Evaluation of Anti-Inflammatory, Anti-diabetic activity of Indian Bauhinia vahlii (stem bark)

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Objective: To evaluate the anti-inflammatory and antidiabetic property of Bauhinia vahlii (stem bark) with preliminary phytochemical profile of the extracts.

Methods: The dried whole plant material (1,400 g) was packed in soxhlet apparatus and extracted successively with Pet. Ether (PE) to defat the drug, petroleum ether was removed from the powdered defatted drug which was then extracted with benzene (BE), chloroform (CE) and 95% of Ethanol (EE) as increasing polarity and all extracts screened for anti-inflammatory and antidiabetic activity using carrageenan induced paw edema and streptozotacin induced diabetic respectively. The toxicity and phytochemical screening were done using standard procedure.

Result: The preliminary phytochemical tests revealed the presence of alkaloids, flavonoids, phytosterol, phenolic compounds, and glycoside. While carbohydrates, protein, gums and amino acids were absent. The acute toxicity study of various extracts of Bauhinia vahlii was conducted and dose of 353 mg/kg is fixed for anti-inflammatory and antidiabetic property. The pet ether, chloroform and ethanolic extract of Bauhinia vahlii significantly decreased the paw edema induced by carrageenin in rats at a dose of 353 mg/kg comparable to standard ibuprofen (100 mg/kg). Similarly in case of antidiabetic property, the ethanolic and chloroform extract of Bauhinia vahlii at a dose level 353 mg/kg, showed significant reduction in blood sugar level from 2 to 24 h in progressive manner comparable to standard glibenclamide (5mg/kg).

1. Introduction

Plants are indispensable sources of medicine since time immemorial. Studies on natural product are aimed to determine medicinal values of plants by exploration of existing scientific knowledge, traditional uses and discovery of potential chemotherapeutic agents. Phytochemicals are used as templates for lead optimization programs, which are intended to make safe and effective drugs [1].

Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses [2]. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can easily be induced [2]. The side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical uses [3]. Therefore, the development of newer and more potent anti-inflammatory drugs with lesser side effects is necessary.

Diabetes mellitus is a group of disorders with different aetiologies. It is characterized by derangements in carbohydrate, protein and fat metabolism caused by the complete or relative insufficiency of insulin secretion and/or insulin action [4]. Approximately 140 million people worldwide suffer from diabetes [5]. The disease becomes a real problem of public health in developing countries, where its prevalence is increasing steadily and adequate treatment is often expensive or unavailable[6]. Alternative strategies to the current modern pharmacotherapy of diabetes mellitus are urgently needed [7], because of the inability of existing modern therapies to control all the pathological aspects of the disorder, as well as the enormous cost and poor availability of the modern therapies for many rural populations in developing countries.

Diabetes mellitus is ranked seventh among the leading
causes of death and third when it’s fatal complications are taken into account [8]. Traditional preparations of plant sources are widely used almost everywhere in the world to treat this disease. Therefore, plant materials are considered to be the alternative sources for finding out new leads for hypo-/anti-hyperglycemic agents.

*Bauhinia vahlii* [9] is a gigantic climbing evergreen tree and largest creeper in India and can grow up to 10–30 m long. The woody stem can get as thick as 20 cm. The spreading stout branches are covered with rusty fine hair. Branchlets [10] densely pubescent and terminating in a pair of revolute tendrils; young branches, tendrils, petioles, underside of leaves especially along the nerves and inflorescence clothed with dense ferruginous tomentum. Leaves [11] very variable in size, often up to 18 in. diam., as broad as long or broader, deeply cordate, 11–15 nerved, cleft through about 1/3 of the length, sub-coriaceous, dark green and glabrescent above more or less downy beneath; lobes obtuse, rounded; petiole 3–6 in. long, stout. Flowers [11] white, on long slender pedicles, in terminal corymbose or corymbose racemes.

