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ANTIDIABETIC ACTIVITY OF COUROUPITA GUIANENSIS LEAF EXTRACTS IN ALLOXAN INDUCED DIABETIC MICE

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ABSTRACT

The present study has been to investigate the possible antidiabetic effect of *Couroupita guianensis* of leaf methanoilc and petroleum ether extracts in Alloxan (200mg/kg b.w.) induced swiss albino mice. A comparision was made between the actions of Methanoic and Petroleum ether leaf (300 mg/kg b.w.) extracts of *Couroupita guianensis* and a known oral hypoglycemic drug Glibenclamide (2 mg/kg, *per os*). To investigate serum blood glucose levels, body weights of all groups of animals and histopathological studies of pancreas. The animals after treatment with leaf extracts of *Couroupita guianensis* significantly reduces blood glucose levels in mice. Further the treatment with leaf extracts of *Couroupita guianensis* significantly decreases the serum blood glucose levels.

Keywords: Glibenclamide, *Couroupita guianensis*, Alloxan.

INTRODUCTION

Importance of Herbal Drugs

In earlier times, most of the drugs were derived from natural substances but later on synthetically derived drugs, based on rational drug design gained popularity. The study of natural products has advantages over synthetic drug design in that it leads optimally to materials having new structural features with novel biological activity i.e., Generation of "Novel lead". There has been a recent revival of interest in herbal medicine and herbs are currently one of the most frequently used forms of complementary and alternative medicine. Interest in medicinal plants as a reemerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well being and the bio-prospecting of new plant derived drugs. Based on current research and financial investments, medicinal plants will seemingly continue to play an important role as a health aid [1-6].

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Diabetes

Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. Hyperglycaemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels. Diabetes disease has afflicted humankind since antiquity. The treasure house of plant kingdom has a number of plants to treat this ailment. The indigenous system of medicine provides an abundant data about plants available for treatment of diabetes. A lot work has been carried out by researches on various plants to evident their effectiveness in diabetes. But still lots many are left which are used in the indigenous system but no systematically studies regarding their pharmacology have been carried out. One such natural medicine in indigenous system claimed to be useful in treatment of diabetes. *Couroupita guianensis* plant has pharmacological importance in regarding diabetes and its information about antidiabetic or hypoglycemic activity was written in some Ayurvedic books [6-12].

MATERIALS AND METHODS

Animal Husbandry

All experiments and protocols described in present study were approved by the institutional animal ethical committee (IAEC) of Vel's College of Pharmacy, Chennai, and with permission from committee for the purpose of Control and Supervision of Experiments on Animals-290/CPSCSEA/12.12.2000 (CPCSEA), ministry of social justice and Empowerment, Government of India.

Animals

Male swiss albino mice (25-30 g) will be housed in group of 6 animals and maintained under standardized condition (12 – hours light / dark cycle, 24ºc) and fed with high diet food and purified drinking water Ad libitum.

Chemicals

Alloxan (SD Fine chemicals), Glibenclamide standard drug (Dianil), Methanol, Petroleum ether, Carboxy Methyl Cellulose, 0.9% Normal Saline all other chemicals used are of analytical grade.

Plant Authentification

The processes of plant authentification want to be done in Institute Of Herbal Botany, Plant Anatomy Research Centre, West Tambaram, Chennai.

Preparation of Leaf Extracts of *Couroupita guianensis*

The fresh and mature leaves of *couroupita guianensis* were collected and dried under shade, made in to coarse powder which is being used for preparation of Methanolic and Petroleum ether extracts. To prepare extracts 50g of plant grain powder in 250 ml of Methanol and Petroleum ether was performed by soxhlet apparatus for 8h at room temperature for 20 days. The residue was removed by filtration. The filtrate was evaporated to dryness at 40-50ºc under reduced pressure in a rotary evaporator. The yield of both Methanolic and Petroleum ether extracts were approximately 10%. The extract was suspended in carboxy methyl cellulose and used for oral administration [13-15].

Preliminary phytochemical screening

Phytochemical screening of leaf extract for all constituents such as alkaloids, carbohydrates, falavonids, saponins, steroids, flavonoids, tannins and protein was done according to the chemical test procedure.

