ANTIDIABETIC AND ANTIHYPERLIPAEMIC EFFECTS OF AQUEOUS NEEM (AZADIRACHTA INDICA) EXTRACT ON ALLOXAN DIABETIC RABBITS

Khalil, Abdullah Khalil (PhD)
Faculty of Medical Sciences. Dept Medical Laboratories. Hodeidah University. Yemen
Khalil_bioc@yahoo.Com

Abstract:

Extracts of various plants material capable of decreasing blood sugar have been tested in experimental animal models and their effects confirmed. Neem or Margose (Azadirachta Indica) is an indigenous plant believed to have antiviral, antifungal, antidiabetic and many other properties. This paper deals with a comparative study of effect of aqueous Neem leaves extract alone or in combination with glibenclamide on alloxan diabetic rabbits. Administration of crude aqueous Neem extract (CANE) alone (1.5 ml/kg/day) as well as the combination of CANE (1.5 ml/kg/day) with glibenclamide (0.25 mg/kg/day) significantly decreased ($P<0.05$) the concentrations of serum lipids, blood glucose and lipoprotein VLDL and LDL but significantly increased ($P<0.05$) the concentration of HDL. The change was observed significantly greater when the treatment was given in combination of CANE and glibenclamid than with CANE alone.

KEY WORDS: Crude Aqueous Neem Leaves Extract (CANE), hypoglycemic, cholesterol, triglycerides, phospholipids

1. Introduction

Diabetes mellitus is a chronic metabolic disorder affecting approximately 5% of the world's population. It is characterized by dysregulation in carbohydrate, protein and fat metabolisms caused by the complete or relative insufficiency of insulin secretion and/or insulin action [1]. According to World Health Organization projections, the diabetic population is likely to increase to 300 millions or more by the year 2025[2].

In recent years, extracts from the seeds of *Neem* plant have emerged as a promising source of natural pesticides owing to their low mammalian toxicity [3, 4]. The medical properties has been well known in the indigenous Indian system of Ayurvedic medicine for many centuries commercially available products such as

tooth paste, bath soaps, skin care, capsules and tonics based on antibacterial[5]. Extracts of various plant material capable of decreasing blood sugar have been tested in experimental animal models and their effects confirmed [6, 7].
Also, *Neem* oil and nimbidin significantly delayed the peak rise in blood sugar after administration of glucose. Further it was found that glucose tolerance curves were similar to that of to lbutamide[8], it has been reported that an aqueous extract of tender leaves of *Neem* tree reduced blood sugar in dogs [9].
The present paper deals with comparative study of effects the aqueous extract of *Neem* leaves alone or with glibenclamide on alloxan diabetic rabbits.

2. Materials and Methods

2.1. Plant material

Neem leaves was collected from Hodeidah, Yemen during 2003, the leaves were removed from their mud and dried at room temperature then blended with a coffee bean blender and stored at 4 °C.

2.2. Preparation of extract

The powder was extracted with distilled water using soxhelt at boiling temperature (100 ◦C) up to 4 hrs. The extract was cooled and filtered to remove the residue. This was designated as crude aqueous *Neem* extract (CANE)[10].Administration was affected in volume of 1.5 ml/kg, once daily for one month orally.

2.3. Animals

2.3.1 Experimental animals

A) Thirty-six adult male albino rabbits (1 and 2 kg), maintained at 25±1°C in a well Ventilated animal house under natural photoperiod conditions were used for study. They were provided with standard diet and water ad libitum.
B) Diabetic was induced in the rabbit by a single I.V injection of alloxan mono hydrated (Hopkins and Williams) in distilled water at the rate of 75mg/kg body weight [11].The animals were fasted for 36 hrs before injection. After fortnight rabbits moderate diabetes having glucosuria (using urine test tab (glucose tests) for detected on glucose in urine[Bohringer-Mannheim,Germany][12].And hyperglycemia (blood glucose 200-250/100ml) were divided into six groups of six animals each as mentioned below.

Group1: control given with saline (1.5ml/kg/day).
Group2: diabetic given with saline (1.5ml/kg/day).
Group3: diabetic treated with (1.5ml CANE/kg/day).
Group4: diabetic treated with glibenclamide (0.5mg/kg/day).
Group5: diabetic treated with (1.5ml CANE/kg/day) plus glibenclamide (0.25mg/kg/day).
Group6: diabetic treated with insulin (8 units/kg/day).