Fruit [12] is a flat woody pod with fine rusty hairs, 20–30 cm long. The plant has been reported to have agathisf flavonoid, flavonoid, betulinic acid, triterpene, campesterol and steroid in leaves [13], catechin, gallic acid methyl ester, benzenoid, moplanol and 4-O-methyl ester [13]. The plant is also reported to contain kaemferol, quercetin, rutin, betulinic acid [14] and Quercitrin, Stigmasterol, Sitosterol [16] in leaves.

The purpose of the present study was to evaluate the anti-inflammatory, anti-diabetic activity of *Bauhinia vahlii* stem bark extracts using carrageenan induced rat paw edema and Streptozotocin induced diabetic. The extracts were also studied for its acute toxicity effects and preliminary phytochemical screening.

### 2. Materials and methods

#### 2.1 Materials

Streptozotocin (STZ) was purchased from Sigma Chemicals Co. (St. Louis, MO, USA), Carrageenan was procured from Himedia laboratories, Mumbai, India. All other chemicals and solvents (Pet ether, Benzene, Chloroform and Ethanol) used were of analytical grade and obtained commercially from Merck–Limited, India, Mumbai.

#### 2.2 Collection and identification of plant material

The Plant *Bauhinia vahlii* were collected in the month of October from Padiabahal, Sambalpur, Odisha, India. The plant material was taxonomically identified by Dr. (Mrs.) Uma Devi, Head, Department of Botany, Govt. Women’s College, Sambalpur, Odisha. A voucher specimen (GWC/B-315/09) has been deposited in the Herbarium of the Department of School of Pharmaceutical Education & Research, Berhampur University, Berhampur–760007, India for future reference.

#### 2.3. Experimental animals

Swiss albino mice (20–25 g) and Male Wistar rats (150–200 g) were purchased from the animal house of Gosh enterprises, Kolkata and housed in polypropylene cages at room temperature with proper ventilation. Prior to the experiments, mice and rats were fed with standard diet for 1 week in order to adapt to laboratory conditions. They were fasted over night but allowed free access to water before the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee (Reg. No.1339/ac/10/ CPCSEA).

#### 2.4. Preparation of plant material

The whole plant was first sun dried for several weeks, crushed by hands and dried again. Then the crushed parts of the plant were ground into coarse powder with the help of a mechanical grinder. By using the concept of the nature of solubility and distribution of the active ingredients, powdered material (1400 g) was packed in soxhlet apparatus [17] and extracted successively with Pet. Ether (60–80), to defat the drug, petroleum ether was removed from the powdered defatted drug which was then extracted with benzene, chloroform and 95% of Ethanol as increasing polarity. The whole each mixture then underwent filtration through what man filter paper. The filtrates (Pet. Ether, Benzene, Chloroform and Ethanol filtrate) obtained were evaporated by rotary evaporator at 5 to 6 rpm and at 40 °C temperature. It rendered a gummy concentrates. The gummy concentrate was designated as crude extract of respective solvent which was then freeze dried and preserved at 4 °C.

#### 2.5. Phytochemical screening

Qualitative phytochemical tests for the identification of alkaloids, flavonoids, steroids, glycosides, saponins, tannins and terpenoids were carried out for all the extracts by standard procedure [18-21] (Table 1).

#### 2.6 Acute toxicity study (LD₅₀)

Acute toxicity study [22–23] was performed according to Organisation for Economic Co–operation and Development was performed as per Karber’s method. Swiss albino mice of either sex weighing between 20–25 g were divided into six groups with six animals each and of 90 d were used to determine LD₅₀ of various extracts of *Bauhinia vahlii*. The gum acacia solution (2%) was used as a vehicle to suspend various extracts. The extracts were administered orally at different dose levels to group of six mice each, which have
been fasted overnight. The LD50 values of various extracts are calculate and dose is fixed as 353 mg/kg.