EXPERIMENTAL DESIGN

The experiment conducted for 30 days, Swiss mice (n=6) are divided in to 5 groups as per following

Group 1: Control
Receive standard food pellets and water ad libitum for the period of 30 days

Group 2: Negative control
Receive standard pellets and water ad libitum for 30 days and alloxan 200 mg/kg i.p. at induction day

Group 3: Positive control
Receive standard food pellets and Glibenclamide (2 mg/kg oral) for the period of 30 days

Group 4: Methanolic
Receive standard food pellets and Methanolic extract (300 mg/kg oral) for the period of 30 days

Group 5: Petroleum ether
Receive standard food pellets and Petroleum ether extract (300 mg/kg oral) for the period of 30 days

Acute Toxicity Studies

Acute oral toxicity study was performed as OECD – 423 guidelines (acute toxic class method), (Ecobichon 1997). Swiss albino mice (n=6) of female selected by random sampling technique were used for acute toxicity study. The animals were kept fasting for providing only water, after which the extracts were administered orally at the dose levels of 2000 mg/kg body weight by oral feeding needle and observed for 24 hours. If mortality was observed in 2 out 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in animal, the same dose was repeated again to conform the toxic dose. If mortality was not observed the procedure was repeated for further higher dose such as 3000 mg/kg of body weight. The Methanolic and Petroleum ether extracts of *couroupita guianensis* was found to be non–toxic up to the dose of 2 g/kg and did not cause any death of the tested animals [16-18].

BIOCHEMICAL STUDIES

Estimation of Glucose Level

The determination of glucose is one of the most frequently performed tests in a clinical laboratory. The test based on the reducing property of glucose do not measure true glucose, as there are many interferences. Subsequently other chemical and enzymatic methods were developed. Enzymatic methods are preferred because of their reliability and safety. Pipette in to clean, dry tubes labeled blank (B) standard (s) and test (T) and add the reagents in the following order. Mix well and incubate at 37°c for 10 min. or at R.T. for 20 min. measure the absorbance of test (T) and standard (S), against blank (B) on the photocolorimeter with green filter or on spectrophotometer at 505 nm [19-22].

$$\text{Glucose in mg \%} = \frac{\text{A of (T)}}{\text{(STD.Conc) A of (s)}} \text{ X100}$$

Histopathological Studies

Control group: Normal cyto-architecture of pancreatic tissue with lower magnification (10X) and higher magnification (40X).

Negative control group: Pancreas tissue shows structural damages like vacuolization and necrosis with lower magnification (10X) and higher magnification (40X).

Methanolic group: Recovery in pancreatic tissue takes place and tissue shows similar to normal cyto-architecture with lower magnifications (10X) and higher Magnification (40X).

Petroleum ether group: Regenerative change takes place in pancreatic tissue with lower magnification (10X) and higher magnification (40X).

Positive control group: Regenerative changes are takes place in pancreatic tissue and shows similar to normal cyto-architecture with lower magnification (10X) and higher magnification (40X) [22-25].

Statistical Analysis

All the data expressed as Mean ± SEM. Statistical significance between more than two groups was tested using one way ANOVA followed by Bonferroni test using computer based fitting program (Prism Graph Pad.). Statistical significance was determined at $p < 0.05$.

RESULTS

The results of Preliminary phytochemical screening of ethanolic leaf extract was shown in table 1.

Table 1. Preliminary Phytochemical Screening of Ethanolic Leaf Extract

S.No	Plantextract/Reagent Used	Obeservation
1	**Alkaloids**	
	Mayer's reagent	+ve
	Dragendoeff's reagent	+ve
	Hager's reagent	+ve
	Wagner's reagent	+ve
2	**Carbohydrates**	
	Molisch's reagent	-ve
	Fehling's solution	-ve
	Barfoed's test	-ve
	Benedict's reagent	-ve
3	**Flavonoids**	
	Ferric chloride test	+ve
	Lead acetate test	+ve
4	**Saponins**	
	Foam test	-ve
5	**Steroids**	-ve
6	**Glycosides**	
	Borntrager's test	+ve
	Baljed test	+ve
	Keller-killiani test	+ve
	Legal's test	+ve
7	**Resins**	-ve
8	**Phytosterols**	+ve
9	**Tannins and Phenolic Compounds**	+ve
10	**Proteins**	-Ve

