Steroidogenetic and Spermatogenetic Activities of Aqueous Extract of *Phragmanthera capitata* in Wistar Rats

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Abstract

This study was carried out to investigate steroidogenetic and spermatogenetic activities of aqueous extract of *Phragmanthera capitata* (AEPC) in male Wistar rats. Healthy adult male Wistar rats of proven fertility weighing 150-200 g were randomized into three groups with body weights measured weekly. Group I (control) received 10 ml/kg saline, Groups II and III (tests) received 150 and 300 mg/kg AEPC respectively per oral per day for 60 days. Mating test was assessed from day 55 with cohabitation with coeval females for 5 days with the resulting sired litters counted and pup weights measured. The males were sacrificed; testes, epididymis, seminal vesicles and ventral prostrate were excised and weighed. Epididymal sperm motility (EPM), epididymal sperm reserve (ESR), daily sperm production (DSP), blood testosterone and cholesterol concentrations were measured. Mean weekly body weight as well as weights of testes, epididymis, seminal vesicles and ventral prostate revealed no significant change as compared to control. Similarly, no significant changes in EPM, ESR and DSP in test groups were observed. However, cholesterol and testosterone levels were significantly ($P < 0.05$) increased while mating test showed a corresponding significant ($P < 0.05$) increase in pup weight of 28.85±0.76 for 300 mg/kg AEPC as compared to 20.53±0.52 for control. Therefore, AEPC enhances steroidogenetic and spermatogenetic activities in male Wistar rats.

Keywords: *Phragmanthera capitata*, steroidogenesis, spermatogenesis, loranthaceae, sired litters.

1. Introduction

The entire male reproductive system is dependent on hormones for the regulation of activities of many different types of cells or organs. Steroidogenesis is biological synthesis of steroids [1] and steroid hormones are derivatives of cholesterol synthesized by a variety of tissues, most prominently adrenal gland and gonads [2]. The primary hormones involved in the male reproductive system are follicle-stimulating hormone, luteinizing hormone and testosterone [3]. Follicle-stimulating hormone is necessary for spermatogenesis and luteinizing hormone stimulates production of testosterone which is also needed to make sperm and also helps to support libido, energy, memory, bone density, and well-being [4, 5]. These hormones deficiency in males can cause low libido, fatigue, mood swings, irritability and thus fertility problems [6-9]. Fertility decline is a major problem in most sub-Saharan African nations for the past two decades though rates vary greatly from country to country [10-14]. In most countries with high rates, declining democratic governance has exacerbated the problem [15] with citizens most concerned in debilitating illnesses and hunger than fertility issues.

Herbs have a long history of usage in Africa for the treatment of various diseases. In some countries, up to 90% of the population still relies mainly on herbal products as a source of medicine because of their availability, effectiveness and cheapness [16]. It is known that about 250,000 flowering plant species occur globally, and approximately half of these are found in the tropical forests. Yet, till date only about 1% of tropical species have been studied for their pharmaceutical potential [17].

Phragmanthera capitata is mistletoe plant of loranthaceae family [18] having woody shrub with stems up to 2 m long. It is found in secondary jungle and bush savanna areas from Sierra Leone to W Cameroons and Fernando Po, and extending across the Congo basin to Zaïre and Angola [19]. The plant is very variable in form, common, widely distributed and often seen with ants' nests [20]. Aqueous extract of *P. capitata* possesses anti-diarrheal properties [21], analgesic and anti-pyretic potentials [22]. Infusion of leaves treats diabetes [23], Chlamydia infection, dysentery, cancer, diabetes, arthritis, epilepsy, gynecological problems and cardiovascular diseases in Cameroon folkloric medicine [24, 25]. Our objectives were, however, to study steroidogenetic and spermatogenetic potentials of AEPC in experimental animal model.

2. Materials and Methods

2.1 Plant material and preparation of extract

The parasitic plant, *Phragmanthera capitata* or Ntsalar, as it is called in Babadjou dialect, was plucked from avocado trees in Konka, Baligham village in North West Region of Cameroon in December 2013. Authentication of the plant was in Cameroon National Herbarium (CNH) with Voucher No. 24673/SRF/CAM. The fresh plant sample was washed in tap water, rinsed in distilled water and shade-dried at room temperature (25±2^{o}C) for fifteen days. The dried plant sample was ground and water macerated (1kg in 7.5 litres) for 3 days, filtered and sun-dried at 43±32^{o}C for another 3 days to obtain chocolate paste-like extract with a yield of 15.5%.