2.7. Experimental design

Thirty-six experimental animals were randomly selected and divided into six groups denoted as Group I, Group II, Group III, Group IV, Group V and Group VI, consisting of 6 Wistar rats in each group. Each group received a particular treatment i.e. control, standard and the four doses of the extract. Prior to any treatment, each rat was weighed properly and the doses of the test samples and control materials were adjusted accordingly. Group III to Group VI received the crude extract orally at the doses of 353 mg/kg of body weight. Group II received intraperitoneal administration of Ibuprofen at a dose of 100 mg/kg as standard for anti-inflammatory study, while Group I was kept as control giving 2% gum acacia solution in normal saline water.

Similarly forty-two animals were selected and divided into seven groups denoted as Group I, Group II, Group III, Group IV, Group V, Group VI and Group VII consisting of 6 Wistar rats in each group. Each group received a particular treatment i.e. normal control, diabetic control, standard and the four doses of the extract. Prior to any treatment, each rat was weighed properly and the doses of the test samples and control materials were adjusted accordingly. Group IV to Group VII received the crude extract orally at the doses of 353 mg/kg of body weight. Group III received intraperitoneal administration of Glibenclamide at a dose of 5 mg/kg as standard drug for anti-diabetic study, while Group II was kept as diabetic control giving 2% gum acacia solution in normal saline water.

2.8. Anti-inflammatory Activity

The anti-inflammatory activity of Bauhinia vahlii was studied using acute (carrageenan induced paw edema) models of inflammation. The experiment protocols were approved by the Institutional Animal Ethics Committee prior to the conduct of the animal experiments (Reg. No.1339/ac/10/CPCSEA).

This model is based on the principle of release of various inflammatory mediators by carrageenan. Edema formation due to carrageenan in the rat paw is biphasic event. The initial phase is attributed to the release of histamine and serotonin. The second phase of edema is due to the release of prostaglandins, protease and lysosome[24,25]. Subcutaneous injection of carrageenan into the rat paw produces inflammation resulting from plasma extravasations, increased tissue water and plasma protein exudation along with neutrophil extravasations, all due to the metabolism of arachidonic acid[26]. The first phase begins immediately after injection of carrageenan and diminishes in two hours. The second phase begins at the end of first phase and remains through third hour up to five hours.

2.9. Method: Carrageenan-Induced Paw Edema in Rats

Anti-inflammatory activity was evaluated using the Carrageenan induced rat paw oedema according to the technique of Winter et al [26-31]. The animals were housed in cages under standard laboratory condition. They had free access to standard diet and water. The animal were divided into 6 groups of six animals each and fasted for 12 h before the experiment. The initial right hind paw volume of the rats were measured using a plethysmometer and then 0.1 mL of 1% w/v carrageenan solution in normal saline was subcutaneously injected into the sub plantar region of the right hind paw. The volume of right hind paw was measured at 1, 2, 3, and 4 h after carrageenan injection, and the paw volume was determined. The data were expressed as paw volume (ml), compared with the initial hind paw volume of each rat. Cosolvent (2% gum acacia solution, p.o) and ibuprofen (10 mg/kg, p.o) [25] was administered 30 min before carrageenan injection. The group received Co solvent was treated as control. The hind paw volume was measured plethysmometrically before and after the carrageenan injection, at hourly intervals for 4 h (Table 2).

\[
\% \text{ inhibition of edema} = \frac{V_c - V_t}{V_c} \times 100
\]

Where, \( V_t \) = mean paw volume of test group
\( V_c \) = mean paw volume of control group

2.10. Anti diabetic Activities

2.11. Induction of diabetes in rats [32-33]

Rats were made diabetic with an intraperitoneal injection of Streptozotocin (STZ 60 mg/kg body weight) dissolved in citrate buffer (0.1 M, pH 4.5). Diabetes was confirmed in STZ rats by measuring the fasting blood glucose level 48 h after the injection of STZ. Rats with blood glucose level above 250 mg/dL were considered to be diabetic and were used in this experiment.

