

SPASMOLYTIC AND HYPOGLYCEMIC EFFECTS OF DIFFERENT FRACTIONS ISOLATED FROM METHANOLIC EXTRACT OF WHOLE PLANT OF *HYGROPHILA AURICULATA* IN WISTAR ALBINO RATS

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Received: 17 Oct 2013, Revised and Accepted: 08 Nov 2013

ABSTRACT

Objective: The present Study was taken identify spasmolytic and hypoglycemic fractions from methanolic extract of *Hygrophila auriculata* whole plant.

Methods: Isolated fractions were investigated based on their capacity of to inhibit rat ileum spontaneous contraction provoked by acetylcholine (Ach) (100 µg). An acute treatment of isolated fractions on STZ induced diabetes wistar rats, monitored blood glucose level at 1st, 2nd, 3rd, 4th and 5th hr time intervals.

Results: The isolated fraction A at dose of 80 mg/kg b.w. produced the maximum fall of 80% in blood glucose level in the STZ induced diabetic rats after 5 hrs of the treatment. The fraction F of methanolic extract of *Hygrophila auriculata* whole plant showed potent inhibitory effect on the response of acetylcholine (Ach 100 µg).

Conclusion: it can be concluded that the antispasmodic action and hypoglycemic of methanolic extract of *Hygrophila auriculata* could be due to terpenoids in the fractions.

Keywords: Methanolic extract of *Hygrophila auriculata* whole plant, Streptozotocin (STZ), Spasmolytic, Hypoglycemia.

INTRODUCTION

Herbal medicines have been used in medical practice for thousands of years and are recognized especially as a valuable and readily available healthcare resource. During the past decades, the contribution of herbal medicines and their preparations has attracted much interest in the pharmaceutical industry. However, most herbal medicines still need to be investigated scientifically, although the experience obtained from their traditional use over the years should not be ignored [1, 2].

Hygrophila auriculata (K. Schum) Heine (syn.) *Asteracantha longifolia* Nees, Acanthaceae are described in ayurvedic literature as Ikshura, Ikshugandha and Kokilasha "having eyes like the Kokila or Indian Cuckoo". The plant is widely distributed throughout India, Sri Lanka, Burma, Malaysia and Nepal [3]. The whole plant has medicinal properties and it is being used in Ayurveda for various ailments like jaundice, hepatic obstruction, rheumatism, inflammation, pain, as a diuretic, aphrodisiac, spermatorrhea [4] and in the treatment of dropsy, scanty urine and ascites [5,6]. The plant is known to possess hypoglycemic activity in human subjects [7], antitumor [8], haematinic [9], anti-nociceptive [10], hepatoprotective [3, 11], free radical scavenging and lipid peroxidation [12] activities. However, naturally occurring terpenoids, flavonoids, steroids and alkaloids, are unique phytoconstituents of the genus *Hygrophila auriculata* (K. Schum) Heine [13]. But there is no direct scientific evidence of the effect of these phytoconstituents regarding their biological potentials. In present studies, the fractionated phytoprinciples from *Hygrophila auriculata* (K.Schum) Heine need to be evaluated in spasmolytic and hypoglycemic activities in experimental animal models.

MATERIALS AND METHODS

Collection of plant material

The Whole plant of *Hygrophila auriculata* was collected from the deciduous forest of Thirumala in Andhra Pradesh State, India. Samples were authenticated by Dr. Madhavasetti, Department of Botany, and Sri Venkateswara University. The Whole plant of *Hygrophila auriculata* were sorted, cleaned and air-dried at room temperature for one week. These were ground to powder using the

laboratory Hammer mill. Powdered samples were collected and stored in air- and water-proof containers protected from direct sunlight and heat until required for extraction.

Preparation of extracts

The powdered materials of *Hygrophila auriculata* (whole plant) were extracted successively with hexane, chloroform and methanol (95%) in soxhlet apparatus, each for 18 hours. The extracts were concentrated to dryness in Rota evaporator till free from the solvents and obtained yield was respectively 1.58 g/kg, 2.85 g/kg, 18.85 g/kg and 28.41g/kg.

