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HYPOGLYCEMIC AND ANTIHYPERLIPIDEMIC ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *AZIMA TETRACANTHA* LAM. ON ALLOXAN-INDUCED DIABETIC RATS

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Abstract: In the present study, the ethanolic leaf extract of Azima tetracantha Lam. was investigated for hypoglycemic and hypolipidemic activity in alloxan-induced diabetic albino rats. Diabetes was induced in rats by administration of alloxan monohydrate (150 mg/ kg, i.p.). Animals were divided into five group (n=6) receiving different treatments: vehicle (control), diabetic control (alloxan monohydrate 150 mg/kg), ethanolic leaf extract (100 mg and 200 mg/kg b.w), and standard antidiabetic drug glibenclamide (5 mg/kg). Blood samples were collected and analyzed for plasma glucose on 1, 7 and 14th day and lipid profile on day 14. The ethanolic leaf extract of Azima tetracantha at the dose of 200 mg/kg produced significant reduction (P<0.001) in plasma glucose and also had beneficial effects (P<0.001) on the lipid profile in alloxan-induced diabetic rats at the end of the treatment period of fourteen day. However, the reduction in the plasma glucose and in the lipid profile was slightly less than that achieved with the standard drug glibenclamide.

Key words: *Azima tetracantha*, Anti-diabetic, Hypercholesterolemia, Arteriosclerosis.

INTRODUCTION

Diabetes mellitus is a heterogenous primary disorder of carbohydrate metabolism with multiple etiological factors; it generally involves absolute or relative insulin deficiency, or insulin resistance, or both. Whatever the cause, diabetes ultimately leads to hyperglycemia, which is the landmark of this disease syndrome. Non-insulin dependent diabetes mellitus has also been associated with an increased risk of premature arteriosclerosis due to increase in triglycerides (TG) and low-density lipoprotein (LDL) levels. About 70-80% of deaths in diabetic patients are due to vascular disease. An ideal treatment for diabetes would be a drug that not only controls the glycemic level but also prevents the development of arteriosclerosis and other complications of diabetes [1]. Long before the use of insulin became common, indigenous remedies were used for the treatment of diabetes mellitus and hyperlipidemia. There has been an increasing demand from patients for the use of natural products with antidiabetic and antihyperlipidemic abilities. This is largely because insulin cannot be taken orally and its injections are associated with the risk of hypoglycemia and impairment of hepatic and other body functions. The undesirable side effects, contraindications of synthetic drugs and the fact that they are not suitable for use during pregnancy, have made scientists to look towards hypoglycemic agents of plant origin [2]. Many herbs and plant

products have been shown to have antihyperglycemic and antihyperlipidemic actions [3-5].

Azima tetracantha Lam (Salvadoraceae) is known as 'Mulsangu' in Tamil and 'Kundali' in Sanskrit. Its root, root-bark and leaves are administered with food as a remedy for rheumatism [6,7]. It is a powerful diuretic given in rheumatism, dropsy, dyspepsia, chronic diarhoea and is considered as stimulant tonic and given to pubertal women immediately after confinement [8]. Its leaves are found to possess azimine, azcarpin, carpine and isorhamnitine-3-0-rutinoside [9,10]. The objective of the present study was to evaluate the hypoglycemic and hypolipidemic activities of an ethanolic leaf extract of *Azima tetracantha* in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Animals: Wistar albino rats of 6-8 weeks age and of either sex, weighing 150-180 g, were used. The animals were kept in clean and dry plastic cages, with 12h: 12h light-dark cycle at 25 ± 2ºC temperature and 45 - 55 % relative humidity. The animals were fed with standard pellet diet and water was given *ad libitum*. This study was carried out in the animal house of Periyar College of Pharmaceutical Sciences for Girls, Tiruchirappalli (Regd. No. 265/CPCSEA). Toxicity study was carried out as per the Organisation for Economic Co-operation and Development (OECD) guidelines. The LD_{50} of the *Azima tetracantha* ethanolic leaf extract as per OECD guidelines falls under class 4, values with no signs of acute toxicity (LD_{50} >2000 mg/kg). Hence the dosage was fixed in 100 and 200 mg/kg b.w.

