV (mg)	Prostrate (mg)
	Before	After		Caput	Cauda			
Group-I	258.14±11.4	266.42±12.3	1.786±0.66	134.13±3.8	279.41±13.2	101.26±11.3	343.36±13.8	169.46±7.3
Group-II	240.19±12.6	252.21±10.1*	1.413±0.40*	119.43±5.2*	245.14±8.6*	90.14±2.5	318.43±17.4	156.56±8.4
Group-III	218.15±11.2	237.14±14.3	1.301±0.90**	116.26±3.1**	233.46±5.4**	96.21±1.6	313.56±12.4	168.26±8.6 NS
Group-IV	216.25± 9.3	214.32±12.3	1.209±0.14***	98.43±1.8***	215.72±1.9***	93.46±2.1	318.23±14.4	153.33±8.4 NS

Each Value is SEM of 5 animals * p < 0.05** p<0.01*** p<0.001 Control vs. Treated

Table – 2 Effect of ethanol extract of *Dioscorea esculenta* on the sperm concentration and motility in the epididymis of adult male albino rats.

Parameter	Treatment Groups								
	Group- I		Group - II		Group - III		Group – IV		
	Caput	Cauda	Caput	Cauda	Caput	Cauda	Caput	Cauda	
Sperm Concentration (Counts x 10 ⁶ mil)	476.34±31.4	502.14±31.09	205.14±16.5*	198.11±22.3*	192.12±15.4**	172.21±18.4**	158.4±16.4***	105.4±19.4***	
Sperm Motility (FMI) @	---	132.4±15.5	---	72.4±4.4*	---	68.31±4.8**	---	48.40±9.2***	
Sperm Abnormality #	Head	----	5.12±0.12	----	43.6±2.49*	-----	68.98±4.23**	-----	95.21±6.5***
	Tail	-----	6.78±0.45	-----	62.34±1.56*	-----	88.98±3.33**	-----	98.56±4.98***

Each Value is SEM of 5 animals * p< 0.05, ** p<0.01, ***p<0.001 Control vs. Treated

@: Motility is movement recorded after 5 min in suspension of caudal epididymal spermatozoa in phosphate buffered solution.

#: Expressed in percentage

Table -3 Effect of ethanol extract of *Dioscorea esculenta* on few serum biochemical profiles of adult male albino rats.

Parameters	Treatment Groups			
	Group I	Group II	Group III	Group IV
Protein (gm/dl)	7.91±0.8	6.6±0.8	7.4±0.7	7.4±0.32
Albumin (gm/dl)	4.90±0.6	3.6±0.14	4.8±1.9	4.2±0.16
Globulin(gm/dl)	3.01±0.5	3.0±0.16	2.6±0.15	3.2±0.24
Urea(mg/dl)	18.33±2.4	20.51±2.23	16.3±1.9	19.1±1.9
Creatinine(mg/dl)	0.67±0.3	1.1±0.84	1.0±0.9	1.8±0.06*
	12.34±0.78	18.44±0.45	21.89±2.87	22.96±1.98
SGPT(U/L)	19.45±0.98	24.21±1.56	22.67±2.08	26.67±6.44
ALP(U/L)	123.42±9.88	129.55±4.32	112.89±5.89	148.48±9.87

Each Value is SEM

Table – 4 Effect of ethanol extract of *Dioscorea esculenta* on sex hormones levels

Treatment Groups	Parameters			
	Testosterone (ng/ml)	LH/ICSH (μ Iu/ml)	Estrogen (pg/ml)	FSH (μ Iu/ml)
Group I	2.14±0.56	1.33±0.21	24.89±0.22	0.92±0.09
Group II	2.04±0.26	1.02±0.36	32.12±0.43	3.21±0.76 *
Group III	1.67±0.95**	0.57±0.19 *	57.43±0.34 **	2.87±0.21 *
Group IV	0.84 ± 0.11***	0.29±0.78 **	88.23±0.98 ***	1.45±0.54

Each Value is SEM of 5 animals* p < 0.05, ** p<0.01, *** p<0.001 Control vs. Treated

Reproductive hormone file

Serum Testosterone level

The ethanol extract of *Dioscorea esculenta* tuber (100, 300, 600 mg / kg body weight) repeated treatment daily for 14 days caused a significant decrease in the serum level of testosterone in male rats. The level of testosterone decrease was dose related (Table 4).

