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EVALUATION OF ANTIOXIDANT, ANTIDIABETIC AND ANTIHYPERLIPIDAEMIC ACTIVITY OF METHANOLIC EXTRACT OF *CASEARIA ELLIPTICA* IN ALLOXAN INDUCED MALE WISTAR DIABETIC RATS

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Abstract:

Background: *Diabetes mellitus is a group of metabolic disorders with the common manifestations, hyperglycemia.. Alloxan causes a massive reduction in insulin release by the destruction of β cells of the islets of langerhans, there by inducing hyperglycaemia. Alloxan has been shown to induce free radical production and cause tissue injury. The aim of the present investigation was to evaluate the antioxidant, antihyperlipidemic and antidiabetic activity of methanolic extract taken from Casearia elliptica.*

Methods: *The methanolic extract of Casearia elliptica whole plant used for the study. The Phytochemical test, acute toxicity study and oral glucose test was performed. Diabetes was induced in rat by single intra-peritoneal injection of alloxan (120 mg/kg). Male albino Wistar rats were divided into five groups each consisting of six animals as follows:* ***Group I****- Administered vehicle serves as Normal control.,* ***Group II****- Administered Alloxan (120 mg/kg sc) serves as diabetic control,* ***Group III****- Administered Reference Standard, (Glibenclamide 10 mg/kg, orally once daily),* ***Group IV****- Diabetic rats treated with Casearia elliptica (250mg/kg b.wt), serves as treated group,* ***Group V****- Diabetic rats treated with Casearia elliptica (500mg/kg b.wt), serves as treated group.. Bodyweight of each rat in the different groups was recorded daily. Biochemical and antioxidant enzyme parameters were determined on day 14. Histology of different organ (heart, liver, kidney, and pancreas) was performed after sacrificing the rats with euthanasia.*

Results: *Preliminary Phytochemical investigation of methanolic extracts of whole plant of Casearia elliptica was carried out and found that it contains fixed oil, saponins, alkaloids and carbohydrates. The maximum tolerated dose of root of Casearia elliptica was found as 2000 mg/kg after completion of toxicity study according to OECD guideline. The methanolic extract of whole plant of Casearia elliptica has potential anti-diabetic action in alloxan induced diabetic rats and the effect was found equally effective with glibenclamide at higher dose. Our study clearly reveals that MECE has some obvious therapeutic implications in treating hyperglycemia, atherogenic lipoprotein profile and also possess antioxidant activity*

Keywords: *Diabetes mellitus, Alloxan, Casearia elliptica, Serum Triglycerides,*

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INTRODUCTION:

Health is defined as a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity by WHO in 1946. It is the level of functional or metabolic efficiency of a living organism. In humans, it is the general condition of a person's mind and body, usually meaning to be free from illness, injury or pain [1].

Diabetes is a multifactorial disease which is characterised by hyperglycemia [2], lipoprotein abnormalities, raised basal metabolic rate, defect in reactive oxygen species scavenging enzymes [3]. Diabetes mellitus is characterized by impaired glucose utilization and is the underlying factors for both hypoglycemia and hyperglycemia. Hyperglycemia is a condition in which blood glucose level is high and there is diminished action insulin either because of decrease in the circulatory concentration of insulin or due to decrease in the response of peripheral tissue to insulin. These abnormalities give rise to altered metabolism of lipids, carbohydrates, and amino acids. All these effects produce hyperglycemia. Chronic hyperglycemia resulting in impaired function or failure of various organs especially eyes, kidney, nerves, heart, and blood vessels [4].

Type II diabetes is a major health problem because of its high frequency, long duration and high risk of chronic complications [5]. A currently available hypoglycemic agent used in allopathic medication has many side effects such as skin rashes, dilutional hyponatremia, transient leucopenia, thrombocytopenia, myocarditis and sever hypoglycemia and increased chances of cardiovascular death [6]. Management of diabetes with agents devoid of any side effect is still challenge to the medical profession. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area for research.

In folklore practice, the decoction of seeds of *Casearia elliptica* willd. has been used in diabetes mellitus[7]. But there is no investigation on plant for anti-diabetic activity until today. Hence, this study has been conducted to evaluate anti-diabetic activity using whole plant of *Casearia elliptica* willd.

MATERIALS AND METHODS:

Plant material-collection and authentication:

The whole plant of *Casearia elliptica* was collected from native species growing in deciduous forests of tirumala region, Andhra Pradesh, India. The whole plant material has been identified taxonomically and authenticated by Dr.S.Madhava Chetty, Associate Professor, Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh.

