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Research Article

Efficacy of *In Vitro* and Native *Tylophora Indica* Leaf Extract against Hyperglycemic Mice Induced with Alloxan through Oral Administration

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ABSTRACT

Tylophora indica (Asclepiadaceae) have long been used for the treatment of asthma, bronchitis, whooping cough, dysentery, rheumatic gouty pains and hydrophobia[1]. In the present study, an assessment of Alloxan induced Swiss albino mice is made by treating with oral administration of leaf extract of native (n) and *in vitro* propagated or micro-propagated (m) *Tylophora indica*. Extracts of both *in-vitro* and native plant are prepared in methanol (MLTLn and MLTLm) for investigation of blood glucose lowering affect and improvement in body weight in diabetic Swiss albino mice .The results obtained are evaluated with respect to known anti diabetic drug glyburide. It is observed that *in vitro* propagated *T.indica* shows better hypoglycaemic effect than native plant. Various factor are monitored e.g. blood glucose level, body weight, mortality rate in different groups.In this study, the anti diabetic activity of herbal drug for treatment of diabetes mellitus is clearly indicated.

Keywords: Hypoglycaemic; *In vitro* propagated; Anti diabetic activity; *Tylophora indica*

1. INTRODUCTION

Diabetes mellitus is a medical disorder characterized by varying or persistent hyperglycemia i.e. high blood glucose levels resulting from insufficient production of a hormone i.e. insulin or defective secretion or lack of responsiveness to insulin which results in factors like stress, hypertension and autoimmunity. It also leads to other diseases like nephropathy, neuropathy and retinopathy[2,3]. Many drugs and intervention are available to manage diabetes like Sulfonylureas and related compounds, biguanides, thiazolidenediones, alpha glucosidase inhibitors, insulin etc. In most the cases they are expensive, have serious side effects, and in addition they are not considered to be safe for use during certain conditions e.g. pregnancy [4, 5, 6]. Chlorpropamide, glibenclamide, glicazide, metformin etc. are also available chemical compounds as drugs for the treatment of diabetes. But researchers are continuously working to find effective drugs originated from plants for the treatment of diabetes. The plants with medicinal properties have been used for the treatment of diseases like rheumatism, skin diseases, ulcers, diabetes etc. by Ayurveda since centuries. The use of herbal medicines for the treatment of diabetes mellitus is also gaining importance throughout the world. In the present study hypoglycaemic effect of native and in vitro or micropropagated *Tylophora indica* against Alloxan induced diabetes in Swiss albino mice are reported.

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2. MATERIALS AND METHODS

2.1 Chemicals

Alloxan was procured from Sigma Chemical Co.(Sigma-Aldrich, USA), Glyburide from (Ranbaxy Pharma. Ltd., New Delhi, India)

and Blood Glucose estimation kit from Entrust Ascentia and MylifePura™. All other chemicals were of analytical grade, available locally.

2.2 Plant material

Native *T. indica* plant was collected from herbal garden of Punjab Agricultural University, Ludhiana. *In vitro* raised *T.indica* plant was collected from Thapar University, Patiala. Leaves were washed, dried, powdered and preserved at 4°C.

2.2.1 Preparation of plant extract

The leaves of native and *in vitro* cultivated *T. indica* plant were washed with water and then dried in an oven at 37° for 3 days. The dried leaves were powdered and weighed. 6g of dried leaf powder was dissolved in 80 ml of methanol. This mixture was agitated on the magnetic stirrer for 48 hours. The obtained extract was then filtered using Whatmann's filter paper and sterilized using millipore filters having pore size 0.22µm. This was then evaporated by using a rotary evaporator to get the crude dried extract. Thereafter the extract was then stored at 4°C until used. The whole procedure yielded 10-11% (w/w) of the extract in terms of dried starting material.

2.2.2 Preparation of Sample suspension

The sample suspension of methanolic *Tylophora indica* leaf extract (MLTLn and MLTLm) was prepared at a dose 100 mg/kg body weight and suspending in 0.1%DMSO.

