M ISSN 1119-8362

Full-text Available Online at
http:// www.bioline.org.br/ja

J. Appl. Sci. Environ. Mgt. 2005
Vol. 9 (1) 85 - 88

Antidiabetic Effect of Chloroform -Methanol Extract of *Abrus Precatorius* Linn Seed in Alloxan Diabetic Rabbit

[1]MONAGO, C C; [2]ALUMANAH, E O

[1.] *Department of Biochemistry, university of Port Harcourt, Port Harcourt, Nigeria.*
[2.] *Department of Biochemistry, University of Nigeria, nsukka.enugu state, Nigeria.*

ABSTRACT: The antidiabetic effect of chloroform – methanol extract of *Abrus precatorious* seed, was studied in alloxan diabetic rabbits. The effect was compared to that of chlorpropamide – a known antidiabetic drug in the class of sulphonylurea and a control group that received normal saline instead of the extract. Normal blood glucose levels drawn before the alloxan injection were 127.80 ± 2.55, 114.30 ± 4.17 and 123.60 ± 1.47 mg/100ml for chloroform – methanol, chlorpropamide and control respectively. When 50mg / kg body weight of chloroform – methanol, chlorpropamide and 5ml of normal saline for control were given orally, blood glucose levels decreased in chloroform – methanol and chlorpropamide groups of alloxan diabetic rabbits but not in control. The percentage reduction of blood glucose of chlorpropamide was 13.8, 32.3, 60.3, 53.5, 46.8, 46.3 and 46.2 after 05, 10, 20, 30, 40, 60 and 68 hours of oral administration respectively, while that of chloroform – methanol extract was 42.9, 58.7, 67.4, 69.1, 67.9, 56.6 and 51.8% respectively. The peak percentage reduction was 69.1% after 30 hrs. and 61.3% after 20 hrs. For chloroform – methanol extract and chlorpropamide respectively. This study therefore has shown that the chloroform – methanol extract of *Abrus precatorius* seed has some antidiabetic properties similar to that of chlopropamide. This is shown in their similar percentage reduction in blood glucose level. @JASEM

Diabetes mellitus is a group of metabolic disorders that result in hyperglycemia due to decreased insulin production or inefficient insulin utilization. The World Health Organization predicted that the number of diabetic patients will double from 143 million in 1997 to about 300 million in 2025 largely because of dietary and other lifestyle factors (Seidell, 2000).

The incidence of type II diabetes is closely linked to choice of diet leading to overweight or obesity (Wannamethee and Shaper, 1999). About 75% of diabetes is type II or non – insulin dependent diabetes (NIDD) (Barnett, 1991) and is associated to other disease conditions like obesity (Wolf and Colditz, 1998); coronary heart, eye, renal, vascular and neurological problems (Miller, 1991). The use of most synthetic antidiabetic drugs like sulfonylurea, biguanides and intravenous insulin injections have their own disadvantages. The most important side – effect of sulfonylureas is hypoglycaemia (Berger, 1985). The severe hypoglycaemia can lead to death. Insulin injection takes place intraveneously. This is because insulin is frequently destroyed in the gastrointestinal tract. Insulin degradation and presence of insulinase were also reported by many authors (Kitabchi and Stentz, 1972; Kahn et al 1976). There is therefore need for oral substitutes for both insulin and severe hypoglycaemic antidiabetic drugs in management of diabetes.

Several botanical supplements have been studied as potential therapeutic agents in the management of diabetes and its related complications. The use of legumes in this effect was reported by many authors. Legumes like *Trigonella foemum graecum* (Festrow and Avila, 1999; Sharma, et al., 1996), *Pterocarpus marsupium*, (Meries and Farnsworth, 1995), *Vigna unquiculata* (Tella and Ojihomon, 1980) have been in use. Jenkins, et al. (1981) showed that legume whole seed or extracts could be helpful in reducing the high blood sugars in diabetes because of their low glycaemic index. *Abrus precatorius* is a tropical legume found in the forests of Nigeria. The seed extracts of *Abrus Precatorius* was reported by Nwodo and Alumanah (1991) as an antidiarrhoeal agent in mice. Uterotonic activity of the seed in rats was reported by Nwodo and Botting, (1983).

