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Research Article

ANTIHYPERGLYCEMIC EFFECT OF DIFFERENT SOLVENT EXTRACTS OF LEAVES OF *CAJANUS CAJAN* AND HPLC PROFILE OF THE ACTIVE EXTRACTS

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ABSTRACT

The present work studies the antihyperglycemic potential of different solvent extracts of leaves of *Cajanus cajan* in alloxan induced diabetic mice. Among the four solvent extract tested Viz.Petrolium ether,Chloroform,Ethyl acetate and methanol extract, two extracts namely ethyl acetate and methanol extract reduced the blood glucose level by 27.09 and 37.68% respectively in diabetic mice after daily oral administration for 10 days at a dose of 250 mg/kg.During the same period of treatment, standard drug Glibenclamide(0.4mg/kg) reduced the sugar level by 30.57%.These two extracts also potentiated the action of insulin. While insulin alone caused fall of blood sugar by 32.66%.Simultaneous administration of the ethyl acetate and methanolic extract caused fall of blood sugar by 43.07% and 48.14% respectively.Body weight of the animals were reduced after induction of diabetes by alloxan but gain in body weights was obsearved after treatment with plant extracts/insulin. No significant changes were observed in haematological parameter (R.B.C count and W.B.C count and Haemoglobin (Hb) content) indicating the safety aspect of the extracts.In acute toxicity study (OECD guidelines) the extract did not show any lethality or toxic symptoms at a dose of 2000mg/kg and hence the extracts of *C. cajan* are considered as safe.The HPLC profiles of the active fractions were carried out, which will be helpful for future identification of the bio-active components.

Keywords: *Cajanus cajan*; Antihyperglycemic activity; Alloxan; Acute toxicity.

INTRODUCTION

Diabetes mellitus is a group of syndromes characterized by hypoglycemia, altered metabolism of lipids, carbohydrates and proteins[1], it is an increased risk of complications from vascular diseases. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications effecting eyes, kidneys, nerves and arteries[2]. The increasing number of aging population, consumption of calorie rich diet, obesity and secondary life style have lead to tremendous increase in the number of type II diabetes worldwide[3]. The global prevalence of diabetes is estimated to increase, from 4percent in 1995 to 5.4 percent by the year 2025[4].

The modern oral hypoglycemic agents produce undesirable side effects. The need of the hour is to shift towards the different indigenous plant and herbal formulation[5].

Cajanus cajan (*L.*) Millsp.(family Fabaceae and sub-family Papilionaceae) is an erect woody and annual or short- lived perennial shrub or small tree that is widespread and cultivated throughout the tropics and subtropics. This plant used in Panamanian folk medicine for the treatment of Diabetes[6.7] . In Africa,Asia and South America different parts of the plant are used in the management of disorders such as ulcer, diarrhoea, pain, diabetes, cough and sores.The plant, often grown as a shade crop is commonly used all over the world for the treatment of diabetes, dysentery, hepatitis, measles, as a febrifuge, and to stabilize menstrual period [8-11]. In Chinese medicine, the leaves of plant has been widely used to relieve pain and kill worms for the treatment of wounds, bedsores and malaria, as well as diet-induced hypercholesterolemia[12-15]. The antioxidant activity and the protective effects of the leaf extracts against hypoxic-ischemic brain damage and alcohol induced liver damage have also been reported[16-18].

MATERIALS AND METHODS

Extraction

The leaves of *Cajanus cajan* were procured from Dibrugarh ,Assam.The plant were identified in Department of Life science,DibrugarhUniversity,Dibrugarh.(Voucher Specimen no.AKD/PS/007). The air dried and powdered leaves of *Cajanus cajan* was successively extracted with Petrolium ether, Chloroform, Ethylacetate and Methanol in a soxhlet extractor.Each extract was concentrated under reduced pressure and then dried in a vaccum desiccator.

Animals

Swiss albino mice aged 8-10 weeks (weight20-30gm) were maintained at 25±2ºCTemperature,50±15ºC relative humidity and normal photoperiod(12h dark/12h light) in plastic cages.The animals were fed standard pellet diet and water *ad libitum*.All the animal experiments were carried out in accordance with the guidelines of CPCSEA and were approved by the Institutional Animal Ethical Committee (Reg. No-35/1999/CPCSEA).

