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Research Article

Anti-Diabetic Activity of Leaf Extract of *Nepenthes khasiana* Hook on Dexamethasone Induced Diabetic Rats

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ABSTRACT

The present research was carried out to investigate the potential hypoglycemic and hypolipidemic effect of leaf extract of *Nepenthes khasiana* Hook in Dexamethazone-induced Hyperlipidemia and Insulin Resistance in Rats. The experiment was done at two different doses of 250 & 500 mg/kg p.o. on day 11 of treatment, blood was collected for the estimation of serum glucose and lipid parameters. The effect of leaf extract was compared with standard glibenclamide at a dose of 500 mg/kg p.o. Leaf extract and glibenclamide significantly decreased (P<0.05) dexamethazone induced elevation of serum glucose when compared to the control group. Leaf extract at a dose of 500 mg/kg showed better activity than standard. In conclusion, the present study indicated that leaf extract of *Nepenthes khasiana* Hook show significant glucose and lipid lowering activity.

Key words: *Nepenthes khasiana* Hook, Dexamethazone, Insulin resistance, Hypolipidemic effect

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INTRODUCTION

Diabetes mellitus is a metabolic disorder initially characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1]. Without enough insulin, the cells of the body cannot absorb sufficient glucose from the blood; hence, blood glucose levels increase, which is termed as hyperglycemia. If the glucose level in the blood remains high over a long period, this can result in long-term damage to organs, such as the kidneys, liver, eyes, nerves, heart and blood vessels. Complications in some of these organs can lead to death [2]. WHO classification of diabetes introduced in 1980 and revised in 1985 was based on clinical characteristics. The two most common types of diabetes were insulin-dependent diabetes mellitus (IDDM) or (type I) and non-insulin-dependent diabetes mellitus (NIDDM) or (type II). WHO classification also recognized malnutrition-related diabetes mellitus and gestational diabetes. Malnutrition-related diabetes was omitted from the new classification because its etiology is uncertain, and it is unclear whether it is a separate type of diabetes [3, 4].

To explore the antidiabetic property of a rarely available unknown plant, we have chosen a steroidal drug which is having diabetic induing property i.e. Dexamethasone, a very potent and highly selective glucocorticoid. People are taken as steroidal supplement, but corticosteroids profoundly affect carbohydrate and protein metabolism. Teleologically, this effect of glucocorticoids on intermediary metabolism can be viewed as protecting glucose-dependent tissues (e.g., the brain and heart) from starvation. They stimulate the liver to form glucose from amino acids and glycerol and to store glucose as liver glycogen. In the periphery, glucocorticoids diminish glucose utilization, increase protein breakdown and the synthesis of glutamine and activate lipolysis, thereby providing amino acids and glycerol for gluconeogenesis. The net result is to increase blood glucose levels [5]. The present study tries to reduce the incidence of haemoglobin during glucororticoid treatment with the develop of a newer drug from *Nepenthes khasiana*. Traditionally it is reported that juice from the unopened pitcher of *Nepenthes khasiana* Hook used for the treatment of diabetes [6].

The Genus *Nepenthes* is a scandent or rarely erect insectivorous herb, subshrub or shrub distributed from Southern china to north eastern Australia and New Caledonia and extending westwards to Seychelles and Malagasy (Madascar). One Species *Nepenthes Khasiana* has been found in North East India (in the Garo Hills and Khasi Jantia Hills of Meghalaya and some part of Assam upto 1200 m). This is also called Leaf plant. The stems of leaf plants are climbing herbs or under shrubs which often climb by means of the tendrillar stalk of the leaf. The leaf itself is a modification of the leaf blade [7].

MATERIALS AND METHODS

Plant Material

The plant *Nepenthes khasiana* was collected during June-August from the Jarain area of Jaintia Hills of Mehghalaya. The plant was identified from the standard literature [8], then confirmed and authenticated by Botanical Survey of India, Shilong. The collected plant material was washed thoroughly with the help of water to remove the earthy matter or adherent impurity and then shade dried. The dried material was powdered by means of mechanical grinder. The resulting powdered material was stored in air tight glass container for further studies.

Extraction of Crude Drugs

About 250g of powdered drug of leaf of *Nepenthes khasiana* was taken in a 5000ml of round bottom flask and extracted for 72 hrs by continuous hot percolation process using different solvent according to the increasing polarity; likewise petroleum ether, benzene, chloroform, acetone, ethyl acetate, methanol, ethanol, water. The extracts were filtered through whatmann filter paper to remove impurities present. The extracts were then concentrated by vacuum distillation, cooled and placed in desiccators to remove the excessive moisture [9].