Isolation of fractions

Thin-layer chromatography method was carried out using silica gel aluminum plate 60F-254, 0.5mm (TLC plates, Merck). The solvent system used for TLC was Petroleum ether/chloroform (9:1). The spots of both marker and extract were applied and the plates were developed and dried with help of a hair dryer. The spots were visualized in UV light and 10% of H₂SO₄ in methanol. The methanolic extract was subjected to column chromatography (silica gel 60-100) for further purification. The column was equilibrated for one hour with petroleum ether at flow rate 5 ml/min. The sample was (2 g dissolve in methanol) was loaded on to the column and five fractions were collected using different eluents (Petroleum ether (two fraction; white powder and another white sticky material), Petroleum ether: Chloroform (9:1) one fraction; green sticky material, Chloroform: Methanol (9:1) one fraction yielded sticky dark brown material, Chloroform: Methanol (9:2) one fraction yielded sticky dark brown material). The yield of five fractions was fraction A 140 mg/g, fraction B 40 mg/g, fraction C 18.8 mg/g, fraction D 32 mg/g and fraction E 20 mg/g (w/w) respectively. From above, fraction C was excluded from pharmacological studies due to low yield. Later two fractions (fraction D and E) were pooled into one fraction (fraction F) based upon their chromatogram on TLC plates. All three fractions were (fraction A, B and F) checked for their spasmolytic and anti diabetic activity.

Phytochemical analysis

Phytochemical analysis of fractions was carried out by different methods [23].

Animals

Albino rats of Wistar strain weighing 150-200 g were purchased from MKM, Hyderabad, India and used for the studies after obtaining the permission from our institutional animal ethical committee (439/PO/01/a/CPCSEA).

Experimental design

Spasmolytic study [22]

Wistar rats (200–250 g) were killed by a blow on the head followed by exsanguinations. A portion of ileum was removed and placed in oxygenated Tyrode's solution at room temperature. The connective tissue was carefully trimmed from the tissue and then suspended in Tyrode's solution at 37°C and bubbled with oxygen. From a resting tension of 1 g, isotonic contractions, elicited by Acetylcholine (Ach) (1 µg, 10 µg, 100 µg) were recorded using kymograph machine. Fractions were added directly to the organ bath in volumes usually not exceeding 5% of bath volume (20 ml organ bath), record responses with Ach 100 µg.

STZ induced diabetic rats [21]

Diabetes was induced in male albino rats by intraperitoneal administration of STZ (a single dose 45 mg/kg b w) dissolved in freshly prepared 0.01 M citrate buffer P^H 4.5. After 72 hrs rats with marked hyperglycaemia (blood glucose ≥ 300 mg/dl) were selected and use for study.

Evaluation of hypoglycaemic effect

The diabetic rats were divided in to nine groups and each group consist six rats.

- Group 1 Normal group (Tween 80)
- Group 2 Diabetic rats (STZ 45 mg/kg)
- Group 3 Diabetic rats + Glybenclamide (20 mg/kg)
- Group 4 Diabetic rats + Fraction F (8 mg/kg)

- Group 5 Diabetic rats + Fraction F (80 mg/kg)
- Group 6 Diabetic rats + Fraction B (8 mg/kg)
- Group 7 Diabetic rats + Fraction B (80 mg/kg)
- Group 8 Diabetic rats + Fraction A (8 mg/kg)
- Group 9 Diabetic rats + Fraction A (80 mg/kg)

Blood sample were collected for the measurement of glucose at 1,2,3,4 and 5 h after administration of the fractions and blood glucose levels were determined by using Accucheck Glucometer.

Statistical analysis

All the values were expressed as Mean ± SEM. The data were statistically evaluated using one way analysis of variance (ANOVA) using Graph Pad Prism 5 computer software.

RESULTS

Preliminary phytochemical screening

Phytochemical screening of the above fractions was performed and details given below.

