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EVALUATION OF ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACTION OF LEAVES OF *RHINACANTHUS NASUTUS (L.)*

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ABSTRACT

Objective: To evaluate the antidiabetic effects of ethanolic extracts of *Rhinacanthus nasutus* leaves (ERN) in Wister rats. Methods: Alloxan induced diabetic test model were performed to evaluate antidiabetic activity of ERN at two different doses 200 and 400 mg/kg respectively. Ethanolic extract of *Rhinacanthus nasutus* leaves (Acanthaceae) was tested for anti-diabetic activity for alloxan induced diabetics in wistar rats. After oral administration of the extract at two different doses (200 and 400mg/kg body weight) for 21 days to alloxan-induced diabetic rats, the blood glucose, level was assayed periodically on 0, 7, 14 and 21th day. After 21 days treatment all biochemical parameters like total cholesterol, triglyceride, total protein levels were checked and compare with control and standard group and body weight was also determine which was compare with initial weight. **Results:** Ethanolic extract shown significant protection and lowered induced blood glucose level to normal in glucose tolerance test on the day 0,7, 14 and 21th compare with diabetic control and standard groups. There was significant control of all biochemical parameters levels like total cholesterol, triglyceride, total protein in extracts of *Rhinacanthus nasutus* leaves treated diabetic rats. Marked body weight loss was observed in diabetic rats. The data obtained from this study showed that the treatment of extracts *Rhinacanthus nasutus* leaves protect the diabetic rats from loss of massive body. **Conclusions:** These results indicate that the *Rhinacanthus nasutus* leaves posses significant anti-diabetic activity.

KEYWORDS: Antidiabetic activity, *Rhinacanthus nasutus*, Acanthaceae, Alloxan- induced diabetis, biochemical parameters.

INTRODUCTION

Diabetes is a major degenerative disease in the world today, affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders. Diabetes mellitus is ranked seventh among the leading causes of death in the world and is considered third when its fatal complications are taken into account. If not cured or controlled it may even lead to acute or chronic complications. By the year 2025, India shall have the maximum number of diabetes in the world making it, the "Diabetes capital of the world". It is a heterogeneous group of metabolic disorders characterized physiologically by dysfunction of pancreatic β cells and deficiency in insulin secretion, insulin activity or both. It is an endocrinological syndrome abnormally having high levels of sugar in the blood. This may be either due to insulin not being produced at all, is not made at sufficient levels, or is not as effective as it should be. Diabetes is still a serious health problem all over the world since it is associated with increased morbidity and mortality rate. When compared with the general population, mortality and morbidity increase in diabetes is mainly due to the associated chronic complications both specific (microvascular) and nonspecific (macrovascular). Since the disease prevails in both genders and in all age groups, the general public has a concern about its control and treatment.[1]

Rhinacanthus nasutus that belongs to the family Acanthaceae is considered to possess great medicinal value found in species for the proposed study that is leaves of *Rhinacanthus Nasutus* collected carefully from the Kollimalai hills, Namakkal Dt, Tamilnadu. *Rhinacanthus nasutus* is used in treatment of common disorders including cancer, fungal infections, eczema, pulmonary tuberculosis and herpes virus infections. *Rhinacanthus nasutus* can be used for health promotion due to its immunomodulating activity. Through various researches, it has been reported that the plant has no toxicity. *Rhinacanthus Nasutus* contains several chemical compounds such as rhinacanthin A, B, C, and D which are active against human cytomegalovirus, microbials, diabetes mellitus, cancers and hypertension. Preparations of *Rhinacanthus Nasutus* are very

effectively used by traditional practitioners in Sri Lanka in treatment of skin diseases.[2]

MATERIALS AND METHODS Plant materials and extraction

The leaves part of *Rhinacanthus nasutus* was collected carefully from the Kollimalai hills, Namakkal Dt, Tamilnadu, The collected leaves were washed with tap water, prior to distilled water, shade dried and coarsely powdered using a cutter mill, extracted with petroleum ether for defatting followed by ethanolic extraction using hot continuous percolation for 6 hrs. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts.

Experimental animals

To evaluate the antidiabetic activity of ethanolic extracts of *Rhinacanthus nasutus* leaves (ERN), Wistar rats weighting between (150-250) gm procured from animal house, JKKMMRF College of Pharmacy, B.Komarapalayam, Namakkal. The temperature in the experimental room was around 25°C. Lighting was natural sequence being 12 hours dark and 12 hours light. All the animals were followed the internationally accepted ethical guidelines for the care of laboratory animals. The animal studies were approved by the JKKMMRFCOP /IAEC/2016/007. Prior to the experiments, rats were fed with standard food for 1 week in order to adapt to the laboratory conditions. All animal procedures were performed after approval from the institutional ethics committee and in accordance with the recommendations for the proper care and use of laboratory animals.