2.12. Estimation of blood glucose

Blood samples were collected from the tail tips at 12, 24, 36 and 48 h after streptozotocin administration and found that stable hyperglycemia produced after 48 h. The blood sugar level was measured by digital display glucometer (One touch –Johnson & Johnson Ltd.). Initial blood sample were taken before the oral administration of the standard drug (Glibenclamide) and extracts. The blood glucose levels were measured at 2, 4, 8, 12 and 24 h after oral administration of Glibenclamide and various extracts of Bauhinia vahlii (Table 3).

2.13. Statistical Analysis

For Anti-inflammatory screening all experimental results were expressed as mean±SEM and data were assessed by
ANOVA Method followed by student’s t–test. P<0.05 was considered as statistically significant. While for Anti–diabetic activity, all results are expressed as mean± standard error. The data was analyzed statistically using ANOVA followed by Dunnett’s Multiple Comparison Test using SPSS 10.0 statistical software. The level of significance was fixed at 5%.

3. Result

3.1. Phytochemical constituents of the plant

The preliminary phytochemical tests revealed the presence of alkaloids, flavonoids, phytosterol, phenolic compounds, and glycoside. While carbohydrates, protein, gums and amino acids were absent (Table 1).

3.2. Acute toxicity study

The acute toxicity study of various extracts of Bauhinia vahlii was conducted and the LD₅₀ of all extracts found to lay between 3 000–4 000 mg/kg body weight. The LD₅₀ is in a higher dose range so the extracts are safe and 1/10 average of all extracts i.e. 353 mg/kg was taken as dose for pharmacological screening procedure.

3.3. Anti–inflammatory activity

The pet ether, chloroform and ethanolic extract of Bauhinia vahlii significantly decreased the paw edema induced by carrageenin in rats at a dose of 353 mg/kg comparable to standard ibuprofen (100 mg/kg) shown in Table 2.

3.4. Antidiabetic activity

The result of antidiabetic effect depicted in Table 3. From the table it is revealed that the ethanolic and chloroform extract of Bauhinia vahlii at a dose level 353 mg/kg, showed significant reduction in blood sugar level from 2 to 24 h in progressive manner comparable to standard glibenclamide.

4. Discussion

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury. Steroidal anti–inflammatory agents will lyse and possibly induce the redistribution of lymphocytes, which cause rapid and transient decrease in peripheral blood lymphocyte counts to affect longer term response. B. vahlii belongs to family Caesalpiniaceae is a common plant of Western Odisha. Phytochemical evaluation of the various extracts of Bauhinia vahlii reveals the presence of alkaloids, flavonoids, phytosterol, phenolic

### Table 1
Preliminary Phytochemical Investigation of various extracts and powder of Bauhinia vahlii.

<table>
<thead>
<tr>
<th>Test</th>
<th>PD</th>
<th>PEE</th>
<th>BE</th>
<th>CE</th>
<th>EE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test for Carbohydrates</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Test for Gums and Mucilages</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Test for Proteins and Amino Acid</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Test for Fixed Oils and Fats</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Test for Phytosterols</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Test for Glycosides</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Tests for Saponins</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Tests for Flavonoids</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tests for Alkaloids</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Tests for Tannins and Phenolic Compounds</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