Table 1: Preliminary phytochemical evaluation

S. No.	Fraction	Presence of Photochemicals
1	A	Terpenoids
2	B	Terpenoids, Sterols
3	F	Terpenoids

Antispasmodic activity

Rat ileum suspended in Tyrode's solution under 1 g tension after 15 min had a stable tension. Ach (1 µg, 10 µg, 100 µg) produced a dose dependent contraction, reaching their maxima within 60 s of contact. Fraction F reversibly inhibited the ileum contraction induced by 100 µg Ach with 59.71 % of inhibition, 48.5 % of inhibition with fraction B and 24.24 % of inhibition with fraction A (Table 2, Fig 1).

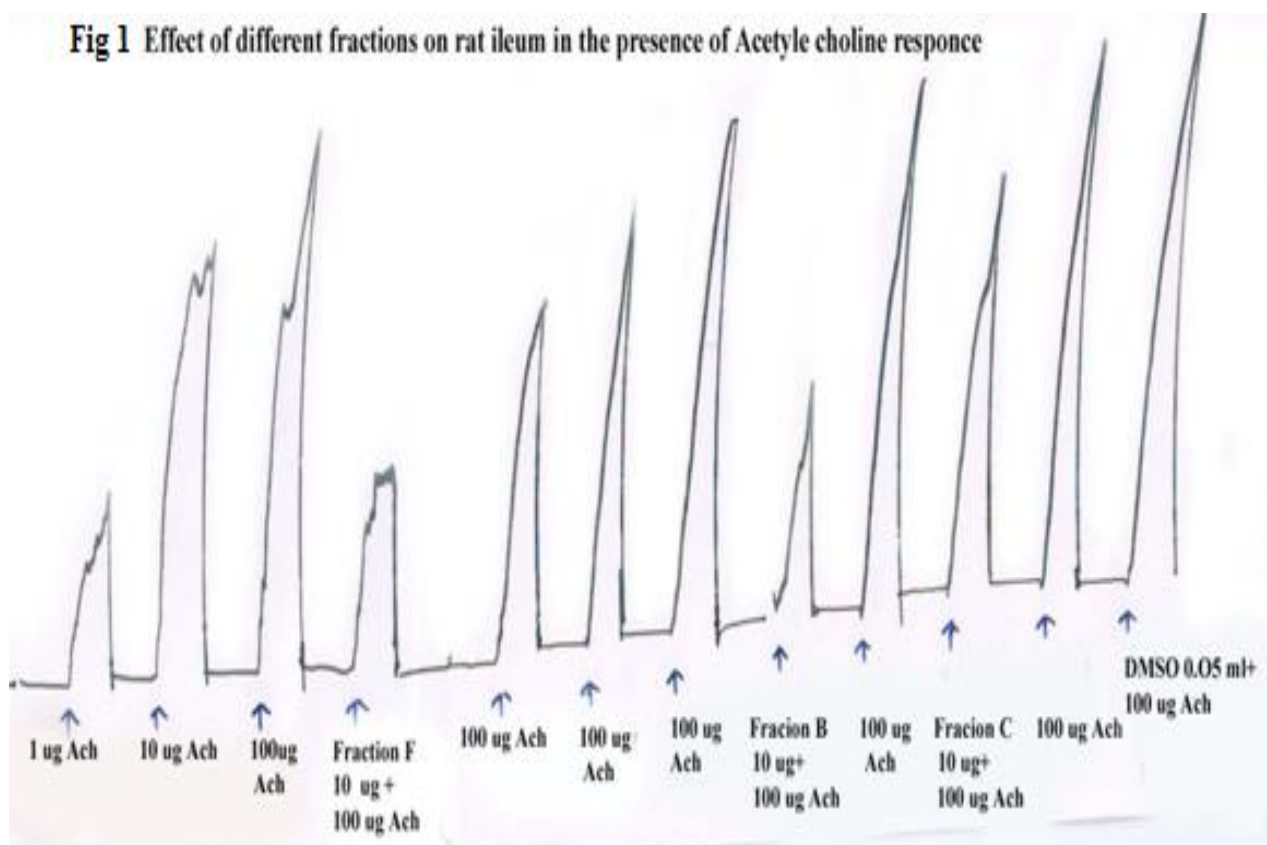


Fig. 1: Effect of different fraction on rat ileum in the presence of acetyl choline response

