ASPECTS ON THE ANTIFERTILITY PROPERTY OF PIPER BETEL LINN. LEAF STALK EXTRACT: EFFECT ON GRAVIMETRIC ANALYSIS AND CAUDA EPIDIDYMAL SPERM PARAMETERS

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ABSTRACT

Although many types of contraceptive agents are available but these have several side effects, there are several medicinal plants known to possess male contraceptive properties either by suppressing spermatogenesis or by spermicidal action. In the present study the betel leaf stalk extract was administered orally at the dosage of 50 mg/kg body weight/day for 15days. The investigations are carried out on sperm analysis and gravimetric analysis. The reduction in the caudal epididymal sperm count, sperm motility as well as sperm viability are clear indications that piper betel extract can affect one or more aspects of spermatogenesis. The increase in the testes weight may be attributed to impaired secretory activity of the testes. Decreased weight of accessory sex glands indicate the atrophy of glandular tissue and diminished secretory ability reflects the decreased level of testosterone as these organs are androgen dependent. Reduction in the weight and weight ratio of accessory sex organs might be due to low levels of androgen, which was not enough to maintain the weight of accessories. The atrophy of glandular tissue and diminished secretory ability reflects the decreased level of testosterone as these organs are androgen dependent.

KEYWORDS: Antifertility, sperm analysis, reproductive tissues.

INTRODUCTION

Fertility regulation comprising contraception and management of infertility forms an important component of reproductive health. There is a growing interest in search of male
contraceptive of natural origin with least side effect. Piper betel leaves are credited with many medicinal properties such as digestive, simulative, carminative and aphrodisiac. However, Sri Lankan P. betle inhibits male sexual behaviour in rats and possesses antiaphrodisiac activity [1] indicating the differences in biological activities of Sri Lankan betel. Further, very few investigations on the activities of P. betle grown in Sri Lanka are reported except the experiments on antifertility effects of male rats [2]. A 10mg dose of Piperine treatment caused a significant reduction in the weights of testis and accessory sex organs. Histological studies revealed that Piperine caused severe damage to the seminiferous tubule, decrease in seminiferous tubular and Leydig cell nuclear diameter and desquamation of spermatocytes and spermatids. The studies on sperm analysis were lagging. The assessment of traditional semen analysis has been the object to obtain a clear indication of fertilization potential. Hence the present study was focused on this direction.

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 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 55-60ºC for 3h. The solvent was distilled off under reduced pressure; the resulting mass was dried under vacuum and kept at 24ºC 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 ± 2ºC 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 gravimetric analysis. Sperm sample were collected from cauda epididymis for sperm analysis like sperm count, sperm motility and sperm viability.
RESULTS & DISCUSSION

The value of traditional semen analysis has been the object of discussion to measure the antifertility potential of the animal. The development of normal mature sperm is the key to optimum male fertility. The production of the sperm cells (spermatozoa) and testosterone in the testis are mainly regulated by the follicle stimulating hormone (FSH) and Luteinizing hormone (LH), which are released from the anterior pituitary [4]. FSH stimulates spermatogenesis in the sertoli cells, while LH stimulates the production of testosterone in Leydig cells of the testis. Spermatogenesis is therefore, a complicated process, covering proliferation of the spermatogonia, long-lasting process of the tissue meiosis and numerous changes in the spermatids during their pre formation [5].

Sperm Analysis is used to determine Motility: movement of the sperm (swimming ability), Morphology: percentage of sperm that have a normal shape, Count: the number of individual sperm present in one ejaculation, Vitality: sperm’s ability to live and endure. The assessment of traditional semen analysis has been the object to obtain a clear indication of fertilization potential [6]. Hence in the present study, the sperm analysis in terms of sperm count, sperm motility, and sperm viability were undertaken in both control and experimental rats. The results revealed that the *Piper betel* leaf stalk extract causes a significant reduction in sperm count, sperm motility as well as sperm viability over control, supported by earlier reports [7, 8, 9, 10]. The sperm count reduction was severe. The analysis of caudal epididymal fluids revealed a concomitant decrease in the sperm concentration which may be due to the inhibition in spermatogenesis, which has been reflected here by the low count. Another possibility of low sperm concentration by the extract administration may be due to oxidative stress [11, 12]. This may be as a result of the ability of the extract at the given dose, to either interfere with spermatogenesis process in the seminiferous tubules, epididymis functions or activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadotropins which may result in alteration of spermatogenesis [13] may leads to oligospermia [5].

Marked inhibition of sperm motility may be due to low level of ATP content [14]. Slight reduction due to alteration in the metabolism of the testes has serious repercussion on sperm motility and fertility rate, since normal internal milieu of epididymis is necessary for proper maturation of sperm [15, 16].
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 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 [5,16].

