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Evaluation of Antioxidant and Antidiabetic Effect of *Ipomoea reniformis* Chois in Alloxan Induced Diabetes in Rats

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ABSTRACT

The objective of the study was to investigate the alcoholic (ALCIPR) and aqueous (AQUIPR) extracts of *Ipomoea reniformis* (Convolvulaceae) leaves for antioxidant and hypoglycemic effects. Antioxidant properties were determined by DPPH and lipid peroxidation method ALCIPR showed significant radical scavenging properties as compared to that AQUIPR. The hypoglycemic effect was determined in normal and diabetic rats. Diabetes was induced in rats by a single dose administration of alloxan (120 mg/ kg, i.p.) for 07 days. In normal rats, ALCIPR and AQUIPR had significantly decreased the blood glucose level (BGL) in a dose-dependent manner after repeated administration for 7 days. In alloxan-induced diabetic rats, both the extracts decreased blood sugar levels with significant improvement in glucose tolerance and body weight at the end of 1st, 2nd and 3rd week after test extract treatment. These results suggest that both extracts possess hypoglycemic activity in normal as well as in diabetic rats. Among ALCIPR and AQUIPR, ALCIPR possesses better antioxidant and hypoglycemic activity than AQUIPR. Preliminary phytochemical investigations revealed that alcoholic extracts contain carbohydrates, Glycosides, flavonoids, saponins, phytosterols, phenolics and tannins, and amino acids. The aqueous extract contains carbohydrates, flavonoids, Phytosterols, phenolics, Tannins and amino acids. These phytoconstituents may be responsible for the antioxidants and hypoglycemic activity of the plant.

1. INTRODUCTION

Diabetes is a major endocrine disorder causing morbidity and mortality of all over the world. The problem of diabetes is particularly relevant to India, as several studies had clearly documented an increased ethnic susceptibility to diabetes in migrant Asian Indians. [1] The recent epidemiological studies have pointed out to growing epidemic of diabetes in India. [2] Indeed, according to the recent Diabetes Atlas produced by the International diabetes federation (IDF), India is home to the largest number of people with diabetes in the world, 40.9 million and these numbers are consistent to increase 69.9 million by 2025. [3]

Diabetes is a chronic disease characterized by high blood glucose levels due to absolute or relative deficiency of circulating insulin levels. [4] Diabetes mellitus could also mean a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. [5] The disease is a major degenerative ailment in the world today, affecting million of people and having complications which include hypertension, atherosclerosis, and microcirculatory disorders. [6]

Generally there are two main basic types of diabetes; Type 1 (insulin dependent diabetes mellitus) characterized by a deficiency of insulin due to loss of the insulin producing beta cells of the islets of Langerhans in the pancreas and Type 2 (non-insulin dependent diabetes mellitus) which is due to insulin resistance or reduced insulin sensitivity combined with relatively reduced insulin secretion which in some cases become absolute. [7] The defective response of the body tissues to insulin is believed to involve the insulin receptors of the cell membranes. Globally, type 2 diabetes mellitus is by far the commonest form of the disease and developing countries are the worst hit as far as this epidemic is concerned. [8]

Currently available treatments for diabetes include insulin and various oral hypoglycemic agents such as sulfonylureas, metformin, glucosidase inhibitors, troglitazone, etc. [9] In conventional therapy, type 1 diabetes is treated with exogenous insulin and type 2 with oral hypoglycemic agents (sulfonylurea, etc.). [10] These drugs are used as monotherapy or in combination to achieve better glycemic control. They have their limitations and are known to produce serious side effects; therefore, the search for safer, specific and effective hypoglycemic agents has

continued to be an important area of research with natural extracts from readily available traditional medicinal plants offering potentials for the discovery of new antidiabetic drugs. [11]

Plants have always been an exemplary source of drugs; many of the currently available drugs have derived directly or indirectly from them. The ethnobotanical information reported about more than 800 plants that may possess antidiabetic properties. [12] Several herbs have shown antidiabetic activity when assessed using presently available experimental techniques. [13] Wide arrays of plant derived active principles representing numerous chemical compounds have demonstrated activity consistent with their possible use in the treatment of diabetes mellitus [14]

Ipomoea reniformis chois (Convolvulaceae) is a much branched creeping herb, rooting at nodes. The leaves are up to 1.9 cm long, kidney shaped or ovate-cordate, broader than long and toothed. The flowers are auxiliary and yellow with hairy sepals, flower in the rainy season. 15] It is widely distributed all over India, especially in a damp place in upper Gangetic plain, Gujarat, Bihar, Chhattisgarh, West Bengal, the Western Ghats ascending up to 900 meters in the hills, Goa, Karnataka, Ceylon and Tropical Africa. [16] The plant extracts were containing resin, glycoside, reducing sugar. [17] Chemical investigation showed that presence of caffeic, P-coumaric, ferulic and sinapic acid ester. [18] It is one of such medicinal plants used in folkloric medicine for the management of diabetes. It is reported to have many important medicinal properties. In indigenous system of medicine, *Ipomoea reniformis* have been claimed a use for cough, neuralgia, rheumatism, diuretics, inflammation, troubles of the nose, and fever due to enlargement of the liver, kidney diseases, and the powder is used for epileptic seizures. [19]

2. MATERIALS AND METHODS

2.1. Plant Material

2.2.1. Collection and identification of plant materials

Ipomoea reniformis chois leaves (Convolvulaceae) were collected from the tribal belt of Kunkuri district Jashpur, a region of Chhattisgarh, India. The plant was identified and authenticated by Dr. H.B. Singh, Scientist, National Institute of Scientific Communication and Research (NISCAIR), New Delhi (India).

