“Preliminary Phytochemical Investigation and Pharmacological Screening of *Casearia elliptica* bark(stem) for Antidiabetic Activity.”

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ABSTRACT:

The metabolic disorder which has become more common in world population is Diabetes mellitus. Ample number of formulations of medicines for Diabetes are available but still this disorder requires a search for a drug with no or considerably less side effects. This thought gave scope for complementary therapies. A study on bark of *Casearia elliptica* was conducted for evaluation of antidiabetic activity in STZ induced diabetic rats. Rats of body weight 180-200gms were administered with STZ dose 60 mg/kg body weight to induce diabetes. Five rats were placed in each group . Rats were grouped into Normal group (Group I) ,Diabetic control treated with STZ (Group II), Diabetic rats treated with Glibenclamide (Group III), Diabetic rats treated with *Casearia elliptica* bark extract(Group IV). Group III is administered with 10mg/kg of standard drug Glibenclamide . The *Casearia elliptica* extract were administered with doses of 200mg/kg & 400mg/kg using CMC solution (5%) as the vehicle. Blood glucose in Group IV,III,II, I, on 14^{th} day were 157.8 ± 4.44 & 138.2 ± 10.64(200&400mg/kg), 98.8 ± 2.59, 291±1.58,107±1.67 respectively .

The Observation showed that standard is one and half times more potent than Group IV in hypoglycemic effect at a dose of 200mg/kg.. Body weights of Group IV , III , II,I and on 14^{th} day were198.22±2.74 & 193.96 ± 3.82, 187.2±0.96, 165.8±1.2,193.5 ±3.43 and with no toxic effects were observed in the Test Group (Group IV). Body weights and blood glucose levels , ALP,SGOT & SGPT were compared with Group II. Proteins , Carbohydrates; secondary metabolites like Alkaloids, Steroids , Saponins and Flavonoids were present in *Casearia elliptica* which were detected by preliminary chemical tests.

INTRODUCTION:

Diabetes is a heterogeneous metabolic disorder with altered carbohydrate, lipid and protein metabolism with hyperglycemia due to insufficient insulin secretion or insulin action or both . As per International Diabetes Federation, 246 million people suffer from diabetes on the globe and this figure will rise to 380 million by the year 2025. Streptozotocin-induced diabetes is one of the widely used model to induce Type I diabetes mellitus in the the experimental animals. The plant selected for antidiabetic activity was taken from the traditional literature .

Casearia elliptica belongs to the family Flacourtiaceae used in bleeding, Root and stem bark paste orally administered for muscular pain[2], Leucorrhea[3], antifungal activity[5],antidiabetic[7], antipyretic, antiulcer.[6]The root gave leucopelargonidin, beta-sitosterol, dulcitol, a flavonoid and arabinose.[4]

Kingdom	**PLANTAE**
Division	**ANGIOSPERMS**[7*]
Order	**MALPIGHIALES**
Family	**FLACOURTIACEAE**
Genus spices	***CASEARIA*** ***ellipica***

INVIVO studies:

Animal model of male Wister rats were selected for the study.Male Rats of body weights 180-200gm were taken and grouped into five groups where each group contain five rats. The rats were housed in polypropylene cages bottomed with husk maintained at temperature conditions of 25±2ºC with 12/12 light and dark hours. Standard pellet diet and water was provided for the animals ad libitum for a period of 7days as acclimatization period. The study was conducted according to ethics of IAEC(with 557/02C/CPCSEA) OGEC guidelines and approval.

- **MATERIALS AND METHODS**

Plant Material:

The plant material required for the antidiabetic activity is authentificated by the Botanist Dr.K. Madhava Chetty , Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. *Casearia elliptica* was collected dried and powdered.

- **Preparation of the extract** :

250grams of bark macerated with methanol for 72hr. The extract was concentrated and dried product is obtained by using rota evaporator. Dried extract is stored till the usage in air tight container.

