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ANTIHYPERGLYCEMIC ACTIVITY OF POLYHERBAL FORMULATION BSL-150 IN STZ-NICOTINAMIDE INDUCED DIABETES IN MICE

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ABSTRACT

Diabetes mellitus is a group of syndrome characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins and an increased risk of complications from vascular diseases. A number of investigations on hypoglycemic agents from plants used in traditional medicine have been conducted and many of them were found with good activity. A polyherbal formulation BSL-150 is a formulation manufactured by Indu Pharma and consists of combination of herbs and various bhasmas. Each tablet of BSL-150 weighing 500 mg contains *Gymnema sylvestre*, *Tinospora cardifolia*, *Embellica officinalis* and various bhasmas. In this study the formulation is screened for its antihyperglycemic activity in Streptozotocin-Nicotinamide induced diabetes in Swiss albino mice. The effect of single and repeated oral administration of BSL-150 on glycemic parameters, lipid profile and histology of pancreas in doses of 250, 500 and 1000 mg/kg is evaluated. The significant ($p < 0.001$) reduction in the glycemic parameters, and lipid profile ($p < 0.05$) in BSL-150 treated groups was observed as compared to diabetic control group. While no significant ($p < 0.001$) change in Serum and Pancreatic insulin level was observed in the animals treated with BSL-150 on the other hand there was significant increase ($p < 0.001$) in Liver glycogen content in animals treated with BSL-150 as compared to the diabetic control group. The histological studies of pancreas also showed the decreased tissue damage in the BSL treated groups as compared to diabetic control group. The results indicate that BSL 150 possesses potential antihyperglycemic activity in STZ- Nicotinamide induced diabetes in mice.

Key Words: Diabetes mellitus, BSL-150, STZ- Nicotinamide, Antidiabetic.

INTRODUCTION

Diabetes mellitus (DM) is a group of syndrome characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins and an increased risk of complications from vascular diseases. It is a chronic disease caused by inherited or acquired deficiency in

insulin secretion and/or by decreased responsiveness of the organs to secreted insulin. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body systems, in particular the blood vessels and nerves (Rother KI, 2007). As per WHO key facts about diabetes are that 346 million people worldwide have diabetes. In 2004, an estimated 3.4 million people died from consequences of high blood sugar. More than 80% of diabetes deaths occur in low- and middle-income countries. WHO projects that death due to diabetes will be double by 2005 to 2030. Healthy diet, regular

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physical activity, maintaining a normal body weight and avoiding tobacco use can prevent or delay the onset of type 2 diabetes (Diabetes Fact sheet N°312). Various types of Oral Hypoglycemic agents such as biguanides, sulphonylureas and insulin are used for the treatment of diabetes mellitus (Holman RR and Turner RC, 1991) but are associated with the side effects (Valithan MS, 1998). There are varieties of glucose-lowering agents available for the treatment of type 2 diabetes with differing mechanisms of action, although side effects, including weight gain and the risk of hypoglycemia, have been the main obstacles hindering achievement of glycemic targets. This treatment gap is highlighted by the recent controversy surrounding the outcome of the Action to Control Cardiovascular Risk in Diabetes (ACCORD) study, in which subjects who received intensive glucose control had increased weight gain, increased risk of hypoglycemia and increased risk of mortality during the study. (The Action to Control Cardiovascular Risk in Diabetes Study Group, 2008). Thus, new strategies are needed for the prevention and treatment of diabetes. Amongst the existing alternative therapies, best is use of herbal remedies, which have been used since ancient times for the treatment of diabetes mellitus.

BSL 150 is a polyherbomineral formulation a proprietary medicine manufactured by Indu Pharma, Jejuri. As shown in the table no. 1, it consist of *Syzygium cumini*, *Gymnema sylvestre*, *Embillica officinalis*, *Tinospora cardiofolia* and bhasmas of tin, gold, iron, lead and mica along with shilajeet. The herbs and bhasmas used in the formulation are reported in traditional medicine to treat diabetes mellitus. But the antidiabetic efficacy of the combination of all above as formulation is not evaluated pre-clinically. The formulation BSL150 was evaluated for antihyperglycemic activity in STZ-Nicotinamide induced diabetic mice in this study.

