Experimental Research

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Effect of Ethyl Acetate Extract of *Melothria Perpusilla* on Oral Glucose Tolerance Test in Albino Rats

Pharmacology Section

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ABSTRACT

Introduction: Diabetes mellitus is a group of common metabolic disorders sharing the phenotype of hyperglycaemia. Certain disadvantages like side effects or less efficacy limit the optimal use of antidiabetic drugs.

Aim: To evaluate the effect of Ethyl Acetate Extract of *Melothria Perpusilla* (EAEMP) on oral glucose tolerance test in albino rats.

Materials and Methods: Six healthy albino rats weighing between 100-150 g were selected. The same set of six animals were used for the experiment throughout and successive tests were conducted after a drug wash out period of 10 days. Fasting blood glucose samples were measured using glucometer. A 2% gum acacia suspension {10 ml/kg per oral (p.o.)} was given in all six albino rats followed by the oral glucose load of 3g/kg. Glucose concentrations were estimated at one hour and two hour after the glucose load. Using the same set of animals, similar tests were repeated with the test dose of 250 mg/kg and 500 mg/kg of EAEMP and glibenclamide (0.5 mg/kg p.o.). In this experiment, glucose was given immediately at the dose of 3 g/kg p.o. after the treatments. Drug wash out period of 10 days was maintained in between the successive tests to avoid the interference of action of the drug with the other. The non parametric data were analysed by Kruskal Wallis test.

Results: EAEMP produced a significant increase in the oral glucose tolerance test when compared with control and standard.

Conclusion: Treatment with *Melothria perpusilla* lowers the blood glucose level due to higher oral glucose tolerance possibly due to release of insulin from the pancreas.

Keywords: Animal experiment, Diabetes mellitus, Glibenclamide, Glucose tolerance

INTRODUCTION

Diabetes Mellitus (DM) is a group of common metabolic disorders characterised by hyperglycaemia and caused by complex interactions between genetics and environmental factors. Reduced insulin secretion, decreased glucose utilisation, and increased glucose production are among the various factors which cause hyperglycaemia [1]. The pathological changes that occur in the tissues in diabetes mellitus is due to hyperglycemia [2]. It is considered to be the key contributor to microvascular complications like retinopathy, neuropathy and nephropathy and is also one of several risk factors associated with cardiovascular disease, insulin resistance or deficiency, free fatty acidemia, hypertension, hyperlipidemia and inflammation [3]. New antidiabetic drugs are currently under development and their application in pharmacotherapy of diabetes is being investigated [4]. Several plants used as antidiabetic remedies are found to possess hypoglycaemic effects and this has been confirmed [5], and studies have been carried out to analyse the mechanism of their hypoglycaemic actions. Besides, they can also be used to treat various types of secondary complications due to DM [5]. The antihyperglycaemic effects of these plants are found to be due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or by inhibiting the intestinal absorption of glucose or due to the facilitation of metabolites in insulin dependent processes. There are more than 400 plant species having hypoglycaemic activity available in literature, but there is still a need to discover new antidiabetic drugs from natural plants as they contain substances which demonstrate alternative and safe effects on DM. These substances include glycosides, alkaloids, terpenoids, flavonoids, cartenoids, etc., [6]. Oral glucose tolerance test provides the information on insulin secretion and action but it does not directly yield a measure of insulin sensitivity [7].

Melothria perpusilla is a medicinal plant endemic in the north eastern part of India including Manipur. People of Manipur have used this plant for the curative purpose of diseases such as jaundice and kidney ailments. Its roots are used with milk for treating fever and diarrhoea while its fruits have antihelminthic activity and are also used for demulcent action [8].

Melothria perpusilla is a non aromatic plant belonging to Cucurbitaceae family. It is monoecious climber with striate and glabrous stem. It has heart shaped leaves and smooth, globose fruits which turn red in colour after they ripe; seeds have smooth appearance and flat structure. Roots are oblong flattened and tuberous. It is popularly known as “Lamthabi” in Manipuri and “Bankundri” in Hindi and has been used traditionally in therapeutics since ages [9]. The active ingredients isolated from *Mellothria perpusilla*, used for the treatment of jaundice in Manipur, are sterols and glycosides [8].

Significant scientific literatures to substantiate the various traditional therapeutic uses of plants are unavailable. Hence, in search of a naturally occurring antidiabetic drug which may be relatively safer with minimal adverse effects, the study was conducted to explore the potential antidiabetic property of *Melothria perpusilla* which is being commonly used as an antidiabetic by the local population of Manipur.

Another part of this experimental study using the same plant species has already been published by the same authors [10]. In that part the effect of EAEMP on intestinal absorption of glucose in albino rats has been studied.

MATERIALS AND METHODS

Place of study: This was an experimental study conducted completely in the chemical laboratory of Department of Pharmacology, Regional Institute of Medical Sciences (R.I.M.S.), Imphal, Manipur.