2.2.2. **Preparation of plant extract**

The shade dried *Ipomoea reniformis* chois leaves were powdered. The coarse powder was subjected to successive extraction with petroleum ether, alcohol (95%) in soxhlet apparatus (at 60-80 ^{0}C) and the marc obtained after alcoholic extraction was macerated with distilled water to obtain an aqueous extract. [20]

2.2.3. **Phytochemical investigation**

The alcoholic (ALCIPR) and aqueous (AQUIPR) extracts of *Ipomoea reniformis* leaves obtained were subjected to various phytochemical tests for identification of secondary metabolites present in them. [21]

2.2.4. **Determination of Tannin**

Tannin contents were determined by spectrophotometer (Shimadzu), using tannic acid (Sigma-Aldrich Mumbai) as standard .1 ml from each solution of standard and test were treated with 0.5 ml tungstophosphoric acid in 10 ml volumetric flask. Volumes were made up to 10 ml with 50 % natrium carbonate. After 120 seconds the absorbance at 750 nm has been read. The concentration of total tannin is expressed as the tannic acid equivalent. The same way of standard solution preparation samples were prepared in triplicate**.** [22]

2.2.5. **Determination of Flavonoid**

Flavonoid contents were measured with the aluminum chloride (Loba Chemie Mumbai) colorimetric assay. Aqueous and alcoholic extracts that have been adjusted to come under the linearity range and different dilution of standard solution (Quercetin) (S. d.-fine chemicals Mumbai) each 4 ml were taken to 10 ml volumetric flask. The above mixture, 0.3ml of 5% $NaNO_2$ was added. After 5 minutes, 0.3ml of 10% $AlCl_3$ was added. After 6 min, 2ml of 1 M NaOH was added and the total volume was made up to 10ml with distilled water. Then the solution was mixed well and the absorbance was measured against a freshly prepared reagent blank at 510 nm. The concentration of total flavonoid is expressed as the quercetin equivalent. All samples were prepared in triplicate. [23]

2.2.6. **Determination of Polyphenols**

The Folin-Ciocalteu method was used for the determination of the total phenolics. In brief, an aliquot (2 ml) of the appropriately diluted extracts was added to a 10 ml volumetric flask, containing 5 ml of distilled water. Then, 0.5 ml of Folin-Ciocalteu (Sigma-Aldrich) reagent was added and the contents mixed. After 3 min, 1.5 ml Na2CO3 solution (concentration 5 g/l) was added and made up to a total volume of 10 ml distilled water. After keeping the samples at 50°C (water bath) for 16 min in sealed flasks and subsequent cooling, their absorbance was read at 765 nm against distilled water as the blank. A calibration curve was constructed using gallic acid (S. d.-fine chemicals Mumbai) standard solutions (1-50µg/l). The concentration of total Phenolic was expressed as the gallic acid equivalent (GAE). All samples were prepared in triplicate. [24]

2.3.**Antioxidant Activity**

2.3.1. **DPPH assay**

A working solution of methanolic DPPH (Sigma-Aldrich) having an absorbance at 516 nm was used (Shimadzu UV-VIS Spectrophotometer). This was prepared by taking 150 µl of stock solution (12.9 mg of DPPH in 10 ml of methanol) in 3 ml of methanol. To 150 µl of DPPH solution in methanol, different concentrations of ascorbic acid were added and the total volume was made up to 3 ml with methanol. DPPH diluted to 3 ml was taken as blank. Decrease in absorbance in the presence of ascorbic acid was noted down at 516 nm after 15 min. A standard graph was plotted between concentration vs absorbance. The test solutions were treated in a similar manner. [25]

% inhibition of free radicals formation was calculated as:

$$\% = \frac{(A_{control} - A_{test})}{A_{control}} \times 100$$

Where the positive control consists of all related reagents except the sample.

2.3.2. **Ferric chloride induced lipid peroxidation.**

Male Wistar rat (200 to 250 gm) was sacrificed by cervical dislocation. The skin over the abdomen was cut open and the liver was perfused with ice cold 0.15 M KCl (S.d-fine chemicals)

via the portal vein. After perfusion, the liver was isolated and 20 % (w/v) homogenate in 0.15 M KCl was prepared using tissue homogenizer under ice-cold (0º-4ºC) conditions. The homogenate was centrifuged at 1500 *g* for 5 min and the clear supernatant was used for further study. Different concentrations of test extracts were taken in test tubes to which 1ml of 0.15 M KCl and 0.5 ml of cell-free homogenate were added.

Peroxidation was initiated by adding 100 µl of 1mM ferric chloride (S.d-fine chemicals). The mixture was incubated for 30 min at 37ºC. After incubation, the reaction was stopped by adding 2 ml of ice cold 0.25 N HCl containing 15 % trichloroacetic acid (TCA) (S.d-fine chemicals), 0.38% thiobarbituric acid (TBA) (S.d-fine chemicals), and 0.5% of 0.05% butylated hydroxyl toluene (BHT) (Loba chemie) The reaction mixture was heated for 60 min at 80ºC. The sample was cooled and centrifuged at 5000 *g* for 15 min and absorbance of the supernatant was measured at 532 nm (Shimadzu UV-VIS Spectrophotometer). A standard graph was plotted between concentration vs absorbance and EC50 values were calculated. The test solutions were treated in a similar manner and the EC50 values were calculated.