MATERIAL & METHODS

Chemicals and Drugs

The BSL 150 was obtained as gift sample from Indu Pharma, Jejuri. The other drug glipizide was obtained as gift samples from Pharmaceutical industry. The chemicals required were purchased from local supplier Research laboratories, Hadapsar, Pune. The composition of BSL 150 is shown in Table No. 1.

Animals

Swiss albino mice bred in the animal house facility of PDEA's SGRS College of Pharmacy were used. The animals were maintained under controlled temperature, humidity and light cycle as per prescribed by the CPCSEA. The standard animal chow and water was provided *ad libitum*. The experimental protocol was approved by the IAEC (SGRS/IAEC/19/2009-10).

Preparation and administration of test drugs

The test drug and reference drugs were triturated and either suspended in 5% carboxymethyl cellulose (CMC) or dissolved in water depending on solubility. The accurately measured dose was administered to animals. (Muralik S *et al.*, 2005).

Induction of diabetes with Streptozotocin – Nicotinamide (STZ-NTM)

In overnight fasted mice Streptozotocin was injected (150 mg/kg, i.p.) 15 minutes after Nicotinamide injection (110 mg/kg) in all the groups except group I which was non-diabetic. Animals were fed with glucose solution (5%) for 12h to avoid hypoglycemia. Hyperglycemia was confirmed after 3 days. Mice having serum glucose >250 mg/dl were selected for the study (Rakieten N *et al.*, 1963).

Group	Name of the Group	Treatment Given
Group-I	Normal Control	vehicle 5% CMC
Group-II	Diabetic Control	vehicle 5% CMC
Group-III	Std.	Nicotinamide (110 mg/kg, i.p.)+ Streptozotocin (150 mg/kg, i.p.)+ Glipizide (10 mg/kg p.o.)
Group IV	Test-I	Nicotinamide (110 mg/kg, i.p.)+ Streptozotocin (150 mg/kg, i.p.)+ BSL-150 (250 mg/kg)
Group V	Test-II	Nicotinamide (110 mg/kg, i.p.)+ Streptozotocin (150 mg/kg, i.p.)+ BSL-150 (500mg/kg)
Group VI	Test-III	Nicotinamide (110 mg/kg, i.p.)+ Streptozotocin (150 mg/kg, i.p.)+ BSL-150 (1000 mg/kg)

Antihyperglycemic study

The mice were divided in six groups (each consisting 6 mice) and treated with test and reference drugs as following. The study was carried out in three ways:

OGTT

The mice were fasted for 12-14 hours. The test drug was administered orally. The blood glucose level was measured and glucose solution (2.5 g/kg body weight) was administered orally in a volume of 1 ml using oral feeding needle. The blood glucose level at 30, 60 and 120 minutes was measured.

Acute study

The mice were allowed free access to tap water and standard laboratory diet except when starvation was

required. The test and reference drug solutions were administered orally according to the body weight of the animals and the blood glucose level at 0, 2, 4, 6 and 24 hr was evaluated.

Sub-acute study

Chronic study involved daily administration of test drug for 28 days (once a day) at predetermined time and blood glucose was determined 7th, 14th, 21st and 28th day.

The Serum Insulin, Pancreatic Insulin, liver glycogen Glycosylated Haemoglobin, and histopathological changes in Pancreas were also evaluated.

Statistical Analysis

The data are presented as the mean \pm SEM. Results were analyzed statistically using the One Way ANOVA followed by Bonferroni multiple comparison tests. The minimum level of significance was set at $p < 0.05$.

RESULTS

Effect of treatment with BSL-150 on OGTT

There was a significant ($p < 0.001$) decrease in glucose clearance observed in group-II (DC) when compared with group-I (NC). On measuring the glucose clearance at 30 min no significant increase in glucose clearance was observed in group-III (Std) when compared with group-II (DC). While on the other hand it has been observed that at 30 min the glucose clearance was significantly ($p < 0.001$) increased in group IV (Test-I) and group V (Test-II.), when compared with group-II (DC). Significant ($p < 0.01$) increase in glucose clearance at 30 min has been also observed in group VI (Test-III.) when compared with group-II (DC). At 60, 90 and 120 min significant ($p < 0.001$) increase in glucose clearance has been observed in all the groups, group-III (Std.), group IV (Test-I.), group V (Test-II.), and group VI (Test-III.) when compared to group-II (DC) as seen in Table 2.

