Impact of Calotropis Gigantea Leaves via Different Routes of Administration in Normal and Alloxan Induced Diabetic Rats

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Abstract- The present study was carried out to evaluate the anti-diabetic activity of water extract of Calotropis gigantea leaves in alloxan n induced diabetic rats for 0, 20, 60, 120, 240, 360 minutes. The water extract at the dose (0.7 gm/kg) exhibited significant anti-hyperglycemic activity. Oral and intraperitoneally administration of the plant produced significant hypoglycemic effect in normal as well as hyperglycemic rats. The water extract of Calotripis gigantea leaves showed hypoglycemic effect in normoglycemic and hyperglycemic rats after both oral and intraperitoneal administration. The effect could be comparable to that of well-known hypoglycemic compound like metformin and glibenclamide used at 11.3 and 0.13 mg/kg, respectively.

Index Terms- Calotropis gigantea; Asclepiadaceae; Alloxan monohydrate ; Route of administration

I. INTRODUCTION

Diabetes mellitus is an endocrine disorder characterized by hyperglycemic effecting nearly 10% of the population all over the world. Insulin and oral hypoglycemic agents like sulphonylureas and biguanides are still the major players in the management of the disease. However, complete cure of the disease has been eluding physicians for centuries and the quest for the development of more effective anti-diabetic agents is pursued relentlessly. Many herbal products, including several metals and minerals have been described for the cure of diabetes mellitus in ancient literature. Herbal preparations alone or in combination with oral hypoglycemic agents sometimes produce a good therapeutic response in some resistant cases where modern medicines alone fail. There is increasing demand by patients to use natural products with anti-diabetic activity due to side effect associated with the use of insulin and oral hypoglycemic agents [1]. The World Health Organization has also recommended the evaluation of the effectiveness of plants in condition where there is a lack of safe made drugs.

Currently available treatment for this disorder is far from satisfactory and expensive. Calotropis gigantea leaves (Family : Asclepiadaceae) is a small tree variety found throughout India. It is commonly called Swallow – Wort. Leaves contain the cardiac glycoside; Calotropin; Uscharin; Calotoxin; Calactin; Uscharidin and gigantin [2-3-4]. It is used widely used for healing of wounds; anthelmintic; expectorant; useful in leprosy scabies ring warm of the scalp; piles, eruptions on the body; asthma; prevention of insulin resistance [5], hepatoprotective [6], anti-diarrhoeal [6], antipyretic and analgesic [7-8], anti-inflammatory[9] and wound healing activity [10]. The calotropin Uscharin and gigantin show digitalis – like action on the heart.

The preliminary phytochemical studies reveal the presence of flavonoids; glycosides; alkaloids; tannins. The focus of the present study is to evaluate water extract of Calotropis gigantea leaves material at various doses in normal and alloxan induced diabetic rats. However, no scientific data are available regarding the effect of water extract of Calotropis gigantean leaves on blood glucose level. The present study is undertaken to explore the effect of water extract of Calotropis gigantea leaves on the blood glucose level of experimental animals and to determine the probable mechanism of action. The effect of water extract of Calotropis gigantea leaves on fasting blood sugar level has been evaluated as compared to the standard drug glibenclamide, both in normal and diabetic albino rats. The effect of Calotropis gigantea extract on glucose uptake by rat hemi-diaphragm and the glycogen content of the liver, skeletal muscle and cardiac muscle are evaluated to study its probable mechanism of action as a hypoglycemic agent.

II. EXPERIMENTAL

2.1. Material required:

Alloxan monohydrate was purchased from Sigma Chemical Co St Louis, USA. All other chemicals were obtained from local sources and were analytical grade. Calotropis gigantean was collected from the forest area of Ghaziabad, U.P., India in March 2008. The plants was identified from the School of Pharmacy, Vishveshwariya Institute of Medical Science, Greater Noida, Gautam Budha, Nagar, India. They were assigned voucher specimen Ref. VIMS/CONSULT/2009/02/10.

2.2. Methods:

2.2.1. Preparation of water extract:

The leaves of Calotropis gigantea was air dried and powdered in a grinder. 300g of Powder mixture of the plant parts was extracted overnight with 360 ml of water with magnetic stirring in cold room (4^{o}C). The water extract was separated and the residue was re-extracted with water. The water extract was concentrated to produce semisolid mass and dried in lyophilizer (Mini Lyotrap, Serial No J8199/5, LET Scientific Ltd UK).

2.2,2 Preliminary phytochemical screening:

The extracts were subjected to preliminary screening for various active phytochemical constituents [11].

2.2,3. Animal and experimental set-up:

Colony bred, healthy male wistar albino rats of either sex weighing 150 – 200 g were taken for the study. The animals fed on standard laboratory diet with water ad libitum and housed at room temperature. The rats were kept fasting overnight with free access to water during the experiment in the ambience. The animals were divided into eight groups of six animals each. 1 ml of blood was taken from the orbital sinus of each rat with the help of a capillary tube for the estimation of blood sugar. The institutional Ethical Committee approved all experiment protocols.