Serum Luteinizing hormone (LH) level

Repeated treatment of male rats with the *Dioscorea esculenta* extract for 14 days caused a dose related decrease in the serum level of LH (Table 4). The level of decrease was statistically significant (p < 0.05).

Serum Estrogen level

The ethanol extract of *Dioscorea esculenta* (100, 300 and 600 mg / kg body weight) caused an increase in the serum level of estrogen in male rats. Doses of 100,

300 and 600 mg / kg body weight administered daily for 14 days caused a sharp rise in the serum level of estrogen (Table 4) whereas the highest dose of 600 mg / kg body weight induced gradual increase.

Serum follicle stimulating hormone (FSH) level

Pretreatment with ethanol extract of *Dioscorea esculenta* tuber for 14 days caused an increase in the serum level of FSH in male rats compared to control (Table 4). The increase in the serum level of FSH in male rats statistically significant (p < 0.05).

Discussion

Studies on the effects of plant products on male reproductive system and fertility are comparatively few and far fetched. From a public health perspective, the need for contraception has never been greater.

The results revealed no change in the body weight of rats treated with *Dioscorea esculenta* ethanol extract of tubers (100, 300, and 600mg / Kg body weight) for fourteen days. The testes and other accessory sex organs decreased significantly during the experiment. Among the accessory sex organs, a significant weight reduction was seen in the testes, caput and caudal epididymal segments and the weight reduction was dose dependent. Reduction in the weight of testes and other accessory sex organs might be due to low level of androgen, which was not enough to maintain the weight of gonads and accessories¹⁸. It is known that the accessory sex organs viz., epididymis and vasdeferens are androgen dependent target organs and manifest differential sensibility to androgens for maintenance of their structure and function. It is also known that, any change in circulating androgens would affect the internal microenvironment of epididymis and thereby lead to alteration in sperm motility and metabolism¹⁹.

In the present study, *Dioscorea esculenta* ethanol extract treated rats decreased the sperm motility and sperm density in cauda and caput epididymal segments (Table 2). Drastic effect on the nature of the normal sperms in the caput and caudal region was observed in *Dioscorea esculenta* tubers treated rats. Further tail region of the sperm in all the treated groups (Groups II, III and IV) were much affected than the head regions (Table 2). The development of normal and mature sperm is the key to optimum male fertility. The production of the sperm cells (spermatozoa) and testosterone in the testis are mainly regulated by the follicle stimulating hormone (FSH) and luteinizing hormone (LH), which are released from the anterior pituitary²⁰. FSH stimulates spermatogenesis in the sertoli cells, while LH stimulates the production of testosterone in the leydig cells of the testes²¹. Many studies on the testes of rat treated with plant extracts has also demonstrated that the inhibitory activity on the proliferation of spermatogonia in mammals^{22, 23, 24}. Spermatogenesis is therefore, a complicated process, covering proliferation of the spermatogonia, long-lasting process of the tissue meiosis and numerous changes in the spermatids during their pre-formation^{15, 16}. The result of the present study suggests that ethanol extract of *Dioscorea esculenta* tuber may affect the normal

function of the sertoli and leydig cells on continuous oral administration for seven days.

Sexual cells can occur during the reproductive phase, mitotic division of the spermatogonia or during the maturation of the spermatozoa, thereby affecting the number and quality of the sperm cells produced in the testes. Among the ethanol extract treated groups III and IV (300mg / kg body weight and 600mg / kg body weight) produced a significant reduction in total sperm count and viable sperms. This may be as a result of the ability of the extract at the given doses, to either interfere with spermatogenetic process in the seminiferous tubules, epididymal functions or activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadotropins which may result in alteration of spermatogenesis^{25, 26}. The presence of immature sperms was also observed in the experimental rats treated with 300mg / kg body weight and 600mg / kg body weight of ethanolic extracts of *Dioscorea esculenta*. This suggests that the 300mg / kg body weight and 600mg / kg body weight dose level could affect the maturation of the spermatozoan in the male rats, which might also be a contributory factor to the decrease in the mean total sperm count. The data generated in the present study, by and large, confirm to those already reported and studied with various plant extracts^{27, 28, 29}. The decrease in the caudal epididymal sperm counts are clear indications that, *Dioscorea esculenta* tuber extract can affect one or more aspects of spermatogenesis as well as spermigenesis. Though a direct effect of *D. esculenta* extract on the cellular mechanisms of spermatogenesis cannot be concluded, it is likely that the impairment of the hormonal mechanisms concerned with the regulation of spermatogenesis may be the underlying cause.

