

## The Influence of *Piper betel* Linn. Leaf Stalk Extract as an Antispermatoxic on some Marker Enzymes of Protein Metabolism in Male Albino Rats

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**Abstract:** The present study was designed to evaluate the effect of *Piper betel* leaf stalk extract on marker enzymes like protease, ATPase and aminotransferases. The betel leaf stalk extract was administered at the dose of 50 mg/Kg/day through oral gavages for 15 days. The reduction of ATPase may be attributed to disturbance in biochemical metabolism which is due to the inhibition of energy transfer, through the inhibition of ATP-ase activity. Also, the reduction of ATP-ase activity might provide an explanation for the reduction of the reproductive capacity of animal. The decline in epididymis could probably be due to loss of epididymal function as a result of low circulating androgen levels in administered rats. Therefore, the *Betel* leaf stalk extract might possibly inhibit the activity of adenosine triphosphate (ATP) in the spermatozoa by uncoupling of oxidative phosphorylation from the respiratory chain and prevent phosphorylation of adenosine diphosphate to ATP and thus renders the spermatozoa immotile.

**Keywords:** Betel leaf stalk, Antifertility, Protease, Aminotransferases, Anti androgenic.

### Introduction

Phytotherapy has a very long tradition, although proper scientific explanation is relatively new. 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. Methanolic extract of *Albizialebeck bark* extract when administered showed anti-spermatoxic and anti-androgenic activities in male albino rats<sup>1</sup>. It was suggested that the role of seed extract of *A.precatorius* as an antifertility agent or contraceptive with a risk of DNA damage in spermatozoa and may lead to teratogenic effect<sup>2</sup>. The plant products which is very commonly used in daily life, *Piper betel* also known to have antifertility effect in rodents<sup>3&4</sup>. Contraceptive like properties have also been reported in women by local tribes of Rajasthan and Bengal region of India as they use these for birth control also<sup>5</sup>. In view of these findings, it is found that there was lagging of biochemical studies on *piper betel* leaf stalk extract, as an antifertility agent. Hence, the present study was undertaken.

### Material and Methods

In the present study healthy adult (3-4 months old, weight 215±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 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 (1983)<sup>6</sup> protocol CG-04. Stalks were shed-dried, powdered and extracted with 95% ethanol (v/v) at 55-

60°C for 3h. The solvent was distilled off under reduced pressure; the resulting mass was dried under vacuum and kept at 24°C 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°C 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. Protease<sup>7</sup>, ATPase<sup>8</sup>, ALAT (Alanine amino transferase)<sup>9</sup>, AST (Aspartate amino transferase)<sup>10</sup> were estimated in both control and experimental rats.

**Table: 1. Effect of *Betel* leaf stalk extract on the levels of Protease, ATPase, Alanine amino transferase and Aspartate amino transferase in testis, Epididymis, Seminal vesicle and prostate gland over control rats.**

Parameter	Control, Betel leaf stalk extract, % change and significance			
	Testis	Epididymis	Seminal vesicles	Prostate gland
Protease (µmoles of tyrosine /mg protein/hr)	0.219±0.012 0.376±0.021 +71.69*	0.392±0.057 0.551±0.032 +40.56*	0.171±0.008 0.298±0.013 +74.26*	0.158±0.012 0.24±0.017 +51.89*
ATPase (µmoles of Pi formed/mg protein/hr)	36.84±2.13 32.61±1.36 -11.48*	24.02±1.11 15.78±1.01 -34.30*	33.34±2.61 26.91±1.86 -19.28*	29.66±1.23 22.50±1.38 -24.14*
Alanine amino transferase (µmoles of sodium pyruvate formed/mg protein/hr)	0.92±0.03 0.73±0.05 -20.65*	0.84±0.05 0.59±0.03 -29.76*	0.65±0.03 0.38±0.02 -41.53*	0.87±0.01 0.53±0.05 -39.08*
Aspartate amino transferase (µmoles of sodium pyruvate formed/mg protein/hr)	0.942±0.06 0.624±0.08 -33.75*	0.732±0.05 0.528±0.03 -27.86*	0.902±0.07 0.715±0.04 -20.73*	0.531±0.03 0.342±0.01 -35.5*

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

## Results

The data represented in table-1 shows the levels of protease activity, ATPase activity, alanine amino transferase activity and aspartate amino transferase in reproductive tissues of control and administered rats. The *Betel leaf* stalk extract showed significant increase of protease activity in all reproductive tissues like testes (+71.69%), epididymis (+40.56%), Seminal vesicle (+74.26%) and prostate gland(+51.89%).

