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Anti-spermatogenic Activity of *Leptadenia hastata* (Pers.) Decne Leaf Stems Aqueous Extracts in Male Wistar Rats

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ABSTRACT

Testis histology and sperm parameters were used to evaluate the antispermatogenic effect of *Leptadenia hastata* aqueous extract in male Wistar rats. 100, 200, 400 and 800 mg kg⁻¹ of *Leptadenia hastata* aqueous extracts were orally administered during 60 consecutive days. *L. hastata* aqueous extract did not have significant ($p>0.05$) effect on body, testis and epididymis weights. Testes histology of rats treated with the plant extract showed the decrease of Leydig cells number and the spermatogenesis was been influenced with high doses of *L. hastata* aqueous extracts. Some sperm parameters as path velocity, progressive velocity, straightness, linearity and motility of spermatozoa were been significantly ($p<0.05$) decreased. The treated rats with the different amount of *L. hastata* aqueous extracts showed a significant ($p<0.01$) reduction in the number of sperm in the testis and the cauda epididymis. Even if its not significant, the extract showed a decrease of testes, epididymis weights and a rarity of Leydig cells. These results confirm the antiandrogenic effect of *L. hastata* and the claims of breeders that the consumption of the leaves of *L. Hastata* reduced the fertility of their animals.

Key words: *Leptadenia hastata*, antispermatogenic, testis histology, sperm motility, sperm parameters

INTRODUCTION

Traditional medicine is a significant element of the African cultural heritage and for a large majority of people a resort for treating health problems. This knowledge is passed down from generation to the next in oral form but very little written informations are available on the active, safe and effective effects of this medicine. *Leptadenia hastata* (Pers.) Decne is an African traditional plant belongs to the family Asclepiaceae. Different parts of the plant have been reported to have several medicinal values. Its leaves and latex have trypanocidal, antimalarial, antibacterial, anti-inflammatory and sterility properties (Nikiema *et al.*, 2001; Bizimana *et al.*, 2006; Magassouba *et al.*, 2007; Kaou *et al.*, 2008; Aderbauer *et al.*, 2008). In the majority of western

Sahel country, the young leaves of *L. hastata* is an important staple when cereal harvests are inadequate to support populations (Sena *et al.*, 1998; Glew *et al.*, 2005; Hassan *et al.*, 2007). However, little attention was paid to the examination of the action of *L. hastata* on the reproductive system of mammals since in northern Burkina Faso, the breeders and local people traditionally believe that *L. hastata* can reduced the fertility of their animals (Arbonnier, 2000).

In previous study, the aqueous extracts of *L. hastata* exhibited an antiandrogenic effect on immature castrated rats by Hershberger assay (Bayala *et al.*, 2011). The present study deals with the effect of the aqueous leaf stems extract of *L. hastata* on the male reproductive organs of the wistar rat. We have evaluated various male reproductive end points such as organs weight, sperm parameters and testis histology.

MATERIALS AND METHODS

Plant collection and preparation of extract: The leaves and stems of *L. hastata* were collected from Kamboinsè (25 km in the North of Ouagadougou), during April and May 2009. The plant was authenticated by the Department of Botany at Ouagadougou University. Herbaria are made and their voucher specimen was deposited in the Department.

The leaf stems were first washed with large amount of water then dried in a ventilated room, away from dust and direct sunlight. 150 g of dried material were coarsely powdered and macerated in distilled water at 40°C. The obtained macerated product was then filtrated, run through Rotavapor (Buchi/R-114), lyophilised and kept in a drier until ready for use. Yield of the extraction was 24.6%.

Animal model: Wistar male rats from Charles River Laboratories (France) were checked without signs of illness and anomalies. Animals were acclimatized to the laboratory environment for 7 days before use. During the experiment, rats were housed six animals per cage in polycarbonate cages under controlled environmental conditions, including a temperature of 22°C, a relative humidity of 55% and a 12 h light cycle/12 h dark cycle. Pellet rodent diet and drinking water were available *ad libitum*. All the experiments have been carried out under approval of institutional ethics committee.