Effect of BSL-150 on Blood Glucose Level in Acute study

As observed in Table 3 the significant ($p < 0.001$) increase in blood glucose was observed in group-II (DC) when compared with group-I (NC). On measuring the blood glucose at 0.5 hrs after administration of test drugs significant ($p < 0.001$) decrease in blood glucose was observed in group-III (Std.) when compared with group-II (DC). With group IV (Test-I.) and group V (Test-II.) significant ($p < 0.01$) decrease in blood glucose was observed at 0.5 hrs when compared with group-II (DC). While with group VI (Test-III.) significant ($p < 0.05$) decrease in blood glucose level was observed at 0.5 hrs when compared with group-II (DC). At 1,2,4,6, and 24 hrs significant ($p < 0.001$) decrease in blood glucose was observed in all the groups, group-III (Std.), group IV (Test-

I.), group V (Test-II.), and group VI (Test-III.) when compared to group-II (DC).

Effect of BSL-150 on Blood Glucose Level in Sub acute study

As seen in table 4, in the sub-acute study conducted for 28 days, significant ($p < 0.001$) increase in the blood glucose was observed in group-II (DC) when compared with group-I (NC). On 7th, 14th, 21st and 28th day the significant ($p < 0.001$) decrease in the blood glucose was observed in all the groups, group-III (Std.), group IV (Test-I.), group V (Test-II.), and group VI (Test-III.) when compared to group-II (DC).

Effect of BSL-150 on Serum Insulin in Sub acute study

Figure 1 shows the effect of treatment on Serum Insulin measured on 29th day of treatment with standard and test drugs. Significant ($p < 0.001$) decrease in the Serum Insulin was observed in group-II (DC) when compared with group-I (NC). The significant ($p < 0.001$) increase in the Serum Insulin was observed in group-III (Std.) when compared with group-II (DC). While on the other hand no significant change in serum insulin has been observed in group IV (Test-I.), group V (Test-II.), and group VI (Test-III.) when compared to group-II (DC).

Effect of BSL-150 on Pancreatic Insulin in Sub acute study

Figure 2 shows the effect of treatment on Pancreatic Insulin measured on 29th day of treatment with standard and test drugs. Significant ($p < 0.001$) decrease in the Pancreatic Insulin was observed in group-II (DC) when compared with group-I (NC). The significant ($p < 0.001$) increase in the Pancreatic Insulin was observed in group-III (Std.) when compared with group-II (DC). While on the other hand no significant change in Pancreatic insulin has been observed in group IV (Test-I.), group V (Test-II.), and group VI (Test-III.) when compared to group-II (DC).

Effect of BSL-150 on Liver Glycogen content in Sub acute study

Figure 3 exhibits the effect of treatment on Liver Glycogen content measured on 29th day of treatment with standard and test drugs. Significant ($p < 0.001$) decrease in Liver Glycogen content was observed in group-II (DC) when compared with group-I (NC). Significant ($p < 0.05$) change in Liver Glycogen content has been observed in group-III (Std.) when compared to group-II (DC). While the significant ($p < 0.001$) increase in Liver Glycogen content was observed in group IV (Test-I.), group V (Test-II.), and group VI (Test-III.) when compared with group-II (DC).

Effect of BSL-150 on Glycosylated Haemoglobin content in Sub acute study

Figure 4 exhibits the effect of treatment on Glycosylated Haemoglobin content measured on 29th day of treatment with standard and test drugs. Significant ($p < 0.001$) increase in Glycosylated Haemoglobin content was observed in group-II (DC) when compared with group-I (NC). While on the other hand significant ($p < 0.001$) decrease in Glycosylated Haemoglobin content was observed in group-III (Std), group IV (Test-I), group V (Test-II), and group VI (Test-III) when compared with group-II (DC).

