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Behara Chandra Rekha
PG student, Department of Pharmacology, Gandhi Medical College, Secunderabad.

Sunitha Tangeti
Assistant Professor, Department of Pharmacology, Gandhi Medical College, Secunderabad.

Lanke Venu Madhavi
Assistant Professor, Department of Pharmacology, Vishwabharathi Medical College, Kurnool

Rama Mohan Pathapati
Associate Professor of Pharmacology, Narayana Medical College, Nellore, A.P

Correspondence
Lanke Venu Madhavi
Assistant Professor, Department of Pharmacology, Vishwabharathi Medical College, Kurnool

Toxicity, Acute and Longterm Anti-Diabetic Profile of Methanolic Extract of Leaves of Pterocarpus Marsupium on Alloxan Induced Diabetic Albino Rats

Behara Chandra Rekha, Sunitha Tangeti, LankeVenu Madhavi, Rama Mohan Pathapati

Abstract
Background- Type-2 Diabetes becomes a real problem of public health in developing and developed countries, where its prevalence is increasing gradually, and suitable treatment is becoming expensive. It is also multifactorial ailment leading to numerous complications, which in turn demands alternative therapeutic approach in view of side effects like hypoglycemia, weight gain, gastrointestinal disturbances, and liver toxicity caused by present drugs available drugs in the market. Conventionally antidiabetic effect of some herbal extracts has been confirmed in human and animal models of type 2 diabetes as an alternative medicine/nutritional supplements. To this rationale, we studied the antidiabetic effect of methanolic extract of leaves of Pterocarpus marsupium on alloxan induced diabetic albino rats.
Methods- Albino rats of Wister strain weighing 180 – 225 gm of either sex, non-pregnant were used for the study, were procured from our animal house. We employed Alloxan monohydrate 5% fresh solution (125mg/kg body weight) administered intraperitoneally to induce diabetes. Animals were divided into four groups with six animals in each group, kept separately in different cages. Group N: - Non-diabetic rats served as control group and received plain water, Group C: -Diabetic control group (Alloxan +2% Gum Acacia), Group G: -Standard group (Alloxan +0.5mg/kg BW Glibenclamide) and Group PM: -Test group (Alloxan +700mg/kg BW Pterocarpus marsupium leaves).
Acute toxicity study was conducted using doses 100-1000 mg/kg body weight of Pterocarpus marsupium on healthy rats, additionally; 24-hour antidiabetic profile was also conducted on animals of four groups. In 6th-week efficacy study after giving the medications under trial, fasting blood glucose levels were recorded on the 1st, 3rd, 7th, 14th, 21st, 28th, 35th$^{\&}$42nd Day. On 42nd day biochemical parameters such as total protein, serum albumin, albumin to globulin ration, Total cholesterol, LDL VLDL, HDL and LDL/HDL ratio were also analysed. After six weeks of study, two animals from each group were sacrificed & the animals were dissected & their pancreas was sent for histopathological examination.
Results-700mg/kg body weight Pterocarpus marsupium leaves showed its peak action in 24-hour anti-diabetic activity and also was found to be safe in acute toxicity study. For this reason, the therapeutic dose for hypoglycemic activity was calculated as 700mg/kg. In 24 Hour antidiabetic study with methanol extract of leaves of Pterocarpus marsupium on alloxan induced diabetic rats: After administration of 700mg/kg methanolic extract of Pterocarpus marsupium leaves, we observed the fall in blood glucose concentrations were 56.12% at a 1st hour & 14.61% at a 2nd hour with ($p < 0.0001$). The maximum percentage reduction in fasting blood glucose value was achieved at 24 hrs., indicates the long acting nature of the extract (i.e.,) >24 hrs. In 42-day study, the percentage reduction in blood glucose with 700mg/kg body weight of the extract was, 14.51%, 50.16%,42.12%,44.49%,52.23%,56.48%,53.26% and 56.32% on 1, 3, 7, 14, 21, 28, 35 & 42 days respectively. Whereas with Glibenclamide it was 40.29%,42,51%, 38.98%,40.10%, 43.49%, 40.39%, 42.24% & 47% on 1, 3, 7, 14, 21, 28, 35 & 42 days respectively. On 42nd day Pterocarpus marsupium showed reduction in total cholesterol (54.51%), LDL cholesterol (58.86%), LDL/HDL ratio (80.47%) and albumin/globulin ratio (63.82%) ($p<0.05$), suggesting its statistical significance on comparison with Glibenclamide which show a marginal raise in above parameters Such as total cholesterol (4.65%), LDL cholesterol (4.35%) & LDL/HDL ratio (10.31%) and albumin/globulin ratio (3.89%) Such a result explains that, in such a long term significant antihyperglycemic activity we observed reductions in the biochemical parameters by the methanol extract apart from their improvement in diabetes.
Conclusion- The above results indicate that methanol extract of leaves of Pterocarpus marsupium plant has significant & sustained oral hypoglycemic activity, comparable with the oral hypoglycemic effect of insulin secretagogue like glibenclamide, a sulphonylurea. Hence, it is concluded, that this antidiabetic effect may be due to increased secretion of insulin. Even it showed significant improvement in lipid profile, serum Albumin/Globulin ratio and beta cell regeneration; still it needs larger studies to confirm.

