Annual Research & Review in Biology
5(3): 246-253, 2015, Article no.ARRB.2015.026
ISSN: 2347-565X

SCIENCEDOMAIN *international*
www.sciencedomain.org

Enzymatic Studies with Reference to Antifertility Potential of *Piper betle* Linn. Leaf Stalk Extract in Male Albino Rats

A. Govardhan Naik[1] and C. Changamma[1*]

[1]Department of Zoology, S. V. University, Tirupati-517 502, A.P., India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2015/11560
Editor(s):
(1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) A L Udupa, Department of Pharmacology, Faculty of Medical Sciences, University of the West Indies, Cave Hill, Barbados; West Indies.
(2) Anonymous, Djillali Liabes University of Sidi-Bel-Abbes, Sidi-Bel-Abbes, Algeria.
(3) Anonymous, University of Rajasthan, India.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=668&id=32&aid=6340

Original Research Article

Received 22nd May 2014
Accepted 3rd July 2014
Published 6th October 2014

ABSTRACT

The betel leaf (Tambula patra brint) is extensively cultivated in warm moist parts of India for its leaves; the antifertility properties of the betel plant were studied in both male and female rats. It was suggested that the contraceptive effect of the extract of leaf stalk of piper betel Linn is mainly on the maturation process of spermatozoa in epididymis without influencing hysteric hormonal profiles. The main aim of this study was to evaluate the antifertility efficacy of piper betel Linn leaf stalk extract with reference to some marker enzymes. In this study healthy male albino rats were administered with betel leaf stalk extract, at the dose rate of 150 mg/Kg/ day through oral gavage, for 15 days. The enzymatic changes were observed over control male rats. The observed reduction in Glucose-6-Phosphate dehydrogenase activity in testis, suggests that decreased level of operation of hexose mono phosphate pathway and also mobilization of carbohydrate reserves in to Hexose mono phosphate (HMP) pathway. Increased Succinate dehydrogenase is an indicative of better energy utilization due to the production of intermediates in the tricarboxylic acid cycle. The significant decrease in testicular Glutamate dehydrogenase activities (a biochemical indicator used to assess injury to the mitochondria) may imply reduction in the amount of energy being made available to the sperm cells. The total phosphorylase activity was highly depleted suggesting the

**Corresponding author: Email: challa1957@gmail.com;*

overall degradation of the enzyme, possibly for inhibiting glycogenolysis in response to betel leaf stalk extract. The depleted aldolase levels indicate decreased mobilization of glucose in to Embden-Mayeroff pathway in testis. Thus the extract administration does not affect the normal energy metabolism; it affects the sperm energy metabolism. Hence, sperm metabolic processes are disrupted. It also brings the alterations in chemical composition of seminal plasma and prostatic fluid leads to sperm anomalies.

Keywords: Spermatogenesis; hexose mono phosphate pathway; tricarboxylic acid cycle; Embden-Mayeroff pathway.

1. INTRODUCTION

Several plant extracts, *Aegle marmelos* [1], *Albizia lebbeck* [2], *Syzygium aromaticum* [3], *Eurycoma longifolia* [4] in rats were exhibit antispermatogenic activity and reduced sperm motility and concentration. Reports on decrease in the number of primary spermatocytes, interference and arrest of spermatogenesis, reduction in male fertility by extract of *Tinospora cordifolia* [5] and *Eurycoma longifolia* [4] have been documented. Antifertility effect of *Piper betle Linn*. Extract on ovary and testis of albino rats have been studied [6]. Chronic administration (subcutaneous) of the extract (stalk) of Piper betle at the dose of 30 mg/kg b. wt. for 21 days have produced significant decrease in oestrogen and androgen dependent organ weights, but increase in cholesterol in adrenal, ovary and testis. There was marked change in morphology of testis and ovary. In treated males number and motility of sperm were also reduced. Antiandrogenic and antifertility properties of ethanolic extract of Piper betle 150 mg/day/rat for 60 days in male albino rats have been reported [7]. The vas deferens sperm motility and sperm count declined significantly leading to negative fertility. Most of the studies were confined to sperm analysis. Hence the present study was focused on some enzymes in *Piper betle Linn.* Extract administered rats.