The lowering of caudal epididymis sperm motility and density suggested an undersupply of testosterone to epididymis there by possibly causing impaired epididymal function. The impaired epididymal function may also be due to the reduced activity of testes, which affect the normal passage of testicular fluid into the epididymis [17]. A marked reduction in the level of seminal vesicular fructose may be another cause of reduction in sperm motility as motile sperm consume fructose after ejaculation [18] which provided energy for sperm motility [17]. Piperine (1-PiperoylPiperidine) is an alkaloid present in Piper species. Piperine is the major pungent substance present in these plants. Piperine could damage the epididymal environment and sperm function [19, 20]. Hence, the sperm viability decreased significantly. The decrease in the caudal epididymal sperm counts are clear indications that Piper betel extract can affect one or more aspects of spermatogenesis as well as spermigenesis.

Gravimetric analysis is a part of quantitative analysis and is defines as the process of weighing an element or a definite compound of the element in as pure form as possible. Gravimetric analyses rely on some final determination of weight as a means of quantifying an analyte, since weight can be measured with greater accuracy than almost any other fundamental property. The insoluble compound is washed to make it free from impurities, dried and weighed. From the weight of the compound, composition and amount of constituent is calculated. Depending on the nature of the sample, results are expressed in terms of percentage weight by weight (w/w) or weight by volume (w/v).

In this administration, there was a gradual increase in the body weight [12]. The findings of the present study showed that the ethanolic leaf stalk extract of P. betel could significantly alter the fertility potential of male rats. The fact that there was significant increase in body weight (+17.31% P< 0.01), doesn’t rule out the possibility of a systemic toxicity at the doses administered due to behavioral alterations observed within the administered groups which showed progressive decrease in agility. The significant increase in the body weight could be
due to the androgenic properties of this plant extract since androgens possess anabolic activity [21].

The weight of reproductive organs like testis (+9.44 P<0.01) was markedly increased. The weight, size and secretary functions of testes are closely regulated by androgens [22]. An increase in organ weight may either indicate inflammation or an increase in the secretory ability of the organ while a reduction in the value of organ weight ratio may imply cellular constriction. Therefore, the increase in the testes weight observed following the administration of the plant extract may be attributed to impaired secretory activity of the testes which is supported by increase in the concentration of cholesterol & protein [23]. However, the testes weight ratio was reduced, which can be attributed for the loss of germ cell [16,24]. Reduction in the weight and weight ratio of accessory sex organs like epididymis (-8.02% P<0.05), Seminal vesicle (-16.18% P<0.001) and prostate gland (-21.11% P<0.001) [20, 25, 26, 27] might be due to low levels of androgen, which was not enough to maintain the weight of accessories [28]. Decrease weight of accessory sex glands indicate the atrophy of glandular tissue and diminished secretory ability reflects the decreased level of testosterone as these organs are androgen dependent. A dose dependent lowering of caudal epididymis sperm motility and density suggested an undersupply of testosterone to epididymis there by possibly causing impaired epididymis function. The impaired epididymis function may also be due to the reduced activity of testes, which affect the normal passage of testicular fluid into the epididymis [17].

It is known that the accessory sex organs are androgen dependent target organs through their specific receptors and manifest differential sensibility to androgens for maintenance of their structure and function [16]. It is also known that, any change in circulating androgens would affect the internal microenvironment of epididymis and thereby lead to alteration in sperm motility and metabolism [5,29]. The atrophy of glandular tissue and diminished secretory ability reflects the decrease level of testosterone as these organs are androgen dependent [17]. Testosterone is required for the growth, development and maintenance of male reproductive organs [7]. An endogenous balance of estrogens and androgens is essential for the maintenance of normal epididymal growth and function [30]. Androgen deprivation not only suppresses spermatogenesis, leading to low sperm concentration, but also alters the epididymal milieu which renders it hostile for maturation and survival of the spermatozoa.
the maintenance of accessory sex organs. The structural and functional integrity of reproductive tissues depends on the circulating androgen and therefore, any small change in testosterone content may result in reduction in the weights of the reproductive organs [31]. The reduced or non-availability of androgens is further supported by the reduction in the weight of accessory organs like epididymis, Seminal vesicle, vas deferens, prostate gland and bulbourethral gland. All these organs play important role in the maturation and mobility of the sperm and formation of semen [32].

There was significant reduction in the dry matter of all reproductive organs except in epididymis where it was elevated, over control. The epididymis, is not in secretary nature, as it is a duct system, due to the debris’s of spermatozoa, there was an elevation in dry matter. But the other organs like testes, Seminal vesicle and prostate gland were secretary in nature. The secretary activities of these organs were altered by the extract administration due to the deprivation of androgens [33]. Hence, there was depression in dry matter. More over the water content does not showed any significant changes in all reproductive tissues.