The various other sperm abnormalities like sluggish motility, coiled tail and sperm maturation are also due to *D. esculenta* toxicity. The hitherto unreported abnormal sperm morphology, coiled tail and malformed head could be attributed to both testicular and epididymal effects of *D. esculenta* tuber extracts. Coiling of the sperm tail is usually the product of abnormal axoneme and / or the outer dense fibril. The outcome of the present study affirms the male reproductive toxic effects of *D. esculenta* when applied as a therapeutic agent. Since male reproductive toxicology and male contraception are two sides of the same coin, the negative consequence of *D. esculenta* on the sperm may be taken as an advantage for further study. By the treatment employed in the study, no toxic

effect was produced in the liver and kidney, neither was it directly involved on the development and functioning of the male reproductive system nor in the reproductive organs.

The present study revealed a decrease in the serum level of testosterone. This observation was similar to the earlier findings of^{30, 31, 32}. The reduction in the serum level of testosterone could probably be due to the decrease of serum levels of LH / ICSH observed in this investigation. Leydig cells secrete testosterone by the stimulatory effect of LH^{33, 32, 34}. In males reduction of testosterone level may impair spermatogenesis and cause male infertility. This study further observed a dose dependent increase in the serum estrogen level. This increase might probably be due to the conversion of testosterone to estrogen^{35,36}.

Treatment with the ethanol extract of *Dioscorea esculenta* (300mg / kg body weight and 600mg / kg body weight) was highly effective in producing reversible functional sterility. The drug treated male rats clearly indicated structural and functional alteration in testes, epididymis and seminal vesicle. Depletion of sperm count and sperm motility in the drug treated rats suggests alteration in sperm production in the testes and maturation in the epididymis. Changes in both sperm count and motility resulted in partial infertility within seven days. This resulted in abnormal sperm functions which ultimately gave rise to complete male sterility. Among the plant based contraceptives, inhibition of male fertility after administration of natural substances has been related to decreased spermatozoa density³⁷. For

male contraception, it is not necessary to stop spermatogenesis, but it is enough to eliminate the fertilizing ability of the spermatozoa by causing changes in the morphology or in the function of the sperm³⁸.

The activity of *Dioscorea* species has been attributed to the action of various steroidal saponins (diosgenin an aglycone) and also to dioscorin(e), dioscin(e) and other alkaloids³⁹. Saponins are important mainly because of their steroid structure. They are precursors for the hemisynthesis of birth control pills (with progesterone and estrogens) as well as similar hormones and corticosteroids⁴⁰. Recently many laboratories are engaged in developing male contraceptives from plants⁴¹. Plant products as contraceptives will be more acceptable for economic reasons in terms of self reliance and the possible practicability for a male pill approach in countries where population pressure is high. Recently extensive efforts have been made to study the antifertility drugs from plants^{42, 19, 43}. In the present study, dose dependent treatment of *Dioscorea esculenta* tuber extract and duration suggests marked alterations in the male reproductive organs. Further studies are needed to prove whether the alterations are reversible or permanent after cessation of treatment and for understanding the exact mechanism.