Preparation of the extract [8]:

The collected plant was washed thoroughly with water and dried in the shade. The dried leaves were ground well to coarse powder (500gms). Methanolic extract was obtained by extracting powder with methanol by soxhlet extraction method for 72hr. After completion of the extraction the solvent was removed by rotary evaporator method. The methanolic extract was used for further study. The yield obtained from the above process was found to be 52.78% w/w. The extracts were preserved in a refrigerator. The Methanolic extract of *Casearia elliptica* was subjected to the following investigations.

Preliminary phytochemical screening [9,10]:

Preliminary phytochemical screening was carried out on *Casearia elliptica* extract for detection of phytoconstituents. Test for the presence of common phytochemicals were carried out by the standard methods described by Dr. C. K. Kokate and K.R. Khandelwal.

ESTIMATION OF *IN-VITRO* ANTIOXIDANT ACTIVITY:

1 DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity [11, 12]:

Procedure:

The scavenging reaction between (DPPH) and an antioxidant (H-A) was shown as below figure. 4.3 mg of DPPH (1, 1-Diphenyl –2-picrylhydrazyl) was dissolved in 3.3 ml methanol; it was protected from light by covering the test tubes with aluminium foil. 150 µl DPPH solution was added to 3ml methanol and absorbance was taken immediately at 517nm for control reading. 25, 50, 75, 100 and 125 µg/ml of various concentrations of test (*Casearia elliptica* methanolic extract) group compounds as well as standard compound (Ascorbic acid) were taken and the volume was made uniformly to 150 µl using methanol. Each of the samples was then further diluted with methanol up to 3ml and to each 150 µl DPPH was added.

(The concentrations were prepared as 1:1 ratio of test extract and DPPH in test group as well as Ascorbic acid and DPPH in Standard group). Absorbance was taken after 15 min. at 517nm using methanol as blank on UV-visible spectrometer Shimadzu, UV-1601, Japan. The DPPH free radical scavenging activity was calculated using the following formula:

% scavenging = [Absorbance of control - Absorbance of test sample/Absorbance of control] X 100

Nitric oxide (NO) free radical scavenging method [13]:

50 µl of each of the concentrations of test group (AM extracts) compounds previously dissolved in DMSO, as well as ascorbic acid (standard compound) were taken in separate tubes and the volume was uniformly made up to 150 µl with methanol. To each tube 2.0 ml of sodium nitroprusside (10 mM) in phosphate buffer saline was added. The solutions were incubated at room temperature for 150 minutes. The similar procedure was repeated with methanol as blank which served as control. After the incubation, 5 ml of griess reagent was added to each tube including control. The absorbance of chromophore formed was measured at 546 nm on UV-visible spectrometer Shimadzu, UV-1601, Japan. Ascorbic acid was used as positive control. The IC_{50} value for each test compound as well as standard preparation were calculated.

% scavenging/Reduction = [Absorbance of control - Absorbance of test sample/Absorbance of control] X 100

Pharmacological Studies:

Experimental Animals [14]:

Albino rats (180±200gms) were procured from Mahaveer Enterprises, Hyderabad, India and used for the experiment. Rats were maintained in an air conditioned room (25±2°C) with a normal night and day cycle. Rats were feed with standard pellet diet and demineralized drinking water ad libitum. The rats were allowed to acclimatize to the laboratory environment for a week before the start of the experiment. All experimental procedures were conducted in conformity with Animal Ethics committee (Reg. No. number285/2011/CPCSEA) for the care and use of animals and were strictly followed throughout the study

Acute Toxicity Studies [15]: Acute toxicity study for the methanol extract of *Caeseria Elliptica* was done according to the OECD guidelines No: 423 and dose was selected for treatment

The overnight fasted rats were divided into 4 groups, each group consisting of 3 female animals. The methanolic extract were given in various doses (5, 50, 300, and 2000 mg/kg) by oral route. After administration of the extract, the animal were observed continuously for the first 2 hours and at 24 hrs.Then intermittently and at the end of 24hrs, the number of deaths were noted to calculate LD [50].

Gross behavioural changes:

Animals were observed for changes in behavioral responses and also for tremors, convulsion, salivation, diarrhoea, lethargy, sleep, and coma and also were monitored up to 14 days for the toxic symptoms and mortality.

Selection of dose:

The acute toxicity studies of the methanolic extract of whole plant of *Casearia elliptica* willd was found to be non-lethal up to dose of 2000 mg/kg body weight of the animals so that 1/8th and 1/4th (i.e. 250 mg/kg and 500 mg/kg orally) was selected for anti-diabetic activity.

Anti diabetic study:

1) **Alloxan induced model [16]**

Grouping of animals:

Male albino Wistar rats were divided into five groups each consisting of six animals as follows:

Group I- Administered vehicle serves as Normal control.