Body weight, urine sugar (qualitative) and fasting blood glucose of all rabbits were determined before the start of the experiment. After 30 days of treatment the body

weight, urine sugar and fasting blood glucose of animal were determined. Total cholesterol, HDL-cholesterol, VLDL, triglyceride and phospholipids were analyzed from the serum by using Lab Kits (Randox, United Kingdom) whereas LDL-cholesterol concentration was calculated according to Friendswood formula:

LDL-cholesterol=Total cholesterol-(TG/5+HDL-cholesterol), results were expressed as mg/dl [13].

Statistical analysis: the results were presented as Mean ± SEM and included student's *t*-test and analysis of variance (ANOVA) test. The significance level for all tests was taken as P value < 0.05.

3. Results

3.1. Blood glucose

In alloxan diabetic rabbits there were a significant increase (P< 0.05) in fasting blood glucose and urine sugar and there was a significant decrease (P<0.05) in body weight and total hemoglobin content. A significant increase in body weight and hemoglobin level were observed, and significant decrease fasting blood glucose (FBG) and urine sugar (Table 1) in diabetic rabbits treated with CANE, glibenclamide, insulin and in combination of CANE and glibenclamide. Though all the antidiabetic drugs used significantly decreased the FBG level, combination therapy of CANE (1.5 ml/kg) and glibenclamide (0.25 mg/kg) produced greater reduction in FBG as compared to all the other groups. There was a significant (P<0.05) a melioration of body weight and total hemoglobin content in the diabetic rabbits given with combination therapy of glibenclamide with CANE.

Table 1. The effect of crude aqueous *Neem* extract (CANE) on blood glucose, change in body weight and urine sugar in normal and diabetic rabbits.
(Values are mean±SEM of six rabbits in each group).

Group	FBG mg/100ml		Change in body weight (g)	Total Hb content	Urine sugar
	Initial	Final			
Control	85.3±17.1	88.9±13.2	34.0±5.2	15.1±4.2	–
Diabetic control	230±10.9*	290±6.5*	-15.2±2.4*	10.8±2.9*	++
Diabetic + (CANE)	223±9.2	156.2±7.4*	+5.9±1.4*	12.6±2.3*	+
Diabetic + Glibenclamide	225±12.5	130±14.2*	+9.1±3.4*	12.4±1.7*	+
Diabetic + (CANE) + Glibenclamide	229.3±14.5	115.4±10.3*	+16.2±1.8*	14.8±1.4*	+
Diabetic + Insulin	220.6±11.2	122±8.4*	+11.0±1.4*	13.0±1.2	+

Group 2 is compared to group 1, Group 3, 4, 5 and 6 are compared to Group 2.
*P Value < 0.05.

3. 2. *Serum cholesterol and triglycerides*

Serum cholesterol and triglyceride levels in all groups of animals are given in Table 2. In alloxane diabetic rabbits there was a significant ($P<0.05$) increase of total cholesterol, triglycerides, phospholipids, LDL-cholesterol and VLDL and the significant decrease ($P<0.05$) in HDL-cholesterol in serum compared to that of normal control. All antidiabetic agents used in the experiments significantly decreased (Table 2) the levels of cholesterol, triglycerides, phospholipids, LDL and VLDL. The significant ($P< 0.05$) fall of the serum lipid parameters was observed in the combination therapy of CANE with glibenclamide when compared with diabetic control, CANE or glibenclamide.

Table 2. The effect of crude aqueous *Neem* extract (CANE) on serum lipid of normal and diabetic rabbits.

(Value expressed as mg/100 ml serum are mean±SEM of six rabbits in each group)

Group	Total cholesterol	triglycerides	Phospholipids	VLDL	HDL	LDL
control	112.4±6.5	72.4±5.5	162±7.7	13.2±0.8	45.2±3.6	50.19±8.56
Diabetic control	280.2±9.2*	260.5±9.2*	210.0±2.8*	37.9±3.6*	22.52±6.93*	81.92±8.5*
Diabetic + (CANE)	200.5±12.8*	96.8±12.1*	178.5±11.2*	20.2±3.8*	40.2±7.9*	68.26±6.6*
Diabetic+Glibenclamid	189.2±11.2*	99.2±10.2*	182.6±12.2*	23.8±2.8*	39.8±2.4*	59.8±6.9*
Diabetic + (CANE) + Glibenclamide	170.2±11.9*	85.4±11.5*	180.8±7.5*	15.5±1.2*	44.3±2.97*	48.96±5.9*
Diabetic + Insulin	205.8±10.2*	106.8±11.2*	188.2±7.4*	30.2±4.2*	37.5±4.86*	60.28±8.9*

Group 2 is compared to group 1, Group 3, 4, 5 and 6 are compared to Group 2.
* P Value < 0.05.