2.3 Experimental Animals

Swiss albino mice aged 6-8 weeks were purchased from Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana. Swiss albino mice (25-30 g) maintained on standard laboratory diet (Kisan Feeds Ltd., Mumbai, India) and water *ad libitum*, housed in the departmental animal house and were exposed to 12 h cycle of light and dark.The experimental protocol was approved by the University Animal Ethical Committee and animals were kept carefully as per the ICMR guidelines recommended by Committee for the purpose of Control and Supervision of Experiments on Animals, Ministry of Environment and Forests, Government of India (Reg no.107/99/CPCSEA/2013-29).

2.4 Toxicity Study

The preliminary experiments were carried out to select the sub-lethal and sub-chronic dose of plant extracts. Acute toxicity of alkaloid present in leaf extract was described by Dikshith[7].Mice were given a single oral dose each of 100-600mg/kg body weight. The dose of plant extract spread which showed 100% survival of inoculated mice after 21 days (3 weeks) was selected as sub-lethal and sub-chronic doses. The treated animals were observed for gross general behaviour, mortality, haematological changes and body weight.

2.5 Induction of Diabetes

Alloxan (2, 4, 5, 6-tetraoxypyrimidine; 2, 4, 5, 6-pyrimidinetetrone) was used for induction of diabetes. Administration of aqueous Alloxan monohydrate in acetate buffer (0.15 M,pH 4.5) in was done in overnight fasting mice[8].Total dose of Alloxan (450 mg/kg,b.wt.) was administered in three injections at intervals of 48 h (150 mg/kg,b.wt. each time) and monitored for increase in glucose level. Animals having blood glucose level more than 120 mg/dl were selected for the study.

2.6 Experimental design of animals

The experimental work was carried out for 4 weeks. The first week was for the induction of diabetic condition in mice and the following 3 weeks were investigational period with plant extract. The animals were grouped according to the following scheme:

Group 1: {**Control group**} Not subjected to any treatment i.e. only diet was provided.

Group 2: {**Hyperglycemic (HYG) Control}** Mice were made hyperglycemic with Alloxan and were fed the basal feed only.

Group 3: {HYG+NativeLeaf Extract (MLTLn)} Hyperglycemic mice which were subjected to native leaf extract (100mg/kg b.wt.) with normal diet.

Group 4: {HYG+Micropropagated Leaf Extract (MLTLm)} Hyperglycemic mice which were subjected to micropropagated leaf extract (100mg/kg b.wt.) with normal diet.

Group 5: {HYG+Glyburide} Hyperglycemic mice were treated with Glibenclamide (10mg/kg b.wt.) with normal diet.

2.6.1 Acute study

The blood glucose level was checked on 1st day of test dosing at 0thhr, 2ndhr, 4thhr, 6thhr, and 24thhr for the acute study to observe effects of doses on the levels of blood glucose.

2.6.2 Sub acute study

Sub acute study was carried out for 30 days checking blood glucose levels on 0th, 6th, 12th, 18th, 24th and 30th day of test dosing to check the significant decrease in blood glucose levels among all the doses. . Body weight of the mice were also noted during the study period of 30 days and represented as mean change in body weights. The death of the mice was also monitored and percentage mortality was calculated.

2.6.3 Analysis of Blood Glucose

For acute and sub acute studies, the blood sample was taken on the above said days from tail vein and glucose levels were checked using Glucometer from Entrust Ascentia and MylifePura™.

2.6.4 Statistical Analysis

Results were expressed as Mean ± Standard Deviation (SD). Data of tests were statistically analyzed using one way ANOVA followed by Tukey's multiple range test, applied for *post hoc* analysis. A value of $p<0.001$ was considered to be statistically significant.

3. RESULTS and DISCUSSION

3.1 Toxicity Study

The dose level of 500mg/kg body weight was safe. However at higher doses, some animals showed lethargic behaviour and some could not sustain.

