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ANTIHYPERLIPIDEMIC ACTIVITY OF *BIOPHYTUM SENSITIVUM* EXTRACTS IN STREPTOZOTOCIN (STZ) INDUCED DIABETIC ALBINO RATS

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ABSTRACT

Hyperlipedimia is common in both insulin dependent and non-insulin dependent diabetes mellitus and are related to the degree of glycemic control. In the present investigation, the antihyperlipidemic activity of *Biophytum sensitivum* extracts on streptozotocin induced diabetic albino rats was carried out. Ethanolic, aqueous ethanolic and ethylacetate extract of whole plant of *B. sensitivum* (200mg/kg body weight) was administered orally to streptozotocin (STZ) (50mg/kg bw) induced diabetic albino rats for 45 days to investigate the antihyperlipedemic activity of the plant. Biochemical parameters were studied including total cholesterol, triglycerides, HDL and LDL in control, treated and diabetic rats. *B. sensitivum* extracts restored to normal levels of lipid profiles in streptozotocin induced diabetic albino rats. The results of the experiments suggest that ethanolic, aqueous ethanolic and ethyl acetate extracts of *B. sensitivum* exerts significant antihyperlipidemic effect in STZ induced diabetic rats.

KEY WORDS: Antihyperlipidemic activity, Antidiabetic activity, streptozotocin (STZ), *Biophytum sensitivum* and *Rattus norvegicus.*

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INTRODUCTION

Diabetes mellitus is a non-communicable disease, which is considered as one of the five leading causes of death in the world. Diabetes mellitus is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin[1]. Due to this disorder, body fails to make proper use of sugar (loss of ability to metabolize sugar). The excessive amount of sugar accumulates in the blood and often assess in the urine. Diabetes mellitus is also often linked with abnormal lipid metabolism[2]. The abnormality of lipid metabolism is common in both insulin dependent and non-insulin dependent diabetes mellitus and is related to the degree of hyperlipidemia. Hyperlipidemia is a major risk factor for initiation and progression of cardiovascular disease (The lipid research clinics program, 1981; National Cholesterol Education Program Expert Panel, 1994). Accelerated cardiovascular disease is a leading cause of both morbidity and mortality in diabetic patients[3]. The investigation on plant drugs will be useful strategy in the discovery of new lead molecules eliciting improved activity by regulating the different mechanisms maintain the lipid metabolism and thus can be used in treating hyperlipidemia of varied etiology[4]. Recently herbal hypolipidemics have gained importance to fill the lacunae created by the allopathic drugs. *Biophytum sensitivum* is an annual herb, medicinal plants belonging to family OXALIDACEAE used in traditional oriental herbal medicine. It is well known for hypoglycaemic[5] and immunomodulatory[6] properties and chemoprotective[6] and antitumor effect[6,7]. The scientific knowledge on antihyperlipidemic efficacy of *B. sensitivum* is very scanty. So the present study was carried out to evaluate the antihyperlipedemic activity of *B. sensitivum* whole plant extracts on STZ induced diabetic albino rats.

MATERIALS AND METHODS

Plant materials

The whole plant of *Biophytum sensitivum* was collected from fallow lands of nearby village *viz.,* Gandhi Nagar, Trichy district, Tamilnadu.

Preparation of extracts

The whole plant of *B. sensitivum* was washed thoroughly with water to remove the soil particles, and then dried under shade and powdered. The plant powder was extracted with different solvents by using Soxhlet apparatus and evaporated by using a vacuum evaporator and stored in a desiccator for subsequent experiments.

Chemicals

Streptozotocin was obtained from Sigma Chemicals, Bangalore, India. All other chemicals and reagents were of analytical grade.

Animals

Healthy male Wistar albino rats, *Rattus norvegicus* (150-200mg/kg b.wt.) were used throughout the study. The rats were obtained from Tamilnadu Veternary and Animal Science University, Chennai brought to the laboratory and were maintained under controlled environment. All animals were fed with standard pellet feed (Sai Durga Feeds & Foods, Bangalore) and water *ad libitum*. The study was approved by the Animal Ethical Committee of the Institute (790/03/ac/CPCSEA).The principles of animal care were followed throughout the experimental period.

