INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF *MICHELIA NILAGIRICA* IN WISTAR ALBINO RATS

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ABSTRACT

Michelia nilagirica belonging to the family Mangoliaceae is commonly used by many traditional healers in most of the herbal preparations for diabetes and kidney diseases. Different fractions isolated from ethanolic extract of whole plant of *Michelia nilagirica* is investigated for the antidiabetic activity in wistar albino rats. An acute treatment of isolated fractions on streptozotocin induced diabetes wistar albino rats, monitored blood glucose level at 1st, 2nd, 3rd, 4th and 5th hr time intervals. 72.6% and 54.7% of glucose reduction with fractions D, A (dose 100 mg/kg of body weight) at 5th hr after oral administration in diabetic rats. It can be concluded that the hypoglycemic activity of ethanolic extract of *Michelia nilagirica* could be due to terpenoids in the fractions.

Keywords: *Michelia nilagirica*, Streptozotocin, Terpenoids.

INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder, has now become an epidemic, with a worldwide incidence of 5% in the general population. The number of people suffering from diabetes has soared to 246 million and the disease now kills more people than AIDS[1]. Decreased physical activity, increasing obesity, stress and changes in food consumption have been implicated in this increasing prevalence in the past two decades[2]. In conventional therapy, Type I diabetes is treated with exogenous insulin and Type 2 with oral hypoglycemic agents (sulphonyl ureas, biguanides etc)[3]. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes, there is an increased demand by patients to use natural products with antidiabetic activity[4]. Since time immemorial, patients with non-insulin dependent diabetes have been treated orally in folk medicine, with a variety of plant extracts. In India, a number of plants are mentioned in ancient literature for the treatment of diabetic conditions.

Flower buds of *Michelia champaca* Linn. belonging to the family Magnoliaceae is commonly used by many traditional healers in most of the herbal preparations for diabetes[5] and kidney diseases[6]. Traditionally, it is being used in fever, colic, leprosy, post partum protection[7] and in eye disorders[8]. It has been reported to possess antipyretic, anti-inflammatory[9], insecticidal[10], antimicrobial[7] and leishmanicidal activities[11]. The active constituents reported in this plant are alkaloids, saponins, tannins, sterols, flavonoids and triterpenoids[7].

In Ayurveda, traditional usages of plants are most commonly in the form of their aqueous extracts only. Concurrently, some of the papers searched focus testing plants in their ethanolic or aqueous extracts and some have also reported activity in petroleum ether, benzene and chloroform extracts[12-14]. Keeping these facts in view, the present study was undertaken to create a scientific base for the use of the extract of *M. nilagirica* as an antidiabetic in diabetes associated complications, and to identify the active antidiabetic.

MATERIALS AND METHODS

Collection of plant material

The whole plant of *Michelia nilagirica* was collected from the deciduous forest of Tirumala Hills in Andhra Pradesh State, India. Samples were authenticated by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, India. The whole plant of *Michelia nilagirica* were sorted, cleaned and air-dried at room temperature for one week. These were ground to powder using the laboratory hammer mill. Powdered samples were collected and stored in air- and water-proof containers protected from direct sunlight and heat until required for extraction.

Preparation of extracts

The powdered materials of *Michelia nilagirica* (whole plant) were extracted successively with petroleum ether, ethyl acetate, chloroform, ethanol and distilled water in soxhlet apparatus, each for 18 hours. The extracts were concentrated to dryness in rota evaporator till free from the solvents.

Isolation of fractions

Thin-layer chromatography method was carried out using silica gel aluminum plate 60F-254, 0.5 mm (TLC plates, Merck). The spots were visualized in UV light and 10% of H_2SO_4 in methanol. The ethanolic extract was subjected to column chromatography (silica gel 60-100) for further purification. The column was equilibrated for one hour with petroleum ether at flow rate 5 ml/min. The sample was (2 gm dissolve in acetone) loaded on to the column, 8 fractions were collected using petrolium ether:ethyl acetate (4:1), petroleum ether:ethyl acetate (1:1), petroleum ether:ethyl acetate (2:3), ethyl acetate (100%), chloroform:methanol (9:1), chloroform:methanol (1:1) and chloroform:methanol (2:8).

Above yielded product were pooled into five fractions based on TLC. The yield and appearance of the five fractions was fraction A 50 mg/gm & yellow, fraction B 300 mg/gm & black, fraction C 150 mg/gm & green, fraction D 200 mg/gm & darkish brown and fraction E 150 mg/gm & saffron.

Phytochemical analysis

Phytochemical analysis of fractions was carried out for the presence of alkaloids, tannins, saponins, glycosides, terpenoids, carbohydrates, flavonoids, proteins, amino acids, fixed oils, steroids & sterols by different methods and the results are shown in the Table 1.