- **Induction of diabetes** : Rats were administered with 60mg/kg STZ intraperitoneally to induce diabetes.[13]
- **Methodology:** Wistar rats of weight ranging from 180-200gms were taken and grouped into four groups by randomization (n=5)., Group I (control), Group II (Diabetic control), Group III(standard drug- Glibenclamide), Group IV A (Casearia elliptica extract 200mgkg) Group IV B. The rats were acclimatized for a week period. The rats were fasted overnight before experimentation. The blood samples were Collected on 1^{st} day,7^{th} day, 14^{th} day from orbital plexus and the blood glucose levels were recorded. No signs of toxicity were found .
- **Phytochemistry :** The preliminary phytochemical screening of different extracts revealed presence of Carbohydrates, Alkaloid, steroids, diterpenoids, sesquiterpenoids, phenylpropanoids. [7]

Preliminary phytochemical tests

A	Test for carbohydrates	
A1	Molisch	+ve
A2	Benedicts (Reducing)	+ve
a1	Test for Glycosides	
i	Baljet	+ve
ii	Keller kiliani test	+ve
iii	Raymonds	-ve
B	Test for Proteins	
B1	Biuret test	+ve
B2	Millon's test	+ve
B3	Xanthoproteic test	-ve
C	Test for steroids	
C1	Libermann burchard(steroids)	+ve
c2	Salkowaski	+ve
c3	Sulphur test	+ve
D	Foam test (Saponin test)	+ve
E	Test for Alkaloids	
E1	Dragendorff 's test (Alkaloid test)	+ve
E2	Mayer 's test	-ve
E3	Hager's test	-ve
E4	Wagner's test	+ve
F	Tests For Tannins and Phenolic compunds	
F1	Lead acetate	+ve
F4	Ferric Chloride test	+ve
F5	Gelatin	+ve
G	Tests for Flavonoids	
	Schinoda test	+ve

+ve = presence of phyto constituent ; ve =Absence of phytoconstituents

The methanolic bark extracts of *Casearia elliptica* was administered to rats with doses of 200mg/kg using CMC solution (5%) as the vehicle by oral gavage . Blood glucose in Groups.

RESULTS:

Statistical analysis: The results were expressed by Mean ± SD . The significance of the data was done by p value using one way ANOVA Dunnets multiple comparison test.

- Table 1 shows the body weights of five groups recorded on 1^{st} ,7^{th}, 14^{th} day of the study.

Table 1 : - Body weight of the rats :-.

Groups	Day 1	Day 7	Day 14
Normal control	191.4± 1.14***	193.52 ± 1.39***	193.4± 3.43***
Induced control(STZ)-	173 ± 1.15	169.2± 1.17	165.8±1.2
Standard group (Glibenclamide)	180.2± 1.64**	182.4±1.12***	187.22 ± 0.93***
casearia elliptica 200mg/kg	197.6± 2.74***	196.08±2.36***	198.2±2.74***
Casearia elliptica 400mg/kg	194.2 ± 5.403***	192.74±4.72***	193.96±3.81***

****P < 0.0001 ; *** P < 0.001compared with STZ using one way Anova Dunnett's Multiple Comparison Test etic,

Table 2 :Oral Glucose Tolerance Test

s.no.	Groups	BLOOD GLUCOSE LEVELS (mg/dl)					
		0 min	30 min	60 min	90 min	120 min	150 min
1	Normal group	112.8 ± 5.71***	116.4±6.35 ***	119± 5.03***	121 ± 4.95***	121.4± 4.39***	123.6 ± 3.911***
2	Induced control(STZ60mg/kg)-	276 ± 5.49	326±6.4	345 ±7.69	373±9.21	326±7.04	314±6.78
3	Standard group (Glibenclamide)	96±0.7***	186±0.7***	246.2±1.7***	278.2±0.83 ***	174.4± 1.14***	126±1.02***
4	*Casearia elliptica* 200mg/kg	153± 2.24**	236.4± 4.77**	277.6± 6.11**	295.6± 4.67**	196.6± 4.39**	189.2± 4.38**
5	*Casearia elliptica* 400mg/kg	142.8 ± 3.11***	217± 4.5***	261± 6.8***	282± 3.16***	185± 7.31***	160.4± 3.36***