Thus the oral administration of leaf stalk extract can lead to fertility control in male rats due to interference at the level of testicular androgens, which arrests the process of spermatogenesis in the testis, apparently without disturbing general metabolism. The relative weight of the testis, epididymis [34] and the caput and cauda of the latter [35] were not affected at any dose [36]. An increase in the testicular weight without accompanying changes in the weights of the secondary sex organs may signify a selective effect of *Piper betel* leaf stalk [37, 38].

**Table 1: Effect of Betel leaf stalk extract on sperm count, sperm motility and sperm viability over control.**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameter</th>
<th>Control</th>
<th>Betel leaf stalk extract administered</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sperm count (millions/ml)</td>
<td>70.45±3.28</td>
<td>47.82±2.74</td>
<td>-32.12*</td>
</tr>
<tr>
<td>2</td>
<td>Sperm motility (%)</td>
<td>68.46±3.42</td>
<td>63.24±2.34</td>
<td>-7.62**</td>
</tr>
<tr>
<td>3</td>
<td>Sperm viability (%)</td>
<td>67.42±4.27</td>
<td>53.24±3.42</td>
<td>-21.03*</td>
</tr>
</tbody>
</table>

Mean± SD of six individual observations. + and – indicates percent increase and decrease respectively over control. *P<0.001, **P<0.01 indicates significant changes.
Table 2: Effect of Betel leaf stalk extract on Body weight (in grams) over Control rats.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameter</th>
<th>Control rats.</th>
<th>Betel leaf stalk extract administered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>% Change</td>
</tr>
<tr>
<td>1</td>
<td>Body weight (g)</td>
<td>201.25 ±19.01</td>
<td>216.45 ±18.25</td>
</tr>
</tbody>
</table>

Mean± SD of six individual observations. + and – indicates percent increase and decrease respectively over control rats. **P<0.01 indicates significant changes. NS- non significant changes.

Table 3: Effect of Betel leaf stalk extract on Organ weight and TSI in Testes, Epididymis, Seminal vesicle and Prostate gland over control rats.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameter</th>
<th>Paired Testes</th>
<th>Paired Epididymis</th>
<th>Paired Seminal vesicle</th>
<th>Prostate gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Organ Weight (g)</td>
<td>2.54±0.12, 2.78±0.11, +9.44**</td>
<td>2.082±0.23, 1.915±0.06, -8.02 ***</td>
<td>1.186±0.04, 0.994±0.06, -16.18*</td>
<td>0.715±0.02, 0.564±0.04, -21.11*</td>
</tr>
<tr>
<td>2</td>
<td>TSI</td>
<td>1.17±0.10, 1.04±0.03, -11.11***</td>
<td>0.96±0.02, 0.72±0.02, -25.00*</td>
<td>0.45±0.01, 0.44±0.02, -2.22 NS</td>
<td>0.30±0.02, 0.26±0.01, -13.33**</td>
</tr>
</tbody>
</table>

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

Table 4: Effect of Betel leaf stalk extract on Dry matter and Water content in Testes, Epididymis, Seminal vesicle and Prostate gland over control rats.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameter</th>
<th>Testes</th>
<th>Epididymis</th>
<th>Seminal vesicle</th>
<th>Prostate gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dry matter (mg/g wet wt.)</td>
<td>305.31±28.43, 272.73±24.18, -10.66***</td>
<td>303.93±27.12, 353.92±32.03, +16.44**</td>
<td>297.52±27.42, 254.52±22.13, -14.45**</td>
<td>343.82±32.29, 308.32±27.35, -10.32***</td>
</tr>
<tr>
<td>2</td>
<td>Water content (mg/g wet wt.)</td>
<td>732.56±70.63, 763.44±75.45, +4.21 NS</td>
<td>707.73±68.47, 736.31±71.43, +4.04 NS</td>
<td>714.37±69.43, 690.43±6793, -3.35 NS</td>
<td>720.24±51.25, 696.7±49.29, -3.26 NS</td>
</tr>
</tbody>
</table>

Mean± SD of six individual observations. + and – percent increase and decrease respectively over control rats. ** P<0.01, ***P<0.05 indicates the levels of significance, NS- non significant changes.
CONCLUSIONS

The oral administration of *Piper betel* leaf stalk extract can lead to fertility control in male rats due to interference at the level of testicular androgens, which arrests the process of spermatogenesis in the testis, apparently without disturbing general metabolism.

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