



Anti-diabetic activity of the leaves extracts of *Wrightia Tinctoria* on alloxan induced diabetic rats

Shruthi A, Latha K. P^{*}, Vagdevi H. M, Pushpa B and Shwetha C

Department of P.G. Studies and Research in Chemistry, Sahyadri Science College (Autonomous)
Kuvempu University, Shivamogga 577203, Karnataka, India

ABSTRACT

In the present study, investigation has been carried out to evaluate the effect of the different extracts of the leaves of *Wrightia tinctoria* on alloxan induced diabetic rats of wistar strain. The experiment was carried out using six groups of albino rats. Chloroform extract showed a significant anti-diabetic activity when compared to the standard drug glibenclamide.

Keywords: *Wrightia tinctoria*, anti-diabetic activity, glibenclamide.

INTRODUCTION

Diabetes mellitus is a metabolic disease as old as mankind and its incidence is considered to be high (4-5%) all over the world [1]. It is a chronic disorder caused by partial or complete insulin deficiency, resulting in hyperglycemia leading to acute and chronic complications [2]. The incidence of diabetes mellitus is on rise all over the world. Over time, uncontrolled diabetes can lead to serious damage to the various body systems [3]. In spite of the introduction of hypoglycemic agents, diabetes and related complications continued to be a major medical problem. Synthetic drugs are likely to give serious side effects in addition they are not suitable for intake during conditions like pregnancy [4-6]. Hence, search for a new drug with low cost, more potential, without adverse effects is being pursued in several laboratories all around the world. Since time immemorial, patients with non-insulin requiring diabetes have been treated orally in folk medicine with a variety of plant extracts. Among plants of economic importance, medicinal and aromatic plants have played a vital role in alleviating human sufferings. Plants are utilized as therapeutic agents since time immemorial in both organized (Ayurveda, Unani) and unorganized (folk, tribal, native) forms. Uses of medicinal plants in the industrialized societies have been traced from the extraction and development of several drugs and chemotherapeutic drugs from these plants as well as from traditionally used rural herbal remedies [7]. In India a number of plants are mentioned in ancient literature (Ayurveda) for the cure of diabetic conditions known as 'madhumeha' and some of them have been experimentally evaluated and the active principles isolated [5, 6, 7, 8, 9]. However, search for new anti-diabetic drugs continue.

Wrightia tinctoria belongs to the family apocynaceae commonly called as 'Jaundice curative tree' in South India [10]. It is a small deciduous tree which grows up to 10 meters and seen throughout India and Myanmar. It is grown well in deciduous forest. Leaves are opposite up to 8-15 cm long and lanceolate. Bark is smooth and ivory colored. Flowers are usually seen in the tip of branches, scattered in the inflorescence and whitish with fragrance. Fruits are

pendulous, long paired dark follicles joined at their tips long up to 50cm. Seeds are 1-2 cm long with white hairs. All parts are having white latex [11]. From a distance, the white flowers may appear like snowflakes on the tree.

The juice of the tender leaves is used efficaciously in jaundice and skin disorders. Crushed fresh leaves when filled in the cavity of decayed tooth relieve toothache. In Siddha system of medicine it is used for psoriasis and other skin diseases [12-13]. The leaves are acrid, thermogenic, hypotensive and are useful in odontalgia, vitiated conditions of vata and hypertension. The bark and seeds are bitter, astringent, acrid, thermogenic, carminative, digestive, stomachic, constipating, depurative, anthelmintic, aphrodisiac and febrifuge [14]. The latex of the ripe and unripe fruits is used by hill tribes for coagulating and solidifying milk [15]. Leaves contain beta-amyrin and an indigo yielding glucoside [16].

Five flavanoid compounds; indigotin, indirubin, tryptanthrin, isatin and rutin were isolated and identified from the leaves [17]. A new sterol, 14 α -methylzymo sterol is isolated from seed lipid [18] and a new terpene Wrightial, cycloartenone, cyclooleucalenol, are extracted from methanolic extract of immature seed pods of *Wrightia tinctoria* [19]. Taking into account, in the present study the anti-diabetic evaluation of *Wrightia tinctoria* leaves extracts was performed.