Phytochemical Analysis

The concentrated extracts were subjected to chemical test as per the methods mentioned in the reference book for the identification of the various constituents [9, 10].

Acute Toxicity Study

Healthy Wister albino rats of either sex weighing 100 ± 20 g were divided into 5 groups of 6 animals each. The animals were housed under standard conditions and room temperature (25 ± 2°C) was controlled. All animals were fed with standard rat pelleted diet and had free access to tab water *ad libitum*. The study has got the approval from the Institutional Animal Ethical Committee (IAEC) of Committee for the Purpose of Control and supervision of Experiments on Animals (CPCSEA). Methanolic extract of leaf was administered orally through gastric intubation in 5% T80 at doses of 500, 1000, 1500, 2000 and 3000 mg/kg bw and control group received 0.5 ml of 5% T80. The animals were observed continuously for 72 hr for any signs of behavioral changes, toxicity and mortality.

Dexamethasone-Induced Insulin Resistance and Hyperlipidemia

Animals were divided in to 5 groups, each consisting of six rats. Rats in the first group received vehicle and served as control group, while the second group of rats received vehicle plus dexamethasone (10 mg/kg s.c.) and served as positive control group. Rats in experimental groups 4-5 were treated with methanolic extract of leaf of *Nepenthes khasiana* (250 & 500 mg/kg p.o.) plus dexamethasone, whereas rats in the 3rd group were treated with standard drug glibenclamide (500 µg/mg p.o.). All the animals received their respective assigned treatment daily for a period of 10 days. Rats of group 2-5 were daily fasted over night before dexamethasone treatment. On day 11, the animals were anesthetized with ether and blood was collected from retero-orbital plexus. Serum was then separated for the estimation of glucose, cholesterol, triglyceride, high density lipoprotein (HDL) and low density lipoprotein (LDL) by using respective kits [11].

Statistics

All the results were expressed as Mean ± SEM and the data were analyzed using one- way ANOVA followed by Student-newman-keuls post-test using Graph Pad Prism software. $P<0.05$ was considered significant.

RESULTS

Phytochemical Screening

Phytochemical screening of plant extracts revealed that the presence of flavonoids, alkaloids, glycoside, tannins, saponins, phytosterols.

Acute Toxicity Study

There was no mortality or any signs of behavioral changes or toxicity observed after oral

administration of methanolic extract of leaf up to the dose level of 3000 mg/kg b.w. in rats.

Dexamethasone Induced Insulin Resistance and Hyperlipidemia

The entire group (extract treatment and standard groups) significantly (P<0.001) decreased dexamethasone-induced elevation of serum glucose, cholesterol, triglyceride and LDL. Methanolic extract of leaf at a dose level of 250 mg/kg decreases cholesterol (P<0.01) and LDL (P<0.05) level when compared with standard treated group. Methanolic extract of leaf at a dose level of 500 mg/kg produced lower effect in LDL (P<0.05) level when compared with standard treated group. The standard and extracts treated group showed significant (P<0.001) increase in the level of HDL when as compared with positive control group (Diabetic control). Table 1 and Figure 1 represent the biochemical parameters.

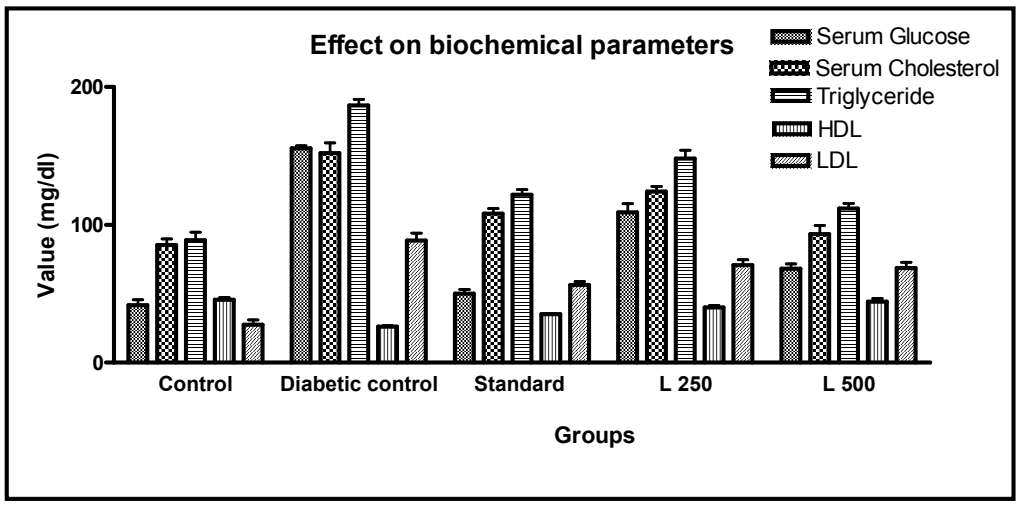
Table 1: Effect of Methanolic Extract of Leaf in Biochemical Parameters