Methodology: Thirty male rats of 25 days old were divided into five groups of six animals each. The control group received distilled water and the other groups received respectively 100, 200, 400 and 800 mg kg⁻¹ of the aqueous extracts of *L. hastata* leaf stems. All the groups were treated by oral gavage during 60 consecutive days. The amount of *L. hastata* extracts was prepared daily prior to administration.

Histology of the testis: During the autopsy, the testis were removed and immediately fixed in Bouins liquid. The testis were remained in Bouin during 48 h and placed in gauze. They were washed with tap water during 2 h and passed into different amounts of alcohol. The testes were cut with paraffin at the thickness of 5 µm and stained with Mayers haematoxylin and eosin for the discrimination of the stages of spermatogenesis. The observations were made with the X40 enlargement (Ocular, X10) with the OLYMPUS BX51 Microscope. The images were captured using the Software SPOT BASIC, Version 4.0.8.

Sperm parameters: Epididymides were removed, isolated, rinsed and dilacerated in order to release the spermatozoa in 500 µL Phosphate Medium Bicarbonate (PMB): 90 mM NaCl, 30 mM

NaHCO₃, 11 mM KCl, 0,8 mM MgSO₄, 7 H₂O, 0,4 mM KH₂PO₄, 0,6 mM Na-acétate, 3,4 mM lactate (1,7 mM Ca²⁺), 2,3 mM Na-pyruvate, 6,6 mM glucose and 20 mM HEPES Buffer with 1 g/100 mL of BSA and adjusted to PH: 7,7. The osmolarity of PBM was 300 mosm kg⁻¹. The maintained in PBM at 37°C was favourable for the survival of spermatozoa. The homogenate was diluted at 1/10 to facilitate the observations. Two drops from 5 to 10 µL of the diluted solution were examined with the sperm analyzer HTM-IVOS (Hamilton Thorne Research, Beverly, MA). The HTM-IVOS permitted the analysis of the following parameters: Path Velocity (VAP), Progressive Velocity (VSL), Track Speed (VCL), Elongation (El), Lateral Displacement (ALH), Beat Cross Frequency (BCF), Straightness (STR), Linearity (L) and the motility of the spermatozoa.

Density of the spermatozoa in the testicles and the cauda epididymis: The testicle and the cauda epididymis were removed, mashed and placed into 5mL NaCl (0.9%). Homogenates were kept refrigerated at 4°C for 24 h to allow sperm to be released from the walls. Then, 5 mL of eosine (2%) was added and vortexed. One milliliter of this mixture is diluted with 2 mL eosine (2%) and a sample is placed in a Neubauer chamber and head sperm counted in 25 squares.

Statistical analyses: Data were analyzed using the Statistical Package SYSTAT (Version 10) and were presented as Mean±standard error (n = 6) of the mean (SEM). If variances were homogeneous, differences between groups were assessed by one-way analysis of variance. Differences between pair of means were assessed by the LSD test. A value of p<0.05 was considered as statistically significant.

RESULTS

Testis and epididymis weight: Sixty days after treatment with *L. hastata* aqueous extracts, there were no significant (p>0.05) differences in the weight of bodies, testes and epididymides in the four treated-groups of rats compared to those in the control group (Fig. 1, 2). However, the different treatment of *L. hastata* aqueous extracts showed the decrease of testis and epididymis weight.

Testis histology: Histological analysis of the testes of control group showed a normal spermatogenesis with a full complement of germinal, Sertoli and Leydig cells (Fig. 3). After 60 days treatment with *L. hastata* aqueous extracts, the number of Leydig cells in intertubellar space decreased significantly (p<0.05) (Fig. 4-7). Seminiferous tubules were found to contain spermatozoa in abundance in control (Fig. 3) and treated groups (Fig. 4-7).