EXPERIMENTAL SECTION

Plant material:

The leaves of *Wrightia tinctoria* were collected in the month of May-June from the fields around Shankaraghatta, Shimoga district, Karnataka. The plant was authenticated by taxonomist Prof. M.S. Pushpalatha, Department of Botany, Sahyadri Science College, Shimoga. Leaves were shade dried and later it was grinded into coarse powder. The powdered material was used for extraction.

Preparation of extracts:

The powdered leaves were extracted with the following solvents based on their polarity such as petroleum ether, chloroform and then with ethanol by soxhlet extraction process. The extract obtained was concentrated in vacuum using rotary evaporator. The dried extract was suspended in 1% Tween-80 and used as vehicle to screen anti-diabetic activity.

Test Animals:

Healthy male and female albino mice weighing (18-23g) and male wistar albino rats (160-200g) were used for the study and were obtained from National College of Pharmacy, Shimoga, Karnataka. These animals were maintained at standard housing conditions and were fed with standard diet (Hindustan lever Ltd., Bangalore) and water ad libitum. All the animal experiments were approved by Institutional Animal Ethics Committee and were done as per their guidelines. (No.- REG. No.144/1999/CPCSEA/dtd:10/04/2000).

Acute Toxicity studies:

Graded doses of 1000, 2000 and 3000mg/kg body weight of the leaves extracts of *Wrightia tinctoria* in normal saline were administered intraperitoneally to albino mice (3 groups of 6 each weighing about 18-23g). They were kept in transparent plastic cages at room temperature. Death was recorded after 24 hr and LD₅₀ values were recorded.

Accordingly, the LD₅₀ of pet ether, chloroform and ethanolic leaves extracts were found to be 2000mg/kg. Hence 1/10th dose of this was considered to be the safe dose for the animals and was selected as the therapeutic dose for the evaluation of anti-diabetic activity of the extracts.

Induction and Screening of anti-diabetic activity using alloxan:

Anti-diabetic effect was evaluated in alloxan-induced diabetic model [20-23]. Male wistar rats were made diabetic by a single i.p. injection of 120mg/kg body weight of alloxan monohydrate in sterile normal saline. The animals were fasted for 16h before experimentation but allowed free access to water. The alloxan induced diabetic rats were divided into six groups. Group I was kept as non-diabetes control, group II served as untreated diabetic control which received vehicle (1% Tween-80 in water), group III, IV and V received pet ether, chloroform and ethanolic extracts at 200mg/kg body weight respectively while group VI received standard drug glibenclamide at 5mg/kg body weight. The diabetic state of the animals was checked by measuring glucose level after 72 hr of alloxan

treatment. The blood samples were drawn from tail vein and glucose levels were determined by one touch profile method using Gluco-meter. The rats with a blood sugar 300mg/dl were selected for the experiment. Blood samples were collected from the tail vein from 0hr, 1hr, 3hr, 5hr and 7hr after drug administration of the dose and were recorded.

Statistical analysis:

