Asian Pacific Journal of Reproduction

Journal homepage: www.apjr.net

Document heading doi:

Effect of *Piper betel* leaf stalk extract on protein metabolism in reproductive tissues of male albino rats

Vengaiah V[1], Govardhan Naik A[2], Changamma C[3*]

Department of Zoology, Sri Venkateswara University, Tirupati−517 502, A.P., India

ARTICLE INFO

Article history:
Received 18 July 2014
Received in revised form 12 February 2015
Accepted 15 March 2015
Available online 20 June 2015

Keywords:
Betel leaf stalk
Proteins
Free amino acids
Antifertility
Spermatogenesis
Anti-androge

ABSTRACT

Objective: To know the impact of *Piper betel* leaf stalk (*P. betel*) extract on Protein and energy metabolism and its role in male albino rats. **Methods:** Healthy adult (3-4 months old) male Wistar strain albino rats were administered with betel leaf stalk extract, at the dose of 50 mg/kg/day through oral gavages for 15 days. Twenty four hours after the last dose, the animals were autopsied. In order to assess antifertility effect in testis, epididymis, seminal vesicle and prostate gland, estimation of total, soluble and structural proteins, free amino acids and DNA, RNA were undertaken. **Results:** The accumulation in proteins indicates the anti-androgenic effect of extract. The reduction in free amino acids will affect the sertoli cell function, results in the damage of spermatogenesis. The significant elevation in testicular DNA content (hyperplasia) was observed. In the present study, *P. betel* leaf stalk extract decreases the concentration of RNA, in testes, seminal vesicle and prostate gland except in epididymis where it was elevated. It indicates the alterations in rate of protein synthesis and growth rate of tissues due to the administration of *P. betel* leaf stalk extraction. However, the RNA: DNA ratio was reduced except in prostate. **Conclusions:** *P. betel* leaf stalk extract exert its anti androgenic effect by alterations in rate of protein synthesis and cellular hypertrophy occur in prostate.

1. Introduction

In our country as well as in the world, there are several medicinal plants associated with antifertility properties. A large number of plant species with antifertility effects have been screened in China and India beginning about 50 years ago and were subsequently fortified by national and international agencies. During the recent past decades a large number of plant species mentioned in old Material, Medica and Ayurvedic literature have been screened and searched thoroughly for their antifertility effect on males. The intraperitoneal administration of 20 and 60 mg/kg of ethanolic seed extract of Abrusprecatorius caused a decrease in daily sperm production. The reversibility in sperm production was observed in all the administered animals after 20 days of withdrawal of treatment[1] suggests that the role of seed extract of *Abrus precatorius* (*A. precatorius*) as an antifertility agent or contraceptive with a risk of DNA damage in spermatozoa and may lead to teratogenic effect. Recently it has been reported that aqueous infusion of *Allamanda cathartica* leaves showed significant decline in sperm motility, viability, and number, and increase in morphologically abnormal spermatozoa in caudal epididymis of male laboratory mice. Also, decrease in fertility and number of viable implants in females sired by administered males was noticed[2]. The plant products which is very commonly used in daily life, *Piper betel* (*P. betel*) also known to have antifertility effect. The *P. betel* plant extract may brought about its effect through pituitary-gonadal axis, which resulted in diminished gonadotropin release, in turn reduced reproductive organ weights. Sri Lankan *P. betel* inhibits male sexual behavior in rats. Hence, the purpose of this study was to assess the *P. betel* leaf stalk extract as an antifertility through different metabolites and enzymatic processes involved in protein and energy metabolism.

*Corresponding author: Changamma C, Department of Zoology, Sri Venkateswara University, Tirupati-517 502, A.P., India.
Email: challa1957@gmail.com
Foundation project: This study was finicially supported by UGC-BSR & RGNF, New Delhi.

