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Pharmacology

Evaluation of the Hypoglycaemic and Hypolipidaemic Activities of the Aqueous Extract of the leaves of Ixora Coccinea Linn in Diabetic Rats

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ABSTRACT

Objective: To evaluate the hypoglycaemic and the hypolipidaemic activity of the aqueous extract of the leaves of Ixora Coccinea Linn in alloxan induced diabetic albino rats.

Materials and methods: Diabetes was induced in the albino rats by the administration of alloxan monohydrate (120 mg/kg, i.p.). The rats were divided into five groups of six animals each. The first group served as the non-diabetic control, the second group as the diabetic control and the third group as the standard and it was treated with 0.1 mg/kg/ day of glibenclamide. Groups four, five and six received 100, 200 and 400 mg/kg body weight of the aqueous extract. The blood samples were analyzed for blood glucose on days 0, 1, 7, 14 and 21 and the lipid profile was assessed on day 21.

Results: The aqueous extract of leaves of Ixora Coccinea showed significant reduction ($p<0.01$) in the blood glucose levels and the serum lipid profile levels, with 400 mg/kg of body weight in the alloxan induced diabetic rats as compared to the controls.

Conclusion: It was concluded that the aqueous extract of the leaves of Ixora Coccinea was effective in controlling the blood glucose levels and in improving the lipid profile in diabetic rats.

Key Words: Hypoglycaemia, Hypolipidaemia, Ixora Coccinea linn, Aqueous leaf extract.

INTRODUCTION

Diabetes is a metabolic disorder of multiple aetiologies, which is characterized by chronic hyperglycaemia, with disturbance in the carbohydrate, fat and protein metabolism, which results from defects in insulin secretion and insulin action or both. [1] India leads the world with the largest number of diabetic patients and it has been termed as the diabetic capital of the world .According to the International Diabetes Federation, the number of the people with diabetes in India is currently around 40.9 million and the number is expected to rise 69.9 million by 2025, unless urgent preventive steps are taken. [2] Diabetes mellitus has been identified by the Indian Council of Medical Research (ICMR) as one of the refractory diseases for which satisfactory treatment is not available and a suitable herbal preparation has to be investigated about. In India, there is a documentation of about 150 plants of various families with hypoglycaemic activity. More than 1200 plant species are being used worldwide for diabetes phytotherapy and experimental studies have proved the hypoglycaemic activity of a large number of these plants. In addition to the correction of the blood glucose levels, several plants have the potential to ameliorate the lipid metabolism abnormalities of diabetes mellitus. In order to reduce the number of diabetes complications and to postpone their development, Savickiene recommended the use of biological active components and plants. Thus, this study may be a new approach in the treatment of diabetes mellitus. [3]

Ixora Coccinea Linn is a small shrub which is cultivated throughout India. (It is called as 'Flame of the Woods' in English, 'Rangan' in Hindi and Bengali and 'Kisukare' in Kannada.) Its roots and flowers are used for the treatment of dysentery, dysmenorrhoea, leucorrhoea, haemoptysis, and catarrhal bronchitis. Its leaves are used for the treatment of diarrhoea. Its roots are also used for the treatment of hiccups, nausea and loss of appetite and externally for the treatment of sores, eczema and chronic ulcers. Its roots contain aromatic acrid oil, tannin and fatty acids. Its leaves yield flavonoids, kaemferol, quercetin, anthrocyanidines, phenolic acids, and ferulic acids. Its flowers yield cyanidins, flaconboides, and cooling materials which are related to quercitin. Its roots are ground into a pulp, mixed with water and are used as a tincture for diarrhoea and dysentery. [4-6] However, there is limited scientific evidence to verify these claims. There is a dearth of reports on the hypoglycaemic and hypolipidaemic effects of the leaves of this plant.

In view of this, the current study was designed to evaluate the hypoglycaemic and the hypolipidaemic activities of the aqueous extract of the leaves of Ixora Coccinea in diabetic rats.

