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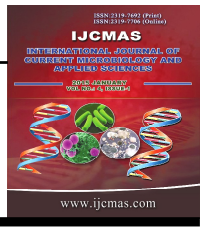

Original Research Article

Study the effect of *Bauhinia variegate* Linn. ethanolic extract on reducing glucose and lipid levels of white albino mice

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Keywords

Bauhinia variegate, hyperglycemia and lipidemia, dexamethzone

A B S T R A C T

This study was carried out in Baghdad/ Biotechnology Center /Al-Nahrain University to evaluate leaf ethanolic extract of *Bauhinia variegata* Linn. (Family; caesalpiniaceae) in normal and dexamethzone induced hyperglycemia and lipidemia in white albino mice. Diabetes was induced by intraperitoneally (i.p) dexamethzone (0.1 ml of 150 mg/kg) in white albino mice (age 8 weeks). Blood glucose levels were determined pre and after injection of *B. variegata* (200 mg/kg b. wt) in diabetic groups. Blood glucose levels were determined on 0, 7, 14, 21 and 28 day and diabetes drug glibenclamide (600 µg/kg) in diabetic mice. Extract effects of *B. variegata* on blood glucose levels and serum lipids, Total cholesterol (TC), Triglycerides (TG), and High density lipoprotein (HDL) were measured in the diabetic and non diabetic mice. There was significant reduction in TC, TG and elevation of HDL in diabetic mice as compared to normal control (NC) and diabetic control (DC). These results indicate that *B. variegata* leaf ethanolic extract possesses a hypoglycemic effect.

Introduction

Diabetes mellitus (DM) is a disease common in all parts of the world [1]. It is seemed to be a group of disorders with different etiologies [2]. This disease is characterized by differences in carbohydrate, protein and fat metabolism caused by the complete or relative insufficiency of insulin secretion and insulin action [3]. Different types of hypoglycemic agents as alternatives with insulin for the treatment of diabetes mellitus, therefore, an increasing demand by patients to use the natural products with antidiabetic activity was appeared [4]. Pharmacological treatment of DM is based on hypoglycemic agents and insulin, Currently, these substances used in clinical practice because some times they do not act in reducing glucose levels in most cases or may be fail after a distinct period of time.

Continuous uses of the synthetic anti-diabetic drugs may be show side effects or toxicity [5, 6]. So there is a requirement for testing more effective and safe hypoglycaemic gents.

Bauhinia variegata Linn. (Family; Caesalpiniaceae) grows as a deciduous tree, distributed in a wide range of geographic locations. Certain *Bauhinia* species have a long history of traditional medicinal applications it is used in the treatment of, inflammatory, diarrhoea, leprosy, tumours, wounds, ulcers, and diabetes [7].

The leaf of *B. variegata* extract is used in the treatment of diabetics, bark also used as an anticarcinogenic and mutagenic, anti-inflammatory, stem used as cytotoxic, hepatoprotective, antitumor. The following compounds were isolated from various parts of this plant; galactoside binding lectine, a new phenanthraquinone from the stem. flavanone and a dihydrodibenzoxepin [8]. Tannins such as tannic acid stimulate the transport of glucose and inhibit adipocyte differentiation. Polyphenols decrease the blood glucose levels [9, 10]. Flavonoids are considered to be one of main components of Bauhinia leaf. The health benefits of flavonoids are well known and are displayed as a remarkable range of biochemical and pharmacological properties [11].

The aim of this study is to detect some active compounds in Bauhinia plant leaf extract and to evaluate hypoglycemic and hypolipidemic effects of leaf ethanolic extract of *B. variegate* (BEE) on Dexamethazone induced diabetic mice.

Materials and Methods

Total duration of the experiment was 28 days, diabetes mellitus was induced for the experimental animals by injecting dexamethzone through intraperitoneal at a dose of 150 ug/kg body weight [12].

Preparation of the extract

The shade dried Bauhinia leaf was crushed in an electrical grinder and then powdered. Out of this powder, 50 g was extracted with 50% ethanol solution (v/v) at 80°C for 8 h. After filtration with Whatman No. 1 filter paper and centrifugation (10000 rpm, 5 min) at 20°C, the solvent was concentrated by evaporation using rotary evaporator apparatus [13].

Chemical detection of active compounds

Detection of some active ingredients was carried out using chemical reagents to determine the presence of the active compounds only without quantification or determination of their structure.

Detection of tannins

The extract of *B. variegata* was boiled in a boiling water bath for 10 minutes, then filtered and the filtrate was treated with 5 drops of 1% lead acetate solution. The development of greenish-blue precipitate is an indicator for the presence of tannins [14].

