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The study of anti-diabetic activity of aqueous extract of root of *Gynandropsis gynandra* in diabetic rats

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ABSTRACT

It has been planned to explore the hypoglycaemic and antihyperglycaemic activity of aqueous extract of root of *Gynandropsis gynandra* herb in normal and diabetic rats.The extract was evaluated for its specific physical and chemical characteristics in order to standardize it. The aqueous extract of root of *G.gynandra* were tested at 3 dose levels (100, 200 and 400 mg/kg each) in rats. In each case the initial test was performed at dose of 100 mg/kg. The actions were compared with standard Tolbutamide drug at a dose of 40 mg/kg. The data obtained was analysed with the one-way analysis of variance (ANOVA), followed by a post hoc of Dunnett's T-test. The study revealed that the *Gynandropsis gynandra* herb was found to have hypoglycemic actions compared with Tolbutamide.

1. INTRODUCTION

Gynandropsis gynandra, Family, Capparidaceaeis an Erect, branched, glandular pubescent herb, 30-70 cm tall. Leaves digitate, **3**-9 foliolate, petioles 4-8 cm long, hairy; leaflets subsessile, obovateoblanceolate, cuneate, hairy, 2-5 x 1.5-3 cm, terminal one largest. Flowers at first corymbose, elongating into bracteate raceme. Petals white or pale pink, up to 1.6 cm long, obovate or suborbicular with a long narrow claw; stamens purple. Capsules 6-10 cm long, tapering both ends. Seeds many, dark brown, muricate.

*G.gynandra*is used as a medicinal plant and can be found in India. It grows as a weed in most tropical countries and grows in paddy fields and also in road sides and in open grass lands. In India it is never cultivated but grows spontaneously everywhere. Different species of *Gynandropsis* can be found in all states of India.

Figure.1.*Gynandropsis gynandra*

Traditional Uses: *G.gynandra* plant is traditionally used in the treatment of pain, wounds, malaria, piles, rheumatism, snake bite, scorpion stings and epilepsy. A decoction or infusion of boiled leaves and/or roots is administered to facilitate childbirth, stomach-ache and constipation, conjunctivitis, severe thread-worm infection and relieve chest pains. Seeds are anthelmintic, antispasmodic, sudoriphic, carminative and rubefacient and used for explosion of round worms, in sprains, convulsive affections, typhus fever and cough. Roots possess mild febrifigal property. Their seeds are used as anthelmintic, antispasmodic, sudorific, carminative and rubefacient. They are used for expulsion of round worms, in sprains, convulsive affections, typhus fever and cough. There has been global resurgence of interest in the herbal drugs in the recent past. Though herbal medicines are effective in the treatment of various ailments very often drugs are unscientifically exploited or improperly used. Therefore herbal drugs deserve detailed studies in the light of modern medicine. A majority of population in India suffer from diabetes disease due to various reasons. The development of effective anti-diabetic drugs is one of the major thrust areas of research currently. The present research work has been undertaken with an objective to investigate selected roots of herbal plants for anti-diabetic activity and to develop herbal formulation for the treatment of diabetes.

2. MATERIALS AND METHODS

Hypoglycaemic & antihyperglycaemic activity of *G.gynandra*: In the present work, the hypoglycaemic & antihyperglycaemic activity of aqueous extracts of *G.gynandra* was studied by measuring blood glucose levels. Three different doses (100, 200 and 400 mg/kg) of plant extract were selected based on acute toxicity studies in mice. The extracts were administered orally to rats, blood samples were collected from retro-orbital fluxes at regular intervals (at 0, 1, 2, 4, 6, 8, 16 and 24 hour) and estimated for serum glucose levels using GOD/POD method.

The animals are divided into seven groups, each consisting of six animals. 1% Sod. CMC used as suspending agent. In all groups zero hour blood sample were collected and estimated for blood glucose levels served as control value.

After treatment (Extracts) as per the experimental protocol blood samples were collected and analyzed for blood glucose levels. Tolbutamide is used as standard drug and served as experimental control. The dose dependent (100, 200 and 400 mg/kg) blood glucose reduction was observed with selected aqueous plant root extract.

