EVALUATION OF ANTIASTHMATIC ACTIVITY OF ETHANOLIC EXTRACT OF GYMNEMA SYLVESTRE L. LEAVES

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ABSTRACT

Present study, ethanol extract of G. sylvestre leaves was evaluated for antiasthmatic activity using histamine and acetylcholine-induced bronchospasm, mast cell degranulation and histamine induced constriction on isolated guinea pig tracheal chain at different dose levels. Student's t-Test and Dunnett's test were used for statistical analysis. The result of present investigation showed that the ethanolic extract of G. sylvestre significantly (P<0.001) decreased the bronchospasm induced by histamine, acetylcholine and protected mast cell degranulation as compared to control groups. It also decreased the histamine induce constriction on isolated guinea pig trachea in dose-dependent manner. The present study concludes that the antiasthmatic activity of ethanolic extract of G. sylvestre leaves may be due to the presence of flavonoids or steroids. Antiasthmatic action of the G. sylvestre could be due to its antihistaminic, anticholinergic and mast-cell-stabilizing property.

KEY WORDS: G. sylvestre, bronchospasm, mast cell degranulation, compound 48/80.
INTRODUCTION
Asthma is one of the most common disorders characterized by airway inflammation. It can be caused by various factors like allergens, drugs, respiratory infection, dust, cold air, exercise, emotions, occupational stimuli, chemicals, histamine, etc. *G. sylvestre* belong to the family Apocynacea, derives from the Greek words "gymnos" (γυμνός) and "nēma" (νῆμα) meaning "naked" and "thread" respectively; the species epithton sylvestre means "of the forest" in Latin. *G. sylvestre* has been used in herbal medicine as a treatment for diabetes for nearly two millennia,[1] and though there is insufficient scientific evidence to draw definitive conclusions about its efficacy,[2] two small clinical trials have shown gymnema to reduce glycosylated haemoglobin levels.[3] Common names include gymnema, cowplant, Australian cowplant,[4] The effects of the herb are not entirely known. *Gymnema* reduces the taste of sugar when it is placed in the mouth. From extract of the leaves were isolated glycosides known as gymnemic acids, which exhibit anti-sweet activity.[5] This effect lasts up to about 2 hours. Some postulate that the herb may block sugar receptors on the tongue. This effect was observed in isolated rat neurons.[8] The active ingredients are thought to be the family of compounds related to gymnemic acid: purified gymnemic acids are widely used as experimental reagents in taste physiology[9] and have also an anti-diabetic effect in animal models,[10] reduce intestinal transport of maltose in rats when combined with acarbose,[11] and reduce absorption of free oleic acid in rats.[12] Historically, the leaves were used for stomach ailments, constipation, water retention, and liver disease; however, these claims are not supported by scientific studies.[13] A water-soluble extract of *G. sylvestre* caused reversible increases in intracellular calcium and insulin secretion in mouse and human β-cells when used at a concentration (0.125 mg/ml) without compromising cell viability. This invitro data suggests that extracts derived from *G. sylvestre* may be useful as therapeutic agents for the stimulation of insulin secretion in individuals with type 2 diabetes[14] the rise in insulin levels may be due to regeneration of the cells in the pancreas.[15] *G. sylvestre* can also help prevent adrenal hormones from stimulating the liver to produce glucose in mice, thereby reducing blood sugar levels.[16-17] Clinical trials with type 2 diabetics in India have used 400 mg per day of water-soluble acidic fraction of the *Gymnema* leaves administered for 18–20 months as a supplement to the conventional oral drugs. During GS4 supplementation, the patients showed a significant reduction in blood glucose, glycosylated haemoglobin and glycosylated plasma proteins, and conventional drug dosage could be decreased. Five of the 22 diabetic patients were able to discontinue their conventional drug and maintain their blood glucose homeostasis with GS4 alone. These data suggest that the beta cells may be
regenerated/repaired in Type 2 diabetic patients on GS4 supplementation. This is supported by the appearance of raised insulin levels in the serum of patients after GS4 supplementation. Though for the moment *G. sylvestre* cannot be used in place of insulin to control blood sugar by people with either type 1 or type 2 diabetes, further evidence of its positive effect is accumulating. Constituents of *G. sylvestre* are a group of oleanane-type triterpenoid saponins known as gymnemic acids. The latter contain several acylated (tigloyl, methylbutyroyl etc..) derivatives of deacetylgumnemic acid (DAGA) which is the 3-O glucuronide of gymnemagenin (3, 16, 21, 22, 23, 28-hexahydroxy-olean-12-ene). The individual gymnemic acids (saponins) include gymnemic acids I-VII, gymnemosides A-F, and gymnemasaponins. The leaves of *G. sylvestre* contain triterpene saponins belonging to oleanane and dammarane classes. The major constituents like gymnemic acids and gymnemasaponins are members of oleanane type of saponins while gymnemasides are dammarane saponins. Other phytoconstituents include anthraquinones, flavones, hentriacontane, pentatriacontane, phytin, resins, tartaric acid, formic acid, butyric acid, lupeol; β-amyl *G. sylvestre* related glycosides, stigmasterol, and calcium oxalate. The presence of alkaloids had been detected in plant extracts. Leaves of *G. sylvestre* have acidic glycosides and anthraquinones and their derivatives. On the basis of above-mentioned scientific evidences, the present study was undertaken to investigate the effect of the leaf extract of *G. sylvestre* L. for its antiasthmatic activity.