Table 2. Blood Glucose Levels of Induction Day

S.No	Experimental groups	Blood glucose levels (mg/dl)
1.	Control group	98.16 ± 3.361 ***
2.	Negative control group	209.5±4.169
3.	Positive control group	200.1±3.351
4.	Methanolic group	204.5±5.620
5.	Petroleum ether group	200.8±4.512

All values shown are mean ± SEM and n=6, *** $P < 0.001$, ** $p < 0.01$, * $p < 0.05$ compared to Negative control group

Serum Blood Glucose Levels

Administration of Alloxan in mice show significant increase in the glucose levels in blood serum compared to respective control group. Treatment with test drugs and Glibenclamide show significant ($p < 0.001$) reduction in the amount of blood glucose levels in Methanolic, Petroleum ether and positive control groups compared to respective Negative control group was shown in table 2-8.

Table 3. Blood glucose levels on 10th day

S.No	Experimental groups	Blood glucose levels (mg/dl)
1.	Control group	92.33±2.124 ***
2.	Negative control group	212.5±4.097
3.	Positive control group	170.16±2.600 ***
4.	Methanolic group	184.33±1.783 ***
5.	Petroleum ether group	175±2.595 ***

All values shown are mean ± SEM and n=6, *** P < 0.001, ** p < 0.01, * p < 0.05 compared to Negative control group

Table 4. Blood glucose levels on 20th day

S.No	Experimental groups	Blood glucose levels (mg/dl)
1.	Control group	83.33±2.591 ***
2.	Negative control group	221.83±2.798
3.	Positive control group	141.83±1.447 ***
4.	Methanolic group	166.33±2.472 ***
5.	Petroleum ether group	149.83±2.561 ***

All values shown are mean ± SEM and n=6, *** P < 0.001, ** p < 0.01, * p < 0.05 compared to Negative control group

Table 5. Blood glucose levels on 30th day

S.no	Experimental groups	Blood glucose levels (mg/dl)
1.	Control group	86.5±2.579 ***
2.	Negative control group	233.83±3.027
3.	Positive control group	121.83±1.352 ***
4.	Methanolic group	142±1.826 ***
5.	Petroleum ether group	131.83±1.537 ***

All values shown are mean ± SEM and n=6, *** P < 0.001, ** p < 0.01, * p < 0.05 compared to Negative control group

Table 6. Body weights on induction day

S.No	Experimental groups	Body weights (gms)
1.	Control group	27.71±0.4778
2.	Negative control group	29.28±0.2496
3.	Positive control group	27.65±0.6286
4.	Methanolic group	28.23±0.5123
5.	Petroleum ether group	28.21±0.3877

All values shown are mean ± SEM and n=6

Table 7. Body weights on 15th day

S.No	Experimental groups	Body weights (gms)
1.	Control group	28.65±1.043
2.	Negative control group	23.85±0.86
3.	Positive control group	23.28±0.74
4.	Methanolic group	23.58±1.273
5.	Petrolium ether group	23.35±1.482