Experimental design

Albino rats of wistar strain weighing 150-200 gm were purchased from National Institute of Nutrition, Hyderabad. The rats were kept in polypropylene cages (3 in each cage) at an ambient temperature of 25±2°C and relative humidity of 55–65%. A 12 h light and dark schedule was maintained in the air conditioned animal house. All the rats were fed with common diets for 1 week after arrival and then divided into groups with free access to food and water.

Streptozotocin induced diabetic rats

Rats were fasted overnight before inducing diabetes with streptozotocin (STZ)[15]. The rats were given an intraperitoneal injection of streptozotocin (45 mg/kg) freshly prepared in 0.1M sodium citrate buffer. The diabetic state was confirmed 48 h after streptozotocin injection[16]. Threshold value of fasting blood glucose was taken as > 350 mg/dl.

Evaluation of hypoglycemic effect

Male wistar normoglycemic[17] rats (150-200 g) were used in the experiment. All experiments were carried out using six animals per group.

Group 1: Normal group (Tween 80)
Group 2: Diabetic rats
Group 3: Diabetic rats + Glibenclamide (20 mg/kg)
Group 4: Diabetic rats + Fraction A (10 mg/kg)
Group 5: Diabetic rats + Fraction A (100 mg/kg)
Group 6: Diabetic rats + Fraction B (10 mg/kg)
Group 7: Diabetic rats + Fraction B (100 mg/kg)
Group 8: Diabetic rats + Fraction D (10 mg/kg)
Group 9: Diabetic rats + Fraction D (100 mg/kg)
Group 10: Diabetic rats + Fraction E (10 mg/kg)
Group 10: Diabetic rats + Fraction E (100 mg/kg)

Blood samples were collected from the tail vein at 0, 1, 2, 3, 4 and 5 hr after treatment administration. Blood glucose concentration was estimated by enzymatic glucose oxidase method using a commercial glucometer (Accu Chek Active). The percentage variation of glycemia for each group was calculated in relation to initial (0 h) level, according to:

$$\% \text{ glycemic change} = G_0 - G_t/G_0 \times 100$$

Where G_0 were initial glycemia value and G_t were the final glycemia value

Statistical analysis

Values are expressed mean±standard error of mean (SEM). In case of *in vivo* studies comparison were made between normal and diabetic, diabetic versus diabetic treated

animals. Changes were considered significant if the *P*-value was less than 0.05. Statistical analysis was performed using Graph pad prism with one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Table 1: Phytochemical screening of the various fractions

S. No.	Phytochemicals	Fraction A	Fraction B	Fraction C	Fraction D	Fraction E
1	Alkaloids	----	----	----	----	+
2	Tanins	----	----	----	----	+
3	Saponins	----	----	----	----	----
4	Glycosides	----	----	----	----	----
5	Terpinoids	----	+	----	+	----
6	Carbohydrates	----	----	----	----	+
7	Flavonoids	+	----	----	----	+
8	Proteins	----	+	----	----	----
9	Aminoacids	+	----	----	----	----
10	Fixed oils	----	----	----	----	----
11	Steroids & Sterols	----	----	----	----	----

Antidiabetic activity

Effect of acute treatment of various fractions isolated from ethanolic extract of *Michelia nilagirica* on STZ induced diabetic rats

Table 2 and Figure 1 shows 72.6% and 54.7% of glucose reduction with fractions D, A (dose 100 mg/kg of body weight) at 5th hr after oral administration in diabetic rats. Treatment of Glibenclamide at a dosage of 20 mg/kg.b.wt. Diabetic rats resulted in 87.9% of fall of blood glucose after 5 hrs.

Table 2: Effect of different fractions of ethanolic extract of whole plant of *Michelia nilagirica* on STZ induced diabetic rats

Group	Treatment	Blood glucose levels at different time intervals (hrs)					
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
1	Normal group (Tween 80)	110±2.4	115±2.8	119±1.9	105±3.1	107±1.3	98±1.5
2	Diabetic rats	401±13.4	398±12.4	381±10.9	380±15.4	379±13.1	385±12.1
3	Diabetic rats + Glibenclamide (20 mg/kg)	459±20.1	432±21.1	343±18.5	178±7.3	85±1.4	53±1 (87.9%)
4	Diabetic rats + Fraction A (10 mg/kg)	420±19.9	389±17.2	356±16.9	347±15.1	339±15.6	321±14.7 (23.5%)
5	Diabetic rats + Fraction A (100 mg/kg)	398±14.6	280±12.2	250±13.1	220±15.3	200±8.3	180±9.3 (54.7%)
6	Diabetic rats + Fraction B (10 mg/kg)	410±20.2	399±19.9	351±17.3	311±16.5	296±16.2	287±14.5
7	Diabetic rats + Fraction B (100 mg/kg)	396±15.6	394±14.3	408±20.4	470±18.5	229±12.3	183±15.4 (53.7%)
8	Diabetic rats + Fraction D (10 mg/kg)	350±20.8	314±17.6	250±16.9	244±15.1	215±14.3	198±16.1 (43.4%)
9	Diabetic rats + Fraction D (100 mg/kg)	376±21.3	349±22.3	250±24.2	180±12.3	154±14.9	103±12.3 (72.6%)
10	Diabetic rats + Fraction E (10 mg/kg)	412±20.8	431±17.3	425±23.2	401±20.4	396±15.1	389±18.1
11	Diabetic rats + Fraction E (100 mg/kg)	376±14.2	583±21.3	589±22.1	456±24.3	448±23.5	418±19.8

