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Antidiabetic Potential of Fractions of Hydro-Ethanol Extract of *Cyperus rotundus* L. (Cyperaceae)

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ABSTRACT

Traditional systems of medicine reported the use of *Cyperus rotundus* in the treatment of diabetes. To substantiate this claim, investigations were carried out using hydro-ethanol extract and the same has been reported (Raut and Gaikwad, 2006). Present study evaluated the antidiabetic activity of fractions of hydro-ethanol extract of *C. rotundus*. Dried powdered material of *C. rotundus* was defatted with petroleum ether 60-80 °C, cold macerated with hydro-ethanol for seven days and then fractionated successively in Soxhlet apparatus with chloroform, ethyl acetate, acetone and methanol. These fractions were screened for antidiabetic activity using alloxan induced diabetes in rats. Diabetes was induced by intra-peritoneal administration of alloxan monohydrate (120 mg/kg) on days 1 and 12 and blood glucose levels were estimated on 15th day. Various oral doses were tried and significant antidiabetic activity ($p<0.001$) was found at a dose of 300 mg/kg in acetone fraction and residue left after successive fractionation. The activities of active extracts were comparable to metformin (450 mg/kg, per oral). The results suggested that, fractions possess antidiabetic activity attributed to the presence of polyphenols and flavonoids.

Keywords: *Cyperus rotundus*, diabetes, antidiabetic, flavonoids, polyphenols

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INTRODUCTION

Traditional medicine is an evolutionary process where in use of natural products in drug discovery remains important because many modern drugs have their origin in ethnopharmacology [1]. According to WHO, as many as 80% of the world's people are dependent on traditional medicine for their primary healthcare, most type of which use remedies obtained from plants [2, 3]. For a long time, diabetes, a chronic metabolic disorder, has been treated with several medicinal plants or their extracts based on folklore medicine [4]. Synthetic hypoglycemic agents can produce serious side effects [5]. Therefore the search for more effective and safer hypoglycemic agents has continued to be an important area of active research. Furthermore, after the recommendations made by WHO on diabetes mellitus [6], investigations on hypoglycemic agents from medicinal plants have become more important.

Cyperus rotundus L. (Cyperaceae), vernacularly called 'Nagarmotha' is an erect perennial glabrous grass like sedge with cylindrical brown edible tubers. It is distributed throughout India, Ceylon and most of the temperate countries. The *C. rotundus* have been reported to contain oils, alkaloids, glycosides, saponins, flavonoids, tannins, starch and carbohydrates [7]. The rhizomes of *C. rotundus* have been used in ancient medicine in India for fever, dysentery, pruritis, pain, vomiting and various blood disorders. Traditional systems of medicine reported the use of *C. rotundus* in the treatment of diabetes [8] and to substantiate the traditional claim, investigations were carried out. The antidiabetic potential of this plant has been reported using hydro-ethanol extract in alloxan induced diabetic rats [7].

In view of the above, the present work aimed to study the antihyperglycemic effect of various fractions of hydro-ethanol extract of rhizomes of *C. rotundus* in alloxan induced hyperglycemia in rats.

MATERIALS AND METHODS

Plant material:

C. rotundus rhizomes, collected locally, were authenticated by the Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University Campus, Nagpur. A voucher specimen has been deposited in the herbarium of Department of Botany, with specimen number RA1223.

Extraction:

Dried powdered material of *C. rotundus* was defatted with petroleum ether 60-80 °C, cold macerated with hydro-ethanol (30:70) for seven days and then fractionated successively in Soxhlet apparatus with chloroform, ethyl acetate, acetone and methanol. All the fractions (chloroform-CCR, ethyl acetate- EACR, acetone- ACR, methanol-MCR and residue left after fractionation –HACR) were concentrated in rotary vacuum evaporator, dried under vacuum and stored in dessicator.

Animals:

Male Sprague–Dawley rats (200–250 g) were used and kept at 25±2 °C in a 12 h light dark cycle with lights on at 07:00 h. They were allowed standard pellet rat diet (Goldmohor Brand, India) and water ad libitum.

Institutional Animal Ethics Committee, constituted under the guidelines of CPCSEA, Ministry of Environment, Govt. of India, New Delhi, approved all the animal experimental protocols.

Evaluation of antihyperglyacemic activity:

Diabetes was induced by intraperitoneal administration of alloxan monohydrate in rats (120 mg/kg) [7] on days 1 and 12. The blood samples were collected from tail vein on day 15 and blood glucose levels were estimated using glucometer.