The report of chemical composition by Nwodo and Botting, (1983) showed that *Abrus precatorius* contain some substances in the chloroform – methanol extract of the seed, that are likely to reduce the blood glucose level. We therefore studied the antidiabetic properties of the chloroform – methanol extract of the seed of this plant. The aim is to know whether it can be used in the management of diabetes melitus

*Corresponding author
Email: commys2000@yahoo.com

MATERIALS AND METHODS

Sample Preparation: Fresh seeds of *Abrus precatorius* were collected from a local source. The seeds were collected from the pods, cleaned and ground to fine powder with a high speed blender (Mill size 8). The ground seed was stored dry and used throughout the study. A known weight of the sample was soaked in chloroform – methanol (2:1) and extracted for 18 hours in a container on a flaskshaker (Gallenkamp). The mixture was filtered and the filtrate was re-extracted with equal volume of water and evaporated to dryness. The crude extract is regarded as chloroform – methanol extract chloroform – methanol. Chlorpropamide a known sulfonylurea was bought from the market and used as a control for antidiabetic drugs.

Treatment of Animal: Three groups of male healthy rabbits (n = 3) with average weight of 1.6kg were used. Food, water, ambient temperature and proper ventilation were allowed throughout the study. Alloxan (180mg/kg body weight) was injected intraperitoneally to all the rabbits. They were allowed for 72 hours for full development of diabetes. After 72 hours, hyperglycaemic glucose level was determined using O-toluidine method. Then oral administration of 50mg/kg body weight of the chloroform – methanol extract, chlorpropamide and 5mls of normal saline as control were given respectively.

Collection and Determination of Blood Glucose Levels: Blood was drawn after 5, 10, 20, 30, 40, 60 and 168 hours of oral administration of chloroform – methanol extract, chlorpropamide and control. The blood was drawn from the ear vein and transferred to NaF/oxalate bottle. The blood was centrifuged at 2000g for 10 minutes. Blood glucose level was determined using O-toluidine method of Frings, et al (1970). Statistical analysis was done using students T – test. Data obtained were analyzed as mean ± standard error of mean. Values were considered significant at $P<0.05$ and $P< 0.001$.

RESULTS AND DISCUSSION

The chloroform – methanol extract of *Abrus precatorious* seed showed a marked blood glucose reduction especially after 30 hours of oral administration. Table 1.0 and fig. 1.0 show the relative blood glucose reduction compared to chlorpropamide a known sulfonylurea and a control group control. The peak percentage reduction of chloroform – methanol was 69.1% after 30 hours while that of chlorpropamide was 61.3% after 20 hours of administration. The blood glucose percentage reduction was 42.9, 58.7, 67.4, 69.1, 67.9, 56.6 and 51.8 after 05,10,20,30,40,50,60 and 168 hours respectively; while that of chlorpropamide was 13.8, 32.3, 61.3,33.5,46.8, 46.5 and 46.2% respectively. There was a significant difference ($P<0.001$) between the reduction parttern of chloroform – methanol to that of chlorpropamide. When chloroform – methanol was however compared to control, there was no significant difference after each time of blood collection except after 05 minutes which was significant at $P<0.001$. Also chlorpropamide did not show any significant difference when compared with control except after 05 ($P<0.05$) and 40 hours ($P<0.001$) of blood collection.