Keywords: Type-2 Diabetes, Glibenclamide, Pterocarpus marsupium, Albino rats

Introduction

Type-2 diabetes mellitus is a metabolic disorder, characterised by persistent hyperglycemia, abnormal carbohydrate, lipid and proteins metabolism. (1) It is estimated in India about 19.4 million individuals were affected with this disease, which is likely to go up to 57.2 million by 2025. (2) DM becomes a real problem of public health in developing and developed countries, where its prevalence is increasing gradually, and suitable treatment is becoming expensive. (3) DM is a multifactorial ailment leading to numerous complications, which in turn demands alternative therapeutic approach in view of side effects like hypoglycemia, weight gain, gastrointestinal disturbances, and liver toxicity caused by present drugs available drugs in the market. [4] Various conventional medicines were derived from medicinal plants; minerals and organic matter used for over thousands of years named as Rasayana. [5, 6] In Indian systems of medicine, most practitioners formulate and dispense their own recipes. [7] Conventionally, the hypoglycemic effect of some herbal extracts has been confirmed in human and animal models of type 2 diabetes as an alternative medicine/nutritional supplements. [8] Madhavulu Buchineni *et al*. in his various herbal studies found, antihyperglycemic and hypolipidemic Murraya and Ocimum sanctum Linn in Wister rats. [9-11] Chakravarty B.K *et al*. study found that flavonoid fraction from Pterocarpus marsupium has been shown to cause pancreatic beta-cell degranulation. [12] To this purpose, we studied the antidiabetic effect of methanolic extract of leaves of Pterocarpus marsupium on alloxan induced diabetic albino rats.

Methods

The study was conducted at Gandhi Medical College Hyderabad; animal ethics committee approved the study proposal. Albino rats of Wister strain weighing 180 – 225 gm of either sex, non-pregnant were used for the study, were procured from our animal house. The animals were housed in polypropylene cages maintained under conditions of 12 hrs light: 12hrs dark cycle, 25±3 degree centigrade. They were fed with standard rat pellet diet (obtained from NIN, Hyderabad, India) and water ad libitum, throughout the experiments. Animals were acclimatised to laboratory conditions before carrying out any experimental work. We employed Alloxan monohydrate 5% fresh solution (125mg/kg body weight) administered intraperitoneally to induce diabetes. Blood samples were collected from the dorsal/lateral tail vein of the rats for the daily fasting blood sugar estimation from the overnight fasted rats and from the retro-orbital plexus puncture method for the investigations done during chronic for toxicity. The ONE TOUCH Ultra ™ glucometer was used for recording the blood glucose. Fasting blood glucose level was estimated at the time of induction of diabetes and regularly after that till stable hyperglycemia was established. Rats were kept for the next 24 hrs on 10% glucose, to prevent hypoglycemia. After 72 hrs of injection, the fasting blood levels in rats began to increase gradually, and animal develops stable hyperglycemia after 4 - 5 days. Diabetic rats were used for the experiment after their fasting blood glucose levels were stabilised.

6 Week Efficacy Study

Animals were divided into four groups, six animals in each group, which were kept separately in different cages. Group N: -Non-diabetic rats served as control group and received plain water, Group C: -Diabetic control group (Alloxan +2% Gum Acacia), Group G: -Standard group (Alloxan +0.5mg/kg BW Glibenclamide) and Group PM: -Test group (Alloxan +700mg/kg BW Pterocarpus marsupium leaves)

1. **Group-N:** Non-diabetic animals were taken as a control group for study. Rats were fed with 0.5 ml of distilled water for 42 days through the oral route. Fasting blood glucose levels were recorded on 1st, 3rd, 7th, 14th, 21st, 28th, 35th, 42nd days.