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References

1. Deshgan, M.H., Daryani, A and Robabeh, D. Histological evidence of male potent reproductive sites by Iranian Botanical *Azadirachta indica* (neem) seed extract. Int.J.Mol.Med.Adv.Sci. 2006. 2, 7-15.
2. Sinha, R. Post- testicular antifertility effects of *Abrus precatorius* seed of extract in albino rats. J. Ethnopharmacology.1990. 28,173-181.
3. Purohit,A and Daradka, H.M.M. Antiandrogenic efficacy of *Curcuma longa* (50% ethanol extract) with special emphasis on testicular cell population dynamics. Indian Drug. 1999, 142-143.
4. Khouri, N.A and Akawi, Z.EL. Antiandrogenic activity of *Ruta graveolens* L. in male albino rats with emphasis on sexual and aggressive behaviour. Neuroendocrinology Letters. 2005, 823-829.
5. Sofowara, A. Medicinal plants and traditional medicine in Africa.Spectrum Books Limited. Nigeria.1993.
6. Olayemi,J.O, and Ajaiyeoba, E.O. Anti-inflammatory studies of yam (*Dioscorea esculenta*) extract on Wistar rats. Afri. J. Biotech. 2007. 6, 1913-1915.
7. Brinda P, Sasikala P, Purushothaman KK: Pharmacognostic studies on *Merugan kizhangu*. Bulletin in Medical Ethnobotanical Research. 1981; 3:84-96.
8. Anonymous: Phytochemical investigation of certain medicinal plants used in Ayurveda.

- Central Council for Research in Ayurveda and Siddha, New Delhi. 1990.
9. Lala PK: Lab manuals of Pharmacognosy CSI Publishers and Distributors, Kolkata. 1993.
 10. OECD, (Organisation for Economic co-operation and Development). OECD guidelines for the testing of chemicals/Section 4: Health Effects Test No. 423; Acute oral Toxicity- Acute Toxic Class method. OECD. Paris. 2002.
 11. Zaneveld, L.J.D. and Polakoski. Collection and physical examination of the ejaculate. In: Hafez, E.S.E. (ed) Techniques in human andrology. vol. 1, Human reproductive medicine. North – Holland Publishing company, Amsterdam, 1977. pp. 147-172.
 12. Linde, R.E., Strader, L.F., Slott, V.L. and Suarez, J.D. End points of spermatotoxicity in the rat after short duration exposures to fourteen reproductive toxicants. *Reprod. Toxicol.* 1992. 6, 491-505.
 13. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ: Protein measurement with the folin's phenol reagent. *Journal of Biological Chemistry.* 1951; 193: 265-275.
 14. Varley, H. Practical clinical biochemistry, Arnold Heinemann Publication Pvt. Ltd. 1976. P. 452.
 15. Owen, J.A., Iggo, J.B., Scangrett, F.J. and Steward, I.P. 1954. Determination of creatinine in plasma serum, a critical examination . *J. Biochem.* 58 : 426-437.
 16. Reitman, S. and Frankel, S.A. Colorimetric method for the determination of Serum glutamic oxalo-acetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.* 1957. 28, 56-63.
 17. King, E.J. and Armstrong, A.R. Determination of serum and bile phosphate activity. *Can. Med. Assoc. J.* 1934. 31, 56-63.
 18. Sharma, N. and Jacob, D. Anti-fertility investigation and toxicological screening of the Petroleum ether extract of the leaves of *Mentha arvensis* L. in male albinomice. *J. Ethnopharmacol.* 2001. 75, 5-12.
 19. Khan, P.K. and Awasthy, K.S. Cytogenetic toxicity of Neem. *Food chem. Toxicol.* 2003. 41, 1325-1328.
 20. Steinberger, E. 1971. Hormonal control of mammalian Spermatogenesis. *Physiology Review*, 5111, 1-22.
 21. Kerr, J.B. and Klester, D.M. Cyclic variation in sertoli cell lipid content throughout the spermatogenic cycle in the rats. *J. Reprod Fert.* 1975. 43, 1-8.
 22. Steinberger, E., Steinberger, A., Perlof, W.H. Initiation of spermatogenesis *in vitro*. *Endocrinology.* 1964. 74, 788.
 23. Mancini, R.E., Castro, A. and Serguer, A.C. Histological localization of follicle-stimulating and luteinizing hormones in the rat testis. *J. Histochem. Cytochem.* 1967. 15, 516-526.
 24. Krueger, P.M., Hodgen, C.D. and Sherins, K.I. New evidence for the role of the sertoli cells and spermatogonia in feed back control of FSH secretion in male rat. *Endocrinology.* 1974. 95, 955-962.
 25. Bowman, W.C.M. and Rand, M.J. The reproductive system and drugs affecting the reproductive systems. Text book of Pharmacology. 1985, 2.
 26. William, K.N. Hormones and Hormone antagonists. In : Remington, The Science and Practice of Pharmacy, 2000.77, 1390-1391.
 27. Njar, V.C., Alao, T.O., Okogun, J.I., Raji, Y., Bolarinwa, A.F., Nduka, E. V. Antifertility activity of *Quassia amara*: quassin inhibits the setroidogenesis in rat Leydig cells *in vitro*. *Planta Med.* 1995. 61, 180-182.
 28. Raji, Y. and Bolarinwa, A.F. Antifertility activity of *Quassia amara* in male rats- *in vivo* study. *Life Sci.* 1997.61, 1067-1074.
 29. Parveen, S., Das, S., Kundra, C.P. and Pereira, B.M. A comprehensive evaluation of the reproductive toxicity of *Quassia amara* in male rats. *Reprod. Toxicol.* 2002. 17, 45-50.
 30. Udoh, P. and Kehinde, A. Studies on Antifertility effects of paw paw seeds (*Carica papaya*) on the gonads of male Albino rats. *Phytother. Res.* 1999. 13, 226-228.
 31. Udoh, P. and Ekipeyong, J. Effects of *Mucuna urens* (Horse eye bean) on the gonads of male Guinea pigs. *Phytother. Res.* 2001. 15, 99-102.
 32. Udoh, P., Essien, I. and Udoh, F. Effect of *Carica papaya* (paw paw) seeds extract on the morphology of pituitary- gonadal axis of male