2. Material and methods

In the present study healthy adult (3-4 months old, weight 215±10 g) male Wistar strain albino rats were used. The rats were purchased from Sri Raghavendra Enterprises, Bangalore, India. The male albino rats were taken and divided in to two groups, each group contains 6 rats. First group rats were control rats administered with 1 mL of distilled water. Second group rats were experimental administered with betel leaf stalk extract, at the dose of 50 mg/kg/day through oral gavages for 15 days. The ethanol extract was prepared according to WHO[3] protocol CG-04. Stalks were shed-dried, powdered and extracted with 95% ethanol (v/v) at 55-60 ℃ for 3h. The solvent was distilled off under reduced pressure; the resulting mass was dried under vacuum and kept at 24 ℃ until use. Animals were housed in a clean polypropylene cage under hygienic conditions in well ventilated clean air conditioned room, with photoperiod of 12 hours light and 12 hours dark cycle, at (25 ± 2) ℃ with a relative humidity of (50±5)%. The rats were fed with standard laboratory feed (Hindustan Lever Ltd, Mumbai) and water ad libitum. Twenty four hours after the last dose, the animals were autopsied. The tissues like testes, epididymis, seminal vesicle and prostate gland were isolated, chilled immediately and used for biochemical analysis. The proteins[4], free amino acids[5] and nucleic acids[6] were estimated.

3. Results

The data represented in Table 1 shows the levels of total proteins, soluble proteins, structural proteins and free amino acids in reproductive tissues like testes, epididymis, seminal vesicle and prostate gland in control and administered rats. Total proteins were significantly increased in all reproductive tissues, testis (+20.67%), epididymis (+21.64%), seminal vesicle (+44.90%) and prostate gland (+36.75%). The levels of soluble proteins were also elevated in all reproductive tissues, testis (+17.07% $P< 0.01$), epididymis (+41.09%), seminal vesicle (+29.44%) and prostate gland (+51.21%). The leaf stalk extract shows significant elevation of structural proteins in all tissues, testis (+30.02%), epididymis (+10.55%, $P<0.05$) seminal vesicle (+61.80) and prostate gland (+18.45% $P<0.01$). The elevation was more in seminal vesicle. In the case of free amino acids the stalk extract shows significant reduction in all tissues in testes (-46.71%), epididymis (-52.20%), seminal vesicle (-51.93%) and prostate (-43.82%).

The data represented in Table 2 shows the levels of DNA and RNA in control and administered rat reproductive tissues. The Betel leaf stalk extract shows significant changes in all tissues. The DNA was elevated in testis (+52.21%). epididymis (+79.18%) and seminal vesicle (+12.74% $P< 0.01$) except prostate gland where it was significantly reduced (-65.50%). The levels of RNA was significantly decreased in all tissues, testes (-25.46%), seminal vesicle (-10.42%) and prostate gland (-16.19%) except in epididymis where significantly increased (+15.23% $P< 0.01$). The RNA/DNA ratio was decreased in all reproductive tissues except in prostate where it was elevated fourfold (+145.83%).

4. Discussion

4.1 Total proteins

Proteins and other molecules required for zygote survival prior to oocyte-to-embryo transition and full activation of embryonic genome. The spermatozoon, in contrast, forfeits most of its cytoplasm and organelles to transform into a motile cell capable of surviving and functioning outside of the male reproductive system.

The growth rate of any organ is proportional to its protein content. The reduced glycogen level could affect protein synthesis; because of protein synthesis in spermatogenic cells was dependent on glucose[7].

Spermatogenesis requires functional integrity and cooperation of the sertoli cells as they occupy the full thickness of the seminiferous tubules and are in close contact with germinal cells. Secretory activity of sertoli cells i.e. A B P (Androgen Binding Protein) production is modulated by germinal cells particularly by pachytene

Table 1

Effect of P. betel leaf stalk extract on total proteins, soluble proteins, structural proteins and free amino acids in testes, epididymis, seminal vesicle and prostate gland over control rats.

Parameter (mg/g wet wt.)	Testis			Epididymis			Seminal vesicles			Prostate gland		
	Control	Extract treament	% change	Control	Extract treament	% change	Control	Extract treament	% change	Control	Extract treament	% change
Total Proteins	164.21±10.73	198.16±12.43	+20.67**	241.71±18.12	294.03±23.72	+21.64**	148.15±10.73	214.67±15.49	+44.90**	186.65±16.21	255.26±21.43	+36.75**
Solubleproteins	62.13±4.32	72.74±6.39	+17.07**	98.14±7.29	138.47±11.47	+41.09**	63.89±4.74	82.7±7.59	+29.44**	76.43±5.27	115.57±8.38	+51.21**
Structural proteins	100.16±9.63	130.83±10.28	+30.02**	140.01±12.47	154.79±11.47	+10.55*	81.48±7.43	131.84±10.13	+61.80**	111.32±8.69	131.86±9.81	+18.45**
Free aminoacids	1.37±0.23	0.73±0.12	-46.71**	1.56±0.12	0.73±0.23	-52.20**	1.29±0.11	0.62±0.01	-51.93**	1.62±0.01	0.91±0.02	-43.82**

Mean ±SD of six individual observations.+ and – percent increase and decrease respectively over control rats. *$P<0.05$, **$P<0.01$, indicates the level of significance.

