EVALUATION OF ANTIDIABETIC AND ANTIOXIDENT ACTIVITY OF ETHANOLIC EXTRACT OF *RHUS MYSORENSIS* IN ALBINO RATS

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ABSTRACT:

One of the greatest health issues facing the globe today is diabetes mellitus, whose prevalence and related mortality are rising. Poor blood sugar control has detrimental effects on one's health. Although traditional anti-diabetic medications work well, they also have unavoidable side effects. However, medicinal plants can serve as a different source of antidiabetic medications. Focus is placed on preclinical and clinical investigations as examples of medicinal plants with potential for treating diabetes are given. The major goal of the current investigation is to assess the antioxidant and anti-diabetic effects of an ethanolic extract of Rhus mysorensis in albino rats.The goal of the study is to identify prospective ethnobotanical herbs for the creation of phyto medicine by investigating the potentials of the bioactive components from Rhus mysorensis and demonstrating their safety and efficacy.

KEY WORDS: Diabetes mellitus, ethanolic extract, Alloxan, antidiabetic, Glucose tolerance and Pathological study.

INTRODUCTION

Diabetes mellitus is a collection of metabolic disorders that affect how fat, glucose, and protein are metabolized. It is caused by abnormalities in insulin secretion, insulin action (sensitivity), or both, and can cause organ failure in the eyes, brain, heart, kidney, and reproductive system. Reduced insulin secretion, decreased glucose utilization, and increased

glucose production are all factors that contribute to hyperglycemia. The secondary pathophysiologic alterations in numerous organ systems brought on by the metabolic dysregulation associated with DM place a heavy strain on both the diabetic and the healthcare system. Free radicals were known to play a definite role in a wide variety of pathological manifestation. Antioxidants fight against free radicals by protecting us from various diseases and scavenge reactive oxygen radicals or protect the antioxidant defense mechanism. DPPH is a stable free radical at room temperature, which produces a violet solution in ethanol.

DPPH is widely used to evaluate the free radical scavenging effect of natural antioxidant. The therapy with ERM considerably (p 0.001) lowered both cholesterol and triglycerides levels in the diabetic rats used in the current investigation who had hypercholesterolemia and hypertriglyceridemia. This implies that ERM can prevent or minimize the lipid profile issues linked to diabetes. As a marker of renal impairment, plasma creatinine levels in the diabetic group significantly increased when compared to control levels, according to the study. Creatinine levels in diabetic rats treated with ERM after being given alloxan were considerably (p 0.001) lower than those in the untreated diabetic group. This study demonstrates that Rhus mysorensis can guard against kidney problems brought on by diabetes.

MATERIALS AND METHODS

The plants Rhus Mysorensis DG don were collected in the chittoor district of Andhra Pradesh region. The collected plant dried in shade. The dried plant was powdered by using a miller. The powdered drug was subjected to solvent extraction by soxhlet apparatus. The whole plant material was grounded with mortar and pestle into the fine powder, ethanolic extract of Rhus Mysorensis was prepared.

Male albino rats weighing 150-200gm were used for the study. The starting dose level of Rhus Mysorensis was 5000, 2500, 1500, 500 mg/kg body weight p.o. Dose volume was administered to overnight fasted rats with were ad libidum. Food was withheld for a further 3-4 hours after administration of Rhus Mysorensis and observed for signs for toxicity. The body weight of the rat before and after administration were noted that changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system and motor activity and behavior pattern were observed and also sign of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity also noted (OECD 2002). They are divided into groups.

Group I: Normal rats (fed with standard food and Water)

Group II: Control (1gm/kg Glucose)

Group III: Glibenclamide (5mg/kg orally)

Group IV: ERM (250 mg/kg orally)

Group V: ERM (500mg/kg orally)

RESULTS

The current study was undertaken to explore the efficacy of antidiabetic activity of Rhus Mysorensis whole plant extract in alloxan induced diabetic rats. The study of antidiabetic activity of the Rhus Mysorensis whole plant part extract was carried out on Male albino rats. Experimental diabetes was induced with alloxan intraperitoneal dose.