****P < 0.0001 ; *** P < 0.001compared with STZ using one way Anova Dunnett's Multiple Comparison Test

Table 3: Blood Glucose Levels

Groups	Day 1	Day 7	Day 14
Normal control	98.8±1.92**	103±1.95***	107±1.67***
Induced control(STZ)-	251.75±1.58	270.2±3.56	291±1.58
Standard group (Glibenclamide)	248.8±2.588	159.2±6.76***	98.8±2.59***
***Casearia elliptica* 200mg/kg**	247±1.58	200.8±6.76***	157.8±4.44***
***Casearia elliptica* 400mg/kg**	250± 3.16	185± 5.52***	138.2± 10.64***

****P < 0.0001 ; *** P < 0.001compared with STZ using one way Anova Dunnett's Multiple Comparison Test

- Results are presented as mean ± standard deviation (SD). The statistical analysis involving two groups was evaluated by means of Student's unpaired t-test, whereas one way analysis of variance (ANOVA) followed by statistical comparison between Streptozocin and other groups.

- **Table 4: Alkaline Phosphotase levels**

Groups	ALP (U/L)
Normal control	126.2 ± 3.56 *
Induced control(STZ)	132±2.38
Standard group (Glibenclamide)	125.8±3.96*
***Casearia elliptica* 200mg/kg**	159±5.09***
***Casearia elliptica* 400mg/kg**	140.6±2.41**

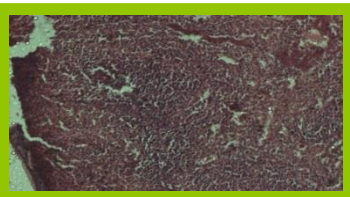
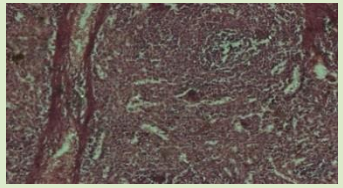
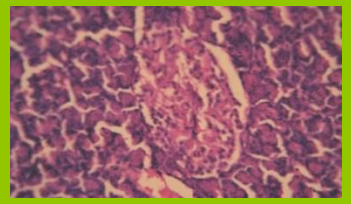
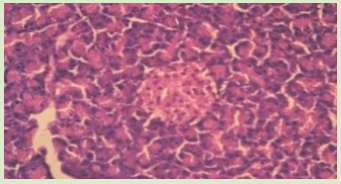
*P <0.05 ; **P < 0.01 ; ***P<0.001 compared with STZ using one way Anova Dunnett's Multiple Comparison Test

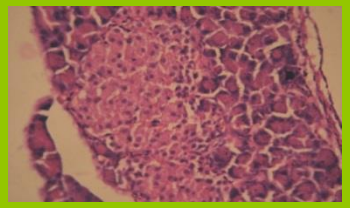
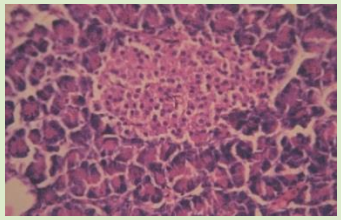
Table 5: SGOT and SGPT Levels:

s.no.	Groups	SGOT (IU/L)	SGPT (IU/L)
1.	Normal control	32.4 ± 4.16***	34.2± 3.42***
2.	Induced control(STZ)	71.4±6.21	82.8±2.39
3.	Standard group (Glibenclamide)	25.2± 1.92***	29.6±2.07***
4.	*Casearia elliptica* 200mg/kg	44 ±1.58***	53.2±1.92***
5.	*Casearia elliptica* 400mg/kg	41.2 ±1.30***	50.2±4.32***

