INTERNATIONAL JOURNAL OF BIOPHARMACEUTICAL & TOXICOLOGICAL RESEARCH

STUDY OF ORAL HYPOGLYCEMIC EFFECT WITH DIFFERENT ETHANOLIC EXTRACTS OF PREMNA INTEGRIFOLIA LINN ON BLOOD GLUCOSE IN ALLOXAN INDUCED TYPE 2 DIABETIC RATS.

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Keywords:
Premna integrifolia, Antidiabetic activity, Alloxan induced diabetes mellitus.

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ABSTRACT:
Ethanopharmacological relevance: Premna integrifolia Linn. (Verbenaceae) is traditionally used to treat diabetes mellitus. The roots of Premna integrifolia are having potential in the development of drug for diabetes due to their antidiabetic activity.
Aim of study: To evaluate the antidiabetic activity of different extracts of roots of Premna integrifolia Linn. (Verbenaceae) in alloxan induced diabetic rats.

Introduction:
Diabetes mellitus is one of the most common chronic diseases in nearly all countries and continue to increase in numbers. The world prevalence of diabetes among adults will be 6.4% affecting 285 million adults in 2010, and will increase to 7.7% and 439 million adults by 2030 (Shaw et al., 2010). Although there are intensive use of current antidiabetic agents but still more than 50% of type 2 diabetes mellitus patients suffering from poor glycaemic control and 18% develop serious complications within six years of diagnosis. Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides, glucosidase inhibitors, which are used as monotherapy or in combination to achieve better glycaemic regulation. Many of these oral antidiabetic agents have a number of serious adverse effects ***(Zhang et al., 2000)***. Clearly, there is a need for new antidiabetic agents ***(Bailey, 2000).***
Diabetes—is a condition in which the body either does not produce enough, or does not properly respond to insulin ***(Mozaffarian et al., 2009).*** Diabetic have significant accelerated levels of oxidative stress and this contributes massively to most neurological, cardiovascular, retinal, renal complications ***(Dubey et***

al., 2008). Clinically, diabetes mellitus is divided into two types. Type 1 diabetes is characterized by an absolute deficiency of insulin secretion, associated with auto-immune destruction of pancreatic β-cells. Type 2 diabetes, which accounts for more than 90% of cases, is caused by a combination of resistance to insulin action and impaired insulin secretion ***(Stephen, 2006).***

Traditional medicines derived mainly from plants play major role in the management of diabetes mellitus ***(Ahmed et al., 2004).*** P.integrifolia Linn (Verbenaceae), commonly known as 'Arni', distributed in Assam, Khassi hills and in Gujarat. It is large shrub or small tree reaching 9 m. high, bark yellowish glabrous, roots are bitter, pungent and traditionally use as laxative, stomachic, diabetes, inflammation and for bronchitis (Nadkarni, 2002). However, there is no scientific evidence to support its antidiabetic potential. So the aim of this study is to provide scientific basis for the use of P.integrifolia in the management of diabetes.

Materials and methods

Collection of plant material

The roots of P.integrifolia were collected during November 2009 from Timba ayurvedic college Timba, Gujarat and identified at Government Agriculture College, Indore, India. A voucher specimen SCOPE/Phcog/01/07-09 is retained in our department for further reference.

Preparation of extracts

The ethanolic extract was prepared by soxhlet extraction method. The extract was concentrated and freeze dried (yield 4.6% w/v), successive solvent liquid-liquid extraction of ethanolic extract was done in a separating funnel using petroleum ether, chloroform and ethyl acetate solvents and then these extracts were collected, concentrated and dried. The yield of petroleum ether extract was 0.4% w/v, chloroform extract was 0.3% w/v and ethyl acetate extract was 0.8% w/v.

Animals

Healthy adult male wistar albino rats aged between 2 and 3 months and weighing 150-200 g were used for the study. Housed individually in polypropylene cages, maintained under standard conditions (12 h light and 12 h dark cycle, 25±30° C, 35-60% relative humidity), the animals were fed with standard rat pellet diet and water ad libitium. The Institutional Animal Ethics Committee (IAEC/SCOPE/07-08/04) approved the study ***(Barik et al., 2008).***

Acute toxicity study

The acute toxicity study was done according to OECD (Organization of Economic Co-Operation and Development) guidelines 420-Fixed Dose Procedure, as in annex 2D. The animals were divided into one group which consists of five mice. The defined or fixed dose level of ethanolic, petroleum ether, chloroform and ethyl acetate extract (2000mg/kg/bw) were given orally to identify a dose producing evident toxicity. The animals were observed continuously for 2 hours for behavioral, neurological and autonomic profiles. The toxicity signs were observed after 24 hours till fourteen days for any lethality or death.