Acute Toxicity Test

A preliminary toxicity study was designed to demonstrate the appropriate safe dose range that could be used for subsequent experiments rather than to provide complete toxicity data on the extract. Acute toxicity studies conducted revealed that the administration of graded doses of ethanol extracts of Rhus mysorensis(up to a dose of 5000 mg/kg) did not produce significant changes in behaviors such as alertness, motor activity, breathing, restlessness, diarrhea, convulsions, coma and appearance of the animals. No death was observed up to the dose of 500 g/kg body weight. The rats were physically active. These effects were observed during the experimental period (72 hrs). The result showed that in single dose; the plant extracts had no adverse effect, indicating that the medium lethal dose (LD50) could be greater than 500 mg/kg body weight in rats.

Table 1: Acute toxicity of ERM

S.No	Animals	Dose(Mg/Kg)	Behavioral Changes
1.	Rats	ERM 500	Normal
2.	Rats	ERM 1500	Normal
3.	Rats	ERM 2500	Normal
4.	Rats	ERM 5000	Normal

Preliminary Phytochemical screening

Phytochemical screening was done using color forming and precipitating chemical reagents on the Rhus mysorensis to generate preliminary data on the constituents of the plant extracts. The chemical tests revealed the presence of major secondary metabolites such as alkaloids, flavonoids, tannin, saponins and sterols.

Table 2 : Results of phytochemical screening of the ethanolic extracts of *Rhusmysorensis*

TEST	REAGENTS	RESULTS
Test for alkaloids	Dragendorff's	+
	Mayer's	+
Test for steroidal Compounds	Acetic anhydride and conc. sulfuric acid	+
	Chloroform and conc. sulfuric acid	+
Test for Phenolic Compounds	Ferric chloride and potassium ferrocyanide	+
Test for flavonoids	10% Lead acetate	+
	Sodium hydroxide	+
	Ethyl acetate	+
Test for Saponnins	Froth test	±
	Ferric chloride	±
	Aqueous hydrochloric acid	±
Test for tannins	Test for tannins	±
Test for anthraquinones	Test for free anthraquinones	–
	Test for o-anthraquinone glycosides	–

Hypoglycemic activity

Table 3: Glucose tolerant test on Non – Diabetic rats by using Ethanolic extract of Rusmysorensis

Test groups	0 hour	1 hour	2 hours	3 hours	4 hours
NORMAL (GLUCOSE)	83.83 ± 1.92	144.8 ± 2.52	113.50 ± 4.14	101.17 ± 3.52	86.00 ± 3.39
STANDARD (GLIMENCLAMIDE)	79.50 ± 1.41	154.83 ± 2.57	131.33 ± 3.05	82.83 ± 3.08*	71.66 ±2.20**
ERM 250 mg/kg	88.17 ± 2.56	137.17 ± 3.63	125.67 ± 3.63	88.17 ± 2.72*	77.00 ± 1.24**
ERM 500 mg/kg	90.16 ± 3.58	151.33 ± 3.12	131.33 ± 4.59	82.17 ± 2.72*	74.18 ± 1.25**

All values are mean ±S.D. (n=6). **p<0.01, *p<0.05, ns-non significant compared with control. (One-wayANOVA followed by Dunnett's t test).

Glucose Tolerance

The effects of extracts of ERM (500 mg/kg and 300 mg/kg) on glucose tolerance test are shown in Figure 2. The supplementation of ERM improved the glucose tolerance in the fasted normal rats. After that serum glucose level was lowered significantly (P <0.05) at 120 minutes and varied significantly (P <0.01) lowered at 180 minutes. Extract also showed significant hypoglycemic effect after 90 minutes of treatment.