2.2 Experimental animals

Eighteen male and 36 coeval female Wistar rats of 3 months old and weighing between 150-200 g were housed in polyvinyl cages and maintained under standard laboratory conditions of relative humidity (50±5%), temperature (28±2^{0}C) and 12 h light : 12 h dark photoperiod. The animals received standard pellet diet (Agro Feeds, Calabar) and water *ad libitum* and were treated according to Guide for the Care and Use of Laboratory Animals [26].

2.3 Phytochemical screening

AEPC was qualitatively assayed for the presence of bioactive compound such as alkaloids, glycosides, flavonoids, tannins, saponins, and anthroquinones following standard tests procedures in our earlier study [21].

2.4 Experimental procedures

2.4.1 Treatment

Method of Pramod *et al* [27] was used with light modification. Briefly, eighteen adult male rats of proven fertility were divided randomly into 3 groups of 6 animals each. Group I (control) received 10 ml/kg saline, Groups II and III received 150 and 300 mg/kg AEPC per oral per day for 55 consecutive days. Extract doses were chosen based on acute toxicity study carried out in our earlier study [21].

2.4.2 Mating test

On day 55, each male rat was caged separately with 2 coeval females for 5 days corresponding to at least 1 oestrous cycle. When the females gave birth, number of sired litters and weights of pups were recorded [28].

2.4.3 Body weight, reproductive and accessory reproductive organs weights

Body weights of all male rats were taken before the start of treatment, during treatment (once weekly) and on day 60 after mating test. Percent change in mean body weight of each group was calculated using the formula:

$$\boldsymbol{mean\ \%\ change\ in\ body\ weight} = \frac{\boldsymbol{M2 - M1}}{\boldsymbol{M1}} \boldsymbol{X\ 100}$$

Where,

M1 = mean body weight taken before treatment and

M2 = mean body weight taken after treatment

The male rats were then sacrificed by an overdose of sodium pentobarbital and testes, seminal vesicles and ventral prostate were isolated and weighed.

2.4.4 Preparation of cell solutions

Epididymis and testis were prepared by method of Robb *et al* [29] with slight modification. Briefly, each tissue weighing 100 mg was isolate, minced with scissors and homogenized separately using semimicro Waring Blender in 20 ml of physiological solution containing 0.5% Triton × 100 for 1 min. The homogenates were quantitatively transferred into glass jars where further dilutions of sperm concentrations were made to convenient levels for accurate hemocytometer counting (400-600 sperm per chamber).

2.4.5 Sperm motility and viability

10µl of each sample of epididymis were applied separately to Neubauer's improved counting chambers. Using light microscope (x40), quantitative motility expressed as percentage was determined by counting motile and immotile spermatozoa per chamber. Quantitative viability expressed as percentage was determined by counting viable and nonviable spermatozoa per chamber. Viable spermatozoa cannot absorb Negrosin stain while nonviable spermatozoa can absorb the stain.

2.4.6 ESR and DSP

10µl of each epididymis and testis were pipetted from their respective jars and applied to Neubauer's improved counting chamber and elongated spermatid nuclei with a shape characteristic of step 17-19 spermatids and resistant to homogenization were counted and counts for 4 chambers were averaged for one rat. The number of spermatid nuclei per gram testis was calculated and values divided by 6.10 days to convert them to daily sperm production [30].

2.4.7 Concentration of testosterone and cholesterol

Blood samples from all groups were collected by cardiac puncture under sodium pentobarbital anesthesia and allowed to clot at room temperature for 30 min. Serum was centrifuged at 2000 rpm for 10 min and separated top layer of serum from each sample was used to determine the concentration of testosterone by Radio immunoassay [31] and cholesterol by Wybenga *et al* [32].

2.5 Statistical analysis

Data were expressed as means ± SEM (n = 6) and were statistically evaluated by one-way analysis of variance (ANOVA) followed by Tukey test for post hoc. Values of P <0.05 were statistically considered significant [32].