Oral glucose tolerance test (OGTT)

Oral glucose tolerance test ***(Shirwaikar et al., 2006; Sy et al., 2005)*** was performed in overnight fasted (18 h) normal rats. Rats divided into ten groups, each consisting of six rats were administered 0.9% (w/v) saline, gilbenclamide 2.5mg/kg, petroleum ether, chloroform, ethyl acetate and ethanolic extracts (200mg/kg/bw and 400mg/kg/bw). Glucose (4gm/kg) was feed 30 min after the administration of extracts. Blood was withdrawn

from the retro orbital sinus under ether inhalation at 0, 30, 60 and 120 min of glucose administration and glucose levels were estimated using glucose oxidase - peroxidase reactive strips and glucometer (Accuchek, Roche Diagnostics, USA).

Induction of non-insulin-dependent diabetes mellitus (NIDDM)

NIDDM ***(Kannur et al., 2006)*** was induced in overnight fasted adult male wistar albino rats weighing 150-200 g by a single interaperitoneal injection of 120 mg/kg alloxan monohydrate (Loba Chemie). Hyperglycemia was confirmed by the elevated glucose levels determined at 72 h. Animals with blood glucose level more than 150 mg/kg were considered as

diabetic. Gilbenclamide 2.5 mg/kg was used as the standard drug.

Antidiabetic study

Animals were divided into five groups, each consisting of six rats. The extracts were administered for 20 days. Group I: Normal control rats administered saline (0.9% w/v); Group II: Diabetic control rats administered saline (0.9% w/v); Group III: Diabetic rats administered gilbenclamide (2.5 mg/kg) daily for 20 days; Group IV: Diabetic rats administered ethanolic extract (400 mg/kg/bw); Group V: Diabetic rats administered ethyl acetate extract (400 mg/kg/bw).

The effects of administration of P.integrifolia extracts in normal and diabetic rats were observed by measuring fasting blood glucose, serum insulin levels, serum lipid profile and changes in body weight. Fasting blood glucose level was estimated on days 0, 5, 10, 15 and 20 of extract administration. The other biochemical parameters were determined on day 20 after the animals were sacrificed. Serum lipid profile was measured by autoanalyzer (Kannur et al., 2006).

Stastical analysis

Data were statistically evaluated using one-way ANOVA, followed by Dunnett test using STAT software. The values were considered significant when $p < 0.05$.

Results

Acute toxicity study

Acute toxicity study revealed the non toxic nature of extracts. There was no lethality or any toxic reactions found of the doses selected until the end of the study period.

Oral glucose tolerance test

Table 1 shows the hypoglycemic effect of extracts administered at dose level of 200 and 400 mg/kg/bw. The maximum fall in blood glucose level (23.2%) was produced by ethyl acetate extract 400 mg/kg at 120 min after glucose administration.

Table 1: Effect of different extracts of *P. integrifolia* on OGTT

Treatment	Dose	Blood glucose concentration (mg/dl)			
n=6	(mg/kg/bw)	0min	30min	60 min	120min
Control		89.1 ± 2.15	131.0 ± 2.15	114.2 ± 2.10	112.2 ± 1.94
EtOH extract	200	90.3 ± 1.10	122.3± 1.99**	103.2± 1.56**	95.2 ± 0.56*
EtOH extract	400	98.5 ±1.64	134.1 ±1.41**	103.5 ±0.84**	91.5 ±0.6**
EtOAc extract	200	97.0 ±1.56	132.7 ±1.82**	101.7 ±1.70**	90.1 ±1.60**
EtOAc extract	400	91.2±1.49*	120.7 ±1.43**	95.0 ± 1.38**	86.2 ± 1.60**
Petroleumether extract	200	91.0 ± 1.55	126.3 ± 2.11	113.1± 2.04	108.2 ± 2.12
Petroleumether extract	400	90.0 ± 2.80	120.5 ±2.31	104.2± 3.01**	95.5 ±2.92
Chloroform extract	200	94.0 ±2.94	123.7 ±3.01*	109.1±1.87**	106.5±1.42**
Chloroform extract	400	87.2 ± 3.11	116.7 ±2.36	99.2 ± 2.06	97.5 ± 1.98
Gilbenclamide	2.5	96.0 ±1.07	116±1.07**	87 ± 1.27**	83 ± 1.19**

Results are expressed as Mean ± S.E.M. n (no. Of animals) = 6.*p < 0.05 compare with control. **p <0.01 compare with control

Antidiabetic study

Table 2 show the results of antidiabetic study, which revealed significant decrease in fasting blood glucose levels, were observed in rats upon treatment with extracts.