Group II- Administered Alloxan (120 mg/kg sc) serves as diabetic control

Group III- Administered Reference Standard, (Glibenclamide 10 mg/kg, orally once daily)

Group IV- Diabetic rats treated with Casearia elliptica (250mg/kg b.wt), serves as treated group

Group V- Diabetic rats treated with Casearia elliptica (500mg/kg b.wt), serves as treated group

The male albino Wistar rats weighing (150-250gm) were fasted for overnight before challenging with single subcutaneous route (s.c.) of alloxan monohydrate, freshly prepared and injected within 5-min of preparation to prevent degradation at a dose of 120 mg/kg body weight after administration of alloxan monohydrate 5% glucose solution was given for 72 h to prevent hypoglycemic shock. Animals had access to feed and water. The development of hyperglycemia in rats was confirmed by fasting serum glucose estimation 72 h post alloxan monohydrate injection where in the animals were fasted again for 14h before blood collection from tail of animal. The rats with fasting serum glucose level of above 200mg/dl at 72h were considered as diabetic and are included in the study. Body weight and glucose levels were estimated on initial 1st, 3rd, 7th

day of treatment. On 15th day, blood samples were collected from overnight fasted rats by retro orbital plexus under diethyl ether anesthesia for biochemical estimations and sacrificed for histopathological studies.

Histopathological studies:

Processing of isolated pancreas:

At the end of the study, the animals were sacrificed and the pancreas of each animal was isolated and was cut into small pieces, preserved and fixed in 10% formalin for two days. Then the pancreas piece was washed in running water for about 12 hours to remove the formalin and was followed by dehydration with isopropyl alcohol of increasing strength (70%, 80% and 90%) for 12 hours each. Then finally dehydration is done using absolute alcohol with about three changes for 12 hours each.

Dehydration was performed to remove all traces of water. Further alcohol was removed by using chloroform and chloroform is removed by paraffin infiltration. The clearing was done by using chloroform with two changes for 15 to 20 minutes each. After paraffin infiltration the pancreas pieces were subjected to automatic tissue processing unit.

Embedding in paraffin vacuum:

Hard paraffin was melted and the hot paraffin was poured into L-shaped blocks. The pancreas pieces were then dropped into the molten paraffin quickly and allow it to cool.

Sectioning:

The blocks were cut using microtome to get sections of thickness of 5µ. The sections were taken on a micro slide on which egg albumin i.e., sticking substance was applied. The sections were allowed to remain in an oven at 60^0C for 1 hour. Paraffin melts and egg albumin denatures, thereby fixing tissue to slide.

Staining:

Eosin is an acid stain, hence it stains all the cell constituents pink which are basic in nature i.e., cytoplasm. Haematoxylin, a basic stain which stains all the acidic cell components blue i.e. DNA in the nucleus.

Statistical analysis:

The data obtained from the present study were subjected to statistical analysis. All the results were expressed as Mean ± Standard Error (SEM). Data obtained from various groups was subjected to one-way analysis of variance (ANOVA) followed by Dunnett's t-test. Significant values were set accordingly.

RESULTS:

Preliminary phytoconstituents

Table No: 01 Preliminary phytochemical screening

S. No.	TEST	Methanolic extract
1.	Carbohydrates	+++
2.	Proteins and Amino acids	+
3.	Tannins	_
4.	Flavanoids	++
5.	Alkaloids	++
6.	Steroids	+
7.	Glycosides	_
8.	Saponins	++
9.	Inulin	_

- indicates absent
+ indicates Presence
++ indicates clarity
+++ indicates better response

***In-vitro* antioxidant parameters**

Nitric Oxide (NO) scavenging

Table No: 02 : Standard Concentrations (Ascorbic acid) Vs % Inhibition

S.No	Concentration	Log concentration	%Inhibition (Mean ± SEM)	IC_{50} and R^2 Value
1	5	0.698	5.03 ± 0.81	IC_{50} = 41.89 R^2 = 0.9813
2	10	1.0	12.12± 1.25	
3	15	1.176	24.81 ± 0.67	
4	25	1.397	43.33 ± 1.09	
5	50	1.698	68.28 ± 1.46	
6	75	1.875	78.56 ± 0.74	
7	100	2.00	80.23 ± 1.21	
8	125	2.09	88.82 ± 1.16	

Values are expressed as Mean ± SEM

Nitric Oxide Method for MECE

TABLE:03

S.No	Concentration	Log concentration	%Inhibition (Mean ± SEM)	IC_{50} and R^2 Value
1	5	0.698	7.13 ± 1.13	IC_{50} = 77.55 R^2 = 0.9946
2	10	1.0	14.12 ±2.16	
3	15	1.176	33.63±1.02	
4	25	1.397	47.33±1.47	
5	50	1.698	69 .28± 1.76	
6	75	1.875	79.56 ± 2.29	
7	100	2.00	84.23 ± 3.20	
8	125	2.09	86.82± 3.76	

