Antidiabetic Potential of *Biophytum Sensitivum* Whole Plant Extracts in STZ Induced Diabetic Rats

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Abstract - Antidiabetic effect of three different extracts (Aqueous, Ethanol and Ethyl acetate) of *Biophytum sensitivum* on streptozotocin (STZ) induced diabetic rats was studied**.** Oral administration of aqueous, ethanol and ethyl acetate extracts of *B. sensitivum* to diabetic rats at a dose of 200 mg/kg body weight resulted in a significant reduction in blood glucose in STZ induced diabetic rats at different treatment period (0^{th} day and 45^{th} day). *B. sensitivum* extracts treated diabetic rats were significantly recovered from diabetic condition such as, polyphagia, poly urea and hypoglycemia. *B. sensitivum* treatment decreased the blood glucose and Glycosylated hemoglobin level, and increased serum insulin level. Present study reveals that the *B. sensitivum* may be used for the treatment of diabetes and its related complications.

Keywords - Antidiabetic, *Biophytum sensitivum*; Streptatozocin; Glycosylated hemoglobin; Serum insulin.

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1 INTRODUCTION

Diabetes mellitus is a major illness with numerous clinical manifestations. It is one of the most common endocrine metabolic disorders, characterized by hyperglycemia due to defects in insulin secretion, action, or both [Schwartz *et al*., 2016]. According the International Diabetes Federation (IFD), the world diabetic population in 2015 was estimated to be 415 million and the prevalence increased from 4.7% in 1980 to 8.5% in 2015 [IDF, 2015]. The International Diabetes Federation (IDF) revealed that, in 2040, diabetes will affect 642 million people making it one of the leading causes of disability and death worldwide [IDF, 2015]. Several approaches of treatment of diabetes available include hormonotherapy (insulin) or by using glucose lowering drugs such as alpha-glucosidase inhibitors, sulfonylureas, biguanides, and thiazolidinediones either in monotherapy or in combination [Patel 2016].However, the increase of side effects is one of the complications in the treatment of any systemic disorder. There is therefore an urgent need to search for new classes of compounds from phytomedicine without side effects for the disease [Li *et al*., 2016]. Thus the present study was carried out to evaluate the anitidiabetic activity of *Biophytum sensitivum* whole plant extracts.

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2 MATERIALS AND METHODS

2.1. Collection of plant materials

In the present study, Sicker bud, *Biophytum sensitivum* was selected to evaluate its effect on treatment of diabetes. The *B. sensitivum* whole plant including aerial parts and roots were collected from fallow lands of nearby villages (Kottathur and Gandhi Nagar, Musiri, Taluk, Tiruchirappalli Districi, Tamilanadu Lat. 11º 06″; Long. 78º 68″). The *B. sensitivum* whole plants were dried under shade and finely powdered.

2.2. Preparation of extracts: The process of extraction and formulation of the traditional remedy are followed as described previously by [Sohni and Bhatt 1995]. The whole plants of *B. sensitivum* were pulverized and extracted as a whole preparation in a Soxhlet apparatus. The ethanolic and ethylacetate extracts were concentrated to a dry mass by vacuum evaporator and stored in refrigerator.

2.3. Experimental animals

Healthy male albino rats, *Rattus norvegicus* (150-200mg/kg body weight) were used for the present study. The rats were obtained from Tamilnadu Veternary and Animal Science University, Chennai. They were brought to the laboratory and maintained under controlled environment. All animals were fed with standard pellet feed (Sai Durga Feeds & Foods, Bangalore) and water *ad libitum*. The principles of animal care were followed throughout the experimental period.

2.4. Experimental design

Antidiabetic effect of *B. sensitivum* whole plant extracts was evaluated on the streptozotocin (STZ) induced albino rats. Healthy male albino rats of seven to eight weeks old and weighing 150 to 200 g were used for the present study. The rats were randomized into control and experimental groups and housed in different rat cages (Tarsons make). The control and experimental rats were provided food and drinking water *ad libitum.* Pellet feeds were used as a basal diet during the experiment.

Experimental set up for streptozotocin (STZ) induced rats: In the antidiabetic evaluation study, the healthy albino rats were used. They were divided into seven groups (each group included six rats) and caged in separate rat cages.

