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Research Article

Evaluation of Hypoglycemic Potential Effect of Phyllanthus Niruri in Alloxan Induced Diabetic Male Swiss Albino Mice

Pabbathi Venkata Sravani*, C. Madhavi Latha, Y. Prapurna Chandra

Department of Pharmacology, Ratnam Institute of Pharmacy, Pidathapolur (V&P), Muthukur (M), SPSR Nellore District – 524 346

Abstract

The present study on diabetes was carried out on the male swiss albino mice. The project was aimed to study the antidiabetic activity of leaf extracts of *phyllanthus niruri* in Alloxan induced diabetes. Serum blood glucose levels were anazyled by using auto analyzer. Histopathological studies were carried out to confirm the biological changes. In the present study administration of Alloxan significant increase of blood glucose levels in Diseased, Standard, Ethanolic and Aqueous groups. Administration of seed extracts of *phyllanthus niruri* shown significant decrease in the ethanolic and Aqueous groups. Thus seed extracts of *phyllanthus niruri* shows hypoglycemic potential effect.

Keywords: *phyllanthus niruri*, antidiabetic activity, Alloxan, hypoglycemic, blood glucose, albino mice

Article Info

Corresponding Author:
Pabbathi Venkata Sravani
Department of Pharmacology,
Ratnam Institute of Pharmacy,
Pidathapolur (V&P), Muthukur (M),
SPSR Nellore District – 524 346

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Contents

1. Introduction

The term diabetes mellitus describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs. The abnormalities of carbohydrate, fat and protein metabolism are due to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin. Chronic hyperglycemia and other metabolic disturbances of DM lead to long-term tissue and organ damage as well as dysfunction involving the eyes, kidneys, and nervous and vascular systems. The

definitions and categories of DM used in this document are based on the most recent classifications reported by the American Diabetes Association.

Fig.1. Phyllanthus niruri plant.

Kingdom – Plantae
Division – Magnoliophyta
Class – Magnoliopsida
Order – Euphorbiales
Family – Euphorbiaceae
Genus – Phyllanthus
Species – Niruri

Bhumyaamalaki (Phyllanthus niruri Schum. & Thonn., Euphorbiaceae), which is widely spread throughout the tropical and subtropical countries of the world including India is most commonly used in the Indian Ayurvedic system of medicine in problems of stomach, genitourinary system, liver, kidney and spleen. Phyllanthus niruri has been described in Ayurveda by the Sanskrit name – Bhoomyaamalakee, Taamalakee and Bhoodhatree. It was described to have the properties of Rasa, Guna, Veerya and Vipaaka. The Ayurvedic literature has shown its uses as Kaasahara (antitussive), Shwaasahara (antispasmodic, antidyspnoic), Kaphapittahara (which relieves the Kapha Pitta Dosha), Pipaasaaghna (which relieves Polydipsia), Raktapittahara (hemorrhage disease), Paanduhara (antianemic), Kaamalaahara (which cures jaundice), Kushthaghna (indicated in leprosy), Daahaghna (refrigerant, relieves burning sensation), Kshatakshayaghna (indicated in Trauma) and Mootrarogahara (which cures urinary disorders).

2. Methodology

Animals: Male Swiss Albino Mice (25-35 g) will be housed in group of 6 animals and maintained under standardized condition (12 – hours light/dark cycle, 24ºc) and fed with high diet food and purified drinking water Ad libitum.

Chemicals: Alloxan (Sigma-Aldrich), Glibenclamide standard drug (Dianil), Ethanol, Distilled water, Carboxy Methyl Cellulose, 0.9% Normal Saline all other chemicals used are of analytical grade.

Instruments

- Auto analyzer (CPC STAT FAX 3000 Plus Awareness Technology INC).
- Magnetic stirrer (REMI)

Kits

- Glucose kit, EXCEL Diagnostics Ltd, Ind.

Plant Authentification

The processes of plant authentification want to be done in Institute Of Herbal Botany, Plant Anatomy Research Centre, West Tambaram, Chennai.