**MATERIALS AND METHODS**

**Plant Material**

Leaves of *G. sylvestre* were collected from local area of Warangal, The plant was identified and authenticated at the Department of Botany, Dr. Raju Kakatiya University, Warangal (T.S), where a plant specimen was deposited.

**Preparation of Extract**

The dried leaves were reduced to coarse powder and macerated with ethyl alcohol for 48 hours and filtrate was evaporated under reduced pressure to obtain dried extract (extractive value 9.28% w/w). Qualitative preliminary phytochemical tests were performed to find the presence of various phytochemicals in the extract. For antiasthmatic evaluation, extract was dissolved in distilled water prior to its use.

**Chemicals**

Histamine dihydrochloride, acetylcholine chloride, ketotifen, compound 48/80 were purchased from Sigma-Aldrich Chemical Co., USA. Egg albumin and other chemicals were
purchased from Himedia Laboratories Pvt. Ltd., India. All the other chemicals were of analytical grade.

**Phytochemical Screening**

Preliminary phytochemical tests were performed on ethanolic extract of *G. sylvestre* L for the presence of various phytoconstituents as per described methods.[24]

**Experimental Animals**

Wister rats (175-200 g) and guinea pigs (400-600 g) of either sex housed in standard conditions of temperature (22 ± 2°C), relative humidity (55 ± 5%) and light (12 hrs light/dark cycles) were used. They were fed with standard pellet diet and water *ad libitum*. The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of CPCSEA, Ministry of Social Justice and Empowerment, Government of India. (Protocol No. 379/01/ab/CPCSEA).

**Acute Toxicity Testing**

No mortality and the sign of toxicity were observed at the dose of 6000 mg/kg.[6] Dose selected for antiasthmatic evaluation was 250 mg/kg and 500 mg/kg.

Histamine and acetylcholine induced bronchospasm in guinea pigs.[25] Guinea pigs of either sex were divided into two groups of six animals each and exposed to 0.1% w/v of histamine dihydrochloride aerosol in histamine chamber. The progressive dyspnea was observed in animals when exposed to histamine aerosol. The end point, preconvulsion dyspnea (PCD) was determined from the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsion. As soon as PCD commenced, the animals were removed from chamber and placed in fresh air. PCD of this time was taken as day 0 value. Both groups of guinea pigs were given ethanolic extract of *G. sylvestre* L at the dose of 250 mg/kg, and 500 mg/kg, p.o. respectively, once a day for 7 days. On the 7 day 2 h after the last dose, the time for the onset of PCD was recorded as on day 0. Same procedure was followed in another set of animals (*n* = 6) for acetylcholine induce bronchospasm study using 0.5% acetylcholine chloride. The percentage increased in time of PCD was calculated using following formula.[26-27]

\[
\text{Percentage increased in time of PCD} = \left(1 - \frac{T_1}{T_2}\right) \times 100
\]

where \(T_1\) = time for PCD onset on day 0, \(T_2\) = time for PCD onset on day 7
Mast Cell Degranulation Studies.\textsuperscript{[28]}