The experimental set up was given below.

Group-I: Normal control (Non-diabetic rats)

Group-II: Diabetic rats (50 mg of STZ / kg body weight)

Group-III: Diabetic and treatment with 99.9% ethanolic extract of *B. sensitivum* (200 mg/kg body weight)

Group-IV: Diabetic and treatment with 70% ethanolic extract of *B. sensitivum* (200 mg/kg body weight)

Group-V: Diabetic and treatment with ethylacetate extract of *B. sensitivum* (200 mg/kg body weight)

Group VII: Diabetic and treatment with dimethyl sulfoxide, DMSO (2% mg/kg body weight)

2.5. Collection of blood

Blood was collected from live rats by sinooccular punching method for preglucose and post glucose estimation. Before diabetic induction and extract treatment, the blood was collected from all groups of rats for preglucose estimation. In post glucose estimation, after 3days, 15 days and 30 days of diabetic induction, the blood was collected from all groups of rats by sinooccular punching method except 45th day estimation. During 45th day, all the rats were sacrificed and blood was collected by heart punching method. The serum was separated by centrifuging the samples at 5000 rpm for 10 minutes and stored in a refrigerator until analyzed.

2.6. Evaluation of antidiabetic activity

Antidiabetic activity of *Biophytum sensitivum* whole plant extracts was evaluated by analyzing abnormalities in serum blood glucose level [Sasaki 1972], serum insulin level [Herbert *et al*., 1965], glycosylated haemoglobin level [Eross *et al*., 1984] and Glycogen is hydrolysed to glucose thus formed is estimated by standard method in the diabetic rats, extracts treated diabetic rats were assessed and were compared with that of normal rats.

2.7. 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).

3. OBSERVATION AND RESULTS

This study is based on blood glucose level which diabetes affected, for further comparison we check blood glucose level of rats before the injection.

3.1. Blood glucose level of animals after STZ injection

The blood sugar level was increased as compared to the rats with normal blood sugar level. Group I rats had normal blood glucose level and they were considered as the non-diabetes group whereas group II-VII were considered as diabetic animals. It was shown that the blood glucose level >250 mg/dl were considered as diabetic. In our study we used 30 animals for the induction of diabetes, 6 animals were died after the injection.

In this study, diabetes was induced in rats by single intraperitoneal injection of streptozotocin (50 mg/kg b.wt.). After 72 hrs rats with marked hyperglycaemia (blood glucose above 250 mg/dl) were selected and used for the study. Antidiabetic effect was evaluated by oral administration of aqueous combined plant extract at doses of 200 mg/kg b.wt. for 45 days. The treatment with aqueous combined plant extract up to 45 days at the dose of 200 mg/kg significantly improve the alterations in blood glucose levels and body weight in streptozotocin induced diabetic rats.

However, at the end of 45th days of treatment, there was a decrease of blood glucose levels with the glibenclamide and aqueous combined plant extract (200 mg/kg) respectively when compared with diabetic control group as shown in Table 1.

3.2. Effect of *B. sensitivum* extracts on serum glucose level in STZ induced diabetic rats

Serum glucose levels were measured in control and experimental rats from first day of experiment, 3rd day of experiment and to the end of experiment with an interval of 15 days (15th, 30th and 45th day of treatment). The effect of *B. sensitivum* extracts on blood glucose level in STZ induced diabetic rats are given in table 1. There was no significant difference in the blood glucose levels in all groups of rats on the initial day of experiment (pre glucose level). However, there was a significant decrease (P<0.05) in the blood glucose levels in all experimental groups rat (groups II, III, IV, V, VI and VII; respectively) after the induction of diabetes (3rd day). There was no change in blood glucose level of control group rats. Decrease in blood glucose level was greater in the glibenclamide treated rats, followed by considerable decrease in the aqueous ethanolic extract, ethyl acetate extract and ethanol extract treated rats on the 15th day of experiment. Similar trend was observed in all groups with further decrease in blood glucose levels on the 30th day of experiment. However, blood glucose level of glibenclamide treated rats was slightly lower than that of control rats. It is interesting to observe that the blood glucose levels of aqueous ethanolic extract treated rats and glibenclamide treated rats was very close to that of control rats during 45th day of experiment. There was no significant difference (SNK multiple comparison test; Table1) among the blood glucose levels in aqueous ethanolic extract treated rats, control rats and glibenclamide treated rats. Thus present result reveals that the aqueous ethanolic extract remarkably recovered the blood glucose level on the 45th day of experiment as very close to the normal value.