- albino rats. *Phytotherapy. Res.* 2005 a. 19, 1065-1068.
33. Udoh, F.V. and Udoh, P.B. Hepatotoxicity of the methanol extract of *Carica papaya* seeds in Wistar Rats. *Pharmaceutical Biol.* 2005.43, 349-352.
 34. Udoh, F.V., Udoh, P.B. and Umon, E.E.. Activity of alkaloid extract of *Carica papaya* seeds on reproductive functions in male Wistar rats. *Pharmaceutical Biol.* 2005 b 43, 563-567.
 35. Carr, B.R. and Blackwell, R.E. Neuroendocrinology of reproduction In: Text book Reproductive Medicine Carr and Blackwell R.E.(eds). Norwalk: Appleton and Lange. 1993. pp. 157.
 36. Chinoy, R.J. and Padman, P. Antifertility investigation and benzene extract of *Carica papaya* seeds in male albino rats. *J. Med. Aromatic Plant Sci.* 1996. 18, 489-494.
 37. Watche, P., Kamtehouing, P., Sokeng, S., Moundipa, P.F., Tantchu, J., Essame, J.L. and Kaveeta, N. Reversible antispermatogenic and antifertility activity of *Mondia whitei* L. in male albino rats. *Phytother. Res.* 2001. 15, 26-9.
 38. Dwivedi, A.K., Chaudhary, M. and Sarine, J.Ps. Standardisation of a new spermicidal agent sapindus saponin and its estimation in its formulation. *Indian J. Pharm. Sci.* 1990. 52, 165-167.
 39. Dweek, A.C. The Wild Yam – review of Dioscorea species. *Personal Care Magazine* – 2002. 3, 7-9.
 40. Crabbe, P. Some aspects of steroid research based on natural product from plant origin. *Bull. Soc. Chim. Belg.* 1979. 88, 5-7.
 41. US National Academy of Sciences, Publication. Neem – a tree for solving global problems. For thousands of years the beneficial properties of Neem office of International Affairs. 1992.
 42. Handelsman, D.J. Hormonal male contraception. In : Puri CP. Van Look PFA. Editors. Current concepts in fertility regulation and reproduction. New Delhi. Wiley Eastern Ltd. 1994. P. 133.
 43. Upadhyay, S.N., Dhawan, S. and Talwar, G.P. Antifertility effects of Neem (*Azadirachta indica*) oil in male rats by single intra-vas administration. An alternate approach to vasectomy. *J. Androl.* 1993. 14, 275-281.