MATERIAL AND METHODS

Plant material:

The leaves of Ixora Coccinea were collected from the garden of S.Nijalingappa Medical college, Bagalkot, Karnataka, India, during the period from March-June 2009. The identity of this plant was authenticated by the botanist, Prof. Jedimath and the voucher specimen was deposited in the Herbarium of the Department of Pharmacology of S.Nijalingappa Medical College, Bagalkot, Karnataka, India.

Preparation of the extract:

The leaves of this plant were subjected to surface sterilization by using 30% alcohol, and they were then dried in the shade. The dried leaves were subjected to size reduction to a coarse powder by using dry grinder and they were passed through a sieve. The powdered sample (50g) was boiled in hot water for 30 minutes, after which it was filtered by using a piece of white cotton gauze. The filtrate was evaporated to dry at 400C, which produced a brown coloured solid residue (yield:35% w/w). The residue was

weighed and stored in air and water proof containers and it was kept in a refrigerator at 40C. From this stock, a fresh preparation was made whenever it was required.

Animals:
Healthy Wistar albino rats of either sex, which weighed about 150-200 g, were used. The animals were housed in poly propylene cages and were maintained under standard conditions (12h light:12h dark cycle;25± 20 C,35-60% humidity).They were fed with a standard rat pellet diet (Hindustan Lever Ltd. Mumbai, India) and water ad libitum .The Institutional Animal Ethical Committee of S.Nijalingappa Medical College (IAEC-reg No:627/02/a CPCSEA), Bagalkot, Karnataka, India approved the study protocol.

Phytochemical screening:
The extract which was obtained, was subjected to various qualitative tests for the identification of the constituents which were present, by using simple and standard qualitative methods as described by Trease and Evans. [7]

Sample collection:
The blood samples were collected by the retro-orbital plexus puncture method from overnight fasted rats under light ether anaesthesia and the blood glucose levels were estimated by using Acucheck Active TM glucose strips in an Acucheck Active TM test meter.

Determination of LD 50 of the extract:
For the acute oral toxicity study and the LD 50 determination, the Organization for Economic Co-operation and development (OECD) guideline 425 [8] was followed.

Induction of diabetes:
A single dose (120 mg/kg, b.w, i.p) of alloxan monohydrate (Sigma Ltd.USA) which was dissolved in normal saline, was used for the induction of type 2 diabetes in rats after overnight fasting. After 1hr of alloxan administration, the animals were fed the standard pellets and water ad libitum. The animals were stabilized for a week and those showing blood glucose levels (estimated by the GOD-POD method) of more than 250 mg/dl were selected for the study.

Experimental design:
The rats who were fasted overnight for 12-24 hours were randomly divided into 5 groups of 6 rats per group. Group 1 served as the normal control or the non diabetic group and was treated with 10ml/kg/day of distilled water orally. Group 2 served as the untreated diabetic control, which received 0.5mg/100g of the vehicle (2% gum acacia). Group 3 served as the standard group and was treated with 0.1mg/kg/day of Glibenclamide. Group 4, 5 and 6 were treated orally with 100mg/kg/day, 200mg/kg/day and 400mg/kg/day of the aqueous extract of the leaves of Ixora Coccinea respectively. The fasting blood glucose estimation was done at 0, 2, 4 and 6 hours after the treatment. The treatment was continued for 21 consecutive days. The fasting blood glucose levels were estimated at 0, 1,7,14 and 21 days.

Estimation of the biochemical parameters:
On day 21, blood was collected from the overnight fasted rats under ether anaesthesia by the retro-orbital plexus puncture method and was it kept aside for 30 min for clotting. By centrifuging the same sample at 6000rpm for 20 min, the serum was separated and it was analyzed for total proteins (by the Biuret method)[9] cholesterol (by the CHOD-PAP method)[10] and triglycerides (by the GPO method). [11]

Statistical analysis:
All the values were expressed as mean ± SEM. The results were analyzed for statistical significance by using one way ANOVA, followed by the Dunnetts test. P values which were <0.05 were considered as significant.