Rats having blood glucose levels above 200 mg/dl were selected for further experiments [7] and divided in thirteen groups of five rats each. Group I (normal rats); group II (diabetic untreated rats); group III (diabetic rats treated with CCR 200 mg/kg), group IV (diabetic rats treated with CCR 300 mg/kg), group V (diabetic rats treated with EACR 200 mg/kg), group VI (diabetic rats treated with EACR 300 mg/kg), group VII (diabetic rats treated with ACR 200 mg/kg), group VIII (diabetic rats treated with ACR 300 mg/kg), group IX (diabetic rats treated with MCR 200 mg/kg), group X (diabetic rats treated with MCR 300 mg/kg), group XI (diabetic rats treated with HACR 200 mg/kg), group XII (diabetic rats treated with HACR 300 mg/kg), and group XIII (diabetic rats treated with 450 mg/kg of metformin).

The fractions were suspended in water using Tween 80 (1% v/v) as a suspending agent for the purpose of oral administration. All groups were treated orally once a day for 7 days. Rats in group I and II were fed with vehicle. The blood glucose levels were evaluated at regular time intervals at 0, 2, 4 and 24 h after the first treatment (acute treatment) and 1 h after the last treatment on day 7 (sub-acute treatment).

Study on normoglycaemic and glucose hyperglyacemic (NG-OGTT) rats:

A methodology described by Aslan et al. (2007) [9] was used to assess the activities of fractions in order to minimize the number of animals. Animals were fasted over night and after taking a baseline (0 min) samples the fractions were administered immediately. The blood glucose levels were determined at 30 and 60 min to assess the effect of the test fractions on normoglycemic animals. The animals were then loaded orally with dose of glucose bolus 2 g/kg of body weight. Blood samples were subsequently collected at 60, 90 and 210 min after the glucose load [9].

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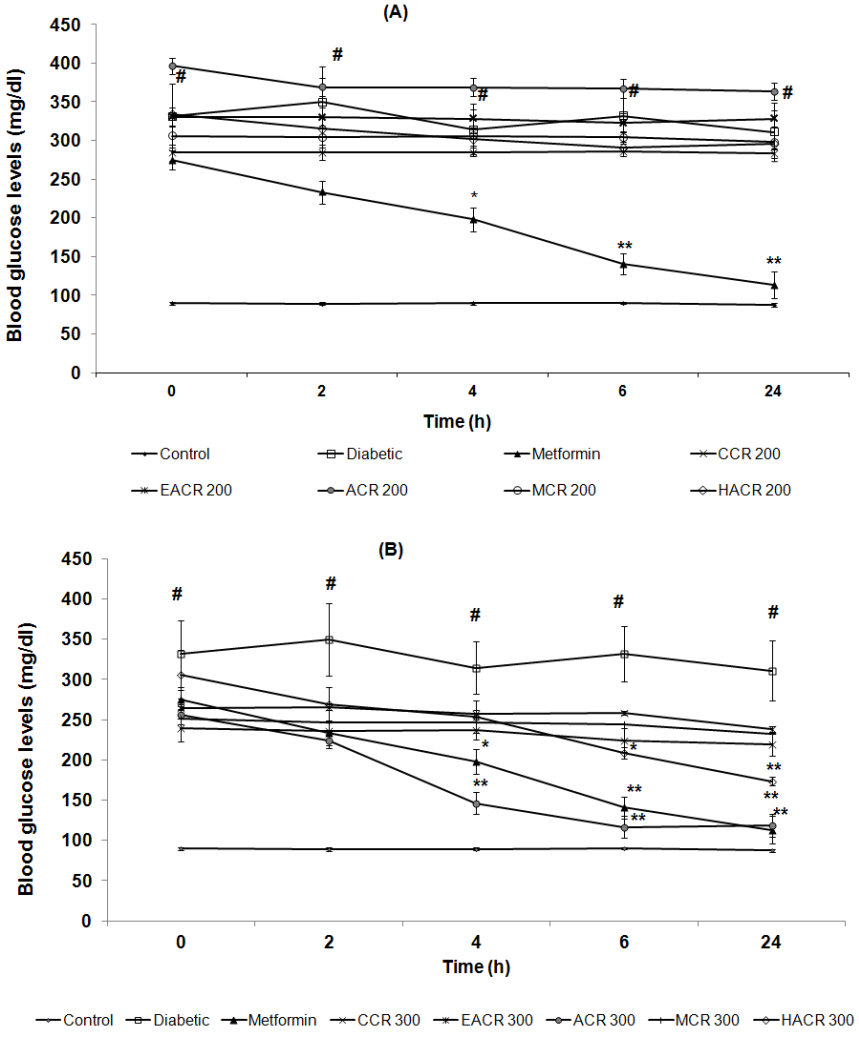
Statistical analysis:

All the data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keul's test for multiple comparisons. $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Various oral doses were tried and significant antidiabetic activity ($p<0.001$) was found at a dose of 300 mg/kg in acetone fraction (ACR) and residue left after successive fractionation (HACR). The activities of active fractions were comparable to metformin (450 mg/kg, per oral) (Figs. 1 and 2).