2. MATERIALS AND METHODS

In the present study healthy adult (3 months old, weight 160±10g) 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 containing 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 150 mg/Kg/day through oral gavages [8] for 15 days. The ethanol extract was prepared according to WHO [9] 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 reproductive tissues and liver were isolated, chilled immediately and used for biochemical analysis. Glucose-6-phosphate dehydrogenase activity [10], Succinate dehydrogenase [11], Lactate dehydrogenase [12], Malate dehydrogenase [12], Glutamate dehydrogenase [13], Phosphorylase [14] and Aldolase [10] levels were estimated both in control and experimental rats.

3. RESULTS AND DISCUSSION

The measurement of the activities of 'marker' enzymes in tissues can be used in assessing the degree of assault and the toxicity of a plant extract on organ/tissues [15]. Measurement of enzyme activities can also be used to indicate tissue cellular damage caused by a plant extract [16,17]. Hence, the present investigation was designed to evaluate the effect of betel leaf stalk extract on selected marker dehydrogenase enzymes. The observed reduction in the G-6-PDH activity Table 1 in testis, (-30.19% $P<0.001$) suggests that decreased level of operation of hexose mono phosphate pathway and also mobilization of carbohydrate reserves in to HMP pathway. There are two distinct phases in the pathway. The first is the oxidative phase, in which NADPH is generated, and the second is

the non oxidative synthesis of 5-carbon sugars. This pathway is an alternative to glycolysis. The primary functions of the pathway are [18] to generate reducing equivalents, in the form of NADPH, for reductive biosynthesis reactions within cells, and to provide the cell with ribose-5-phosphate (R5P) for the synthesis of the nucleotides and nucleic acids. Although not a significant function of the pentose phosphate pathway (PPP), the pathway can operate to metabolize dietary pentose sugars derived from the digestion of nucleic acids as well as to rearrange the carbon skeletons of dietary carbohydrates into glycolytic gluconeogenic intermediates located exclusively in the cytoplasm. The pathway is one of the three main ways with which the body creates molecules with reducing power, accounting for approximately 60% of NADPH production in man [19,20].

G-6-PDH is an important enzyme in steroidogenesis as it is an interstitial enzyme. Androgen production depends on the side chain splitting enzyme of cholesterol and on the microsomal hydroxylase which are NADPH dependent. G-6-PDH is a potential generator of NADPH in the testis. HMP pathway provides the necessary raw material for the lipogenesis and also ribose sugars for nucleic acid synthesis [21].

The germ cells have some pentose phosphate pathway activity, as indicated by glucose 6-phosphate dehydrogenase activity. As it is a cytosolic enzyme, glucose 6-phosphate was naturally found to have a greater activity in spermatocytes [22] G-6-PDH, present in Leydig, sertoli and spermatogenic cells, is more active in Leydig cells. The activity of G-6-PDH is associated with the function of Leydig cells. In this study, the G-6-PDH activity decreased dramatically suggesting that leaf stalk extract can injure the function of Leydig cells [23].

The elevation of G-6-PDH in prostate gland represents the alterations in oxidative metabolism which leads to changes in chemical composition of prostatic secretions, with the result of administration. Hence the betel leaf stalk extract administration alters HMP pathway and oxidative metabolism as an antifertility agent.

In the present study the betel leaf stalk extract enhanced testicular succinate dehydrogenase activity. Increased Succinate dehydrogenase is an indicative of better energy utilization due to the production of intermediates in the tricarboxylic acid cycle [24,25]. It is associated with the maturation of the germinal epithelial layer of seminiferous tubule [23]. SDH, mainly located in chondriosome of sertoli cells and spermatogenic cells, plays an important role in energy metabolism of sperms. Therefore, the extract administration does not affect the normal energy metabolism; it affects the sperm energy metabolism. Hence, sperm metabolic processes are disrupted [26]. No changes in epididymal SDH activity. But in secretary glands, reduced SDH activity levels were noticed which was in consonance with previous studies [27]. The lowered SDH activity in seminal vesicle and prostate gland indicates reduction in the conversion of succinate to fumarate and also depressed oxidative metabolism in mitochondria, reflects the turnover of carbohydrates and energy output [28].

In this study, Lactate dehydrogenase activity Table 1 reduced in testes and sexual accessories like epididymis and seminal vesicle but enhanced in prostate gland. Testicular LDH is an essential component of the metabolic machinery of spermatozoa and involved in the energy generation processes. The decrease in LDH activity in leaf stalk extract administered rats points towards the interference of stalk extract with the energy metabolism in testicular tissues [29,30]. The correlation between LDH and motility and living sperm could be a sign that extracellular LDH ensures metabolism of spermatozoa, perhaps even in anaerobic conditions. This hypothesis is underlined by the significant negative correlation between LDH and path morphology of sperm [31]. Notably, decline in serum testosterone level was observed with reduced reproductive organ weights, which means that male reproductive toxicity induced by extract would be augmented by decreased serum testosterone level as well as a decreased function of Sertoli cells and Leydig cells, in addition to the direct Cytotoxicity effect on germ cells [32].