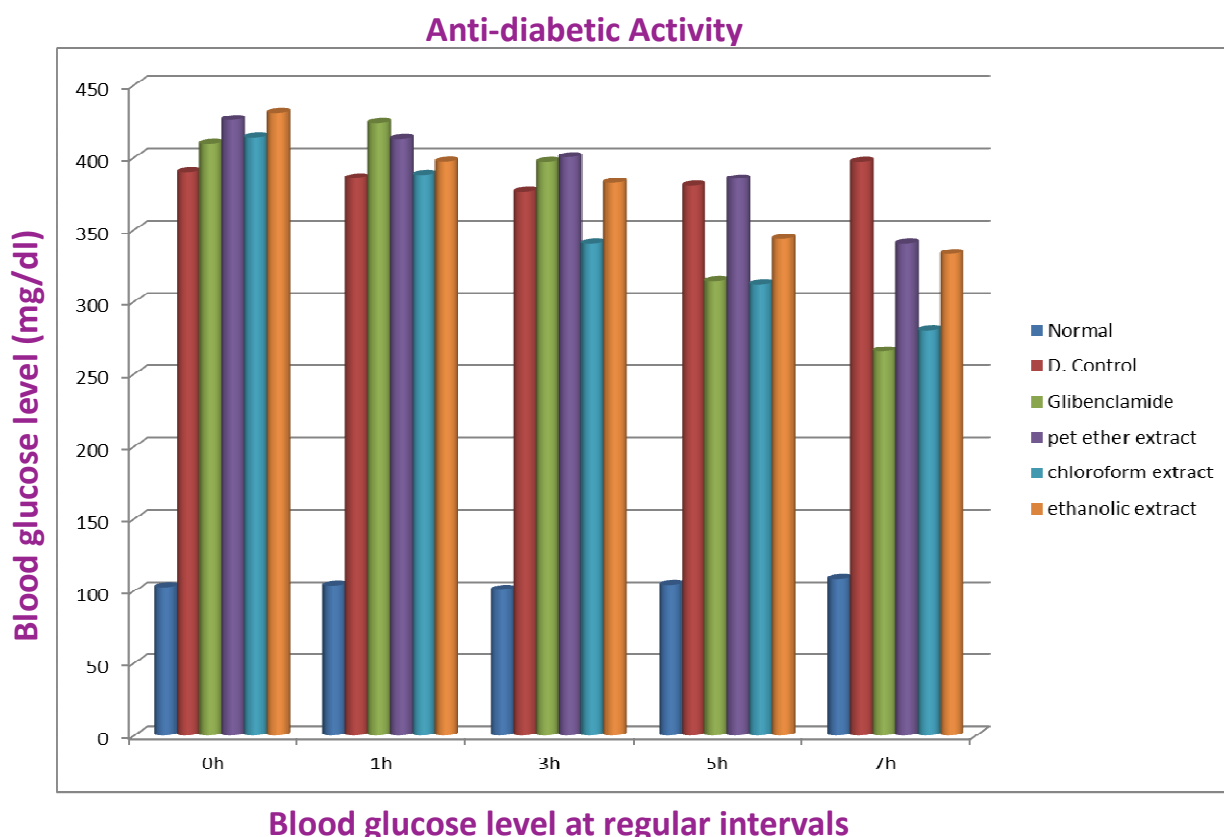
The results are expressed as mean S.E.M. the significant of various treatments was calculated using students t-test and were considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

The effect of leaves extracts of *Wrightia tinctoria* for anti-diabetic activity is tabulated in table 1 and depicted in fig 1. The pet ether and ethanolic leaf extracts of the plant administered at the dose of 200mg/kg body weight did not showed significant anti-diabetic activity in the alloxan induced diabetes rats whereas chloroform extract showed considerable reduction in blood glucose levels. The reduction in glucose levels is significant ($p < 0.001$) in the treated animals at 0hr, 1hr, 3hr, 5hr and 7hr after drug administration.

Table I: Anti-diabetic activity of different extracts of leaves of *Wrightia tinctoria*

Groups	Treatment	Blood glucose level (mg/dl) Time				
		0hr	1hr	3hr	5hr	7hr
1	Normal	102.87±2.67	104±2.50	101.05±3.13	104.5±4.34	108.5±3.69
2	Diabetic Control	390.10±7.65	385.78±8.12	376. ±8.85	380.12±7.84	396.84±7.20
3	Diabetic rats with pet ether extract	425.86±4.43	412.47±13.61	400.23±12.05	385.02±9.5	340.16± 9.32
4	Diabetic rats with chloroform extract	413.41±5.75	388.16±8.59	340.16±9.32	312.13±9.50	280.13±9.46
5	Diabetic rats with ethanolic extract	430.75±2.58	397.00±14.23	382.08±7.94	343.41±17.02	333.0±13.94
6	Diabetic rats with Glibenclamide	409.29±6.82	423.57±14.28	396.84±7.20	314.29±10.63	266.0±8.7



CONCLUSION

Wrightia tinctoria leaves are claimed to be useful in diabetes. Results of anti-diabetic activity of the leaves extracts established the scientific basis for the utility of this plant in the treatment of diabetes. All the three extracts of leaves produced significant reduction in blood glucose level in a dose dependent manner. The chloroform extract showed maximum anti-diabetic activity and is comparable to the hypoglycemic activity of glibenclamide in the diabetic rats. Thus the claims made by the traditional Indian systems of medicine regarding the use of leaves of this plant in the treatment of diabetes were confirmed.

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