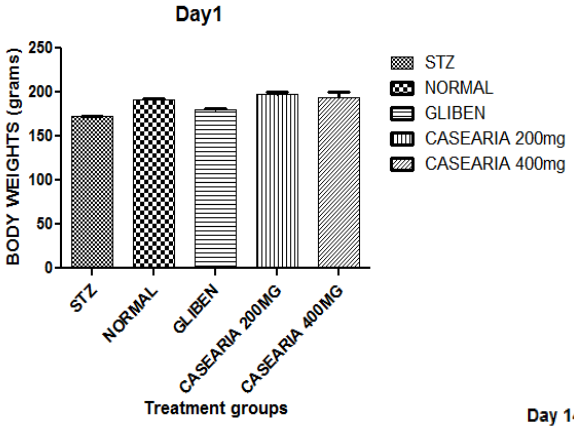
****P < 0.0001 ; *** P < 0.001compared with STZ using one way Anova Dunnett's Multiple Comparison Test

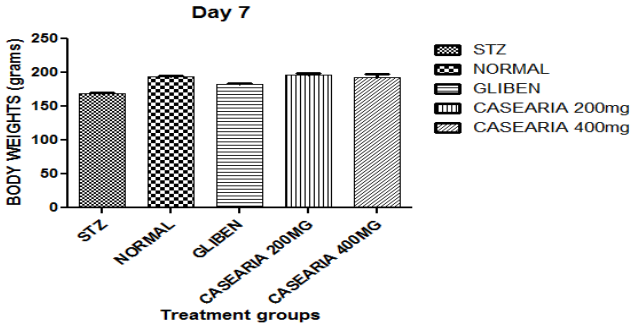
HISTOPATHOLOGY

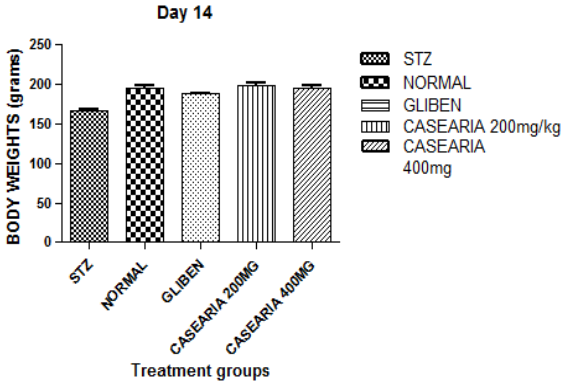
Induced control(STZ) -	**stained section shows damaged and atrophic islet**	
	stained section shows damaged and atrophic islet	
***Casearia elliptica* 200mg/kg**	**stained section shows damaged pancreatic islet cells.**	
***Casearia elliptica* 400mg/kg**	stained section shows preserved pancreatic islet cells.	

Standard group (Gliclamide)- H	stained section shows preserved pancreatic islet cells.	
B	stained section shows preserved pancreatic islet cells.	

Graphical Representation of changes in body weights of rats:

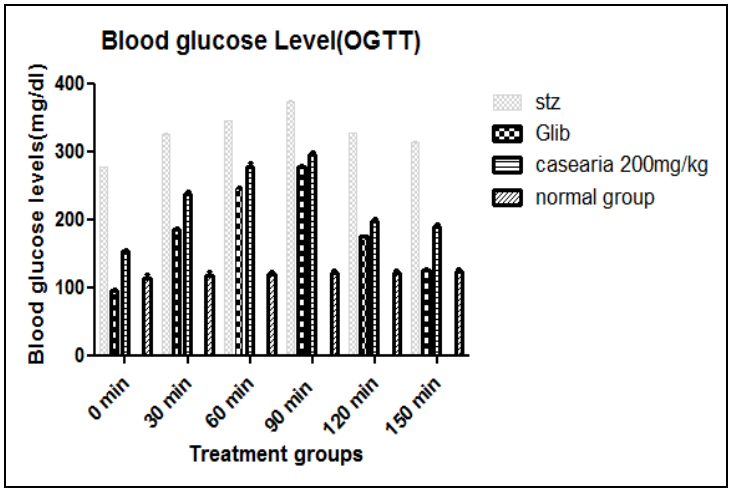


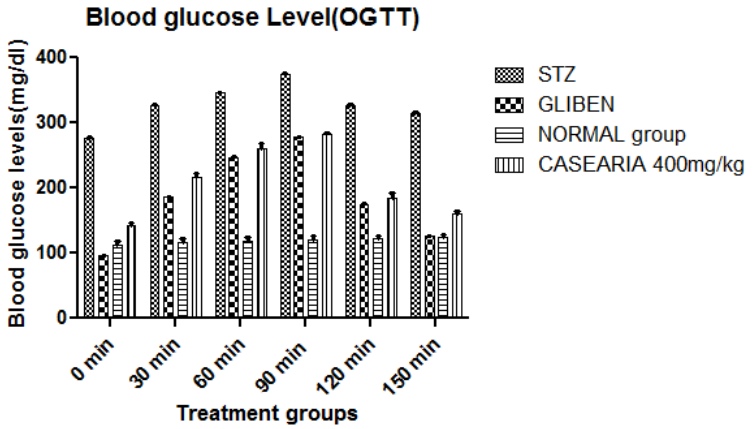




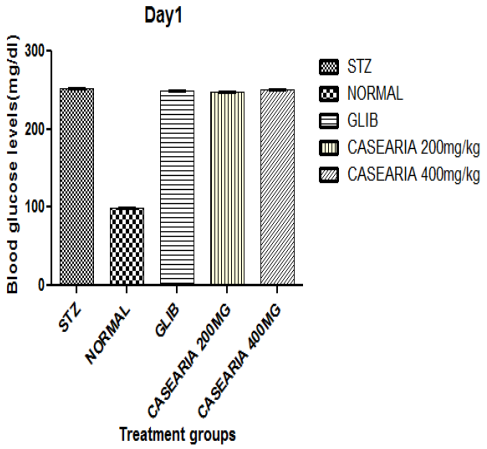
Graphical representations :