Preparation of the extract: Fresh leaves of *Azima tetracantha* were collected in Ponnamaravathi (Pudukkottai District) during the months of November-December. The plant was authenticated by Botanist at the Ranipet Herbarium and Centre for Molecular Systemics, St. Joseph's College Campus, Tiruchirappalli. Plant material was dried under shade at room temperature, pulverized by a mechanical grinder and sieved through 40 mesh. The powdered material (100 g) was extracted with 95% ethanol by hot continuous percolation method in a Soxhlet apparatus. The extract was then concentrated and dried under reduced pressure. The ethanol free semi solid mass obtained (13.65 g) was used for the experiment.

Drug administration: The quantities of the individual drug to be administered were calculated and suspended in vehicle (1% W/V suspension of carboxymethyl cellulose (CMC) in water 10ml/ kg b.w). The drug was administered continuously for 14 days orally using an infant feeding tube. The results were compared with that of the standard drug glibenclamide, which was also given regularly for 14 days.

Introduction of experimental diabetes: A single dose (150 mg/kg b.w, i.p.) of alloxan monohydrate (1%) dissolved in sterile normal saline was used for induction of diabetes mellitus in the rats. Diabetes was confirmed 1 week after alloxan injection by determining the plasma glucose concentration; only animals with plasma glucose of 200-300 mg/dl (mild diabetes) were used for the experiment. The diabetic animals were allowed free access to tap water and pellet diet and were maintained at room temperature in plastic cages.

Collection of blood and experimental setup: Animals were classified into five groups of six rats each. Group I served as control and received 1% w/v suspension of CMC in water at a dose of 10 ml/ kg b.w. Group II treated with alloxan monohydrate 150 mg/kg served as diabetic control. Group III and Group IV received ethanolic leaf extract of *Azima tetracantha* in 1% CMC at a dose of (100 mg/kg and 200 mg/kg. b.w) respectively. Group V treated with glibenclamide (5 mg/kg, b.w) and served as reference standard. Plasma glucose was estimated before starting the treatment weekly and up to the end of treatment period (7 and 14 days). Lipid profile was estimated at the end of the study (14 day).

Estimation of plasma glucose and lipid profile: Plasma glucose [11] total cholesterol (TC) [12] plasma TG [13] and high-density lipoprotein (HDL) cholesterol [14] was determined by using commercially available kits. VLDL (very low density lipoproteins) cholesterol was calculated as: TG / 5; LDL cholesterol was calculated by the equation: LDL cholesterol = TC – (HDL + VLDL). All estimations were done using the auto analyzer.

Statistical analysis: All the values were expressed as mean ± standard error mean (SEM). The differences were compared using one-way analysis of variance (ANOVA) followed by students 't' test.

P values < 0.001 were considered as significant. The minimum level of significance was fixed at P<0.01.

RESULTS

The hypoglycemic activity of ethanolic leaf extract of *Azima tetracantha* is shown in table 1. A significant reduction in plasma glucose levels was observed at the end of the second week (Fourteenth day) of treatment with ethanolic leaf extract of *Azima tetracantha* (100 mg/kg and 200 mg/kg b.w) in the alloxan induced diabetic rats. The maximum reduction in plasma glucose level was seen at a dose of 200 mg/kg (P<0.001) the fall being 118.3 ± 8.2, 92.3 ± 8.2 respectively after 7 days and 14 days when compared with alloxan induced diabetic control group which showed 262.1 ± 21.2, 262.8 ± 18.8 after 7 days and 14 days treatment respectively. However the hypoglycemic effect of the test drug 200 mg/kg was significantly higher than that of the standard drug glibenclamide.The activity of the standard drug glibenclamide showed significant reduction of plasma glucose level at the end of seventh and fourteenth days viz., 110.5 ± 9.7 and 93.5 ± 8.2 respectively. On regular daily administration of leaf extract for fourteen days, a significant decrease in plasma glucose of the alloxan induced diabetic rats was seen as compared to the control group.