Effect of BSL-150 on Histology of Pancreas in STZ-NTM induced diabetic mice

Pancreas was observed for following findings under 400X after staining with hematoxylin stain.

- i) Beta cells (slightly elongated cells blue arrow with bright pink colored cytoplasmic granules
- ii) Loss of cytoplasmic granules The histopathological studies showed the more than 75% pathological changes in Pancreas (Fig. 5) in group II (DC) as compared to group I (NC). The Group III (std), IV (Test-I), V (Test-II), and VI (Test-III) showed less pathological changes (50%) as compared to Group II (DC) suggesting that treatment with the test drug is decreasing tissue dam

Table 1. Composition of BSL-150

Sr.No.	Common Name	Quantity	Botanical Name
1	Jambhul beej	150 mg	<i>Syzygium cumini</i>
2	Madhunashini	100 mg	<i>Gymnema sylvester</i>
3	Amalaki	100 mg	<i>Embillica officinalis</i>
4	Guduchi Ghana	50 mg	<i>Tinospora cardifolia</i>
5	Shuddha Shilajit	40 mg	Mineral
6	Abhrak Bhasma	10 mg	Bhasma of mica
7	Naag Bhasma	10 mg	Bhasma of Lead
8	Jasad Bhasma	10 mg	Zinc Oxide
9	Kantloha Bhasma	10 mg	Iron
10	Vang bhasma	10mg	Bhasma of Tin
11	Suvarna makshik Bhsama	10 mg	Bhasma of Gold

Table 2. Effect of treatment with BSL-150 on OGTT in STZ-NTM induced diabetic mice

Groups	Treatment	Blood Glucose Level (mg/dl)				
		0 min	30 min	60 min	90 min	120 min
I	Normal Control	95.3± 1.44	131±1.93	156.6±4.60	127±2.27	94.6±1.89
II	Diabetic Control	361.6± 0.82	415.5±9.40 ^{***}	448 ±16.30 ^{***}	416.3±18.11 ^{***}	383.8±17.31 ^{***}
III	Std	352.8±5.72	393.1±2.42 ^{NS}	314.1±4.04 ^{***}	270.3± 8.67 ^{***}	236.6±9.25 ^{***}
IV	Test-I	305.6±11.36	354.5±13.01 ^{***}	311.5±11.16 ^{***}	250.6±6.58 ^{***}	184.5±3.94 ^{***}
V	Test-II	316.8±15.43	345.1±13.85 ^{***}	308.5±13.76 ^{***}	283.8±12.29 ^{***}	225±12.40 ^{***}
VI	Test-III	311±12.04	366±10.45 ^{**}	323±27.48 ^{**}	291±21.07 ^{***}	248±22.15 ^{***}

All values are expressed as mean ± SEM. n = 6. All data are subjected to One Way ANOVA followed by Bonferroni's multiple comparison test using GraphPad Prism 5 software; When group-II is compared with group I; and group-III, group-IV, group-V and group-VI are compared with group-II. P value are expressed as ***- $p < 0.001$, **- $p < 0.01$, *- $p < 0.05$ and NS is not significant.

Table 3. Effect of BSL-150 on Blood Glucose Level in STZ-NTM induced diabetic mice (Acute study)

Groups	Treatment	Blood Glucose Level (mg/dl)						
		0 hr	0.5 hr	1 hr	2 hr	4 hr	6 hr	24 hr
I	Normal Control	90.83±2.40	91.33±2.34	90.67±2.40	90.83±2.4	91.67±1.54	90.83±2.02	91.5±2.17
II	Diabetic Control	375.16± 8.72	376.5±7.38 ^{***}	374.8±8.33 ^{***}	376.5±7.63 ^{***}	375.5±7.27 ^{***}	375±7.86 ^{***}	376±8.20 ^{***}
III	Std.	346.3± 9.97	319.3±9.10 ^{***}	293.8±8.59 ^{***}	275.3±8.76 ^{***}	253.3±8.29 ^{***}	226.3±8.40 ^{***}	217.3±9.03 ^{***}
IV	Test-I	343.1± 7.32	332.1±7.15 ^{**}	36.3±5.16 ^{***}	298.1±4.26 ^{***}	283.6±4.20 ^{***}	234±3.80 ^{***}	197.1±1.57 ^{***}
V	Test-II	350± 7.85	336.6±7.74 ^{**}	322.8±7.62 ^{***}	308±6.38 ^{***}	290.3±4.72 ^{***}	248.3±3.87 ^{***}	205±3.89 ^{***}
VI	Test-III	353.5± 11.51	342.3±11.43 [*]	327±11.46 ^{***}	313.8±11.75 ^{***}	299.8±10.91 ^{***}	260.8±12.19 ^{***}	219.6±9.73 ^{***}