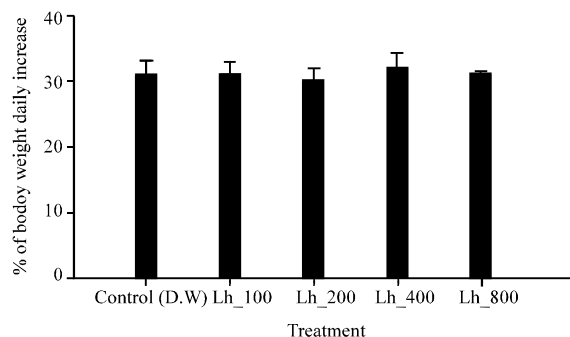


Fig. 1: Effect of *L.hastata* aqueous extract on body weight after sixty day of treatment

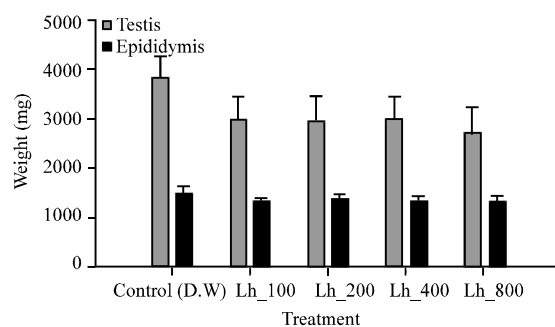


Fig. 2: Effect of *L. hastata* aqueous extract treatment on testis and epididymis weight

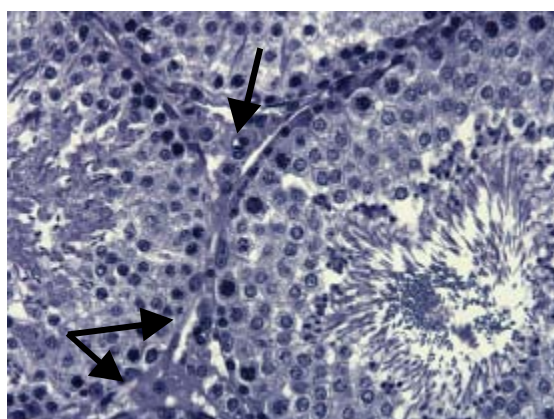


Fig. 3: Testis of rat control group treated distilled water during 60 consecutive days. Intertubular space of untreated testis presenting normal Leydig cells (arrow) (Mag. X400)

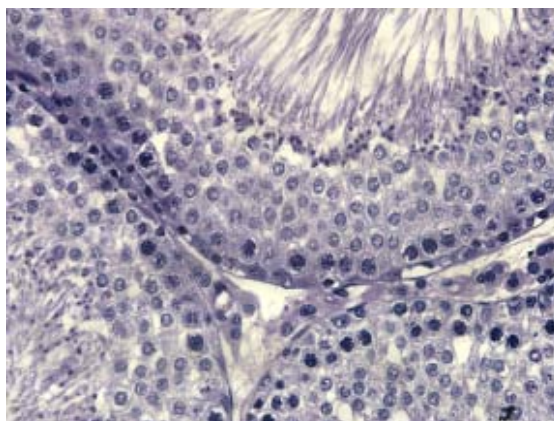


Fig. 4: Testis of rat group treated with 100 mg kg⁻¹ of *L. hastata* aqueous extracts during 60 consecutive days (Mag. X400)

Spermatic parameters and sperm density in testis and cauda epididymis: After sixty days of treatment, the different amounts of *L. hastata* aqueous extracts showed a significant ($p < 0.05$)

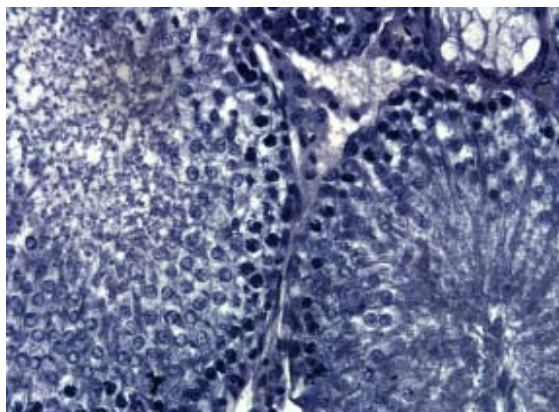


Fig. 5: Testis of rat group treated with 200 mg kg⁻¹ of *L. hastata* aqueous extracts during 60 consecutive days (Mag. X400)