Table 2

Effect of *P. betel* leaf stalk extract on DNA, RNA in testes, epididymis, seminal vesicle and prostate gland over control rats.

Parameter (mg/g wet wt.)	Testis			Epididymis			Seminal vesicles			Prostate gland		
	Control	Extract treament	% change	Control	Extract treament	% change	Control	Extract treament	% change	Control	Extract treament	% change
DNA	4.73±0.34	7.20±0.34	+52.21**	3.94±0.24	7.06±0.53	+79.18**	4.63±0.25	5.22±0.32	+12.74**	4.32±0.19	1.49±0.01	-65.50**
RNA	2.16±0.12	1.61±0.04	-25.46**	2.38±0.15	2.74±0.12	+15.23**	2.11±0.13	1.89±0.09	-10.42**	2.10±0.15	1.76±0.02	-16.19**
RNA/DNA Ratio	0.45	0.22	-51.10**	0.80	0.38	-52.50**	0.45	0.36	-20.00**	0.48	1.18	+145.83**

Mean±SD of six individual observations.+ and – percent increase and decrease respectively over control rats. *P<0.01, **P<0.01 indicates the level of significance.

and early spermatids. Alteration of sertoli cells affects the production of ABP, which in turn leads to the arrest of spermatogenesis. There is also evidence that the disturbance of sertoli functions results in the damage of spermatogenesis[8].

In view of these importances of proteins, the present study was focused on protein metabolism. These analyses could possibly identify potential biomarkers for male infertility.

The *P. betel* L. leaf stalk extract showed significant elevation in total proteins, soluble and structural proteins in all reproductive organs like testes, epididymis, seminal vesicle, prostate gland and also in liver and serum[9].

The increased protein might be due to the presence of steroids and other antioxidants, which favours protein metabolism by depressing oxidative damage. The weight gain in the plant extracts administered group is mainly due to protein mass. The observed increase in testicular protein content and weight may be the result of testosterone action[10]. The testicular secretary constituents of protein concentration can give useful information on the androgenic and or anti-androgenic potential of plant extracts. This parameter can also be used to evaluate normal functioning capacity of the testes[11,12]. Testicular proteins are one of the constituents that ensure the maturation of spermatozoa. Increased weight and high protein concentration of the testes indicates enhancement of testicular growth as follicle stimulating hormone is also essential for protein synthesis in the gonads. Alterations in the secretion and functions of these proteins which are maintained by androgens may impair sperm maturation.

Seminiferous tubule fluid is thought to be a major source of nutritional support for germ cells. This fluid bathes developing germ cells with proteins including transferrin[13] and androgen binding protein, in other cell systems, microtubules are necessary for protein secretion. Likewise, in the Sertoli cell, the extensive array of microtubules may be involved in protein secretion and seminiferous tubule fluid formation [14]. Hence, the accumulation in proteins indicates the anti androgenic effect of extract.

Protein level is directly correlated with the secretory activity of the testis and accessory glands, which in turn depends on the androgen levels. The most pronounced general metabolic action of the androgen is the promotion of protein anabolism[15]. The accessory sex organs possess 5 alpha reductase activities, which converts testosterone to dihydrotestosterone, the active hormone[16].

The spermatozoa, during their epididymal maturation, are altered with respect to the principle cells of the initial segment and caput; they secrete several proteins, some of which get translocated on the spermatozoa. It is already known that changes in the sperm surface protein and the pH of the medium can cause sperm agglutination[17]. The epididymis also synthesized epididymal-specific secretary proteins[18]. During the transit through epididymis, sperm undergo maturational changes necessary for them to acquire these attributes. Due to the extract administration an accumulation of proteins occurs in epididymis, where upon the sperm entering epididymis fail to undergo adequate maturation which would lead to infertility[19]. So the accumulation in protein content indicates the changes in sperm anomalies and decrease in sperm concentration which were coincide by the earlier reports[20].