Ethical clearance: Approval was obtained prior to the initiation of the study from “Institutional Animal Ethics Committee” (IAEC), R.I.M.S., Imphal, Manipur. Registration No. 1596/GO/a/12/CPCSEA.

Experimental animals: Six healthy adult albino rats of either sex weighing between 100-150 gm were obtained from the animal house located in R.I.M.S., Imphal. Rats were housed in animals cages and acclimatised in the departmental animal house at room temperature, relative humidity (55±5%), and light (12 hour light/dark cycles) for complete one week before using in experimental procedure. Standard pellet diet was used for feeding animals and water ad libitum.

Collection and identification of plant: *Melothria perpusilla* plants were collected from R.I.M.S., compound located in Lamphel area of Imphal West District, Manipur during the months of June-August, 2015. Prof. H. Nandiram Sharma, Professor (retired) of Botany, Department of Life Sciences, Manipur University, Canchipur identified and authenticated the plants.

Preparation of plant extract: *Melothria perpusilla* plants were collected from R.I.M.S., compound located in Lamphel area of Imphal West District, Manipur. The aerial portions of the plant were separated and completely air dried in the shade, which was powdered and then subjected to defatting process using petroleum ether solvent {Boiling Point (B.P.) 40°-60°}. Plant material was then emptied, washed thoroughly with 95% ethanol and adherent solvent was evaporated by spreading the plant materials over a large surface area. The dried material was later repacked and final plant extract for the experiment was obtained using ethyl acetate solvent [11]. Soxhlet apparatus was used for obtaining the plant extract [12]. The calculated yield at the end of extraction was 0.36%.

Phytochemical studies: EAEMP was subjected to preliminary qualitative phytochemical screening. Tests to detect flavonoids include formation of a yellow precipitate on addition of few drops of lead acetate solution to a small quantity of the extract or formation of yellow precipitate on addition of few drops of sodium hydroxide to the extract which become colorless on addition of dilute acid.

Acute toxicity study: Local Meitei population used *Melothria perpusilla* since thousands of years for treatment of various common diseases. Hence, we directly conducted the limit test of the test plant extract. As there was no mortality at a single dosage of 2000 mg/kg p.o.of EAEMP when given to the albino rats, dosages of 250 mg/kg and 500 mg/kg were fixed for the study [13].

Experimental treatment of animals: Six healthy albino rats weighing between 100-150 g were selected for this study and the test was conducted by the method adopted by Puri D and Baral N [14]. In this study, the same set of six animals were used for the experiment throughout and the successive tests were conducted after a drug wash out period of 10 days in between the administration of the drugs, to avoid the interference of the action of one particular drug with the other. Fasting blood glucose samples were collected and measured using glucometer. 2% gum acacia suspension (10 ml/kg p.o.) was given in all six albino rats followed by the oral glucose load of 3 g/kg. A 2% gum acacia was used as suspending agent for uniform distribution of the test drug in distilled water due to greasy nature of the test drug [15]. Glucose concentrations were estimated at one hour and two hour after the glucose load. Using the same set of animals, similar tests were repeated with the test dose of 250 mg/kg and 500 mg/kg of EAEMP and glibenclamide (0.5 mg/kg p.o.). In this experiment, glucose was given immediately at the dose of 3 g/kg p.o. after the treatments. Drug wash out period of 10 days was maintained in between the successive tests.

Blood collection: Blood was collected from the orbital sinus by capillary tube following engorgement of retro-orbital sinuses by pressing the jaw behind the angle with the thumb. The capillary tube was inserted at medial canthus into retro-orbital plexus with the gentle rotation for free flow of blood [16].

Blood glucose estimation: The blood glucose level was measured using glucometer (ACCU-CHEK® Active strips in Accu-Chek® Active test meter, Roche Diagnostics, Germany). Blood sample required for the estimation of glucose level in glucometer is as small as 1-2 microlitres and the time for the result with test strip in the glucometer is approximately five seconds.

STATISTICAL ANALYSIS

The blood glucose levels were expressed as Mean±Standard deviation. Kruskal Wallis test was used for the interpretation of non parametric data. IBM Statistical Package for Social Sciences (SPSS) software version 23.0 was used for analysis of the data and $p<0.05$ was considered statistically significant.

RESULTS

The effect of EAEMP on oral glucose induced hyperglycaemia was studied in the albino rats.