Effect of ethanolic leaf extract of *Azima tetracantha* on lipid profile is shown in table 2. A significant decrease in the plasma level of TG, VLDL, LDL and TC was observed at the end of the second week (Fourteenth day) of treatment in the alloxan induced diabetic rats which showed 93.32 ± 5.83, 18.66 ± 1.02, 51.08 ± 2.31, 108.57 ± 5.39 respectively as compared to diabetic control which showed 116.48 ± 8.5, 23.30 ± 1.7, 171.07 ± 2.27, 219.3 ± 10.49 respectively. A significant increase in plasma level of HDL was observed, at a dose level of 200 mg/kg b.w ethanolic leaf extract treated group (40.71 ± 3.52) compared to diabetic control group (24.93 ± 6.52).The standard drug glibenclamide (5 mg/kg) also showed significant decreased level of plasma TGL, VLDL, LDL and TC and significant increase of HDL viz., 88.58 ± 6.7, 17.71 ± 1.28, 33.8 ± 1.91, 184.71 ± 8.20, 33.32 ± 2.93 mg/dl respectively. The leaf extract and the standard drug glibenclamide (5 mg/kg) b.w led to significant decrease in the plasma level of TG, TC, LDL, VLDL, and significant increase in the plasma level of HDL in the diabetic rats.

DISCUSSION

Diabetes mellitus is a chronic disorder caused by partial or complete insulin deficiency, which produces inadequate glucose control and leads to acute and chronic complications. Premature and extensive arteriosclerosis, involving renal, peripheral and cardiovascular vessels, remains the major complication of diabetes mellitus. Alteration in the serum lipid profile is known to occur in diabetes and this is likely to increase the risk of coronary heart disease. A reduction in serum lipids, particularly of

Table 1: Hypoglycemic activity of ethanolic leaf extract of *Azima tetracantha* in alloxan-induced diabetic rats. Values are expressed as Mean ± SE. n=6 by students 't' test. *P<0.01 Vs control, **P<0.001 Vs control.

S. No	Groups	Dose mg/kg	Treatement (Days) (Mean ± SE, n = 6)		
			1	7	14
1.	Control (Normal saline)	2 ml/kg	90.3 ± 4.1	90.8 ± 3.9	91.2 ± 3.3
2	Diabetic control (Alloxan)	150	258.3 ± 13.8	262.1 ± 21.2	262.8 ± 18.8
3	Ethanolic leaf extract of *Azima tetracantha*	100	259.4 ± 16.0	163.3 ± 10.3*	112.8 ± 7.9**
4	Ethanolic leaf extract of *Azima tetracantha*	200	263.2 ± 15.2	118.3 ± 8.2**	92.3 ± 8.2**
5	Glibenclamide	5	260.3 ± 14.3	110.5 ± 9.7**	93.5 ± 8.2**

Table-2 Effect of ethanolic leaf extract of *Azima tetracantha* on lipid profile in alloxan-induced diabetic rats. Values are expressed as Mean ± SE. n=6 by students 't' test. *P<0.01 Vs control, **P<0.001 Vs control.

Treatment	Triglycerides (TG) mg (dl)	High- density lipoprotein (HDL) mg/dl	Very low -density lipoprotein (VLDL) mg/dl	Low- density lipoprotein (LDL) mg/dl	Total cholesterol (TC) mg/dl
Control (Normal saline)	75.15 ± 5.03	25.6 ± 1.83	15.03 ± 1.006	41.9 ± 4.37	82.53 ± 7.21
Diabetic control (Alloxan 150 mg/kg)	116.48 ± 8.5	24.93 ± 6.52	23.30 ± 1.7	171.07 ± 2.27	219.3 ± 10.49
Azima tetracantha Ethanolic leaf extract (100 mg/kg)	112.81 ± 7.79	38.83 ± 2.31*	22.56 ± 1.58	65.81 ± 5.12**	129.1 ± 3.88**
Azima tetracantha ethanolic leaf extract (200 mg/kg)	93.32 ± 5.38*	40.71 ± 3.52**	18.66 ± 1.02**	51.08 ± 2.31**	108.57 ± 5.39**
Glibenclamide (5 mg/kg)	88.58 ± 6.7*	33.32 ± 2.93*	17.71 ± 1.28	33.8 ± 1.91**	84.71 ± 8.20**

the LDL and VLDL fraction and TG, should be considered as being beneficial for the long-term prognosis of these patients [15]. The lowering of plasma glucose and lipid levels through dietary modification and drug therapy seems to be associated with a decrease in the risk of vascular disease.