### Table 2
Anti–inflammatory activity of various extracts of Bauhinia vahlii (Stem bark).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(2% gum acacia)</td>
<td>−</td>
<td>0.48±0.03</td>
<td>0.78±0.09</td>
<td>0.85±0.12</td>
<td>0.89±0.14</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>100</td>
<td>0.29±0.07 (39.6)</td>
<td>0.28±0.07 (64.1)</td>
<td>0.24±0.06 (71.80)</td>
<td>0.23±0.13 (74.2)</td>
</tr>
<tr>
<td>Pet.Ether. Ext</td>
<td>325</td>
<td>0.33±0.13 (31.25)</td>
<td>0.41±0.09 (47.4)</td>
<td>0.49±0.01 (42.4)</td>
<td>0.42±0.07 (52.8)</td>
</tr>
<tr>
<td>Benzene. Ext</td>
<td>325</td>
<td>0.45±0.09 (6.3)</td>
<td>0.73±0.12 (6.4)</td>
<td>0.78±0.15 (8.2)</td>
<td>0.78±0.11 (12.3)</td>
</tr>
<tr>
<td>Chloroform. Ext</td>
<td>325</td>
<td>0.36±0.07 (25)</td>
<td>0.34±0.09 (56.4)</td>
<td>0.35±0.07 (58.8)</td>
<td>0.32±0.11 (64)</td>
</tr>
<tr>
<td>Ethanol. Ext</td>
<td>325</td>
<td>0.36±0.12 (25)</td>
<td>0.42±0.13 (46.2)</td>
<td>0.57±0.17 (33)</td>
<td>0.50±0.09 (43.8)</td>
</tr>
</tbody>
</table>

Each value is mean±SEM (n=6), *Denotes significant difference compared to control value at P<0.05.
compounds, and glycoside. Here anti-inflammatory activity was performed based on the folk lore information by using the method, Carrageenan induced inflammation model for the estimation of anti-inflammatory effect. The development of oedema in the paw of the rat after the injection of Carrageenan is due to the release of histamine, serotonin, and prostaglandin [38–39]. Stem bark extracts of B. vahlii showed significant anti-inflammatory activity. This significant anti-inflammatory effect may be due to the inhibition of any inflammatory mediators by the glycosides or steroids [39] present in the extract. Similarly the treatment of diabetes with medicines of plant origin that proved much safer than synthetic drugs is an integral part of many cultures throughout the world and gained importance in recent years. India has a rich history of using various potent herbs and herbal components for treating various diseases diabetes [34] and several species of plants have been described as having a hypoglycemic activity [35–37].

The anti-inflammatory activity in carrageenan–induce rat paw edema and antidiabetic activity in streptozotocin–induced diabetic rats of Bauhinia vahlii were monitored at a dose level 353 mg/kg for a time interval of 1 hr to 4 h and 2 h to 24 h respectively. From evaluation it was concluded that the Pet.ether, chloroform and Ethanol extract shows significant reduction of inflammation as compared to Ibuprofen (P<0.05) and Chloroform and Ethanol extracts cause a significant decrease in blood glucose level as compared to standard drug Glibenclamide (P<0.05).

The present result indicates the efficacy of Bauhinia vahlii as an effective therapeutic agent in the treatment of acute inflammations and diabetic and also the present study authenticifies the folk lore information on the anti-inflammatory and antidiabetic property of the plant Bauhinia vahlii. Further and detailed studies are in process for the isolation of active constituent responsible for this property and to identification of the possible mechanism for the said properties.

Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Time (h)</th>
<th>Blood Sugar level in mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 h</td>
</tr>
<tr>
<td>I</td>
<td>Normal control</td>
<td>–</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>2% Gum acacia solution</td>
</tr>
<tr>
<td>III</td>
<td>Glibenclamide</td>
<td>5mg / kg /Day</td>
</tr>
<tr>
<td>IV</td>
<td>Pet. Ether Ext</td>
<td>320mg/kg</td>
</tr>
<tr>
<td>V</td>
<td>Benzene. Ext</td>
<td>320mg/kg</td>
</tr>
<tr>
<td>VI</td>
<td>Chloroform. Ext</td>
<td>320mg/kg</td>
</tr>
<tr>
<td>VII</td>
<td>Ethanol. Ext</td>
<td>320mg/kg</td>
</tr>
</tbody>
</table>

All values are expressed as mean± SEM, n=6, *P<0.05 significant compared to control.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgement

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