2. **Group-C:** Diabetic rats were fed with 0.5 ml of distilled water orally, daily for 42 days. Fasting blood glucose levels were recorded as like control group.

3. **Group-G:** Diabetic rats were fed with Glibenclamide suspended in 0.5 ml of gum acacia, in a dose of 0.5 mg/kg body weight orally through an intra-gastric tube (80). The drug was administered daily once in the morning for 42 days. On the first day, repeated blood glucose levels were recorded every half an hour for 3 hrs and at the end of 24 hr. To know the onset & peak hypoglycemic activity. Afterwards fasting blood glucose levels were recorded on the 1st, 3rd, 7th, 14th, 21st, 28th, 35th&42nd Day.

4. **Group (PM):** Diabetic rats were fed with 700mg/kg body weight methanol extract of leaves of Pterocarpus marsupium suspended in 2% of gum acacia was given orally through the intra-gastric tube was used as a suspending agent. The test drug was administered daily once in the morning for 42 days. On the first day, repeated blood glucose levels were recorded every half an hour for 3 hrs and at the end of 24 hr. To know the onset & peak hypoglycemic activity. Afterwards fasting blood glucose levels were recorded on the 1st, 3rd, 7th, 14th, 21st, 28th, 35th&42nd Day.

Biochemical Estimation: On the day 42, blood samples were collected from retro-orbital plexus of overnight fasted rats. Serum was separated by centrifuging the sample at 6000 rpm for 20 minutes. The serum was analysed for total protein, serum albumin, albumin to globulin ration, Total cholesterol, LDL VLDL, HDL and LDL/HDL ratio.

Histopathology After six weeks of study, two animals from each group were sacrificed& the animals were dissected & their pancreas was sent for histopathological examination.

Statistical Analysis

Data was presented as mean, standard deviation, numbers and percentages. Inferential statistics was computed using One Way ANOVA followed by post hoc Dennett's multiple comparison tests where test group & standard group are compared with diabetic control group. Two-tailed P- values were significant at <0.05 level.

Results

24-hour acute toxicity study

Normal healthy rats were divided into five groups of 6 animals each. Different doses (100mg, 300mg, 500mg, 700mg, 1000mg/kg body weight) of methanol extract of plant Pterocarpus marsupium leaves suspended in 2% of gum acacia were administered orally through the orogastric tube. The animals were observed continuously at one hr, two hr, 3hr & 24hr for any signs of intoxication like general appearance, the activity of the animals, behavioural, neurological, autonomic profiles & for any lethality & mortality after 24 hrs up to 72 hrs.

24-hour acute anti-diabetic study

We selected 700mg/kg body weight from a range of doses from 100mg to 1000mg in diabetic rats after conducting an experiment in diabetic rats. In this study, we collected the fasting blood glucose levels were recorded every half an hour for 3 hrs at the end of 24 hr on the first day to know the onset of action & peak hypoglycemic effect. 700mg/kg body weight showed its peak action and was found to be safe in acute toxicity study. For this reason, the therapeutic dose for hypoglycemic activity was calculated as 700mg/kg

Table 1: 24-hour effect of Methanol extract of leaves of Pterocarpus marsupium and Glibenclamide on blood glucose levels in Alloxan (125 mg/kg IP) are induced diabetic. Data presented as Mean ± SEM

Groups	0 hr.	1 hr.	2 hr.	3 hr.	24 hrs.
N	80.00±2.58	80.50±2.45	78.67±2.47	79.17±2.8	76.33±2.37
C	225.3±7.98	229.5±3.70	233.3±4.12	226.3±3.28	224.7±8.50
PM	84.33±3.12	100.7±3.20	199.2±14.09	185.3±12.75	85.33±3.25
G	120.8±6.14	101.2±4.46	87.83±3.54	93.17±3.46	116.5±8.88
P value	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001
G	-46.38%	-55.90%	-62.35%	-58.82%	-48.06%
PM	-62.65%	-56.12%	-14.61%	-18.11%	-61.95%