All values are expressed as mean ± SEM. n = 6. All data are subjected to One Way ANOVA followed by Bonferroni's multiple comparison test using GraphPad Prism 5 software; When group-II is compared with group I; and group-III, group-IV, group-V and group-VI are compared with group-II. P value are expressed as ***- $p < 0.001$, **- $p < 0.01$, *- $p < 0.05$ and NS is not significant.

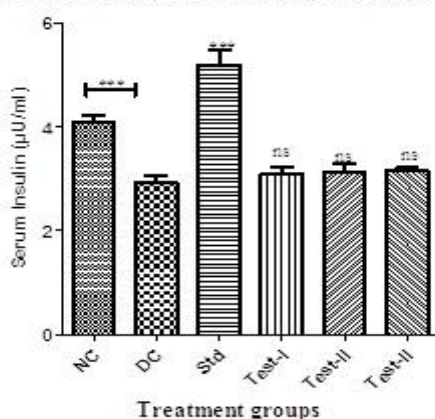
Table 4. Effect of BSL-150 on Blood Glucose Level in STZ-NTM induced diabetic mice (Sub- acute study)

Groups	Treatment	Blood Glucose Level (mg/dl)				
		0 day	7 day	14 day	21 day	28 day
I	Normal Control	97± 2.39	97.5±2.31	96.16±2.98	95.33±2.45	97.83±3.04
II	Diabetic Control	357.1±4.45	359.6±4.54***	359±4.12***	357.1±4.24***	356.1±3.54***
III	Std.	349.6±9.62	244.1±6.03***	202.5±2.36***	159.5±4.63***	100.33±3.06***
IV	Test-I	347±7.74	263.5±8.04***	221.5±4.07***	164.3±4.14***	114.8±3.90***
V	Test-II	345.3±6.06	266.3±3.53***	224±8.19***	165.66±5.17***	120.5±3.53***
VI	Test-III	342.8±6.48	264.5±2.27***	227.8±1.70***	167±3.45***	123.66±3.14***

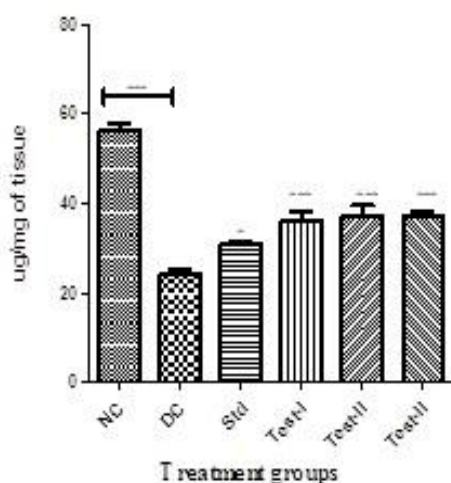
All values are expressed as mean ± SEM. n = 6. All data are subjected to One Way ANOVA followed by Bonferroni's multiple comparison test using GraphPad Prism 5 software; When group-II is compared with group I; and group-III, group-IV, group-V and group-VI are compared with group-II. P value are expressed as ***-p<0.001 and NS is not significant.