LDH activity of the treated rats indicate the reduction of substrate lactate, suggesting an anaerobic type of respiration or altered physiological/metabolic activity which may have a definite influence on androgen regulated glycolytic enzyme activities in the male accessory organs, thereby indirectly affecting the secretary activities of these tissues. The elevation of LDH in prostate gland represents the elevation of the substrate lactate level as LDH is one of the key enzymes in Embden-Mayeroff pathway of carbohydrate metabolism [33].

The significant decrease in testicular Glutamate dehydrogenase activities (a biochemical indicator used to assess injury to the mitochondria) [17] may imply reduction in the amount of energy being made available to the sperm cells. This decrease in GDH activity may have been due to the instability of energy metabolism, the impairment of glutamate transport, or the activation of lipid peroxidation [25].

Glutamyl transferase is a membrane bound enzyme which catalysis the transfer of glutamyl group between peptides and amino acids and is considered, a 'marker' enzyme of sertoli cell function of testes. Thus, extract showed its effect on sertoli cell functions. The spermatogenic and androgenic potential of the testis has been associated with oxidative metabolism. The observed reduction in GDH levels in testis indicates the inhibition of oxidative metabolism, might indicate the possible action of the functions concerned with seminiferous tubules and Leydig cells and anti androgenic property of leaf stalk extract on sexual accessory organs.

In the present study the activity levels of Malate dehydrogenase (Table 1) showed inhibited pattern of response in tissues. The drop in MDH activity denotes fluctuations of oxidative metabolism and also reflects the turnover of carbohydrates and energy output [34]. The activity of MDH depends on the rate of formation of oxaloacetate and phosphoenol pyruvate from malate. The reduced oxidation of malate as indicated by decreased MDH activity in the tissues of experimental animals, indicate the possible diversion of malate undergoing decarboxylation leading to the formation of phosphoenol pyruvate for its rapid utilization through glycolytic pathway and reduction of pyruvate feeding in TCA cycle and decrease of Succinate dehydrogenase activity in tissues. Reduction in MDH activity may also be due to the inhibition exerted by oxaloacetate because decrease in the activity of TCA cycle dehydrogenase is consistent with the disintegration of mitochondria for CO_2 formation from acetate. This results in the accumulation of oxaloacetate which in turn inhibits NAD specific MDH. Another reason for the decreased levels of MDH suggests that there is a shift in the respiratory metabolism towards anaerobic nature that results in the decreased oxidative metabolism and decreased MDH activity [34].

The total phosphorylase active-inactive & phosphorylase active activity were elevated in liver with depletion in phosphorylase b. The proportion of phosphorylase in the active form can thus be used as an indirect monitor of changes in the concentration of 3', 5'-cyclic AMP in the liver. The increase in phosphorylase activity in the liver indicates that an increase in the concentration of 3', 5'-cyclic AMP. Thus the betel leaf stalk extract does not show any alteration in enzyme synthesis. Hence, there is no sign of hepatotoxicity.

The depleted aldolase levels indicate decreased mobilization of glucose in to EMP path way in all reproductive tissues (Table 2). Aldolase activity was inhibited; this type of inactive condition of the metabolism indicates the inefficient functional condition of epididymis. Since epididymis is involved in sperm maturation as well as storage these two functions seem to be having been inhibited, which can be attributed to the decreased circulation of testosterone in the body. Thus maturation of sperms in epididymis was affected by betel leaf stalk extract. This represents the antifertility efficacy of this extract. In secretary glands like seminal vesicle and prostate gland the depleted aldolase levels with elevated lactic acid content represents the antiandrogenic property of the extract administered. Hence depleted lactic acid in liver, in spite of elevated aldolase activity (Table 3), indicating the increased glycolytic pathway as the lactic acid was depleted in the liver. Acid content in the light of elevated activities of phosphorylase and aldolase possess contradictory situation. In such a condition active mobilization of lactic acid in to the oxidative metabolism of the tissue can be expected. In view of elevated aldolase activity, activated hexose diphosphate pathway and the mobilization of glucose in to the same can be envisaged.