Effect of ERM on glucose level of alloxan induced rats

The fasting mean blood glucose level did not showed in the normal rats when compared with diabetic control .On treatment with the ERM (250 & 500 mg/kg) pasting blood glucose level on Day 1 (after being Diabetic) is 237.00 ± 3.651 & 249.667 ± 6.211 are reduced to 86.33 ± 1.926 &81.330 ± 1.978 respectively , which showed a significant of (P < 0.01) when compared with diabetic control. The fasting mean blood glucose level of diabetic rats treated with standard drug glibenclamide showed a reduction of blood glucose from the initial day (233.833 ± 5.890) to final day (83.500 ± 2.141) with a significance of (P<0.01) when compared with diabetic control .Improvement in the blood glucose Homeostasis was in a dose dependent manner .After 14 days treatment of ERM (at a dose of 500 mg/kg) is significant when compared with dose of 300 mg/kg .It was found that, there occurs selective decrease in hyperglycaemic state after the administration of ERM in alloxan induced diabetic rats. The results suggested that ERM has more favourable effect on decreasing the glucose level compared with glibenclamide. The present study suggests that Rhus mysorensis can be successfully utilized for the management of diabetes.

Table 4 : Effect of ERM on glucose level of alloxan induced rats .

1	**NORMAL**	93.00 ± 3.540	90.833 ± 2.023	86.669 ± 2.171	87.833 ± 1.579	84.667 ± 2.076
2	**DIABETIC CONTROL**	245.007 ± 7.746	251.667 ± 6.912	271.167 ± 7.016	277.667 ± 5.493	290.667 ± 3.084
3	**STANDARD (GLIBENCLAMIDE)**	233.833 ± 5.890***	158.500 ± 28.016***	148.833 ± 2.040***	103.169 ± 3.851***	83.500 ± 2.141***
4	**TEST 1 ERM 250 mg/kg**	237.00 ± 3.651***	189.667 ± 2.011***	143.00 ± 2.066***	107.00 ± 3.307***	86.33 ± 1.926***
5	**TEST 2 ERM 500 mg/kg**	249.667 ± 6.211***	190.167 ± 2.432***	133.167 ± 3.260***	98.80 ± 1.40***	81.330 ± 1.978***

All values are mean ±S.D. (n=6). **p<0.001 significant compared with control. (One-way ANOVA followed by Dunnett's t-test).

Effect of ERM on body weight allaxon induced diabetic rats :

The body weights of all rats of different groups from the initial day to the final day of the study are in the below table .The body of normal rats were unchanged when compared with diabetic control.The body weight of diabetic controlled rats were decrease during this study , initialday (198.11 ± 1.02) and final day (184.12 ± 1.02). On treatment with ERM (250 & 500 mg/kg), the body weight on intial day (after rats beingdiabetic) 98.00 ± 0.66 , 99.00 ± 1.33 are respectively increased to 205.12 ± 0.56 , 210.13± 0.66 respectively. The body weight of diabetic rats were treated with standard drug glibenclamide showed increased in body weight from initial day (198.12 ± 1.33) to final day (210.23 ± 1.44) with a significance of (**P < 0.01) when compared with diabetic control .

Table 5: Effect of ERM on body weights in Alloxoan induced Diabetic rats

TEST GROUP	0 DAYS	7 DAYS	14 DAYS
NORMAL	210.21 ± 1.43	211.11 ± 1.28	210 ± 1.02
DIABETIC CONTROL	198.11 ± 1.02	193.123 ± 1.28	184.12 ± 1.02
STANDARD (GLIBENCLAMIDE)	198.12 ± 1.33**	204.12 ± 0.12**	210.23 ± 1.42**
ERM 250 gm/kg	198.00 ± 0.66**	204.12 ± 0.12**	205.12 ± 0.56**
ERM 500 gm/kg	199.00 ± 1.33**	204.12 ± 0.12**	210.13 ± 0.66**

Table 6: Effect of ERM on Bio-chemical parameters in Alloxon induceddiabetic rats

ANIMAL GROUPS	SERUM CHOLESTEROL	TRIGLYCERIDES	HDL	LDL	VLDL	CREATINE
NORMAL	100.85±2.0***	138.50±1.2***	61.25±0.6***	14.44±2.0***	27.85±0.3***	0.96±0.1***
CONTROL	251.66±3.0***	345.83±2.3***	38.83±0.4***	146.10±4.1***	65.83±3.3***	2.15±0.0***
STANDARD	129.00±4.5***	146.83±1.9***	50.35±1,3***	46.10±4.6***	29.63±0.1***	0.88±0.7***