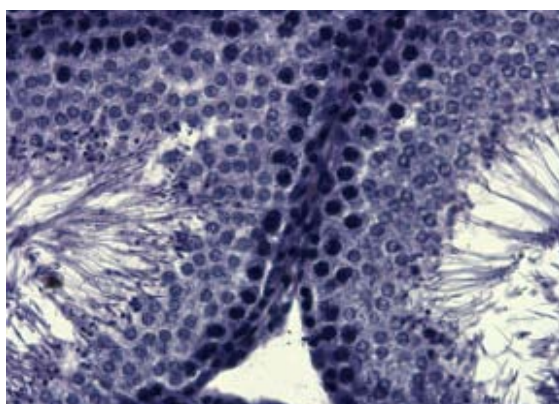


Fig. 6: Testis of rat group treated with 400 mg kg⁻¹ of *L. hastata* aqueous extracts during 60 consecutive days. Intertubular space of treated testis presenting the rarity of Leydig cells (Mag. X400)

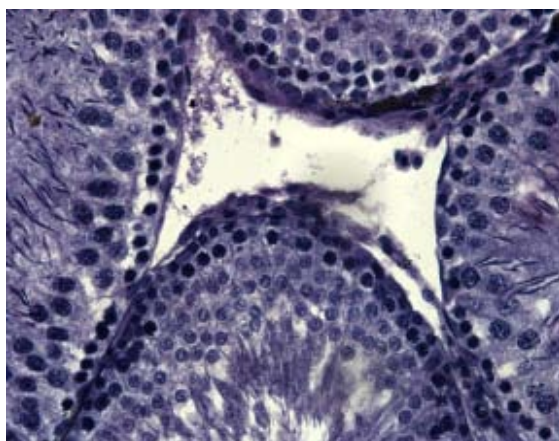


Fig. 7: Testis of rat group treated with 800 mg kg⁻¹ of *L. hastata* aqueous extracts during 60 consecutive days. Intertubular space of treated testis presenting the rarity of Leydig cells (Mag. X400)

Table 1: Effect of *L. hastata* aqueous extracts on some sperm parameters

Treatments	Sperm parameters								
	VAP ($\mu\text{m sec}^{-1}$)	VSL ($\mu\text{m sec}^{-1}$)	VCL ($\mu\text{m sec}^{-1}$)	ELO (%)	ALH (μm)	BCF (Hz)	STR (%)	LIN (%)	Motility (%)
Control (DW)	276.83 $\pm$ 3.57	195.83 $\pm$ 10.25	451.50 $\pm$ 20.66	25 $\pm$ 2.26	17 $\pm$ 0.57	21.33 $\pm$ 0.95	64.5 $\pm$ 0.61	49.66 $\pm$ 0.95	91.66 $\pm$ 2.66
Lh_100	208.6 $\pm$ 6.82*	148.60 $\pm$ 6.02	394.50 $\pm$ 14.95	27.60 $\pm$ 1.36	16.20 $\pm$ 0.80	19.80 $\pm$ 1.15	63.80 $\pm$ 1.62	34.80 $\pm$ 2.39	85.20 $\pm$ 3.21
Lh_200	204.57 $\pm$ 9.93*	139.42 $\pm$ 9.90*	385.14 $\pm$ 14.56	24.85 $\pm$ 1.05	16.40 $\pm$ 0.98	21.14 $\pm$ 0.67	40.28 $\pm$ 0.74*	33.57 $\pm$ 0.42*	74.42 $\pm$ 1.52*
Lh_400	238.66 $\pm$ 7.57	137.66 $\pm$ 8.54*	338.50 $\pm$ 6.18**	26.16 $\pm$ 1.24	17.83 $\pm$ 0.47	20.83 $\pm$ 1.62	42.33 $\pm$ 1.66*	31.16 $\pm$ 1.32*	74.83 $\pm$ 4.29*
Lh_800	210.80 $\pm$ 8.94*	125.80 $\pm$ 6.33*	320.00 $\pm$ 4.87**	22.00 $\pm$ 1.30	14.60 $\pm$ 0.67	18.90 $\pm$ 1.28	46.20 $\pm$ 1.56*	32.60 $\pm$ 1.12*	72.20 $\pm$ 3.92*