4. Discussion

Rabbits were used as animal models and alloxan was used a diabetogenic agents. Alloxan administration has been found to lead to long-last in diabetes in many animals species. A number of studies have shown that alloxan disrupts the integrity of the beta cell plasma membrane. Some evidences indicate that alloxan acts at the site of sugar transport in to the cell [14].

Bequest has postulated that alloxan acts by inhibiting a mitochondrial transport system for inorganic phosphate leading to fall intracellular pH and cell death [15].

It has also been proposed that alloxan leads to mitochondrial dysfunction and interferes with intracellular glucose oxidation [16].

Most early evidences were based on the fact that alloxan can produce D.M. in laboratory animals by destroying beta-cells of the pancreas through oxidative stress [17].

Inductions of diabetes in these animals were confirmed by significant rise in blood glucose and fall in the liver glycogen level [18]. The lowering activity of the glycolytic activity of various isoenzymes of LDH within single metabolic pathway possible points to decrease supply of glucose to the cell during alloxan induced diabetes. Excess of fatty acids in plasma produced by the alloxan induced diabetes promotes the liver conversion of some fatty acids into phospholipids and cholesterol. These two substances along with excess triglycerides formed at the same time in the liver may be discharge into the blood in the lipoproteins [19].

In the present study CANE alone or in combination with glibenclamide produced a marked fall of serum glucose, cholesterol, triglycerides, phospholipids, LDL cholesterol, VLDL cholesterol and increase serum HDL cholesterol. The effect was more pronounced with combination the therapy of CANE and glibenclamide compared to CANE alone which is less pronounced. The hypoglycemic and anti hyperlipaemic effect of CANE with glibenclamide may be a synergistic combination. The maximum effect of reduction was observed on LDL cholesterol and increase in HDL cholesterol after treatment with CANE plus glibenclamide. High LDL and low HDL levels are usually associated with atherosclerosis [20]. High HDL levels reduced the risk. The possible mechanism of the CANE may be due to increase in the liver LDL receptor activity[21]and decreased triglycerides synthesis[22].This would be useful in disease like diabetes mellitus and coronary heart disease (CHD) because of their inverse relationship[23].

Hypertriglyceridemia is one of the risk factors in coronary heart disease. CANE alone or in combination with glibenclamide reduced triglycerides in alloxan induced diabetic rabbits and may be prevent the progression of CHD. Sharma, et al have found higher levels of serum lipids in both insulin dependent diabetes mellitus (IDDM) and non insulin dependent diabetes mellitus (NIDDM)[24].CANE used alone or in combination with glibenclamide may be used to counter the high serum lipids produced by the above conditions.

The antihyperlipaemic effect of CANE may be due to the down regulation of NADPH and NADH a cofactor in fat metabolism. When glycoly sis slows down because of cellular inactivity, the pentose phosphate pathways still remains active in liver to breakdown glucose that continuously provides NADPII which convert acetyl radicals into long fatty acid chains. CANE may be capable of oxidizing NADPH and enhancing the turnover of HDL ratio. Serum glucose level was reduced in rabbits treated with CANE suggests greater uptake of glucose from the blood by the liver cells. The reduction of serum lipids may be due to decreased synthesis or increased

excretion through intestinal tract by HMGCoA reductase, which is the important enzyme in the formation of cholesterol. One of the possible actions of CANE may be its inhibition of endogenous synthesis of cholesterol and enhancement of the degradation of formed cholesterol by increasing the excretion through intestinal tract. The reduction of serum glucose may be due to enhancing greater uptake of glucose from the blood by liver cells.

5. Conclusions

From this study we can conclusively state that CANE alone or in combination with glibenclamide significantly reduces the levels of serum glucose, lipids and lipoproteins which are actually raised in alloxan diabetic rabbits. CANE alone or in combination with glibenclamide has beneficial effect on serum HDL-cholesterol concentration. Moreover its antihyperlipaemic effect could represent a protective mechanism against the development of atherosclerosis. It is well known that hyperlipidemia has an association with atherosclerosis and the incidence of atherosclerosis is vastly increased in diabetics [25]. CANE in combination with glibenclamide may be utilized for the prevention or management of diabetic induced atherosclerosis in diabetic mellitus patients.

6. References

1- O'Brien, R.M., Granner, D.K., 1996. Regulation of gene expression by Insulin. Physiological Reviews 76, 1109–1161.