There was 62% elevation in structural proteins of seminal vesicle by the administration of betel leaf stalk extraction. Seminal plasma contains unique proteins necessary for sperm function and survival[21]. Seminal plasma proteins play a variety of roles they help protect the sperm by binding to the sperm surface during ejaculation and play a key role in capacitation, acrosome reaction, and sperm-egg fusion. Thus, seminal plasma proteins can serve as important biomarkers for male infertility[22]. Seminal plasma is a very rich source of protein with concentration ranges from 35 to 55 g/L[21]. It provides a safe environment for spermatozoa to carry out their physiological functions. Hence, the elevation in protein concentration represents the changes in chemical composition of seminal plasma. Thus alterations at the molecular level in spermatozoa and the seminal plasma may contribute to male infertility.

The soluble proteins were increased more in prostate gland when compare to other organs. Prostate gland is a major contributor to seminal plasma. Prostate specific antigen is a serine protease that cleaves semenogelin by hydrolysis and thus liquefies the semen coagulum and facilitates sperm motility and capacitation[23]. Both Sg I and II are the major proteins of the coagulum. They represent 20%-40% of the seminal plasma proteins[22]. Furthermore, one of the proteins-prostatic acid phosphatase (PAP) was significantly increased in azoospermic men compared to oligozoospermic men and asthenozoospermic men[21]. The increased expression of semenogelin I suggests that the accessory gland secretions have a profound impact on oligozoospermic men with abnormal morphology[22]. Hence the extract influences the prostatic acid

phosphatase and also semenogelin I, causes the changes in sperm count, thus infertility.

4.2. Amino acids

Amino acids are the structural units (monomers) that make up proteins. The *P. betel* L. leaf stalk extract reduced free amino acids significantly in all reproductive organs like testes, epididymis, Seminal vesicle and prostate gland. The reduction in free amino acids may be due to accumulation of testicular proteins. Sertoli cells support spermatogenesis both spatially and energetically. Sertoli cells use alanine, leucine, valine, and glycine as energetic substrates[24]. Thus reduction in free amino acids will affect the sertoli cell function, results in the damage of spermatogenesis [8]. Alanine aminotransferase (ALT) and aspartate amino transferase (AST) play an important role in the mobilization of amino acids into gluconeogenesis[25]. Hence, reductions in free amino acids were noticed in the present study.

The decreased amino acids in accessories decrease the alkalinity of the semen. So, it affects the motility and survival of the sperm and protection of the sperm genetic material which would lead to infertility[19].

4.3. Nucleic acids

Experimental studies of nucleic acids constitute a major part of modern biological and medical research, and form a foundation for genome and forensic science, as well as the biotechnology and pharmaceutical industries[26].

Many temporarily functioning proteins are generated during the replacement of nucleoproteins in the nuclei of late spermatids and seem to be degraded in the nucleus during spermiogenesis, a number of chromatin rearrangements occur in the nuclei of rat spermatogenic cells and, as spermiogenesis progresses, testis-specific histones are synthesized[27]. In late steps of spermiogenesis, almost all histones displace to transition proteins, and the latter are then replaced by protamines during nuclear condensation of spermatid and spermatozoa[28]. During replacement of nucleoproteins, many temporarily functioning proteins are formed and might be degraded. Thus, efficient degradation machinery is required for the removal of these proteins. The exact reproduction of cellular composition is controlled by genes through the action of RNA and DNA. Hence the present study was focused on estimation of nucleic acids.

In the present study a significant elevation in testicular DNA content (hyperplasia) was observed. The primary spermatocytes are the most mature germ cells to synthesize DNA in preparation for meiosis I and II. Moreover, these germ cells synthesize four times more DNA than is found in the haploid state. As the primary spermatocytes prepare for and enter the long prophase of meiosis I, they move from the basement membrane of the seminiferous tubule toward its lumen. At this point in the spermatogenesis process, germ cells bind to the sertoli cells through specialized cellular junctions and remain so until the immature spermatozoa are released from the seminiferous epithelium. The spermatid begins life as a simple round cell but rapidly undergoes a series of complex morphological changes. The nuclear DNA becomes highly condensed and elongated into a head region which is covered by a glycoprotein acrosome coat while the cytoplasm becomes a whip-like tail enclosing a flagellum and tightly-packed mitochondria. Hence, the extract shows its effect on sertoli cells and also at the stage of spermatid formation during spermatogenesis.

