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Hypolipidemic Properties of *Rhinacanthus nasutus* in Streptozotocin Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus is one of the widespread and severe metabolic disorders in humans all over the globe. Various medicinal plants have effectively been used to conquer this global health problem. *Rhinacanthus nasutus* is widely used in Thai medicine and various parts of the world to treat different diseases i.e., cancers, skin diseases, ringworm etc. The methanolic extract of *R. nasutus* was administered to streptozotocin induced (50 mg kg⁻¹ b.wt.) diabetic male albino wistar rats for 30 days to identify its effect on serum and hepatic cholesterol levels. Oral administration of *R. nasutus* (200 mg kg⁻¹ b.wt.) significantly decreased the serum total cholesterol, triglycerides, LDL-cholesterol and increased the HDL-cholesterol levels. The hepatic total cholesterol, triglycerides, phospholipids were also decreased after administration of *R. nasutus* in diabetic rats. The results of this experimental study indicate that *R. nasutus* possesses hypolipidemic activity without evident toxic effects to the experimental animal.

Key words: Diabetes, *Rhinacanthus nasutus*, cholesterol, triglycerides, lipid profile

INTRODUCTION

Diabetes Mellitus (DM) is one of the most major metabolic diseases and cause of death in modern society. Diabetes mellitus clutches a group of chronic disorders characterized by disarrangement in carbohydrates, proteins and fat metabolisms caused hyperglycemia or reduced insulin secretion or action or both (American Diabetes Association, 2007). As the disease progresses tissue or vascular damage ensues leading to severe diabetic complications such as retinopathy, neuropathy, nephropathy, cardiovascular complications and ulceration. In addition to hyperglycemia, diabetic individuals endure from a group of problems such as cardio vascular risks, most importantly the altered lipid profile. It is characterized by increased levels of moving Free Fatty Acids (FFAs) and Triglycerides (TGs). There is a decline in High-Density Lipoprotein Cholesterol (HDL-C) along with overload fat deposition in various tissues including the liver (Ahmadi *et al.*, 2008). An unusual deposition of fat in the liver and muscle elicits insulin resistance that terminates in β -cell reduction in type 2 diabetes. Therefore, successful management of diabetes mellitus depends on diminishing the glucose levels in the blood and along with lipid homeostasis that could influence patients to cardiovascular obstacles. Thus, diabetes covers a broad range of heterogeneous diseases. The World Health Organization (WHO) predicts that 300 million people will have diabetes by the year 2025 (WHO and IDF, 1999). India is one of the developing countries for the number of people with diabetes mellitus and it is expected that diabetes will affect about

69.9 million nations by the year 2025 (Saravanana and Ponmurugan, 2011). Controlling diabetes mellitus is considered as a serious global health problem and triumphant treatment is yet to be discovered. Besides the synthetic drugs which are widely used for the treatment of diabetes (Sulfonylureas, Biguanides, Thiazolidinediones and Insulin), several medicinal plant species have been proved to have hypoglycemic and anti hyperglycemic activity. Some phytochemicals have either antioxidant or insulinotropic activity on the existing β -cells (Karthikesan *et al.*, 2010). In the last few years there has been an exponential growth in the field of herbal medicine. These herbal drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects than synthetic ones and relatively low cost (Pari and Srinivasan, 2010). A large number of crude extracts and purified phytochemicals have been tested in clinical trials for treatment of diabetes.

Phenolic compounds, flavonoids are secondary metabolites which are present in the plants and act as antioxidants and thereby help in preventing different types of diseases including diabetes (Pandey and Rizvi, 2009). *Rhinacanthus nasutus* (Linn) belongs to Acanthaceae family (Sattar *et al.*, 2004). *R. nasutus* is being used as Thai medicine for different types of cancers. Various parts of this plant have been used for the treatment in a number of other diseases such as eczema, pulmonary tuberculosis, herpes, hepatitis, diabetes (Rao and Naidu, 2010a) hypertension and various skin diseases. It has been found to possess antimicrobial properties against different types of Gram positive and Gram negative bacteria and fungi of *A. niger* (Rao *et al.*, 2010) and *in vitro* antioxidant activity was also reported (Rao and Naidu, 2010b). However, so far there are no systemic reports of *R. nasutus* on hypolipidemic activity. Hence, the present study was aimed at assessing the hypolipidemic effects of *R. nasutus* in STZ induced diabetic rats.

