



RESEARCH ARTICLE

ANTIFERTILITY EFFICACY OF BETEL LEAF STALK EXTRACT IN MALE
ALBINO RATS

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Corresponding Author*Govardhan Naik A****Abstract**

The present study was designed to study the impact of *Betel leaf stalk* extract on glucose, glycogen, pyruvic acid and lactic acid contents in reproductive tissues to understand the carbohydrate metabolism and its role in male albino rats. The administration of *Betel leaf stalk* extract, brings physiological changes in carbohydrate metabolism in reproductive tissues of rats. In order to assess antifertility effect in testis, epididymis, seminal vesicle and prostate gland, estimation of glucose, glycogen, lactic acid and pyruvic acid has been undertaken. The result showed testicular glucose and glycogen levels were not significantly affected by betel leaf stalk extract. The deprivation of ABP levels and the concentration of dihydrotestosterone may be the reason for elevation in glucose levels in epididymis. The accumulation of lactic acid in testes is the direct evidence for the antispermatogenic effect of betel leaf stalk extract. Higher pyruvic acid content, probably due to higher glycolysis.

*Copy Right, IJAR, 2013.. All rights reserve***INTRODUCTION**

Fertility regulation with plants or plant preparations has been reported in the ancient literature of indigenous systems of medicine. 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. However, the search for an orally active, safe and effective plant preparation or its compounds is yet to be needed for fertility regulation due to incomplete inhibition of fertility or side effects due to synthetic antifertility compounds.

Several plant species have been described as antifertility agents (Lin, 1992). Several medicinal plants have been used as dietary adjunct and in the treatment of numerous diseases including for inducing infertility without proper knowledge of their function. Although several herbal plants possess different types of antifertility activities such as anti implantation, Abortifacient, Ecobolic, Oestrogenic and Spermicidal, a large number of medicinal plants possess some degree of toxicity (Marles and Farnsworth, 1994).

The betel leaf (*Tambula patrabrint*) 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 (Tewari *et al.*, 1970). 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. Withdrawal of the extract restored all altered parameters including organ weights and fertility after 60 days (Sarkar *et al.*, 2000). Antifertility effect of alcoholic seed extract of *Abrus precatorius* Linn. in male albino rats were suggested that decrease in fertility rate with reduced sperm morphology in epididymis (Rao *et al.*, 1987). However, most of the studies were confined to sperm analysis, the biochemical studies were lagging. Hence the present study was focused on carbohydrate metabolism.

MATERIAL AND METHODS

In the present study healthy adult (3 months old, weight 160 ± 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 are control rats administered with 1 ml of distilled water. Second group are experimental, administered with betel leaf stalk extract, at the dose rate of 150 mg/Kg body weight/day through oral gavages method (Mohammad Husain Dehgan *et al.*, 2005) for 15 days. 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 $25 \pm 2^\circ\text{C}$ with a relative humidity of $50 \pm 5\%$. 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, prostate gland were isolated, chilled immediately and used for biochemical analysis. Glucose was estimated by the method of Mendal *et al.*, (1954) and glycogen by the method of Kemp and Van Heijningen (1954). Lactic acid in the tissue was estimated by the method of Barker and Summerson (1941) as modified by Huckabee (1961). Pyruvate was estimated by the method of Friedeman and Hangen (1942).

RESULTS AND DISCUSSION

The present study was focused in order to find out the effect of betel leaf stalk extract on these substrates. The testicular glucose and glycogen levels were not significantly affected by betel leaf stalk extract. But in duct system, in epididymis ($+87.90$ $P < 0.001$) glucose content become double over control concentration. In secretory organs elevation in seminal vesicle ($+30.25$ $P < 0.001$) and reduction in prostate gland (-18.54 $P < 0.001$) were observed.

Testis energy is provided mostly by glucose, which is the preferred substrate in mammals and most germ cell protein synthesis is under control of glucose metabolism (Miki, 2007). Glucose acts both as a source of energy and as a source of starting material for nearly all types of biosynthetic reactions (Chen *et al.*, 2003). It has been proposed that sertoli cells “nurse” spermatogenic cells by releasing L-lactate as an end product of their glycolytic metabolism (Robinson Fritz 1981; Mita and Hall, 1982). Glucose can cross the hematotesticular barrier (Turner *et al.*, 1983) and be taken up by the spermatogenic cells (Nakamura *et al.*, 1986), because it is a glycolytic substrate of meiotic and postmeiotic spermatogenic cells (Mita and Hall, 1982; Nakamura *et al.*, 1982; Grootegoed *et al.*, 1986; Nakamura *et al.*, 1986; Bajpai *et al.*, 1998).

No significant changes in testicular glucose and glycogen levels over control. Moreover, glucose is present in very low concentration in the lumen of the seminiferous tubules most spermatid ATP is therefore synthesized through the degradation of lactate and pyruvate produced massively by sertoli cells (Grootegoed *et al.*, 1989; Courtens and Ploen, 1999). Glycolytic enzymes are present in germ cells of the ad luminal compartment of the seminiferous tubules, but they are probably not active in the normal testis (Grootegoed *et al.*, 1984; Hammerstedt and Andrews, 1997). Hence, the testicular glucose levels were not affected by the administration of betel leaf stalk extract which in turn results no influence on the testes energy.