An identical experiment (control induced) was performed in the absence of test compounds to determine the amount of lipid peroxidation obtained in the presence of inducing agents without any test compounds. [26] A standard graph was plotted between concentration vs absorbance.

% Anti-lipid peroxidation effect (% ALP) was calculated by the following formula

$$\% \text{ ALP} = \frac{(A_{\text{control}} - A_{\text{test}})}{A_{\text{control}}} \times 100$$

2.4.**Antidiabetic Activity**

2.4.1. **Experimental Animals**

Adult Wister rats of either sex weighing (150–200 g) from Zydus Research centre, Ahmedabad were used. All the animals were housed in the animal house of C.U. Shah College of Pharmacy and Research, Wadhwan and were maintained at a standard room temperature of 25±1^{0}C relative humidity of 45-55% and a 12:12 h light/dark cycle for 7 days with free access to standard rat

pellets and water adlbitum under hygienic conditions. The animals were selected based on the model criteria and care was taken in such a way that all the animals were used only once. Animals were accustomed to laboratory conditions for 48 h before the initiation of the experiment. The approval of the Institutional Animal Ethical Committee (IAEC) of C.U. Shah College of Pharmacy and Research, Wadhwan (Gujarat) was taken before the initiation of the experiments. All the protocols and experiments were conducted in compliance with the ethical principles and guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

2.4.2. **Acute oral toxicity**

Acute oral toxicity of the ALCIPR and AQUIPR was determined by using female, nulliparous and non pregnant Wister rats weighing 180-200 gm. The animals were fasted for 3 h prior to the experiment. Procedure OECD guideline No. 423 was adopted for toxicity studies. Animals were administered with single dose of extract and observed for their mortality during 24 h (acute) and 14 days (chronic). [27]

2.4.3.**Assessment of hypoglycemic activity in normal rats** [28]

Fasted Wister rats were divided into five groups consisting of six animals in each group. Group 01 vehicle treated (2 % acacia in distilled water), orally in a volume of 10 ml/kg, which served as normal control. Group 02 received Glibenclamide (10 mg/kg/p.o.) as a standard drug suspended in the vehicle. ALCIPR and AQUIPR suspended in the vehicle were administered at the doses of 200 and 400 m/kg, p.o. in a volume of 10 ml/kg to the rats of groups 3, 4 and 5, respectively. Blood samples were collected from the tail vein just prior to (0 h) and at 1, 2, 3, and 6 h after dosing for acute studies and glucose were estimated. For sub-acute studies blood sample was removed on the 0, 4^{th} and 7^{th} days after 16 h of overnight fasting for glucose level estimation. Oral glucose tolerance test (OGTT) was carried by administering glucose (2 g/kg, p.o.), 30 min after the extract or standard drug administration after 7 days of the pre-treatment period. Blood samples were collected from tail vein for glucose analysis prior to glucose administration ((0 h) and at 1, 2, 3, and 6 h after glucose loading.

2.4.4. **Assessments of anti-diabetic activity in alloxan-induced diabetic rats** [28]

Assessments of anti-diabetic activity in alloxan-induced diabetic rats. Alloxan monohydrate was dissolved in saline and administered intravenously into fasted rats at a dose of 120 mg/kg body wt. The solution should be freshly prepared just prior to the administration. The rats were given 5% (w/v) glucose solution in feeding bottles for next 24hr in their cages to prevent hypoglycemia after alloxan injection. After 72 h rats with BGL greater than 200 mg/dl and less than 400 mg/dl were selected and observed for consistent hyperglycemia (fasting blood glucose level greater than 200 mg/dl and lesser than 400 mg/dl) up to 7 days. Such animals were divided into six groups as follows:

The groups and the design of the experiment were as follows:

Group 01 :	Received 2% acacia (Chemdyes Ahmedabad) (10ml/kg/p.o.). (Normal Control)
Group 02 :	Alloxan Control (Sigma Aldrich Mumbai) (120 mg/kg/i.p.)
Group 03 :	Standard group received glibenclamide (Zydus Cadila) (10 mg/kg/p.o.)
Group 04 :	Alloxan+ALCIPR 400 mg/kg/in 2% acacia (10ml/kg/p.o.)
Group 05 :	Alloxan+ALCIPR 200 mg/kg/in 2% acacia (10ml/kg/p.o.)
Group 06 :	Alloxan+AQUIPR 400 mg/kg/in 2% acacia (10ml/kg/p.o.)

Blood samples were collected by a retro-orbital puncture at 0, 1, 2, 4 and 6 h after the administration. The treatment was continued for the next 21 days and blood samples were collected on 0, 4th, 7th, 14th and 21st days after 1 h administration. Blood glucose level (BGL) was estimated at various time intervals GOD/P.O.D. kit (Span Diagnostics Ltd). Oral glucose tolerance test (OGTT) on day 8^{th} and15^{th} was carried out. Body weights of all animals were measured on the 0, 4th, 7th, 14th and 21st days after 1 h of treatment with the extracts/Glibenclamide/vehicle. The change in body weight was also observed.

