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A Study on Antidiabetic potency of mixture of powders of dried fruits of *Eucalyptus globules* and rhizomes of *Curcuma zedoaria*.

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ABSTRACT

The antidiabetic potency of the aqueous extract of the powder mixture of dried fruits of *Eucalyptus globules* and rhizomes of *Curcuma zedoaria* in a ratio of 10:1 was investigated using streptozotocin as the diabetogenic agent. The extract produced a significant antihyperglycemic activity in dose dependant manner. The highest oral dose tested (600mg/kg) produced significant antihyperglycemic activity when compared with that of standard Glibenclamide (180µg/kg). The results of biochemical estimations were reported as mean±S.E.M. The total variation present in the data was analyzed by one way Analysis of Variance. It may be concluded that from this study that the extract prepared from the above mentioned formulation possess potential antihyperglycemic activity.

Keywords*:* Antidiabetic, Eucalyptus globules, Curcuma zedoaria, Streptozotocin

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INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, altered carbohydrate, protein and lipid metabolism [1]. Though several reasons have been quoted for its pathogenesis, the ultimate defect encountered is either decreased synthesis or release of insulin. As on date about 1.7% of the world population has been estimated to suffer from diabetes mellitus and is expected to rise to 3.6% by the year 2025 [2].

In uncontrolled diabetic subjects, the major problem is secondary complications, such as diabetic retinopathy, nephropathy, ketoacidosis and foot ulcers etc [3]. Hence, there is very much need to keep tight glycemic control to avoid secondary complications. In this regard several attempts have been made and are still continuing to combat the deadly disease. In spite of tremendous strides in the modern medicine there is no effective remedy by which tight glycemic control is possible without adverse affects [4]. Hence, there is a need to explore other alternatives therapies like ayurveda, unani, homeopathy, siddha etc. which are believed to be effective, safer and economical.

Plants have always been a very good source of drugs and many of the present drugs have been extracted directly or indirectly from them. Present days widely used hypoglycemic drug like ***metformin*** was extracted from a plant named ***Galega officinalis***.

In some cases different parts of a single plant possesses antidiabetic activity and on other hand a mixture of two or more different plant parts shows antidiabetic activity along with increased potency.

Eucalyptus globules is a big tree, though indigenous to Tasmania it is also found densely in sub-tropical countries including India. The biological activities reported contained in the different parts of this plant are mainly the leaves, flowers and bark. However, no literature is available about any biological activity contained in the fruits of this plant. The literature survey about biological activity of this plant mainly highlights the anti-diabetic activity contained in leaves. Hence it is also probable that the fruits may also contain the proposed activity.

Curcuma zedoaria is dried rhizome and its plant is a small deciduous which has its stem grown under soil commonly known as rhizomes. The other varieties of this plant are used as condiments, flavouring agent and colouring agent. The essential oil contained in the rhizome processes antimicrobial and antifungal activity.

In this context an effort has been proposed to evaluate the anti-diabetic potency of mixture of powders of *Eucalyptus globules* fruits and *Curcuma zedoaria* rhizomes (10:1). It is one of the most widely used remedy for diabetes mellitus in unani practice, but no scientific validation has been attempted as on date.

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In view of this, the present study was taken up for the evaluation of antidiabetic activity in Streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Plant Material

The fruits of *Eucalyptus globules* and rhizomes of *Curcuma zedoaria* were collected locally. The specimen authentication was done by Prof. Veda Vyas, Dept. of Botany, L.V.D. College, Raichur, Karnataka and the voucher specimen is deposited in the Department of Pharmacology, N.E.T. Pharmacy College, Raichur for future reference. The coarse powder of the dried bark was subjected to exhaustive continuous hot extraction using Soxhlet apparatus using Water. The obtained masses were dried and stored in an airtight container in refrigerator for further use.

Test Animals

About 30 albino rats (either sex) of mean weight 150-200gm were used in these experiments. The animals identified to be used in any particular study were acclimatized to laboratory conditions for one week and then included in the experimental groups. During the period, the animals were allowed free access to food and water. Rats were randomly allocated to groups of 6 animals each. The animal experiments were approved by the ethics committee of the institute.