Fig 1: Effect of oral acute treatment of various doses of fractions of hydro-ethanol extract of *C. rotundus* on alloxan induced diabetes in rats (A) Dose 200 mg/kg (B) Dose 300 mg/kg



Results are expressed as mean±SEM (n=5). # p<0.001 Vs control, * p<0.01 Vs diabetic, ** p<0.001 Vs diabetic.

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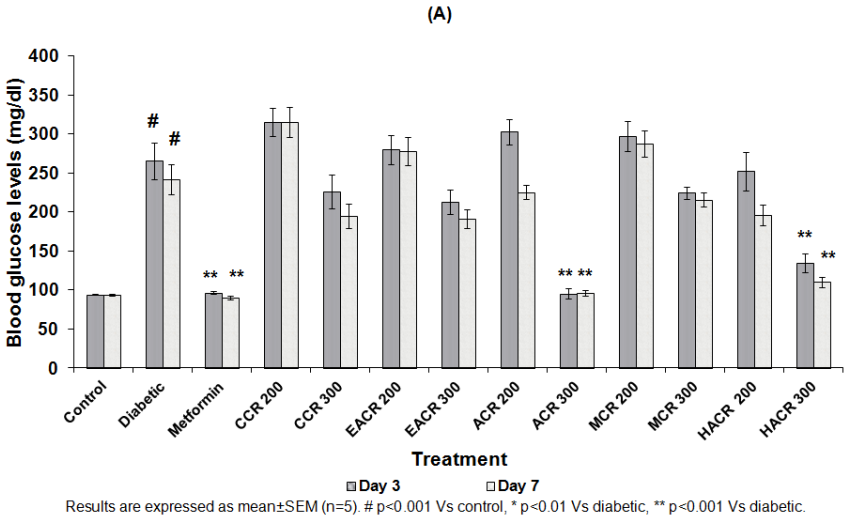
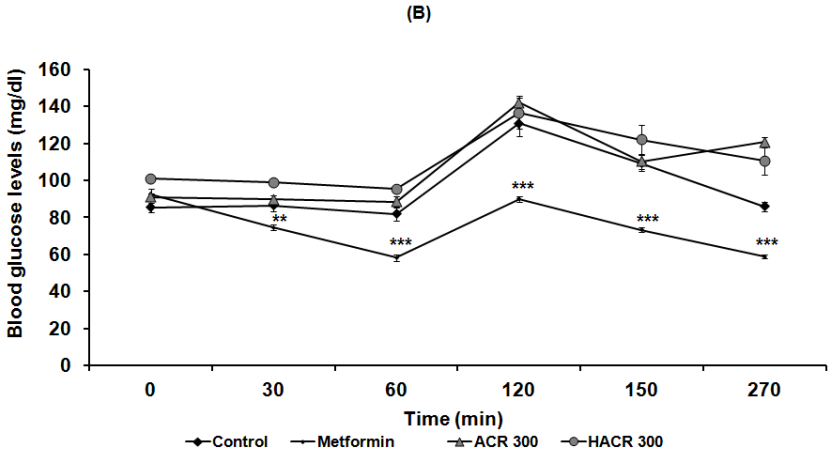
Fig 2: (A) Effect of oral sub-acute treatment of various doses of fractions of hydro-ethanol extract of *C. rotundus* on alloxan induced diabetes in rats

(B) Effect of oral treatment of various active fractions of *C. rotundus* on blood glucose levels in normal and 2 g/kg of glucose loaded hyperglycaemic (NG-OGTT) rats



Results are expressed as mean±SEM (n=5). # p<0.001 Vs control, * p<0.01 Vs diabetic, ** p<0.001 Vs diabetic.



Results are expressed as mean±SEM (n=5) ** p<0.01 and *** p<0.001 Vs control

ACR and HACR did not lowered blood glucose levels in normoglycaemic rats. Metformin was found to be more effective in the NG-OGTT experiment than in acute and sub-acute diabetic tests (Fig. 2). As these fractions did not lower the blood glucose levels in NG-OGTT experiment, it indicates that these fractions are not acting through the mechanism by which metformin produces its effect. Therefore, the possibility of other mechanisms including release of insulin is needed to be studied. From the results of phytochemical investigations and pharmacological screening, it was indicated that, fractions having tannins, polyphenols, flavonoids and saponins possessed good antidiabetic activity which is evident from the literature [10, 11].

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CONCLUSION

Being the weed, *C. rotundus* is always troublesome for the farmers. The results obtained in the present study suggested that, fractions possess antidiabetic activity which can be attributed to the presence of polyphenols and flavonoids. Therefore, further exploration of antidiabetic activity of such weed can be boon for the society.

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