2.4.5.**Statistical analysis**

The values were expressed as mean ± SEM Data were analyzed using One-way ANOVA followed by Tukey test. The analysis was carried out using graph gad prism software V.5. P values less than 0.5 were considered to be statistically significant.

3. RESULTS

3.1 Determination of Phytochemical

3.1.1. Preliminary phytochemical

Phytochemical investigation of *Ipomoea reniformis* chois revealed that petroleum ether extract contains fixed oils. ALCIPR contains carbohydrates, Glycosides, flavonoids, saponins, phytosterols, phenolics and tannins, and amino acids. AQUIPR contains carbohydrates, flavonoids, Phytosterols, phenolics, Tannins and amino acids. The percentage yield of petroleum ether, alcoholic, and aqueous extract were found to be 0.80, 5.57and 2.50, respectively. The % yield of petroleum ether extract was not sufficient. Hence was not used for further experimentation.

3.1.2. Quantitative estimation of phytochemical

The Quantitative estimation of phytochemical of *Ipomoea reniformis* (Leaves) Extracts revealed that ALCIPR contains a significant amount of tannins, Flavonoids, and Polyphenols as compared to AQUIPR. The results of Quantitative estimation of phytochemical were shown in Table 01.

Table 01: Quantitative estimation of different phytochemical of *Ipomoea reniformis* Leaves extracts

Extracts	Tannins		Flavonoids		Polyphenols	
	Tannic Acid equivalent (µg/ml) (n=3)	% (n=3)	Quercetin Equivalent (µg/ml) (n=3)	% (n=3)	Gallic acid equivalent (µg/ml) (n=3)	% (n=3)
ALCIPR	12.164± 0.0462	30.41± 0.1155	11.28± 0.0505	28.19± 0.1264	18.33± 0.0548	61.11± 0.1829
AQUIPR	2.250± 0.0675	5.62± 0.1688	2.55± 0.0505	6.37± 0.1264	5.75± 0.0721	19.15± 0.2406

3.1.3. **Antioxidant Activity**

The extracts containing varying quantities of tannins, flavonoids and total polyphenols were comparatively studied for their antioxidant potentialities. Two different in vitro methods namely DPPH assay and lipid peroxidation assay were employed, the ascorbic acid used as a standard. In DPPH assay, the ALCIPR significantly decreased the absorbance produced by the DPPH and it was found to possess more significant antioxidant activity, however, the activity is lesser then ascorbic acid. Whereas the AQUIPR has moderately decreased the absorbance due to DPPH and which is not significant when compare to ALCIPR and ascorbic acid. In lipid peroxidation assay, the ALCIPR and ascorbic acid have significantly inhibited of lipid peroxidation by decreasing the absorbance of the supernatant, whereas it is not significant with AQUIPR when compare to ALCIPR and ascorbic acid. The results of antioxidant studies were shown in Table 02.

Table 02: EC50 values of *Ipomoea reniformis* Leaves extracts in antioxidant studies.

S.No.	Extract/Standard	EC50 valves	
		DPPH assay	Lipid peroxidation assay
1.	Ascorbic acid	2.278	66.45
2.	ALCIPR	2.392	68.11
3.	AQUIPR	2.951	100

3.2.**Acute oral toxicity test**

The acute oral toxicity in rats produced no death or signs of toxicity even at the highest dose of the extract (2000 mg/kg). Accordingly, toxicity selected dose were 200mg/kg/po /b.w. and 400mg/kg/bow.

3.3.**Antidiabetic activity**

3.3.1. **Effect of extracts by single dose administration on fasting blood glucose level in normal rats zero day**

The hypoglycemic activity of extracts was performed in normal rats, the overnight fasted blood glucose of all the animals was found in a range of 95-97mg/dl. Animals treated with glibenclamide showed a significant reduction in blood glucose level at 2, 3 and 6hr compared to normal control, whereas treatment with extracts had not shown any significant reduction in blood glucose level at different time intervals, indicating that the extracts are not possessing significant hypoglycemic activity after single administration. The results are summarized in Table no.03

Table 03: Effect of *Ipomoea reniformis* extracts by single dose administration on fasting blood glucose level in normal rats zero day

Group	Treatment	00 Hour	01 Hour	02 Hours	03 Hours	06 Hours
1	Normal control (Acacia 2% in water)	97.00± 0.8082	96.92± 0.3862	97.5± 0.4781	96.37± 0.2901	96.37± 0.2901
2	GB (10 mg/kg/p.o.)	97.03± 0.3906^{ns}	94.84± 0.482^{ns}	82.97± 0.4744***	75.96± 0.5565***	73.96± 0.6154***
3	ALCIPR (200 mg/kg/p.o.)	96.38± 0.4644^{ns}	96.92± 0.5164^{ns}	95.9± 0.4226^{ns}	96.19± 0.3107^{ns}	96.19± 0.3107^{ns}
4	ALCIPR (400 mg/kg/p.o.)	95.51± 0.5006^{ns}	96.17± 0.3325^{ns}	95.56± 0.7577^{ns}	94.88± 0.4587^{ns}	95.48± 0.4619^{ns}
5	AQUIPR (400 mg/kg/p.o.)	96.54± 0.9656^{ns}	96.9± 0.4857^{ns}	97.01± 0.5421^{ns}	95.74± 0.3445^{ns}	95.74± 0.3445^{ns}