Values are expressed as Mean ± SEM

DPPH:

Table No: 04: Standard (Ascorbic acid) Concentrations Vs % Inhibition

S.No	Concentration	Log concentration	%Inhibition (Mean ± SEM)	IC_{50} and R^2 Value
1	5	0.698	7.25 ± 0.13	IC_{50} =69.28 R^2 =0.9914
2	10	1.0	12.50 ± 1.14	
3	15	1.176	20.75± 1.78	
4	25	1.397	36.06 ± 1.10	
5	50	1.698	55.65± 1.77	
6	75	1.875	66.52± 1.53	
7	100	2.00	68.04 ± 1.57	
8	125	2.09	72.12 ± 1.93	

Values are expressed as Mean ± SEM

DPPH Method for MECE
TABLE:05

S.No	Concentration	Log concentration	%Inhibition (Mean ± SEM)	IC_{50} and R^2 Value
1	5	0.698	5.65±1.16	
2	10	1.0	13.75±1.21	
3	15	1.176	17±1.89	IC_{50}=61.11
4	25	1.397	39±1.51	R^2=0.9850
5	50	1.698	49.98± 2.06	
6	75	1.875	53.69± 1.69	
7	100	2.00	59 ±2.12	
8	125	2.09	63± 2.08	

Values are expressed as Mean ± SEM

Table No: 6
Effect of MECE on Body weights in Alloxan induced diabetic rats:

Groups	Treatment	Body weight of animals (g)			
		'0' day	3rd day	7th day	14th day
G I	Normal control	224.17±15.94	245.8±15.49	252.5±14.8	266.66±13.34
G II	Diabetic control	247±11.8	230.33±15.52	221±4.09	204±9.57
G III	Alloxan+ Glibenclamide (10 mg/kg)	225.83±11.9	245.16±5.062	249.5±9.514	254.5±8.156
G IV	Alloxan + methanolic extract (250 mg/kg)	234.83±15.40	224.8±7.97	229±8.99	232.83±5.82
G V	Alloxan+ methanolic extract (500 mg/kg)	242.33±13.92	231.6±7.06	238.3±9.27	241.16±11.64

Values are expressed as Mean ± SEM; n=6

Table No: 7
Effect of MECE on Blood Glucose levels in Alloxan induced diabetic rats:

Groups	Treatment	Blood Glucose levels (mg/dl)			
		'0' day	3rd day	7th day	14th day
G I	Normal control	69.16±6.77	76.33±3.75**	83.83±1.79**	95.5±1.76**
G II	Diabetic control	74.16±2.72	454.83±6.49	498.83±4.97	548.83±2.109
G III	Alloxan + Glibenclamide (10mg/kg)	74.5±5.06	311±17.69**	255.5±5.88**	120.66±1.80**
G IV	Alloxan + methanolic extract (250 mg/kg)	66.33±2.57	400±10.76*	374.16±7.42**	190±1.75**
G V	Alloxan+ methanolic extract (500 mg/kg)	74±3.46	339.83±14.63**	330.5±5.92**	166.16±3.06**

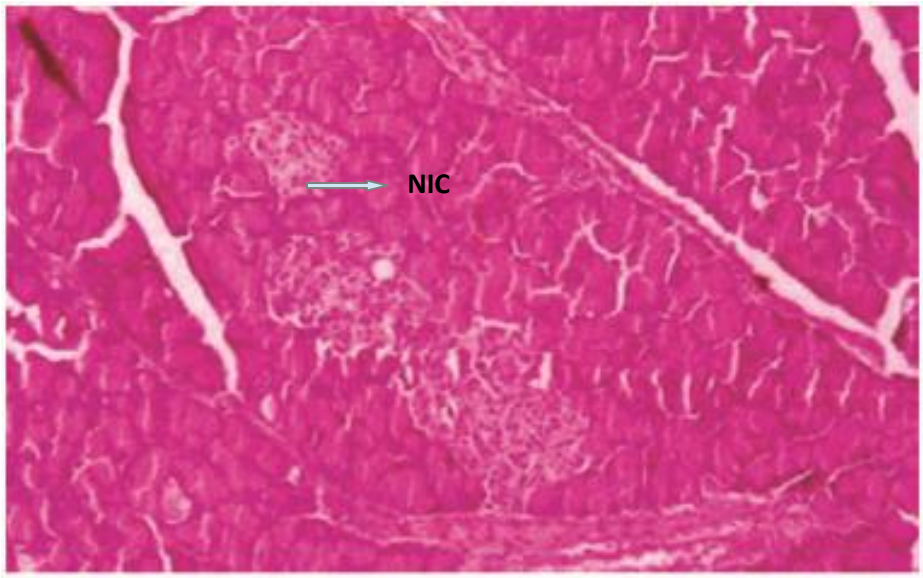
Values are expressed as Mean ± SEM; n=6
*P < 0.05 and ** P < 0.01 Vs Diabetic control
Statistical analysis is done by ANOVA followed by Dunnett's t-test