3. Results

3.1 Body weight, reproductive organ and accessory reproductive organs weights

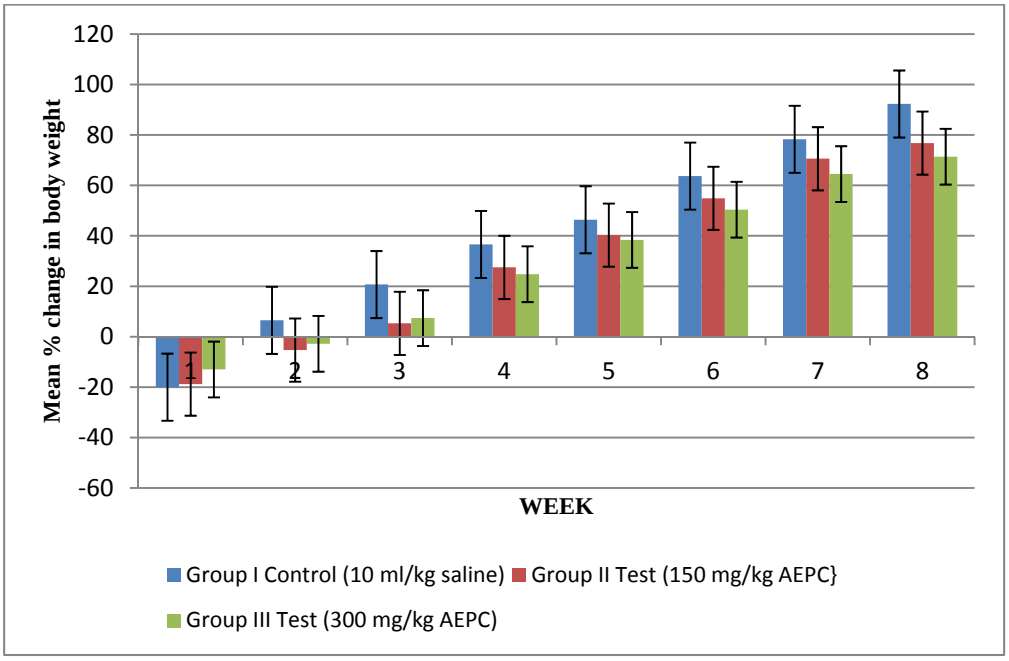
Body weights were taken weekly as shown in Fig. 1. Week 1 showed a decrease in mean body weight in all the groups. Week 2 showed total recovery of mean body weight lost only in the control group while from week 3, all the groups showed mean weight gains though no statistically significant (P >0.05) as compared to control.

Reproductive organ and accessory reproductive organs were isolated and weighed at the end of 60 days of treatment as shown in Fig. 2. There were also no significance differences (P >0.05) between control and treated groups vis-à-vis testes, epididymis, seminal vesicles and ventral prostate.

3.2 Sperm parameters, testosterone and cholesterol concentrations and fertility

Sperm parameters, fertility parameters, cholesterol and testosterone concentrations were evaluated and presented in Table 1. Treated groups showed significant difference (P <0.05) at 300 mg/kg as compared to control group vis-à-vis weight of pups. All other parameters had no significant change.

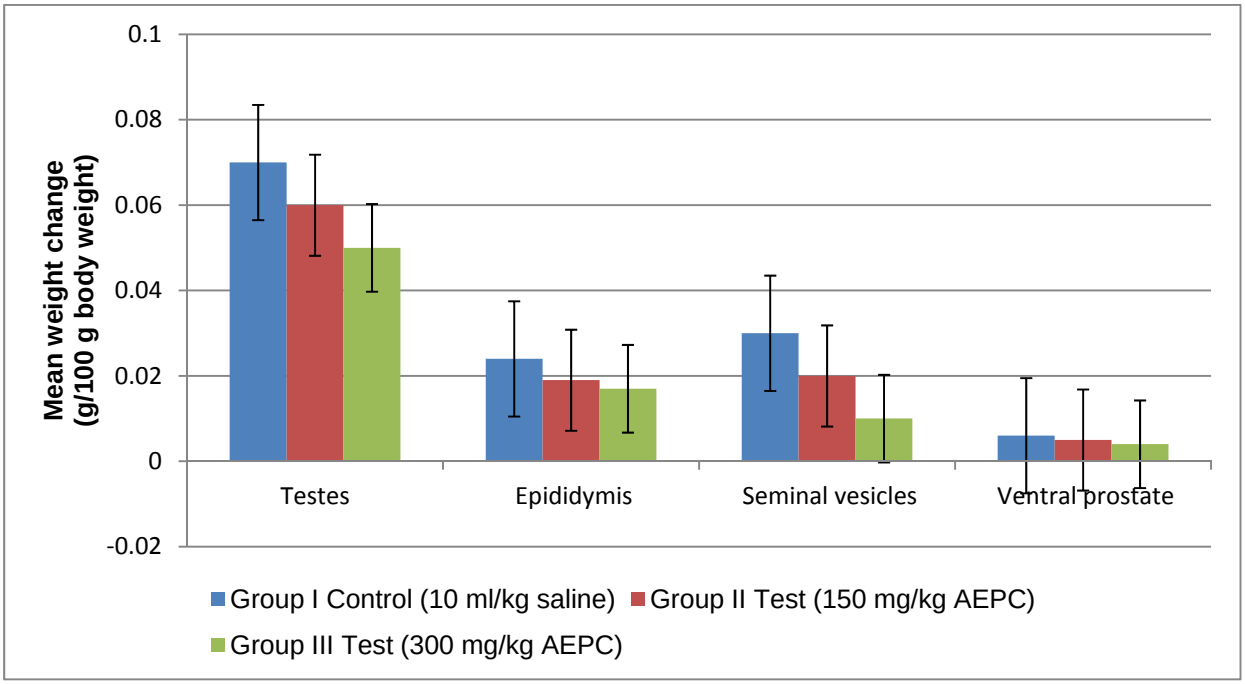
Fig. 1: Effect of AEPC on mean body weight of male Wistar rats after 60 days of treatment.