All the values are expressed as Mean± SEM, n= 6. ***P< 0.001 vs. normal control group, **P< 0.01 vs. normal control group and *P< 0.05 vs. normal control group. $^{###}$P< 0.001 vs Alloxan induced group, ##P< 0.01 vs Alloxan induced group and $^{#}$P< 0.05 vs. Alloxan induced group, ns - Non-significant

3.3.2. **Effect of Extracts by Repeated Dose Administration on Fasting Blood Glucose Level in Normal Rats 7 Days**

The results are summarized in repeated administration up to 7 days, the extracts at dose 200 mg/kg and 400 mg/kg have significantly decreased the blood glucose level on 4th and 7th day when compared to normally treated group of animals. The results are summarized in Table no. 04

Table 04: Effect of *Ipomoea reniformis* Extracts by Repeated Dose Administration on Fasting Blood Glucose (FBG) Level in Normal Rats 7 Days

Group	Treatment	00 Day	04 Day	07 Day
1	Normal Control (Acacia 2% in water)	97.03± 0.1541	96.5± 0.2995	95.84± 0.5591
2	GB (10 mg/kg/p.o.)	96.89± 0.3994^{ns}	57.48± 0.336^{***}	53.63± 0.5932^{***}
3	ALCIPR (200 mg/kg/p.o.)	96.69± 0.5484^{ns}	91.74± $0.2805^{###}$	88.41± $0.2663^{###}$
4	ALCIPR (400 mg/kg/p.o.)	96.29± 0.3027^{ns}	86.74± $0.2682^{###}$	77.3± $0.3192^{###}$
5	AQUIPR (400 mg/kg/p.o.)	95.63± 0.3834^{ns}	94.34± $0.3585^{#}$	91.82± $0.3133^{###}$

All the values are expressed as Mean± SEM, n= 6. ***P< 0.001 vs. normal control group, **P< 0.01 vs. normal control group and *P< 0.05 vs. normal control group. $^{###}$P< 0.001 vs Alloxan induced group, ##P< 0.01 vs Alloxan induced group and $^{#}$P< 0.05 vs. Alloxan induced group, ns - Non-significant

3.3.3. **Repeated dose administration of effect of extracts with 2 gm/kg/p.o. Body wt of glucose on 8th days**

Administration of extracts and Glibenclamide orally 1 hr prior to glucose load showed improved glucose tolerance in normal rats. In control animals the in blood glucose significantly increasingly in 60 min. whereas treatments with extracts (dose 200mg/kg and 400mg/kg) and glibenclamide (10 mg/kg) increase in the blood glucose significantly decreases.

Similarly at 120 min after treatment with extracts and glibenclamide, the blood glucose level of normal control were increased and whereas the extracts ((dose 200mg/kg and 400mg/kg) and glibenclamide treated animals were showed blood glucose level significantly decreases, the third and sixth hours also blood glucose significantly decreasing except of AQUIPR400. The results are summarized in Table no. 05

Table 05: Repeated Dose Administration of Effect of *Ipomoea reniformis* Extracts with 2 gm/Kg Body weight of Glucose on 8 Days

Group	Treatment	00 Hour	01 Hour	02 Hours	03 Hours	06 Hours
1	Normal Control (Acacia 2% in water)	96.81± 0.4093	144.9± 1.252	121.2± 0.5991	103.4± 0.5285	98.76± 0.4706
2	GB (10 mg/kg/p.o.)	55.07± 0.4211***	72.79± 0.616***	63.61± 0.7153***	56.59± 0.6682***	54.47± 0.5302***
3	ALCIPR (200 mg/kg/p.o.)	90.59± 0.311$^{###}$	125± 0.4982$^{###}$	105.9± 0.4882$^{###}$	94.17± 0.4044$^{###}$	93.23± 0.2725$^{###}$
4	ALCIPR (400 mg/kg/p.o.)	70.9± 0.4963$^{###}$	95.57± 0.5221$^{###}$	82.19± 0.9125$^{###}$	72.35± 0.6433$^{###}$	72.03± 0.4349$^{###}$
5	AQUIPR (400 mg/kg/p.o.)	95.41± 0.695^{ns}	134.7± 0.3176$^{###}$	115.3± 0.4945$^{###}$	105.6± 0.455^{ns}	96.96± 0.4944^{ns}

All the values are expressed as Mean± SEM, n= 6. ***P< 0.001 vs. normal control group, **P< 0.01 vs. normal control group and *P< 0.05 vs. normal control group. $^{###}$P< 0.001 vs Alloxan induced group, $^{##}$P< 0.01 vs Alloxan induced group and $^{#}$P< 0.05 vs. Alloxan induced group, ns – Non-significant

3.3.4. **Effect of extracts by single dose administration on Fasting Blood Glucose (FBG) in Alloxan induced diabetic rats.**

Alloxan administration at a dose of 120 mg/kg i.v. showed significant hyperglycemia, the normal FBG values 97 to 99 mg/dl had raised to values of 252 to 266 mg/dl by 72 hours. These values on subsequent days got stabilized by day 7 on an average between 255 mg/dl.