4. CONCLUSION

The observed reduction in the G-6-PDH activity in testis, epididymis, and seminal vesicle suggests that decreased level of operation of hexose mono phosphate pathway and also mobilization of carbohydrate reserves in to HMP pathway. The elevation of G-6-PDH in prostate gland represents the alterations in oxidative metabolism which leads to changes in chemical composition of prostatic secretions, with the result of administration. Increased SDH is an indicative of better energy utilization due to the production of intermediates in the tricarboxylic acid cycle. The total phosphorylase 'ab'

Table 1. Effect of betel leaf stalk extract on G-6-PDH, SDH, LDH, GDH, MDH activity levels in testis, epididymis, seminal vesicle and prostate gland over control male albino rats

S. No	Parameter	Control, betel leaf stalk extract administered, % change and significance			
		Testis	**Epididymis**	**Seminal vesical**	**Prostate gland**
1.	Glucose-6-P Dehydrogenase	0.414±0.028	0.188±0.013	0.130±0.009	0.125±0.006
	(µmoles of formazan formed/mg protein /hr)	0.289±0.019	0.144±0.012	0.110±0.008	0.141±0.008
		-30.19*	-23.40*	-15.38**	+12.8**
2.	Succinate dehydrogenase	0.214±0.016	0.120±0.08	0.062±0.002	0.071±0.003
	(µmoles of formazan formed/mg protein /hr)	0.246±0.018	0.107±0.009	0.048±0.001	0.051±0.002
		+14.53**	-10.83NS	-22.58*	-28.16*
3.	Lactate dehydrogenase	0.524±0.036	0.353±0.028	0.039±0.002	0.060±0.003
	(µmoles of formazan formed/mg protein /hr)	0.344±0.019	0.231±0.017	0.016±0.001	0.088±0.006
		-34.35*	-34.56*	-58.97*	+46.66*
4.	Glutamate Dehydrogenase	0.149±0.008	0.101±0.009	0.026±0.001	0.046±0.002
	(µmoles of formazan formed/mg protein /hr)	0.073±0.003	0.066±0.002	0.011±0.001	0.019±0.001
		-51.00*	-34.65*	-57.69*	-58.69*
5.	Malate dehydrogenase	0.051±0.001	0.059±0.002	0.024±0.001	0.023±0.001
	(µmoles of formazan formed/mg protein /hr)	0.042±0.001	0.024±0.001	0.010±0.001	0.013±0.001
		-17.64*	-59.32*	-58.33*	-43.47*

*Mean±SD of six individual observations. + and – indicates percent increase and decrease respectively over control. *indicates P<0.001, ** indicates P<0.01, *** indicates P<0.05 the level of significance*

Table 2. Effect of betel leaf stalk extract on phosphorylase – ab, phosphorylase – a, phosphorylase – b, aldolase activity levels in testis, epididymis, seminal vesicle and prostate gland over control male albino rats

S. No	Parameter	Control, betel leaf stalk extract administered, % change and significance			
		Testis	Epididymis	Seminal vesicle	Prostate gland
1	Phosphorylase – ab	0.457±0.022	0.517±0.032	0.260±0.021	0.167±0.011
	(µmol pi formed/mg protein/hr)	0.320±0.012	0.172±0.013	0.179±0.015	0.176±0.012
		-29.97*	-66.73*	-31.15*	+5.38***
2.	Phosphorylase – a	0.373±0.031	0.417±0.033	0.205±0.011	0.171±0.014
	(µmol pi formed/mg protein/hr)	0.297±0.012	0.141±0.011	0.137±0.009	0.164±0.012
		-20.37*	-66.18*	-33.17*	-4.09NS
3.	Phosphorylase – b	0.084±0.005	0.031±0.001	0.055±0.003	0.010±0.001
	(µmol pi formed/mg protein/hr)	0.023±0.001	0.101±0.01	0.042±0.002	0.012±0.001
		-72.61*	-69.30*	-23.63*	+20.0*
4.	Aldolase	0.688±0.061	0.725±0.051	0.310±0.022	0.177±0.001
	(µmol FDP cleaved/mg protein/hr)	0.632±0.057	0.264±0.022	0.201±0.017	0.156±0.001
		-8.139***	-63.58*	-35.16*	-11.86*

*Mean±SD of six observations. + and – indicates percent increase and decrease respectively over control. *indicates P<0.001, ** indicates P<0.01, *** indicates P<0.05 the level of significance. NS- no significant changes*