Preparation of aqueous extract: The powdered plant material was macerated with chloroform: water (1:9) for seven days in round bottom flask with occasional shaking. Chloroform-water was used to prevent the growth of microorganism in the extract. The extract was filtered and concentrated to dryness at room temperature to avoid the decomposition of the natural metabolites.

Preliminary qualitative phytochemical tests: The aqueous extract of *G.gynandra* was subjected for qualitative chemical tests to find out the functional groups presence such as sterols, glycosides, saponins, carbohydrates, alkaloids, flavonoids, tannis, proteins and phenolic steroids.

Determination of Total Phenolic Compounds: The total phenolic compounds in plant extract were determined by spectrophotometric method. 1 mg/ml concentration of extract is prepared by using methanol. 0.5 ml of methanolic solution and 2.5ml of 10% Folin-Ciocalteu's reagent were mixed initially and then diluted with water. Finally 7.5% NaHCO3 was added to the mixture. The samples were for incubated for 45 minutes at 45^0c and absorbance was recorded at 765 nm. The total phenolic content was expressed in terms gallic acid equivalent (Ga/g). The phenolic content of extract was 4.56 mg of GA/g of extract.

Determination of Superoxide Radical Scavenging Activity: For the assessment of free radicals scavenging activity, the aqueous extracts of plant extract was dissolved in water and is depends on light induced superoxide generation by photo reduction of riboflavin. The different concentrations of plant extract (50, 100, 250, 750, 1000 µg) were prepared and ethylenediamine tetra acetic acid, nitro blue tetrazolium and riboflavin were transferred to a test tube.The percentage inhibition of superoxide production by the extract was calculated using the formula: Inhibition ratio= $(A_0-A_1)\times100/A_0$

Where A_0 is the absorbance of control

A_1 is the absorbance with addition of plant extract/ ascorbic acid.

In the present study, the aqueous extract of roots of *G.gynandra* was found to possess concentration dependent scavenging activity on superoxide generated by photo reduction of riboflavin.The mean IC_{50} values of superoxide radical scavenging activity of aqueous extract of roots of *G.gynandra* were found to be 420.90µg.

Determination of 1,1-diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity: In DPPH assay method is following the procedure given by Braca *at el* (2003) and it is based on the reduction of aqueous DPPH solution (dark blue in colour) in the presence of hydrogen donating antioxidant converted to the radical form of yellow colored 1,1-diphenyl-2-picrylhydrazine.

Experiment: The albino rats (150-250g) were randomly selected and divided into ten groups, each consists of six animals. They were maintained under standard conditions (Room temperature at 25 ± 2^0C, 12 h light/dark and free access to food along with water up to 2 weeks before the experiment to adapt to laboratory conditions. The animals were deprived of food for 18 h and water allowed *ad libitum* prior to experiment.

Group A: Received vehicle for 24 hours (Normal)

Group B: STZ (45 mg/kg i.p) induced Diabetic rats received 1% Sod.CMC orally

Group C: Received Tolbutamide 40 mg/kg p.o. (Normal)

Group D: Received Plant Extract 100 mg/kg p.o. (Normal)

Group E: Received Plant Extract 200 mg/kg p.o. (Normal)

Group F: Received Plant Extract 400 mg/kg p.o. (Normal)

Group G: Received Tolbutamide 40 mg/kg p.o. (Diabetic)

Group H: Received Plant Extract 100 mg/kg p.o. (Diabetic)

Group I: Received Plant Extract 200 mg/kg p.o. (Diabetic)

Group J: Received Plant Extract 400 mg/kg p.o (Diabetic)

3. RESULTS AND DISCUSSION

The animals in Group D (Normal) received aqueous extract of root of *G.gynandra* at a dose of 100 mg/kg and were found to produce maximum decrease in blood glucose levels at 6^{th} hour (79.21 ±0.89 mg/dl). The percentage reduction in blood glucose was found to be 19.51% at 6^{th} h. The results clearly indicated that the aqueous extract of *G.gynandra* possess hypoglycaemic effect.

The animals in Group E and F (Normal) received aqueous extract of root of *G.gynandra* at a dose of 200 and 400 mg/kg respectively. In the animals in group E the maximum decrease in blood glucose levels was observed at 6th hour (74.19±0.55 mg/dl). The percentage blood glucose reduction at 6th hour was found to be 21.25%.

