Antioxidant Property of the Root of Asparagus Gonoclados Baker (Liliaceae)

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Abstract
The present investigation was aimed at evaluating the antioxidant potential of the alcohol and aqueous extracts of root of Asparagus gonoclados Baker by in vitro and in vivo methods. The ability of extracts to scavenge DPPH and superoxide radicals was evaluated by in vitro methods. In vivo evaluations were done by CCl4 induced hepatotoxicity. Hepatotoxicity was induced in Wistar rats by administration of CCl4 1.5 mL/kg i.p. following extract treatment for 15 days. Total proteins, catalase and SOD were estimated in liver and MDA was estimated in brain. The studies showed that the alcohol and aqueous extracts at dose levels of 200 and 400 mg/kg exhibited significant increase in catalase and superoxide levels (p < 0.01) in CCl4 intoxicated rats. Simultaneously, the levels of MDA in brain were significantly decreased (p < 0.01) in the extract treated groups when compared with the positive control. Preliminary phytochemical screening of the extracts was carried out to detect the presence of various phytoconstituents. HPTLC fingerprint profiles of the detected phytoconstituents were also obtained. Acute toxicity studies revealed that both the alcohol and aqueous extracts were safe up to the dose of 3000 mg/kg on oral administration.

Key Words: Asparagus Gonoclados, Catalase, Superoxide Dismutase, Malondialdehyde

1. INTRODUCTION
Shatavari is a well known drug in Ayurveda [1] and its accepted botanical source is Asparagus racemosus L. [2]. However, many other species of Asparagus L. including Asparagus gonoclados Baker are used as Shatavari [3, 4]. Root tuber of Shatavari (A. racemosus) is considered as one of the Rasayana (adaptogenic) drugs, having cooling, diuretic, emollient, rejuvenating, and stomachic properties [5]. It is useful in the treatment of nervous system disorders, dyspepsia, diarrhea, dysentery, tumors, inflammation, tuberculosis, epilepsy and fatigue [6].

A. gonoclados contains phytoconstituents like apigenin, kaempferol, rutin and chalcone glycoside, anthocyanin and malvin4. The diuretic, galactogogue, antiulcer and antioxidant properties of A. racemosus have been reported [7-10]. Pharmacognostical studies on root tubers of A. gonoclados have also been reported [11]. In the present work, the effect of alcohol and aqueous extracts of root tuber of A. gonoclados on certain liver antioxidant enzymes and brain antioxidant status have been investigated besides assessing the free radical scavenging of the extracts.

2. METHODOLOGY

2.1 Plant Material
The plant material was collected from the forests situated between Madikeri and Sakleshpur, during March 2006. The plant material was identified and authenticated by Dr. S.N. Yoganarasimhan, Taxonomist and Research coordinator following local floras [12, 13].

2.2 Preparation of Extracts
Alcohol extract was prepared by soxhlation and aqueous extract was prepared by cold maceration.

2.3 Animals
Swiss albino mice of either sex, in the weight range of 18 – 25 g, were used for acute toxicity studies and albino rats of Wistar strain, of either sex in the weight range of 180 – 200 g were used for the in vivo antioxidant activity studies. Animals were maintained in accordance with CPCSEA regulations and the study protocol was approved by the Institutional Animal Ethics Committee of M. S. Ramaiah College of Pharmacy.

2.4 Phytochemical Analysis
Preliminary phytochemical analysis and HPTLC studies were carried out on the methanol and aqueous extracts [14, 15].

2.5 In Vitro Antioxidant Activity Studies
DPPH and super oxide scavenging assays were performed [16, 17] to evaluate free radical scavenging ability of the extracts.

2.6 Acute Toxicity Studies
The acute toxicity study was carried out following Ghosh [18].

2.7 In Vivo Antioxidant Activity
Albino rats of Wistar strain weighing 180 – 200 g of either sex were used for the study. The animals were divided in to 7 groups containing 6 animals each. Group I was maintained as normal control. Group II was maintained as positive control and administered with CCl4 at a dose of 1.5 mL / kg, i.p. Group III was administered with standard Vit E at a dose of 50 mg/kg body weight, orally. Group IV and V animals were administered with alcohol extract at doses 200 and 400 mg/kg respectively orally.
Group VI and VII were administered with aqueous extract at doses 200 and 400 mg/kg respectively orally. The treatment schedule was once daily for 15 days. On 15th day, 6 h after the last dose of extract, CCl4 (1.5 ml/kg) was administered to all animals by i.p. injection.

Six hours after CCl4 administration, all the animals were sacrificed, for the isolation of liver and brain. The liver was isolated, perfused with ice cold saline to remove contaminated blood. The isolated liver was divided into 2 parts for preparation of homogenates. A 5 % w/v homogenate of liver was prepared in 0.15 M potassium chloride and centrifuged at 8000 rpm for 10 min. The supernatant thus obtained was used for estimation of total proteins [19] and catalase [20]. Another 5 % w/v homogenate of liver was prepared using 0.25 % w/v solution of sucrose in 5M phosphate buffer, centrifuged at 8000 rpm for 10 min. The supernatant was used for estimation of super oxide dismutase [21].

Brain was isolated and homogenized, for the estimation of MDA. The isolated brain was washed with ice cold potassium chloride solution (1.15%w/v) and sliced. The sliced rat brain was used for preparing homogenate in the ratio, of 0.75:9.25 ml potassium chloride and centrifuged at 8000 rpm for 10 min. The supernatant was used for estimation of super oxide dismutase [21].