1. Normal Non-diabetic-N, Diabetic Control-C, Glibenclamide- Pterocarpus marsupium -PM. Data presented as Mean ± SEM. % was calculated comparing Control group. % reductions were calculated comparing Control group.
2. During 24hour period, control and normal rats showed <1 % fluctuation in fasting blood glucose. The average reductions in with Pm extract and Glibenclamide were 42.69% and 54.30% respectively.
3. Minus indicates drop while plus indicates raise
4. 24 Hour antidiabetic study with methanol extract of leaves of Pterocarpus marsupium on alloxan induced diabetic rats: After administration of 700mg/kg methanolic extract of Pterocarpus marsupium leaves, we observed the fall in blood glucose concentrations were 56.12% at 1st hour & 14.61% at 2nd hour with (p <0.0001). The maximum percentage reduction in fasting blood glucose value was achieved at 24 hrs, indicates the long acting nature of the extract (i.e.,) >24 hrs. (Table-1)

Table 2: 42-day antidiabetic study with methanol extract of leaves of Pterocarpus marsupium on alloxan induced diabetic rats

Day	N	C	PM	G	P	PM	G
1	80.83+ 2.72	196.3+22.77	117.2 + 3.24	167.8+ 7.36	<0.0001	-14.51%	-40.29%
3	79.0+ 4.56	192.71+ 24.67	111.7 + 3.68	96.83+ 6.58	<0.0001	-50.16%	-42.51%
7	85.83+. 892	188.0+ 15.01	114.7+ 5.85	108.8+ 6.27	<0.0001	-42.12%	-38.98%
14	76.83+. 088	187.0+ 13.11	112.0+ 4.05	103.08+ 18.5	<0.0001	-44.49%	-40.10%
21	86.83+ 3.73	198.2+ 17.79	112.0+ 4.06	94.67+ 7.60	<0.0001	-52.23%	-43.49%
28	85.67+ 2.72	207.2+ 16.32	123.5+ 2.52	90.17+ 5.88	<0.0001	-56.48%	-40.39%
35	94.83+ 3.25	209.9+ 14.58	120.7+ 3.87	97.67+ 9.46	<0.0001	-53.26%	-42.24%
42	89.67+ 3.47	216.0+ 14.28	114.5+ 4.90	94.33+ 4.00	<0.0001	-56.32%	-47.00%

1. Normal Non-diabetic-N, Diabetic Control-C, Glibenclamide- Pterocarpus marsupium -PM.
2. Data presented as Mean ± SEM. % reductions were calculated comparing Control group.
3. The percentage reduction in blood glucose with 700mg/kg body weight of the extract was, 14.51%, 50.16%,42.12%,44.49%,52.23%,56.48%,53.26% and 56.32% on 1, 3, 7, 14, 21, 28, 35 & 42 days respectively. Whereas with Glibenclamide it was 40.29%,42,51%, 38.98%,40.10%, 43.49%, 40.39%, 42.24% & 47% on 1, 3, 7, 14, 21, 28, 35 & 42 days respectively
4. Maximum reduction in fasting blood glucose values was seen after 3 weeks with Glibenclamide and whereas it was at the end of 4 weeks with rats.
5. Minus indicates drop while plus indicates raise

Table 3: 42nd-day metabolic changes with methanol extract of leaves of Pterocarpus marsupium on alloxan induced diabetic rats

Rats	Total Cholesterol	LDL Cholesterol	LDL/HDL Ratio	Albumin/Globulin Ratio	P Value
Non-diabetic	140.3 + 4.40	115.5+4.42	2.18+0.26	1.24+0.16	<0.0001
Diabetic	208.5 + 7.70	140.2+5.75	5.65+0.17	2.14+0.18	<0.0001
Glibenclamide	218.2+7.90	146.3+6.72	6.23+0.25	2.05+0.23	<0.0001
% Change	+4.65%	+4.25%	+10.31%	-3.89%	>0.05
Pterocarpus	94.83+4.167	57.67+8.50	1.10+0.07	0.78+0.08	<0.0001
% Change	-54.51%	-58.86%	-80.47%	-63.82%	<0.05

Data presented as Mean ± SEM. % reductions were calculated comparing Control group. Minus indicates drop while plus indicates raise

Pterocarpus marsupium showed reduction in total cholesterol (54.51%), LDL cholesterol (58.86%), LDL/HDL ratio (80.47%) and albumin/globulin ratio (63.82%) (p<0.05), suggesting its statistical significance on comparison with glibenclamide which show a marginal raise in above parameters such as total cholesterol (4.65%), LDL cholesterol (4.35%) & LDL/HDL ratio (10.31%) and albumin/globulin ratio (3.89%) Such a result explains that in such a long term significant antihyperglycemic activity we observed reductions in the biochemical parameters by the methanol extract apart from their improvement in diabetes.