Lh: *Leptadenia hastata*, VAP: Path velocity, VSL: Progressive velocity, VCL: Track speed, ELO: Elongation, ALH: Lateral displacement, BCF: Beat Cross Frequency, STR: Straightness, LIN: Linearity, DW: Distilled water, *: $p < 0.05$; **: $p < 0.001$

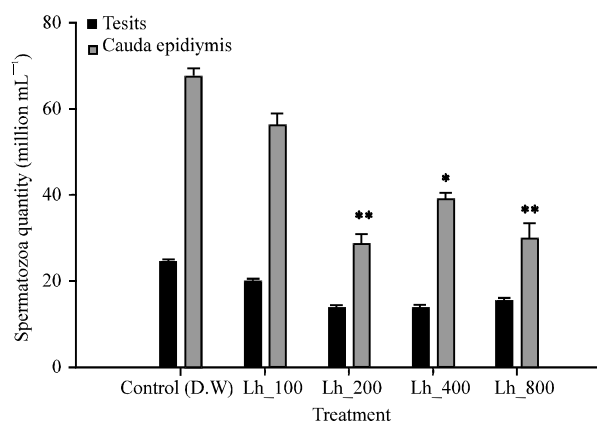


Fig. 8: Effect of *L. hastata* aqueous extract on sperm number in testis and cauda epididymis
*: $p < 0.05$; **: $p < 0.001$

decrease of path velocity, progressive velocity, track speed, straightness, linearity and motility of spermatozoa compared to control group, elongation, lateral displacement and beat cross did not showed a significant ($p > 0.05$) difference compared to control group (Table 1).

The treated rats with the different amounts of *L. hastata* aqueous extracts showed a significant ($p < 0.01$) reduction in the sperm concentration of testis and cauda epididymis compared to control group. The decreased is significantly ($p < 0.05$) important in cauda epididymis compared to testis (Fig. 8).

DISCUSSION

The results of the present study indicate that *L. hastata* treatment did not cause alterations in body weight of the treated animals, suggesting that the treatment had no systemic toxic effect in rat. These results confirm the works of Tamboura *et al.* (2004) which showed on mice the non toxic effect of *L. hastata* aqueous extracts. Furthermore the present results indicated that the treatment caused a non significant decrease of testis and epididymis weights. In the testis, though the histology showed the decrease of the number of Leydig cells after the treatment, the spermatogenesis was been influenced with high doses of *L. hastata* aqueous extracts (Homady, 2001; Al-Majed, 2007; Nejad *et al.*, 2008).

In this study, treatment with the extracts resulted in a decrease of the number of sperm. It is well established that androgens and gonadotrophins are essential for the spermatogenesis and the function of male reproductive system (Leidl *et al.*, 1982). The decrease of the number of sperm in

rats treated with plant extracts may be derived from a hormonal imbalance including serum levels of testosterone, prolactin and Luteinising Hormone (LH). Low cauda epididymal and testis sperm count and a reduction in the epididymal and testis weight imply that *L. hastata* aqueous extract effect might be caused by several factors. One factor may be that *L. hastata* extract interferes with enzymatic reactions including the oxidative phosphorylation uncoupling (Abou-Donia and Dieckert, 1974; Ke and Tso, 1982). It is a well-known and widely accepted concept that LH is basically responsible for testosterone production (Ewing *et al.*, 1980; Arafa, 2010). It is probable that the primary step in the mechanism of the effects on testis induced by the *L. hastata* extract was the suppression of LH. At the testicular level, the absence of stimulation by LH would secondarily cause Leydig cell dysfunction, thereby resulting in decline in testosterone secretion which is responsible for diminished spermatogenesis and hence, reduction in sperm counts (Chase *et al.*, 1992; Shan and Hardy, 1992; Verhoeven, 1992; Wanichacheewa *et al.*, 2001).