In the present study, treatment with *Azima tetracantha* ethanolic leaf extract (200 mg/kg b.w) in alloxan induced diabetic rats produced more significant decrease in blood glucose level. The hypoglycemic effect may be due to increased secretion of insulin from the beta cells of pancreas, i.e., pancreatotrophic action [16]. The results were comparable to that of glibenclamide, which acts by stimulation of insulin release [17] thus further confirming that the extract lowers blood glucose by a pancreatotrophic action.

Moreover, *Azima tetracantha* produced significant beneficial effects in the lipid profile in alloxan induced diabetic rats by reducing TG, TC, LDL and VLDL and increasing HDL, significantly. The leaf extract may increase the secretion of insulin from beta cells of pancreas; this increased secretion of insulin stimulates fatty acid biosynthesis and also the incorporation of fatty acids into TG in the liver and adipose tissue [18].

Alloxan, a beta cytotoxin, induces "chemical diabetes" in a wide variety of animal species by damaging the insulin-secreting cells of the pancreas [18]. Literature sources indicate that alloxan rats are hyperglycemic [19]. The use of lower doses of alloxan (120 mg/kg b.w) produced a partial destruction of pancreatic beta cells even though the animals became permanently diabetic [20]. Thus, these animals have surviving beta cells and regeneration is possible [21]. It is well known that the sulfonylureas (glibenclamide) act by directly stimulating the beta cells of the Islets of Langerhans to release more insulin and these compounds are active in mild alloxan induced diabetes [22]. Since our results show that glibenclamide reduced the plasma glucose levels in the diabetic animals, the extentof diabetes is not severe.

Diabetic rats were observed to have increased plasma lipids, which are responsible for several cardiovascular disorders [25]. The higher lipid level, seen in diabetic rats, was due to increased mobilization of free fatty acids from peripheral depots and also due to lipolysis caused by hormones [26,27]. The leaf extract leads to regeneration of the beta cells of the pancreas and potential of insulin secretion from surviving beta cells; the increase in insulin secretion and the consequent decrease in blood glucose level may lead to inhibition of lipid peroxidation and control of lipolytic hormones. In this context, a number of other plants have also been reported to have antihyperglycemic antihyperlipidemic, and insulin stimulatory abilities [28-30]. It is well known that LDL plays an important role in arteriosclerosis and that hypercholesterolemia is associated with a defect relating to the lack of LDL receptors. The decrease of cholesterol, TG and LDL levels achieved by administration of leaf extract, demonstrates a possible protection against hypercholesterolemia. The hypoglycemic effect of *Acacia catechu* may be due to the presence of flavonoids, which act as insulin secretagogues. Epicatechin a flavonoid compound, is reported to promote regeneration of beta cells of the Islets of Langerhans [31-34]. The leaf extract and a purified fraction-1 of stem of *Bauhinia purpurea* exhibited anti diabetic property in alloxan-induced diabetic rats and may be ascribed to the presence of flavonoids, which have been shown to inhibit cyclooxygenases and promote beta cell regeneration besides having insulin secretory property [35]. The *Pongamia pinnata*, commonly known as "Karanj", consists of several phytoconstituents belonging to the of category flavonoids and fixed oils. *Pongamia pinnata* flower shows significant anti-.hyperglycemic and anti-lipidperoxidative effect and enhancement in antioxidant defense system in alloxan-induced diabetic rats [36]. Since the leaf extract of *Azima tetracantha* is rich in flavonoid, isorhamnetin and 3-0-rutinoside, they may acts as insulin regulator to promote regeneration of beta cells of the Islets of Langerhans.

From overall study it is concluded that the ethanolic leaf extract of *Azima tetracantha* showed a significant anti-hyperglycemic and anti-hyperlipidemic activities in the model of alloxan-induced diabetic rats. Therefore, this treatment can safely be considered to be an alternative antihyperglycemic drug for diabetic patients. However further studies are needed.

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