2.2.4. Hypoglycemic effect in normal rats [12-13]:

Groups of six rats each (fasted for18 hrs) received 10 ml/kg infusion, intragastrically or intraperitoneally (i.p.) Blood samples were drawn by puncture from the tail immediately before administration in the time intervals of 20, 60, 120, 240 and 360 min later. Control group received an equal volume (10 ml/g) of normal saline, glibenclamide (0.13 mg/kg) and metformin (11.3 mg/kg), calculated on the basis of the daily doses [14].

2.2.5. Hypoglycemic effect on alloxan-diabetic rats [15]:

Chronically hyperglycemic rats were obtained by i.p. injection of 150 mg/kg of alloxane dissolved in distilled water [16]. After 8 hrs administration, the hyper-glycemic rats were selected (plasma glucose level 2-2.8 g/L) and used in the experiments. The same experimental protocol described above was then adopted.

2.2.6. Glucose tolerance test (GTT) in rats [17-18]:

A polyethylene cannula was injected into the jugular vein under ethyl carbonate anesthesia. Another catheter was injected into right carotid. All rats received orally 10 ml/kg of 25% glucose solution. One group of animals received the plant infusion (10 ml/kg) through the venous catheter, while the control group received normal saline. Blood samples (0.2 ml) were taken from the carotid catheter at time intervals of 5, 10, 20, 30, 40, 50 and 60 min after injection. The coefficient of glucose assimilation (K_G) was determined with the formula.

$$K_G = (\log C - \log C/_2) \quad t½ = 0.639\ t½$$

Where : C = glycaemia (g/L); t½: Time for the blood glucose concentration $C/_2$.

2.2.7. Statistical analysis:

Results are reported as mean ± SEM statistical analysis was carried out using analysis of variance (Anova). The difference of the means was calculated using Newman – Keuls test. P values of 0.05 or less were taken as significant.

Table 1.

Effect water extract of Calotropis gigantea leaves on plasma glucose levels after intragastric (p.o) and intraperitoneal (i.p.) administration to normoglycaemic rats.

Treatment	Route	Plasma glucose (g/L) at time (min) after treatment					
		0	20	60	120	240	360
Control(Saline.10 ml/kg)	p.o.	0.99±0.05	0.99±0.08	0.98±0.09	0.90±0.16	0.95±0.06	0.96±0.07
	i.p.	0.98±0.04	0.96±0.06	0.89±0.08	0.93±0.09	0.93±0.08	0.94±0.08
Glibenclamide(0.13 mg/kg)	p.o.	0.96±0.09`	0.69±0.11*	0.46±0.07++	0.57±0.07+	0.73±0.06	0.83±0.05
	i.p.	0.98±0.08	0.73±0.1*	0.48±0.06++	0.63±0.05+	0.75±0.06*	0.93±0.13
Metformin(11.3 mg/kg)	p.o.	0.99±0.05	0.73±0.06*	0.65±0.1*	0.54±0.08+	0.75±0.06*	0.93±0.13
	i.p.	0.93±0.04	0.79±0.05*	0.57±0.06+	0.71±0.05*	0.74±0.05	0.80±0.05
C. gigantea(0.7 g/kg)	p.o.	0.93±0.05	0.59±0.06++	0.58±0.14++	0.51±0.07++	0.83±0.13	1.02±0.06
	i.p.	0.98±0.07	0.68±0.06++	0.56±0.22++	0.54±0.19++	0.63±0.09+	0.67±0.08+

[a] values are mean ± SEM, n= 8; *p< 0.05; +p<0.01; ++p<0.001 vs. Control; Anova and Newman - Keuls test.

Table 2.

Plasma insulin in normoglycaemic rats after intragastric (p.o.) and intraperitoneal (i.p.) administration water extract of Calotropis gigantea leaves.

Treatment	Route	Insulinmia (12.42 µlU/mL) at time (min) after treatment					
		0	20	60	120	240	360
Control(Saline.10 ml/kg)	p.o.	58.08±4.41	68.56±10.26	63.11±8.42	56.64±11	60.06±10	72.32±6.16
	i.p.	88.88±14.96	71.89±8.25	60.41±7.89	61.57±9.15	67.27±8.01	84.94±7.91
C. gigantea(0.7 g/kg)	p.o.	82.06±14.35	68.96±8.52	54.14±9.56	51.88±3.03	63.17±19.04	75.03±19.42
	i.p.	64.22±8.56	78.07±12.62	90.19±7.16*	73.02±16.9	121.42±10.01++	113.37±20.52+

Values are mean ± SEM, n= 8; *p< 0.01; ++p<0.001 vs. Control; Anova and Newman - Keuls test.

Table 3.

Effect water extract of Calotropis gigantea leaves on Plasma glucose levels after intragastric and intraperitoneal (i.p.) administration to alloxan-diabetic rats.

