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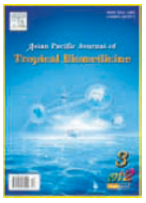

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Antihyperglycemic Effect on Chronic Administration of Butanol Fraction of Ethanol Extract of *Moringa Stenopetala* Leaves in Alloxan Induced Diabetic Mice

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ABSTRACT

Objective: The present study was conducted to evaluate the antihyperglycemic activity on chronic administration of the butanol fraction of the ethanol extract of *Moringa Stenopetala* leaves in alloxan induced diabetic mice. **Methods:** The mice were grouped in four groups; Normal control, Diabetic control, Butanol fraction treated and standard drug treated groups. The Diabetic mice received the butanol fraction of *Moringa stenopetala* daily for 28 days. **Results:** The butanol fraction of *Moringa stenopetala* treatment resulted in significant reduction of fasting blood glucose level, serum total cholesterol and triglycerides level. This fraction also showed a tendency to improve body weight gain in diabetic mice. Its oral LD50 was found to be greater than 5000mg/Kg indicating its safety in mice. **Conclusions:** Though the mechanism of action of *Moringa stenopetala* seems to be similar to that of sulfonylureas, further studies should be done to confirm its mechanism of antidiabetic action. Furthermore the active principle(s) responsible for the antidabetic effects should also be identified.

1. Introduction

Diabetes mellitus (DM) is a systemic metabolic disease characterized by hyperglycemia, hyperlipedemia, hyperaminoacidemia and hypoinsulinaemia, and leads to reduced insulin secretion, insulin action, or both [1]. It is frequently associated with development of micro and macrovascular diseases which include neuropathy, nephropathy and cardiovascular as well as cerebrovascular diseases [2]. The disease is associated with reduced quality of life and increased risk factors for mortality and morbidity. The long-term hyperglycemia is an important factor in the development and progression of micro and macrovascular complications [3].

The local communities residing in the biodiversity-rich areas of the southern region of Ethiopia have traditionally used and relied on plants for treating various ailments. In many cases, local knowledge of medicinal plants remains poorly documented in scientific literature. These plants have found a prime place in the indigenous system of medicine and are in focus for evaluation of their active ingredients. *Moringa stenopetala* is one of these medicinal plants which is widely used for antidiabetic purpose in the area and supported by Makonnen and coworkers for its hypoglycemic effect [4].

In the present study bioactivity guided fractionation and antihyperglycemic activity evaluation; acute oral toxicity test; change in body weight; serum triglyceride and total cholesterol were carried out in butanol fraction in long term use.

2. Methods and materials

2.1. Collections and preparation of plant materials

The leaves of *Moringa stenopetala* was collected from Wolaitta zone, South Nation's Nationalities Peoples Region, 400 kilometer south of Addis Ababa. After collection, the

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plant was identified and authenticated by Dr Getachew Addis, a taxonomist, and deposited in herbarium of Ethiopian nutrition and health research institute (ENHRI) with a voucher number AL-001. It was then dried under shade and crushed to powder for extraction. The study was carried out in EHNRI from November 2010 to April 2011

2.2. Chemicals and instruments

Alloxan(Sigma, Alderich, Germany), Ethanol(Alpha chemicals, India),n-Hexane(wagtech international Ltd, England), Glibenclamide(glitisol, Cyprus), n-Butanol(Blulex, India), Dichloromethane(Alderich, Germany), Humaster 80 automated chemistry analyzer (Humostar 80, Germany), Rota vapor (buchi rota vapor vac R-500, Switzerland), GLAB glucometer(Roche diagnostic, Germany) and GLAB active glucose test strip(Rochi diagnostic, Germany) were used in this study.

2.3. Preparation of plant material extract

The powdered leaves (1.2 Kg) were extracted by percolation using 70 %(v/v) ethanol, and the mixture was then filtered using Whatmann filter paper no. 1. The extract was dried by evaporation using rotary vaporizers under reduced pressure at a temperature of 40-45℃. The residue filtrate obtained was then dried by steam bath at 40℃ and kept in refrigerator at 8℃. The yield of the extract was 19.6% in weight in weight (w/w).

2.4. Solvent-solvent fractionation of the total ethanol extract

The procedure for solvent-solvent separation was adopted from Ranjan (2002) with some modification. Ten percent (w/v) of ethanol extract of the plant was prepared with mild hot distilled water. The dissolved ethanol extract was partitioned with n-hexane (3 x 50), dichloromethane (3 x 50) and n-butanol (3 x 50) using separatory funnel successively until the extracting solvent become colorless. After completing the separation process, the solvents were recovered by Rota Vapor. The separates were dried by steam bath at 40℃ and kept in the refrigerator for the experiments. The yields of n-hexane, dichloromethane, and n- butanol were 1.6%, 0.4%, and 7.1% (w/w), respectively.

2.5. Pharmacological and Toxicological evaluation

2.5.1. Animals

The Swiss albino mice of both sexes weighing 18-25g each were used for the study. The animals were obtained from animal department of ENHRI, kept under standard conditions (at a temperature of 22 ± 2 ℃, and with 12 hr light/ 12 hr dark cycle) and provided with free access to standard pellet laboratory diet and water ad libitum. The experimental protocol was approved by the Institutional review board (IRB) of Addis Ababa University, School of medicine with protocol number 097/10/pharm.

2.5.2. Induction of experimental diabetes

Eight mice were randomly selected as normal controls; the remaining mice were fasted overnight with free access to water, and then injected intraperitonially with alloxan 150mg/kg body weight dissolved in normal saline solution. All the animals had free access to water and pellet diet after thirty minutes of administration of Alloxan. Seven days latter, the fasting blood glucose levels of mice were determined using glucose oxidase method with glucose analyzer. A blood glucose level greater than 200mg/dl was defined as DM. Alloxan induced diabetic mice were selected and divided in three groups; negative control, positive control, and test group.

2.5.3. Study on long-term effect of n-butanol fraction on blood glucose levels

The alloxan induced diabetic test group mice were administered with 500mg/kg body weight of butanol fraction of *Moringa stenopetala* daily for 28 days via oral gavage. The normal control mice were given 10ml/kg of body weight of normal saline via oral gavage. The negative control and positive controls among the diabetic mice were given 10ml/Kg body weight of normal saline and 0.66mg/kg of body weight of Glibenclamide via oral gavage, respectively. On days 0, 7, 14, 21, and 28 the blood samples were collected from tail vein following overnight fasting, and blood glucose levels were measured. The body weight of each mouse was also measured.

2.5.4. Assay of serum triglyceride (TG) and total cholesterol (TC) level

On day 29, the mice were fasted overnight, blood samples were collected in sterile tubes by cardiac puncture under ether anesthesia and left to stand at room temperature for 2h, then centrifuged at 1500xg for 15 minutes at 4℃. The supernatant was immediately separated from the whole blood to prepare serum samples in order to determine TG and TC using automated chemistry analyzer (Humostar 80, Germany).

2.5.5. Acute Toxicity studies with butanol fraction

Acute toxicity study was performed on Mice of either sex selected at random. The animals were kept fasting over night providing only water. They were divided in to four groups, six animals in each group (three males and three females), and then the fraction was administered orally in an increasing dose level of 300, 2000, 5000mg/kg via oral gavage according to the guidelines of the Organization for Economic cooperation and Development [5]. Animals were kept under close observation for 4 hours after administering the fraction for behavioral, neurological and autonomic profile and then they were observed for any change in the general behavior