The blood glucose levels of the animals in Group F were found to be 70.46±0.50 mg/dl at 6thhr and the percentage blood glucose reduction was observed to be 24.59%. The aqueous root extract of *G.gynandra* showed dose dependent blood glucose reduction (shown in Fig No.4) and maximum percentage blood glucose reduction was observed with 400 mg/kg dose. Thus the results clearly indicated aqueous root extract of *G.gynandra* possess hypoglycemic activity in normal rats and the results are given Table No.1.

The animals in group H (Diabetic) received aqueous extract of root of *G.gynandra* at a dose of 100 mg/kg. In this group, the maximum reduction in blood glucose was observed at 6th hour (252.77±2.67 mg/dl) and percentage blood glucose reduction was found to be 22.51%. The animals in group I and J (Diabetic rats) received aqueous extract of root of *G.gynandra* at a dose of 200 and 400 mg/kg respectively. The animals in group I showed maximum blood glucose reduction at 6th hour (206.80±2.61 mg/dl) and percentage blood glucose reduction was found to be 31.95%.

The animals in Group J have shown the maximum blood glucose reduction at 6th hour (170.52±1.82 mg/dl) and percentage blood glucose reduction was found to be 54.47%. The results are given in Table No. 2 and shown in Fig. No 7. The aqueous of G.*gynandra* has anti-diabetic dose dependent activity and shown in Fig No.8

Table.1.The hypoglycaemic activity of aqueous extract of *G.gynandra* in normal Rats

Groups	The percentage of reduction of blood Glucose levels mg/dL (hr)						
	1	2	4	6	8	16	24
Normal	0.38	1.94	3.31	4.21	4.91	6.97	9.61
Tolbutamide	7.12	12.53	20.62	32.05**	25.34	15.42	11.96
G.gynandra 100 mg/kg	2.40	6.73	9.55	19.55**	14.50	10.54	7.39
G.gynandra 200 mg/kg	4.22	9.93	13.74	21.25**	16.97	11.20	8.54
G.gynandra 400 mg/kg	5.34	7.57	20.25	24.59**	18.42	14.35	10.66

Values are expressed in Mean±SEM, n=6, P<0.05*, 0.01**, 0.001*** Statistical analysis by paired‘t’ test.

Table.2.The antihyperglycaemic activity of aqueous extract of *G.gynandra* against STZ induced diabetic Rats

Groups	The percentage of reduction of blood Glucose levels mg/dL(hr)						
	1	2	4	6	8	16	24
Diabetic	3.48	5.00	4.47	3.96	2.57	3.10	2.31
Tolbutamide	11.67	17.86	34.77	45.40**	36.11	19.86	11.99
G.gynandra 100 mg/kg	3.31	7.58	13.80	22.51**	18.69	8.96	3.19
G.gynandra 200 mg/kg	5.47	12.84	20.37	31.95**	18.03	14.14	8.34
G.gynandra 400 mg/kg	9.09	25.28	39.01	54.47**	43.07	25.74	12.03

Values are expressed in Mean±SEM, n=6, P<0.05*, 0.01**, 0.001*** Statistical analysis by paired‘t’ test.

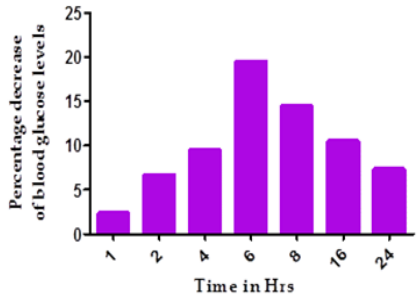

Figure.1.The effect of aqueous extract of roots of *G.gynandra* (100 mg/kg) on Blood Glucose levels in Normal Rats

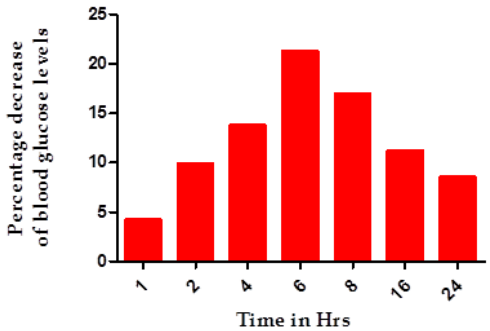

Figure.2.Effect of The effect of aqueous extract of roots of *G.gynandra* (200 mg/kg) on Blood Glucose levels in Normal Rats