2- Boyle, J.P., Honeycutt, A.A., Narayan, K.M., Hoerger, T.J., Geiss, L.S., Chen, H., Thompson, T.J., Projections of diabetes burden through 2050: impact of changing demography and disease prevalence in the US. Diabetes Care (2001), 24: 1936–1940., King, H., Aubert, R.E., Herman, W.H., Global burden of diabetes (1998), 1995–2025 prevalence, numerical estimates and projections. Diabetes Care 21, 1414–1431.

3-Bhushan, M. N. pests and their control, CRC press, Florida, (1988): pp3-10

4- Sahai, O. and Knuth, M. Biotechnology progress, (1985): 1, 1-9

5- Singh, N. and Sastry M. S. Antimicrobial activity of *Neem* oil, Indian J. Pharmacy. (1981), 13: 102.

6- Agacnkar, S. S. In: Ancient Indian medicine and diabetes mellitus in developing countries, Bajaj JS, editor, New Delhi; Interprint, (1984): 3-10

7- Upadhyay, P. Pandey, Ayurvedic approach to diabetes mellitus and its management by countries, Bombay: popular Books Depot, (1984): 375-377

8- Pillai, N. R. Santhakumari, G. Hypoglycemic activity of melia Azadirachta lindica (*Neem*). Indian J. Med. Res. (1981): 931-933.

9- Murthy, K. S., Rao, D. N.; Murthy, L. B. G.; A preliminary study on hypoglycemic effects of Azadirachta indica. Indian, J. Pharmacology. (1978): 10: 47-48.

10- Zheng-Mu, M., Sakai, Y.; et al antimutagenic activity by the medicinal plants in traditional chines medicines, Snoyakuhak, (1990), 44: 225-229.

11- Erenmenmisoglu, A.; Doagan, O. Z.; Saaymen

12– Harold Varley, Alan Gowenio ck, Masvice Bell. Practical clinical Biochemistry. 6th ed. London. Heinemann Medical Book, (1988): 330-331

13-Burtis, C.A and Ashwood (EDS): Teits Test Book of clinical chemistry .3rd Ed, W.B.Saunders comp. (1999):p469.

14- Bell, R.; Handtlye, R. J.: Animal models of diabetes mellitus physiology and pathology. J. Surgical Res. (1983), 35: 433-451

15- Boquist, L.: A new hypothesis for alloxan diabetes. Acta Pathol. Microbial. Econd. (A). (1980), 88: p201

16- Borg, L. A. H., Effect of alloxan on islets of langerhans possible mechanism of diabetogenic action, Act. Biol. Med Ger. (1980), 40: 71

17- Montella, P. l., Vavagas, J. F; Tunes, I. F; Munzo, M. C. and sress in diabetic carbena, E. S.: Oxidative sress in diabetic rats Induced by sterptozotoun, J. Pineal. Res. (1998), 25(2): 94-100

18- Mishra, G.; Behera, H. N.; Alloxan induced changes in the college characteristics in the skin of male garden lizards, Calotes Versicolor from three age groups. Arch Gerantol Geniator (1986), 5: 9-11

19- Bopanna, K. N.; Bhagyalakshmi, N.; Rathod, S. P.; J. Cell culture derived Hemidesmus indicus in the prevention of hypercholesterololemia in normal and hyperlipidemic rats Indian J. Pharmacology(1988),15(2):97-102

20- Artisharma, R. M., Dixit, V. P. Prevention of hyperchesterolemia and atherosclerosis in rabbits after supplementation of *Myristica fragens* seed extract. Indian J. Physical Pharmacol (1995), 39: 407-410

21- Zheng-Mu, M., Sakai, Y., Et al Antimutagenic activity by the medicinal plants in traditional chines medicines (1990), 44:223-229

22- Wong, S., Nestel, P. J., Trimble, R. P. The adaptive effects of dietary fish safflower oil on lipid and lipoprotein metabolism in perfuse liver. Biochemist. Biophysics. Acta., (1984), 792: 103-109

23- Austin, M. A., Hankinson, J. E., Epidemiology of triglycerides small dense low density lipoprotein as risk factor for coronary heart disease. Med. Clic. North Am. (1994), 78: 99-115

24- Sharma, R. D., Raghuram, T. C., Hypoglycemic effects of fenugreek seeds in non insulin dependent diabetic subjects. Nutr. Res. (1990), 10: 731-739

25- Kannel, W. B., McGee, D. L., Diabetes and cardiovascular risk factors, the Framingham study, Circulation (1979), 59: 8-13