Albino rats of either sex were divided into 6 groups of six animals each and sacrificed by cervical dislocation. The animals were immediately injected with 15 ml of prewarmed (37°C) buffered salt solution (NaCl 137 mM; KCl 2.7 mM; MgCl$_2$; 1 mM; CaCl$_2$ 0.5 mM; NaH$_2$PO$_4$ 0.4 mM; Glucose 5.6 mM; HEPES 10 mM) into the peritoneal cavity, and massaged gently in this region for 90 s, to facilitate cell recovery. A midline incision was made and the peritoneum was exposed. The pale fluid was aspirated using a blunted plastic Pasteur pipette, and collected in a plastic centrifuge tube. The fluid was then centrifuged at 1000 rpm for 5 min, and the supernatant was discarded to reveal a pale cell pellet. The cell pellets were resuspended in fresh buffer and recentrifuged.

The peritoneal cell suspension divided in six parts viz. – ve control, +ve control, reference standard (Ketotifen 10 μg/ml) and ethanolic extract of \textit{G. sylvestre} L at different concentration i.e., 600, 800, 1000 μg/ml, each containing 0.1 ml of cell suspension and incubated at constant temperature 37°C in water bath for 15 min. Then 0.1 ml of compound 48/80 was added in all samples except in – ve control group and suspensions were further incubated for 10 min at 37°C. The cells were then stained with 10% of toludine blue solution and observed under the higher magnification by microscope. The percent granulated and degranulated mast cells were counted in each group.

Guinea Pig Tracheal Chain Preparation.\textsuperscript{[29]}

Guinea pigs of either sex (200-500 g) were divided into 4 groups. Each group contains six animals and were allowed to starve overnight and free access to water. The animals were killed by a blow on the head and exsanguinated. The isolated trachea was mounted in a 30-ml organ bath containing Tyrode solution, maintained at 37 ± 1°C and gassed with air. The tissue was equilibrated for 45 min during which the bath solution was replaced every 10 min. At the end of the equilibration period, histamine (0.5 μg/ml)-induced contraction as well as effect of extract (up to 1000 μg/ml) was recorded. A drug tissue contact time of 1 min was maintained. The percent response of each groups were calculated from the height of the peaks obtained.

Statistical Analysis

The results of various studies were expressed as Mean ± SEM and analyzed using one-way ANOVA followed by Student's \textit{t}-Test to find out the level of significance. Data were considered statistically significant at minimum level of \(P<0.001\).
RESULTS
Preliminary qualitative phytochemical screening of ethanolic extract of *G. sylvestre* L showed the presence of flavonoids, phenols, steroids, tannins, terpenes, xanthoproteins and sugar.*G. sylvestre* L significantly (*P*<0.001) increased the preconvulsive dyspnoea time following exposure to histamine and acetylcholine aerosols induced bronchospasm in guinea pigs, (Table 1). It also inhibited *in vitro* rat peritoneal mast cell degranulation significantly (*P*<0.001) induced by compound 48/80 as compared to base line value(Table 2). The isolated guinea pig tracheal chain preparation showed dose dependent significant (*P*<0.001) inhibition of the contraction of tracheal muscles induced by histamine as compared to control group (Table 3.)

DISCUSSION
Bronchodilating effect of ethanolic extract of *G. sylvestre* L was evaluated by observing its effects on the time of PCD. In our study we found that the time of occurrence of PCD was significantly increased suggestive of bronchodilating activity of *G. sylvestre* L against spasmogens. Mast cell degranulation is important in the initiation of immediate responses following exposure to allergens. An attempt was made to find out whether ethanolic extract of *G. sylvestre* L has any effect on the rate of disruption of mast cells following exposure to compound 48/80, an agent which causes histamine release. The present study, *G. sylvestre* L offered significant protection against Compound 48/80 induced mast cell degranulation by stabilizing it, which is responsible for the decreasing airway inflammation by preventing release of various inflammatory mediators. The relaxant effects of ethanolic extract of *G. sylvestre* L on tracheal chains of guinea pigs might be produced by different mechanisms including stimulation of β-adrenergic receptors, inhibition of histamine H1 receptors, or an anticholinergic property of this plant. The relaxant effects of all concentrations of the extract of *G. sylvestre* L obtained were significantly lower than control group. These findings suggest probable b-adrenergic stimulatory, muscarinic and/or histamine H1 blocking properties of the plant extract. *G. sylvestre* leaves contain triterpene saponins belonging to oleanane and dammarene classes. Oleanane saponins are gymnemic acids and gymnemasaponins, while dammarene saponins are gymnemasides. Besides this, other plant constituents are anthraquinones, hentrioccontane, pentatricontane, α and β-chlophyll, phytin, resins, D-quercitol, tartaric acid, formic acid, butyric acid, lupeol, beta-amyrin –related glycosides and stigmosrerol. Our data suggest that the ethanolic extract of the leaves of *G. sylvestre* L possesses significant antianaphylactic, anticholinergic and antihistaminic activity.
However, further studies are needed to establish molecular mechanism and to isolate and characterize the active principles, which are responsible for the antiasthmatic action.

