Therasilin Louis *et al.* / *International Journal of Biological & Pharmaceutical Research.* 2015; 6(5): 327-331.

e- ISSN 0976 - 3651
Print ISSN 2229 - 7480

International Journal of Biological & Pharmaceutical Research
Journal homepage: www.ijbpr.com

IJBPR

ANTI DIABETIC AND ANTI HYPERLIPIDEMIC ACTIVITIES OF DIFFERENT EXTRACTS OF *AILANTHUS MALABARICA* STEM BARK IN ALLOXAN INDUCED DIABETIC RATS

Therasilin Louis*[1], P.Yuvaraj[1], V. Madhavachandran[1], R. Anandan[2]

[1]Experimental Pharmacology Unit, Nagarjuna Herbal Concentrates Ltd, Kalayanthani, Thodupuzha, Idukki-685 588, India.
[2]Biochemistry & Nutrition Division, Central Institute of Fisheries Technology, Cochin, Kerala -682029, India.

ABSTRACT

To evaluate the anti diabetic and anti hyperlipidemic activities of different extracts of *Ailanthus malabarica* stem bark in alloxan induced diabetic rats. Different extracts (hexane, ethanol and water, 50 mg/kg) of *A. malabarica* stem bark were administered to alloxan-induced diabetic rats for 21 days and blood glucose levels of the diabetic rats were monitored at one week intervals. Lipid profiles of the treated diabetic rats were determined after the period of treatment. Treatment of alloxan-induced diabetic rats with ethanol extract of *A. malabarica* stem bark caused a significant ($P<0.05$) reduction in fasting blood glucose levels of the diabetic rats in 21 days treatment. The ethanol extract showed a comparable action with the reference drug, glibenclamide. The ethanol extract exerted a significant reduction in the levels of serum total cholesterol, triglycerides, LDL, VLDL and phospholipids, and increase in HDL levels of the diabetic rats. However, water and hexane extract of *A. malabarica stem bark* did not show any such beneficial effects. . These results suggest that the ethanol extract of *A. malabarica* stem bark possesses anti diabetic effect on alloxan induced diabetic rats and this justifies its usage in ethno medicine and can be exploited in the management of diabetes.

Key Words: Antidiabetic, Antihyperlipidimic, *Ailanthus malabarica, stem bark.*

INTRODUCTION

Diabetes mellitus is a chronic disease, that occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces (Ugochukwu *et al*., 2003). This cause metabolic disorder of carbohydrate, fat and protein, affects a large number of populations in the world (Pareek *et al*., 2009). Diabetes mellitus has caused significant morbidity and mortality due to microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications (Craig *et al*., 2009).

Corresponding Author

Therasilin Louis
Email: dr.therasilin@gmail.com

It is considered as one of the five leading causes of death in the world (Devendra *et al*., 2004) To date, the treatment for diabetes including insulin, metformin, and sulfonylureas was found to cause various side effects especially the development of resistance after a certain period of time (Noor *et al*., 2008). Thus, efforts to search for alternative and novel therapies to manage diabetes are still receiving great attention.

Ailanthus malabarica DC (Simaroubaceae) is a large tree distributed in India and Indo- China and is regarded as an important medicinal plant useful for the treatment of dysentery, dyspepsia, febrifuge, asthma and bronchitis (Airtikar and Vasu, 1935). Novel nortriterpinoids, Malabanones A and B were isolated from the stem bark of *Ailanthus malabarica* DC (Hitotsuyanagi *et al*., 2001). Joshi identified cycloapotirucallane

triterpenoid ailanthol from stem bark of *A. malabarica* (Joshi *et al.,* 1985). In the light of the literature on *A. malabarica*, an attempt has been made, for the first time, to study the anti-diabetic effect of *A. malabarica* in experimentally induced diabetic rats. The present study evaluated the anti-diabetic and anti hyperlipidimic effects of *A. malabarica*. Also, we tried to determine the physiochemical constituents the active extract of this herb

MATERIALS AND METHODS

Plant material

Stem bark of *A. malabarica* was collected freshly from Kottayam District, Kerala, India and dried under shade. The plant was identified and authenticated at the Herbarium and was deposited in the Department of Pharmacognosy, Nagarjuna Herbal Concentrates Ltd, Thodupuzha, Kerala, India.

Preparation of plant extracts (Hexane, ethanol and water)

A. malabarica stem bark was cut into small pieces, shade dried and powdered. The dried stem bark powder was extracted separately in n-hexane, ethanol and water as described elsewhere (Subramoniam *et al.,* 1998). The ethanol (AMSE) and hexane (AMSH) extracts of *A. malabarica* stem bark were dried using a rotary evaporator under reduced pressure at 40°C. The water extract (AMSW) was freeze dried. The percentages of yield of n-hexane, ethanol and water extracts were determined.

