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Research Article

EVALUATION OF ANTI-DIABETIC ACTIVITY OF *DERRIS BREVIPES* STEM BARKS ON STZ INDUCED DIABETIC RATS

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Abstract: The anti-diabetic potential of ethyl acetate extract of *Derris brevipes* stem barks was screened on Streptozocin induced diabetes in Wister rats for 9 weeks. The fasting blood sugar levels, cholesterol level, urine analysis and organ weight were performed to assess anti-diabetic activity. *Derris brevipes* ethyl acetate extract at dose level 200 and 400 mg/kg dose level showed significant decrease in blood glucose level and cholesterol level. *Derris brevipes* extract, the level was found to return back to its normal value for sodium, urea and creatinine which were significantly increased in diseases control group. In in-vitro studies on inhibitory activity of glucose-6-phasphatase was increasing with concentration of the compound using human urinary amylase. Further Studies at molecular level will explain more about the mechanism of the anti diabetic activity of our extract.

Key words: *Derris brevipes*, Streptozotocin (STZ), Antidiabetic effect, glucose-6-phasphatase, blood glucose level.

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease affecting more than 30 millions Indians and would may go up to 74 million by 2025 AD.[1] Diabeties mellitus mainly inherited and/ or acquired deficiency in production of insulin by the pancreas or by ineffectiveness of the insulin produced and Indians are genetically more susceptible to diabetes.[2] Apart from currently available therapeutic options, many herbal medicines have been recommended for treatment of diabetes. Furthermore after the recommendation made by WHO on diabetes mellitus, investigation on hypoglycemic agent from medicinal plants has become more important[3].

Derris is one of the largest genus of family leguminosae consisting 70-80 species.[4] Derris are popular insecticide, pesticides, molluscide and have antibacterial properties. Literature review revealed that Derris species mainly contain flavanoids, rotenoids, coumarins, triterpinoids, sterols, glycosides .[5] Derris species from India are known for medicinal value and different parts of species have been used in folk medicine for bronchitis, cough, rheumatoid arthritis, diabetes and anti-fertility.[6-9]

MATERIALS AND METHODS

Collection of plant material:

The stem barks of Plant were collected from Nursery. Plants were identified by Dr. Jayaraman, Director (PARC) medicinal plant research unit, Tambaram, Chennai. The voucher specimen (8765/plant/crc) was deposited at the herbarium, chromosoft research centre, Chennai, India. The stem barks were powdered, pulverized and used for extraction.

Preparation of Extract

The *Derris brevipes* stem barks were cut into pieces and shade dried at room temperature. The dried pieces were subjected to size reduction to a coarse powder by using dry grinder and passed through sieve**.** This powder was packed into soxhlet apparatus and extracted successively with water and ethyl acetate (50:50). Finally residues are dried by lyophilization process.

Preliminary Phtochemical Investigations

The ethyl acetate extract of *Derris brevipes* was screened for phytochemicals like alkaloids, flavonoids, glycosides, lignin, phenols, saponins, sterols and tannins and used for the pharmacological investigations.

Experimental Animals

Wistar Albino Rats (150–200g) were obtained from the Animal House, CRC group, Hyderabad. Rats were maintained on standard pellet diet and tap water *ad libitum*. They were kept in clean cages under a 12 hour light/dark cycle, at room temperature 22–24^{o}C and were acclimatized

to the environment for 2 weeks prior to experimental use. This study was conducted according to the guidelines approved by the Institutional Animal Ethics Committee: (WERT/PHAR/CRC8868)

Acute Oral Toxicity Study

The acute oral toxicity study is conducted using the limit test procedure according to OECD Test Guidelines 423 using female rat. Four groups at the dose levels of 5, 50, 300, 2000 mg/kg consisting of 03 animals in each group are used; animals received a single dose by intragastric intubations start with 5 mg/kg of extracts (AE) dissolved in distilled water and are observed for mortality, signs of gross toxicity or behavioral changes (excitability, convulsions, lethargy, sleep) one to four hour post dosing and at least once daily for 14 days for the immediate and delayed toxicity during the observation period.