RESULTS

Phytochemical analysis:
The phytochemical analysis of the extract showed the presence of tannins, alkaloids, flavonoids, saponins, anthrquinones, anthracyanosides and reducing sugars.

Determination of LD_{50} of the aqueous extract of the leaves of Ixora Coccinea.
Administrations of a single dose of extract (500mg/kg, b.w., and p.o.) did not produce any mortality. The animals were alive, healthy and active during the observation period of 14 days. Use of the AOT 425 software was made to obtain higher doses for LD_{50} determinations as per the OECD guidelines. The results indicated that doses up to 2000mg/kg were non lethal.

Effects of the aqueous extract of the leaves of Ixora Coccinea on diabetic rats.
The hypoglycaemic activity of the aqueous extract of Ixora Coccinea on the fasting blood sugar levels of diabetic rats is shown in [Table / Fig-1]. Acute and chronic treatments with the dose of 400mg/kg b.w., in alloxan induced diabetic rats showed a significant ($p<0.01$) decrease in the elevated blood glucose levels as compared to the controls. The doses 100mg/kg b.w. and 200mg/kg b.w. of the extract did not reduce the sugar levels to normal .But the extract in the dose of 100mg/kg b.w. showed significant ($p<0.01$) hypoglycaemic activity on day 14 and the dose of 200mg/kg b.w., showed significant ($p<0.01$) results on day 7.

Biochemical parameters:
A decrease in the serum lipid profile and the serum protein levels ($p<0.01$) was noted with 400mg/kg b.w. of the aqueous extract of Ixora Coccinea in diabetic rats as compared to the diabetic controls and normal rats, as shown in [Table/Fig-2].

DISCUSSION
The present study showed the hypoglycaemic and the hypolipidaemic effects of the aqueous extract of the leaves of Ixora Coccinea in alloxan induced diabetic rats. The diabetic rats which were treated with the extract showed a 4.15%, 6.52% and 8.56% decline in the blood glucose levels in 2, 4 and 6 hours. They showed a 12.63%, 22.38%, 30.14% and 38.19% decline in the blood glucose levels on days 1,7,14 and 21 respectively. There were significant differences in the serum cholesterol, triglycerides and the total protein levels.

Alloxan induces diabetes by destroying the insulin producing beta cells of the pancreas. In vitro studies have shown that alloxan is selectively toxic to the pancreatic beta cells, leading to the induction of cell necrosis. [12] This action is mediated by reactive oxygen species with a simultaneous massive increase in the calcium concentrations, leading to a rapid destruction of the beta cells.

Group	Treatment	Blood glucose Conc. (mg/dl)							
		0 hr	2 hr	4 hr	6 hr	1 day	7 day	14 day	21 day
I	Normal control (Vehicle 2% gum acacia)	87.5±1.6	87.6±1.3	87.9±1.9	88.2±1.5	88.3±1.5	88.8±1.3	89.7 ±1.9	90.4±1.3
II	Diabetic control (vehicle 2% gum acacia)	262.1 ± 5.3	266 ± 5.1**	269.5 ± 3.7**	270.6 ± 3.1**	272.6 ± 2.7**	280 ± 1.2**	293 ± 2.2**	313.2 ± 3.6**
III	Alloxan (120 mg/kg i.p.) +0.1 mg/kg Glibencl-amide	272.2 ± 6.4	239.6 ± 1.9*	228.4 ± 2.3**	221.3 ± 1.5**	280.1 ± 3.6**	165.3 ± 4.2**	107.7 ± 3.2**	95.5 ± 1.4**
IV	Alloxan (120 mg/kg i.p.) + C.P. 100 mg/kg.	272.3 ± 5.8	271.7 ± 5.5	270.7 ± 5.4	270.3 ± 5.8	269.7 ± 5.5	270.1 ± 6.1	270 ± 5.2*	269.3 ± 4.9**
V	Alloxan (120 mg/kg i.p.) + C.P. 200 mg/kg	278.7 ± 7.9	273 ± 7.0	269.4 ± 7.3	266.2 ± 7.6	256.8 ± 6.4	242.4 ± 4.8**	230.7 ± 5.2**	221.4 ± 4.3**
VI	Alloxan (120 mg/kg i.p.) + C.P. 400 mg/kg.	245.3±11.8	235.1±10.4**	229.3±10.1**	224.3±9.5**	214.3±11.2**	190.4±9.7**	170.7±5.8**	151.6±2.6**