Group 2 vs Group 1 P<0.001, Group 2 vs Group 3 P<0.05, Group 2 vs Group 4 P<0.05, Group 2 vs Group 5 P<0.05, Group 2 vs Group 7 P<0.05

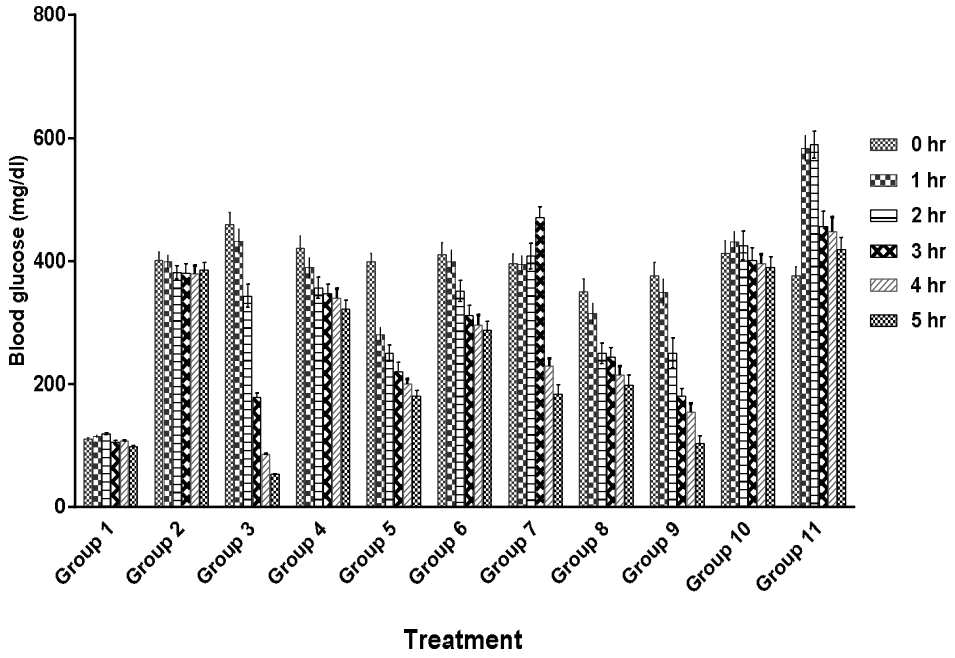

Fig. 1: Effect of different fractions of ethanolic extract of whole plant of *Michelia nilagirica* on STZ induced diabetic rats

Values are given as mean±SEM.
Values were significant between Group 1 and Group 2 P< 0.001, P<0.01 and P< 0.005.
Values were significant between Group 2 and Group 3 P<0.01 and P<0.005.
Values were significant between Group 2 and Group 9 P< 0.01 and P< 0.005.

The present study was taken to examine the antidiabetic activity of different fractions isolated from ethanolic extract of whole plant of *Michelia nilagirica* in wistar albino rats. Fraction D obtained from ethanolic extract of *Michelia nilagirica* whole plant has produced maximum antihyperglycemic activity (72.6%). The onset of antidiabetic activity was observed from second hour after treatment and was continued till the end of fifth hour. The antihyperglycemic activity of the fraction D 100 mg/kg. b.w significantly higher than other fraction of ethanolic extract of *Michelia nilagirica* whole plant.

The phytochemical analysis of fraction D revealed the presence of terpenoids. They have a broad range of biological activities. They function as a powerful anti oxidants and some are reported to have antidiabetic activity. The fraction D which has shown the maximum anti hyperglycemic action was considered as the active fraction containing the active principle of antihyperglycemic activity.

CONCLUSION

The results revealed that *Michelia nilagirica* ethanolic extract possess significant antidiabetic activity in STZ induced diabetic mice. Potent hypoglycemic activity of fraction D could be attributed to valuable terpenoids in ethanol extracts of *Michelia nilagirica* whole plant. Further studies are necessary to elucidate in detail the mechanism of action of the medicinal plant at the cellular and molecular levels.

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