Effect of MECE on lipid profile levels in Alloxan induced diabetic rats:
TABLE:8

Groups	Treatment	14 days TG	CHOL	LDL	HDL	SGOT	SGPT
G I	Normal control	113.09 ±5.72**	138.5 ±4.62**	77.41 ±2.74**	38.48 ±1.83**	53.56 ±0.56**	59.81 ±0.66**
G II	Diabetic control	187.22 ±5.64	292.21 ±7.29	228.53 ±4.15	26.24 ±1.39	77.16 ±1.10	81.23 ±0.55
G III	Alloxan + Glibenclamide (10mg/kg)	116.63 ±7.19**	151.79 ±6.82**	78.67 ±4.13**	49.58 ±1.18**	57.43 ±0.62**	64.48 ±0.68**
G IV	Alloxan + methanolic extract (250 mg/kg)	148.34 ±4.99**	203.72 ±5.81**	126.98 ±6.37**	47.79 ±1.32**	67.71 ±0.57**	75.56 ±0.69**
G V	Alloxan+ methanolic extract (500 mg/kg)	119.58 ±7.39**	158.25 ±6.44**	86.75 ±4.57**	48.07 ±1.41**	61.76 ±0.47**	66.08 ±1.01**

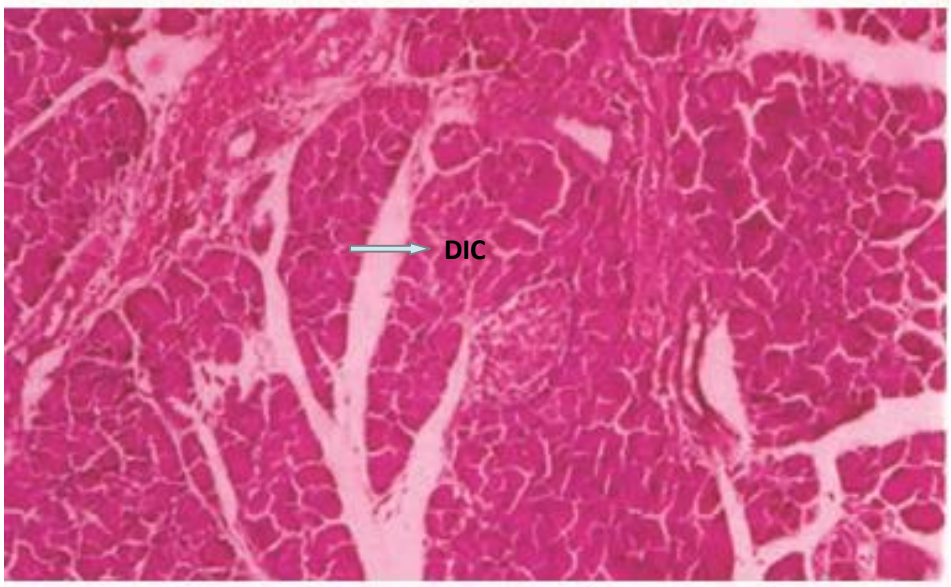
Fig No: 16

Photomicrographs of pancreas of alloxan induced diabetic rats:
Normal pancreas



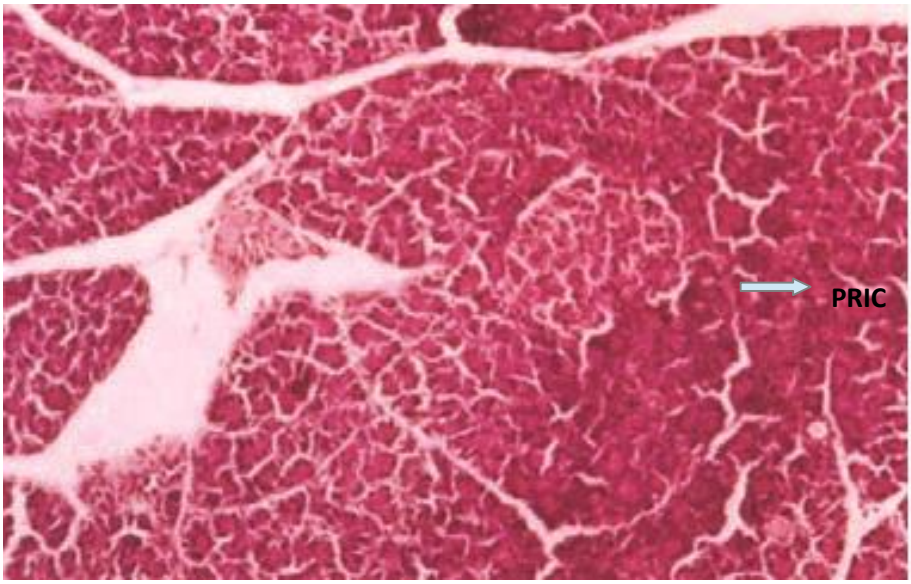
Photomicrographs of normal healthy control group rat showing normal globules of acini with normal islet cells (NIC), stained with hematoxylin and eosin