Chemicals and Drugs

Ethanol (Nice Chemical, Limited, Mumbai), Streptozotocin (Aurobindo pharmaceuticals, Hyderabad), Gum Acacia powder (Sd fine-chemicals, Mumbai), Glibenclamide (Zydus Cadila, Ahemadabad).

Preparation of extract

Plant material was air-dried and all the following extractions were done using known amounts of dried samples. For the extracts a differential solvent system using a soxhlet extractor was used. 200 ml of water was used to extract 40 g of moderately fine powdered drug for 8 hrs. The extracts were concentrated in a rotary evaporator under reduced pressure until dryness at 40°C. The yield was: 3.75 gms. The extract were suspended in 2% of acacia gum and sonicated to facilitate dissolution before being administered.

EXPERIMENTAL

All the animals were deprived of food for 18 h but water was allowed *ad libitum*, and then randomly divided into five groups each group consists of 6 animals: Normal control,

Diabetic control, Standard (Glibenclamide), High, medium and low depending upon dose of aqueous extract. All the groups except the Normal control were administered with Streptozotocin and 14 days after administration of diabetogenic agent the animals shows increased blood glucose levels (Diabetic condition). Further, one group is separated and kept as it is and does not receives any treatment(Diabetic control) and out of four groups one group receives Standard(glibenclamide) and the three groups receives High , Medium and Low doses of aqueous extract.

After the induction of diabetes, animals were administered with calculated amount of drugs i.e. glibenclamide (180µg/kg), high dose of aqueous extract (600mg/kg), medium dose of aqueous extract (300mg/kg) and low dose of aqueous extract (200mg/kg) respectively. Blood was withdrawn from the animals after the administration of the drug at regular intervals i.e. 0hr, 1hr, 2hrs, 4hrs, 8hrs, 12hrs, 18hrs and 24hrs and blood glucose was estimated by using GOD/POD method.

Statistical analysis

The statistical analysis was carried out using one-way ANOVA followed by Dennett's multiple comparisons for the data, which are normally distributed. All the results obtained in the study were compared with the vehicle control group. P values <0.05 were considered statistically significant.

RESULTS

Phytochemical screening

Preliminary phytochemical screening was carried out for the presence or absence of various phytoconstituents in the test extracts of mixture of powders of *Eucalyptus globules and Curcuma zedoaria.* Carbohydrates, glycosides, alkaloids and tannins are present.

Effect of aqueous extract of EG and CZ on increased blood glucose levels

After the induction of diabetes with streptozotocin the blood glucose levels were found high and the animals with higher blood glucose levels (>250mg/dl) were considered diabetic and selected for the experiment. From all the animals of all the groups' blood was withdrawn and estimated, It is denoted by the time 0 hr. Then the animals were administered with standard and different doses of aqueous extract (high, medium and low) then subsequently blood was withdrawn at regular intervals i.e. 1hr, 2hrs, 4hrs, 8hrs, 12hrs, 18hrs and 24hrs and was estimated. The results produced by aqueous extract at a dose of 600mg/kg (high dose) were very near to the standard (glibenclamide). The combined values are shown in table no: 1 and figure no: 1