Table 2: Effect of different extracts of *P. integrifolia* on blood glucose level in diabetic rats

Treatment	Fasting blood glucose level (mg/dl)				
n=6	0 Day	5 Day	10 Day	15 Day	20 Day
Normal	91.1 ± 1.25	91.6 ± 0.62	93.0 ± 0.47	92.3 ± 0.62	94.1±0.6
Diabetic Control	195.5±5.55	191.0 ±4.54	195.6 ±4.29	201.3 ±4.36	203.0±0.40
Standard Control	204.6±2.39	168.0±2.85	127.3 ±2.39	106.6 ±1.55	103.3±1.03
EtOH extract (400 mg/kg)	191.0±5.31	161.1±2.30**	134.2±1.49**	115.3±1.42**	110.1±1.26**
EtOAc extract (400 mg/kg)	198.0±2.21	160.5±1.50**	129.6±1.37**	111.6±0.67**	108.3±0.74**

Results are expressed as Mean ± S.E.M. n (no. Of animals) = 6.*p < 0.05 compare with control. **p < 0.01 compare with control

Serum lipid profile

Table 3 shows the results of serum lipid profile, which revealed that significant decrease in serum triglycerides, total cholesterol, LDL (Low density lipids) and VLDL (Very low-density lipids) and significant increase in HDL (High density lipids) were observed in rats treated with extracts.

Table 3: Effect of different extracts of *P. integrifolia* extracts on serum lipid profile in diabetic rats

Groups	TG (mg/dl)	TC (mg/dl)	HDL(mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
Normal control	87.3□1.05	74.3□0.40	41.2□0.15	19.3□0.20	14.6□0.10
Diabetic control	143.0□1.50**	123.8□0.50**	14.7□0.40**	31.3□0.15**	50.2□0.45**
Standard control	99.1□5.00**	83.4□0.85**	23.6□0.35**	18.2□0.25*	17.9□0.35**
EtOH (400 mg/kg)	112.7□1.00**	106.5□0.71**	20.7□0.15**	19.9□0.10	21.7□0.56**
EtOAc (400mg/kg)	104.5□1.00**	88.7□0.20**	19.8□0.55**	21.6□0.10**	23.3□0.49**

Results are expressed as ± S.E.M. n (no. Of animals) = 6.*p < 0.05 compare with control. **p < 0.01 compare with control.

Body weight and serum insulin level

Table 4 and Fig. 1 show the results of body weight changes and serum insulin levels, which revealed that a better control in loss of body weight as compare to control group rats were observed and significant difference was also observed in serum insulin in extracts treated rats compare to control group rats.

Table 4: Effect of different extracts of *P. integrifolia* extracts on body weight changes in diabetic rats

Groups	Body weight changes (gm)				
	0 Day	5 Day	10 Day	15 Day	20 Day
Normal control	145±2.75	145±2.54	146±2.42	147±2.69	147±2.50
Diabetic control	171±1.8**	153±3.30	142±3.81	136±3.94	131±3.78**
Standard	196±3.6**	192±3.05**	187±3.72**	185±3.81**	184±3.48**
EtOH(400 mg/kg)	163±2.42**	158±2.31**	155±2.46*	151±2.73*	150±2.70*
EtOAc (400 mg/kg)	158±2.91*	153±2.90*	151±2.71*	148±2.51*	146±2.54

Results are expressed as ± S.E.M. n (no. Of animals) = 6.*p < 0.05 compare with control. **p < 0.01 compare with control.

Discussion and conclusion

Diabetes is a major endocrine disorder, affecting a considerable part of the world population. It is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by pancreas or by ineffectiveness of the insulin produced ***(Nagappa et al., 2003).*** Though different types of oral hypoglycemic agents are available along with insulin for treatment of diabetes still there is growing interest in herbal remedies due to the side effects associated with these agents ***(Rao et al., 2003).***

In anti-diabetic study, oral glucose tolerance test was performed to evaluate the hypoglycemic effect of plant. Results of OGTT indicate that both ethanolic and ethyl acetate extracts produced significant hypoglycemic effect thus these extracts were further used for anti-diabetic study. The diabetogenic agent alloxan monohydrate was used to induce diabetes in rats. Alloxan is a hydrophilic and chemically unstable pyrimidine derivative, which is toxic to pancreatic beta cells because it can generate toxic free oxygen radicals ***(Wilson et al., 1984).*** Over production of glucose and decreased utilization by the tissue is the fundamental basis of hyperglycemia in diabetes mellitus ***(Chattopadhyay et al., 1993)***. The results of present study revealed that maximum reduction in fasting blood glucose (45.5%) was produced by ethyl acetate extract (400 mg/kg). Thus administration of extract to diabetes rats showed significant decreased in blood glucose level and an increased in serum insulin levels.

The rise in blood sugar is accompanied with increase in TG, TC, VLDL and fall in HDL. Administration of extracts normalized serum lipids. Diabetes induced hyperlipiemia is attributable to excess mobilization of glucose ***(Krishnakumari et al., 2000)***. The body weight was decreased in alloxan induced diabetic rats ***(Al- Shamaony et al., 1994).*** This reduction in body weight was due to increased muscle wasting ***(Swanton et al., 1990).*** Diabetic rats treated with extracts showed an increase in body weight as compared to control group rats.

It is concluded from the data of our study that ethanolic and ethyl acetate extracts of P.integrifolia possesses significant anti-diabetic effect and it may be prove effective in treatment of diabetes mellitus.

Acknowledgement

The authors sincerely thank Madhya Pradesh Council of Science and Technology, India, for providing financial assistance in Carrying out the study.

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