Table 1: Effect of ethanolic extract of *G. sylvestre* (p.o for 7 days) on histamine and acetylcholine – induced bronchospasm in guinea pigs

<table>
<thead>
<tr>
<th>Group</th>
<th>Histamine- Induced bronchospasm</th>
<th>Acetyl choline –induced bronchospasm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment (control)</td>
<td>After treatment</td>
</tr>
<tr>
<td>Ethanolic extract of <em>G. sylvestre</em> (250 mg/kg)</td>
<td>129.89+_1.53</td>
<td>390.36+_2.66*</td>
</tr>
<tr>
<td>Ethanolic extract of <em>G. sylvestre</em> (500 mg/kg)</td>
<td>131.66+_1.61</td>
<td>601.36+_3.25*</td>
</tr>
</tbody>
</table>

Values are expressed as MEAN+_SEM. N=6, P<0.001, as compared to control group.

Table 2: Effect of ethanolic extract of *G. sylvestre* on induced mast cell degranulation in albino rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Con (ug/ml)</th>
<th>Mast cells</th>
<th>Granulated %</th>
<th>Degranulated %</th>
</tr>
</thead>
<tbody>
<tr>
<td>-ve control</td>
<td>-</td>
<td>91.58+_1.69</td>
<td>9.41+_1.74</td>
<td></td>
</tr>
<tr>
<td>+ve control</td>
<td>-</td>
<td>24.52+_1.55</td>
<td>75.41+_1.59</td>
<td></td>
</tr>
<tr>
<td>Ketotifen</td>
<td>10</td>
<td>79.10+_3.56*</td>
<td>21.21+_3.16</td>
<td></td>
</tr>
<tr>
<td>EEGS</td>
<td>600</td>
<td>38.21+_2.65#</td>
<td>62.10+_2.24</td>
<td></td>
</tr>
<tr>
<td>EEGS</td>
<td>800</td>
<td>50.23+_2.83*</td>
<td>49.76+_2.58</td>
<td></td>
</tr>
<tr>
<td>EEGS</td>
<td>1000</td>
<td>61.72+_2.81*</td>
<td>39.29+_2.58</td>
<td></td>
</tr>
</tbody>
</table>

Ethanolic extract of *G. Sylvestre*, Values are expressed as MEAN+_SEM. N=6, *P<0.001, #P<0.01 as compared to positive control group.

Table 3: percent inhibition of *G. Sylvestre* on histamine induced contraction on isolated guinea pig trachea

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Con(um/ml)</th>
<th>Peak height</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>0.5</td>
<td>2.65+_0.10</td>
<td>-</td>
</tr>
<tr>
<td>Histamine+EEGS</td>
<td>600</td>
<td>1.69+_0.06*</td>
<td>29.65</td>
</tr>
<tr>
<td>Histamine+EEGS</td>
<td>800</td>
<td>1.51+_0.05*</td>
<td>40.66</td>
</tr>
<tr>
<td>Histamine+EEGS</td>
<td>1000</td>
<td>0.99+_0.06*</td>
<td>64.23</td>
</tr>
</tbody>
</table>

Ethanolic extract of *G. Sylvestre* Values are expressed as MEAN+_SEM. N=6, *P<0.001, #P<0.01 as compared to histamine induced group.
CONCLUSIONS
The present study concludes that the antiasthmatic activity of ethanolic extract of *G. sylvestre* leaves may be due to the presence of flavonoids or steroids. Antiasthmatic action of the *G. sylvestre* could be due to its antihistaminic, anticholinergic and mast-cell-stabilizing property.

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