

Antihyperglycemic effect of *Diospyros melanoxylon* (Roxb.) bark against Alloxan-induced diabetic rats

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Abstract: The antihyperglycemic activity of *Diospyros melanoxylon* (Roxb.) bark was evaluated with scientific approach including biochemical parameters and histopathological studies of pancreas. The ethanolic extracts of the powdered bark was tested for its efficacy in alloxan-induced diabetic rats. The extracts were also evaluated for acute oral toxicity studies and its effect on different biochemical parameters. An effect of extracts was compared to that of standard glibenclamide. It was revealed that ethanolic extracts has significantly ($p < 0.01$) reversed the diabetes-induced hyperlipidemia compared to standard drug. Histopathological studies of pancreas revealed its significant effects on β -cells count. The extracts showed significant antihyperglycemic activity as compared to standard drug. Ethanolic extract (200mg/kg) showed beneficial effects on blood glucose and hyperlipidemia associated with diabetes, which might be due to presence of steroids, tannins, alkaloids and triterpenoids present in that extract. Thus ethanolic extract could serve as good adjuvant to other oral hypoglycemic agents and seems to be promising for the development of phytomedicines for diabetes mellitus.

Key words -Antihyperglycemic, *Diospyros melanoxylon*, Glibenclamide, Alloxan-induced.

Introduction:

According to WHO, the prevalence of diabetes is likely to increase by 35%. Currently there are over 150 million diabetics worldwide and this is likely to increase to 300 million or more by the year 2025. Statistical projection about India suggests that the number of diabetics will rise from 15 million in 1995 to 57 million 2025 making it the country with the highest number of diabetics in the world^{1,2}. Diabetes is a serious metabolic disorder with micro and macrovascular complication that results in significant morbidity and mortality³. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries⁴. Recently, the search for appropriate hypoglycemic agents has been focused on plants used in traditional medicine partly because of leads provided by traditional medicine to natural products that may be better treatments than currently used drugs⁵. The

indigenous system of medicine (Ayurveda) made good number of plants for the cure of diabetes and some of them have been experimentally evaluated and the active constituents were also isolated^{6,7,8}.

According to the history, may be of Unani, Ayurveda, Siddha or Homeopathic, it has been well documented that illness can be managed purely by herbal preparations. And thus, the diabetic individual could lead a healthy life as non-diabetics. Experiments and clinical trials conducted worldwide have provided dependable evidences on the effects of various herbal formulations in the maintenance of normal blood sugar level. These invaluable findings are now conclusively processed in the backdrop of Ayurveda, Unani and Siddha system⁹.

In present study plant selected for screening of antihyperglycemic activity is *Diospyros melanoxylon* (Roxb.), which was selected on the basis of its abundantly availability in Maharashtra state and on

the basis of ethnomedicinal information that the tribal of Chotta Nagpur region (Orissa) use it extensively as antidiabetic¹⁰. It is well known world wide for its leaves (Tendu patta) used for making Bidi and also for ebony yielding property¹¹. Here, the antihyperglycemic activity of *Diospyros melanoxylon* Roxb ethanolic extract is evaluated in detail with scientific approach including its effects on biochemical parameters and histopathological studies of pancreas.

Materials and Methods:

The species for the proposed study, *Diospyros melanoxylon* (Roxb.) bark was collected from the forest of Ballarpur area, Dist- Chandrapur, Maharashtra, authenticated and used. Glibenclamide was obtained from Lupin laboratories, Alloxan monohydrate was obtained from SD fine chemicals Pvt. Ltd. Biosar., and other chemicals were purchased from local market and were of analytical grade.

Preparation of extracts: The bark collected was shade dried completely. The dried bark was then coarsely powdered and was sieved (sieve # 60) to get uniform coarse powdered. The extract was prepared by continuous hot extraction using ethanol as a solvent. Extracts obtained was concentrated, dried kept in a desiccator for further use. The yield was found to be 14.72%w/w. Experimental Models:

Male Wistar albino rats of 150-200gm. were selected of either sex, for studies they were kept in a standard polypropylene cage at room temperature of 27±2°C, relative humidity 60-70% and well ventilated. They were fed a standard rat pellet and water. Animals were deprived of food initially for 16hrs but had free access to water. Acute Oral Toxicity Studies were performed for ethanolic extract following OECD guidelines-420. Where fixed dose levels of extract starting from 50,100,200,500,1000, increasing up to 2000mg/kg body weight was given, sign and symptoms of toxicity was observed for next 48 hrs. No toxicity or death was observed in experimental rats¹². Screening of Antihyperglycemic Activity¹³⁻¹⁹:

Diabetes was induced by single intraperitoneal injection of freshly prepared aqueous solution of alloxan monohydrate 150mg/kg, to overnight fasted rats. After 48hrs of alloxan injection, the animals which did not developed hyperglycemia i.e. glucose level > 200mg/dl, were rejected and replaced with new animals.