It is known that the structure and function of the epididymis are dependent on androgens (Cooper, 1992). In the present investigation, a dose-related suppression of cauda epididymal sperm motility in treatment groups suggests an undersupply of testosterone to epididymis and therefore, an impaired epididymal function. The impaired epididymal function may also be due to reduced activity of the testis which affects the normal passage of testicular fluid into the epididymis (Lohiya *et al.*, 1992; Gupta *et al.*, 1993; Ansari *et al.*, 1998). This is also confirmed by reduced epididymal weight even if it's not significant.

CONCLUSION

In conclusion, the oral administration of aqueous extract of *L. hastata* leaf stems to male rats produced dose related effects on reproduction. The effects may have an inhibitory influence on gonadotrophin release which may be responsible for the decline in testosterone production, leading to changes in spermatogenesis. Further long term studies are in progress for the evaluations of antifertility with this extract.

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REFERENCES

- Abou-Donia, M.B. and J.W. Dieckert, 1974. Gossypol uncoupling of respiratory chain and oxidative phosphorylation. *Life Sci.*, 14: 1955-1963.
- Aderbauer, B., P.H. Clausen, O. Kershaw and M.F. Melzig, 2008. *In vitro* and *in vivo* trypanocidal effect of lipophilic extracts of medicinal plants from Mali and Burkina Faso. *J. Ethnopharmacol.*, 119: 225-231.
- Al-Majed, A.A., 2007. Effect of valerian on spermatogenic, genotoxic, reproductive and biochemical changes in sex cells after chronic treatment in male Swiss albino mice. *J. Medical Sci.*, 7: 276-283.
- Ansari, A.S., Y. Kumar, S. Srivastava and N.K. Lohiya, 1998. Longterm sequelae of toluidamine on male reproduction and general body metabolism in rabbits. *Contraception*, 57: 271-279.