RME (250mg/kg)	129.00±4.3***	149.16±1.4***	58.26±0.4***	40.90±4.1***	29.83±0.1***	0.93±0.1***
RME (500mg/kg)	124.50±5.6***	149.16±1,4***	60.83±0.3***	35.37±4.2***	29.63±0.1***	0.96±0.7***

Table 7 : Hydroxyl radical scavenging activity of *Rhus mysorensis* ethanol extract

Sample	Conc(µg/ml)	% inhibition	IC_{50} (µg/ml)
ERM	50	28.05± 0.802	172± 0.50
	100	35.70±1.018	
	150	47.37±1.344	
	200	58.40±1.534	
	250	67.70±1.71	
Quercetin	50	22.93±1.25	190± 0.71
	100	38.72±0.98	
	150	42.52±0.70	
	200	50 ± 0.5657	
	250	58.82 ±1.131	

Table 8: Superoxide radical scavenging assay using *Rhus mysorensis* methanol extract

Sample	Conc(µg/ml)	% inhibition	IC_{50} (µg/ml)
ERM	20	24.6±0.848	59± 0.33
	40	37.25 ± 0.35	
	60	51.4 ±1.273	
	80	56.65 ±0.91	
	100	67.9 ± 0.707	
Ascorbic acid	20	47.35±1.612	39±0.49
	40	51.37±1.259	
	60	63.32±2.121	
	80	77.85±1.464	
	100	81.15±1.88	

Table 9: Reducing power assay of Rhus mysorensis ethanol extract

Sample	Conc(µg/ml)	Absorbance
ERM	50	0.1770±0.005
	100	0.2377±0.00416
	200	0.324±0.001
	400	0.4287±0.0152
	800	0.5387±0.0143
BHT(Butylated hydroxyl tolue)	50	0.2297±0.01662
	100	0.342±0.0185
	200	0.4753±0.0113
	400	0.9847±0.01206
	800	01.73±0.03647

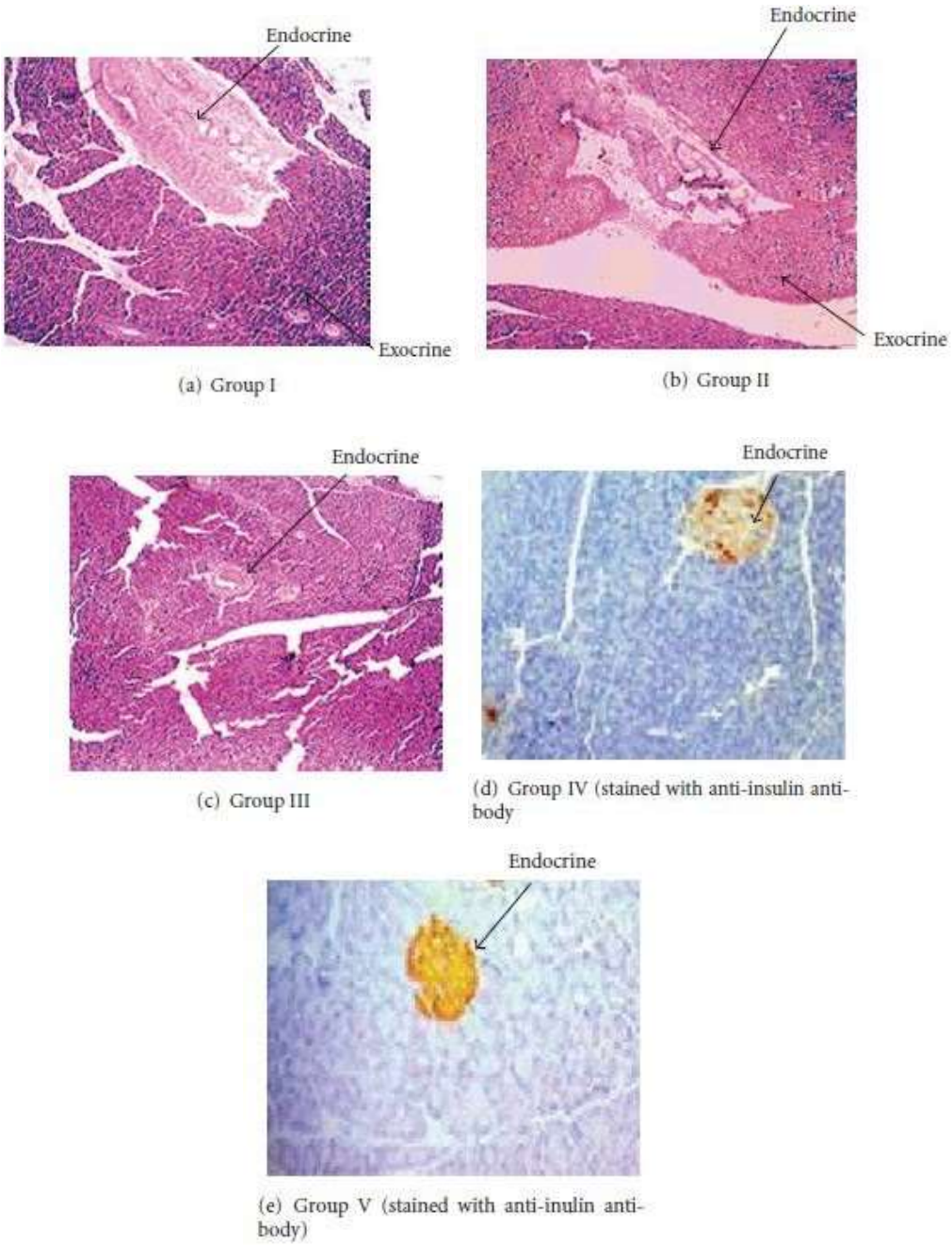
Table 10: Nitric oxide radical scavenging assay using ethanol extract of *Rhus mysorensis*