Phytochemical Screening

Phytochemical screening of the active extract was carried out employing standard procedures (Trease and Evans *et al.,* 1989), to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, saponins, anthraquinones, reducing sugars, cardiac glycosides and others.

Animals

Adult male Wistar albino rats, weighing 180-200 g bred in Nagarjuna Herbal Concentrates Ltd, Thodupuzha, Kerala, India, were used. All animal experiments were approved by the Institutional Animal ethics committee, Nagarjuna Herbal Concentrates Ltd and were maintained in accordance with the guidelines of the CPCSEA. The animals were housed in polypropylene cages in a room with a 12 hour day-night cycle and were maintained on a standard pelleted feed and free excess of water was given *ad libitum*.

Experimental induction of diabetes in rats with alloxan

The rats were injected with alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg body weight, intraperitoneally. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution (15–20 ml) intraperitoneally after 6 hours. The rats were then kept for the next 24 hours on 5% glucose solution in their cages to prevent hypoglycemia (Gupta *et al.,* 1984). After a fortnight, rats with moderate diabetes having glycosuria (indicated by Benedict's test for urine) and hyperglycemia with blood glucose range of 200–260 mg/100 ml were used for the experiment.

Experimental design

For this experiment 36 rats (30 diabetic surviving rats, six normal rats) were used. The rats were divided into six groups after the induction of alloxan diabetes.

In this experiment, six rats were used in each group

Group I Normal Control (Vehicle treated)

Group II Diabetic control (Vehicle treated)

Group III Diabetic rats given hexane extract (AMSH, 50 mg/kg p.o.) in 5% tween 80 for 3 weeks.

Group IV Diabetic rats given ethanol extract (AMSE, 50 mg/kg p.o.) in 5% tween 80 for 3 weeks

Group V Diabetic rats given water extract (AMSW, 50 mg/kg p.o.) in 5% tween 80 for 3 weeks.

Group VI Diabetic rats given Glybenclamide tablet (0.6 mg/kg p.o.) for 3 weeks.

Blood samples were collected at one week intervals till the end of experimental period. At the end of the study, all the rats were sacrificed by decapitation (Pentobarbitone sodium anaesthesia, 60 mg/kg body weight) and blood was collected.

Statistical analysis

Results were expressed as mean ± S.D. Data were statistically analyzed by one way ANOVA, followed by Duncan multiple range test, with the level of significance set at $p<0.05$

RESULT AND DISCUSSION

Effect of different extracts of A. malabarica stem bark on blood glucose levels of alloxan induced diabetic rats

Table 1 shows the effect of various extracts of *A. malabarica* on blood glucose levels. ethanol extract of *Ailanthus malabarica* stem bark showed an evident blood glucose lowering effect from the first week onwards; decrease in blood sugar was maximum on completion of third week (237.2 mg/dl to 116.2 mg/dl) ($p<0.05$). On the other hand, water and hexane extract did not show any significant changes in blood glucose, when compared to ethanol extract. Similar to ethanol extract, Glibenclamide treatment also significantly decreased ($p<0.05$) the blood glucose level (241.0 mg/dl to 114.7 mg/dl) until end of third week. The maximum reduction in glucose levels was observed in groups receiving ethanol extract at the dose of 50 mg/kg. This anti hyperglycemic action exhibited by ethanol extract might be due to protection of β-cells against the cytotoxic action of alloxan.

Effect of different extracts of A. malabarica stem bark on body weight of alloxan induced diabetic rats

The changes in animal body weight are shown in figure 1. The body weight of normal control, diabetic control and treatment group rats were not different at the start of experiment (150±10 g). Body weight of the diabetic control rats group was significantly reduced in every week of the experimental period. Loss of body weight may be due to excessive breakdown of tissue lipids and proteins during diabetes (Chatterjee and Shinde, 2002). The body weight of the ethanol extract group and control group rats were not significantly ($p<0.05$) different from each other on the 21st day. Body weight of ethanol extract treated rats was found to be significantly higher, when compared with hexane or water extract treated animals. The similar body weight gain was observed in glibenclamide group. This result proves that continuous administration of ethanol extract (50 mg/kg/per day) for 21 days effectively protects tissue damage in the diabetic rats.

Effect of different extracts of A. malabarica stem bark on haemoglobin, glycosylated haemoglobin (HbA_1C) and plasma insulin of alloxan induced diabetic rats

Table 2 represents the effect of different extracts of *A. malabarica* (hexane, ethanol and water, 50 mg/kg, p.o.), during three weeks treatment, on the levels of haemoglobin, Glycosylated haemoglobin and plasma insulin in normal and diabetic rats. The level of plasma insulin was significantly decreased in diabetic control rats, compared to normal control rats. Oral administration of *A. malabarica* ethanol extract (50 mg/kg) and glibenclamide (0.6 mg/kg) to diabetic rats significantly reversed plasma insulin levels near to normal.