Preparation of Legume Extracts of *Phyllanthus Niruri*

The fresh and mature leaves of ***Phyllanthus Niruri*** were collected and dried under shade, made in to coarse powder which is being used for preparation of Ethanolic and Aqueous extracts. To prepare extracts 50g of plant grain powder in 250 ml of Ethanol and Distilled water was performed by soxhlet apparatus for 8h at room temperature for 25 days. The residue was removed by filtration. The filtrate was evaporated to dryness at 40-50ºc under reduced pressure in a rotary evaporator. The yield of both Ethanolic and Aqueous extracts were approximately 10 and 16%. The extracts were suspended in carboxy methyl cellulose and used for oral administration.

Experimental Design

The experiment conducted for 28 days

Swiss mice (n=6) are divided in to 5 groups as per following

Group 1: Normol

Receive standard food pellets and water ad libitum for the period of 28 days

Group 2: Diseased

Receive standard pellets and water ad libitum for 28 days and alloxan 200 mg/kg i.p. at initial day.

Group 3: Standard

Receive standard food pellets and Glibenclamide(2mg/kg oral) for the period of 28 days.

Group 4: Aqueous

Receive standard food pellets and Aqueous extract (500 mg/kg oral) for the period of 28 days.

Group 5: Ethanol

Receive standard food pellets and petroleum ether extract (500 mg/kg oral) for the period of 28 days.

Acute toxicity studies:

Acute oral toxicity study was performed as OECD – 423 guidelines (acute toxic class method), (Ecobichon 1997). Swiss albino mice (n=6) of female selected by random sampling technique were used for acute toxicity study. The animals were kept fasting for providing only water, after which the extracts were administered orally at the dose levels of 2000 mg/kg body weight by oral feeding needle and observed for 24 hours. If mortality was observed in 2 out 3 animals, then the dose administered was assingned as toxic dose. If mortality was observed in animal, the same dose was repeated again to confirm the toxic dose. If mortality was not observed the procedure was repeated for further higher dose such as 3000 mg/kg of body weight. The Ethanolic and Aqueous extracts of *Phyllanthus Niruri* was found to be non–toxic up to the dose of 2 g/kg and did not cause any death of the tested animals. Animals were observed continuously for 24 hours under the following aspects

1. Behavioral profile: alertness, restlessness, irritability and fearfulness

2. Neurological profile: spontaneous activity, touch, response and gait
3. Autonomic profile: defecation and urination

Biochemical Studies: Parameters Measured

- Serum analytical methods
- Estimation of blood glucose levels
- Physical Methods
- Body weights
- Histopathology
- Pancreas

Estimation of glucose level:

The determination of glucose is one of the most frequently performed tests in a clinical laboratory. The test based on the reducing property of glucose do not measure true glucose, as there are many interferences. Subsequently other chemical and enzymatic methods were developed. Enzymatic methods are preferred because of their reliability and safety. This glucose kit is based on Trinder's method in which Glucose Oxidase and Peroxidase enzymes are used along with the Chromogen 4-Aminoantipyrine and phenol. The method is one step, simple and rapid. It does not have any interference due to reducing substances or hemoglobin, etc.,

Principle: Glucose is oxidized by the enzyme Glucose Oxidase (GOD) to give D-gluconic acid and hydrogen peroxide. Hydrogenperoxide in presence of enzyme Peroxidase (POD) Oxidizes phenol which combines with 4-aminoantipyrine to produce red coloured quinoneimine dye. The intensity of the coloured developed is proportional to glucose concentration in the sample.

Procedure:

Pipette in to clean, dry tubes labeled blank (B) standard (s) and test (T) and add the reagents in the following order.

S.No		B	S	T
1	Glucose reagent (ml)	1.0	1.0	1.0
2	Distilled water (ml)	0.01	---	---
3	Standard	---	0.01	---
4	Serum/plasma/CSF (ml)	---	---	0.01

Mix well and incubate at 37° c for 10 min. or at R.T. for 20 min. measure the absorbance of test (T) and standard (S), against blank (B) on the photo colorimeter with green filter or on spectrophotometer at 505 nm.