Evaluation of antihyperlipidemic activity

The animals were divided into seven groups of six rats each. The first group was given *ad libitum* of standard pellet diet and water. All other 4 group rats were given a single dose of diabetogenic agent, streptozotocin (STZ) (50 mg/kg b.w, with Citrate buffer pH 4.5) to induce diabetes. The second group was given a single dose of diabetogenic agent, streptozotocin (STZ) and given normal food for the entire

period. The extracts were orally administered after 48 hours of injection of STZ. The third group was given 99% ethanolic extract of *B. sensitivum* (200 mg/kg body weight) for 45 days. The fourth group was given 70% ethanolic extract of *B. sensitivum* (200 mg/kg body weight) for 45 days. The fifth group was given an ethyl acetate extract of *B. sensitivum* (200 mg/kg body weight) for 45 days. The sixth group was given standard synthetic drug Glibenclamide for 45 days. The seventh group was given DMSO (drug carrier) for 45 days.

Biochemical estimation

All the rats were sacrificed with a mild dose of chloroform and blood samples were collected from heart. Blood was immediately centrifuged (2500 rpm for 10 min) and serum was separated and total cholesterol, triglycerides, HDL and LDL were analysed by Star 21plus biochemical auto-analyser.

Statistical analysis

Values were represented as Mean ± Standard Error. To compare the means of different experimental groups with normal groups, One Way Analysis of Variance (ANOVA) was performed. The post hoc test (Student-Newman Keuls test; SNK) was performed to investigate the influence of the *B. sensitivum* whole plant extracts on various biochemical parameters in the extract treated rats. All statistical analyses were performed by using Windows based SPSS package (Statistical Packages for Social Sciences and now it is called Statistical Product and Service Solutions).

RESULTS

Antihyperlipidemic activity of B. sensitivum on STZ induced diabetic rats

Antihyperlipidemic activity of *B. sensitivum* was evaluated by analyzing abnormalities in serum cholesterol level, triglyceride level, HDL level and VLDL levels in diabetic rats, herbal extract treated rats and were compared with the normal rats. Lipid profiles were measured in control and all experimental rats on the 45th day of treatment. Effect of *B. sensitivum* whole plant extracts on the serum cholesterol, triglyceride, HDL and VLDL levels in STZ induced diabetic rats are presents in the table 1 and the results of one way ANOVA and post hoc SNK test are given in table 2.

Effect of B. sensitivum extracts on serum cholesterol level

The diabetic control rats (152.6 ± 2.68mg/dl) showed a drastic raise in serum cholesterol level on 45th day of experiment. However, the *B. sensitivum* extracts treated rats (group III and group V) exhibited a significant reduction in the serum cholesterol level 92.4 ± 1.23mg/dl and 91.0 ± 2.28mg/dl, respectively compared to that of diabetic control rats. Moreover, aqueous ethanolic extract treated rats showed significantly reduction (77.5 ± 1.46mg/dl) in serum cholesterol levels when compared to that of diabetic control rats (group II; 152.6 ± 2.68mg/dl) and also control rats (79.2 ± 1.62mg/dl).

Effect of B. sensitivum extracts on triglyceride level

Triglyceride levels in diabetic control rats were observed to be high (148.0 ± 1.22mg/dl) when compared to that of control group rats (77.4 ± 1.99mg/dl). After the continuous treatment of the diabetic rats with ethanolic extract, aqueous ethanolic extract and ethyl acetate extract of *B. sensitivum* resulted significant reductions in triglyceride level to 94.5 ± 2.1, 89.1 ± 2.21, 98.5 ± 1.41 and 79.7 ± 2.08mg/dl, respectively. Aqueous ethanolic extract remarkably reduced the triglyceride level.

Effect of B. sensitivum extracts on HDL level

There was a significant (P<0.05) decrease in the level of serum HDL level (20.2 ± 0.40mg/dl) in diabetic control rats when compared to control rats (40.0 ± 1.08mg/dl). After the treatment of diabetic rats with (200 mg/kg body weight) ethanolic extract, aqueous ethanolic extract and ethylacetate extract of *B. sensitivum* resulted significant increase in the level of HDL as about 26.6 ± 3.48, 35.4 ± 0.59, 28.0 ± 1.06 and 34.4 ± 0.91mg/dl, respectively. However, they did not reach the control value

and they were highly significant when compared to that of control rats.

Effect of B. sensitivum extracts on VLDL level

The VLDL levels of diabetic control rats were significantly higher (29.6 ± 0.24mg/dl) than that of control rats (15.4 ± 0.39mg/dl). However, the treatment of the diabetic rats with (200 mg/kg body weight) ethanolic extract, aqueous ethanolic extract and ethyl acetate extract of *B. sensitivum* resulted significant reductions (P<0.05) in VLDL levels to 18.9 ± 0.43, 17.8 ± 0.44 and 19.7 ± 0.28mg/dl, respectively.