Streptozotocin induced diabetes

The albino rats weighing 200---300 g of either sex were allowed to fast for 24 h prior to experimentation and rendered diabetic by a single dose of IP injection of STZ. 60 mg/ kg body weight. After one hr of STZ induction the animals were given feed and libitum and 5% dextrose solution for a day to avoid early hypoglycemic phase. The hypoglycemic activity on these animals was carried out after one week of STZ injection when the stabilization of diabetes was ensured. [10]The animals with sugar level more than 240mg/dl were selected for the study[11].

Experimental Design

The diabetic rats were divided into 3 groups. One normal control group was included

Group 1: Control rats received distilled water, Oral

Group 2: Diabetic rats received distilled water, Oral

Group 3: Diabetic rats received plant extract 200 mg/kg, Oral

Group 4: Diabetic rats received plant extract 400 mg/kg, Oral

The vehicle and plant extracts were administered to the respective group animals for 9 weeks. Throughout the study, plant extract was freshly suspended in vehicle before to the administration. The fasting plasma glucose level and fasting plasma cholesterol level were estimated on 0, 2, 4, 6, 8 and 9 week periodically. Urine parameters were analyzed from the urine sample. At the end of experimental period, rats were sacrificed and the organs including eye, liver, kidney and sciatic nerve are separated and weighed.

In-vitro* glucose 6-phosphatase inhibitory activity of D*erris brevipes

Mode of inhibition of *Derris brevipes* ethyl acetate extract towards alpha amylase activity was determined. Briefly, 100 µl of the *Derris brevipes* ethyl acetate (5 mg/ml) extract was pre-incubated with 200µl of urinary amylase for 15 minutes at 37ºC in one set of tubes. In the other set of tubes urinary amylase was pre-incubated with 100 µl of phosphate buffer, pH 6.9. Four hundred micro liters of potato starch at increasing concentrations (0.15 – 5.0 mg/ml) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated for 20 minutes at 37oC, and then boiled at 100oC for 15 minutes after addition of 2 ml of DNS to stop the reaction.

The amount of reducing sugars released was determined spectrophotometrically using a maltose standard curve and converted to reaction velocities. A double reciprocal plot (1/v versus 1/[S]) where v is reaction velocity and [S] is substrate concentration was plotted.[12]

Statistical Analysis:

The quantitative measurements were made on six animals in each group & the values of biochemical estimations were expressed as mean ± SEM. The data obtained were subjected to one way ANOVA by Dunnet multiple comparison tests.

RESULT AND DISCUSSION

Result of Preliminary phytochemical investigation

The phytochemical screening of the ethyl acetate extract of *Derris brevipes* showed the presence of alkaloids, falvanoids, carbohydrates, tannins, phenolic compunds and saponins.

Results of Acute Oral Toxicity Study

There was no mortality recorded at a dose of 2000 mg/kg of the Plant extract. The LD50 of the Plant extract as per OECD guidelines falls under category 5 (LD50>2000 mg/kg).

Results of *in-vivo* studies

Effect *of derris brevipes* on fasting glucose level

The effect of *derris brevipes* on fasting glucose level on STZ induced diabetic animals shown Table-1. The blood glucose level of Diabetes induced animals was found to be increased significantly in diseased rats from 2nd week onwards. The increment of glucose level was found to be extended up to 9th week. No significant decrease in glucose level was observed up to 9th week against 4th week (treatment starts) of animals in diseased rats. The Diabetic animals were administered with extract from 5th week onwards. This drug was found to decrease the level of glucose significantly ($p<0.05$). The lower dose

of extract itself exhibits its activity and the effect was observed to be dose dependently. The hypoglycemic activity of *Derris brevipes has* been reported earlier by many scientists.