3. Results and Discussion

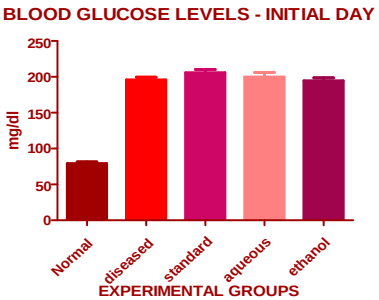

Fig.2. Blood Glucose Levels - Induction Day

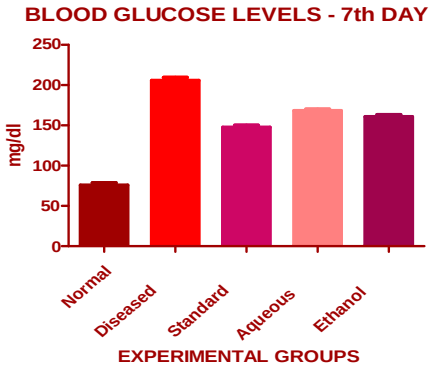

Fig.3: Blood Glucose Levels on 7th Day

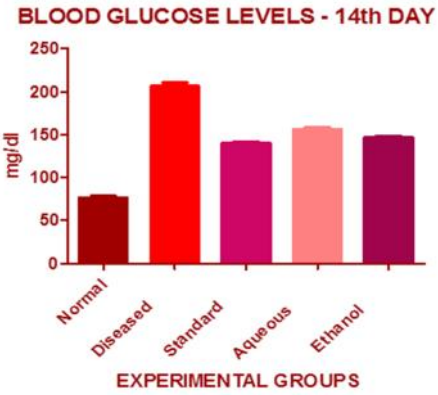

Fig.4. Blood Glucose Levels on 14th Day

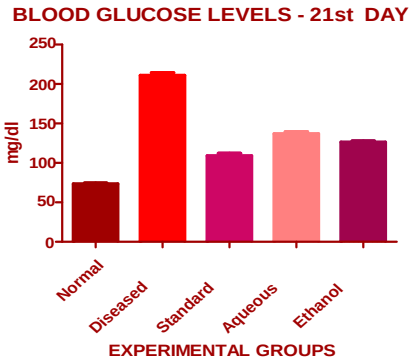

Fig.5. Blood glucose levels on 21st day

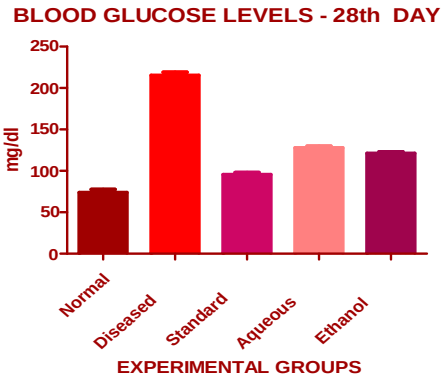

Fig.6: Blood Glucose Levels on 28th Day

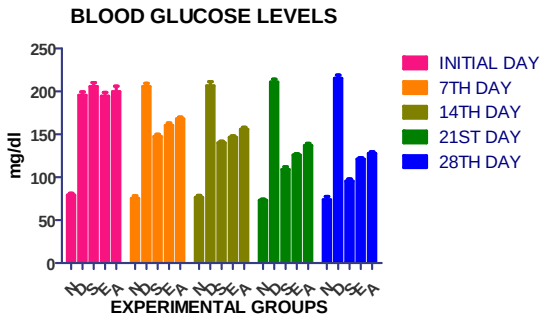

Fig.7: Serum Blood Glucose Levels of All Groups

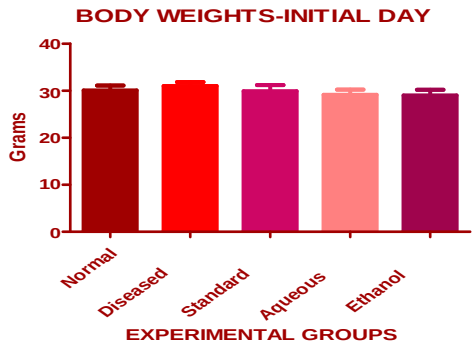

Fig.8: BODY WEIGHTS - INITIAL DAY

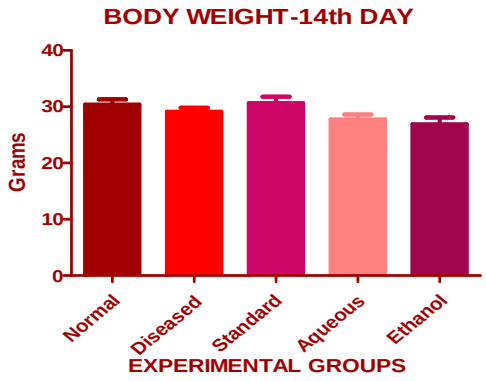

Fig.9: Body weights – 14th day

Discussion

People on all continents have used hundreds to thousands of indigenous plants for treatment of ailments since prehistoric times. According to World Health Organization, about 80% of the world's population presently uses phytotherapy for some aspect of primary health care system. A large number of world's population who live in developing countries can not take the benefits of modern pharmaceuticals as those are very expensive. Hence, phytotherapy is still a popular means of primary healthcare for which people bear a little or no cost. Among the 120 active compounds currently isolatedfrom the higher plants and widely used in modern medicine today, 80 percent show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived. Approximately 25 percent of modern drugs used in the United States have been derived from plant origins. So, research on phytotherapy has got great momentum in recent years to find out noble pharmaceuticals.38-39

Diabetes mellitus is an metabolic disorder with multiple etiology characterized by hyperglycemia disturbances of carbohydrate, protein and fat metabolism, resulting from inadequate insulin secretion or inadequate insulin supply to the target tissues or combination of both. Diabetes is also considered to be ice-berg disease. Diabetes has plagued humans from antiquity and constitutes a major health problem may leads to so many complications. Despite dramatic progress in both medical areas still management of diabetes not complete. The goal of the medical treatment is the controlling of diabetes with