Table 1. Effects of cm, cp and co in alloxan diabetic rabbits blood glucose levels (mg/100ml)

SAMPLE	Hyperglycemic level	TIME – (HOURS)						
	0	5	10	20	30	40	60	168
CM	261.50 ±2.08^{c}	149.46 ± 4.74^{cf}	108.00 ± 720^{bf}	85.20 ± 2.08^{a}	81.00 ± 2.56^{c}	83.70 ±2.85^{cf}	113.40 ±1.63^{b}	126.00 ±2.01^{a}
Reduction from baseline	-	11204	153.50	176.30	180.50	177.80	148.10	
% Reduction	100	429	587	674	691	679	566	518
CP	234.03 ±0.80	201.60 ±1.09^{df}	158.40 ±1.05^{f}	92.90 ±1.29	108.80 ± 1.01	124.40 ±2.09^{f}	125.60 ±2.06	126.01 ±1.02
Reduction from baseline	0.00	32.43	75.63	141.13	125.25	109.63	180.43	107.02
% Reduction	0.00	13.8	32.3	61.3	33.5	46.8	46.5	46.2
CO	207.60 ±0.21	204.80 ±2.08	208.00 ±0.68	207.20 ±5.50	205.80 ± 2.55	206.40 ±5.09	202.20 ±1.63	203.80 ±2.09
Reduction from baseline	0.00	2.80	1.60	0.40	1.20	1.20	5.40	4.20
% Reduction	0.00	2.0	0.01	0	0.01	0.01	1.2	1.3

Remarks: Results represents Mean ± SEM of N =3a =Significant values of P<0.05 when CM was compared with CP
b =Significant values of P<0.01 when CM was compared with CPc =Significant values of P<0.001 when CM was compared with CP
d =Significant values of P<0.05 when CP or CM was compared to COe = Significant values of P<0.01 when CP or CM was compared to COf = Significant values of P<0.001 when CP or CM was compared to CO

The chloroform – methanol extract of *Abrus precatorious* was able to reduce alloxan hyperglycaemic blood glucose levels. The extract was seen to be slightly more potent than chlorpropamide -a known antidiabetic drug in the class of sulfonylurea. The potency was measured in-terms of longer time of action and higher percentage reduction in blood glucose levels.

Many bioactive compounds have been isolated from plants and have been used as antidiabetic agents. Examples are *Trigonella foenum-graecum* which is also a legume (Fetrow and Avila 1999). The mechanism of action of the hypoglycaemic effect of this plant was attributed to inhibition effects of mucilaginous fibers on glucose absorption (Sharma et, al. 1996). Most legumes have low glycaemic index and thus reduces glucose availability. Diabetic subjects were receiving 80gm of fiber per day (Sharma, et, al 1996). This was able to reduce blood sugar level by 30%. The chloroform-methanol extract i.e 50mg per kilogram body weight of *Abrus precatorious* was able to reduce blood glucose level by 69% after 30 hours of exposure which shows that, there are some antidiabetic substances in this extract. This compares well with the above-mentioned legume, since only 50 mg per kilogram body weight of this extract was used as against 80gm of *Trigonella foenum-graecum*. Another leguminosae in this class called *Pterocarpus marsupium*, given as 2-4 grams per day was able to reduce blood sugar by 30mg/dl (Manickam, et, al., 1997) which is still a higher value (2-4 gm per day) than the chloroform – methanol extract (50mg per kilogram body weight for 168 hrs).

The chloroform – methanol extract comprised most of the lipid – soluble compounds in the seed. The mechanism of blood glucose reduction of this extract may be as a result of the ability of the fat soluble extract to bind to receptor sites especially the peroxisome proliferator – activated receptors. These receptors are the chief regulators of glucose metabolism. They have the ability of binding to lipid – soluble substances because they are steroid group of receptors i.e. they are lipophilic in nature. (Vanden, 1999). The binding of chloroform – methanol extract, to this receptors may activate the receptors. Which act on glucose metabolizing pathways and thus reduce or reverse the glucose circulation in dithizone induced diabetes. Activation of these receptors also can be used to regulate gene expression, since the active form of these receptors can bind to DNA and modulates gene expression, and thus tissue – specific expression. Diabetes mellitus having a genetic origin can be attacked at this molecular level. Also since these receptors are found in almost all the tissues, all other tissues diseases, associated with diabetes can also be checked. It is also interesting to note that insulin generates its intracellular effects by binding to a plasma membrane receptor, which is the same in all cells. The receptor is a disulfide – bonded glycoprotein, thus lipid – soluble chloroform – methanol extract can also easily bind to this receptor site at the plasma membrane, this decreases glucose transport by decreasing the number of glucose transport molecules in the plasma membrane (McCallium and Epand, 1995). Membrane – associated signaling processes or alterations in membrane physical properties by chloroform – methanol extract may be another possible mechanism of glucose reduction in this study. Many membrane bound glycolytic enzymes may be also affected, for example, insulin induces the uptake of glucose by fusion of intracellular glucose transporter – containing vesicles to the plasma membrane, this facilitates the phosphorylation of membrane phosphoinositides by activation of phosphoinositide 3 – kinase (Carpenter and Cantley, 1996) which mobilizes critical signaling enzymes and proteins.