3.3. Effect of *B. sensitivum* extracts on serum insulin in STZ induced diabetic rats

Serum insulin levels were measured in control and experimental rats on the 45th day of treatment. The effect of *B. sensitivum* extracts on serum insulin level in STZ induced diabetic rats are given in tables1. Serum insulin level in diabetic control rats was observed to be very low when compared to that of control rats. Conversely, the administration of aqueous ethanolic extract, ethylacetate extract and ethanolic extract of *B. sensitivum* whole plant restored to bring serum insulin level towards close to normal level (Table 2). Nevertheless, the serum insulin level did not reach the normal value. There was no significant difference (P>0.05; SNK multiple comparison test, Table 2) between the insulin level of glibenclamide treated rats and the control group rats. It exhibited that the administration of glibenclamide remarkably increased the insulin level and reached to the normal level. Among the extracts, aqueous ethanolic extract of *B. sensitivum* was greater in increasing the serum insulin level of diabetic rats.

3.4. Effect of *B. sensitivum* extracts on HbA1C in STZ induced diabetic rats

Glycosylated haemoglobin (HbA_1C) levels were measured in control and all experimental rats on the 45th day of treatment. The effect of *B. sensitivum* extracts on HbA_1C level in STZ induced diabetic rats are given in tables 2. There was a significant elevation (P<0.05; SNK multiple comparison test, Table 2) in HbA1C level in STZ diabetic rats and DMSO treated rats, compared with that of control rats on 45th day of experiment. In contrast, administration of aqueous ethanol extract of *B. sensitivum* whole plant tended to bring HbA1C level almost near to control level and the administration of ethanol and ethylacetate extract of *B. sensitivum*. Administration of glibenclamide significantly reduced the HbA_1C levels to below control level.

3.5. Effect of *B. sensitivum* extracts on liver glycogen in STZ induced diabetic rats

Liver glycogen levels were measured in control and all experimental rats on the 45th day of treatment. The effect of *B. sensitivum* extracts on liver glycogen level in STZ induced diabetic rats are given in tables 2. The liver glycogen level in STZ induced diabetic control rats was lesser than that of control rats. The treatment of aqueous ethanol, ethylacetate and ethanolic extract of *B. sensitivum*) markedly increased the liver glycogen level when compared to that of diabetic control rats. Aqueous ethanolic extract of *B. sensitivum* decreased in greater level of liver glycogen when compared to other extracts. Similarly, the glibenclamide treated rats also significantly increased the liver glycogen level towards very close (P>0.05) to that of control rats.

DISCUSSION

Experimental diabetes induced by streptozotocin in rats is a model widely used to study various aspects of the disease. Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and abnormal lipid and protein metabolism along with specific long-term complications affecting the retina, kidney, and nervous system [Dzeufiet Djomeni, 2009]. Plants were an important source for the discovery of novel pharmacologically active compounds, with many blockbuster drugs being derived directly or indirectly [Cordell, 2005]. During the twentieth century, the emphasis gradually shifted from extracting medicinal compounds from plants to making these compounds or their analogues synthetically. This has led to the belief that natural products are safe because they are more harmonious with biological systems. In addition, the cost of administering modern antidiabetic drugs is beyond the reach of people in the low income group in those living in the rural areas. In the present study, antidiabetic activity of *Biophytum sensitivum* whole plant extracts on the artificially induced diabetic rats were evaluated. The oral administration of different doses of *Biophytum sensitivum* whole plant extracts and glibenclamide to diabetic rats after 45 days decreased significantly the levels of blood glucose.

Among all extracts, aqueous ethanolic extract was suitably recovered the blood glucose level as normal level in STZ induced diabetic rats.

