ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Hypoglycemic and Hypolipidemic effect of *Azadirachta indica* seed oil and Mehani (Polyherbal Formulation) on Alloxan induced Diabetic Albino Rats

S Saleem Basha[1], Mriganka Baruah[2*], Anand Shaker[3], Suresh Babu Kondaveeti[1] and Satya Narayana [1]

[1] Tutor, Department of Biochemistry, Melmaruvathur Adhiparasakthi Institute of Medical Science, Tamil Nadu
[2] Asst. Prof, Dept of Biochemistry, Melmaruvathur Adhiparasakthi Institute of Medical Science, Tamil Nadu
[3] Asso. Prof, Dept of Biochemistry, Melmaruvathur Adhiparasakthi Institute of Medical Science, Tamil Nadu

ABSTRACT

The present study aims to investigate the hypoglycaemic and hypolipidemic effect of *Azadirachta indica* seed oil and Mehani (Poly Herbal Formulation) in alloxan induced diabetic wistar albino rats. The rats were divided into 6 groups, each consisting of 6 rats: Control (Normal) rats, diabetic induced rats received alloxan (150/ Kg in 3 doses), diabetic induced rats treated with *Azadirachta indica* seed oil (5ml/Kg orally for 21 days), Normal rats treated with *Azadirachta indica* seed oil, Diabetic induced rats treated with Mehani (2gm/60Kg orally for 21 days) and Normal rats treated with Mehani. The rats were monitored for plasma glucose, glucose metabolising enzymes hexokinase and glucose-6-phosphatase and Lipid profile. Alloxan induced diabetic rats shows a high blood glucose with altered lipid profile and altered level of glucose metabolising enzymes which was counteracted upon treatment with *Azadirachta indica* seed oil and Mehani which shows significant ($p<0.01$) reduction in blood glucose, serum cholesterol, serum triglyceride and glucose-6-phosphatase level and significant ($p<0.01$) increase in the level of hexokinase and serum HDL suggesting its hypoglycaemic and hypolipidemic role. Again in comparison to *Azadirachta indica* seed oil, Mehani (Poly Herbal Formulation) have more hypoglycaemic and hypolipidemic effect.

Keywords: *Azadirachta indica* seed oil, Mehani (Poly Herbal Formulation), Diabetes, Lipid profile, Liver enzymes.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Diabetes Mellitus is a heterogeneous disease primarily due to disorder in carbohydrate metabolism due to deficiency or diminished effectiveness of insulin resulting in hyperglycemia and glycosuria with secondary disturbance in the metabolism of protein, lipids, water & electrolytes and in tissue/organ sometimes resulting with grave consequences. [1]. Diabetes, a disease of pandemic prevalence with its microvascular and macrovascular complication and concern regarding its high morbidity and mortality, has always been a challenge to every physician despite impressive advancement in diagnosis and treatment. The conventional way of treatment with synthetic hypoglycaemic agent and Insulin and observing its side effects and insulin resistance [2], search of a natural and effective hypoglycemic agent with minimum side effects and capable in controlling associated symptom of lipid and protein metabolism is always being a quest of all researcher.

Azadirachta indica also known as Neem, an evergreen fast growing plant widely used in ayurveda for its wide spread medicinal effect. All parts of the plant have shown medicinal property and curing various human diseases including diabetes and heart diseases [3]. Polyherbal formulation are considered more effective in treatment of diabetes mellitus as different plant formulation and different combined extract of plant are thought to have a synergist effect of the combined prepared drug. [4]. Mehani (Polyherbal formulation) having 5 ingredients all having medicinal effect composed of *Salacia oblonga* [5], *Emblica officinale* [6], *Trigonella foenumgracum* [7], *Curcuma longa* [8] and *Tinospora cardiofolia* [9] were combined and were evaluated for its hypoglycemic and hypolipidemic activity .

Thus the present study aims to compare and evaluate the hypoglycemic and hypolipidemic activity of *Azadirachta indica* seed oil and Mehani (Polyherbal formulation).

MATERIALS AND METHODS

Plant collection

The Plant material of *Azadirachta indica* seed oil and Mehani (a polyherbal Formulation) used for investigation was obtained commercially from Astanga ayurvedic Pvt Ltd, Trichy (Dist.), Tamil Nadu. The present study is conducted in the department of biochemistry following approval of Institutional ethical committee and accepted guidelines for animal studies using animals were followed.