Table 3. Effect of betel leaf stalk extract on phosphorylase-ab, a, b and aldolase activity levels in liver over control male albino rats

S. No	Parameter	Control	Betel leaf stalk administered	% Change
1.	Phosphorylase – ab (µmol pi formed/mg protein/hr)	0.179±0.014	0.227±0.017	+26.81*
2.	Phosphorylase – a (µmol pi formed/mg protein/hr)	0.142±0.011	0.212±0.016	+49.29*
3.	Phosphorylase – b (µmol pi formed/mg protein/hr)	0.037±0.001	0.015±0.001	-59.45*
4.	Aldolase (µmol FDP cleaved/mg protein/hr)	0.246±0.01	0.344±0.01	+39.83*

*Mean±SD of six observations. + and – indicates percent increase and decrease respectively over control. *indicates P<0.001 the level of significance*

activity was highly depleted suggesting the overall degradation of the enzyme, possibly for inhibiting glycogenolysis in response to leaf stalk extract. The total phosphorylase 'ab' & phosphorylase 'a' activity were elevated in liver with depletion in phosphorylase 'b'. The proportion of phosphorylase in the active form can thus be used as an indirect monitor of changes in the concentration of 3', 5'-cyclic AMP in the liver. The depleted aldolase levels indicate decreased mobilization of glucose in to EMP path way in all reproductive tissues.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chauhan A, Agarwal M. Reversible changes in the antifertility induced by *Aegle marmelos* in male albino rats. Syst Biol Reprod Med; 2008:54(1):240-246.
2. Gupta RS, Kanwar M, Rehwani H, Verma SK, Dobhaal MP. Contraceptive efficacy of *Strychnos potatorum* seed extract in male Albino rats. Asian J Exp Sci; 2006:20(1):181-187.
3. Mishra RK, Singh SK. Reversible antifertility effect of aqueous rhizome extract of *curcuma longa* L. in male laboratory mice. Contraception. 2009;79(2):479–87.
4. Wahab NA, Mokhtar NM, Halim WN, Das S. The effect of *Eurycoma longifolia* Jack on spermatogenesis in estrogen-treated rats. Clin (Sao Paulo). 2010;65(1):93-98.
5. Gupta RS, Sharma A. Antifertility effects of *Tinospora cordifolia* (Wild) stem extract in male rats. Indian J Exp Biol. 2003;41(1):885-889.
6. Adhikary P, Banerji J, Chowdhury D, Das AK, Deb CC, Mukherjee SR, Chatterjee A. Antifertility effect of *Piper betle* Linn. Extract on ovary and testis of albino rats. Indian J. Exp. Biol. 1989;27(10):868-70.
7. Sharma JD, Sharma S, Jain R. Antifertility activity of *Cuminum cyminum* on reproductive organs of male albino rats (*Rattus norvegicus*), XIX Natl. Symp, on Repro. Biol. Comp. Endocrine. Baroda, FR–01, P. 69. 2001:17-19,
8. Dehghan MH, Martin T, Robabeh Dehghanan MA. Antifertility effect of Iranian neem seed alcoholic extract on epididymal sperm of mice. Iranian Journal of Reproductive Medicine. 2005;3(2):83-89.
9. WHO: Protocol CG-40. Preparation of alcoholic extract for bioassay and phytochemical studies. Geneva, World Health Organization. 1983;(APJF/IP, 1001 A).
10. Bruns FH, Bergmeyer HU. In: methods of enzymatic analysis, acad. Press, New York. 1965;724-731.
11. Nachlas MM, Margulies SI, Seligman AM. A colorimetric method for the estimation of succinic dehydrogenase activity. J Biol Chem. 1960b;235(20):499-503
12. Reddanna P, Govindappa S. Effects of *in vivo* muscular stimulations III some aspects of carbohydrate metabolism of cardiac tissue. Curr sci; 1978:47(1):531-539.
13. Lee YL, Lardy HA. Influence of thyroid hormones on L- Glycophosphate dehydrogenase and other dehydrogenases in various organs of rat. J Biol chem. 1965;240(1):1427-28.
14. Cori GT, Illingworth G, Keller PJ. In: Colowick SP, Kaplan NO (Eds). Methods in enzymology, Academic press, New York. 1995;1:200-205.
15. Yakubu MT Bilbis LS, Lawal M, Akanji MA. Evaluation of selected parameters of rat and kidney function following repeated administration of yohimbine. Biochemistry. 2003;15:50–56.
16. Akanji MA. A comparative biochemical study of the interaction of some trypanocides with rat tissue cellular system. Ph.D. Thesis. University of Ife, Ile-Ife, Nigeria; 1986.
17. 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. Journal of Ethno pharmacology. 2008;111:288–292.
18. Beutler E, Vulliamy T, Luzzatte L. Hematologically important mutations; Glucose-6-phosphate dehydrogenase. Blood cells molecules Dis. 1996;22(1):49-76.
19. Zhang Z, Apse K, Pang J Stanton RC. High glucose inhibits glucose-6-phosphate via cAMP in aortic endothelial cells. J. Med Genet. 2000;16(1):431-434.
20. David AJ, Adefolaju GA, Enaibe BU, Ajao MS, Caxton-Martins EA. LDH and G-6PDH activities in the ovaries of adult female Wistar rats following the administrative aqueous extracts of neem (*Azadirachta*