MATERIALS AND METHODS

Plant materials: The fresh leaves of *Rhinacanthus nasutus* were collected from Tirumala Hills, Tirupati, Chittoor district of Andhra Pradesh in the month of July-October 2010 and identified by a Botanist, Department of Botany, S.V. University, Tirupati. The leaves were shade dried and ground into fine powder and used for preparation of the extracts.

Preparation of extract: Fresh leaves of *Rhinacanthus nasutus* (L) were shade dried and milled to fine powder using a mechanical grinder. The powdered plant material (200 g) was soaked in 1 kg of methanol for 72 h with intermittent shaking and after filtering through funnel it was then concentrated using vacuum rotary evaporator at 40°C. The concentrated crude extract was then layered on aluminum foil for further use.

Chemicals: Streptozotocin was purchased from Sigma (Germany) and all other chemicals and reagents used in this study were of analytical grade. Glibenclamide was purchased from a local drug store.

Induction of experimental diabetes: Diabetes was induced by a single intraperitoneal injection of a freshly prepared Streptozotocin (STZ) solution (50 mg kg⁻¹ in citrate buffer 0.01 M, pH 4.5) to overnight-fasted rats (Ramesh *et al.*, 2009). Control rats received normal water alone. Diabetes was identified by polydipsia, polyurea and by measuring non-fasting blood glucose levels 48 h after injection of STZ. Animals which show blood glucose levels more than 250 mg dL⁻¹ were considered as diabetic rats and used as the experimental animals.

Experimental design: Adult, male rats of Wistar strain weighing 150-180 g obtained from Sri Venkateswara Enterprises, Bangalore-21, were chosen as animal model for present study. They were housed individually in clean, sterile, polypropylene cages under standard conditions (12 h light/dark cycles) with free access to standard chow (Hindustan Lever Ltd., Bangalore, India) and water *ad libitum*. The animals were acclimatized to the laboratory for one week prior to the start of experiments. The animal experiments were designed and performed in accordance with the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (Resolution No. 05/ (i) /a/CPCSEA/IAEC/SVU/ MDN-PVR/dt.13.09.2010).

The rats were divided into 5 groups comprising of 6 animals in each group as follows:

- Group I** : Normal rats (controls)
- Group II** : Normal+*R. nasutus* treated rats (200 mg kg⁻¹ b.wt./day)
- Group III** : Diabetic untreated rats
- Group IV** : Diabetic+*R. nasutus* treated rats (200 mg kg⁻¹ b.wt./day)
- Group V** : Diabetic+Glibenclamide treated rats

Measurement of serum cholesterol levels: Estimation of serum cholesterol was carried out by the method of Zlatkis *et al.* (1953). Serum triglycerides were estimated by the method of Foster and Dunn (1973) and HDL cholesterol was estimated by the method of Burstein *et al.* (1970). The VLDL cholesterol was calculated using the formula, TG/5 mg dL⁻¹. The serum LDL cholesterol was estimated by the method of Friedewald *et al.* (1972).

Estimation of triglycerides (TG): Triglycerides in the liver tissue were estimated by modified version method of Haux and Natelson (1971) with slight modifications as given below. Triglycerides were assayed by hydrolyzing them to glycerol and the liberated glycerol was determined. Tissue homogenates were prepared in 1N H₂SO₄ and to it 4 mL of chloroform was added. The 0.5 mL of tissue homogenate was taken and 0.5 mL of 1N H₂SO₄ and 4 mL of chloroform were added to it. The contents were centrifuged at 1000 rpm for 15 min. The 0.5 mL of chloroform layer was taken and to it 0.4 mL of methanol and 0.1 mL of alkaline barium solution were added and the contents were heated for 30 min at 80°C, the total volume was made up to 1 mL with 2N H₂SO₄ and centrifuged for 10 min at 1000 rpm. The 0.5 mL of this supernatant was taken and 0.1 mL of sodium periodate was added and shaken well for 1 min, 0.1 mL of sodium arsenate and 5 mL of chromotropic acid reagent was added and heated for 30 min and cooled. The samples were read a 575 nm is Spectrophotometer against the reagent blank. The results were finally expressed in mg of triglycerides/gram wet weight of the tissue.