Animals

Wistar strains of male albino rats of 4-6 weeks old weighing 150- 200gm were used in the experiment. These animals were kept in well ventilated cages and maintained under controlled conditions of light (12h/24h) and temperature (23+1^{o}C) and were fed with commercial pellets rats chow (Hindustan Lever Ltd., India) and tap water were provided ad libitum. All animals were carefully monitored and experimental protocols were in accordance

with the recommendations of the institutional Animal Ethical Committee (King Institute, Chennai, India)

Drugs & Chemicals

The comparative study of antidiabetic Activity of *Azadirachta indica* seed oil and Mehani (a polyherbal Formulation) were analysed after inoculating alloxan [10] in the albino rats as per as the experimental design. *Azadirachta indica* seed oil is administered in the dose of 5ml/Kg body weight orally for 21 days and Mehani (a polyherbal Formulation) is administered in the dose of 2gm/60Kg body weight orally for 21 days.

Experimental Design

Following six groups of rats, six rats in each group were considered for the study
Group I : Control (Normal)
Group II: Diabetic induced rats received Alloxan in three doses (150/Kg body weight)
Group III: Diabetic induced rats + *Azadirachta indica* seed oil (5ml/Kg) orally for 21 days.
Group IV: Normal rats treated with *Azadirachta indica* seed oil orally for 21 days.
GroupV: Diabetic induced rats + Mehani (Polyherbal Formulation) (2gm/60Kg) orally for 21 days.
Group VI: Normal rats treated with Mehani (Polyherbal Formulation) (2gm/60Kg) orally for 21 days.

Diabetes were induced via three doses of subcutaneous injection of alloxan monohydrate, freshly prepared in 0.154M sodium acetate buffer (pH 4.5) was given at a dose of 150 mg /Kg body weight. [11]. The rats were monitored for plasma glucose and Lipid profile levels at the end of 48 h after Alloxanisation. The animals were later sacrificed by cervical decapitation after the experimental period of collection of blood. Liver was dissected out, washed in ice cold saline and homogenized in 0.1M Tris-HCl buffer, pH 7.4 and homogenised sample were used for estimation of glucose metabolising enzymes - Hexokinase and Glucose-6-phosphatase.

Biochemical Estimation

Taking all aseptic and antiseptic precautions, 0.05 ml of blood was drawn from the tail vein of albino rats. Estimation of serum blood glucose [12] , total cholesterol [13], triglyceride [14] and HDL [15] was done by Colorimetric method using a Colorimeter and liver homogenised sample were used in estimation of Hexokinase, [16] and Glucose 6 phosphatase [17] activity in liver via Colorimetric method .

Statistics

After the biochemical estimations, the results obtained was statistically analyzed by using statistical software SPSS 16.0 and then compared between different groups of the study

ISSN: 0975-8585

by applying Students T test. The results were expressed as Mean± SD and were taken as significant when the probability (p value) is less than 0.05 as percentage of the observing values of 't' at a particular degree of freedom.

RESULTS

There were observable changes in the blood glucose and liver enzyme Hexokinase and Glucose-6-phosphatase level on alloxan induced diabetic rats being treated with *Azadirachta indica* seed oil and Mehani (Polyherbal Formulation) for 21 days as seen from our results from table 1. Group III and Group V significantly ($p<0.01$) lowers the blood glucose in alloxan induced diabetic rats (GroupII). There is also significant difference between Group III and Group V compared to Group I. Group III and Group V also shows significant differences but no differences is noted in Group IV and Group VI which resembles with the normal (GroupI) . Figure 1 shows the mean glucose level in the different groups.

Table 1: Effect of *Azadirachta indica* seed oil and Mehani (Polyherbal Formulation) on blood glucose and enzyme levels of Alloxan induced diabetic rats

	#Plasma Glucose (mg/dl)	#Hexokinase (nmoles of G-6-P formed/hr/mg protein)	#Glucose-6-phosphatase (nmoles of Phosphorous liberated/mg protein)
Group I	88.25±0.90	170.70±8.1	0.22±.30
Group II	280.4±14.2 $^{**\ a}$	80.9±5.61 $^{**\ a}$	0.30±0.8 $^{**\ a}$
Group III	126.32±6.20 $^{**\ b}$	165.6±9o $^{**\ b}$	0.18±0.8 $^{**\ b}$
Group IV	89.31±0.80 $^{NS\ a}$	169±7.21 $^{NS\ a}$	0.21±0.9 $^{NS\ a}$
Group V	112.33±8.10 $^{**\ b\ c_2}$	168.1±11 $^{**\ b\ c_2}$	0.19±0.6 $^{**\ b\ c_2}$
Group VI	87.44±0.90 $^{NS\ a}$	166.8±4.8 $^{NS\ a}$	0.21±0.1 $^{NS\ a}$