Effect of B. sensitivum extracts on LDL level

The LDL level of diabetic control rats were observed to be high (102.7 ± 2.64mg/dl) when compared to control rats (23.7 ± 2.41mg/dl). After the treatment of the diabetic rats with ethanolic extract, 70% ethanolic extract and ethylacetate extract of *B. sensitivum* resulted significant reductions (P<0.05) in LDL level as about 46.8 ± 1.10; 24.3 ± 1.78 and 43.3 ± 1.56mg/dl, respectively. The activity of *B. sensitivum* extracts on recovering of lipid profile on STZ induced diabetic rats was found to be following order: Aqueous Ethanol extract > Ethylacetate > Ethanol extract.

Table 1

Effect of B. sensitivum extracts on serum cholesterol, triglyceride, HDL, VLDL and LDL levels (Mean ± SE) in STZ induced diabetic rats (Values in the parentheses are range of the respective mean).

*Groups	Serum Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
I	79.2 ± 1.62 (67.0 – 88.0)	77.4 ± 1.99 (65.0 – 86.0)	40.0 ± 1.08 (33.0 – 47.0)	15.4 ± 0.39 (13.0 – 17.2)	23.7 ± 2.41 (2.8 – 34.8)
II	152.6 ± 2.68 (140.0 – 168.0)	148.0 ± 1.22 (140.0 – 155.0)	20.2 ± 0.40 (17.0 – 23.0)	29.6 ± 0.24 (28.0 – 31.0)	102.7 ± 2.64 (89.2 – 117)
III	92.4 ± 1.23 (85.0 – 98.0)	94.5 ± 2.1 (85.0 – 105.0)	26.6 ± 3.48 (25.0 – 28.0)	18.9 ± 0.43 (17.0 – 21.0)	46.8 ± 1.10 (39.6 – 51.9)
IV	77.5 ± 1.46 (68.0 – 87.0)	89.1 ± 2.21 (76.0 – 100.0)	35.4 ± 0.59 (30.0 – 39.0)	17.8 ± 0.44 (15.2 – 20.0)	24.3 ± 1.78 (12.8 – 35.0)
V	91.0 ± 2.28 (70.0 – 99.0)	98.5 ± 1.41 (85.0 – 105.0)	28.0 ± 1.06 (24.0 – 34.0)	19.7 ± 0.28 (17.0 – 21.0)	43.3 ± 1.56 (28.1 – 48.8)
VI	74.2 ± 2.05 (60.0 – 84.0)	79.7 ± 2.08 (69.0 – 90.0)	34.4 ± 0.91 (29.0 – 39.0)	15.9 ± 0.41 (13.8 – 18.0)	23.9 ± 2.05 (11 – 35.2)
VII	147.0 ± 2.89 (140.0 – 165.0)	151.2 ± 1.95 (140.0 – 160.0)	20.5 ± 0.44 (18.0 – 23.0)	30.2 ± 0.39 (28.0 – 32.0)	96.2 ± 2.92 (88.1 – 116)

Groups
I = Control (non-diabetic rats); II = Diabetic control; III= Diabetes + ethanol extract
IV = Diabetes + 70 % ethanol extract; V = Diabetes + ethyl acetate extract;
VI = Diabetes + glibenclamide; VII = Diabetes + DMSO

Table 2

Student-Newman Keuls Post hoc test results showed the variations and similarities in the serum cholesterol, triglyceride, HDL, VLDL and LDL levels among the different groups of rats. Mean values are arranged in ascending order.

Parameters	*Groups						
Cholesterol (mg/dl)	74.2 (VI)	77.5 (IV)	79.2 (I)	91.0 (V)	92.4 (III)	147.0 (VII)	152.6 (II)
Triglyceride (mg/dl)	77.4 (I)	79.7 (VI)	89.1 (IV)	94.5 (III)	98.5 (V)	148.0 (II)	151.2 (VII)
HDL (mg/dl)	20.2 (II)	20.5 (VII)	26.6 (III)	28.0 (V)	34.4 (VI)	35.4 (IV)	40.0 (I)
VLDL (mg/dl)	15.4 (I)	15.9 (VI)	17.8 (IV)	18.9 (III)	19.7 (V)	29.6 (II)	30.2 (VII)
LDL (mg/dl)	23.7 (1)	23.9 (VI)	24.3 (IV)	43.3 (V)	46.8 (III)	96.2 (VII)	102.7 (II)

Horizontal lines connect similar means.