Treatment	Route	Plasma glucose (g/L) at time (min) after treatment					
		0	20	60	120	240	360
Control(Saline.10 ml/kg)	p.o.	2.82±0.2	2.57±0.14	2.14±0.72	2.20±0.45	2.35±0.39	3.00±0.48
	i.p.	2.71±0.2	2.61±0.18	2.59±0.65	2.15±0.5	2.28±0.49	2.92±0.51
Glibenclamide(0.13 mg/kg)	p.o.	2.88±0. 2	2.15±0.26	1.44±0.39+	0.91±0.08++	1.44±0.22++	1.70±0.34+
	i.p.	3.01±0.03	2.36±0.35	1.21±0.47*	0.88±0.11*	1.44±0.22*	1.70±0.34
Metformin(11.3 mg/kg)	p.o.	2.96±0.1	2.66±0.2	1.21±0.09+	0.99±0.06+	1.37±0.25+	2.00±0.47
	i.p.	3.01±0.09	1.30±0.4	0.74±0.41*	1.63±0.25*	1.50±0.35*	1.80±0.30*
C. gigantea(0.7 g/kg)	p.o.	2.90±0.08	2.02±0.53*	1.05±0.16++	1.01±0.07++	1.03±0.12++	0.92±0.20+yx
	i.p.	2.86±0.3	1.74±0.25+	1.60±0.22+	0.79±0.3++	1.189±0.21++	1.32±0.19++bx

Values are mean ± SEM, n= 8; *p< 0.05; +p<0.01; ++p< 0.001 vs. control; ^{b}p<0.05 vs. glibenclamide; ^{x}p<0.05; ^{y}p<0.01 vs. metformin; Anova and Newman - Keuls test. Ajay[1]

Table 4.

Blood glucose in glucose loaded (0.25 g/kg) rats before and after the intravenous administration water extract of Calotropis gigantean leaves.

Treatment	Blood glucose (g/L) at time (min) after load							
	0	5	10	20	30	40	50	60
Control(0.25g/kg glucose)	0.99±0.08	1.26±0.08	1.47±0.11	1.59±0.14	1.71±0.09	1.69±0.11	1.46±0.08	1.33±0.07
C. gigantea(0.7 g/kg)	0.98±0.09	1.15±0.1	1.35±0.06	1.48±0.1	1.51±0.08*	1.31±0.1+	1.25±0.11+	1.16±0.13++

Values are mean ± SEM, n= 5; *p< 0.05; +p<0.01; ++p<0.001 vs. Control; Anova and Newman - Keuls test.

III. Result and Discussion

The infusion of Calotropis gigantea exhibited a remarkable hypoglycemic action 20 min after oral and i.p. administration to normal rats. (Table 1.). Blood glucose level reached a mean value of 0.59 and 0.68 g/L, respectively as compared to 0.99 g/L and 0.96g/L. respectively obtained in the control group. The lowest hypoglycemic effect was observed after 2 hr of administration. After 4 hrs of administration, the blood glucose level reached nearly to the initial glycemic values for orally treated animals, while i.p. administration still showed hypoglycemic effect even after 6 hr. Calotropis gigantea hypoglycemic effect was comparable and sometimes higher than that obtained with 0.13 mg/kg of glibenclamide or 11.3 mg/kg of metformin. After i.p. administration, the variation in insulin plasma levels showed an opposite trend to that of glucose (Table 2). The increase became significant after 1 hr of administration and persisted for at least 6 hr. Plasma insulin reached a maximum level (12.42 µlU/mL) 4hr after i.p. administration. On the other hand, no variation in blood insulin level was found in normal rats orally treated with the water extract of Calotropis gigantea leaves. When compared with control, Calotropis gigantea (Table 3) significantly reduced the blood glucose levels in diabetic rats. The maximum decrease observed 2 hr after the administration in plasma glucose level recorded as 1.01 g/L (-69.96%) and 0.79 g/L (-53.29%), respectively after oral and i.p. treatment. In a glucose tolerance test, intravenous treatment with Calotropis gigantea plasma glucose level significantly reduced at time intervals of 40, 50 and 60 min as compared to plasma glucose level induced in control by a glucose load administration (Table 4). Glycemic values returned to basal levels more rapidly than in control group. The coefficient of glucose assimilation (K_G) showed significant increase in treated rats compared to control (8.17×10^{-3} vs 6.96×10^{-3}).

IV. Conclusion

The Calotropis gigantea showed hypoglycemic effect in normoglycemic and hyperglycemic rats after both oral and intraperitoneal administration. The effect could be comparable to that of well-known hypoglycemic compound like metformin and glibenclamide [19] used at 11.3 and 0.13 mg/kg, respectively. As far as the mechanism of action is concerned, in the light of the obtained results it can be speculated that Calotropis gigantea activity could be due to enhancement of peripheral metabolism of glucose. An increase of insulin release can not be excluded. Further studies to identify the active constituents of Calotropis gigantea and their mechanism of action are in progress.

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