Legend #Values are given as mean ± S.D for 6 rats in each group. Values are statistically significant at **p<0.01 and NS-non significant. Significance compared with in the groups are as follows: **a**. Group I vs. Group II, Group IV and Group VI ; **b**. Group II vs. Group III and Group V; **c_1.** Group III vs. Group V non significant; c_2**.** Group III vs. Group V significant

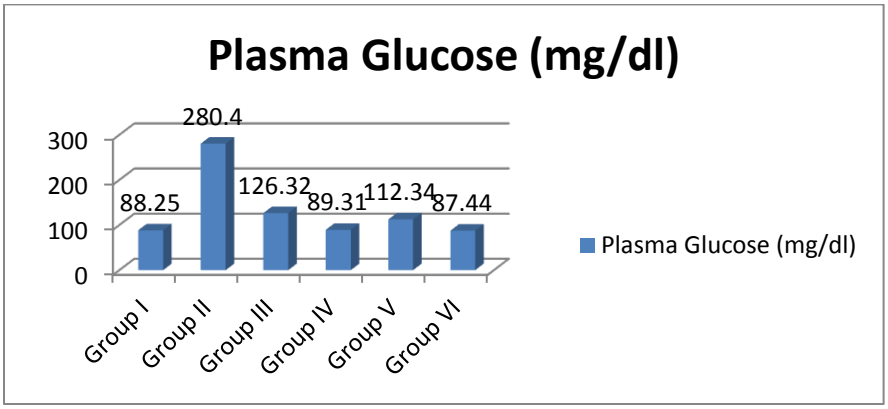

Figure 1: Effect of *Azadirachta indica* seed oil and Mehani (Polyherbal Formulation) on blood glucose of Alloxan induced diabetic rats

ISSN: 0975-8585

Table 2: Effect of *Azadirachta indica* seed oil and Mehani (Polyherbal Formulation) on Lipid Profile of Alloxan induced diabetic rats

	#Serum Cholesterol(mg/dl)	#Serum Triglycerides(mg/dl)	#Serum HDL (mg/dl)
Group I	120.1±7.1	75.9±5.07	30.39±0.40
Group II	170±6.1$^{**\ a}$	93.3±6$^{**\ a}$	18.91 ± 3.18$^{**\ a}$
Group III	149±7.0$^{**\ b}$	75.8±3.6$^{**\ a}$	32.61±0.32$^{**\ b}$
Group IV	121.3±3.8$^{**\ a}$	76.10±6.9$^{**\ a}$	31.22±0.20$^{**\ a}$
Group V	140±7.8$^{**\ b\ c}_{2}$	80.1±3.3$^{**\ a}$	33.96±0.22$^{**\ b\ c}_{2}$
Group VI	122±7.1$^{**\ a}$	77.6±7.1$^{**\ a}$	33.44±0.40$^{**\ a}$

Legend #Values are given as mean ± S.D for 6 rats in each group. Values are statistically significant at **p<0.01 and NS-non significant. Significance compared with in the groups are as follows: **a**. Group I vs. Group II, Group IV and Group VI ; **b**. Group II vs. Group III and Group V; **c.** Group III vs. Group V