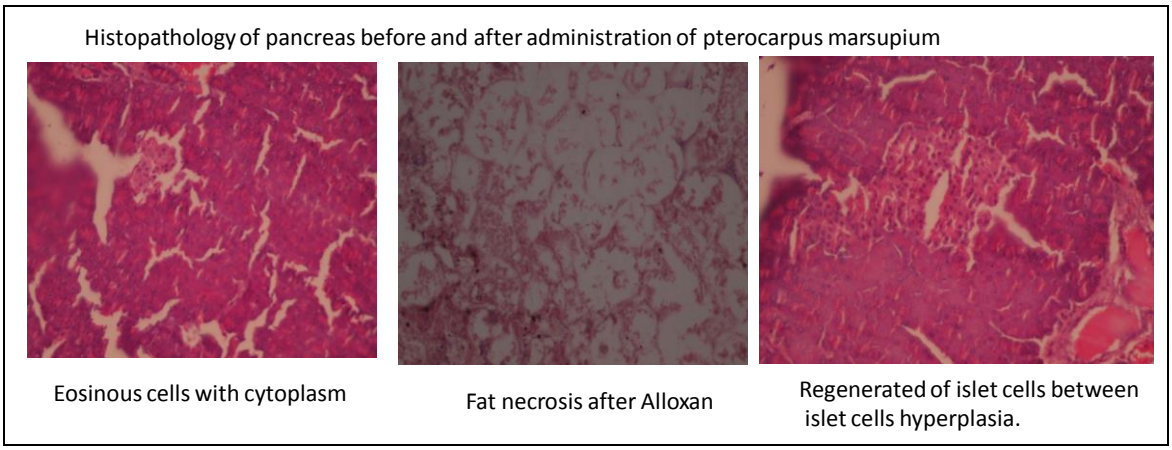

Fig 1: Histopathology of pancreas before and after administration of methanol extract of leaves of Pterocarpus marsupium

Discussion

The blood glucose levels of rats were raised due to permanent destruction of their pancreatic
β cells. Alloxan is a beta cytotoxic, mediated their action by generating reactive oxygen species that cause rapid destruction of β cells and resulting in a diabetic state. [13] Accordingly, in the light of these developments, leaves of Pterocarpus marsupium have been investigated for their hypoglycemic activity and this activity has been compared with that of established oral hypoglycemic drug Glibenclamide. Marsupium Pterostilbene is a constituent derived from wood of this plant caused hypoglycemia in dogs. [14, 15] Showed that the hypoglycemic activity of this extract is because of the presence of tannates in the extract. Flavonoid Fraction from *Pterocarpus marsupium* has been shown to cause pancreatic beta-cell degranulation. [16] Marsupin, pterosaurian and liquiritigenin obtained from this plant showed anti-hyperlipidemic activity. [17]
In alloxan induced diabetic rats significant damage of pancreatic beta cells was seen. The histopathological examination of the sections taken from the pancreas of methanol extract treated rats showed healing of pancreas by significant beta cell regeneration, for which further evaluation of the compound responsible for beta cell regeneration and antidiabetic activity is needed and also its possible mechanism of action. It is to be seen whether the antidiabetic effect of methanol extract of leaves of Pterocarpus marsupium may be due to increased insulin secretion, similar to secretagogue Glibenclamide or it may be due to decreased absorption of glucose from the intestine which may be evaluated further.

Conclusion

The above results indicate that methanol extract of leaves of Pterocarpus marsupium plant has significant & sustained oral hypoglycemic activity, comparable with the oral hypoglycemic effect of insulin secretagogue like Glibenclamide, a sulphonylurea. Hence, it is concluded, that this antidiabetic effect may be due to increased secretion of insulin. Even it showed significant improvement in lipid profile, serum Albumin/Globulin ratio and it also showed beta cell regeneration which needs further evaluation.

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