Acute oral toxicity :

Acute oral toxicity study was performed as per OECD-423 guidelines(Acute toxic class method).Female Swiss albino mice(n=3)selected by random sampling technique were acclimatized for 7 days.The animals were kept fasting for overnight providing water *ad libitum*,after which each extract(in 1%Na-CMC) was administered orally at a dose of 2000mg/kg body weight by intragastric tube and observed for 14 days[19.20].

Induction of Diabetes:

Diabetes was induced in mice by intraperitonial administration of ice-cold aqueous alloxan monohydrate at a dose of 120 mg/kg body wt.After one hour interval of alloxan administration, the animals were fed pellet diet and5% glucose solution *ad libitum* for 24 hrs and then pellet diet and water *ad libitum*.After 14 days, the animals with moderate hyperglycemia(Fasting blood glucose> 150 mg/dl) were selected and used for the study.

Evaluation of anti-hyperglycemic activity:

The mice were divided into 7 groups of 10 mice each.

Group I : Normal mice receiving vehicle (1% Na-CMC)
Group II : Diabetic mice receiving vehicle (1% Na-CMC)
Group III : Diabetic mice treated with standard drug Glibenclamide(0.4mg/kg)
Group IV : Diabetic mice treated with Petrolium ether extract(250mg/kg)
Group V : Diabetic mice treated with Chloroform extract (250mg/kg)
Group VI : Diabetic mice treated with Ethyl acetate extract (250mg/kg)
Group VII : Diabetic mice treated with Methanol extract (250mg/kg).

After overnight fast,the extracts suspended in 1% Na-CMC was fed to the animals by gastric intubolation using a force feeding needle.

All the treatments were continued once daily for 10 days. Blood samples were collected through retro orbital plexus just prior to the start of the treatment and then 2 hrs after the last administration. The blood glucose level of each sample was determined by using glucose estimation kit(Span Diagonostics) based on glucose oxide-peroxidase method[21].

Evaluation of synergistic effects of active extracts on exogenous insulin administration in diabetic mice

The mice were devided of 5 groups of 10 mice each.

Group I : Normal mice receiving vehicle (1% Na-CMC)
Group II : Diabetic mice receiving vehicle (1% Na-CMC)
Group III : Diabetic mice receiving vehicle and insulin (0.1 IU/kg,s.c.) .
Group IV : Diabetic mice receiving insulin(0.1 IU/kg,s.c.) and ethyl acetate extract in 1% Na-CMC(250 mg/kg) and
Group V : Diabetic mice receiving insulin(0.1 IU/kg,s.c.) and methanolic extract in 1% Na-CMC(250 mg/kg) .

All the treatments were continued once daily for 10 days. Blood glucose level,R.B.C count, W.B.C count,Haemoglobin content and body wt. of each animal were determined[22].

HPLC fingerprinting (Profile) of the active extracts

Two active extracts(Ethyl acetate and Methanolic extracts) were filtered through cellulose acetate membrane and injected in Varian HPLC system*(Prostar* model 210SDM).Different parameters were optimized and they are-Mobile phase, Methanol:Water at a ratio of (85:15),Flow rate 0.8ml/min; Column 250x4.6 mm with packing material Microsorb 300-5,C-18, Injection volume:20µL,Temperature 25±1ºC. Detector wavelength was 293 nm for ethyl acetate extract and 272nm for methanol extract. Detector wavelength was fixed on the basis of UV-Visible Spectrophotometer (Hitachi,U-2001) scan report of the respective extract using HPLC mobile phase as blank.

RESULT AND DISCUSSION

In acute toxicity study,different solvent extracts of *C.cajan* at the dose level of 2000mg/kg of body weight did not exhibit any lethality or toxic symptoms. According to OECD guidelines, an LD_{50} dose of 2000mg/kg and above category is classified as unclassified and hence all the extracts are found to be safe.

The antihyperglycemic effects of different extracts of *C. cajan* on the blood sugar levels of alloxan induced diabetic rats are presented in Table1.Administration of alloxan lead to over two fold elevation of blood glucose level($P<0.001$).After 10 days of daily treatment with different extracts, Methanol extract caused maximum fall in blood glucose level by 37.68% followed by treatment with Ethyl acetate by 27.09%.However,Petrolium ether extract and Chloroform extract did not show any significant reduction in blood glucose. Treatment with standard drug Glibenclamide at a dose of 0.4mg/kg, resulted in 30.57% fall in blood glucose level of diabetic mice.