Group	Serum Glucose mg/dl	Serum Cholesterol mg/dl	Triglyceride mg/dl	HDL mg/dl	LDL mg/dl
Control	41.5 ± 4.12	85.33 ± 4.45	88.83 ± 5.79	45.5 ± 1.72	27.66 ± 3.27
Diabetic control	155.5± 1.91[a]	152 ± 7.38[a]	186.6 ± 4.35[a]	26 ± 0.85[a]	88.5 ± 5.48[a]
Standard	50 ± 3.02[¥]	108.16± 3.62[¥]	121.83± 3.81[¥]	35 ± 0.81[*]	56.33 ± 2.65[¥]
L250	109 ± 6.26[¥]	124.16± 3.62[*]	148 ± 6.01[¥]	40 ± 1.49[¥]	70.83 ± 3.73[b]
L500	68 ± 3.75[¥]	93.3 ± 6.30[¥]	111.83± 3.71[¥]	44.33 ± 2.31[¥]	68.66 ± 4.05[b]

All value expressed in Mean ± SEM, One-way ANOVA followed by Student-Newman-Keuls Method. [a] P<0.001 when compared with Control, [¥] P<0.001 when compared with Diabetic control,

* P<0.01 when compared with Diabetic control, [b] P<0.05 when compared with Diabetic control, ns Non significant

Fig. 1 Effect on biochemical parameters



DISCUSSION

Acute toxicity study revealed that the methanolic extract of leaf doesn't shows any toxicity and mortality at dose level of 3000 mg/kg bw. Insulin resistance in type 2 diabetes is not only associated with hyperglycemia, but also with hyperlipdemia and atherosclerosis [12, 13]. Insulin resistance in humans has been shown to be present in conditions like NIDDM, obesity and dyslipidemia. Thus interventions to decrease insulin resistance may postpone the development of NIDDM and its complications. Treatment with natural herbs is likely to be fraught with lesser side effects compared to the presently used synthetic oral antidiabetic agents.

In the present study, it has been found that the elevation of serum glucose and abnormal changes in the lipid profile in dexamethasone treated rats indicating the hyperglycemia and hyperlipidemia caused by this drug. Previous reports also show the same findings in this model [14, 15]. Dexamethasone increases triglyceride levels, causing an imbalance in lipid metabolism leading to hyperlipidemia [16] and an increase in glucose levels leading to hyperglycemia [11]. Pharmacological doses of glucocorticoids induce ob gene expression in rat adipocyte tissue within 24 h. This is followed by complex metabolic changes resulting in decrease in food consumption; reduction in body weight, profound obesity often accompanied by diabetes and development of insulin resistance with enhanced blood glucose and triglyceride levels. Methanolic extract of leaf at the dose level of 250 & 500 mg/kg prevented the rise in triglyceride, glucose, cholesterol and LDL caused by dexamethasone. Previous study in this same plant by using extract of pitcher also shows the same effect in this model [17]. Further, this also prevented the progressive decrease in HDL and body weight caused by dexamethasone. The phytochemical screening shows the presence of flavonoids in the extract might be a responsible active principle for these effects.

CONCLUSION

In conclusion, oral administration of methanolic extract of leaf reduces serum glucose, triglyceride, cholesterol and LDL concentration and improve the concentration of HDL in dexamethasone-administered rats. The methanolic extract showed significant anti-diabetic effect in rats after oral administration. Thus the claim made by the traditional Indian systems of medicine regarding the use of this plant in the treatment of diabetes stands confirms. The results suggest the presence of biologically active principle flavonoids which may be worth further investigation, elucidation.

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