Detection of terpenes and steroids

One milliliter of ethanol extract was participated in a few drops of chloroform, then one drop of acetate anhydride and 1 drop of concentrated sulfuric acid were added, brown precipitate appeared which representing the presence of terpene, and the appearance of dark blue color after 4-5 minutes would ensure the present of steroids [14].

Detection of flavonoids

Ethanol extract was partitioned with petroleum ether using Buckner funnel; the aqueous layer was mixed with the ammonia solution. The appearance of dark color is an evidence for the presence of flavonoids [14].

Detection of alkaloids

The extract (10) gm was boiled with 50 ml of distilled water and 4% of hydrochloric acid was added, then the solution was filtered and cooled. 0.5 ml of the supernatant was tested with Mayer solution, appearance of white precipitate indicates the presence of alkaloids [15].

Detection of Saponins

Saponins were detected by two methods: The first method, aqueous extract of *B. variegata* powder was shaken vigorously with distilled water in a test tube. The formation of foam standing for a time indicates a positive result. The second method, five milliliters of aqueous extract of the plant was added to 1-3 drops of 3% ferric chloride solution, a white precipitate was developed which indicates a positive result [15].

Basal diet elements

Table (1) shows the nutrient elements of the basal diet for the mice nutrition during the experiments.

Selection of experiment animals

Mice of original Swiss albino strain (number of.20 mice), eight weeks of age, weighing 25 g were selected and housed in plastic cages at an a temperature of 25 ± 2°C with 12 h light and 12 h dark cycle. They were fed basal diet, nutrient elements of the basal diet. The animals were randomly divided into four groups (n = 5 mice per group) as given below:

Group 1;Normal Control group (NC)
Group 2 ;Diabetes Control group (DC)
Group 3;Diabetic mice treated with Glibenclamide 600 ug/kg body weight (DGC)
Group 4;Diabetic mice treated with 200 mg/kg Bauhinia ethanolic extract (DBE)

The experiment was carried out according to the guidelines of the institutional animal ethical committee [17].

Induction of diabetes mellitus

Experimental mice were starved for 24 h, then groups 2, 3 and 4 were injected with a intraperitoneally injection of 0.1 ml dexamethazone solution at a dose of 150 mg/kg body weight respectively. Diabetes was confirmed by measuring blood glucose levels from the tail vein on the third day after injection of dexamethzone. The mice having blood glucose levels greater than or equal to 100 mg/dl were considered diabetic. Group 3 of mice were treated with an oral dose of glibenclamide (600 ug/kg) once every day to the end of the experiment, and group 4 were treated with 200 mg/kg of mice body weight with Bauhinia ethanolic extract (BEE), while groups 1 and 2 were received only 1 ml of distilled water as control.

Measuring of blood glucose level

During supplementation of BEE for 28 days, Blood was collected from the tail vein of the mice and fasting blood glucose level was measured every 7 days using a glucose analyzer [18].

Investigation of lipid profile

After 28 days, the mice were sacrificed and blood samples were collected from dorsal aorta. Serum was separated by centrifugation for 5 min, kept at -20°C for the biochemical assay of total cholesterol (TC), high-density lipoprotein cholesterol (HDL) and triglyceride (TG). Enzymatic methods were used to determine TC, TG, and HDL [18].

Statistical analysis

All the data were expressed as mean ± S.D. Statistical analysis was conducted by SPSS program, significance was calculated using analysis of variance (ANOVA). Significance was accepted at the $p < 0.05$.

Results and Discussion

Chemical detection of some active compounds

The chemical test indicated that the leaf ethanolic extract of *B. variegata* contains flavanoids, glycosides, saponins, alkaloids, terpenes and steroids.

Effect of BEE on fasting blood glucose levels in mice

Blood glucose levels, estimated in 20 diabetic mice at the beginning of the experiment (0 day), then measured every 7 days till 28th days. The results were showed in table 2. The blood glucose levels in the treated groups with glibenclamide (GC) and Bauhinia ethanolic extract (BEE) showed significant effect along with the experimental period (28 days), these groups were recorded lower values than that in the Diabetic control (DC). On the end of the experiment, blood glucose levels in the DBE and DGC groups decreased to 64 mg/dl and 70 mg/dl, respectively as compared to the normal control (NC) group and DC group. These results indicated that BEE decreases blood glucose levels in diabetic mice.