Sample	Conc(µg/ml)	% inhibition	IC_{50} (µg/ml)
ERM	50	32.86 ± 0.5657	247± 0.42
	150	39.185± 1.308	
	250	51.050 ± 2.277	
	350	58.315± 1.407	
	450	66.855 ± 2.020	
	100	38.72±0.98	
	150	42.52±0.70	
	200	50 ± 0.5657	
	250	58.82 ±1.131	
Curcumin Standard			147± 0.54

Histopathological pancreas

Histopathological pancreas slides of different group rats are given below, all the slidesshowed the cellular population in the islets of Langerhans in the pancreas.

Fig 1 :Histopathological slides of pancreas



Histopathological studies of pancreas: Group I (Control), Group II (Alloxan 150 mg/kg), Group III (Alloxan + Whole Plant 250 mg/kg), Group IV (Alloxan +Whole Plant 500 mg/kg) and Group V (Alloxan + glibenclamide (5mg/kg).

CONCLUSION

As this Rhus mysorensis consists of all these bioactive compounds, it may act by various mechanisms and helps in preventing Diabetes & halting Diabetic complications. The data indicates that ERM significantly reduced hyperglycaemia in 14days diabetic studies. The efficacy of the ERM was comparable to standard Glibenclamide and hence it was also designed to evaluate the effect of ethanolic extract of *Rhus Mysorensis* on late in

the treatment of diabetes. The present study showed treatment with ERM showed anti-diabetic activity and reduced its complications. This was evidenced by the levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart diseases. This abnormal high level of serum lipids is mainly due to decrease in the action of lipolytic hormones on the fat depots mainly due to the action of insulin.

In the present study the diabetic rats showed the hypercholesterolemia and hypertriglyceridemia and the treatment with ERM decreased both cholesterol and triglycerides levels significantly($p<0.001$). This implies that ERM can prevent in reducing the complications of lipid profile seen in the diabetes. The results showed significant increase in the level of plasma creatinine which is the marker of renal dysfunction in the diabetic goup compared to control levels. After treatment of alloxan induced diabetic rats with ERM the levels of creatinine were significantly ($p<0.001$) decreased compared to those in untreated diabetic group. Rhus mysorensis can prevent diabetic associated renal complications, ethanolic extract of Rhus Mysorensis (ERM*)* has potent anti-diabetic and antioxidant activity in Alloxan induced diabetic rats.

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