Excel data

-20	-18.8	-13
6.5	-5.3	-2.8
20.7	5.3	7.4
36.6	27.5	24.8
46.4	40.3	38.4
63.7	54.9	50.4
78.3	70.6	64.5
92.3	76.8	71.4

Fig. 2: Effect of AEPC on weights of testes, seminal vesicles and ventral prostate of male Wistar rats after 60 days of treatment.



Excel data

0.07	0.024	0.03	0.006
0.06	0.019	0.02	0.005
0.05	0.017	0.01	0.004

Table 1: Effect of AEPC on sperm parameters, testosterone concentration and on fertility of male Wistar rats after 60 days of treatment.

Group	ESR (million)	DSP (million)	Sperm motility (%)	Sperm viability (%)	Cholesterol conc. (mg/ml)	Testosterone conc. (ng/ml)	Fertility		
							Number pregnant (n=12)	Number of litters	Pup weight (g)
Control (saline) (10 ml/kg)	292.12 ± 3.67	49.82 ± 0.99	80.41± 2.21	76.21± 1.40	74.66±2.12	12.38±0.03	10.21± 0.21	10.52± 0.22	20.53±0.52
AEPC (150 mg/kg)	296.21± 3.74	52.64±1.31	90.31± 2.42	81.03± 2.21	100.66± 4.14	16.23±0.04	10.24± 0.11	10.81± 0.22	21.44±0.53
AEPC (300 mg/kg)	307.74± 3.89	55.71±1.09	98.21± 2.44	85.21± 2.52	120.66± 5.22*	18.11±0.05*	11.32± 0.31	10.12± 0.21	28.85±0.76 *

Data represent the Mean ± SEM (n=6 per group).*$P < 0.05$

4. Discussion and conclusion

The internal organs of the male reproductive system are also called accessory organs and they include: epididymis, vas deferens, ejaculatory ducts, urethra, seminal vesicles, prostate gland and Bulbourethral glands [3]. These organs are morphologically and physiologically dependent upon the production of androgenic hormones [33] which maintain the structural and functional properties of sex accessory organs and stimulate spermatogenesis in the testes [34]. Androgenic hormones are derivatives of cholesterol synthesized by a variety of tissues, most prominently adrenal gland and gonads [2]. Administration of AEPC to male Wistar rats showed no significant change in body weight and weights of reproductive organs (testes, epididymis, seminmal vesicles and ventral prostate). This shows that the extract had no structural interference in these organs though there was an initial insignificant decreased in the first week of administration of extract. This could be attributed to stress associated with initial use of oral feeding canula which probably evoked hemodynamic, metabolic and hormonal responses characterized by altered protein homeostasis, hypermetabolism, altered carbohydrate metabolism, increased lipolysis and sodium and water retention [35-37]. All these responses will certainly lead to reduction in weight as seen in all groups of animals with test groups showing some mild form of adaptogenic activity of the extract. Gradual recovery in weekly weight gain was observed thereafter throughout the experimental period. The theorized mechanism of action involving weight gain is through the inhibition of circulating angiotensin I converting enzyme by ER-40133 [38] which suggests that AEPC did not interfere in the inhibitory process.