Table1.Antihyperglycemic effects of different solvent extracts of *C.cajan* in alloxan induced diabetic mice

Group	Treatment	Blood glucose(mg/dl) B.T	A.T	% Reduction or Increase
I	Normal	74.19±0.631	75.45±0.455	**+**1.69
II	Diabetic control	161.2±0.433	163.47±0.518	+1.40
III	Glibenclamide(0.4mg/kg)	182.2±1.194**	126.59±0.260**	-30.57
IV	Pet.ether ext.(250mg/kg)	171.18±1.160**	162.13±0.721**	-5.28
V	Chloform extract(250mg/kg)	167.21±0.597**	157.43±0.429**	-5.84
VI	Ethylacetate extract(250mg/kg)	174.6±0.646**	127.36±0.263**	-27.09
VII	Methanol extract(250mg/kg)	169.3±0.374**	105.54±0.424**	-37.68

Values are expressed as mean±S.D (n=10),SignificanceP<0.01(*), P<0.001(**) as compared to diabetic control.B.T=Before treatment,A.T=After treatment.

Alloxan a β-cytotoxin induces chemical diabetes in a wide variety of animal species by damaging the insulin secreting cells of pancreas[23,24].It is well established that suphonylureas produce hyperglycaemia by increasing the secretion of insulin from pancreas.[25,26]and these compounds are active in mild alloxan induced diabetes whereas they are inactive in intense alloxan diabetes where nearly all β-cells have been destroyed.Reduction of blood glucose by Ethyl acetate and Methanol extract of *C.cajan* and also by Glibenclamide indicate some β-cells were still active to exert their insulin releasing effect by the treatment. Administration of Ethyl acetate and Methanol extracts of *C.cajan* caused statistically significant decrease in the blood glucose levels of diabetic rats as compared to diabetic control group. Insulin primarily controls the glycolytic pathway by regulating the cell entry of glucose and its phosphorylation for further metabolism .A number of plants have been observed to exert hypoglycemic activity through insulin-release stimulatory effects[27].

Blood glucose level, R.B.C count and W.B.C count, Haemoglobin (Hb) content and Body weights of animals before and after simultaneous administration of plant extract and insulin are presented in Table 2.Administration of insulin alone resulted in 32.66% reduction in blood glucose whereas insulin and Ethyl acetate extract caused 43.07% reduction and insulin and Methanol extract caused 48.14% reduction of blood glucose in diabetic animals. Induction of diabetes by alloxan leads to loss of body weight due to dehydration and catabolism of fats and proteins. After 10 days of extract treatment, gain in body weights was observed in diabetic rats (Table 2) indicating the reversal of action of alloxan. No significant changes in the values of R.B.Cs, W.B.Cs and Haemoglobin content of experimental animals were observed, when compared to those of control group. It may be concluded that the extracts of *C.cajan* has no toxicity in mice at a dose of 250 mg/kg treatment for a period of 10 days.

HPLC profile of Ethyl acetate and Methanolic extract are presented in Fig.1 and Fig.2. Ethyl acetate extract revealed six major peaks and three minor peaks. Methanolic extract showed three major and one minor peaks.Peak no.1 of both the extracts have closely similar retention time(2.53min and 2.46 min) indicating that they are of similar chemical structure which may be responsible for biological activity. These HPLC profile will be helpful for future identification of the bio-active components.

It may be concluded that Ethyl acetate and Methanol extracts of *C.cajan* may contain novel bioactive principles with hypoglycaemic activity. Further study is required for evaluation of active principle(s) in different animal models.

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Table 2: Synergestic effects of plant extracts with exogenous insulin in diabetic mice.

Group	Treatment	Blood glucose(mg/dl)		% Reduction or Increase	Body weights(gms)		R.B.C Count (million per cu mm of blood)		W.B.C Count (Per cu mm of blood)		Haemoglobin content	
		B.T	A.T		B.T	A.T	B.T	A.T	B.T	A.T	B.T	A.T
I	Normal	79.1±.37	78.2±0.44	-1.13	28.7±1.54	30.3±1.77	3.2 ±0.22	3.34 ±0.17	5955±317.1	5717±269.1	13.25±0.503	13.5±0.43
II	Diabetic control	164.1±.31**	171±0.41**	+4.20	27.8±1.32*	24.2±1.47**	3.00 ±0.23*	3.08±0.19**	6100±340.3*	6162±394.0**	12.86±0.602**	12.7±0.57**
III	Insulin	175.2±.33**	118.0±0.72*	-32.66	29.4±1.75**	32.6±1.32*	2.98±0.25**	3.0±0.17*	5737±400.3**	5400±467.9**	13.60±0.929**	12.9±0.34**
IV	Insulin+Ethyl acetate extract	169.0±.99**	96.2±0.61**	-43.07	28.6±1.66**	33.2±1.13**	3.03±0.20**	3.16±0.19**	5975±220.0*	5400±467.9**	13.5±0.453*	13.5±0.43**
V	Insulin+Methnol extract	178.0±.24**	92.3±0.55**	-48.14	26.8±0.89*	32.6±1.13**	3.22±0.20**	3.17±0.17**	5977±240.0**	5776±397.0*	12.4±0.574*	12.8±0.43*

