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Research Article

ANTI-DIABETIC ACTIVITY OF *SPHAERANTHUS INDICUS* LINN. EXTRACTS IN ALLOXAN-INDUCED DIABETIC RATS

RITESH KHARKAR[1], DEEPAK P. PAWAR[2], PRASHANT B. SHAMKUWAR*[2]

[1]Sharad Pawar College of Pharmacy, Wanadongri, Hingna road, Nagpur, [2]Government College of Pharmacy, Thiba Palace, Ratnagiri India. *Email: shamkuwarp@rediffmail.com

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder. A study was conducted to screening of flowering tops extracts of *Sphaeranthus indicus* for antidiabetic activity in alloxan induced hyperglycemia. The oral administration of ethyl acetate, methanolic and hydroalcoholic extracts of *Sphaeranthus indicus*, given orally at doses of 200 mg/kg/day for 15 days, were found to be produced significant antihyperglyceamic action in alloxan induced diabetic rats, using Glibenclamide as standard. The measurement of produced lipid peroxides (expressed as the amount of thiobarbituric acid (TBA) reactive substance, nmol TBARS/ml serum) indicated antiperoxidative activity of *Sphaeranthus indicus* extracts. The oral administration of methanolic extract to alloxan-diabetic rats significantly decreased the lipid peroxides. All results were compared with the diabetic control groups. The findings obtained in the experiments demonstrated that Gorakhmundi (*Sphaeranthus indicus* L.) possess potent antihyperglycaemic and antioxidant activity.

Keywords: Antihyperglyceamic, Antiperoxidative, Lipid peroxidation, *Sphaeranthus indicus,* Alloxan-induced diabetic rats.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder affecting approximately 5% of the world's population. It is characterized by dysregulation in carbohydrate, protein and fat metabolisms caused by the complete or relative insufficiency of insulin secretion and/or insulin action [1]. According to World Health Organization projections, the diabetic population is likely to increase to 300 million or more by the year 2025 [2]. Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides, glucosidase inhibitors which are used as monotherapy or in combination to achieve better glycaemic regulation. Many of these oral antidiabetic agents have a number of serious adverse effects [3]. Thus, the management of diabetes without any side effects is still a challenge. There is a growing interest in herbal remedies, the use of medicinal plants for the treatment of diabetes mellitus dates back from the Ebers papyrus of about 1550 B.C. A multitude of herbs, spices and other plant materials have been described for the treatment of diabetes throughout the world [4] [5]. Because of perceived effectiveness, minimal side effects in clinical experience and relatively low cost, herbal drugs are widely prescribed even when their biologically active compounds are unknown.

Sphaeranthus indicus Linn belongs to family *Asteraceae*. The plant is commonly known as *Gorakhmundi* in Hindi. It is an annual spreading herb, which grows approximately 15-30 cm in height. The plant is distributed throughout the plains and wet lands in India, Sri Lanka and Australia [6]. All the parts of the plant have medicinal uses. In folk medicine, the plant is reportedly used in treating epileptic convulsions, mental illnesses and hemicranias [7]. According to Ayurveda, this herb is used in medaroga, laxative, digestible, tonic, alterative, anthelminitic and alexipharmic [8]. It is used to treat vitiated conditions of hemicranias, jaundice, hepatopathy, diabetes, leprosy, fever, pectoralgia, cough, gastropathy, hernia, hemorrhoids, helminthiasis, dyspepsia and skin diseases. It is also used as a nervine tonic. The oil prepared using the plant roots are reportedly useful in treating scrofula and as an aphrodisiac. The external application of a paste of this herb is beneficial in treating pruritus and edema, arthritis, filariasis, gout and cervical adenopathy. It also treats piles and hepatitis [9]. A large number of constituents have been isolated from the extracts of the whole herb, flowers and leaves. Essential oil, obtained by steam distillation of the whole herb, contains ocimene, α-terpinene, methyl-chavicol, α-citral, geraniol, α-ionone, β-ionone, d-cadinene, p-methoxycinnamaldehyde and an alkaloid sphaeranthine [10, 11]. The alcoholic extract of powdered caputula contains stigmasterol, β-sitosterol, hentriacontane, sesquiterpene lactone, sesquiterpine glycoside, sphaeranthanolide, flavone and isoflavone glycosides [12, 13, 14, 15]. Recently, many medicinal properties have been attributed to the extracts, fractions and isolated constituents of *S. indicus* flowers, which include hypotensive, peripheral vasodilatory and cathartic activity of alcoholic extract, antimicrobial activity of alkaloidal and nonalkaloidal fractions of alcoholic extract and sesquiterpine isolated from petroleum ether extract [16, 17, 18]. Essential oil, obtained from leaves, possesses antifungal properties [19].

Meanwhile, in diabetics, oxidative stress has been found to be mainly due to an increased production of oxygen free radicals and a sharp reduction of antioxidants defenses and the tissue antioxidant status were an important factor in the development of diabetic complications. On the other hand, a variety of antioxidants scavenges free radicals and prevents oxidative damage to biological structures. Hence, the effect of S. indicus on the levels of oxidative stress parameters like lipid peroxide (LPO) in blood of alloxan - induced diabetic rats were also determined.