The anti-hyperglycemic effect of extracts on fasting blood glucose levels in alloxan induced diabetic rats were assessed at different time intervals. All extracts didn't produce a significant reduction in blood glucose level compare to diabetic control animals. Glibenclamide 10 mg/kg had produced a significant reduction in BGL after 1, 2, 3 and 6th hour of treatment in diabetic rats. The results are summarized in Table no.06

Table 06: Effect of *Ipomoea reniformis* extracts by single dose administration on Fasting Blood Glucose (FBG) in Alloxan induced diabetic rats.

Group	Treatment	00 Hour	01 Hour	02 Hours	03 Hours	06 Hours
1	Normal Control (Acacia 2% in water)	97.0± 0.8082	96.25± 0.19	97.09± 0.3039	95.9± 0.3919	95.48± 0.381
2	Alloxan (120 mg/kg/i.v.)	265.0± 0.4555***	266.1± 0.6562***	267.2± 0.6319***	257.6± 0.3155***	257.2± 0.5634***
3	GB (10 mg/kg/p.o.)	252.6± 0.5471$^{###}$	240.6± 0.4253$^{###}$	204.5± 0.4211$^{###}$	172.7± 0.2907$^{###}$	180.5± 0.3344^{ns}
4	ALCIPR (200 mg/kg/p.o.)	264.4± 0.2493^{ns}	261.9± 0.5657$^{###}$	260.7± 0.3894$^{###}$	260.8± 0.4842^{ns}	261.4± 0.2767^{ns}
5	ALCIPR (400 mg/kg/p.o.)	259.3± 0.319$^{###}$	250.8± 0.4691$^{###}$	249.3± 0.3313$^{###}$	248.4± 0.443$^{###}$	252.5± 0.3757^{ns}
6	AQUIPR (400 mg/kg/p.o.)	263.0± 0.6085^{ns}	264.4± 0.8704^{ns}	263.9± 0.2534$^{##}$	263.3± 0.5599^{ns}	263.6± 0.2562^{ns}

All the values are expressed as Mean± SEM, n= 6. ***P< 0.001 vs. normal control group, **P< 0.01 vs. normal control group and *P< 0.05 vs. normal control group. $^{###}$P< 0.001 vs Alloxan induced group, $^{##}$P< 0.01 vs Alloxan induced group and $^{#}$P< 0.05 vs. Alloxan induced group, ns - Non-significant

3.3.5. **Repeated Dose Administration on FBG In Alloxan Induced Diabetic Rats Treated For 21 Days**

On repeated dose administration of extract at a dose of (200 mg/kg and 400 mg/kg) significant decrease on day 4,7,14 and 21st day of treatment when compare to the Alloxan (120 mg/kg i.v.) treated group. The results are summarized in table no. 07

Table 07: Effect of *Ipomoea reniformis* extracts repeated Dose Administration on FBG in Alloxan Induced Diabetic Rats Treated for 21 Days

Group	Treatment	00 Day	04 Days	07 Days	14 Days	21 Days
1	Normal Control (Acacia 2% in water)	97± 0.6599	96.01± 0.228	96.53± 0.7276	96.33± 0.6491	96.61± 0.411
2	Alloxan (120 mg/kg/i.v.)	265± 0.3719***	282.1± 0.553***	280.6± 0.5206***	287.6± 0.7265***	290.8± 0.4234***
3	GB (10 mg/kg/p.o.)	252.6± 0.4467$^{###}$	151.3± 0.5454$^{###}$	104.6± 0.7465$^{###}$	97.12± 0.4841$^{###}$	89.34± 0.6646$^{###}$
4	ALCIPR (200 mg/kg/p.o.)	264.4± 0.2035^{ns}	222.9± 0.5397$^{###}$	186.3± 0.8383$^{###}$	162.4± 0.6363$^{###}$	149.8± 0.9646$^{###}$
5	ALCIPR (400 mg/kg/p.o.)	259.3± 0.2605$^{###}$	178.5± 0.7102$^{###}$	143.2± 0.7786$^{###}$	125.9± 0.7591$^{###}$	116.7± 1.406$^{###}$
6	AQUIPR (400 mg/kg/p.o.)	263± 0.4968$^{#}$	232.6± 1.142$^{###}$	194.5± 0.6885$^{###}$	176.7± 0.6207$^{###}$	160.1± 1.027$^{###}$

All the values are expressed as Mean± SEM, n= 6. ***P< 0.001 vs. normal control group, **P< 0.01 vs. normal control group and *P< 0.05 vs. normal control group. ###P< 0.001 vs Alloxan induced group, ##P< 0.01 vs Alloxan induced group and #P< 0.05 vs. Alloxan induced group, ns - Non-significant.