Diabetic pancreas



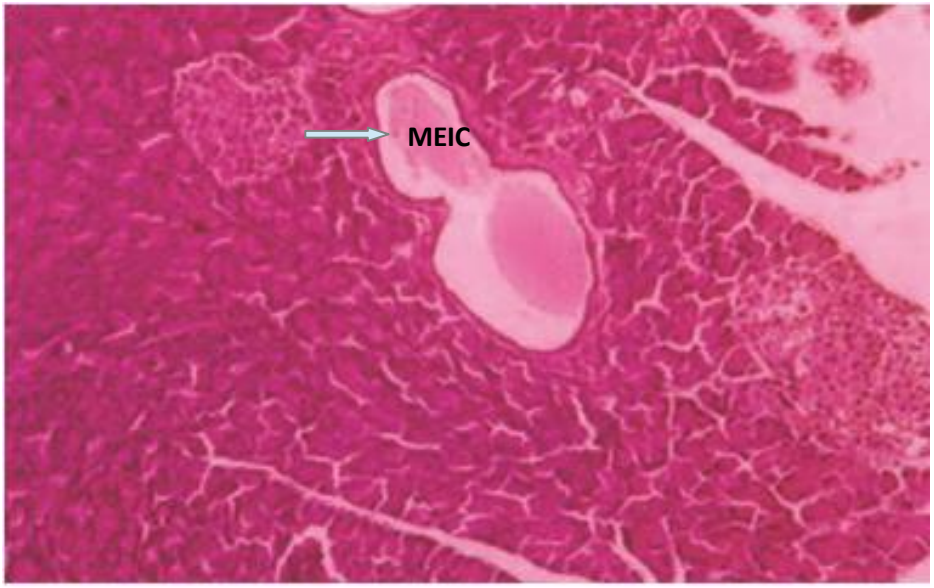
Photomicrographs of diabetic control group rat showing damaged islet cells (DIC) stained with haematoxylin and eosin

Test I (250 mg/kg b.wt)



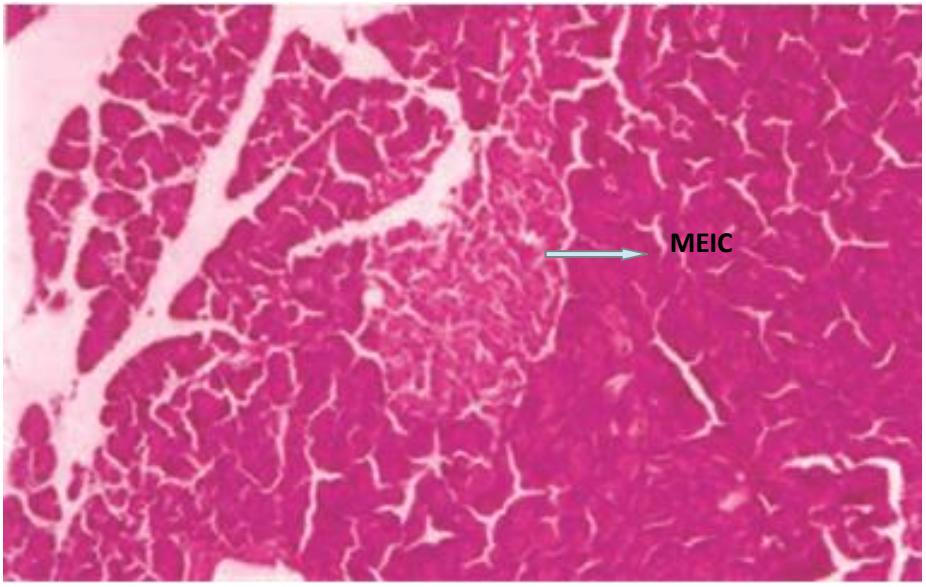
Photomicrographs of methanolic extract (250 mg/kg)-treated group rat showing partial restoration of islet cells (PRIC) stained with hematoxylin and eosin

Test II (500mg/kg b.wt)



Photomicrographs of methanolic extract (500 mg/kg)-treated group rat showing moderate expansion of islet cells (MEIC) like glibenclamide slide, stained with hematoxylin and eosin

Glibenclamide treated:



Photomicrographs of standard (glibenclamide 45 g/mg) treated group rat showing moderate expansion of islet cells (MEIC), stained with hematoxylin and eosin

DISSCUSION:

The preliminary phytochemical analysis of methanolic extract of whole plant of *Casearia elliptica* willd revealed that the presence of carbohydrates, proteins, flavanoids, alkaloids, steroids and saponins

Nitric oxide (NO) scavenging:

The MECE showed promising free radicals action against NO induced release of free radicals at the concentration of 125µg/ml showing (86.82± 3.76) of NO inhibition. The reference standard ascorbic acid also demonstrates significant ($p<0.01$) free radical scavenging potential in the concentration of 125µg/ml (88.82 ± 1.16). The free radical scavenging effect of NO was in a concentration dependent manner.

DPPH:
The MECE showed a significant ($p<0.01$) effect of free radical scavenging of DPPH at a concentration of 125µg/ml (63±2.08). The reference standard ascorbic acid also showed significant free radical scavenging of DPPH at a concentration of 125µg/ml (72.12±1.93).

a) Acute toxicity studies
The MECE found to be safe since no animal died even at the maximum dose of 2000 mg/kg when administered orally.

b) Gross behavioural changes
The animals did not show any gross behavioural changes.

Anti diabetic activity:

Effect on Body weight in alloxan induced diabetic rats:
In groups treated with alloxan (G-II) (120 mg/kg, single dose) a significant decrease in the body weights of animals on the 3rd, 7th and 14th day (230.33±15.52, 221±4.09 and 204±9.57) was observed when compared to the 0th day body weight (247±11.8). This indicates that alloxan reduced the body weights persistently.

Methanolic extract (500 mg/kg) treated group showed significantly prevented reduction in body weights compared to Group-II and Group-IV. Although there was a marginal reduction in weight of animals on the 3rd, 7th and 14th day (231.33±7.06, 238.3±9.27 and 241.16±11.64) in these groups, compared to initial weight (242.33±13.92), the decreased reduction in body weight was significant when compared to diabetic control rats in alloxan induced model.

Effect on Serum Glucose levels in alloxan-induced diabetic rats:
In groups treated with alloxan (G-II) (120 mg/kg, single dose) a significant increase ($p<0.01$) in the serum glucose levels on the 3rd, 7th and 14th day (454.83±6.49, 498.83±4.97 and 548.83±2.1) was observed when compared to the normal animals (G-I) respectively. This indicates that alloxan induces persistent diabetes mellitus.

In the group III, that received standard drug (glibenclamide, 10 mg/kg, p.o, once daily) there was significant decrease ($p<0.01$) in the serum glucose levels on the 3rd, 7th and 14thday (311±17.64, 255.5±5.88 and 120.66± 1.80) respectively when compared to the diabetic control group. In standard group, the serum glucose levels decreased significantly by day 14.

On administration of MECE (250mg/kg, p.o, once daily) there was a significant ($p<0.01$ & $p<0.005$) decrease in the serum glucose levels on 3rd, 7th and 14thday (400±10.76, 374.16±7.42 and 190±1.75) when compared to diabetic control group (II) respectively. The other group receiving MECE (500mg/kg, p.o, once also showed a significant decrease ($p<0.01$) in serum glucose levels on 3rd, 7th and 14thday (339.83±14.63, 330.5±5.92 and 166.16±3.06) when compared to diabetic control group. In both the groups (IV and V), thus the drug treatment restored the serum glucose levels almost nearer to standard drug values on day 14.

A dose related decrease in serum glucose levels was observed in the groups IV and V. This result suggests the anti-diabetic activity of *Casearia elliptica*.

Effect on serum triglycerides in alloxan induced model:
Rats treated with alloxan (G-II) showed a significant increase in ($p<0.01$) serum TG levels on 14th (187.22±5.64) when compared to normal group (113.09±5.72).

The group (III) rats treated with standard drug (glibenclamide, 10mg/kg, p.o, once daily) had a triglyceride levels of (116.63±7.19) when measured on 14th day. This was significantly lower ($p<0.01$) when compared to diabetic control.

Diabetic rats treated with stem MECE (250mg/kg, p.o, once daily) showed a significant decrease ($p<0.00I$) in triglyceride levels on 14th (148.34±4.99) when compared to the diabetic control. The other group receiving MECE (500mg/kg, p.o, once daily) also showed a Significant decrease ($p<0.01$) in serum TG on 14th day (119.58±7.39) when compared to diabetic group (II) respectively.

These values suggest that glibenclamide and *Casearia elliptica* had triglyceride lowering activity and the methanolic at high dose (500mg/kg) had

exhibited almost similar effect on TG as that of glibenclamide.