Fig 1. Effect of BSL-150 on Serum Insulin in STZ-NTM induced diabetic mice

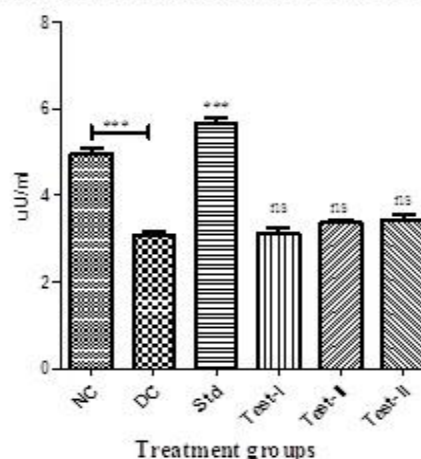
Effect of BSL-150 on serum Insulin level in STZ-NTM induced diabetic mice (Sub-acute study)

**Fig 3. Effect of BSL-150 on Liver glycogen in STZ-NTM induced diabetic mice**

Effect of BSL-150 on Liver Glycogen Content in STZ-NTM induced diabetic mice (Sub-acute Study)

**Fig 2. Effect of BSL-150 on Pancreatic Insulin in STZ-NTM induced diabetic mice**

Effect of BSL-150 on Pancreatic Insulin level in STZ-NTM induced diabetic mice (Sub-acute study)

**Fig 4. Effect of BSL-150 on Glycosylated Haemoglobin in STZ-NTM induced diabetic mice**

Effect of BSL-150 on Hb1Ac in STZ-NTM induced diabetic mice (Sub-acute study)

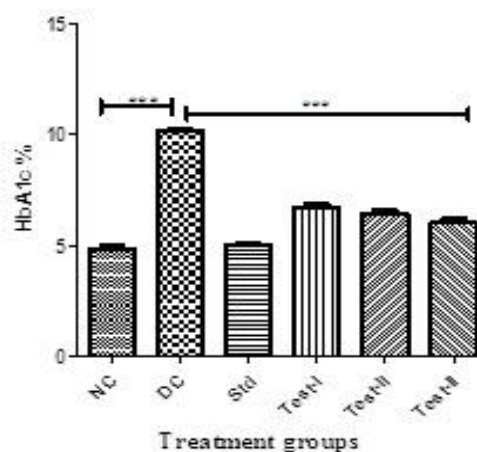
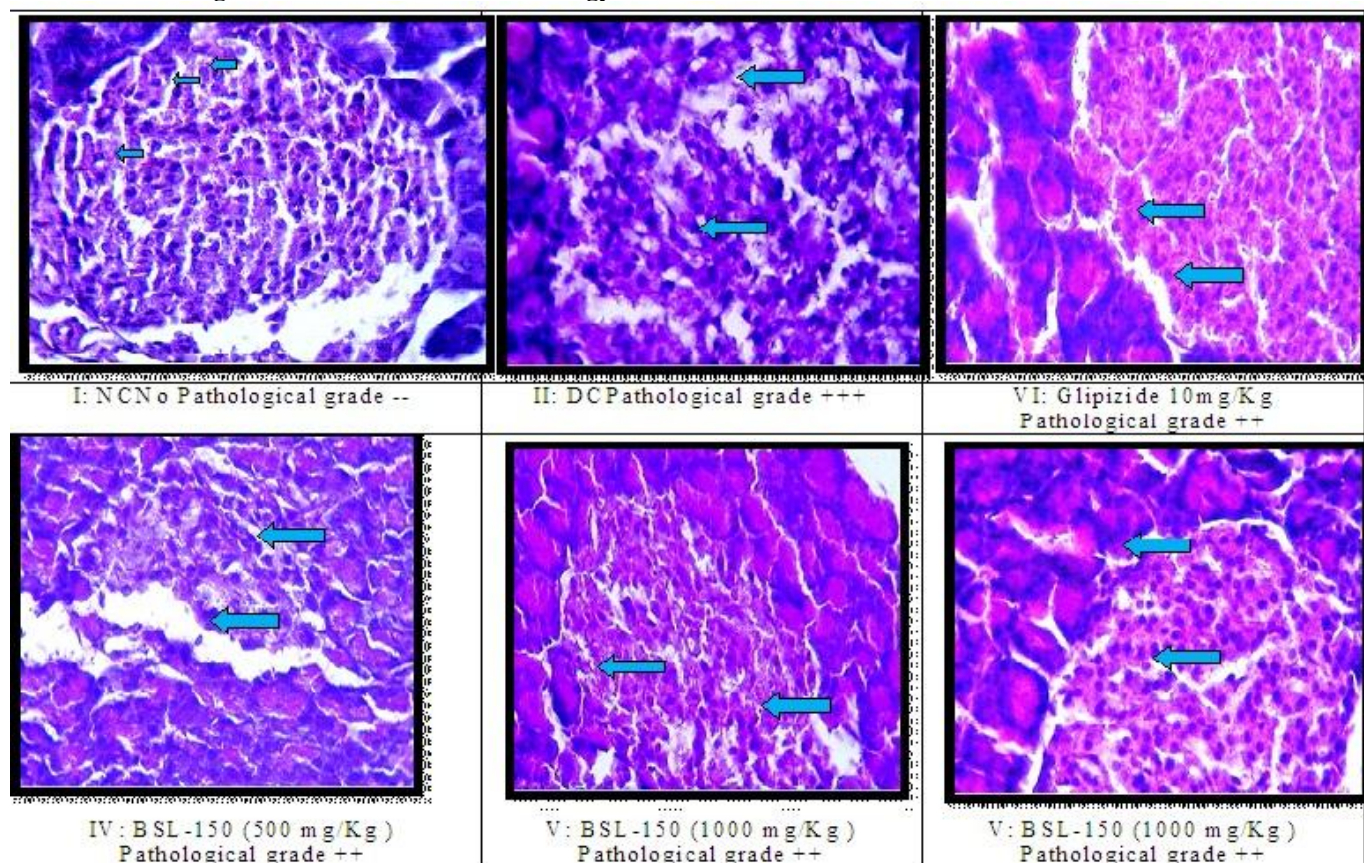


