INTRODUCTION

Prostatitis is a progressive pathologic condition associated with aging men and characterized by proliferation of prostatic tissues, prostate enlargement and lower urinary tract symptoms (Briganti et al., 2009). It is also associated with complex histological changes involving glandular and stromal hyperplasia, fibrosis and prostatitis (Chapple and Smith, 1994; Barnes, 2002). The prostate gland is a major secondary endocrine organ of males whose development and growth depends on androgen stimulation especially by dihydrotestosterone (DHT), an active metabolite product from the conversion of testosterone by...
steroid 5 \(^{-}\) -reductase. It is documented that androgens and possibly estrogens constitute the primary factors responsible for prostate diseases (Shin et al., 2012a; De Nunzio and Tubaro, 2011; Farley, 2011). There is an increased accumulation of DHT in the prostate with aging which results in increased cell growth and hyperplasia (Carson and Rittmaster, 2003). Prostatitis also involves increased adrenergic tone in prostate smooth muscle mediated by \(^{\text{1}}\) -adrenoceptors (Michel et al., 1998). The drugs used in treatment of prostatitis include steroid 5 \(^{-}\) -reductase inhibitors (finasteride) and \(^{\text{1}}\)-adrenoceptor antagonists such as alfuzosin and terazosin (Gravas and Oelke, 2010).

Prostate-Specific Antigen (PSA), a glycoprotein in humans encoded by the KKL3 gene and a member of the kallikrein-related peptidase family is secreted by the prostatic epithelial cells and performs various functions during copulation and fertilization (Menez et al., 2008). Serum PSA levels are often elevated in prostate disorders such as BPH, prostatitis and even in prostate cancer and are used as a clinical marker for disease prognosis (Takizawa et al., 2010). Raised levels of serum PSA may also be suggestive of prostate cancer. prostatitis is not a known risk factor for prostate cancer but may increase the chance of its occurrence (Chang et al., 2012). The etiology of prostate inflammation is complex and not completely elucidated but involves age-related hormonal alterations, metabolic syndrome and inflammation (Thompson and Yang, 2000). Many plants have been identified as good sources of natural antioxidants which protect against degenerative diseases and cancer (Javanmardi et al., 2003; Arabshahi et al., 2007).

Aluminum has possible to induce toxic effects in humans or laboratory animals exposed through inhalation, oral, or dermal exposure. It is widely accepted that nervous system is the most sensitive target of aluminum toxicity and it may induce cognitive deficiency and dementia when it enters the brain. Besides this, Aluminium ingestion in excessive amount leads to accumulation in target organs and has been associated with damage of testicular tissues of both humans and animals. High Aluminium contents in human testes, Leydig cells, spermatozoa, seminal plasma, blood and urine, were associated with impaired sperm quality and viability (Dawson et al., 2000; Hovatta et al., 1988, Reusche, e et al., 1994). Deterioration in spermatogenesis and sperm quality; interruption in sex hormone secretion (Gup.U et al., 2005) are several of the aspects suggested that Aluminium exposure causes adverse impact on male reproduction.

Several phytotherapies are used for prevention and treatment of prostate disorders. This paper reviews the phytomedicines used in Africa, Western countries, India and China as treatment of BPH, prostatitis and prostate cancer. Herbs which hold potential promise are mentioned, although much research is still required. Whole parts of *Evolvulus alsinoides* possess valuable medicinal properties and plant is widely used in ayurveda. The plant reported to contain Shankapushpina, Betaine & also contains volatile oils. The presence of the several active compounds *Evolvulus alsinoides* possess some pharmacological activities which can be used for the cure of several diseases. In the present study *Evolvulus alsinoides* leaf extract has been selected to work for its the therapeutic effect of *Evolvulus alsinoides* Linn in experimental prostatitis.

**MATERIALS AND METHODS**

**Collection of plants:**

The fully mature *Evolvulus alsinoides* Linn. whole plants were collected from marungulam, Thanjavur District, Tamil Nadu, India from a single herb. The collected leaves were identified and authenticated by a Botanist Dr. S.John Britto S.J, The Director, The Rapinat Herbarium and Center for molecular systematic, st.Joseph’s College, Tiruchirappalli, Tamil Nadu. A Voucher specimen has been deposited at Tamil University Herbarium. The plants were cut into small pieces and shade dried and powdered finely then used for extraction.

**Animals:**

Male albino rats of Wistar strain approximately weighing 190-200g were used in this study. They were healthy animals purchased from the Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2° C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided ad libitum. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

**Preparation of plant extract:**

The *Evolvulus Alsinoidei* leaves were washed well and dust was removed from the plant. Leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with 70% methanol for 48 hours. A semi solid extract was obtained after complete elimination of
alcohol under reduced pressure. The extract was stored in refrigerator until used.

**Preparation of Aluminum chloride (AlCl₃)**

Two grams of aluminum chloride was dissolved in 100 ml distilled water to prepare a stock solution (20 mg/ml). The solution was prepared weekly and kept in a plane bottle at 4°C. AlCl₃ was daily administrated to rats (0.1ml (2mg)/100gm) orally.