3.3.6. **Repeated dose administration on OGTT on 8th day in diabetic rats**

All extracts have significantly reduced the BGL on repeated administration with doses (200 and 400 mg/kg) on 1st and 2nd hr and in animals treated with Glibenclamide (10 mg/kg) had significantly reduced the increased BGL at 1st, 2nd ,3rd and 6th hour after glucose loading (2 g/kg) in alloxan induced diabetic rats. The detailed results are summarized in table no. 08

Table 8: Effect of *Ipomoea reniformis* extracts repeated dose administration on OGTT on 8th Day in Diabetic rats

Group	Treatment	00 Hour	01 Hour	02 Hours	03 Hours	06 Hours
1	Normal Control (Acacia 2% in water)	96.81± 0.8143	135.3± 1.432	111.9± 1.014	101.6± 1.53	95.95± 0.6977
2	Alloxan (120 mg/kg/i.v.)	287.9± 0.4209***	391.5± 1.05***	365.5± 0.8713***	311.2± 1.233***	296.5± 0.7666***
3	GB (10 mg/kg/p.o.)	98.4± 0.7518###	123.2± 0.8294###	109.2± 0.9952###	103± 1.13###	97.55± 0.6778###
4	ALCIPR (200 mg/kg/p.o.)	164± 1.128###	234.2± 0.9728###	214.3± 0.7399###	192.8± 1.009###	170.2± 1.023###
5	ALCIPR (400 mg/kg/p.o.)	127.8± 0.8632###	156.9± 1.508###	144.8± 1.713###	134.2± 1.212###	126.9± 1.364###
6	AQUIPR (400 mg/kg/p.o.)	178.7± 0.6848###	253.3± 1.162###	241.5± 1.082###	216.3± 0.768###	187.8± 0.9189###

All the values are expressed as Mean± SEM, n= 6. ***P< 0.001 vs. normal control group, **P< 0.01 vs. normal control group and *P< 0.05 vs. normal control group. $^{###}$P< 0.001 vs Alloxan induced group, $^{##}$P< 0.01 vs Alloxan induced group and $^{#}$P< 0.05 vs. Alloxan induced group, ns – Non-significant.

3.3.7. **Repeated dose Administration on OGTT on 15th day In diabetic rats**

The animals treated with extracts (200 and 400 mg/kg) and Glibenclamide (10 mg/kg) after repeated administration (15 days) significantly suppressed the increase in BGL at 1st , 2nd ,3rd and 6th hour after glucose loading (2 g/kg) in alloxan induced diabetic rats. The results are summarized in Table No.09

Table 09: Effect of *Ipomoea reniformis* repeated dose administration on OGTT on 15th day of diabetic rats

Group	Treatment	00 Hour	01 Hour	02 Hours	03 Hours	06 Hours
1	Normal Control (Acacia 2% in water)	96.81± 0.8143	135.3± 1.432	111.9± 1.014	101.6± 1.530	95.95± 0.6977
2	Alloxan (120 mg/kg/i.v.)	287.9± 0.4209***	391.5± 1.050***	365.5± 0.8713***	311.2± 1.233***	296.5± 0.7666***
3	GB (10 mg/kg/p.o.)	98.40± 0.7518$^{###}$	123.2± 0.8294$^{###}$	109.2± 0.9952$^{###}$	103.0± 1.130$^{###}$	97.55± 0.6778$^{###}$
4	ALCIPR (200 mg/kg/p.o.)	164.0± 1.128$^{###}$	234.2± 0.9728$^{###}$	214.3± 0.7399$^{###}$	192.8± 1.009$^{###}$	170.2± 1.023$^{###}$
5	ALCIPR (400 mg/kg/p.o.)	127.8± 0.8632$^{###}$	156.9± 1.508$^{###}$	144.8± 1.713$^{###}$	134.2± 1.212$^{###}$	126.9± 1.364$^{###}$
6	AQUIPR (400 mg/kg/p.o.)	178.7± 0.6848$^{###}$	253.3± 1.162$^{###}$	241.5± 1.082$^{###}$	216.3± 0.7680$^{###}$	187.8± 0.9189$^{###}$

All the values are expressed as Mean± SEM, n= 6. ***P< 0.001 vs. normal control group, **P< 0.01 vs. normal control group and *P< 0.05 vs. normal control group. $^{###}$P< 0.001 vs Alloxan induced group, $^{##}$P< 0.01 vs Alloxan induced group and $^{#}$P< 0.05 vs. Alloxan induced group, ns - Non-significant.

3.3.8. **Repeated dose treatment of *Ipomoea reniformis* of body weight in alloxan-induced diabetic rats**

Repeated administration of glibenclamide (10 mg/kg) had prevented the reduction in body weight on 4th, 7th, 14th and 21st day in diabetic rats, whereas ALCIPR (400 mg/kg) and ALCIPR (00 mg/kg) has significant improvement in the body weight on 14th and 21st day whereas AQUIPR (400 mg/kg) has not shown significant improvement in body weight. The results are summarized in Table No 10

Table 10: Effect of repeated dose treatment of *Ipomoea reniformis* of body weight in alloxan induced diabetic rats.

Group	Treatment	00 Day	04 Days	07 Days	14 Days	21 Days
1	Normal Control (Acacia 2% in water)	177.18± 3.105	180.76± 2.492	183.04± 2.004	185.53± 1.843	187.71± 1.699
2	Alloxan (120 mg/kg/i.v.)	178.19± 1.509^{ns}	167.67± 1.506*	164.4± 1.214***	159.99± 1.43***	156.75± 1.22***
3	GB (10 mg/kg/p.o.)	179.79± 1.445^{ns}	177.7± 1.072^{ns}	175.03± 1.535$^{#}$	174.01± 1.428$^{###}$	173.19± 1.309$^{###}$
4	ALCIPR (200 mg/kg/p.o.)	174.61± 2.285^{ns}	172.21± 2.019^{ns}	169.97± 1.959 ^{ns}	168.38± 1.763^{ns}	166.8± 1.455$^{##}$
5	ALCIPR (400 mg/kg/p.o.)	178.03± 2.38^{ns}	176.01± 2.585 ^{ns}	173.89± 2.298 ^{ns}	172.17± 2.209$^{##}$	171.03± 2.314$^{###}$
6	AQUIPR (400 mg/kg/p.o.)	174.16± 2.681^{ns}	169.94± 1.918^{ns}	167.28± 1.401 ^{ns}	165.54± 0.8989^{ns}	164.14± 0.8409^{ns}