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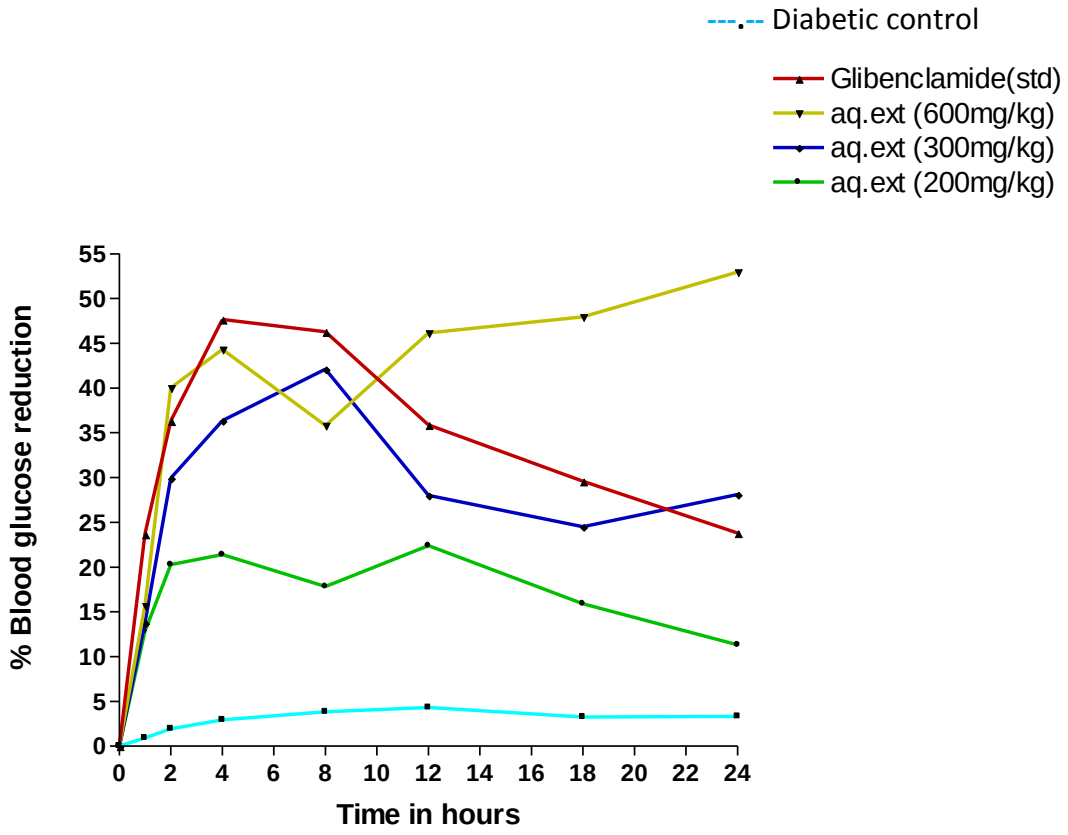
Table No 1: Combined data of % blood glucose reduction of all the extracts, standard (glibenclamide) and Gum acacia in Diabetic induced rats

Sl. No.	Time in hrs.	Aqueous Extract (600mg/kg)	Aqueous Extract (300mg/kg)	Aqueous Extract (200mg/kg)	Glibenclamide (standard)	2%Gum acacia.
01	**1hr**	15.67±6.62	13.76±1.15	12.82±0.94	9.91±3.00	0.90±0.35.
02	**2hrs**	40.02±5.24*	29.93±3.8	20.26±0.31	23.67±0.56*	1.95±0.67.
03	**4hrs**	44.31±5.85*	36.36±4.2*	21.4±0.49*	36.33±1.90*	2.95±0.69.
04	**8hrs**	35.74±7.60	42.1±0.47*	17.83±0.06*	47.67±0.22*	3.83±0.77.
05	**12hrs**	46.16±4.57*	28.0±0.78*	22.40±0.02*	46.29±0.44*	4.33±0.80.
06	**18hrs**	47.95±3.78*	24.5±0.19*	15.9±0.27*	35.89±1.27*	3.27±0.66.
07	**24hrs**	52.88±3.46*	28.11±0.15*	11.33±0.04*	29.50±0.97*	3.32±0.71.

***P<0.05,** significant when compared with Diabetic control.

Figure No: 01: Comparison of % blood glucose reduction produced by different drugs with diabetic control rats.



Effect of aqueous extract of EG and CZ on the Histopathological studies of Pancreas

In histopathological studies the islets are clearly seen in the pancreas of Normal animals (Figure no: 02) whereas islets are completely destroyed in diabetic animals (Figure no: 03), restored islets are seen with hyperplasia in standard drug treated animals (Figure no: 04) and possible restoration of islets are seen in animals treated with aqueous extract (Figure no: 05).