A healthy level of cholesterol enhances Leydig cells to actually produce and release testosterone [39] which is a hormone associated with sexual performance, reproductive function, muscle mass, aggressive and competitive behaviors [40, 41].

This suggests that the extract possesses phytoestrogens that bind to estrogen receptors thereby interfering with regulating system of hypothalamic-pituitary-gonadal (HPG) axis thus altering gonadotropins release [40]. Phytochemical analysis of the plant extract revealed the presence of flavonoids which are mostly implicated in HPG axis regulation (42).

Therefore, AEPC possesses bioactive molecules which enhance steroidogenesis and spermatogenesis in male Wistar rats. However, further research into isolation and purification of the bioactive molecules in recommended.

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References

[1] Available at: http://www. dictionary.reference.com/browse/steroidogenesis
[2] N. Rouzier. How to achieve healthy aging. 2nd ed. Salt Lake City: Worldlink Medical Publishing; 2007.
[3] Available at: http://www.webmd.com/sex-relationships/guide/male-reproductive-system?.
[4] M. Carruthers. Time for international action on treating testosterone deficiency syndrome. Aging Male, 2009, 12(1):21-8.
[5] A. B. Araujo et al. Prevalence of symptomatic androgen deficiency in men. J Clin Endocrinol Metab, 2007, 92(11):4241-7.
[6] S. M. Harman. Testosterone in older men after the Institute of Medicine Report: where do we go from here? Climacteric, 2005, 8(2):124-35.
[7] M. M. Miner et al. 12-Month observation of testosterone replacement effectiveness in a general population of men. Postgrad Med, 2013, 125(2):8-18.
[8] H. Lejeune et al. [Hypoactive sexual desire and testosterone deficiency in men]. Prog Urol, 2013, 23(9):621-8.
[9] S. E. Borst et al. Testosterone replacement therapy for older men. Clin Interv Aging 2007;2(4):561-6.
[10] D. Kirk and B. B. Pillet. Fertility levels, trends and differentials in sub-Saharan Africa in the 1980's and 1990's. Studies in Family Planning, 1998, 29(1):1-22.
[11] D. Shapiro and T. Gebreselassie. Fertility Transition in Sub-Saharan Africa: Falling and Stalling. Paper presented at the 2007 PAA, New York, 2007.
[12] D. Tabutin and B. Schoumaker. The Demography of Sub-Saharan Africa from the 1950s to the 2000s. A Survey of Changes and a Statistical Assessment. Population-E, 2004, 59(3/4): 455-555.
[13] M. Garenne and V. Joseph .The Timing of the Fertility Transition in Sub-Saharan Africa. World Development, 2002, 30 (10): 1835-43.
[14] J. Bongaarts. The Causes of Stalling Fertility Transitions, Paper presented at the IUSSP International Population Conference, Tours, France, 2005.
[15] R. C. Reginald, M. B. Bella, M. S. F. Paul et al. Estrogenic Activity of Pueraria phaseoloides Roxb. Benth Evaluated in Ovariectomized Rats Philippine Journal of Science, 2006, 135(1): 39-48.
[16] K. Hostettmann, A. Marston, K. Ndjoko, J. L. Wolfender. The Potential of African Plants as a Source of Drugs. Curr Org Chem, 2000, 4: 973.
[17] S. M. Jachak, A. Saklani. Challenges and opportunities in drug discovery from plants. Current Science, 2007, 92: 1251-7.
[18] Available at: http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?435150
[19] Available at: http:// www.plants.jstor.org/upwta/3_945
[20] R. Mony, S. D. Dibong, J. M. Ondoua, I. J. Boussim, A. Amougou and B. Bilong. Ants and Phragmanthera capitata (Sprengel), Balle (Loranthaceae) impacts on considerable damages caused on fruit trees of the Ndogbong (Douala, Cameroon) chieftaincy's orchard. Journal of Agricultural Extension and Rural Development, 2010, 2(3):48-53.
[21] L. P. Takem, B. A. S. Lawal and J. A. Lennox. Anti-diarrhoeagenic Properties of Aqueous Extract of Phragmanthera capitata S. Balle in Albino Rats. European Journal of Medicinal Plants, 2014, 4(6):743-52.
[22] L. P. Takem, N. P. Abe and O. J. Ogbonna. Anti-Pyretic and Analgesic Potentials of Aqueous Extract of Phragmanthera capitata S. Balle in albino rats. American Journal of Pharmacy and Pharmaceutical Sciences 2014. In Press.