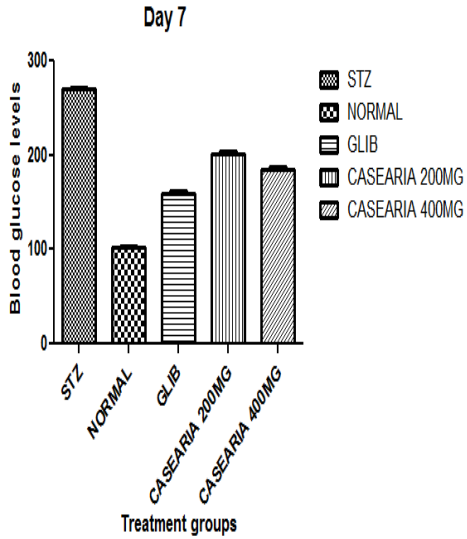
Graph A : - Oral Glucose Tolerance Test

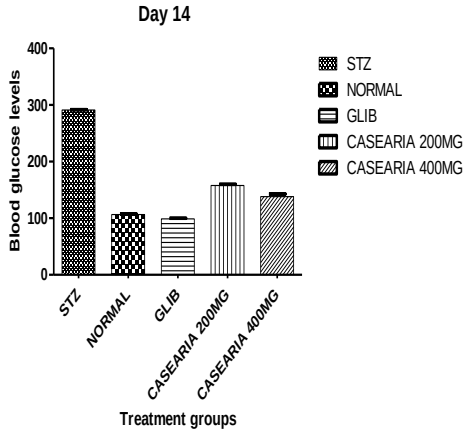




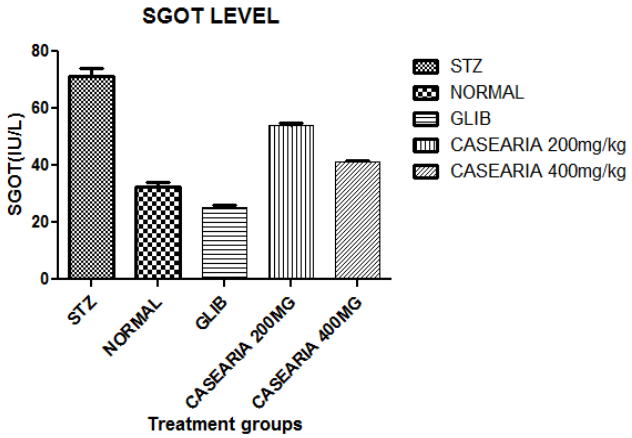
Graphs B :Blood glucose levels on 1,7,14th day

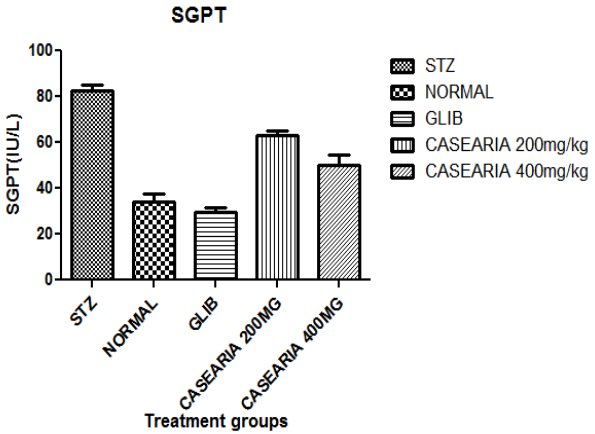




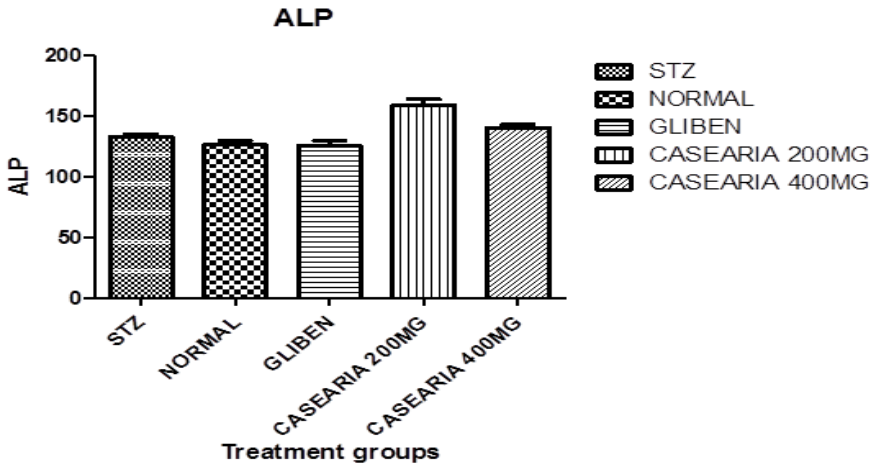


Graph c : SGOT & SGPT





Graph D :- Alkaline phosphatase levels



Conclusion:

The study was conducted to evaluate the antidiabetic activity of *Casearia elliptica* bark . Invivo study was done and the results of the research showed that *Casearia elliptica* had got good antidiabetic activity. Blood glucose levels were decreased by one and half folds of Diabetic rats (291mg/dl) and had got near activity at 400mg/kg (138mg/dl) as that of Glibenclamide (99mg/dl) where the normal blood glucose level was 107mg/dl. The SGPT (40IU/l)and SGOT (53IU/l)levels were near to normal levels indicating no toxicity. Alkaloid, steroids, diterpenoids,Glycosides were of pharmacological ly significant constituents.

Further this crude drug is analysed for synergic activity as a part of my research proposal in polyherbal preparation for registering potent antidiabetic activity.

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Any queries or suggestions

Thank you