Hence, this study is planned to establish scientific data on the validity of the claimed antidiabetic and antioxidant property.

MATERIAL AND METHODS

Plant collection and extraction

Sphaeranthus indicus, collected in November 2006 at Bhandara District, Maharastra and identified taxonomically by Department of Botany; Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur. A voucher specimen has been kept in the same and in our laboratory for future references.

The flowers were dried in shade and pulverized, The powder was treated with petroleum ether for dewaxing in soxhlet apparatus. The defatted powder material again subjected to soxhlet apparatus for successive solvent extraction (ethyl acetate EAE and methanol MAE). After successive solvent extraction, the powder material were kept for maceration using methanol:water (HAE)(1:1), for 7 days, with daily 2 hr. stirring with a mechanical stirrer. After 7 days the extract was filtered through muslin cloth and marc was discarded and its filtrate dried in a hot air oven at 45°C till semisolid mass, was produced. The extracts were concentrated in vacuum giving a brown residue (EAE; yield: 2.88% d.w.), a yellow residue (MAE; yield: 3.78% d.w.), a brown residue (HAE; yield: 4.34% d.w.). Qualitative phytochemical analysis EAE indicated the presence sterols and tannins. MAE indicated the presence of alkaloids, tannins, sugar and proteins and HAE indicated the presence of alkaloids, tannins, saponins, proteins and sugars.

Animals

Albino rats of either sex weighing between 200-250 g were procured from Mahaveera Enterprises, Hyderabad, and were used for the present investigation. Animal Ethical Committee had approved experimental protocol under guidelines of CPCSEA, New Delhi. Animals were housed under standard environmental conditions of temperature, humidity and light; and provided with diet in form of pellets and water *ad libitum.*

Chemicals

A diagnostic kit used for estimation of glucose was obtained from Euro Diagnostic Systems Pvt. Ltd., Chennai.

Instrument

UV spectrophotometer (Schimadzu: UV-1701).

Experimental Design

All the animals were randomly divided in the six groups with six animals in each group. Group I, II and III were normal group administered saline only, diabetic control, and standard drug (glibenclamide, 10 mg/kg per day p.o.) control, respectively. Group IV, V, VI were treated with flower EAE, MET and HAE extracts, administered orally at a dose of 200 mg/kg p.o. in 2% acacia emulsion, respectively.

Assessment of Extracts on Alloxan-Induced Diabetic Animals

Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (Loba Chemie, Bombay: 150 mg/kg) [20]. Alloxan was first weighed individually for each animals according to the weight and solubilized with 0.2 ml saline (154 mM NaCl) just prior to injection. Two days after alloxan injection, rats with plasma glucose levels of >140 mg/dl were included in the study. Treatment with plant extracts was started 48 hours after alloxan injection. Blood sample were drawn from retro. orbital flexus at weekly intervals, till end of study (i.e. 3 weeks). Fasting blood glucose estimation and body weight measurement were done on day of 1, 7 and 21 of the study.

At the 1, 7, 21 day, animals After an overnight fasting, blood samples were withdrawn for biochemical estimations from retro orbital flexus by heparinised heamatocrit capillaries puncture rats under diethyl ether as anesthesia. The blood samples were collected in a clean and dry semi-micro centrifuge tube. The blood was centrifuged at 2500 rpm for 10 min and plasma was separated. The plasma glucose was estimated spectrometrically by using Span Diagnostic Kit (GOD-POD method), Lipid peroxides (thiobarbituric acid reactive substance, TBARS production) were determined according to the previous method [21, 22, 23].

Statistical Analysis

All the values of body weight, fasting blood sugar, biochemical estimations were expressed as mean ± S.E.M. Statistical significance were determined using the one-way ANOVA test followed by Dunnett's test. Values are mean ± S.E.M. when compared with diabetic control, the level of significance was considered at $p<0.05$.

RESULTS

Administration of alloxan (150 mg/kg, i.p) led to 1.5-fold elevation of fasting blood glucose levels, which was maintained for period of 3 weeks. Three weeks of daily treatment of extracts led to a dose-dependent fall in blood sugar levels by 25-62%. Effect seems to reach maximum after 15 days of treatment and remained constant in third week.

Vehicle control animals were found to be stable in their body weight while diabetic rats showed significant reduction in body weight during 21 day. Alloxan caused weight reduction, which was reversed by and methanolic and hydroalcoholic extracts of *Sphaeranthus indicus* after 21 days of treatment (Figure 1).

