RESEARCH ARTICLE

Effect of *Bauhinia purpurea* Linn. on Alloxan-induced diabetic rats and isolated Frog's heart

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In the present study, the ethanolic extract and a purified fraction-1of stem of *Bauhinia purpurea* Linn. (BP) were investigated for anti-diabetic activity and adrenergic property. The anti-diabetic activity of the extract and fraction-1 was compared with standard insulin and adrenergic property was compared with sympathetic agonists and its blocker. The results demonstrated that the percentage of changes of FBS at the dose of 100 mg/kg. (i.p.) of ethanolic extract of BP and fraction-1 were statistically significant ($p < 0.001$). The same fraction exhibited excellent adrenergic activity (10 mg/ml). This was further confirmed as its action was blocked by an adrenergic β_2-blocker (propranolol).

Key words: Adrenaline, anti-diabetic activity, alloxan, *Bauhinia purpurea* Linn., insulin, propranolol

INTRODUCTION

Bauhinia purpurea Linn. (Leguminosae) is a medium-sized deciduous tree, sparingly grown in India. Traditionally this plant is used in the treatment of dropsy, pain, rheumatism, convulsions, delirium, septicemia, etc.[1] The bark of the plant is used as an astringent in the treatment of diarrhoea. Its decoctions are recommended for ulcers as a useful wash.[2] The aerial parts of the plant are reported to contain flavanone glycosides, foliar flavonoids, 6-butyl-3-hydroxy flavanone, amino acids, phenyl fatty ester, lutine and β- sitosterol.[3-8] Flavonoids are polyphenolic compounds, widely distributed in the plant kingdom. They are reported to exhibit various pharmacological activities such as CNS activity, cardiotonic activity, lipid-lowering activity, anti-oxidant activity, hepatoprotective activity, hypoglycemic activity, etc.,[9] These active constituents and the above-mentioned activities in turn appear to correlate with some other biological activities. Our literature survey revealed that the anti-diabetic and cardiac activities were not investigated; hence these activities have been investigated in the present study.

MATERIALS AND METHODS

Plant Material

The plant *Bauhinia purpurea* Linn. (BP) was identified (voucher specimen: 5/2005 and preserved in the Department of Pharmacognosy, Bapuji Pharmacy College, Davangere.) by the taxonomist of Botany department of DRM Science College, Kuvempu University and Department of Pharmacognosy, Bapuji Pharmacy College, Davangere, India. The stem portions of the plant were collected from Alagilawada, Davangere District, Karnataka State and air dried. Subsequently, it was coarsely powdered and used for obtaining the extracts.

Preparation of the Extract

The coarse powder of the air-dried stem was subjected to successive solvent extraction method using solvents of increasing polarity [petroleum ether - (60°-80°C), chloroform, ethanol] in a Soxhlet extraction unit till exhaustion, and finally, an aqueous extract was prepared using chloroform water by simple maceration at room temperature. Each extract was carefully evaporated in a rotary evaporator under controlled temperature and reduced pressure to obtain the dried extract and the yield is shown in Table 1.

Phytochemical screening[10-12] - Each extract was subjected to phytochemical screening, and the results are shown in Table 2.

Fractionation

The ethanolic extract (30 g) was dispersed in 250 ml of distilled water and subjected to fractionation using ethyl acetate, n-butanol and ethyl methyl ketone. The yields were 30, 40, 20 and 10% (residue) respectively with reference to the ethanolic extract. Then the n-butanol fraction (11.8 g) was subjected to chromatography over silica gel column with a length and diameter of 55 cm and 6 cm, respectively. Elution was carried out with solvent

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Received: 21-11-2007; **Accepted:** 22-01-2007

Table 1: Yield, colour and consistency of the extracts

Extract	Yield/kg	Colour	Consistency
Petroleum ether	4.5 g	Dark brown	Semisolid
Chloroform	3 g	Dark brown	Amorphous powder
Ethanolic	22 g	Reddish brown	Powder
Aqueous	33 g	Reddish brown	Powder