**Experimental design**

Body weights of the animals were recorded and they were divided into 4 groups of 6 animals each as follows. First group; was negative control administrated 3 ml distilled water orally once daily. Second group; was positive control group (AlCl₃ group) administered aluminium chloride (20 mg/kg bw), the LD 50 of AlCl₃ when administered orally to rats was reported to be (380 - 400 mg/kg bw (Krasovskii et al., 1979). Third group; was administered *Evolvulus alsinoides* leaves extract (EALE) (75 mg/kg bw) which dissolved in 3ml distilled water orally once daily according to Lekshmi and Reddy, (2011). Fourth group; was co administered with AlCl₃ and EALE in the same doses in 2nd and 3rd groups. Doses were given once daily via gavage for 70 consecutive days, for completion of the spermatogenic cycle and maturation of sperms in epididymis (Sarkar et al., 2003).

**Collection of blood and preparation of serum sample**

At the end of the experimental period, the animals were weighed once after an overnight fasting. The animal weight was noted. The blood sample was collected by cervical dislocation for Prostate Specific Antigen (PSA) hormones and seminal determinations.. The blood was allowed to clot by standing at room temperature for 30 minutes and then refrigerated for another 30 minute. The resultant clear part was centrifuged at 3000 rpm for 10 minutes and then the serum (supernatant) was isolated and stored at refrigerated until required for biochemical analysis.

**Physical parameters:**

After blood collecting, the animals were sacrificed by cervical dislocation and the Kidney, Liver, Heart, Testes, and Prostate were dissected out, washed with ice-cold physiological saline. The required amount was weighed

**Determination of Serum Prostate specific antigen (PSA):**

The serum Prostate Specific Antigen (PSA) levels were determined with a PSA ELISA kit according to the manufacturer’s instructions (Rapid Labs. Ltd, Colchester, Essex, UK) (Nilsson *et al.*, 1997). The absorbance was measured at 450 nm using a microplate ELISA reader (Bio-Rad Laboratories, Inc.). The values were expressed as ng protein mL⁻¹.

**Determination of Hormones:**

The hormones levels in serum were determined with a ELISA kit according to the manufacturer’s instructions (Rapid Labs. Ltd, Colchester, Essex, UK) (Nilsson *et al.*, 1997). The absorbance was measured at 450 nm using a microplate ELISA reader (Bio-Rad Laboratories, Inc.). The values were expressed as ng protein mL⁻¹.

**Statistical analysis**

Values were expressed as mean ± SD for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey’s test for multiple comparisons (Harvey and Paige, 1998). The results were statistically analyzed by using SPSS (Statistical Packages for Social Studies) version 20 was used and p< 0.05 was considered to be significant.

**RESULTS AND DISCUSSION**

**Physical parameters:**

The effect of *Evolvulus alsinoides* leaf extract (EALE) on final body and organ weight and weight gain in AlCl₃ toxicity of male rats were investigated. Oral administration of EALE had no effect on body weight gain of rats, indicating its safe use under the experimental conditions, while there were highly significant decrease in final body weight and body weight gain (p <0.05) in AlCl₃ group as compared with control group. On the other hand, there were highly significant decreased in final body weight and weight gain in AlCl₃ group treated with EALE as compared with untreated AlCl₃ group. The increased liver, heart and kidney weight while decreased the testes and prostate in experimental rats. On treatment with EALE restored the organ weight. Results were shown in Table 1 and 2.

**Effect of *Evolvulus alsinoides* leaves on PSA and hormones in experimental rats**

In the present study to investigate Testosterone, LH, FSH, Estradial, Progesterone and Prostate Specific antigen (PSA). Results showed highly significant decreased in serum Testosterone, LH, FSH, Estradial and Progesterone concentration
(p < 0.05) and increased in PSA in AlCl₃ group compared to control group, while orally treatment with EALE induced highly significant elevation in serum testosterone concentration (p < 0.05) and alleviated the negative effects of AlCl₃ as compared with untreated AlCl₃ group. Results were shown in Table 3.

**Effect of *Evolvulus alsinoides* leaves on Sperm count, motility, morphology and viability in experimental rats**

Epididymal sperm count, sperm motility, viability and abnormal sperm are investigated for AlCl₃ and/or EALE groups. EALE group did not differ significantly from the control in terms of sperm motility, sperm viability and abnormal sperm rates, but had highly significant elevation in sperm count (p < 0.01) as compared with AlCl₃ group. AlCl₃ group had significantly lower sperm count, motility and viability than the control group. On treatment with EALE restored the sperm count, sperm motility, viability and sperm morphology. Results were shown in Table 4.

**Conclusion:**

In conclusion, consumption of *Evolvulus Alsinoides* leaves appear to be protective against prostate inflammation and are a promising candidate for further laboratory and clinical research on prostate related diseases including prostate cancer.