Table 2: Effect of different fractions of methanolic extract of *Hygrophila auriculata* whole plant in the presence of acetylcholine on rat ileum

S. No.	Drug (dose in µg)	Response (Height in cm)	% of inhibition
1	Ach (1µg)	2.5
2	Ach (10µg)	5.4
3	Ach (100µg)	6.6
4	Ach (100µg) + Fraction F (10µg)	2.5	59.71
5	Ach (100µg) + Fraction B (10µg)	3.4	48.5
6	Ach (100µg) + Fraction A (10µg)	5	24.24

Anti diabetic activity

Table 3, Fig 2 showed the effect of three different fractions of methanolic extract of *Hygrophila auriculata* whole plant on the glucose levels of STZ induced diabetic rats. Among the three

fractions only fraction A (80 mg/kg) showed significant anti hyperglycemic activity (80%) after 5hr of the treatment in diabetic rats. Other fractions gave 42% (at 2nd hr) and at 45% (at 5th hr) of blood glucose reduction, respectively with fraction B and F.

Table 3: Effect of different fractions of methanolic extract of whole plant of *Hygrophila auriculata* on STZ induced diabetic rats

Groups	Treatment	Blood glucose levels at different time intervals (hrs)					
		0 hr	1 hr	2hr	3hr	4hr	5 hr
1	Normal group (tween 80)	112±3	120±2	123±1.5	120±2.2	121±2.4	115±3.3
2	Diabetic rats (STZ 45 mg/kg)	320±14	340±4.3	342±4.4	355±2.3	351±3.4	348±2.2
3	Diabetic rats+ Glybenclamide (20mg/kg)	435±13	321±21.3	159±13.4	53±11.3	39±4.1 (91%)	46±2.3
4	Diabetic rats+ Fraction F(8 mg/kg)	341±23.4	302±13.2	267±4.8	253±12.4	263± 3.6	289±9.5
5	Diabetic rats + Fraction F (80 mg/kg)	350±15.2	314±7.8	250±4.3	244±5.7	198±4.6	190±3.5 (45%)
6	Diabetic rats + Fraction B (8 mg/kg)	342±23.1	268±13.2	287±23.2	307±12.3	312±12.1 8	320±12.4
7	Diabetic rats + Fraction B (80 mg/kg)	324±21.2	247±13.2	183± 2.9(42%)	192±15.2	197±13.8	240±11.2
8	Diabetic rats + Fraction A (8 mg/kg)	330±19.6	278±14.2	290±15.8	321±14.6	332±13.2	324±11.3
9	Diabetic rats + Fraction A (80 mg/kg)	311±19.2	362±8.6	296±10.2	167±13.2	71±9.3	60±8.3 (80 %)

Values are given as mean ± SEM.

Values were significant between Group 1 and Group 2 P< 0.001, P<0.01 and P< 0.005.

Values were significant between Group 2 and Group 3 P<0.01 and P<0.005.

Values were significant between Group 2 and Group 9 P< 0.01 and P< 0.005.