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2.8 Statistical Analysis
The values of all groups were compared with positive control (CCl4 treated) and data expressed as Mean values ± S.E.M and tested with one way ANOVA followed by Dunnett multiple comparison test for in vivo and in vitro antioxidant activity.

3. RESULTS AND DISCUSSION
Preliminary phytochemical analysis revealed carbohydrates, flavonoids, glycosides, phenolic compounds, saponins, alkaloids and tannins in alcohol and aqueous extracts; proteins, amino acids, gums and mucilage in aqueous extract. HPTLC fingerprinting of Shatavarin IV in alcohol and aqueous extracts was developed. Alcohol extract revealed 9 phytoconstituents while aqueous extract revealed 7 phytoconstituents. The peak with Rf 0.34 in both extracts correspond to that of standard Shatavarin IV (Rf 0.35). The specificity was confirmed by overlaying the spectra of standard Shatavarin IV (λmax 426 nm), with the absorption spectrum obtained from the corresponding band in alcohol and aqueous extracts (Fig. 1).

Alcohol and aqueous extracts of A. gonoclados were subjected to in vitro antioxidant activity studies. Both extracts exhibited significant free radical scavenging effects against DPPH and superoxide radicals (Table 1).

Acute toxicity studies revealed that both the alcohol and aqueous extracts were safe up to the dose of 3000 mg/kg on oral administration. The in vivo antioxidant activity studies on the root tubers showed that the alcohol and aqueous extracts in the dose levels of 200 and 400 mg/kg exhibited significant increase in catalase and superoxide levels (p < 0.01) in CCl4 intoxicated rats. Simultaneously, the levels of MDA in brain were significantly decreased (p < 0.01) in the extract treated groups when compared with the positive control (Table 2).

A. gonoclados contains phenolic compounds, flavonoids saponins, alkaloids, glycosides and tannins. HPTLC fingerprinting revealed the presence of Shatavarin IV in alcohol and aqueous extracts of A. gonoclados. It was reported that saponins “Shatavari I-IV”, phenolic compounds and alkaloids are present in A. Racemosus [10], which also possesses antioxidant properties [9, 22]. Further, the phytochemical constituents of A. gonoclados (present study) and A. racemosus are also similar; the present findings thus support the earlier findings on in vitro and in vivo studies [23, 24].

4. CONCLUSION
A. gonoclados can be a good substitute for A. racemosus, which is the accepted botanical source of Shatavari.

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

Table 1. In vitro antioxidant activity of alcohol and aqueous extracts of A. gonoclados

<table>
<thead>
<tr>
<th></th>
<th>IC50 (µg/ml) DPPH</th>
<th>IC50 (µg/ml) Superoxide anion</th>
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<tbody>
<tr>
<td>Alcohol ext</td>
<td>Aqueous Ext</td>
<td>Ascorbic acid</td>
</tr>
<tr>
<td>150.61±1.065</td>
<td>171.27±0.95</td>
<td>3.24± 0.19</td>
</tr>
</tbody>
</table>
Fig 1. Spectrum showing λmax at 425 nm for Shatavarin IV standard and in extract of Asparagus gonoclados

Table 2. In vivo antioxidant activity of alcohol and aqueous extracts of A. gonoclados
Values expressed as Mean ± SEM. Results obtained by comparison with positive control.
ONE WAY ANOVA  p value 0.01; Dunnett multiple comparison test * * p < 0.01

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA NMOL/100mg</th>
<th>Catalase µm/min/mg</th>
<th>SOD U/mg proteins</th>
<th>Total protein mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>991.253 ± 1.58</td>
<td>64.385 ± 1.326</td>
<td>49.483 ± 2.884</td>
<td>0.691 ± 0.012</td>
</tr>
<tr>
<td>Positive control (CCLi ip)</td>
<td>1874.75 ± 9.428</td>
<td>36.758 ± 0.980</td>
<td>31.665 ± 0.635</td>
<td>1.383 ± 0.047</td>
</tr>
<tr>
<td>Standard, (Vit E, 50 mg/kg)</td>
<td>1208.35 ± 1.449**</td>
<td>60.343 ± 0.551**</td>
<td>51.145 ± 1.935**</td>
<td>0.766 ± 0.015**</td>
</tr>
<tr>
<td>Alc Extract (200 mg/kg)</td>
<td>1285.013 ± 0.565**</td>
<td>42.896 ± 0.691**</td>
<td>41.008 ± 1.851**</td>
<td>0.82 ± 0.029**</td>
</tr>
<tr>
<td>Alc Extract (400 mg/kg)</td>
<td>1291.076 ± 43.43**</td>
<td>55.061 ± 0.582**</td>
<td>43.821 ± 2.279**</td>
<td>0.78 ± 0.026**</td>
</tr>
<tr>
<td>Aq Extract (200 mg/kg)</td>
<td>1283.63 ± 3.604**</td>
<td>43.22 ± 0.750**</td>
<td>38.665 ± 2.151**</td>
<td>0.816 ± 0.023**</td>
</tr>
<tr>
<td>Aq Extract (400 mg/kg)</td>
<td>1262.048 ± 3.554**</td>
<td>56.41 ± 0.815**</td>
<td>39.856 ± 2.334**</td>
<td>0.773 ± 0.021**</td>
</tr>
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REFERENCES