Table 2: Summary of phytochemical investigations

Extract	Constituents present
Petroleum ether extract	Steroids
Chloroform extract	Steroids, Free Anthraquinones
Ethanolic extract	flavonoids, tannins, steroids, coumarins carbohydrates, reducing sugars
Aqueous extract	Carbohydrates, reducing sugars flavonoids, tannins

mixtures of increasing polarities. Fractions were collected in 100 ml portions and monitored with TLC[12] (silica gel G as adsorbent; solvent system - ethyl acetate:formic acid: glacial acetic acid:water = 100:11:1:27; UV fluorescence was used for visualization) and the fractions showing similar spots were pooled together. Fraction-1 which was eluted with ethyl acetate: methanol (80:20) had shown positive Shinoda test, and its UV spectrum had shown the peaks at 279.40, 324.40, and 223.40 nm, which are matching with the literature values of flavanones.[11] Further studies on the characterization of this isolated compound based on spectral analysis are under progress.

The extract and fraction-1 were dissolved in distilled water and used for investigating anti-diabetic activity and adrenergic activity.

Animals

Wister rats (either sex) weighing between 150 and 200 g were used for anti-diabetic activity and frogs were used for isolated heart experiment. Toxicity study was carried out on Albino mice (25-30 g) as per the OECD (No. 420) guidelines and the study was approved by the Institutional Animals Ethics Committee (CPCSEA).

Anti-diabetic Activity

Wister rats were made diabetic by injecting alloxan monohydrate intraperitoneally with a dose of 60 mg/kg, body weight in chilled citrate buffer (pH 4.5). After 48 h, the rats showing blood glucose levels of 250-350 mg/dl were considered as diabetic and were employed in the study. The rats were housed in polyethylene cages and divided into 6 groups of six animals each.

Group I: Served as solvent control.
Group II: Served as diabetic control (Alloxan-induced)
Group III: Received insulin 0.6 U/kg, S.C.,
Group IV: Received ethanolic extract of BP (100 mg/kg, i.p.)
Group V: Received ethanolic extract of BP (50 mg/kg, i.p.)
Group VI: Received fraction - 1(100 mg/kg, i.p.) for 2 weeks.

Collection of Blood and Determination of Serum Glucose

On the 0, 1 and 2 weeks, the animals were fasted for 8 h and the blood samples were drawn by orbital sinus puncture under mild ether anaesthesia. The blood samples were collected in Eppendroff's tubes that contained 50 µl of anti-coagulant (EDTA). Plasma was separated by centrifugation at 5000 rpm for 10 min and analyzed for glucose content in Autoanalyser Microlab by enzymatic method[13,14] (GOD/POD method-Beacon-Diagnostic PVT.LTD, India).

Statistical Analysis

The results were expressed as Mean ± SEM. Comparison between the groups was made by Analysis of variance (ANOVA), followed by Dunnett's test. A value of $p < 0.001$ was considered significant.

Cardiac Activity[15,16]

Heart and great vessels of a pithed frog were exposed. Pericardium was removed. Through the apex of the heart, a bent pin was passed and connected to startling heart lever with thread. Sinus venous was exposed. Sym's cannula was introduced into the sinus and ligature was applied with the help of aneurysm needle. The cannula was directed towards the heart and the ligature was tightened firmly at the neck of the cannula. The cannula was then connected to Mariotte flask with a rubber tube and perfusion was started using with Frog's ringer solution and the preparation was allowed to stabilize. Normal contractions were recorded, followed by perfusion, with:

1. Epinephrine (10 µg/ml).
2. Nor-epinephrine (10 µg/ml).
3. Calcium chloride (10 mg/ml).
4. Potassium chloride (10 mg/ml).
5. Ethanolic extract of B.P (10 mg/ml).
6. Ethanolic extract of BP + adrenaline and
7. Ethanolic extract + propranolol (10 mg/ml).

Finally, normal contractions were recorded with frog's ringer.