All values shown are mean ± SEM and n=6

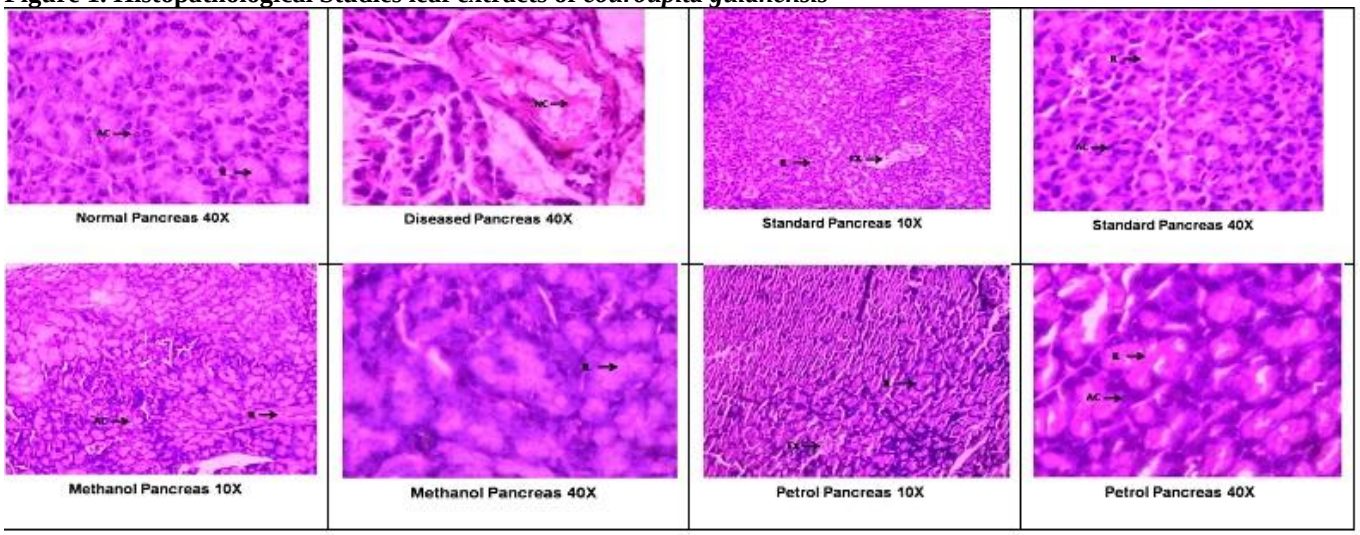
Table 8. Body weights on 30th day

S.No	Experimental groups	Body weights (Gms)
1.	Control group	29.3±0.62
2.	Negative control group	21.55±0.58

3.	Positive control group	26.7±1.012
4.	Methanolic group	26.26±0.96
5.	Petroleum ether group	27.05±0.76

All values shown are mean ± SEM and n=6

Figure 1. Histopathological Studies leaf extracts of *couroupita guianensis*



DISCUSSION

Diabetes mellitus is an metabolic disorder with multiple etiology characterized by hyperglycemia disturbances of carbohydrate, protein and fat metabolism, resulting from inadequate insulin secretion or inadequate insulin supply to the target tissues or combination of both. Diabetes is also considered to be ice-berg disease. Diabetes has plagued humans from antiquity and constitutes a major health problem may leads to so many complications. Despite dramatic progress in both medical area still management of diabetes not complete. The goal of the medical treatment is the controlling of diabetes with synthetic drugs. Each suffers from their own disadvantages and side effects, still offering a wide scope of research in this particular area. Many research laboratories are pursuing investigations in antidiabetic or hypoglycemic in both the preclinical and clinical areas. The aim of present study is also an attempt in the direction.

Despite considerable effort on the part of a number of investigators, there has been only a limited success in developing an ideal animal model of diabetic disease that faithfully mimics hyperglycemia. Various procedures have reported by numerous investigators from time to time such as drug induced diabetes in experimental models.

The experimental model selected for the present study is administration of Alloxan 200 mg/kg body weight through intraperitoneal route for two day because to reduce the mortality rate.

The present study on diabetes was carried out on the male swiss albino mice. The project was aimed to study the antidiabetic activity of leaf extracts of *couroupita guianenesis* in Alloxan induced diabetes. Serum blood glucose levels were anazyled by using auto analyzer. Histopathological studies were carried out to conform the biological changes was shown in figure 1. In the present study administration of Alloxan significant increase of blood glucose levels in Negative control, Positive control, Methanolic and Petroleum ether groups. Administration of leaf extracts of *couroupita guianensis* shown significant decrease in the methanolic and petroleum ether groups. Thus leaf extracts of *couroupita guianensis* shows antidiabetic activity.

CONCLUSION

All synthetic drugs like sulfonyl ureas and biguanides have their own adverse effects like Nausea, Vomiting, Cholestatic jaundice, Skin rashes, Anaemia, Leucopenia, Hypoglycaemia, Intolorance to alchol , GIT disturbances, Visual disturbances, Anorexia, Abdominal discomfort, Metalic taste in mouth. The present study finds out the role of antidiabetic activity of *couroupita guianensis*. Administration of Alloxan increases blood glucose levels. The high blood glucose levels are observed in Negative control group after induction. The treatment with leaf extracts of *couroupita guianensis* significantly reduces blood glucose levels in mice. Further the treatment with leaf extracts of *couroupita guianensis* significantly decreases the serum blood glucose levels. Hence from present study we conclude that the leaf extracts of *couroupita guianensis* may be useful in the management of diabetes. Further studies on *couroupita guianensis* individual or combined with other herbs may be useful in future.

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