In conclusion therefore, chloroform – methanol extract of *Abrus precatorius* has shown to have some antidiabetic properties in alloxan diabetes in rabbit. Further investigations are therefore needed to elucidate the active ingredients in this extract.

REFERENCES

Berger, W (1985).Incidence on severe side effects during therapy with sulfonylureas and biguanide. Horm metab Res. Suppl. 15:111-115.

Carpenter, CL; Cantley, LC (1996). Phosphoinositide kinases. Curr. Pini Cel Boil 8, 153 - 158

Fetrow, CW; Avila, JR (1999) Professional's Handbook of Complementary and Alternative Medicines Springhouse, P.A. Springhouse corporation.

Frings CS; Tatliff, AN; Dunn, BT (1970). Glucose determination by O-toluidine methid using acetic acid in: Clinical chem.. By Toro. C. and Ackerman. P.G. Little Browing and Company. Boston 115 Clin. Chem. 16: 282.

Jenkins, DJ; Nolever, TM Toyler, IH (1981). Glycaemic index of foods: a physiologic basic for carbohydrate exchange: Am. J Clin. Invest. 50:526 - 530

Kahn, CT; Megyesi, K; Roth, J (1976) Non-supressible insulin-like activity of human serum,a potent inhibitor of insulin degradation. J-Clin.Invest. 57:526-530.

Kitabch, AE; Stentz, FB; Duckworth, WC (1972). Accelerated Insulin degradation. An alternate mechanism for insulin resistance. Diabetes Care 2: 414 – 417.

Manickam, M; Ramanathan, M; Ray, AB (1997). Antihyperglycemic activity of phenolics from *Pterocarpus marsupium*. J Nat. Prod. 60: 609-610.

McCallium, CD; Epand, RM (1995). Insulin receptor authophosphonylation and signallin is altered by modulation of membrane physical properties. Biochemistry 34: 1815 – 1824.

Meries, RJ; Farnsworth, NR (1995). Anti-diabetic plants and their active constituents. Phytomedicine 2:137-189.

Miller, GT (1991). Coronary-heart disease and associated characteristics in tropical development community.Trans-R.Soc.Trop.Med.Hyg.85:324-335.

Nwodo, OF; Botting, JH (1983). Uterotonic activity of extract of the seeds of *Abrus precatorius*. Planta Medicus 47: 230 –233.

Nwodo, OF; Alumanah, EO (1991). Studies on *Abrus precatorius* seed II. Antidiarrhoeal activity. Journal of Ethnopharmacology, 31: 395 - 394

Seidell, JC (2000). Obesity insulin resistance and diabetes-a worldwide epidermic. British Journal of Nutrition Millennium. Annual Review Pharmacol. Toxicol 40:177-191.

Sharma, RD; Sarkar, A; Hazra, K; Maheshwari, DM (1996). Use of fenugreek seed powder in the management of non-insulin dependent diabetes mellitus. Nutri Res. 16:1331-1339.

Tella, AF; Ojihomon, OO (1980). An extraction method for evaluating the seed proteins of cowpea [*Vigna unguiculata*] Journal of Sci. Food and Agric 31:1268-1274

Wannamethee, SG; Shaper, AG (1999). Weight change and duration of overweight and obesity in the incidence of type 11 diabetes. Diabetes Care 22: 1266 – 1272.

Wolf, MA; Colditz, GA (1998). Current estimates of the economic cost of obesity in the United States. Obesity Research, 6:97-106.