synthetic drugs. Each suffers from their own disadvantages and side effects, still offering a wide scope of research in this particular area. Many research laboratories are pursuing investigations in antidiabetic or hypoglycemic in both the preclinical and clinical areas. The aim of present study is also an attempt in the direction. Despite considerable effort on the part of a number of investigators, there has been only a limited success in developing an ideal animal model of diabetic disease that faithfully mimics hyperglycemia. Various procedures have reported by numerous investigators from time to time such as drug induced diabetes in experimental models.

Our present study revealed that methanol leaves extract of PHYLLANTHUS NIRURI has significant effect in lowering fasting blood glucose level in Alloxan induced diabetic mice. The experimental model selected for the present study is administration of Alloxan 200 mg/kg body weight through intraperitoneal route for two day because to reduce the mortality rate. The present study on diabetes was carried out on the male swiss albino mice. The project was aimed to study the antidiabetic activity of leaf extracts of Phyllanthus Niruri in Alloxan induced diabetes. Serum blood glucose levels were anazyled by using auto analyzer. Histopathological studies were carried out to conform the biological changes. In the present study administration of Alloxan significant increase of blood glucose levels in Diseased, Standard, Ethanolic and Aqueous groups. Administration of seed extracts of PHYLLANTHUS NIRURI shown significant decrease in the ethanolic and Aqueous groups. Thus seed extracts of Phyllanthus Niruri shows hypoglycemic potential effect.

4. Conclusion

The aqueous and ethanolic extracts of *phyllanthus niruri* had hypoglycemic potential effect. The results revealed that *phyllanthus niruri* possess significant hypoglycemic potential effect in alloxan induced diabetic mice. Futher studies are necessary to elucidate in detail the mechanism of action of the medicinal plant at the cellular and

molecular levels. These extracts also showed improvement in parameters like body weights and serum blood glucose levels.

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