Fig 5. Effect of BSL-150 on Histology of Pancreas in STZ-NTM induced diabetic mice.



Pathological grade: Grade: -- No injury; Grade: +++ severe injury; Grade: ++ moderate injury; Grade: + mild injury

DISCUSSION

The World Health Organization (WHO) has recommended the evaluation of efficacy of plants (Day C, 1998) in various conditions. Most often herbal formulations are prepared from a combination of two or more plant products which many a time contain active constituents with multiple physiological activities (Pieme CA *et al.*, 2006). These formulations are administered in most disease conditions over a long period of time without proper dosage monitoring and consideration of toxic effects that might result from such prolonged usage (Ogbonnia SO *et al.*, 2009).

BSL 150 a polyherbomineral proprietary medicine manufactured by Indu Pharma, Jejuri. consist of *Syzygium cumini*, *Gymnema sylvestre*, *Embillica officinalis*, *Tinospora cardifolia* and bhasmas of tin, gold, iron, lead and mica along with shilajeet. The herbs and bhasmas used in the formulation are used in traditional medicine to treat diabetes mellitus. From all over the world, the fruits of *Syzygium cumini* have been used for a wide variety of ailments, including cough, diabetes, dysentery, inflammation and ringworm (Reynertson KA *et al.*, 2005). *Gymnema sylvestre* has been reported for the treatment of diabetes since 2,000 years. (Saneja A *et al.*, 2010;

Vaidyaratnam, 1995). *Embillica officinalis* has its beneficial role in cancer, diabetes, liver treatment, heart trouble, ulcer, anemia and various other diseases. (Khan KH, 2008). While *Tinospora cardifolia* is widely used in veterinary folk medicine/ ayurvedic system of medicine for its general tonic, antiperiodic, anti-spasmodic, anti-inflammatory, antiarthritic, anti-allergic and anti-diabetic properties (Zhao TF *et al.*, 1991). But as the efficacy of the formulation is not evaluated pre-clinically the formulation BSL150 was evaluated for antihyperglycemic activity in the present study.