Table-1: Effect of ethyl acetate extract of *Derris brevipes* on fasting glucose

Groups	Fasting plasma glucose level (mg/dl)					
	0 week	2 week	4 week	6 week	8 week	9 week
Normal control	78.90 ± 2.2	75.95 ± 5.1a	83.65 ± 11.8	82.75 ± 0.8	83.10 ± 1.2	86.47 ± 1.3
Diseased Control (STZ) 60 mg/kg, i.p	83.55 ± 11.8	468.09 ± 0.2b	526.32 ± 7.0	552.39 ± 8.7	583.11 ± 6.1	604.74 ± 20.9
SBE 200mg/kg, p.o.	74.19 ± 5.0	489.50 ± 0.2 b	585.11 ± 4.0	160.52 ± 9.5	115.64 ± 5.9	93.15 ± 4.3
SBE-400mg/kg, p.o.	68.36 ± 2.8	496.25 ± 0.6	556.00 ± 1.2	210.42 ± 18.3	105.55 ± 3.5	86.35 ± 2.5

The results are expressed as Mean ± S.E.M (standard error mean) for six animals in each group and statistical significance was calculated by ANOVA followed by Dunnett's test.

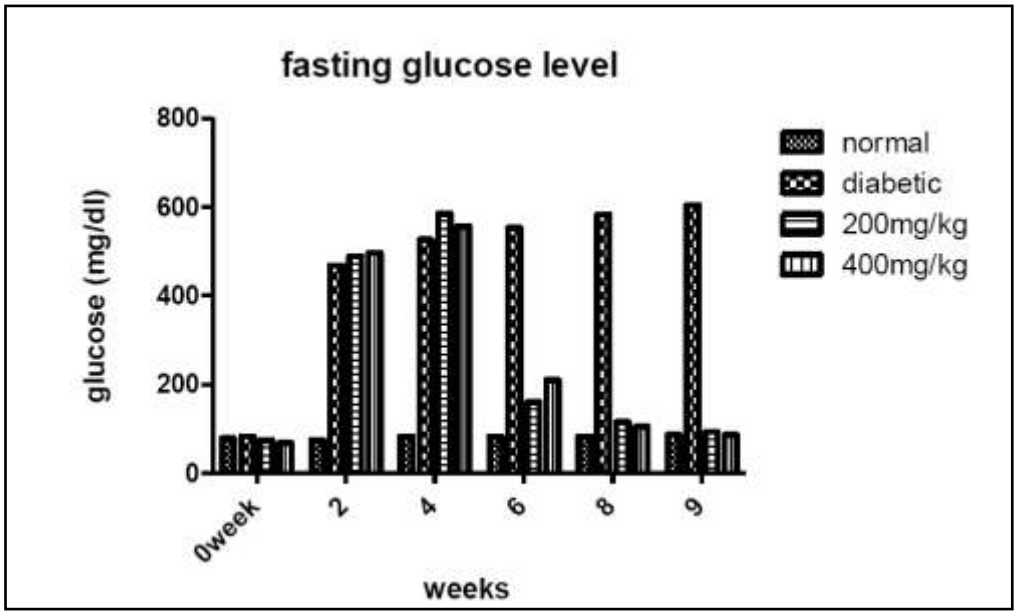

Fig No. 1: Effect of *Derris brevipes* extract on fasting glucose level of STZ induced diabetic animals

Effect of *derris brevipes* on fasting cholesterol level

In diseased rats, the cholesterol level was found to be increased significantly compared to normal rats (Table 2). Moreover, the cholesterol level was found to increase from 2nd week onwards. Moreover, the cholesterol level was returned back to normal level at 9th week. This indicates that the ethyl acetate extract of *Derris brevipes* has favorable effect on the lipid metabolism of diabetic rats also. There are reports that some plants with hypoglycemic constituents also have hypolipidemic effect**.** Earlier report also shows that *Derris brevipes* was used for the treatment of hyperlipidemia. The lower dose of extract itself exhibits its activity and the effect was observed to be dose dependent. It is now established that the majority of diabetic patients with poor glycemic control suffer from increased risk of diabetic complications. They manifest in a variety of progressive disorders affecting circulation, eyes, kidneys, and peripheral nervous system**.**

Table-2: Effect of ethyl acetate extract of *derris brevipes* on fasting cholesterol level of STZ induced diabetic animals