Groups

I = Control (non-diabetic rats); II = STZ induced Diabetic control; III = STZ induced Diabetes + ethanol extract; IV = STZ induced Diabetes + 70 % ethanol extract; V= STZ induced Diabetes + ethyl acetate extract; VI = STZ induced Diabetes + glibenclamide; VII = STZ induced Diabetes + DMSO.

DISCUSSION

Prolonged hyperglycemia ends with severe complications which generate more oxidative stress. Free radicals react with lipids and cause lipidperoxidation. Manimegalai *et al* (1993) reported that the increased level of oxidative stress will increase the hyperlipidemia in animals. The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilisation of fatty acids from the peripheral depots, since insulin inhibits the hormone sensitive lipase[9]. Cholesterol is a sterol useful in cell membrane integrity and precursor for steroid hormones. In the present study, serum cholesterol level is increased in diabetic group when compared with control rats. Diabetes mellitus lead with impaired carbohydrate metabolism and increased lipolysis causing accumulation of acetyl CoA. Increased availability of acetyl CoA leads to the synthesis of cholesterol which causes hyperlipidemia. Insulin deficiency results with hypercholesterolemia due to metabolic abnormalities[10]. Shirwaikar *et al* (2004) have reported the level of serum lipids increased by lipolysis due to insulin deficiency in diabetic rats. In general, insulin increases the lipogenesis and decreases lipolysis and ketogenesis. In the preset investigation, the increased serum cholesterol levels were restored in the STZ induced diabetic rats treated with *B. sensitivum* extracts as control rats. The *B. sensitivum* extracts treated rats exhibited a significant reduction in the serum cholesterol level. Moreover, aqueous ethanolic extract treated rats showed significant reduction in serum cholesterol levels of STZ induced diabetic rats. The abnormalities in lipid metabolism lead to an elevation in the levels of serum lipid and lipoprotein that in turn play an important role in occurrence of premature and severe atherosclerosis, which affects patients with diabetes[12]. Hence, measurements of biochemical parameters such as triglycerides, LDL, VLDL and HDL are necessary to prevent cardiac complications in diabetes condition. Thus, in the present study, triglycerides, LDL, VLDL and HDL were measured along with total cholesterol. Tryglycerides are neutral fats, major energy reserve for the body stored at adipose tissue. Diabetic condition increases the lipolysis and produces more free fatty acids. Increased release of free fatty acids increases the production of ketone bodies and triglyceride synthesis. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia [13,14]. In the present study, triglycerides are increased significantly in the STZ induced diabetic rats. However, the aqueous ethanolic extract strongly reduced the triglyceride level as such of normal rat in the STZ induced diabetic rats. The abnormal high concentration of serum lipids in diabetes is mainly due to the increase release of free fatty acids from the peripheral tissues. Lipolysis is also enhanced by glucagon and catecholamines[15]. LDL is commonly called as bad cholesterol because it has a significant role in atherosclerosis and other related diseases and it is formed from VLDL-cholesterol. The raised total cholesterol and LDL concentrations are having a negative correlation with HDL cholesterol[16]. Increase in HDL cholesterol level is associated with a decrease in coronary risk[17, 18]. In the present study, a remarkable elevation in the triglycerides, LDL and VLDL were observed in the STZ induced diabetic rats. Further, there was a significant reduction in the HDL levels in diabetic rats also. The *B. sensitivum* extracts showed significant reduction in total cholesterol, tryglycerides, LDL and VLDL levels, and increased levels of HDL in diabetic rats. However, the increased HDL (cardioprotective lipid) level by *B. sensitivum* extracts was comparable to the control rats. Therefore, *B. sensitivum* has potential role to prevent formation of atherosclerosis and coronary heart disease. Several authors reported that secondary metabolites, such as saponins, flavonoids, phenolic compounds and triterpenoids have hypolipidemic activity[19]. Hence, the hypolipidemic properties of *B. sensitivum* extracts may be due to different types of active secondary metabolites such as saponins, flavonoids and phenolic compounds

each with a single or diverse range of biological activities.

CONCLUSION

In the present findings, it is well documented that the extracts of *B. sensitivum* have the potential to counteract the hyperlipidemic condition which occurs in STZ induced diabetic rats. Thus *B. sensitivum* may be used for managing diabetes related hypolipidemia.

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