**Table 1 : Effect of *Evolvulus alsinoides* leaves on body weights of experimental rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial weight (gm)</th>
<th>Final weight (gm)</th>
<th>Weight gain (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>196.6±13.32</td>
<td>277.5±19.15</td>
<td>80.9±5.67</td>
</tr>
<tr>
<td>Group II</td>
<td>200.8±14.00</td>
<td>240.9±16.80⁻</td>
<td>-40.1±2.91⁻</td>
</tr>
<tr>
<td>Group III</td>
<td>195.3±12.34</td>
<td>275.7±19.25⁻</td>
<td>80.4±5.60⁻</td>
</tr>
<tr>
<td>Group IV</td>
<td>198.9±13.87</td>
<td>276.2±19.11⁻</td>
<td>77.3±5.41⁻</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six rats in each group.

⁻Significantly different from group I (p < 0.05)

⁻⁻Significantly different from group II (p < 0.05)

**Table 2: Effect of *Evolvulus alsinoides* leaves on organ weights of experimental rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Kidney (gm)</th>
<th>Liver (gm)</th>
<th>Heart (gm)</th>
<th>Testes (gm)</th>
<th>Prostate (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>1.160±0.113</td>
<td>5.460±0.382</td>
<td>0.720±0.050</td>
<td>1.485±0.103</td>
<td>0.400±0.030</td>
</tr>
<tr>
<td>Group II</td>
<td>1.560±0.112⁻</td>
<td>7.370±0.515⁻</td>
<td>1.100±0.077⁻</td>
<td>1.355±0.094⁻</td>
<td>0.850±0.061⁻</td>
</tr>
<tr>
<td>Group III</td>
<td>1.030±0.070⁻</td>
<td>4.110±0.287⁻</td>
<td>0.920±0.064⁻</td>
<td>1.630±0.114⁻</td>
<td>0.495±0.035⁻</td>
</tr>
<tr>
<td>Group IV</td>
<td>1.220±0.086⁻</td>
<td>4.570±0.319⁻</td>
<td>0.790±0.055⁻</td>
<td>1.450±0.101⁻</td>
<td>0.630±0.045⁻</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six rats in each group.

⁻Significantly different from group I (p < 0.05)

⁻⁻Significantly different from group II (p < 0.05)
Table 3: Effect of *Evolvulus alsinoides* leaves on hormones and PSA in serum of experimental rats

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Testosterone (ng/ml)</td>
<td>2.69 ±0.18</td>
<td>1.84 ±0.10</td>
<td>2.87 ±0.21</td>
<td>2.61 ±0.21</td>
</tr>
<tr>
<td>2.</td>
<td>LH (ng/ml)</td>
<td>3.89 ± 0.28</td>
<td>2.14 ±0.15</td>
<td>3.85 ±0.25</td>
<td>3.70 ±0.28</td>
</tr>
<tr>
<td>3.</td>
<td>FSH (ng/ml)</td>
<td>1.67 ±0.14</td>
<td>1.13 ±0.09</td>
<td>1.58 ±0.14</td>
<td>1.54 ±0.18</td>
</tr>
<tr>
<td>4.</td>
<td>Estradioal (pg/ml)</td>
<td>7.98 ± 0.56</td>
<td>4.87 ± 0.29</td>
<td>7.95 ±0.57</td>
<td>7.93 ±0.54</td>
</tr>
<tr>
<td>5.</td>
<td>Progesterone (ng/ml)</td>
<td>13.472 ±0.98</td>
<td>8.75±0.58</td>
<td>14.86 ±1.23</td>
<td>12.64 ±0.087</td>
</tr>
<tr>
<td>6.</td>
<td>Prostate Specific antigen (PSA) (ng/ml)</td>
<td>0.52 ± 0.03</td>
<td>3.86±0.24</td>
<td>0.63±0.45</td>
<td>0.65±0.45</td>
</tr>
</tbody>
</table>

Values are expressed a Mean ± SD for triplicates 

a Significantly different from Group I (P < 0.05)  b Significantly different from Group II (P < 0.05)

Table 4: Effect of *Evolvulus alsinoides* leaves on Sperm count, motility, morphology and viability in experimental rats

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>Sperm count (million/cu. mm)</th>
<th>Sperm motility (%)</th>
<th>Sperm morphology (%)</th>
<th>Sperm viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>152±10.71</td>
<td>79±5.46</td>
<td>71.32 ±4.98</td>
<td>80.84±5.67</td>
</tr>
<tr>
<td>Group-II</td>
<td>61±4.2 a</td>
<td>52±3.71 a</td>
<td>32.65±2.24 a</td>
<td>36.35±3.12 a</td>
</tr>
<tr>
<td>Group-III</td>
<td>169±9.66 b</td>
<td>82±5.69 b</td>
<td>72.84±5.21 b</td>
<td>83.56±6.24 b</td>
</tr>
<tr>
<td>Group-IV</td>
<td>150.75 ±9.65 b</td>
<td>74.56 ±4.35 b</td>
<td>70.65±4.91 b</td>
<td>78.65 ±5.46 b</td>
</tr>
</tbody>
</table>

Values are expressed a Mean ± SD for triplicates 

a Significantly different from Group I (P < 0.05)  b Significantly different from Group II (P < 0.05)

REFERENCES


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Conflict of interest: None declared