In sex accessories, significant elevation in epididymis, seminal vesicle (hyperplasia) and reduction in prostate gland. This is also confirmed by the present results showing increased protein, which in turn may be related to the more increased DNA, possibly prevents the reactive oxygen species from acting on DNA[29]. Reactive oxygen species (ROS) are a double edged sword; they serve as key signal molecules in physiological processes but also have a role in pathological processes involving the reproductive tract. ROS are involved in the damage of spermatozoa, which may result in male infertility[30]. In prostate inhibited synthesis of DNA was observed.

Biochemical growth-rate indicators, such as RNA concentration or the RNA: DNA ratio, are routinely used for estimating growth rates and nutritional condition of the organism. RNA is an essential component of protein synthesis. Its concentration in tissue often reflects the rate of protein synthesis. The RNA: DNA ratio provides an index of protein synthetic capacity per cell since the amount of DNA per cell is assumed not to vary with condition or with growth rate. The RNA content of tissue is related to growth rate, food density, and temperature and may also be affected by gamete production and developmental stage. In vertebrates, total DNA content is considered to represent the number of cells in the organism, regardless of growth rate or condition. That is, all cells should contain roughly the same amount of DNA, so variations in DNA content should reflect changes in the number of cells.

In the present study, betel leaf stalk extract decreases the concentration of RNA, in testes, Seminal vesicle and prostate gland except in epididymis where it was elevated. It indicates the alterations in rate of protein synthesis and growth rate of tissues due to the administration of betel leaf stalk extraction. However, the RNA: DNA ratio was reduced except in prostate. Thus cellular hypertrophy was not noted in testes, epididymis, and seminal vesicle but did occur in prostate[31]).

Declare of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors were grateful to UGC-BSR & RGNF, New Delhi for financial support.

References

[1] Jahan S, Rasool S, khan MA, Ahmad M, Zafar M, Arsahd M, et al. Antifertility effects of ethanolic seed extract of Abrus precatorius L. on sperm production and DNA integrity in adult male mice. *J Med Plant Res* 2009; **3**(10): 809-814.

[2] Singh A, Singh SK. Evaluation of antifertility potential of Brahmi in male mouse. *Contraception* 2009; **79:** 71-73.

[3] WHO: Protocol CG-40. *Preparation of alcoholic extract for bioassay and phytochemical studies. APJF/IP, 1001 A*. Geneva: World Health Organization; 1983.

[4] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193:** 265-270.

[5] Colowick SP, Kaplan NO. Methods in enzymology. New York: *Academic Press*; 1957; **3:** 50-54.

[6] Gills KW, Myers A. An improved diphenyl amine method for the estimation of Deoxy ribonucleic acid. *Nature* 1965; **206:** 93-94.

[7] Thejashwini MS, Krishna Ram H, Shivabasavaiah. Reversible antifertility effect of cyamposis psoralioides in male swiss albino mice. *Int J Adv Boil Res* 2012; **2**(4): 657-665.

[8] Gupta RS, Kachhawa JBS, Rakhi Sharma. Antispermatogenic effects of Nyctanthesarbortristis in male albino rats. *Pharmacologyonline* 2006; **2:** 261-273.

[9] Muthulakshmi A, Jothibai Margret R, Mohan VR. Antifertility effect of ethanol extracts of Feronia elephantum correa leaf and bark on male Albino rats. *Int J Pharmace Sci & Drug Res* 2013; **5**(1): 23-27.

[10]Gupta RS, Kachhawa JBS, Chaudhary R. Antifertility effects of methanolic pod extract of *Albizzia lebbeck* (L.) Benth in male rats. *Asian J Androl* 2004; **6:** 155-159.

[11]Yakubu MT, Akani MA, Oladiji AT. Male sexual dysfunction and methods used in assessing medicinal plants with aphrodisiac potentials. *Pharmacog Rev* 2007; **1:** 49-56.

[12]Yakubu MT, Akanji MA, Oladiji AT. Effect of oral administration of aqueous extract of Fadogia agrestis stem on some testicular function indices of male rats. *J Ethnopharmacol* 2008; **111:** 288-292.

[13]Hagenas L, Ritzen EM. Sertoli cell origin of testicular androgen-binding protein (ABP), *J Mol Cell Endocrinol* 1975; **2:** 339-350.