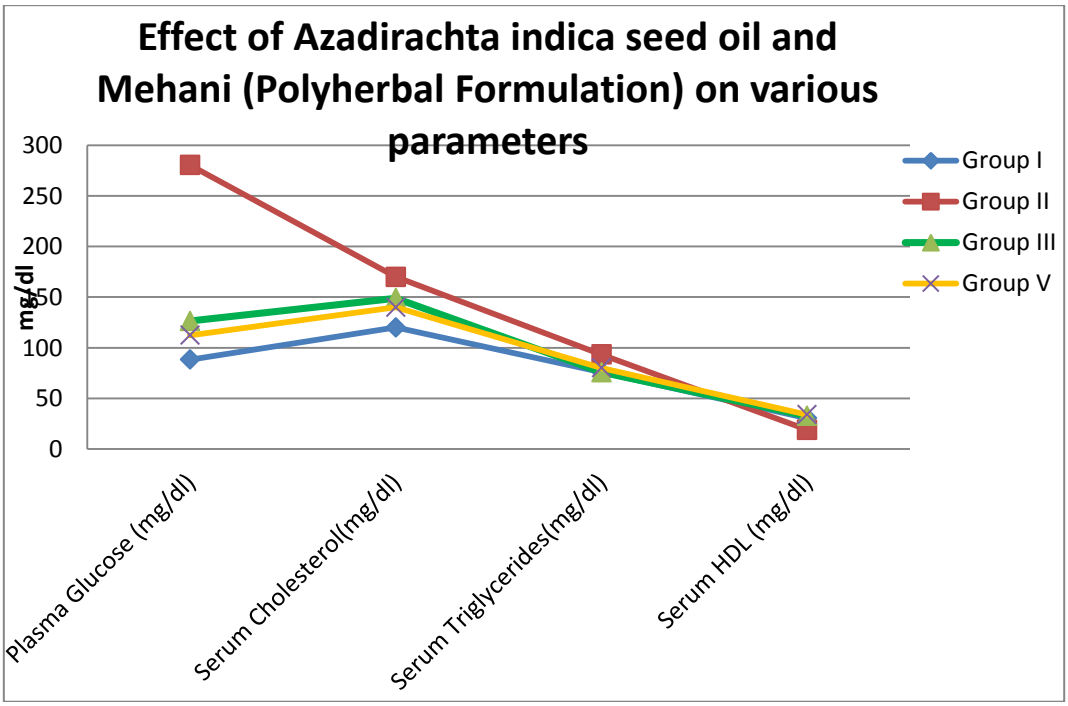

Figure 2: Comparision between *Azadirachta indica* seed oil and Mehani (Polyherbal Formulation) on blood glucose levels and Lipid profile

Results in Table 2 shows total cholesterol and triglyceride values increased significantly and HDL level decreased significantly in Group III and Group V compared to alloxan induced diabetic rats (Group II). Group III and Group V also shows significant difference compared to Group I but no differences is noted in Group IV and Group VI which resembles with the normal (GroupI).

Figure 2 depicts mean blood glucose, serum cholesterol, serum triglyceride and HDL levels between Control (group I), the alloxan induced diabetic group (group II), *Azadirachta indica* seed oil treated group (group III) and Mehani treated (group V) and provides a comparision between Group III and Group V where Group V is shown to be more effective.

DISCUSSIONS

From ancient time onwards ayurvedic medicine are being used for curing various kinds of human ailments. Several traditional medicinal plants and herbs having potential medicinal effects are being used to lower blood sugar, blood lipids and even few of them have proved remarkable well in curing diabetes, heart diseases and its complications. The current paper is aimed at showing the hypoglycemic and hypolipidemic effect of *Azadirachta indica* seed oil and Mehani (Polyherbal Formulation) and providing a comparison between the two based on effect produced on alloxan induced hyperglycaemic rats.

Alloxan is one of the most widely used chemical in inducing diabetes via damaging the insulin secreting cells [18] in experimental animals. Chemically it is 2,4,5,6 tetra-octa-hexahydro pyrimidine , freely soluble in water and slightly acidic with PH 6.63 [19] .

Our Study from table 1 shows increasing levels of hexokinase and lowering of glucose-6-phosphatase in the liver both by *Azadirachta indica* seed oil and Mehani, which will cause an increase in Glucose-6-phosphate level thus stimulating Glycolytic pathway, TCA cycle, HMP shunt and also prevents the formation of free Glucose and all contributing in lowering of the Blood Glucose level on Alloxan induced Diabetic Albino Rats. It also shows lowering of Blood Glucose level directly by *Azadirachta indica* seed oil [20] and Mehani showing their hypoglycaemic effect. In comparison to the *Azadirachta indica* seed oil, Mehani (polyherbal formulation) have more hypoglycaemic effect as seen from Figure 2.

An alteration in lipid profile is a known complication in diabetes mellitus and which leads to atherosclerosis and Heart disease [21, 22] including Coronary artery disease and Myocardial Infarction. Thus hypolipidemic action have to be definitely considered beneficial in the management of Diabetes and its complication.[23].