Values are expressed as mean±S.D (n=10),SignificanceP<0.01(*), P<0.001(**) as compared to diabetic control. B.T=Before treatment,A.T=After treatment.

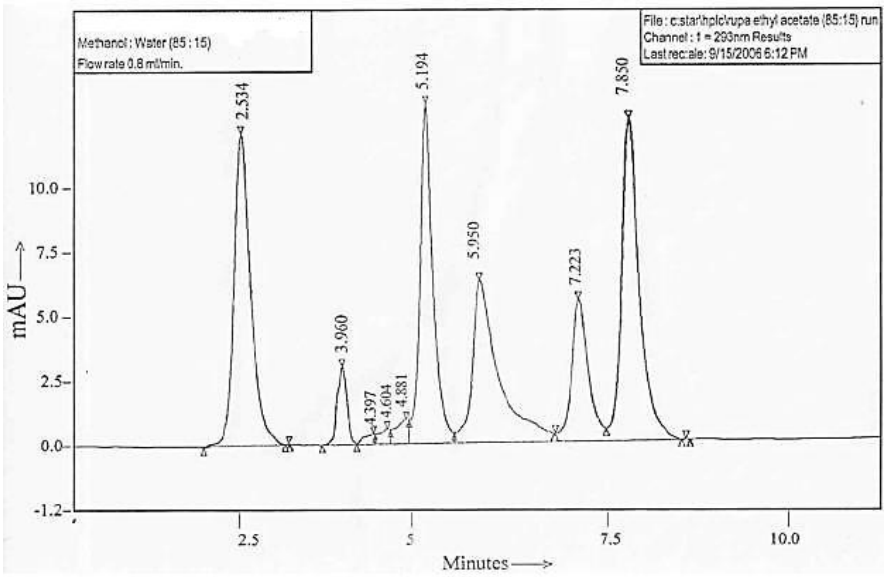

Fig1: HPLC chromatogram of the Ethyl acetate extract .

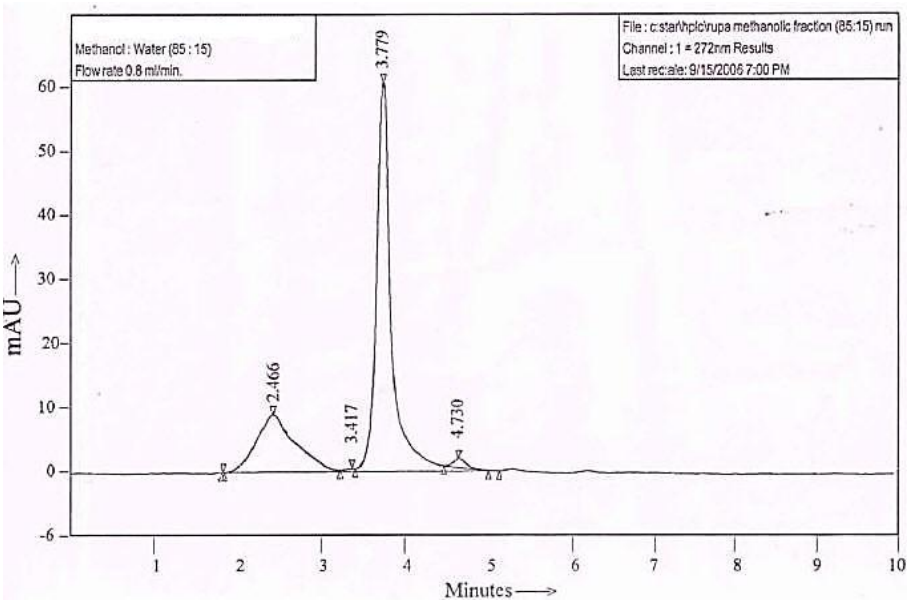

Fig 2: HPLC chromatogram of the Methanolic exract.

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