indica) leaves. African Journal of Biotechnology. 2009;8(7):1310-1313.
21. Changamma C, Lakshman J, Hasim Basha S, Govardhan Naik A. Effect of 5Thio-D-Glucose on testicular metabolism of albino rat. Journal of Applied Sciences Research. 2011;7(2):98-101.
22. Bajpai M, Gupta G, Setty BS. Changes in carbohydrate metabolism of testicular germ cells during meiosis in the rat. European Journal of Endocrinology. 1998;138:322–327.
23. Bo zhang, Sen Lin. Effects of 3, 4-Dichloroaniline on testicle enzymes as biological markers in rats, biomedical and environmental sciences. 2009;22(1):40-43.
24. Singh SN, Suri S. Effect of an antidiabetic extract of *Catharanthus roseus* on enzymic activities in streptozotocin induced diabetic rats. Journal of Ethno pharmacology. 2001;76(3):269–277.
25. Pasupuleti Visweswara Rao K, Madhavi M, Dhananjaya Naidu M, SiewHua Gan, Rhinacanthus nasutus ameliorates cytosolic and mitochondrial enzyme levels in streptozotocin-induced Diabetic rats evidence-based complementary and alternative medicine, 2013;6.
26. Yuhui HAO, Rong LI, Yanbing LENG, Jiong REN, Jing LIU, Guoping LI, Hui XU, Yongping SU, Tianmin Cheng. The reproductive effects in rats after chronic oral exposure to low-dose depleted uranium. J Radiat Res. 2012;53(1):377–384.
27. Adedapo AA, Omoloye OA, Ohore OG. Studies on the toxicity of an aqueous extract of the leaves of Abrus precatorius in rats. On derstepoort J Vet Res. 2007;74(1):31–6.
28. Raghavendra L, Ksheerasagar, Basappa B, Kaliwal. Temporal effects of Carbosulfan on testicular biochemical parameters and enzyme activities in albino mice. Int J Pharm Bio Sci. 2013;4(1):173 -181.
29. Mollenhauer HH, Morre DJ, Rowe LD. Alteration of intracellular traffic by monensin: Mechanism, specificity and relationship to toxicity. Biochem Biophys Acta.1990;1031225-246.
30. Afaf A, El- Kashoury, Afrah Salama F, Adel Selim, Rania Mohamed A. Chronic exposure of dicofol promotes reproductive toxicity in male rats. Life Science Journal. 2010;7(1):3-6.
31. Kamp G, Busselmann G, Lauterwein J. Spermatozoa: Models for studying regulatory aspects of energy metabolism. Experientia; 1996;52(1):487-494.
32. Ravindranath H, aladakatti, Mukhtar Ahmed G, Ghodesawar, Mukhtar Ahmed, Tulasigeri M. Totiger, Sannadurgappa D. Effect of lyophilized *Azadirachta indica* leaf powder on biochemical parameters of testis and epididymis in albino rats. Int. J. Biol. Chem. Sci. 2010;4(1):75-87.
33. Murray RK, Grammar DK, Mayes PA, Road well VW. Harpers Biochemistry; Longer medical Publicative Appleton and Large, California. 25thedn. 1995;710-11
34. Murthy AS, Rajabhusanam BR, Ramani AV, Christopher KI. Toxicity of fenitrothion to fish *Mystus cavasius* and *Labeo rohita*. Environ pollution (Ser.A). 1983;30(1):225-232.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=668&id=32&aid=6340