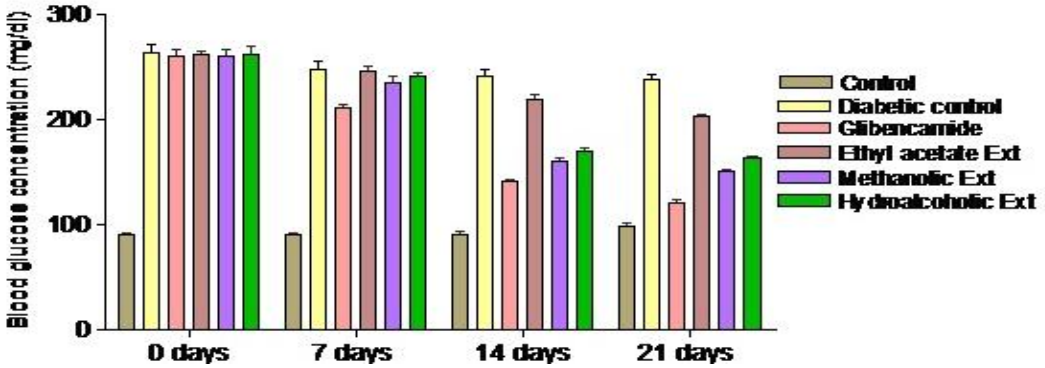

Fig. 1: Comparative effect of extracts of *Sphaeranthus indicus* on blood glucose level in alloxan an (150 mg/kg) induced diabetes in rats

The production of lipid peroxides was significantly decreased in methanolic and hydroalcoholic extracts treated Alloxan-induced diabetic rats from 7.53 to 5.74 nmol TBARS/ml, serum (Figure 2).

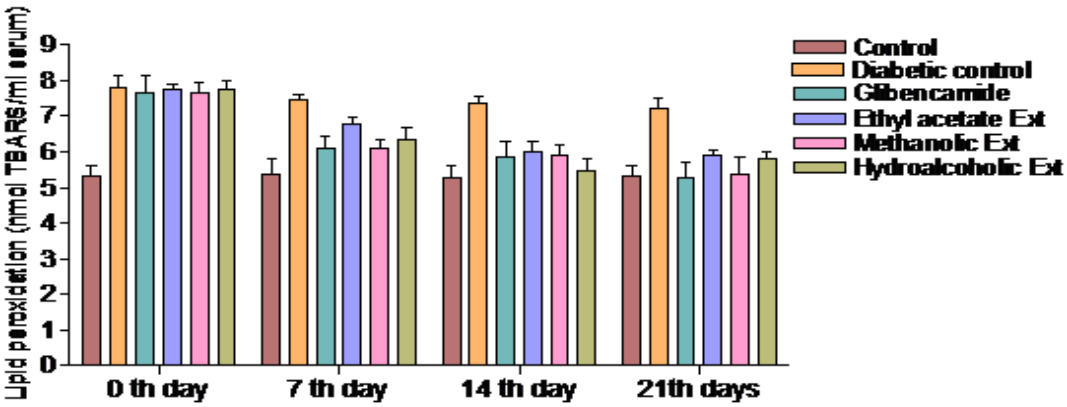

Fig. 2: Comparative effect of *Sphaeranthus indicus* extracts on Lipid peroxidation level in alloxan (150 mg/kg) induced diabetes in rats

DISCUSSION

The comparable effect of the *S. indicus* extract with glibenclamide (Figure 1) may suggest similar mode of action, since alloxan permanently destroys the pancreatic β-cells and the extracts lowered blood glucose level in alloxan induced rats to significant level. This indicates that the extracts possess extra pancreatic effects. *S. indicus* (200 mg/kg) has shown beneficial effects on blood glucose level. As expected in the diabetic control there was severe hyperglycemia as compared to the normal animals. Comparing with the diabetic control all the three extracts EAE, MET and HAE significantly lowered the elevated blood glucose levels. It was observed that the standard drug Glibenclamide in 2% acacia emulsion (10 mg/kg p.o.) lowered the blood glucose level significantly bringing it nearly back to normal. The effect of extracts on the blood glucose (BGL) values are mean ± S.E.M. $p< 0.001$ when compared with diabetic control, $p<0.05$ when compared with diabetic control. From these results, it can be concluded that the extracts of the flower of *Sphaeranthus indicus* possess antihyperglyceamic action against alloxan induced hyperglycemia. These results seem to confirm the alleged antidiabetic activity by the traditional medicine.

Increased lipid peroxidation impairs membrane function by decreasing membrane fluidity and changing the activity of membrane bound enzymes and receptors [24, 25]. Its products are harmful to most of the cells in the body and associated with variety of disease [26, 27]. Our present study showed significant elevation of TBARS content in diabetic rats. The increased TBARS content of diabetic rats suggest that peroxidative injury may be involved in the development of diabetic complications. The extract could significantly reduce the lipid peroxidation product levels in diabetic rats (Figure 2). This indicates that methanolic and hydroalcoholic extract is a potent inhibitor of oxidative damage of cardiac tissues. Upon glibencamide administration, the lipid peroxidation levels are decreased. This indicates that *S. indicus* extract could inhibit or reduce the oxidative stress in diabetes. The antidiabetic potency of the aqueous extract was almost similar to that of glibenclamide with regards to its effect on antioxidant status.

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