[14]Elizabeth M. Edwards, JL, Dacheux, Waites GMH. Effects of α -chlorohydrinon the metabolism of testicular and epididymal spermatozoa of rats. *J Reprod & Fertil* 1976; **48:** 265-270.

[15]Ravindranath H. Aladakatti, Sukesh B, Umadevi C. Jadaramkunti, Hiremath MB. Aspects of the antiandrogenic/antifertility property of Azadirachtin-A from Azadirachta indica leaves in male albino rats. Effect on the biochemical and cauda epididymalsperm parameters. *Rec Res Sci Technol* 2011; **3**(2): 34-46.

[16]Pierre Watcho, Mireille M. Donfack, Fabien Zelefack, Telesphore B, Nguelefack, Sylvie Wansi,et al. Effects of the Hexane extract of Mondia Whitei on the reproductive organs of male rat. *Afr J Tradit Complement Altern Med* 2005; **2**(3): 302-311.

[17]Gunasekaran Balamurugan, Muralidharan, Satyanarayana Polapala. Aphrodisiac activity and curative effects of Pedalium murex (L.) against ethanol-induced infertility in male rats. *Turk J Boils* 2010; **34:** 153-163.

[18]Mohammad Hossein Dehghan, Thomas Martin, Robabeh Dehghanan. Antifertility effect of Iranian neem seed alcoholic extract on epididymal sperm of mice. *Iran J Reprod Med* 2005; **3**(2): 83-89.

[19]Hashim Basha S, Lakshman J, Govardhan Naik A, Changamma C. Impact of *Carica papaya* Linn. Seed extract on reproductive metabolic profiles in male albino rat. *J Endocrinol Reprod* 2010; **14**(2): 49-52.

[20]Lohiya NK, Mishra PK, Pathak N, Manivannan B, Bhande SS, Panneerdoss S, et al. Efficacy trail o the purified compounds of the seeds of Carica papaya for male contraception in Albino rat. *Reprod Toxicol* 2005; **20**(1): 135-148.

[21]Davalieva K, Kiprijanovska S, Noveski P, Plaseski T, Kocevska B, Broussard C, et al. Proteomic analysis of seminal plasma in men with different spermatogenic impairment. *Andrologia* 2012; **44:** 256-264.

[22]Rakesh Sharma, Ashok Agarwal, Gayatri Mohanty, Rachel Jesudasan, Banu Gopalan, Belinda Willard, et al. Functional proteomic analysis of seminal plasma proteins in men with various semen parameters. *Reprod Biol & Endocrinol* 2013; **11**(38): 1-20.

[23]Mitra A, Richardson RT, Orland MG. Analysis of recombinant human semenogelin as an inhibitor of human sperm motility. *Biol Reprod* 2010; **82:** 489-496.

[24]Kaiser GR, Monteir SC, Gelain DP, Souza LF, Perry ML, Bernard EA. Metabolism of amino acids by cultured rat Sertoli cells. *Metabolism* 2005; **54**(4): 515-521.

[25]Reddy KVR, Gupta SM, Aranha CC. Effect of Antimicrobial peptide, nisin, on the reproductive functions of rats. *ISRN Vet Sci* 2011; **32:** 1-8.

[26]Budowle B, van Daal A. Extracting evidence from forensic DNA analyses: future molecular biology directions. *Bio Techniques* 2009; **46**(5): 339-40, 342-50.

[27]Meistrich ML, Bucci LR, Trostle-Weige PK, Brock WA. Histone variants in rat spermatogonia and primary spermatocytes. *Dev Biol* 1985; **112:** 230-240.

[28]Dadoune JP. Expression of mammalian spermatozoal nucleoproteins. *Microsc Res Tech* 2003: **61:** 56-75.

[29]Azza M. El-Wakf, EL-Said M. Elhabiby, Waffa M. El-kholy, Eman Abd El-Ghany. Use of tumeric and curcumin to alleviate adverse reproductive outcomes of water: Nitrate pollution in male rats. *Nat & Sci* 2011; **9**(7): 229-239.

[30]Manawadi SI, Kaliwal BB. Methomyl induced gonadal dysfunction, biochemical contents and enzyme activities in male albino mice. *Int J Biotechnol Appli* 2010; **2**(2): 20-32.

[31]Heyns CF, Human HJ, De Klerk DP. Hyperplasia and hypertrophy of the gubernaculum during testicular descent in the fetus. *J Urol*. **135**(5): 1043-7.