The levels of ATPase activity shows significant reduction in all tissues, testes (-11.48%), epididymis (-34.30%), Seminal vesicle (-19.28%) and prostate (24.14%).

The levels of alanine amino transferase activity showed significant reduction in all tissues, in testes (-20.65% P< 0.01), epididymis (-29.76%), seminal vesicle (-41.53%) and prostate gland (-39.08%).

The levels of aspartate amino transferase activity showed significant reduction in all tissues in testes (-33.75%), epididymis (-27.86%), seminal vesicle (-20.73%) and prostate (-35.5%) over control rats.

## Discussion

Protease enzymes are involved in a multitude of physiological reactions from simple digestion of food proteins to highly regulated cascades (e.g., the blood clotting cascade, the complement system, apoptosis pathways and the invertebrate prophenoloxidase activating cascade). Proteases can either break specific peptide bonds (limited proteolysis), depending on the amino acid sequence of a protein, or break down a complete peptide to amino acids (unlimited proteolysis). The activity can be a destructive change, abolishing a protein's function or digesting it to its principal components; it can be an activation of a function, or it can be a signal in a

signaling pathway. Proteases are used throughout an organism for various metabolic processes. The *Piper Betel* leaf stalk extract showed significant increase in protease activity levels in all reproductive organs like testes, epididymis, seminal vesicle, prostate gland. The enhanced protease levels leads to elevation in protein content in these tissues.

Depleted ATPase activity levels were observed over controls. *Piper Betel* leaf stalk extract might possibly inhibit the activity of adenosine triphosphate (ATP) in the spermatozoa by uncoupling of oxidative phosphorylation from the respiratory chain and prevent phosphorylation of adenosine diphosphate to ATP and thus renders the spermatozoa immotile. The reduction in sperm motility in cauda epididymis is of importance with regard to fertilization. A decrease in sperm reserve may be a responsible cause for reduction in the weight of epididymis. The significant decrease in testosterone level in the administered animals supports this view<sup>11-13</sup>.

Spermatogenic cells obtain 90% of their ATP from oxidative phosphorylation and glycolysis alone cannot provide enough energy for prolonged motility. This may be due to intracellular communication between sertoli cells, because spermatogenesis required direct intracellular communication between sertoli cells<sup>14</sup>. An important role of gap junction is the regulation of cell growth and differentiation by controlling the passage of small molecules between adjacent cells. Alteration in sertoli cells function may lead to impaired spermatogenesis<sup>14&15</sup>.

ATP is the source of energy for sperm motility. ATP is hydrolysed by ATPase activity. The activity of this enzyme in all reproductive organs like testis, epididymis, seminal vesicles and prostate gland of administered rat was significantly reduced. Decrease in ATPase activity could be attributed to androgen dependent parameters. However, the fertility rate was also significantly reduced which can be due to the decrease in caudal epididymal sperm motility and their morphological abnormalities<sup>16</sup>. The depletion in ATPase activity was noticed in all reproductive tissues due to administration of extract. These results are supported by other findings<sup>17&18</sup>. The reduction of ATPase may be attributed to disturbance in biochemical metabolism which is due to the inhibition of energy transfer, through the inhibition of ATPase activity. Also, the reduction of ATPase activity might provide an explanation for the reduction of the reproductive capacity of animal<sup>19</sup>. The decline in epididymis could probably be due to loss of epididymal function as a result of low circulating androgen levels in administered rats<sup>20</sup>. Therefore, the betel leaf stalk extract might possibly inhibit the activity of adenosine triphosphate (ATP) in the spermatozoa by uncoupling of oxidative phosphorylation from the respiratory chain and prevent phosphorylation of adenosine diphosphate to ATP and thus renders the spermatozoa immotile<sup>21</sup>. The reduction in sperm motility in caudal epididymis is of importance with regard to fertilization<sup>22&12</sup>.

Changes in aminotransferase activities could be expected to occur in association with a pathology involving necrosis in liver. AST and ALT are the important aminotransferase and are widely distributed in mitochondrion, which is associated with the integrality of spermatozoa acrosome and cells stress<sup>23</sup>. The present results showed that the activities of AST and ALT were obviously decreased by administration of *Piper Betel* leaf stalk extract in all reproductive organs<sup>24&18</sup>.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) play an important role in the mobilization of amino acids into gluconeogenesis<sup>25</sup>. Hence, reduction in free amino acids may cause reduction in aminotransferases. It is also possible that changes in hormone levels affect the release of alanine<sup>26&18</sup>.

## Acknowledgements

The authors were grateful to UGC –BSR and RGNF, New Delhi for financial support.

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