The results suggest the insulin secretagogue activity of *A. malabarica*. The possible mechanism of action of extract could be correlated with the reminiscent effect of the hypoglycemic sulphonylureas that promote insulin secretion by closure of K^+-ATP channels, membrane depolarization and stimulation of Ca^{2+} influx, an initial key step in insulin secretion. Haemoglobin levels were decreased while Glycosylated haemoglobin levels were increased significantly in diabetic control rats. Administration of ethanol extract (50 mg/kg) and glibenclamide (0.6 mg/kg) to diabetic rats significantly ($p<0.05$) increased the haemoglobin levels and significantly decreased the glycosylated haemoglobin levels.

Glycosylated haemoglobin level was increased over a long period of time in diabetes (Bunn *et al.*, 1979*)*. Therefore, measurement of Glycosylated haemoglobin is supposed to be very sensitive index for glycemic control. In uncontrolled or poorly controlled diabetes, there is increased glycosylation of a number of proteins including haemoglobin and α-crystalline of the lens. During long-term diabetes the glycosylated form of haemoglobin has altered affinity for oxygen and this may be a factor in tissue anoxia (Inouye *et al.*, 1998). In diabetes, the glycation and subsequent browning (glycoxidation) reactions are enhanced by elevated glucose levels and there is some evidence that glycation itself may induce the formation of oxygen derived free radicals (Koening *et al.*, 1978). The levels of Glycosylated haemoglobin are monitored as a reliable index of glycemic control in diabetics (Jackson *et al.*, 1979). The levels of Glycosylated haemoglobin follow a similar pattern in the current study, they were elevated in diabetic control rats and the amount of this increase is directly proportional to the fasting blood glucose level (Venkateswaran and Pari *et al.*, 2002) .

In this context, several medicinal plants were also reported to have the capacity of decreasing the level of Glycosylated haemoglobin and increasing plasma insulin and haemoglobin in diabetic (Latha and Pari, 2000, Leite *et al*., 2007). The present data on the effect of *A. malabarica* extract on alloxan-induced diabetic rats indicates that decreased blood glucose and glycosylated haemoglobin levels have been corrected by the insulin secretary effect and by the presence of some active constituents in the extract.

Effect of various extract of A. malabarica stem bark on lipid profile alloxan-induced diabetic rats

Figure 2 demonstrates the level of serum lipids like total cholesterol, triglycerides, phospholipids, serum high density lipoprotein-bound cholesterol (HDL-c), very low density lipoprotein-bound cholesterol (VLDL-c) and low-density lipoprotein-bound cholesterol (LDL-c). The rise in blood sugar is accompanied by the increase in total cholesterol, triglycerides, phospholipids, LDL-c, VLDL-c and fall of HDL-c in diabetic rats. The levels of serum total cholesterol, triglycerides, phospholipids, VLDL-c and LDL-c were significantly ($p < 0.05$) increased in diabetic rats when compared to those of normal control rats, while the level of serum HDL-c was significantly ($p < 0.05$) decreased in diabetic rats when compared to that of normal control rats. The serum lipids like, triglycerides, phospholipids, VLDL-c and LDL-c were significantly ($p < 0.05$) decreased and HDL-c was significantly ($p < 0.05$) increased in ethanol extract (at the dose of 50mg/kg) or glibenclamide treated diabetic rats , but the reverse action was not observed in water extract and hexane extract.

The effect of ethanol extract was predominant than that of glibenclamide. The abnormal high concentration of serum lipids is mainly due to increase in the mobilization of free fatty acids from the peripheral fat deposits, because insulin inhibits the hormone sensitive lipase production. However, administration of ethanol extract and glibenclamide to diabetic rats tends to bring the values to near normal. Thus, ethanol extract 50 mg/kg treatment exhibited hypocholesterolemic, hypotriglyceridemic and hypophospholipidaemic effects while at the same time increasing the HDL-c. Therefore, the elevated level of serum lipids in diabetes mellitus

causes the risk of coronary heart disease (Jackson *et al*., 1979). It has been well established that diabetics mellitus alters the normal metabolism of tissues like liver, kidney and heart. Like diabetic rats treated with glibenclamide, the administration of ethanol extract to diabetic rats tends to bring the values to near normal. *A. malabarica* reduce the susceptibility of lipids to oxidation and stabilize the membrane lipids thereby reducing oxidative stress.

Phytochemical screening

Phytochemical analysis of active extract (AMSE) of the plant was confirmed to contain Carbohydrates, Phenols, Steroids, Glycosides, Tannins and Terpenoids. In conclusion, the results of this study show that ethanolic extract of *A. malabarica* possessed anti-diabetic and anti hyperlipidemic properties. This confirmation justifies its use in ethnomedical medicine for the treatment of diabetes.