Estimation of total cholesterol: The total cholesterol content of liver tissue was estimated using Liebermann Burchard reaction as described by Haux and Natelson (1971).

Estimation of phospholipids: Phospholipids (PL) in the liver tissue were estimated by the method of Zilversmit and Davis (1950).

Statistical analysis: The results were expressed as Mean±SD (n = 6). Statistical analysis was performed using one-way Analysis of Variance (ANOVA) followed by Tukey's test. The p<0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

There was a significant decrease in serum HDL cholesterol levels and a significant elevation in the total cholesterol, triglycerides and LDL-cholesterol levels in diabetic rats when compared to normal rats. Oral administration of *R.nasutus* for 30 days brought back the levels of serum lipids to near normal levels in diabetic rats. The hepatic total cholesterol, triglycerides and phospholipids also elevated in the diabetic rats and they were restored after administration of *R.nasutus* for a period of 30 days. The total cholesterol, triglycerides and phospholipids of normal rat hepatic tissue are 50.88 ± 0.83 , 1.43 ± 0.12 and 7.58 ± 0.20 whereas, in diabetic rat these levels are raised to 61.87 ± 2.42 , 2.57 ± 0.51 and 10.72 ± 0.45 . The restoration of these levels with the plant extract treatment is found in the hepatic tissue to 54.65 ± 1.63 , 1.84 ± 0.40 and 8.61 ± 0.07 , respectively (Table 1). The total cholesterol, triglycerides, LDL-C, VLDL-C levels of normal rat serum is 85.64 ± 3.02 , 109.5 ± 4.59 , 64.27 ± 3.28 and 21.9 ± 0.91 whereas, in diabetic rat these levels are raised to 129.97 ± 2.85 , 212.66 ± 10.93 , 113.60 ± 4.32 and 42.53 ± 2.18 . The restoration of these lipid levels with the plant extract treatment is found in the serum to 87.92 ± 1.61 , 112.33 ± 5.64 , 64.25 ± 3.40 and 22.46 ± 1.12 , respectively. The HDL-C levels of normal rats were 43.16 ± 1.16 . The levels were decreased significantly in the diabetic rats to 26.16 ± 1.72 . The levels of HDL-cholesterol were regained with the *R. nasutus* treatment to 48.66 ± 1.36 (Table 2).

The management of diabetes is a major worldwide problem. The currently using synthetic drug regimens for management of diabetes mellitus have certain adverse effects and therefore there is a need to develop safer and more effective anti diabetic drugs. Consequently, treatment with drugs isolated from plants has an effect on shielding β cells and leveling the oscillation in glucose levels (Fatima *et al.*, 2010). Elevated serum or tissue lipids and lipoproteins are characteristics of uncontrolled diabetes. Type 2 diabetes mellitus is commonly associated with dyslipidemia which is a significant risk factor for the development of cardiovascular diseases (Karthikesan *et al.*, 2010). This is in support with our present study results.

In the present study a marked increased in the lipid content of serum and liver were found in STZ induced diabetic rats (Group III) that is mainly due to the increased mobilization of Free Fatty

Table 1: Effect of *Rhinacanthus nasutus* on the concentrations of total cholesterol, triglycerides and phospholipids in the liver of normal and experimental rats

Parameters	Total cholesterol	Triglycerides	Phospholipids
Normal	50.88 ± 0.83	1.43 ± 0.12	7.58 ± 0.20
Normal+PE	47.05 ± 1.31^a	1.32 ± 0.15^a	7.33 ± 0.28^a
Diabetic control	61.87 ± 2.42^b	2.57 ± 0.51^b	10.72 ± 0.45^b
Diab+PE	54.65 ± 1.63^c	1.84 ± 0.40^c	8.61 ± 0.07^c
Diab+Gli	51.33 ± 3.64^c	1.68 ± 0.37^c	8.42 ± 0.12^c