[Table/Fig-1]: Effect of aqueous extract of I.C. on blood glucose level in alloxan (120/kg. i.p.) induced diabetes rats.
*All values are expressed as mean ± SEM (n = 6); Group II was compared with group I; Groups III-VI were compared with group II; * p < 0.05, ** p < 0..1.*

Group	Treatment	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Total protein (g/dl)
I	Normal control (vehicle)	86.65 ± 1.83	85.62 ± 3.22	7.20 ± 0.23
II	Diabetic control (vehicle)	153.88 ± 4.19**	189.86 ± 1.74**	4.73 ± 0.53**
III	Alloxan (120 mg/kg i.p.) + Glibenclamide(0.1mg/kg)	92.03 ± 1.09**	94.13 ± 1.20**	6.95 ± 0.07**
IV	Alloxan (120 mg/kg i.p.) + aqueous extract (400 mg/kg)	135.32 ± 4.04**	145.18 ± 3.67**	6.05 ± 0.8**

[Table/Fig-2]: Effect aqueous extract of Ixora Coccinea linn on biochemical parameters in alloxan – induced diabetic rats.
*A All values are expressed as mean ± SEM (n = 6); Group II is compared with group I. Groups III and IV are compared with group II. ** p < 0.01*

[13] The use of lower doses of alloxan (120mg/kg b.w.) produced a partial destruction of pancreatic beta cells, even though the animals became permanently diabetic. Thus, these animals had surviving beta cells and a regeneration was possible. [14]

Glibenclamide, the second generation sulfonylurea, is known to mediate the hypoglycaemic effect by stimulating insulin release from the pancreatic beta cells, reducing the hepatic clearance and suppressing the secretion of glucagon. [15] Sulfonylurea have been shown to suppress gluconeogenesis.

The hypoglycaemic effect of the aqueous extract may be due to the enhanced secretion of insulin from the beta cells of the pancreas or it may be due to an increased tissue uptake of glucose by the enhancement of the insulin sensitivity.

Elevated plasma cholesterol and triglyceride levels are major risk factors of cardiovascular disease. The existing hypoglycaemic agents allow a sharp control of the blood glucose levels, but only an insufficient correction of the lipid abnormality, especially in hypertriglyceridaemia. [16] Diabetic rats showed elevated plasma cholesterol and triglyceride levels due to hyperglycaemia and insulin resistance. [17] The aqueous extract, in the dose of 400mg/kg b.w., reduced the triglyceride and the cholesterol levels along with a reduction in the blood glucose levels. Some studies have reported a similar hypolipidaemic activity in experimentally induced diabetic rats. [18]

The active constituents which are responsible for the hypoglycaemic and hypolipidaemic activities of the extract are not known. Phytochemical analyses showed the presence of alkaloids, tannins, saponins, flavonoids, anthraquinones, anthracyanosides and reducing sugars in the extract. The presence of any of these phytocomponents may be responsible for the hypoglycaemic and hypolipidaemic activities of the extract in diabetic rats. Some of the studies have reported that these activities are due to the flavonoids, alkaloids and the tannins which are present in the extract. [19]

Since many anti-diabetic drugs do not correct dyslipidaemia, the observed hypolipidaemic effects of this plant extract in diabetic rats makes Ixora Coccinea quite important in the management of diabetes. Further investigations are needed to elucidate the mechanism of action, particularly the bioactivity guided fractionation, isolation and the identification of the constituents of the plant extract, which are responsible for the observed pharmacological activities.

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