Chemicals

Alloxan monohydrate (LOBA Chemie, Mumbai, India) was purchased, preserved at 25ºC and used for this study. Glibenclamide is an oral antidiabetic preparation with an efficient hypoglycemic action. Diaonil (Glibenclamide) (S.K.Prasad et.al, 2009) manufactured by Aventis Pharma Ltd. Goa, India, was collected from market and preserved at room temperature.

Experiments

The adult albino- wistar rats (150-250gm) were overnight fasted and determine the fasting blood glucose level. The sequence blood glucose level of animals were selected and except group I animals used to induce diabetes by single i.p injection of 120 mg/kg of Alloxan monohydrate was dissolved in normal saline (pH-4.5). Animals were fed with 5% glucose solution in order to prevent hypoglycemic shock for 18 hrs (Prince PSM et al., 1989). Hyperglycemia is to be confirmed the elevated blood glucose levels, determined at 72 hrs and then on day 0 after injection. The threshold value of fasting blood glucose level >200mg/dl was taken as diabetic animal and rats found with permanent diabetes were used for the antidiabetic study.

Group I: Normal control rats fed with vehicles only. (Normal saline with 1%CMC)

Group II: Diabetic controls rats (Alloxan monohydrate 120mg/kg body weight of rats, once i.p injection).

Group III: Diabetic rats treated with standard drug, Glibenclamide 3mg/kg per oral body weight.

Group IV: Diabetic rats treated with ethanolic extract of Rhinacanthus Nasutus 200mg/kg, per oral, dissolved in 1% carboxy methyl cellulose (CMC).

Group V: Diabetic rats treated with ethanolic extract of RhinacanthusNasutus400mg/kg, per oral, dissolved in 1% carboxy methyl cellulose (CMC).

STATISTICAL ANALYSIS

All the values of body weight, fasting blood glucose level, and biochemical parameter estimations were expressed as mean ± standard error of mean (S.E.M) and was analyzed for significance by ANOVA and groups were compared by Tukey-Kramer multible comparison test, using InStat v.2.02 software (GraphPad Software Inc.). Differences between groups (p Value) were considered significant at $P<0.05$ level.

RESULTS

Ethanolic extract of *Rhinacanthus nasutus* leaves was subjected to anti-diabetic activity in rats where Alloxan monohydrate (120 mg/kg b.w., i.p.) was used as the diabetogenic agent. A marked rise in blood glucose level was observed in diabetic control compared to normal control rats. Ethanolic extract of *Rhinacanthus nasutus* (200 and 400 mg/kg) exhibited a dose dependent significant anti-hyperglycaemic activity on 0, 7, 14, 21th day post treatment as compare with reference standard, Glibenclamide. The result is depicted in Table 1 and Fig. 1. Total cholesterol, Triglyceride, Total protein, levels were decreased significantly in a dose related manner by ethanolic extract of *Rhinacanthus nasutus* (200 and 400 mg/kg) after 21 days of treatment, compare to diabetic control group (Table 2, 3, 4 and Fig.2, 3, 4). Changes in initial and final body weight of normal control and experimental groups are shown in Table 5 and Fig 5. Marked body weight loss was observed in diabetic rats.

Table No.1 Effect of Rhinacanthus Nasutus ethanolic extract of and Glibenclamide on blood glucose level.

Group	Treatment	Blood glucose level (mg/dl)			
		Day 0	Day 7	Day 14	Day 21
I	Normal control rats (vehicles only)	70.65 ± 1.42	80±2.34	78.83 ± 2.36	72.33 ±1.82
II	Diabetic controlrats	380.6±13.57 a	336.84±7.18a	354.84±10.81a	369.32±12.91a
III	Diabetic group + Glibenclamide3mg/kg	313.6±9.09 a	281.34±9.56a	233.65±5.42 a	147.67±8.05 a
VI	Diabetic group + EERN (200mg/kg)	334.66±8.90 c	285±13.26 a	174.82±8.91 a	161±10.81 a
V	Diabetic group + EERN (400mg/kg)	321.84±12.16b	286±5.08 b	157.82±7.30 a	160.5±7.74 a

Table No: 2 Body weight changes in ethanolic extract of Rhinacanthus Nasutus and Glibenclamideon control and experimental groups of rats.