All the values are expressed as Mean± SEM, n= 6. ***P< 0.001 vs. normal control group, **P< 0.01 vs. normal control group and *P< 0.05 vs. normal control group. $^{###}$P< 0.001 vs. Alloxan induced group, $^{##}$P< 0.01 vs. Alloxan induced group and $^{#}$P< 0.05 vs. Alloxan induced group, ns - Non-significant

4. DISCUSSION

The present study was aimed to explore the anti-diabetic properties of *Ipomoea reniformis* in alloxan induced diabetes. *Ipomoea reniformis* was used alone or in combination with other remedial measures against diabetes mellitus in Chhattisgarh by traditional healers.

Alloxan causes a massive reduction in insulin release by the destruction of the β- cells of the islets of langerhans, inducing hyperglycaemia. [29] The diabetic rats (after administration of alloxan) showed a persistent rise in BGL after 7 days with the characteristic features of diabetes mellitus [28]. Plants are used throughout the world for treatment of diabetes. The study of *Ipomoea reniformis* might offer a natural key to unlock a diabetologist's in future. In light of this, an attempt was done to reveal the effect of *Ipomoea reniformis* in normoglycaemic and hyperglycaemic rats.

Both the extracts of *Ipomoea reniformis* were able to decrease the BGL in alloxan induced diabetic rats. Single dose administration of both the extracts on the first day found to be ineffective for normoglycaemic rats.

The significant anti-hyperglycemic activity was attained on repeated administration of the ALCIPR and AQUIPR from the 7th day by controlling the elevated BGL compared with the other group. In the case of OGTT, both the extracts on repeated administration improved glucose tolerance in diabetic rats on 8th and 15th days as compared to diabetic control. Impaired glucose tolerance is attained due to lack of insulin in alloxan-induced diabetic rats by destructing the β-cells which lead to type I diabetes. [30] From the present study it indicates that these extract can improve the glucose tolerance.

Glucosuria is the common symptom of diabetes. It arises due to the increase in BGL above 250 mg/dl in diabetic animals. In our study, it was observed that the administration of both extracts to

diabetic rats reversed their blood glucose. It may be due to insulin mimetic activity or improved glucose utilization mechanism. [31]

The possible mechanism by which the extracts bring about their anti-hyperglycaemic action may be by increasing either pancreatic secretion of insulin from β-cells or its release from the bound form. [32]

It is well established that sulfonylureas produce hypoglycemia by increasing the secretion of insulin from the pancreas and these compounds are active in mild alloxan induced diabetes, whereas they are inactive in intense alloxan diabetes (nearly all the beta cells have been destroyed). [33] In our work, no histological study was carried out to explain the mechanism of antidiabetic action of *Ipomoea reniformis*. However, our results showed that Glibenclamide reduced BGL in mild hyperglycemic animals. The diabetic rats receiving the extracts of *Ipomoea reniformis* showed normalization of BGL compared to diabetic control. This could be due to the possibility that some beta cells are still surviving to exert their insulin releasing effect by *Ipomea reniformis*. Moreover, extracts produced hypoglycemia in normal rats also. This suggests that extracts are probably mediated by enhanced secretion of insulin like, sulfonylureas.

However, the possibility of enhanced tissue uptake by *Ipomoea reniformis* cannot be ruled out. In addition, the glucose lowering effect of extracts was more powerful when compared to normal rats suggesting that it could be caused by an increase in peripheral glucose consumption, this reinforces the hypothesis that the hypoglycemic mechanism involves insulin-like effect through peripheral glucose consumption, delay in insulin catabolism or inhibition of glucose reabsorption by the kidney. The fact that some herbal preparations enhance the beta cell regeneration and peripheral glucose utilization in Alloxan and Streptozotocin induced diabetic rats supports the above assumption. [34] [35] It is well known that oxygen free radicals are involved in the diabetogenic action of alloxan and plants containing flavonoids, tannins and polyphenols have been shown to be effective in diabetes due their antioxidants property that was estimated DPPH assay and lipid peroxidation. [36] This suggest that the anti-hyperglycaemic activity of *Ipomoea reniformis* may be due to free radical scavenging activity which enhances the beta cell regeneration against alloxan induced free radicals. However, from the present experimental data,

it is difficult to say how exactly the mechanism of anti-diabetic activity of extracts of *Ipomea reniformis*. It needs more elaborate study.

One of the major complication of type I diabetes is weight loss. It arises due to the impairment in insulin action in the conversion of glucose into glycogen and catabolism of fats, inhibition of lipolysis due to its unavailability because of the destruction of beta cells. [37] Which results in a decrease in the body weight of the animals and in death. Treatment with extracts ALCIPR and AQUIPR has substantially prevented the body weight loss and mortality produced by alloxan.

5. CONCLUSION

In conclusion, ALCIPR and AQUIPR had shown a significant antioxidant activity and hypoglycemic activity in both normal as well as alloxan induced diabetic rats.

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