Pari L et al in 2003 [24] in their studies have shown that hyperlipidemia is a common occurrence of Alloxan-induced diabetes mellitus in experimental rats. Our results from table 2 shows both *Azadirachta indica* seed oil and Mehani are significantly effective in lowering the serum cholesterol, serum triglyceride and elevating serum HDL level in alloxan induced rats conforming to the studies done by Biswas Kausik et al 2002 [25] and showing its hypolipidemic role. Mehani has more hypolipidemic effect compared to the *Azadirachta indica* seed oil, as seen from Figure 2 probably due to the multiple ingredients and conglomerated effect produced from its different comp onents.

ISSN: 0975-8585

CONCLUSION

In present study, a significant and potent hypoglycemic and hypolipidemic activity is seen in *Azadirachta indica* seed oil and Mehani. But Mehani (Polyherbal formulation) is seen to be more effective in compared to *Azadirachta indica* seed oil.

Thus this study is undertaken to provide a better understanding of the role of *Azadirachta indica* seed oil, Mehani (polyherbal formulation) and to provide a comparison between the two in terms of hypoglycemic and hypolipidemic effect . Although a more elaborate study carried out on various biochemical parameters would have been more enterprising in establishing the actual role and relationship between the two but paucity of time, limited resource may be taken as limitation of our study. It is hoped that the present study will encourage new studies related to the above topic in a bigger way.

REFERENCES

[1] Ebelt H, Brome HJ. J Ethanopharmacol 2000; 12.
[2] V Mohan, S Sandeep, R Deepa, B Shah & C Varghese. Indian J Med Res 2007; 125: 217-230.
[3] Biswas Kausik, Chattopadhyay Ishita, Banerjee Ranjit, Bandyopadhyay Uday. Curr Sci 2002; 82(11): 1336-1345.
[4] Kumar Jaya. Int J Diabetes Dev Ctries 2010; 30: 111-112.
[5] Williams JA, Choe YS, Noss MJ, Baumgartner CJ, Mustad VA. Am J Clin Nutr 2007; 86(1): 124-30.
[6] Akhtar MS, Ramzan A, Ali A, Ahmad M. Int J Food Sci Nutr 2011; 62(6): 609-16.
[7] Hannan JM, Ali L, Rokeya B, Khaleque J, Akhter M, Flatt PR, Abdel-Wahab YH. Br J Nutr 2007; 97(3):514-21.
[8] Kuroda M, Mimaki Y, Nishiyama T, Mae T, Kishida H, Tsukagama M, Takahashi K, Kawada T, Nakagawa K, Kitahara M. Biol Pharm Bull 2005; 28(5): 937-9.
[9] P Stanely Mainzen Prince, Venugopal P. Phytother Res 2003; 4:410-413.
[10] Sharma SB, Nasir A, Prabbu KM, Murthy PS, Dev B. J Ethnopharmcol 2003; 85: 65-67.
[11] Trinder P. Ann Clin Biochem 1996; 6: 24-27.
[12] Rifai N, Bachorik PS, Albers JJ. Lipids, lipoprotein and apolipoprotein. Tietz textbook of clinical chemistry 3rd ed. Philadelphia, W.B. Saunders Company, 1999, 806-61.
[13] Bucolo G, David H. Clin Chem 1973; 19: 476-482.
[14] Sugiuchi H, Uji Y, Okabe H, Irie T, Uekama K, Kayahara N, et al. Clin Chem 1995; 41:717-23.
[15] Katsumate K, Katsumate Y. Horm metab Res 1994; 508-10
[16] Long C. Biochem J 1952; 50: 407.
[17] Taussky HH and Shorr E. J Biol Chem 1953; 202: 675-685.
[18] Bopanna KN, Kannan J, Sushma G, Balaraman R, Rathod SP. Indian J Pharmacol 1997; 29: 162–167.
[19] Labes R and Ferisburger H. Arch Exp Pathol Pharmacol 1936; 156: 226.
[20] Murty KS, Rao DN, Rao DK, Murty LBG. Indian J Pharmacol 1978;10: 247-250.

[21] Steiner G. Diabetes Care 1999; 22(3): C6-C9.
[22] Massing MW, Sueta CA, Chowdhury M, Biggs DP, Simpson RJ Jr. American J Cardiol 2001;87:646-664.
[23] Sweta Shailey , Seemi Farhat Basir . Journal of Pharmacy Research 2011; 4(3): 810-812.
[24] Pari L, Saravanan R. Diabetes Obesity and Metabolism 2003; 5: 156–162.
[25] Biswas Kausik, Chattopadhyay Ishita, Banerjee Ranjit, Bandyopadhyay Uday. Current Science 2002; 82(11): 1336-1345.