The accumulation of glucose in epididymis & seminal vesicle was observed (values $+87.90$ $P < 0.001$ $+30.25$ $P < 0.001$), two fold increment in epididymis. The epididymis is a long, coiled tube that rests on the backside of each testicle. It transports and stores sperm cells that are produced in the testes. It also is the job of the epididymis to bring the sperm to maturity, since the sperm that emerge from the testes are immature and incapable of fertilization. The epididymis, like the accessory sex glands, is an androgen-dependent organ and other testicular products, e.g. rete testis fluid and/or spermatozoa, may also affect epididymal physiology (Yakubu *et al.*, 2008) and spermatozoa present in the epididymal tubules.

A major role played by the epididymis is to regulate the movement of molecules into and out of the lumen. This ensures that as spermatozoa progress along the duct they are exposed to a continually changing, but optimal environment necessary for their maturation and survival. Peak levels of androgen binding protein (ABP) in the caput are seen (Hansson *et al.*, 1974) and the concentration of dihydrotestosterone within the epididymal tissue is more in adult age (Calandra *et al.*, 1974; Yakubu *et al.*, 2008). The sperm-free fluid contained less protein, phosphate, glucose, triglyceride and cholesterol than serum but more testosterone and chloride than peripheral blood. It contained no epididymal secretion products. (Cooper *et al.*, 1992). The uptake of sugars by epididymal tissue occurs by a specific process which may play a role in the regulation of glycolysis in tissue from androgen (Brooks, 1979). Therefore, the deprivation of ABP levels and the concentration of dihydrotestosterone (Calandra *et al.*, 1974; Yakubu *et al.*, 2008) may be the reason for elevation in glucose levels.

Sperm obtained directly from the epididymis contains hardly any fructose. During the passage through the male generative tract the semen acquires fructose from the accessory glands of reproduction, of which the seminal

vesicles are the chief contributors of fructose. Glucose is first phosphorylated by adenosine triphosphate and the monophosphohexose thus formed is further metabolized through diphospho fructose, phosphotriose, phosphoglyceric acid and pyruvic acid to lactic acid. (Priyanka Raghuvanshi *et al.*, 2013). Thus, the glucose phosphorylation is affected by the betel leaf stalk extract; leads to elevation in glucose levels. Therefore, Betel leaf stalk extract may be liable for changes in carbohydrates (Karmanshai *et al.*, 2001).

The elevated and reduced glucose in seminal vesicles & prostate gland represents some alterations in the chemical composition of prostatic fluid due to the administration of leaf stalk extract. The prostatic secretion contains simple sugars; proteins include proteolytic enzymes, prostatic acid phosphatase and prostate specific antigen (Wilke *et al.*, 2009). The reduction in prostatic glucose in turn reduces the sugars in prostatic fluid, alterations in chemical composition of seminal plasma.

There were no significant changes in testicular glycogen content due to the administration of leaf stalk extract. Glycogen, a reserve carbohydrate found in sertoli cells and spermatogonia in testis, serves as a source of glucose, which is an energy supplier to the tubular cells (Sharanabasappa Patil & Saraswati Patil, 2011). The glycogen content in the cells indicates energy storage. Sertoli cells and spermatogonia often contain glycogen, secrete substrates from the blood and provide source of reserve carbohydrate for seminiferous tubular cells and the glycogen level has been found to be directly proportional to the steroid hormones (Satishgoud *et al.*, 2009). Hence, the testicular energy storage in the form of glycogen is not disturbed by the extract administration.

But in sex accessories there was some alterations, the epididymal glycogen levels were slightly increased (+15.85 P<0.01) while drastic reduction in secretory glands like Seminal vesicle and Prostate gland over control (-54.90% P<0.001, -29.89% P<0.001). Glycogen in the epididymal epithelium has been considered as an energy reserve (Brooks, 1976) and as a source of the glucose-6-phosphate needed for pentose phosphate activity in this organ (Sholl & Leathern, 1973; Cooper *et al.*, 1992). The significant reduction in the weight of reproductive organs indirectly supports the reduced availability of androgen (Zeherea *et al.*, 1998). The significant reduction of testosterone level in serum (Govardhan Naik, 2014), which was sufficient for normal mating behavior, but insufficient for the maintenance of fertilizing ability of the epididymal spermatozoa (Bhasin *et al.*, 1988). Androgen deprivation not only suppresses spermatogenesis, leading to low sperm concentration, but alters the epididymal milieu also, which renders it hostile for physiological maturation and survival of the spermatozoa (Setty, 1979; Thejashwini *et al.*, 2012). Therefore, the reserved energy may not be utilized by converting into glucose for epididymal sperm maturation and capacitation. Hence accumulations of glycogen by extract administration in epididymis.