Group	Treatment	Body weight changes (g)	
		Day 0	Day 21
I	Normal control rats (vehicles only)	145±7.67	204.15± 11.94
II	Diabetic control rats	162.5±8.54[b]	129.18± 7.67[b]
III	Diabetic group + Glibenclamide3mg/kg	150±6.44 a	208.37± 12.37[a]
IV	Diabetic group + EERN(200/kg)	154.17±7.67[b]	200± 6.46[b]
V	Diabetic group + EERN(400mg/kg)	162.6 ±14.05[c]	187.6± 17.98[c]

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = p<0.001; b=** = p<0.01; c=*=p<0.05. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests).

Table No: 3 Effect of ethanolic extract of Rhinacanthusnasutus and Glibenclamide in Total cholesterol, Triglycerides levels of control and experimental groups of rats

Group	Treatment	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)
I	Normal control group (vehicles only)	109.82±0.47	91.16±1.71
II	Diabetic control rats	212.82±1.84 a	184±2.63 a
III	Diabetic group + *glibenclamide*(3mg/kg)	122.17±1.94 a	132±2.63 a
IV	Diabetic group + EERN (200mg/kg)	134.6±2.11 a	62.83±1.45 a
V	Diabetic group + EERN(400mg/kg)	131.67±2.94 b	80.83±2.11 a

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = p<0.001; b=** = p<0.01; c=* =p<0.05. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests).

Table No: 4 Effect of ethanolic extract Rhinacanthus Nasutus of and Glibenclamide on LDL, VLDL, HDL of control and experimental group of rats.

Group	Treatment	LDL Cholesterol(mg/dl)	VLDL Cholesterol (mg/dl)	HDL Cholesterol (mg/dl)
I	Normal control group (vehicles only)	49.92±1.19	18.24±0.34	41.65±0.87
II	Diabetic control rats	155.4±2.15 a	36.7±0.52 a	21±1.06 a
III	Diabetic group + glibenclamide (3mg/kg)	56.74±1.67 a	26.52±0.52 a	38.84±1.07 a
IV	Diabetic group + EERN (200mg/kg)	83.1±2.80 b	12.55±0.27 a	38.82±1.07 a
V	Diabetic group + EERN (400mg/kg)	85.32±1.85 a	16.17±0.44 a	31.84±0.82 a

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = p<0.001; b=** = p<0.01; c=* =p<0.05. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests).

Table No. 5 Effect of ethanolic extract of Rhinacanthus nasutus and Glibenclamide in Total protein, Albumin of control and experimental groups of rats.

Group	Treatment	Total protein (mg/dl)	Albumin(mg/dl)
I	Normal control group (vehicles only)	8±0.13	3.867±0.10
II	Diabetic control rats	5.44±0.19 a	1.77±0.11 a
III	Diabetic group + *glibenclamide*(3mg/kg)	7.67±0.14 a	3.22±0.12 a
IV	Diabetic group + EERN (200mg/kg)	7.3±0.13 a	3.08±0.09 a
V	Diabetic group + EERN (400mg/kg)	8±0.18 a	3.26±0.08 a

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = p<0.001; b=** = p<0.01; c=* =p<0.05. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests).

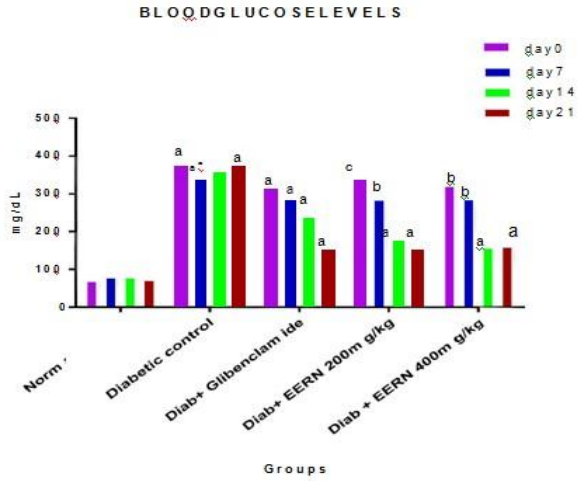

Figure No: 1 Effect of ethanolic extract of RhinacanthusNasutus and *Glibenclamide* on blood glucose level.

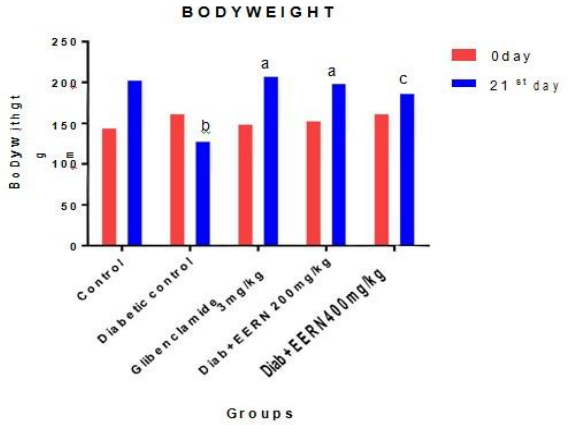

Figure: 2 Body weight changes in ethanolic extract of and *Rhinacanthus Nasutus Glibenclamide* on control and experimental groups of rats.