Blood glucose level (mg%) in the overnight fasted albino rats of (a) control, (b) test 1 (250 mg/kg), (c) test 2 (500 mg/kg), (d) standard drug (glibenclamide 0.5 mg/kg) groups were 71.64±1.54, 71.83±1.74, 69.33±1.96, 70.67±2.88 respectively. Blood glucose level (mg%), after one hour treatment of control (gum acacia followed by oral glucose load) and then after test 1 (250 mg/kg EAEMP), test 2 (500 mg/kg EAEMP) and standard (glibenclamide 0.5 mg/kg) which were administered after the drug wash out period of 10 days on each occasion were 108.83±7.18, 95.17±3.27, 89.17±5.91, 87.83±4.23 respectively. The increase in value from the fasting blood glucose level was 37.19 in the control group and 23.34, 19.84, 17.67 in the test 1, test 2 and standard group respectively. The increase in the blood glucose level after two hour of treatment conducted in the same way was 40.19 in the control group and 19, 15.84, 12.5 in the test 1, test 2 and standard group respectively.

Test 1 and Test 2 showed significant reduction in the blood glucose level ($p<0.05$) when compared with control at two hour. The standard group also showed significant reduction in blood glucose level ($p<0.05$) when compared with the control [Table/Fig-1].

	Blood glucose (Mean±SD)		
	Fasting	1 hour	2 hour
Control	71.64±1.54	108.83±7.18*	111.83±2.65*
Test 1	71.83±1.74	95.17±3.27	90.83±4.54†
Test 2	69.33±1.96	89.17±5.91†	85.17±6.94†
Standard	70.67±2.88	87.83±4.23†	83.17±5.38†

[Table/Fig-1]: Effect of EAEMP on oral glucose tolerance test.
Values are Mean±SD
* $p<0.05$ when compared with standard
† $p<0.05$ when compared with control (Kruskal Wallis test)

DISCUSSION

In the above study, 250 mg/kg and 500 mg/kg of EAEMP caused significant reduction in the blood glucose level ($p<0.05$) when compared with control (2% gum acacia suspension (10 ml/kg p.o.) at two hour. Glibenclamide (0.5 mg/kg p.o.) also caused significant reduction in blood glucose level ($p<0.05$) when compared with the control. The blood glucose level was measured using glucometer which is portable, easy, convenient and relatively cheaper for use. Oral glucose load is considered to be the best test for the endocrine pancreatic function [17]. In the above method, the same animals served as their controls and this method may be considered to be more meaningful and sensitive one. The blood glucose lowering effect of EAEMP was tested using the glucose load (3 g/kg p.o.), a strong stimulus to stimulate pancreatic beta cells to secrete insulin. Plasma insulin level following oral glucose is approximately twice as great as following intravenous glucose [18]. The test provides an indirect idea about insulin secretion. The antidiabetic potential of natural products classified into terpenoids, alkaloids, flavonoids, phenolics, etc. is through the insulinomimetic activity of the plant extract. Flavonoids and polyphenols were found to be effective due to some other extrapancreatic mechanisms [5]. Glibenclamide, an insulin secretagogue was used as a standard drug at the dose 0.5 mg/kg p.o. The statistically significant reduction of one hour of blood glucose in test 2 as compared to control and glibenclamide treated group after glucose load, indirectly proved its insulin secretagogue

activity. Patane G et al., in their study found that beta cells become more sensitive to glucose after a short exposure to glibenclamide and concluded that this effect may contribute to the mechanism of action of this drug in stimulating insulin release [19]. Normally, after glucose load, glucose concentration peaks within one hour and then comes down to normal fasting level by 2-2.5 hours [20]. Test 1 (250 mg/kg p.o.) failed to produce significant reduction in the one hour time duration after the glucose load in comparison with the control. The findings possibly indicated that the test 1 (250 mg/kg) did not possess glibenclamide like activity. Reduction in the blood glucose level after one hour of giving EAEMP-500 mg/kg was found to be comparatively more than that of EAEMP-250 mg/kg. At two hour blood glucose estimation, both test 1 and test 2 showed significant reductions in the blood glucose level and increased the glucose tolerance possibly by the release of insulin from pancreas. Many traditional plants used for treatment of diabetes are considered to be less toxic and free from side effects than synthetic medications. Based on the World Health Organisation (WHO) recommendations, hypoglycaemic agents of plant origin used in traditional medicine are considered important. Most of these plants cause hypoglycaemic effects due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or a decrease in the intestinal absorption of glucose, thereby having an effect on protecting β-cells and smoothing out fluctuation in glucose levels. Though the specific modes of action in the treatment of diabetes is not known, most plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc., that are frequently implicated as having antidiabetic effects [6].

LIMITATION

Small sample size due to non availability of a large number of animals and issues of animal cruelty was the limitation of our study. Further studies on a larger scale may help to substantiate our findings.

CONCLUSION

In the above study, the EAEMP at the dose of 250 mg/kg and 500 mg/kg was found to cause significant increased tolerance for glucose, possibly by the release of insulin from pancreas. Projects funded by the Government to explore the traditionally used medicinal plants for DM may be undertaken which may bring about a turning point towards the discovery of a safe and effective drug for the disease, the incidence of which is rapidly increasing day by day with its significant morbidity and mortality.

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