Immediately after confirmation of diabetes, rats were classified into five groups of six rats each. Test extracts were prepared as Standard drug (glibenclamide 2.5mg/kg), ethanolic test extracts (200mg/kg) in 2%

Carboxy Methyl Cellulose (CMC). Evaluation of antidiabetic effect of test extracts was done by taking six rats in each five groups as: Group I served as normal control (saline); Group II served as diabetic control (alloxan induced); Group III received ethanolic extract (200mg/kg) and Group IV served as reference standards (Glibenclamide, 2.5mg/kg). Treatment was continued for 14 consecutive days, with twice a day dose (morning and evening). Before the treatment (0 day) and at the end of 7th and 14th day, blood samples were collected from the tip of the tail of each rat under mild ether anesthesia in 1ml Eppendorf tubes containing 50ul of anticoagulant (heparin) and serum separated by centrifugation of blood at 4000rpm for 10mins was subjected for estimating glucose by Glucose oxidase method using semi auto-analyzer. Estimation of biochemical parameters were done with 1ml of blood withdrawn on 14th day, from all five groups of rats (normal, diabetic control, extracts and standard treated) under mild anesthesia, where serum was separated by centrifugation of sample at 4000rpm for 10min and stored in a refrigerator until analyzed. And the serum was subjected for the estimation of triglycerides (TGL), HGL, LDL, VLDL and total cholesterol level. Results were analysed by students's t-test. The minimum level of significance was fixed at $p < 0.01$ ²⁰.

Histopathological Studies:

All the animals were sacrificed on 14th day by cervical dislocation; pancreases were excised, isolated and were subjected to histopathological studies and microscopical findings were noted.

Results:

Acute oral toxicity studies following OECD guidelines-420, fixed dose procedure, showed that both ethanolic extracts upto 2000mg/kg are non-toxic and safe. Blood glucose levels in alloxan induced diabetic rats were above 250mg/dl, which was considered as sever diabetes. In the standard drug (glibenclamide 2.5mg/kg) and ethanolic extract (200mg/kg) treated groups, the peak values of blood sugar significantly decreased to 90.8mg/dl (65.07%) and 109.1mg/dl (59.33%) simultaneously on the 14th day. Thus, the ethanolic extract was found to be almost significant as standard drug in lowering blood glucose level. Ethanolic extract has shown positive test for presence triterpenoids, steroids, alkaloids, flavonoids and tannins, which may be an active ingredients in a group or as an individual responsible for activity (Table 1.).

Table -1:Effect of ethanolic extracts of bark of Diospyros melanoxylon (Roxb.) in alloxan induced diabetic rats.

Treatment and Groups	Dose mg/kg	Blood glucose concentration (mg/dl)			Percentage Activity
			7 th day	14 th day	
Control (Normal saline)	2ml/kg	90.3 ± 4.6	90.8 ± 3.6	90.8 ± 6.6	+0.55
Diabetic control (Alloxan)	150	263.3 ± 18.4	262 ± 19.2	262.3 ± 17.7	-0.37
Ethanolic extract	200	268.3 ± 18.9	114.3 ± 8.1*	109.1 ± 7.3**	-59.33
Glibenclamide	2.5	260 ± 14.3	110 ± 9.2*	90.8 ± 6.2**	-65.07

n = 6; Data are expressed as Mean ± S.E, * p> 0.01 Vs Control, **p > 0.001 Vs Control by students 't' test. ("+" denotes increase and "-" denotes decrease in blood glucose level)

Table- 2:Effect of ethanolic extracts of bark of Diospyros melanoxylon (Roxb.) on biochemical parameters

Treatment and doses	TGL mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl	Total Cholesterol
2ml/kg Control (Normal saline)	76.12 ± 4.12	24.5 ± 1.32	39.12 ± 4.13	15.22 ± 0.82	78.84 ± 6.27
Diabetic control	114.2 ± 7.43	73.79 ± 4.7	145.41 ± 1.2	22.8 ± 1.48	242 ± 7.38
Ethanolic extract (200mg/kg)	88.42* ± 6.9	28.2* ± 1.9	51.1** ± 0.04	17.70 ± 1.38	97.0 ± 3.32
(% Reduction)	(22.58)	(61.78)	(64.85)	(22.36)	(59.91)
Glibenclamide (2.5)	83.74* ± 4.9	34.4* ± 2.8	29.8** ± 1.2	16.74 ± 0.98	80.94 ± 4.98
(% Reduction)	(26.68)	(53.38)	(79.50)	(26.57)	(66.55)

n = 6; Data are expressed as Mean ± S.E, * p> 0.01 Vs Control, **p > 0.001 Vs Control by students 't' test

Effect of the plant extracts on different biochemical parameters: Ethanolic extracts has significantly (p<0.01) reversed the diabetes-induced hyperlipidemia compared to standard drug. A significant percentage reduction of total cholesterol level (59.91%), HDL (61.78%), LDL (64.85%), TGL (22.58%) and VLDL (22.36%) in ethanolic extract treated was comparative to standard drug treated groups, total cholesterol level (66.55%), HDL (53.38%), LDL (79.50%), TGL (26.68%) and VLDL (26.57%) and reached normal value. The observation showed decrease ratio of TGL/HDL cholesterol (Atherogenic index) lessens the risk of heart disease (Table 2.).