Values with same superscript in a row do not differ significantly from each other at $p < 0.05$

Table 2: Effect of *Rhinacanthus nasutus* on the concentrations of triglycerides, total cholesterol, HDL-C, LDL-C and VLDL-C in serum of normal and experimental rats

Parameters	Triglycerides	Total cholesterol	HDL-C	LDL-C	VLDL-C
Normal	109.50 ± 4.59	85.64 ± 3.02	43.16 ± 1.16	64.27 ± 3.28	21.90 ± 0.91
Normal+PE	103.83 ± 3.18^a	84.71 ± 1.26^a	44.83 ± 3.31^a	60.64 ± 3.65^a	20.76 ± 0.63^a
Diabetic control	212.66 ± 10.93^b	129.97 ± 2.85^b	26.16 ± 1.72^b	113.60 ± 4.32^b	42.53 ± 2.18^b
Diab+PE	112.33 ± 5.64^c	87.92 ± 1.61^c	46.16 ± 2.63^c	64.25 ± 3.40^c	22.46 ± 1.12^c
Diab+Gli	116.5 ± 5.71^c	93.95 ± 1.84^c	48.66 ± 1.36^c	68.62 ± 1.10^c	23.30 ± 1.14^c

Values with same superscript in a row do not differ significantly from each other at $p < 0.05$

Acids (FFAs) from peripheral depots (Krishnaveni *et al.*, 2010). Interestingly, most of the studies with different plant extracts in diabetic rats were supportive with our results (Ladan *et al.*, 2007; Subash-Babu and Ignacimuthu, 2007; Nirmala *et al.*, 2008). The rise in serum triacylglycerols, cholesterol and LDL-cholesterol levels in the present study indicate derangement of lipid metabolism and amplified incidence of cardiac dysfunction in diabetic rats. Rise in serum lipids indicates either the defective overproduction or removal (or both) of one or more lipoproteins (Okokon *et al.*, 2007; Fernandes *et al.*, 2010). An oral administration of *R. nasutus* for a period of 30 days restored the altered levels of lipids (Triglycerides, total cholesterol, phospholipids) in liver tissue as well as in serum. The decreased levels i.e., restoration levels of cholesterols and triglycerides are due to the presence of glycosides in the *R. nasutus* extract. This is also in support with our results in another plant (Zanna *et al.*, 2008). The elevated concentrations of cholesterol can enhance the risk of oxidative disease process due to susceptibility of cholesterol to oxidation while it is in circulation. Insulin deficiency or insulin resistance may be responsible for dyslipidemia, because insulin has an inhibitory action on HMG-coA reductase, a key rate-limiting enzyme which is responsible for the LDL particle metabolism with cholesterol rich content (Tobias and Stellan, 2009). A shortage of insulin is associated with raise in cholesterol levels due to the increased mobilization of lipids from the adipose tissue to the plasma. The enlarged concentration of FFAs in liver may be due to lipid catalysis which leads to enhanced generation of NADPH and activation of NADPH dependent microsomal lipid peroxidation (Banu *et al.*, 2009). In addition, phospholipids are vital components of biomembranes and play an essential role in the Triglycerides transport (Arulmozhi *et al.*, 2010). In diabetic rats, the gigantic levels of PLs may be due to the high levels of FFAs and total cholesterol (Rajagopal and Sasikala, 2008).

CONCLUSION

Our present study shows that *R. nasutus* restored the activities of regulatory enzymes involved in lipids and lipoprotein metabolism. Hypolipidemic activities of *R. nasutus* were found in diabetic rats without any hypoglycemic condition in normal rats and found to be non toxic. Therefore an established asserts is systematically confirmed using animal experiments. Further studies are in progress of isolating the active fraction or compound(s) is responsible for the hypolipidemic activity.

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