Groups	Fasting plasma cholesterol level (mg/dl)					
	0 week	2 week	4 week	6 week	8 week	9 week
Normal control	80.25 ± 2.5	100.25 ± 0.3	114.75 ± 0.5	123.25 ± 0.2	133.75 ± 4.6	148.39 ± 0.2
Diseased Control (STZ) 60 mg/kg, i.p.	86.70 ± 0.2	265.60 ± 5.6	556.03 ± 0.6	556.03 ± 0.6	658.25 ± 3	687.16 ± 0.6
SBE 200 mg/kg, p.o.	100.02 ± 0.4	275.00 ± 3.0	463.40 ± 0.3	258.24 ± 5.6	128.56 ± .6	94.68 ± 3.4
SBE 400mg/kg, p.o.	101.90 ± 2.6	291.80 ± 3.1	465.20 ± 3.2	236.69 ± 5.9	116.84 ± 2.6	92.56 ± 2.6

The results are expressed as Mean ± S.E.M (standard error mean) for six animals in each group and statistical significance was calculated by ANOVA followed by Dunnett's test.

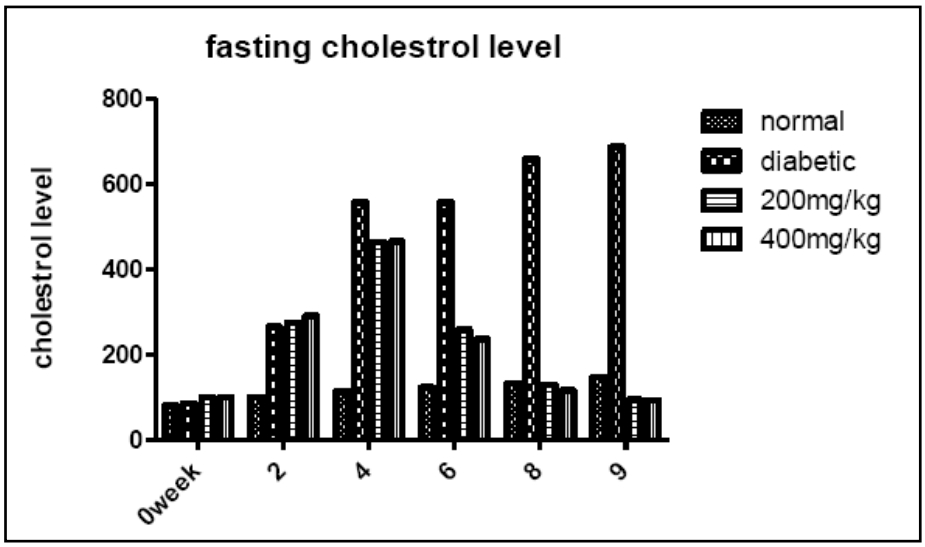

Fig-2: Effect of ethyl acetate extract of *derris brevipes* on fasting cholesterol level of STZ induced diabetic animals

Effect of *derris brevipes* extract on organ weight of STZ induced diabetic animal

The weight of different organs like liver, kidney, eye and sciatic nerve was observed (Table 3). Earlier references showed that the kidney weight was found to be increased in diseased rats. But no much significant difference in the organ weight was observed in our present study. Prolonging the duration of experiment might cause some changes in kidney weight. During diabetes, there is a decrease in liver weight due to enhanced catabolic processes such as glycogenolysis.

Table-3: Effect of ethyl acetate Extract of *Derris brevipes* on organ weight in STZ induced Diabetic rats

Group	Organ weight (gram)			
	Eye	Liver	Kidney	Sciatic nerve
Normal control	0.2525 ± 0.02 ns	9.0375 ± 0.9 ns	1.805 ± 0.5 ns	0.0475 ± 0.001 ns
Diseased Control (STZ) 60 mg/kg, i.p.	0.245 ± 0.02 ns	8.24 ± 0.8 ns	1.825 ± 0.5 ns	0.0175 ± 0.002 ns
SBE 200 mg/kg/p.o	0.236 ± 0.02 ns	8.702 ± 0.9 ns	1.826 ± 0.5 ns	0.18 ± 0.002 ns
SBE 400 mg/kg/p.o	0.223 ±0.02 ns	8.986 ± 0.9 ns	2.03 ±0.8 ns	0.043 ± 0.001 ns

The results are expressed as Mean ± S.E.M (standard error mean) for six animals in each group and statistical significance was calculated by ANOVA followed by Dunnett's test.