[23] N. Din, S. D. Dibong, M. E. Mpondo, R. J. Priso, N. F. Kwin and A. Ngoye. Inventory and Identification of Plants Used in the Treatment of Diabetes in Douala Town (Cameroon). European Journal of Medicinal Plants, 2011, 1(3):60-73.
[24] S. D. Dibong, N. L. E. Obiang, N. Din, R. J. Priso, V. Taffouo, H. Fankem, G. Salle, A. D. Missoup, I. J. Boussim, A. Amougou. An assessment on the uses of Loranthaceae in ethnopharmacology in Cameroon: A case study made in Logbessou, North of Douala. J. Med. Pl. Res, 2009, 3(8):592-595.
[25] S. D. Dibong, M. E. Mpondo, A. Ngoye, R. J. Priso. Modalities of exploitation of medicinal plants in Douala's region. American Journal of Food and Nutrition, 2011, 1(2):67-73.
[26] Available at: http://www.nap.edu/catalog/12910.html
[27] K. V. Pramod, A. Sharma, M. Annu, S. Prachi, R. S. Gupta, S. C. Joshi, V. P. Dixit Effect of Sarcostemma acidum stem extract on spermatogenesis in male albino rats. Asian J Androl, 2002, 4: 43-7.
[28] R. E. Linder, L. F. Strader, W. K. McElroy. Measurement of epididymal sperm motility as a test variable in the rat. Bull Environ Contam Toxicol, 1986, 36:317-24.
[29] G. W. Robb, R. P. Amann, G. J. Kllian. Daily sperm production and epididymal sperm reserves of pubertal and adult rats. J Reprod Fertil, 1978, 54:103-107
[30] R. P. Amann, L. Johnson, D. L. Thompson, Jr & B. W. Pickett. Daily spermatozoal production, epididymal spermatozoal reserves and transit time of spermatozoa through the epididymis of the rhesus monkey. Biol Reprod, 1976, 15: 586-92.
[31] E. F. Richard, A. V. Valbandov. Radio immunoassay of peripheral plasma testosterone in male from eight species using a specific antibody without chromatography. Endocrinology, 1974, 95(5):1 466-8.
[32] D. R. Wybenga, V. J. Pileggi, P. H. Dirstine and J. D. Giorgio. Direct manual determination of serum total cholesterol with a single stable reagent. Clinical Chemistry, 1970, 16(12):980-984.
[33] M. Tetsuhisa .Controversy over multiple comparisons in agronomic research. Japanese J. Appl. Statist, 1997, 26:99-109.
[34] H. G. Williams-Ashman and A. H. Reddy. Androgenic regulation of tissue growth and function. In: Biochemical actions of hormones. G. Litwack Eds. Vol.2, Academic Press, New York, 1972.
[35] C. D. Turner and J. T. Bagnara In: "General Endocrinology" W.B. Saunders Eds. Philadephia: London, 1976.
[36] J. M. Kinney. The application of indirect calorimetry to clinical studies: Assessment of energy metabolism in health and disease, Report of First Ross Conference on Medical Research. Edited by Kinney JM. Columbus, Ross Laboratories, 1980, 42-5
[37] M. Imamura, G. H. A. Clowes Jr, G. L. Blackburn. Liver metabolism and gluconeogenesis in trauma and sepsis: Surgery, 1975, 77:868-80.
[38] R. M. Z Siti, Z. O. Siti, L. A. K. Norhayati, I. M. Nurul, D. Srijit and M. K. Normadiah. Effects of the phytoestrogen genistein on the development of the reproductive system of Sprague Dawley rats. Clinics (Sao Paulo), 2013, 68(2):253–62.
[39] N. Toritsuka, K. Tsukidate and T. Igarashi. Mechanism of weight gain suppressing effect of ER-40133, an angiotensin I converting enzyme inhibitor, in growing rats. Journal of Toxicological Sciences, 1999, 249(1):45-53.
[40] J. C. Buckingham. Hypothalamo-pituitary responses to trauma. Br Med Bull 1985; 41: 203-11.
[41] N. Bassil, S. Alkaade, J. E. Morley. The benefits and risks of testosterone replacement therapy: A review. Ther Clin Risk Manag, 2009, 5 (3):427–48.
[42] Available at: http://www.wikihow.com › Home › Categories › Health › Men's Health
[43] Y. Fatih. Phytoestrogens in Functional Foods, Taylor & Francis Ltd. pp. 3–5, 210–211, 2005.