3.2 General Observations

Hyperglycemic Mice cages were to be cleaned frequently due to offensive smell induced by frequent urination and water consumption of these animals was on higher side as compared to the Control group. These findings were noted approximately after 24 hours of diabetes mellitus injection. After introduction of the extract, the symptoms stated above subsided and the offensive urine smell diminished such that no cage cleaning is required.

3.3 Body Weight

Alloxan induced diabetic rats showed a significant decrease ($P<0.001$) in body weight compared to control group. Oral administration of leaf extract at the dose of 100 mg/kg showed a significant increase ($P<0.001$) in body weight on 12th ,18th,24th and 30thday of post induction when compared to control and hyperglycaemic control Swiss albino mice. So, the treatment of diabetic animals with MLTLn and MLTLm (100 mg/kg, b.wt.) prevented the decrease in bodyweight showing the advantageous outcome of extract administration.

Table 1: Effect of Methanolic leaf Extract of *T.indica (native and* micro propaagated*)* on Body Weight (g) in Alloxan-Induced Diabetic Mice

Groups	Day 0	Day 6	Day 12	Day 18	Day 24
Control	32±0.32	32 ± 0.48	32.5 ± 0.56	32.5 ± 0.61	33 ± 0.73
HYG Control	32±0.63	26 ± 2.0a	24 ± 0.60 a	22 ± 0.11a	19 ± 0.46 a
HYG + L.E(n)	31±0.41	28 ± 0.22 a	29 ± 2.35a	29 ± 1.61a	30 ± 1.91
HYG + L.E(m)	30±0.81	29 ± 0.56 a	29 ± 1.42a	30 ± 1.50a	31 ± 0.95b
HYG + Glyburide	31±0.92	29 ± 0.85 a	30 ± 0.66a	30 ± 0.67a	31 ± 1.22 b

Note: *The results are presented as Mean ± S.D (n = 6), data were analyzed by one-way ANOVA followed by post hoc Tukey's multiple range test. All the values were significant ($p<0.001^a$ and $p<0.005^b$) as compared with control and HYG Control group.*

3.4 Blood Glucose Level

During Acute study administration of both native and micropropagated leaf extract

(100mg/kg, b.wt.) and glyburide (10 mg/kg) significantly reduced ($p<0.001$) the blood glucose levels at 2, 6h and 24h. The onset of anti-hyperglycemic effect of glyburide was 2 h (Table 2). The peak of the effect was attained at 4 h but the effect diminished at 24 h. The micropropagated leaf extract extract exerted the antihyperglycemic effect at 2 h and showed better reduction in glucose (110.5 ± 3.31) than native leaf extract (114.2±5.51).

Sub acute Administration (Table 3) of the both extracts and Glyburide caused a significant ($p<0.001$) reduction in blood glucose level as compared to control and hyperglycaemic control However, the micropropagated leaf extract resulted in a better and significant ($p<0.001$) response than native leaf extract in terms of reduction in blood glucose levels. The blood glucose level on day 30th showed by the micropropagated leaf

extract was 106 ±3.12 mg/dl whereas that of glyburide treated group was 102± 1.87 mg/dl.

Table 2: Effect of Acute Treatment of Methanolic Leaf Extract of *Tylophoraindica*(native andand*invitro*micropropagated) and glyburide on Blood Glucose Level in Alloxan-induced Diabetes in Mice

ACUTE STUDY	0 hr	2hr	4hr	6hr	24hr
Control	88.5 ± 3.12	86.5 ± 2.47 [a]	88 ± 2.12 [a]	88.5 ± 1.76 [a]	87.5 ± 3.18 [a]
HYG Control	158.8 ± 2.12	149 ± 4.94 [a]	152.5 ± 3.88 [a]	149 ± 4.24 [a]	147 ± 1.41 [a]
HYG+L.E (n)	170 + 3.32	114.2 + 5.51 [a]	158 + 2.26	140 + 1.39 [a]	126.5 + 4.59 [a]
HYG+L.E (m)	171.7 ± 2.39	110.5 ± 3.31 [a]	157 ± 2.68 [b]	139 ± 1.15 [a]	122 ± 1.39 [a]
HYG+Glyburide	158.7 ± 5.03	132.7 ± 3.72 [a]	148.7 ± 1.93 [b]	136 ± 4.79 [a]	118 ± 2.32 [a]