Effect of *derris brevipes* extract on urine constituent in STZ induced diabetic rats

In diabetic induced rats, sodium, urea and creatinine level of urine was found to be increased in-significantly against normal animals ($p<0.05$) (Table 4). On treating animals with *Derris brevipes* extract, the level was found to return back to its normal value ($p<0.05$). The potassium level of diseased rats was found to be decreased and it was increased in treatment ($p<0.05$). These results reveal that the extract is found to exhibits its activity in diabetic nephropathy.

Table-4: Effect of ethyl acetate Extract of *Derris brevipes* on urine constituent in STZ induced diabetic rats

Group	Urine Parameters (ml)			
	Na+ ppm	K+ ppm	Urea (mg/dl)	Creatinine (mg/dl)
Normal control	35.2 ± 1.2	46.8 ± 5.2	23.4 ± 2.3	0.5 ± 0.01
Diseased Control (STZ), 60 mg/kg, i.p.	64.4 ± 2.5	23.4 ± 2.6	43.9 ± 4.5	2.43 ± 2.6
SBE 200 mg/kg/p.o	36.8 ± 3.5	39 ± 2.5	23.6 ± 2.3	1.82 ± 0.2
SBE 400 mg/kg/p.o	36.86 ± 2.14	38.04 ± 3.5	23.7 ±3.5	1.65 ± 0.26

The results are expressed as Mean ± S.E.M (standard error mean) for six animals in each group and statistical significance was calculated by ANOVA followed by Dunnett's test.

***In vitro* studies**

Glucose 6-phosphatase inhibitory effect of *Derris brevipes* ethyl acetate extract

The ethyl acetate extract of *Derris brevipes* was determined the inhibitory activity glucose -6-phasphate, the study was done by using human urinary amylase, the absorbance of the compound was taken according its serial dilution, and the increasing of the absorbance according to its concentration was observed. In this study the inhibitory activity on the glucose-6-phasphate of *Derris brevipes* plant was done using in vitro bioassay using human urinary amylase.

Table-5: Absorbance different concentrations of *Derris brevipes* ethyl acetate extract

S. No	Concentration	Absorbance
1	6.25	0.8
2	12.5	0.92
3	25	1.15
4	50	1.28
5	100	1.41

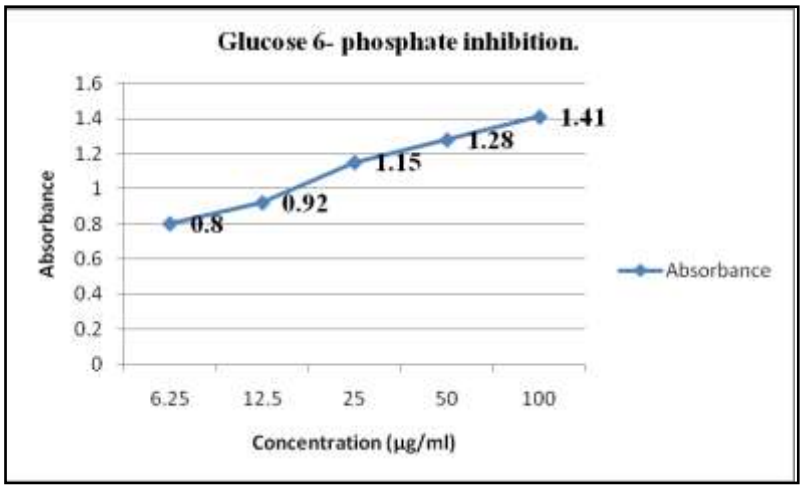

Fig-3: Glucose 6-phosphatase inhibitory effect Of *Derris brevipes* ethyl acetate extract

CONCLUSION

The present study suggests the anti-diabetic properties of Ethyl acetate Extract of *Derris brevipes* on STZ induced diabetic rats. After comparing the treated animal with diseased animal the glucose level was decreased and the cholesterol level also decreased. And the urine parameters of sodium potassium were increased. The inhibitory activity of glucose-6-phasphatase was increasing with concentration of the compound using human urinary amylase. Further Studies are in progress at molecular level to explain more about the mechanism of the anti diabetic activity of our extract.

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