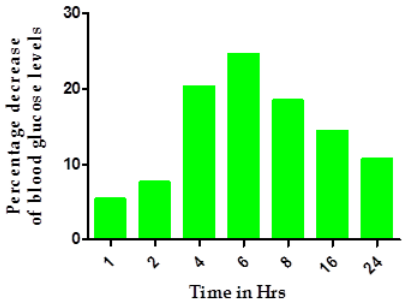

Figure.3.Effect of The effect of aqueous extract of roots of *G.gynandra* (400 mg/kg) on Blood Glucose levels in Normal Rats

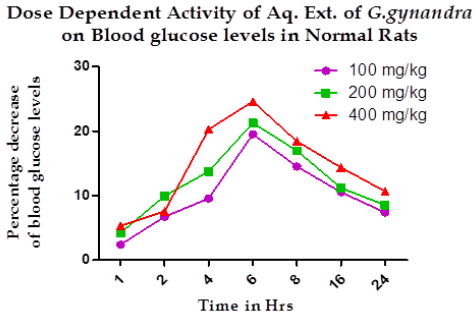

Figure.4.Dose dependent activity of aqueous extract of roots of *G.gynandra* on Blood Glucose levels in Normal Rats

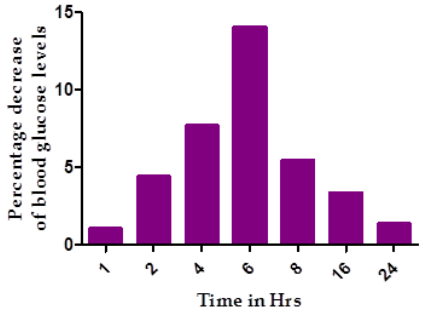

Figure.5.The effect of aqueous extract of roots of *G.gynandra* (100 mg/kg) on Blood Glucose levels in diabetic Rats

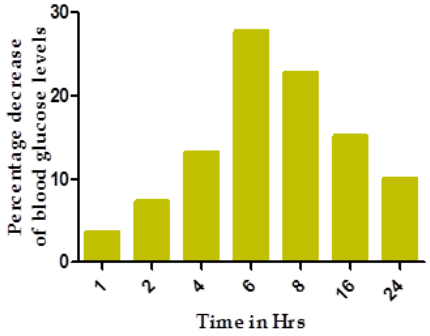

Figure.6.Effect of The effect of aqueous extract of roots of *G.gynandra* (200 mg/kg) on Blood Glucose levels in diabetic Rats

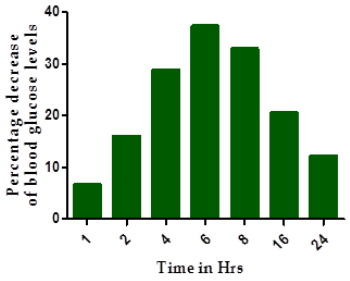

Figure.7.Effect of The effect of aqueous extract of roots of *G.gynandra* (400 mg/kg) on Blood Glucose levels in diabetic Rats

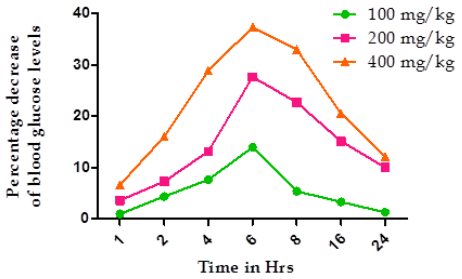

Figure.8.Dose dependent activity of aqueous extract of roots of *G.gynandra* on Blood Glucose levels in diabetic Rats

4. CONCLUSION

Qualitative chemical investigations on aqueous Root Extracts of *G.gynandra* revealed the presence of secondary metabolites like flavonoids, phenolic compounds, glycosides, sterols, phenolic steroids and carbohydrates. The plants extracts showed no toxicity at a dose of 2000 mg/kg. The plant extract also possessing very good antioxidant activity. Based on reduction of blood glucose levels, the aqueous root extracts of *G.gynandra* produced dose dependent hypoglycemic activity and antihyperglycaemic activity.

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