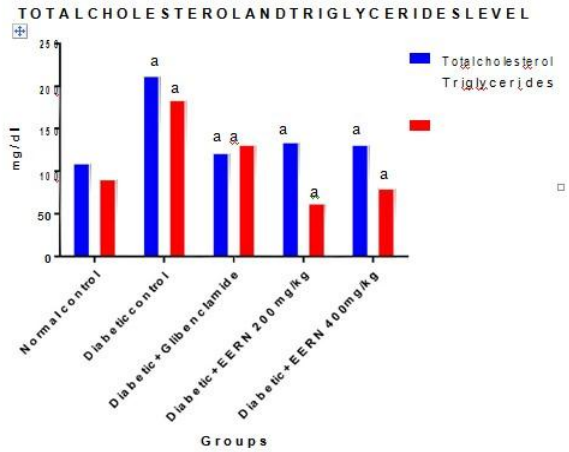

Figure No. 3 Effects of ethanolic extract of *RhinacanthusNasutus* and *glibenclamide*on total cholesterol, triglycerides levels of control andexperimental groups of rats.

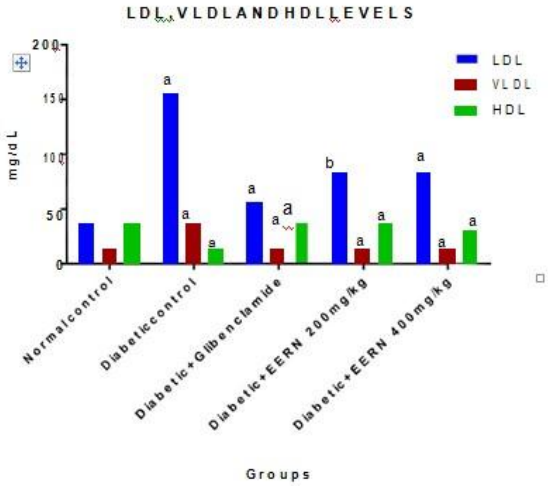

Figure No: 4 Effects of ethanolic extract of *Rhinacanthus Nasutus* and *Glibenclamide* on LDL cholesterol, VLDL cholesterol, HDL cholesterol of control and experimental groups.

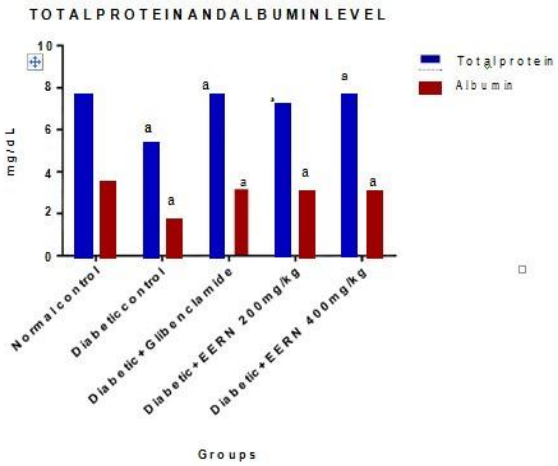

Figure No. 5: Effect of ethanol extract of *Rhinacanthus Nasutus* and *Glibenclamide* on Total protein and Albumin levels of control and experimenta lgroups of rats.

DISCUSSION

Current study focused the effect of different doses of *Rhinacanthus nasutus* leaves extract and comparison of the effects with those of a single dose standard antidiabetic drug in induced diabetic condition. Presence of phytochemical metabolites in extract was also assessed to assume their role in antihyperglycemic activity. The possible mechanism of action of extracts could be correlated with the suggestive effect of the reference antidiabetic drug glibenclamide that promotes insulin secretion by closure of K+-ATP channels,

membrane depolarization and stimulation of Ca2+ influx, an initial key step in insulin secretion.[9] Other possible mechanisms by which the plant extracts lowered blood glucose may be by increasing glycogenesis, inhibiting gluconeogenesis in the liver, or inhibiting the absorption glucose from the intestine. It is noted that the induction with alloxan of same dose to different groups of rat is also varied. The same object may be implied to the administration and effect of extracts and glibenclamide**.**

The result of the present study shows that the ethanol extract exerts anti-diabetic Rhinacanthus Nasutus effect against alloxan induced diabetes.

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