The reduction in Seminal vesicle and Prostate gland which are secretory in nature, due to either increased glycogenolysis or decreased glycogenesis. The decreased glycogen in seminal vesicle indicates the deficiency of nutrients for the development of sperms, the decreased secretory activity of seminal vesicle (Anitha and Indira, 2006). The main function of the prostate is to store and secrete a slightly alkaline fluid that usually constitutes semen along with spermatozoa and seminal vesicle fluid. The prostatic secretion contains simple sugars; proteins include proteolytic enzymes, prostatic acid phosphates and prostate specific antigen (Wilke *et al.*, 2009). Therefore some alterations in chemical composition of semen due to administration.

The accumulation of lactic acid in testes is the direct evidence for the antispermatogenic effect of betel leaf stalk extract. Due to the administration, lactate is not utilized as energy substrate for spermatocytes and spermatids. It alters the micronutrients of the testes, causing a deficiency in the nutrients for the proper maturation and functioning of sperms.

The lowering of the lactic acid in epididymis indicates; extract affecting physiological maturation of the sperm (Ravindranath *et al.*, 2011). The elevation in seminal vesicle and prostate gland represents a definite influence of extract on androgen-regulated glycolytic enzyme activities in the male accessory organs, thereby indirectly affecting the secretory activities of these tissues. (Kumari and Govindarajulu, 1980). Seminal vesicles and ventral prostate being androgen dependent, the regressive changes, suggests the antiandrogenic property of the betel leaf stalk extract (Kasutri *et al.*, 1997).

The pyruvic acid content was increased in all reproductive tissues, probably due to higher glycolysis. Spermatocytes were observed to use pyruvate as much as lactate, but in amounts much greater than those of glucose or fructose. Pyruvate and lactate maintained ATP levels in spermatocytes, while only lactate did in round spermatids and residual bodies. Pyruvate did not inhibit ATP synthesis from lactate in spermatocytes, differing from its behavior in spermatids. Pyruvate was oxidized in the Krebs cycle for further oxidative phosphorylation and was not altered by addition of glucose. Pyruvate and lactate were interconverted, but the intracellular level of pyruvate remained unchanged when either pyruvate or lactate was added to the medium. The maximal activity of lactate dehydrogenase (LDH) of spermatocytes occurred at 0.3-0.7 mM pyruvate, and this enzyme promoted the conversion of pyruvate to lactate rather than the reverse reaction (Nakamura *et al.*, 1984). Therefore, from this information it is

concluded that the spermatocytes are not utilized pyruvate and lactate (where these were accumulated) for its development in testes due to the antispermatogenic effect of the extract administered.

The reduction in epididymal lactate leads to elevated pyruvate levels as lactate is converted to pyruvate. The accumulation of both lactate and pyruvate results the changes in the composition of secretions of seminal vesicle and prostate gland by the administration.

Table: 1 Effect of betel leaf stalk extract on Glucose, Glycogen, Lactic acid and Pyruvic acid in reproductive tissues of male albino rats.

S.No	Parameter	Control, Betel leaf stalk extract, % Change			
		Testis	Epididymis	Seminal vesicle	Prostate gland
1.	Glucose	1.080 ± 0.01	0.810 ± 0.01	1.160 ± 0.07	2.793 ± 0.19
		1.120 ± 0.07	1.522 ± 0.09	1.511 ± 0.08	2.275 ± 0.16
		+3.70NS	+87.90*	+30.25*	-18.54*
2.	Glycogen	4.126 ± 0.31	2.321 ± 0.16	2.001 ± 0.09	2.057 ± 0.14
		4.166 ± 0.32	2.689 ± 0.18	0.902 ± 0.02	1.442 ± 0.03
		+0.97NS	+15.85**	-54.90*	-29.89*
3.	Lactic acid	11.940 ± 1.12	9.452 ± 0.071	4.477 ± 0.022	4.770 ± 0.021
		12.935 ± 1.21	7.960 ± 0.053	5.024 ± 0.033	6.965 ± 0.041
		+8.33NS	-15.78*	+12.21*	+45.83*
4.	Pyruvic acid	25.82 ± 1.5	29.20 ± 1.6	19.43 ± 0.8	20.79 ± 1.5
		28.23 ± 1.6	33.65 ± 2.4	26.69 ± 1.4	29.88 ± 2.2
		+9.33 *	+15.23**	+37.36*	+43.72*

Mean ± SD of six individual observations. + and – percent increase and decrease respectively over control.*

P<0.001, ** P<0.01 indicates the level of significance.

NS- non significant changes.

CONCLUSIONS:

The betel leaf stalk extract brings its antifertility effect by deprivation of androgen binding protein levels and the concentration of dihydrotestosterone. Seminal vesicles and ventral prostate being androgen dependent, the regressive changes, suggests the antiandrogenic property of the betel leaf stalk extract. The antispermatogenic effect of betel leaf stalk extract due to higher pyruvic acid content, probably due to higher glycolysis.

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