Figure 1. Effect of various extract of *A. malabarica* stem bark on body weight of alloxan induced diabetic rats

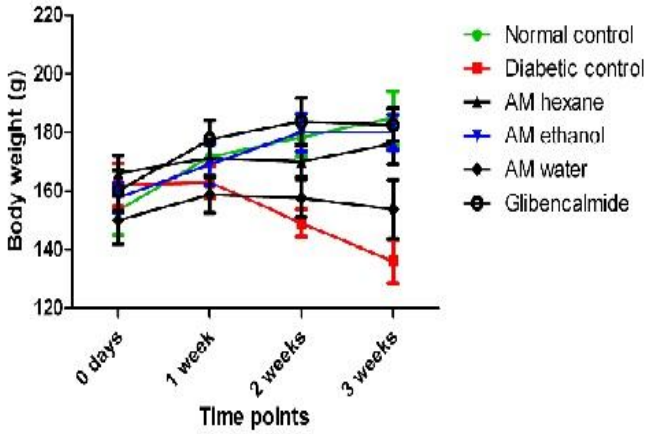
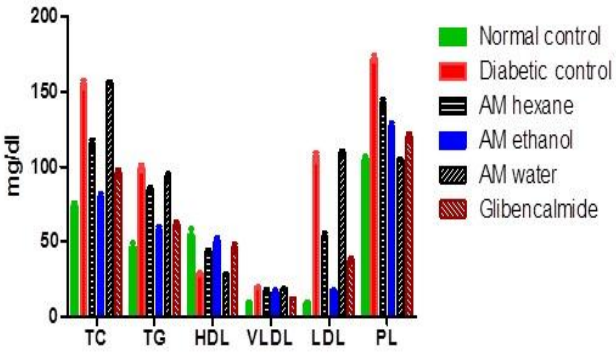


Figure 2. Effect of various extract of *A. malabarica* stem bark on lipid profile alloxan-induced diabetic rats



The statistical analysis was performed using one way ANOVA analysis. * represent statistically significant p<0.05 (DMRT). AM- *Ailanthus malabarica*

Table 1. Effect of blood glucose levels of *A. malabarica* stem bark different extracts on alloxan induced diabetic rats

Groups	Blood glucose levels (mg/dl)			
	Initial	1st week	2nd week	3rd week
Normal control	81.6 ± 4.0[a]	79.6 ± 5.0 [a]	71.0 ± 2.6 [a]	79.6 ± 9.0 [a]
Diabetic control	241.0 ± 19.7[b]	251.2 ± 10.3 [b]	249.7 ± 9.9 [b]	263.2 ± 5.5 [b]
AMSH(50 mg/kg)	231.5 ± 6.6 [b]	225.2 ± 7.97 [b]	215.0 ± 9.6 [c]	212.7 ± 7.9 [c]
AMSE (50 mg/kg)	237.2 ± 9.9 [b]	189.2 ± 9.2 [d]	149.6 ± 10.6[e]	116.2 ± 11.0[f]
AMSW (50 mg/kg)	237.7 ± 11.0[b]	243.0 ± 5.7[b]	247.0 ± 6.3[b]	243.5 ± 5.5[b]
Glibenclamide (0.6 mg/kg)	241.0 ± 14.6[b]	172.0 ± 6.7[d]	134.5 ± 5.5[e]	114.7 ± 5.5[f]

Values are given as mean ± S.D.E for six rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05. AMSH – *A. malabarica* stem hexane extract; AMSE - *A. malabarica* stem ethanol extract; AMSW - *A. malabarica* stem water extract.

Table 2. Effect of *A. malabarica* extracts on Haemoglobin, glycosylated haemoglobin and plasma insulin in normal and diabetic rats

Groups	Hb (g/dl)	HbA1C (mg/g (Hb)	Insulin (µU/ml)
Normal control	12.1±0.4[a]	0.25±0.01[a]	17.0±0.28[a]
Diabetic control	8.1±0.5[b]	0.80±0.01[b]	4.8±0.42[b]
AMSH(50 mg/kg)	8.8±±0.4[c]	0.62±0.02[c]	8.05±0.21[c]
AMSE (50 mg/kg)	10.6±0.8[d]	0.28±0.0[d]	13.0±0.7[d]
AMSW (50 mg/kg)	8.3±0.5b,[c]	0.72±0.01[e]	6.2±0.42[e]
Glibenclamide (0.6 mg/kg)	10.9±0.3[d]	0.33±0.02[f]	11.95±0.77[f]

Values are given as mean ± S.D.E for six rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05. AMSH – *A. malabarica* stem hexane extract; AMSE - *A. malabarica* stem ethanol extract; AMSW - *A. malabarica* stem water extract.

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