RESULTS AND DISCUSSION

The Effect of Ethanolic Extract and Purified Fraction-1 of BP on Alloxan-induced Diabetic Rats

The extract and the fraction-1 exhibited anti-diabetic property in alloxan-induced diabetic rats, as evident from the serum glucose levels. The hypoglycemic activity may be ascribed to the presence of flavonoids, which have been shown to inhibit cyclooxygenases[9] and promote β-cell regeneration besides having insulin secretary property.[17-19]

Table 3: Hypoglycemic activity of ethanolic extract and purified fraction-1 of BP

Group		Serum glucose mg/dl				
		0 week	1st week	% reduction	2nd week	% reduction
I	Normal control	81.8 ± 1.2	81.3 ± 0.8	0.5	83.2 ± 1.0	+1.8
II	Diabetic control (60 mg/kg, i.p.)	255.0 ± 2.2	285.0 ± 2.2	−11.8	331.7 ± 7.5	−30
III	Diabetic control + insulin (0.6 U/kg, s.c.)	258.3 ± 3.1	154.2 ± 2.0**	40.3	119.2 ± 2.0**	54
IV	Ethanolic extract of BP (100 mg/kg, i.p.)	249.2 ± 2.7	174.0 ± 5.3**	30.2	125.8 ± 1.5**	49.5
V	Ethanolic extract of BP (50 mg/kg, i.p.)	249.2 ± 2.7	212.5 ± 4.0**	14.7	200 ± 2.6**	19.7
VI	Fraction-I (100 mg/kg, i.p.)	251.7 ± 3.1	152.3 ± 1.8**	39.5	125.0 ± 1.8**	50.3

One-way ANOVA F 2.04, 271.2, 570.0 p 0.12, NS $P < 0.001$, $P < 0.001$, Values are mean ± SEM: $n = 6$ in each group. Df = 4, 25 **$P < 0.001$ as compared to diabetic control

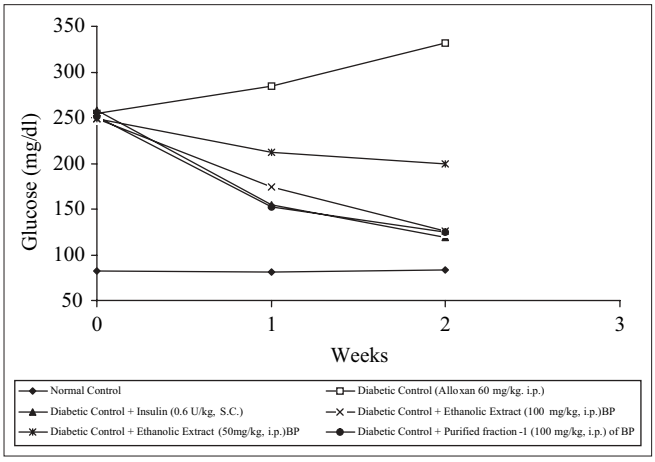

Figure 1: The effect of ethanolic extract of BP and its purified fraction-1 on FBS in alloxan-induced diabetic rats

The results of the present study suggested that ethanolic extract and fraction-1 illustrate significant hypoglycemic activity ($P < 0.001$)- Table 3 and [Fig. 1].

The Effect of the Purified Fraction-1 of Ethanolic Extract of BP on Isolated Frog's Heart

The tracing shows that epinephrine and nor-epinephrine produced an increase in the heart rate and force of contraction. Epinephrine is a hormone, secreted by the adrenal medulla, released predominantly in response to hypoglycaemia. As the test sample had exhibited the hypoglycaemic activity that indicates the release and potentiation of the action of epinephrine. The test sample drastically increased the oxygen demand and lead to tachycardia. Its action was blocked by the β_2-adrenargic blocker propranolol [Fig. 2]. The cardiotonic activity exhibited by the fraction-1 is probably due the presence of flavonoids as they have been reported to have significant action on heart.[20,21] Similarly, the cardiotoxicity of any compound may be inhibited by the flavonoids. Based on these results, it is indicative that the fraction-1 has exhibited positive inotropic and chronotropic effect on an isolated frog's heart. The characterization of the isolated compound based on structural studies is under progress; moreover, it promises a lot of scope for further envisage on its cardiac activity.

N = normal
1 = Epinephrine 10 µg/ml.
2 = Nor-epinephrine 10 µg/ml.
3 = Calcium chloride 10 mg/ml.
4 = Potassium chloride (10mg/ml).
5 = Ethanolic extract of B.P (10 mg/ml).
6 = Ethanolic extract of BP + adrenaline.
7 = Ethanolic extract + propranolol (10 mg/ml)

Figure 2: Effect of fraction-1 of ethanolic extract of BP on isolated frog's heart

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Source of Support: Nil, **Conflict of Interest:** None declared.

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