Effect on serum cholesterol in alloxan induced model:
A significant increase in serum cholesterol ($p<0.01$) was observed in rats treated with alloxan (GII) (292.21±7.29) when measured on 14th day when compared to normal group (I) (138.5±4.62).

The group-III rats treated with standard drug (glibenclamide, 10mg/kg, p.o, once daily) showed a significant decrease in serum cholesterol levels (151.79±6.82) when measured on 14th day when compared to the diabetic control group (II).

The groups (IV and V) receiving MECE (250mg/kg & 500mg/kg p.o, once daily) showed a significant decrease ($p<0.01$) in serum cholesterol levels (203.72±5.81) and (158.25±6.44) respectively when compared to diabetic control group (II).

The serum cholesterol levels were decreased from day 3rd to day 14th in groups III, IV and V. These values suggest that glibenclamide and MECE had cholesterol lowering activity.

Effect on serum LDL in alloxan induced model:
A significant increase in serum LDL ($p<0.01$) was observed in rats treated with alloxan (GII) (228±4.15) when measured on 14th day when compared to normal group (I) (77.4±2.74).

The group-III rats treated with standard drug (glibenclamide, 10mg/kg, p.o, once daily) showed a significant decrease in serum LDL levels (78.67±4.13) when measured on 14th day when compared to the diabetic control group (II).

The groups (IV and V) receiving MECE (250mg/kg & 500mg/kg p.o, once daily) showed a significant decrease ($p<0.01$) in serum LDL levels (126.98±6.37) and (86.75±4.57) respectively when compared to diabetic control group (II).

The serum LDL levels were decreased from day 3rd to day 14th in groups III, IV and V. These values suggest that glibenclamide and MECE had hypo-lipidemic activity.

Effect on serum HDL in alloxan induced model:
A significant decrease in serum HDL ($p<0.01$) was observed in rats treated with alloxan (GII) (26.24±1.39) when measured on 14th day when compared to normal group (I) (38.48±1.83).

The group-III rats treated with standard drug (glibenclamide, 10mg/kg, p.o, once daily) showed a significant increase in serum HDL levels (49.58±1.32) when measured on 14th day when compared to the diabetic control group (II).

The groups (IV and V) receiving MECE (250mg/kg & 500mg/kg p.o, once daily) showed a significant increase ($p<0.01$) in serum HDL levels (47.79±1.41) and (48.07±1.18) respectively when compared to diabetic control group (II).

The serum HDL levels were increased from day 3rd to day 14th in groups III, IV and V. These values suggest that glibenclamide and MECE had hypo-lipidemic activity.

Effect on SGOT in alloxan induced model:
Rats treated with alloxan (G-II) showed a significant increase ($p<0.01$) in SGOT levels (77.16±1.10) when compared to normal levels (53.56±0.56) when measured on 14th day.

The group (III) rats treated with standard drug (glibenclamide, 10mg/kg, p.o, once daily) showed a significant decrease ($P<0.01$) in SGOT levels (57.43±0.62) when compared to diabetic control (G-1I).

The groups (IV and V) receiving MECE (250mg/kg & 500mg/kg p.o, once daily) showed a significant decrease ($p<0.01$) in SGOT levels (67.71±0.57) and (61.76±0.47) when measured on 14th day respectively when compared to diabetic control group (II).

Effect on SGPT in alloxan induced model:
Rats treated with alloxan (G-II) showed a significant increase ($p<0.01$) in SGPT levels (81.23±0.55) when compared to normal levels (59.81±0.66) when measured on 14th day.

The group (III) rats treated with standard drug (glibenclamide, 10mg/kg, p.o, once daily) showed a significant decrease ($P<0.01$) in SGPT levels (64.48±0.68) when compared to diabetic control (G-1I).

The groups (IV and V) receiving MECE (250mg/kg & 500mg/kg p.o, once daily) showed a significant decrease ($p<0.01$) in SGPT levels (75.56±0.69) and (66.08±1.01) when measured on 14th day respectively when compared to diabetic control group (II).

Histopathological Studies:
Histopathological studies of pancreas of Group (I) animals showed normal acini ducts with β islet cells,

while diabetic control (group II) showed reduction in β islet cells with dilated and atrophic islets. Groups treated with standard drug (Group III) showed proliferative hyperplastic β islet cells and groups treated with MECE at 250mg/kg showed β cells with increase in islets size, while MECE treated at the dose of 500mg/kg showed increase in islets size and acini with large hyperplastic cells. Histopathological studies revealed treatment by MECE reduced pancreatic damage caused by alloxan.

CONCLUSION:

Our study clearly reveals that MECE has some obvious therapeutic implications in treating hyperglycemia, atherogenic lipoprotein profile and also possess antioxidant activity. MECE with its multiple beneficiary properties would seem useful as an adjuvant for the prevention and/or management of diabetes.

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