Histopathological studies- Multiple section of pancreas were taken and studied for any histological changes. The pancreas present in the group of animals treated with the ethanolic extract, 200mg/kg showed normal appearance of pancreatic lobules, acini and cells. The islets were normal in size, shape and number comparatively similar to that of standard treated. (Figure 1, 2, 3, and 4).

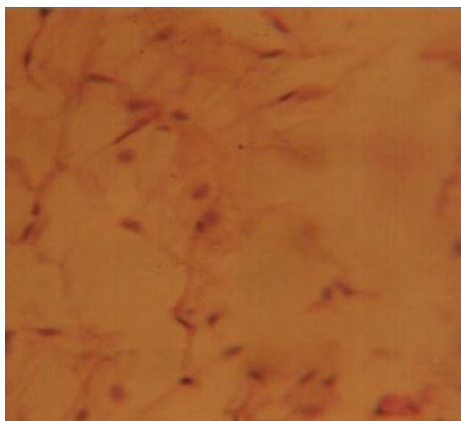


Figure 1: Section of Diabetic control pancreas

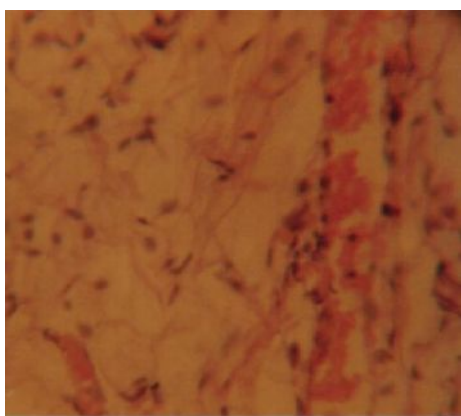


Figure 2: Section of Normal (control)

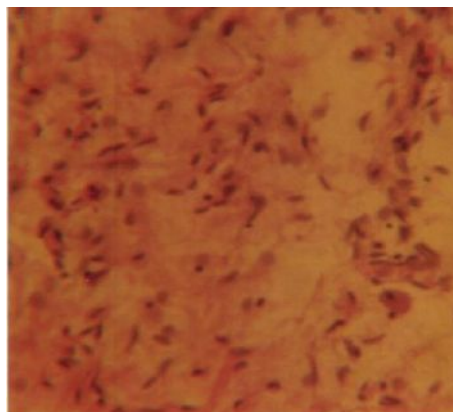


Figure 3: Section of pancreas treated with Glidenclamide (2.5mg/kg)

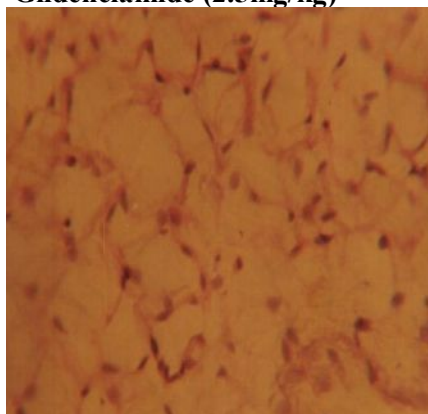


Figure 4: Section of pancreas treated with Ethanolic extract (200mg/kg)

Discussion:

Alloxan is widely used to induce diabetes in experimental animals. The mechanism of action in p-cells of the pancreas has been intensively investigated and now is quite well understood. The cytotoxic action of alloxan is mediated by reactive oxygen species. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by Fenton reaction. The actions of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration cause rapid destruction of P-cells and thus increase the blood sugar level.

According to earlier studies, plant extracts cause antihyperglycemic effect by promoting regeneration of p-cells or by protecting the cells in pancreas from destruction, by restricting glucose load as well as by promoting unrestricted endogenous insulin action or it effect p-cells to release insulin and activate the insulin receptors to absorb the blood sugar (eg. sulphonylureas). The comparable effect of the extract with glibenclamide may suggest similar mode of action, since alloxan permanently destroys the pancreatic p-cells and the extracts lowered blood glucose level in alloxanised rats to significant level,

indicating the extracts possesses extra pancreatic effects. Ethanolic extract (200mg/kg) has shown positive test for triterpenoids, steroids, alkaloids, flavonoids and tannins, which may be an active ingredients in a group or as an individual responsible for activity. The ethanolic extract (200mg/kg) showed beneficial effects on blood glucose and hyperlipidemia associated with diabetes, thus it could serve as good adjuvant to other oral hypoglycemic agents and seems to be promising for the development of phytomedicines for diabetes mellitus. Thus, results of present studies justify the use of plant for treating diabetes as suggested in folklore remedies.

The present investigation shows the therapeutic efficacy of ethanolic extract of *Diospyros melanoxylon* (Roxb) at a dose of 200mg/kg/day gives significant antidiabetic activity in alloxan induced diabetic rats. The active constituents present in the plant, which are responsible for the above said activity and its exact mechanism of action.

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