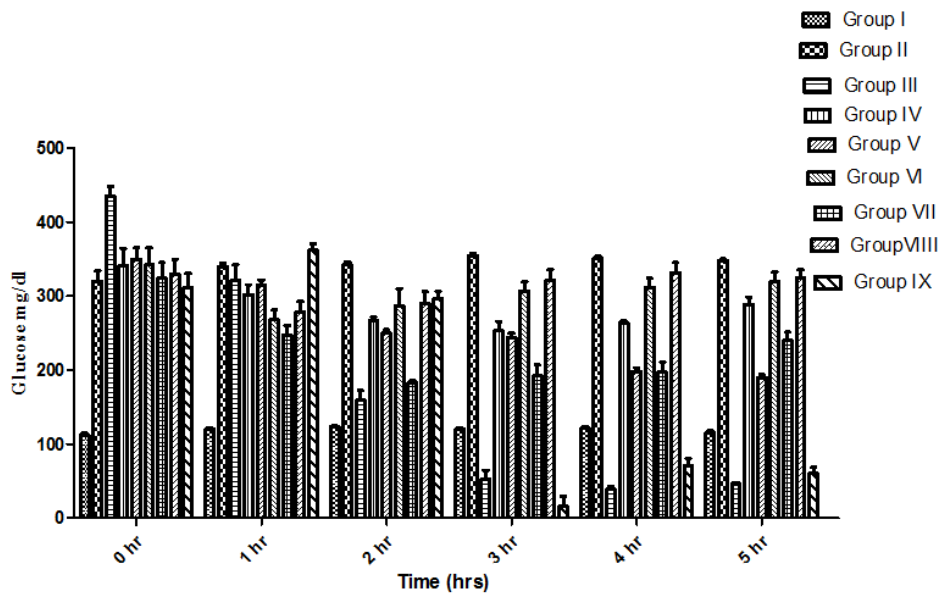


Fig. 2: Effect of different fractions of methanolic extract of whole plant of *Hygrophila auriculata* on STZ induced diabetic rats.

DISCUSSION

Spasmolytic activity of the different fractions

The objective of this work was to investigate the spasmolytic effect of these three fractions isolated from methanolic extract of *Hygrophila auriculata* whole on ileum (Fig. 1). Ach have functional roles in natural contraction of gastrointestinal tract. Ach is a neurotransmitter at post-ganglionic parasympathic neurons that innervate the gut. The response to Ach is mediated by activation of two types (M2 and M3) of muscarinic receptors [15, 16]. Activation of these receptors results in an increase in intracellular Ca²⁺, an effect mediated by inositol triphosphate acting on internal calcium stores [17, 18, and 19]. In this present study fraction F posses potent inhibitory effect on Ach (100 µg) contraction on rat ileum than other fractions. The phytochemical revealed that fraction F contained terpenoids. Some studies they have cholinergic receptor and/or Ca²⁺ channel blockade effect [20]. All the fractions showed smooth muscle relaxant activity, being fraction F the most active fraction. Functional experiments suggest that Fraction F relaxant effect is by possible muscarinic receptors antagonism. The spasmolytic activity induced by the fraction was not significant.

Anti hyperglycemic activity of the different fractions

Earlier studies shown that the methanolic extract of *Hygrophila auriculata* whole plant at a dose of 250 mg/kg.b.w possess significant anti hyperglycemic activity [12] in streptozotocin induced diabetic rats. In present study, fraction A obtained from the methanolic extract of *Hygrophila auriculata* whole plant has produced maximum anti hyperglycemic activity (80 %). The onset of antidiabetic action was observed from second hour after treatment and it was continued till end of the 5hour. The anti hyperglycemic activity of the fraction A 80 mg/kg.b.w significantly higher than other fraction of methanolic extract of *Hygrophila auriculata* whole plant (Table 3 and Fig 2).

The phytochemical analysis of fraction A revealed the presence of terpenoids. They have a broad range of biological activities. They function has a powerful anti oxidants and some are reported to have anti diabetic activity [14]. The fraction A which has shown the maximum anti hyperglycemic action was considered as the active fraction containing the active principle of anti hyperglycemic activity.

CONCLUSION

Potent hypoglycemic activity of fraction A could be attributed to valuable terpenoids in methanolic extracts of *Hygrophila auriculata* whole plant. Antispasmodic action of fraction F of methanolic extract of *Hygrophila auriculata* whole plant could be presence of another valuable terpenoids.

Conflict of interest

Authors declare that there are no conflicts of interest.

ACKNOWLEDGEMENT

We are grateful to the Dr. D Basava raj, Professor, Department of PharmaceuTicalsciences, Shri Vishnu College of Pharmacy, Bhimavaram for his encouragement. The authors are thankful to the Shri Vishnu College of Pharmacy, for its financial support.

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