- Arafa, N.M.S., 2010. Efficacy of echinacea on the action of cyproterone acetate in male rats. *Pak. J. Biol. Sci.*, 13: 966-976.
- Arbonnier, M., 2000. Trees, Shrubs and Lianas of the Dry Zones of West Africa. CIRAD, Margraf Publishers, Paris, France.
- Bayala, B., M.T. Pellicer-Rubio, I.H.N. Bassole, R. Belemtougri, H.H. Tamboura and B. Malpaux, 2011. Effects of aqueous extracts of *Leptadenia hastata* (Pers.) Decne. (Asclepiadaceae) on male reproductive functions using castrated immature rats. *Res. J. Med. Plant*, 5: 180-188.
- Bizimana, N., U. Tietjen, K.H. Zessin, D. Diallo, C. Djibril, M.F. Melzig and P.H. Clausen, 2006. Evaluation of medicinal plants from Mali for their *in vitro* and *in vivo* trypanocidal activity. *J. Ethnopharmacol.*, 103: 350-356.
- Chase, D.J., J.A. Karle and R.E. Fogg, 1992. Maintenance or stimulation of steroidogenic enzymes and testosterone production in rat Leydig cells by continuous and pulsatile infusions of luteinizing hormone during passive immunization against gonadotrophin releasing hormone. *J. Reprod. Fertil.*, 95: 657-667.
- Cooper, T.G., 1992. The Epididymis as a Site of Contraceptive Attack. In: *Spermatogenesis Fertilization, Contraception*, Nieschlag, E and U.F. Habenicht (Eds.). Springer, Berlin, pp: 419-460.
- Ewing, L.L., J.C. Davis and B.R. Zirkin, 1980. Regulating testicular function: A spatial and temporal view. *Int. Rev. Physiol.*, 22: 41-115.
- Glew, R.S., D.J. Vanderjagt, L.T. Chuang, Y.S. Huang, M. Millson and R.H. Glew, 2005. Nutrient content of four edible wild plants from west Africa. *Plant Foods Hum. Nutr.*, 60: 187-193.
- Gupta, G., A.K. Srivastava and B.S. Setty, 1993. Androgenic regulation of glycolytic and HMP pathway in epididymides and vas deferens of rhesus monkey. *Ind. J. Exp. Biol.*, 31: 305-311.
- Hassan, S.W., R.A. Umar, I.K. Matazu, H.M. Maishanu, A.Y. Abbas and A.A. Sani, 2007. The effect of drying method on the nutrients and non-nutrients composition of leaves of *Leptadenia hastata* (Asclepiadaceae). *Asian J. Biochem.*, 2: 188-192.
- Homady, M.H., 2001. Changes in the testicular and preputial gland structures of mice related to influence of *Ferula hormonis* extract. *J. Medical Sci.*, 1: 108-112.
- Kaou, A.M., V. Mahiou-Leddé, S. Hutter, S. Ainouddine and S. Hassani *et al.*, 2008. Antimalarial activity of crude extracts from nine African medicinal plants. *J. Ethnopharmacol.*, 116: 74-83.
- Ke, Y.B. and W.W. Tso, 1982. Variations of gossypol susceptibility in rat spermatozoa during spermatogenesis. *Int. J. Fertil.*, 27: 42-46.
- Leidl, W., U. Braun, J. Braun and G. Buck, 1982. The Testis. In: *Handbook of Endocrinology*, Gass, G.H. and H.M. Kaplan (Eds.). CRC Press, Boca Raton, pp: 145.
- Lohiya, N.K., R.B. Goyal, D. Jayaprakash, S. Sharma, M. Kumar and A.S. Ansari, 1992. Induction of reversible antifertility with a crude ethanol extract of *Carica papaya* seeds in albino male rats. *Int. J. Pharmacognosy*, 30: 308-320.
- Magassouba, F.B., A. Diallo, M. Kouyate, F. Mara and O. Mara *et al.*, 2007. Ethnobotanical survey and antibacterial activity of some plants used in Guinean traditional medicine. *J. Ethnopharmacol.*, 114: 44-53.
- Nejad, D.M., J.S. Rad, L. Roshankar, M. Karimipor, A.A. Ghanbari, A. Aazami and M.R. Valilou, 2008. A study on the effect of thiotepa on mice spermatogenesis using light and electronic microscope. *Pak. J. Biol. Sci.*, 11: 1929-1934.

- Nikiema, J.B., R. Vanhaelen-Fastre, M. Vanhaelen, J. Fontaine, C.D. Graef and M. Heenen, 2001. Effects of antiinflammatory triterpenes isolated from *Leptadenia hastata* latex on Keratinocyte Proliferation. *Phytother. Res.*, 15: 131-134.
- Sena, L.P., D.J. Vanderjagt, C. Rivera, A.T.C. Tsin and I. Muhamadu *et al.*, 1998. Analysis of nutritional components of eight famine foods of the republic of iger. *Plant Foods Hum. Nutr.*, 52: 17-30.
- Shan, L.X. and M.P. Hardy, 1992. Developmental changes in levels of luteinizing hormone receptor and androgen receptor in rat Leydig cells. *Endocrinology*, 131: 1107-1114.
- Tamboura, H.H., B. Bayala, M. Lompo, I.P. Guissou and L. Sawadogo, 2004. Ecological distribution, morphological characteristics and acute toxicity of aqueous extracts of *Holarrhena floribunda* (G. Don) Durand and schinz, *Leptadenia hastate* (Pers) Decne and *Cassia sieberiana* (D.C.) used by veterinary healers in Burkina Faso. *Afr. J. Trad. Comp. Alt. Med.*, 2: 13-24.
- Verhoeven, G., 1992. Local control systems within the testis. *Baillieres Clin. Endocrinol. Metab.*, 6: 313-333.
- Wanichacheewa, S., T. Singtripop, S. Sassa, S. Sakamoto and T. Mori, 2001. Decrease in the number of sperm associated with decreased blood testosterone levels in male rats treated with extracts from seven plants consumed by natives of northern Thailand. *Environ. Toxicol. Pharmacol.*, 10: 1-4.