In the present study, insulin level is increased in *B. sensitivum* extracts treated STZ induced diabetic rats which was more or less similar to the diabetic treated rats treated with glibenclamide and control rats. Similarly, the administration of aqueous ethanolic extract, ethylacetate extract and ethanolic extract of *B. sensitivum* whole plant increased the serum insulin level however, which did not reach the normal value. The increased insulin level in *B.sensitivum* extracts treated diabetic rats may be due to increased pancreatic secretion from existing β cells. The antihyperglycemic effect of herbal plants is due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose (saponin reduces glucose uptake) or the facilitation of metabolites in insulin dependent processes.

Glycosylated haemoglobin (HbA_1C) is now considered as the most reliable marker of glycemic control in diabetes mellitus [Halim Esharat, 2003] and is used to identify the degree of oxidative stress in diabetic condition [Gupta,1997]. The increased level of blood glucose stimulates no-enzymatic protein glycation which can lead to irreversible modification observed with the characterization of glycosylated haemoglobin [Cohen and Wu, 1994]. The glycosylated haemoglobin level is very much higher in diabetic patients [Wolffenbuttel *et al*., 1996]. Diabetic rats showed higher levels of glycosylated haemoglobin indicating their poor glycemic control. Diabetic control group (STZ induced diabetic rats) shown increased level of blood glucose accompanied by elevated glycosylated haemoglobin and decrease in insulin level and liver glycogen in diabetic rats suggest poor glycemic control mechanism.

In the present study, oral administration of *B. sensitivum* extracts recovered the glucose homeostasis during diabetic condition as evidenced by restoration of blood glucose, serum insulin and glycosylated haemoglobin as well as liver glycogen content. Glycogen is the primary intracellular storable form of glucose. The synthesis of glycogen by the liver is impaired in diabetic condition. Notable decrease of liver glycogen is found in diabetic rats which are proved by [Bollen, 1998], [Welihinda and Karunanayake, 1986] also reported about the significant reduction in glycogen level in liver and skeletal muscle in diabetic rats. In the present study, the glycogen level was restored after the treatment *B. sensitivum* extracts in STZ induced diabetic rats which might be due to the increased secretion of insulin.

CONCLUSION

The data obtained from this study indicates that the aqueous ethanolic (70%) extract of the whole plant *B. sensitivum* is capable of exhibiting significant antidiabetic in STZ induced diabetic rats. It is significant to proceed further in this path for the isolation of active principles responsible for antidiabetic activity and other pharmacologic investigations.

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Table 1: Post hoc test (Student-Newman Keuls Test) showed the similarities in the blood glucose level among the different group of rats.

Parameters	*Groups						
Pre glucose level (mg/dl)	83.7 (II)	85.7 (VII)	86.3 (IV)	87.9 (V)	88.7 (I)	89.5 (III)	89.5 (VI)
Post glucose level (mg/dl)	83.5 (I)	294.9 (III)	295.5 (VI)	299.8 (II)	302.0 (IV)	304.1 (VII)	305.8 (V)
15th day glucose level (mg/dl)	85.0 (I)	112.9 (VI)	161.5 (IV)	195.4 (V)	212.4 (III)	306.4 (VII)	307.6 (II)
30th day blood glucose level (mgdl)	84.8 (I)	90.8 (VI)	114.1 (IV)	123.0 (V)	137.9 (III)	312.6 (VII)	314.0 (II)
45th day blood glucose level (mg/dl)	82.9 (I)	84.5 (VI)	86.0 (IV)	97.8 (III)	105.5 (V)	317.9 (VII)	333.3 (II)

***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: Post hoc test (Student-Newman Keuls Test) showed the similarities among the insulin, HbA1C and liver glycogen level of diabetic and non diabetic rats.

Parameters	*Groups						
Insulin (µU/ml)	14.5 (II)	15.5 (VII)	23.4 (III)	29.9 (V)	33.4 (IV)	36.5 (VI)	37.3 (I)
HbA1C (%)	2.6 (VI)	3.2 (I)	3.3 (IV)	5.7 (V)	5.9 (III)	7.1 (VII)	7.3 (II)
Liver glycogen (mg/g)	8.5 (II)	9.0 (VII)	10.7 (IV)	11.9 (V)	12.4 (III)	13.5 (VI)	15.3 (I)

***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