Effect of BEE on blood lipids levels in mice

Blood lipid profile was tested in the normal mice and diabetic mice. The results were showed in Table 3. The levels of TC and TG were significantly elevated and the levels of HDL were decreased in the DC group as compared to the NC group. After treatment with the BEE, the alteration in lipid metabolism was attenuated, while decreased TG and TC levels and increased HDL concentration in experimental mice. The effect was clear in the DBE group compared with the DGC group and DC group as a control. These results indicate that the leaf ethanolic extract of *B. variegata* Linn. at the concentration of 200 mg/kg of body weight possess a hypolipidemic activity.

Diabetes mellitus appears as an alarming disease worldwide affecting human health much. Currently traditional drugs against the disease reduce the suffering of people to some extent; but still it is costly, and associated with side effects. Therefore, it is useful for search of more efficacious drugs with less or no side effects. There is a growing interest in anti-diabetic agents from natural products, derived from plants. Effective control of the blood glucose level is a key step in preventing diabetic complications and improving the life quality of types 1 and 2 diabetic patients [19].

Antihyperglycemic activity of the BEE in diabetic mice has been indicated in this study by the fasting blood glucose levels as the basal parameter for monitoring of diabetes at the concentration of 200 mg/kg which is effective. Hyperlipidemia is also associated with diabetes [20], the levels of TC and TG have been decreased significantly in diabetic mice after the BEE treatment. These effects may be due to low activity of cholesterol biosynthesis enzymes and low level of lipid metabolism or enzymatic analysis which are under the control of insulin [21]. The BEE treatment results to the significant effect in the level of HDL due to the effect of this extract. There are reports that other medicinal plants have hypoglycemic and hypolipidemic effects to prevent or to reduce the complications of lipid profile [22].

It was concluded that this study indicates the beneficial effects of BEE as hypoglycemic and antihyperglycemic agents in the same time. Other experiments are necessary to determine the active compounds that show this effect. Tannins, polyphenols, flavonoids are reported to have antidiabetic effects [23], and it is known from the detection procedure that BEE are rich in these substances and it can be concluded that BEE extract showed an antidiabetic effect because of the tannins and polyphenols therein. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in alloxan-induced diabetic rabbits. Flavonoids are naturally occurring phenolic compounds with a broad range of biological activities and the beneficial effects of flavonoids have been studied in relation to diabetes mellitus, either through the inhibition of intestinal α-glucosidase enzyme or through their capacity to avoid glucose absorption and to improve glucose tolerance [24, 25].

Treatment with BEE significantly reduced the total cholesterol and triglyceride in plasma as compared to the diabetic group. Currently, reviewed on the mode of action of flavonoids including cellular and molecular mechanism. In their review, the authors thoroughly discussed about the various effects of the drug candidates in regulating diabetic syndromes. It has been demonstrated that flavonoid compounds act against diabetes mellitus either through their capacity to avoid glucose absorption or to improve glucose tolerance [26].

Table.1 Nutrient elements (%) for the mice during the experiment period

Element	Percent %	Element	Percent %
Rice	16	Micro elements	0.1
Corn starch	20	Calcium	3
Soybean oil	20	Bran	19
Bone powder	3	Yeast extract	2.3
Salt	0.5	Fish powder	16
Vitamins	0.1		

* Zhou et al., 2009 [16].

Table.2 Effect of Bauhinia variegata leaves ethanolic extract on blood glucose levels (mg/dl) of albino mice

Treatment (Group), n=5	**Dose**	**Blood glucose level (mg/dl)**					
		0 day	**7 days**	**14 days**	**21 days**	**28 days**	
Normal control (NC)	-	70	65	68	72	66	
Diabetic control (DC)	-	162	165	165	170	180	
Diabetic with Glibenclamide (DGC)	600 ug/kg	72	67	63	65	64	
Diabetic with Bauhinia Extract (DBE)	200 mg/kg	69	68	70	64	71	
* n= number of mice for each group							

Table.3 Effect of Bauhinia variegate Linn. leaf ethanolic extract on blood lipids (mg/dl) of albino mice

Treatment (Group), n=5	**Dose**	**Lipid profile levels (mg/dl)**				
		TC	**TG**	**HDL**		
Normal Control (NC)	-	87	69.3	45.6		
Diabetic mice (DC)	-	107.3	109.6	43.3		
Diabetic with Glibenclamide (DGC)	600 ug/kg	1 01.3	75.3	48.6		
Diabetic with Bauhinia Extract (DBE)	200 mg/kg	102.6	77.6	46.3		
* n= number of mice for each group						

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