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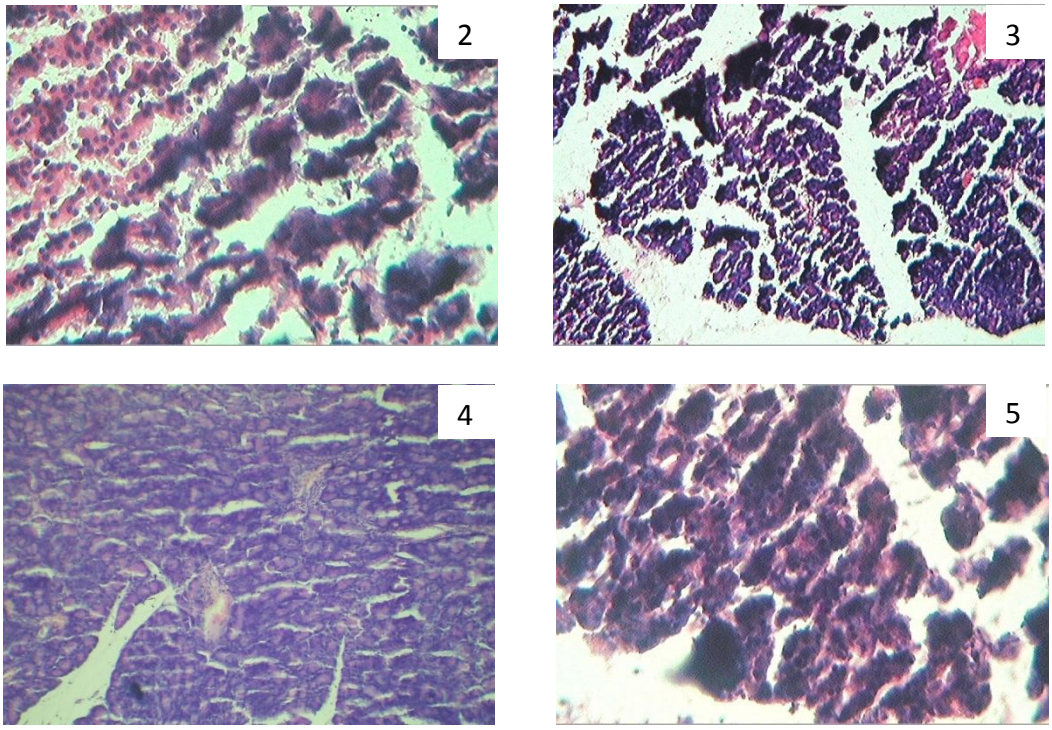

Histopathological studies of Pancreas

DISCUSSION AND CONCLUSIONS

In this present study, five groups of animals were selected and to all these groups diabetes was induced using Streptozotocin as the diabetogenic agent. Then one group of animals were kept as diabetic control which receives only 2%Gum acacia and the second group receives glibenclamide which are kept as standard and the rest three groups receives three different doses of aqueous extract i.e. 600mg/kg, 300mg/kg and 200mg/kg. Blood was withdrawn at regular intervals i.e. at 0hr, 1hr, 2hrs, 4hrs, 8hrs, 12hrs, 18hrs and 24hrs and estimated for glucose concentration. The results produced by the aqueous extract at a dose of 600mg/kg were found very near to the standard Glibenclamide and the results are shown in the tables no: 01.

From the above discussion it can be attributed that the antihyperglycemic activity of the extracts are associated with pancreatic and extra-pancreatic effects in animals [5]. The pancreatic effects may result because of stimulation of islets of Langerhans and which results in the release of Insulin into the blood stream as that of sulfonylurea's, the second possible mechanism may be that they delay the uptake of the glucose from the intestine as that of Biguanides, or the other possible mechanism is that it sensitizes the Insulin which enhances the action in liver and muscles as that of the Thiazolidinedione derivatives, etc

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Streptozotocin has been shown to induce free radical production and cause tissue injury [6]. The pancreas is especially susceptible to the action of Streptozotocin induced free radical

damage. The literature survey reveals that *Eucalyptus globules* possess antihyperglycemic [5], antibacterial [7], anti-inflammatory [8], and *Curcuma zedoaria* possesses antifungal [9], antiallergic [10] and antitumor [11] activities.

Our results have shown that the crude extracts of 10:1 ratio powders mixture of fruits of *Eucalyptus globules* and rhizomes of *Curcuma zedoaria* possess blood glucose lowering effect in Streptozotocin induced hyperglycemic rats significantly. Thus the traditional use of this plant in the treatment of diabetes mellitus may be validated by this investigation.

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