Note: *Blood glucose levels were assessed at regular interval of 0, 2, 4, 6 and 24 h hour after administration of native , micropropagated extract and glyburide.The results are presented as Mean ± S.D (n = 6). p<0.001 [a] comparison with control and HYG control; p<0.05 [b] comparison with HYG control.*

Table 3: Effect of Sub acute Treatment of Methanolic leaf Extract of *Tylophora indica* (native and micropropagated) and glyburide on Blood Glucose Level in Alloxan-induced Diabetes in Mice

SUBACUTE STUDY	0th day	6th day	12th day	18th day	24th day	30th day
Control	99 ± 5.88	97 ± 6.32 [a]	96.5 ± 4.05 [a]	96 ± 5.38 [a]	95.5 ± 4.97 [a]	94.5 ± 3.1 [a]
HYG Control	93 ± 2.32	153 ± 2.02 [a]	147 ± 1.94 [a]	140 ± 2.60 [a]	142 ± 1.05 [a]	141 ± 2.24 [a]
HYG+MLTL(m)	85 ± 1.67	161 ± 2.89 [a b]	140 ± 4.04 [b]	132 ± 1.67 [a]	125 ± 1.90 [a]	110 ± 2.89 [a]
HYG+MLTL(n)	87 ± 1.85	160 ± 2.45 [a c]	137 ± 2.09 [a]	128 ± 3.10 [a]	120 ± 2.26 [a]	106 ± 3.12 [a]
HYG +Anti-diabetic drug	84 ± 2.45	159 ± 3.24 [a c]	135 ± 2.34 [a]	124 ± 3.95	115 ± 2.10 [a]	102 ± 1.87 [a]

Note: *Blood glucose levels were assessed on day 0, 6, 12, 18, 24 and 30 after simultaneous administration of native, micropropagated extract and glyburide. The results are presented as Mean ±S.D (n = 6). p<0.001 [a] comparison with untreated control; p<0.01 comparison with HYG control; p<0.05 comparison with HYG control.*

3.5 Mechanism of action:

Alloxan causes specific destruction of cells of islets of pancreas and result an increase in blood glucose levels in Swiss albino mice. The effect of administration of aqueous leaves extract of *Tylophora* on blood glucose in normal hyperglycaemic control and hyperglycaemic treated Swiss albino mice is reported. Exact mechanism of reducing blood glucose is not well understood. The probable cause of reduction of blood glucose might be due to increase uptake of glucose peripherally and increased sensitivity of insulin receptor in case of *Tylophora indica* extract.It is also observed that hypoglycaemic activity in various plants is assumed to be due to the active principles present. Compounds like polysaccharides[9], flavonoids, terpenoids, tannins[10]and alkaloids[11] etc. have been reported to be responsible for hypoglycemic effect. The preliminary phytochemical studies indicate the presence of phytosterols, flavonoids, alkaloids and glycosides in methanolic extract of *T.indica* leaf. *Tylophora indica* have been used in the Ayurvedic system of medicine and its various extracts are known to have antiasthmatic and antiallergic properties [12-15].

4. CONCLUSIONS

The methanolic extract of *Tylophora indica* leaf exhibited significant hypoglycemic activity in Alloxan induced diabetic Swiss albino mice. From the phytochemical analysis it was found that the major chemical constituents of the leaf extract were alkaloids, flavonoids and glycosides. On the basis of above evidence it is possible that the presence of flavonoids may be responsible for the observed anti diabetic activity. Furthermore, the study suggested that *in vitro* raised plant gave better results in controlling diabetes than that of native plant.

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