Streptozotocin is an antibiotic derived from *Streptomyces achromogenes* and structurally is a glucosamine derivative of nitrosourea. Rakieten and his associates first demonstrated the diabetogenic property of STZ in dogs and rats in 1963. Like alloxan, it causes hyperglycaemia mainly by its direct cytotoxic action on the pancreatic beta cells. The evidences are accumulating on the mechanisms associated with diabetogenicity of STZ. Its nitrosourea moiety is responsible for beta cell toxicity, while deoxyglucose moiety facilitates transport across the cell membrane. Like alloxan, the involvement of free radicals generation and resulting alteration of endogenous scavengers of these reactive species have been reported in

STZ diabetogenicity. Further, STZ causing alkylation or breakage of DNA strands and a consequent increase in the activity of poly-ADP-ribose synthetase, an enzyme depleting NAD in beta cells finally leading to energy deprivation and death of beta cells is reported (Srinivasan K & Ramarao P, 2007).

Recently, a new animal model of type 2 diabetes has been produced by combination of STZ and NAD administration in adult rats. The rats administered NAD (230 mg/kg, ip) 15 min before STZ (65 mg/kg, iv) has been shown to develop moderate and stable non-fasting hyperglycaemia without any significant change in plasma insulin level. As NAD is an antioxidant which exerts protective effect on the cytotoxic action of STZ by scavenging free radicals and causes only minor damage to pancreatic beta cell mass producing type 2 diabetes. Therefore, this model is found to be an advantageous tool for investigation of insulinotropic agents in the treatment of type 2 diabetes (Masiello P *et al.*, 1998). Recently, moderate insulin deficiency and type 2 diabetes was developed in a non-rodent model of Gottingen pig by combination of STZ and NAD, which provides good opportunity to investigate diabetes in much closely similar pathophysiological situation as in human (Larsen MO *et al.*, 2002).

Glucose tolerance test is a standard procedure that addresses how quickly exogenous glucose can be cleared from blood. Specifically, uptake of glucose from the blood by cells is regulated by insulin. Impairment of glucose tolerance (i.e, longer time to clear given amount of glucose) indicates problems with maintenance of glucose homeostasis (insulin resistance, carbohydrate metabolism, diabetes, etc).

The components of BSL-150, *Gymnema sylvestre* (Mishra B and Pancholi SS; 2013), *Tinospora cardifolia*, (Verma RK *et al.*, 2013), *Syzgium cumuni* (Sharma AK *et al.*; 2012) and *Embellica officinalis* (Rajathi D & Daisy P, 2011) are proven to be hypoglycemic when used individually. As seen in Table 1, OGTT studies of the BSL 150 in dose of 250 mg/kg showed statistically significant ($P < 0.001$) increase in glucose clearance when compared to diabetic control group indicating increased glucose uptake by cells. This suggests that the BSL -150 is clearing the glucose from blood which might be due to enhanced uptake of glucose from the cells which is regulated by insulin. The results also point out that the components of BSL-150 are not antagonizing the hypoglycemic effect of each other even in combination.

The acute study / single dose administration study gives the idea about time of onset of action and duration of action. There was a consistent decrease in the blood glucose level in acute study conducted in STZ-NAD induced diabetic mice as shown in table 2. In the groups treated with glipizide and BSL 150 at the doses of 250, 500, 1000 mg/kg of the body weight reduction in blood glucose level was statistically significant ($p < 0.001$) till 24

hrs after administration. At 4 hrs after administration of BSL 150 more than 25% reduction in blood glucose was observed so this can be considered as onset of action of BSL 150 however consistent decrease in blood glucose was observed till the end of 24 hrs, so time of peak action could not be determined from this study. The results obtained suggest that the drug should be administered 4 hrs before meal so that it will effectively start its action and will help to maintain the blood glucose level. The reason behind occurrence of action at 4 hrs may be time required for the release of active constituents from the components of BSL 150, their systemic absorption and then reaching to site of action.

As shown in Table 3. In the Sub acute study conducted in STZ-NAD induced diabetic mice the consistent decrease in the blood glucose level was observed in the groups treated with glipizide and BSL 150 at the doses of 250, 500, 1000 mg/kg of the body weight till 28 days of administration. A marked decrease in blood glucose was started after 7 days of drug administration. On 14th day more than 25 % reduction in blood glucose was observed. On 28th day of administration the blood glucose was found closer to normal blood glucose level. This suggests that the BSL 150 required the duration of about 28 days to reduce the blood glucose level to physiological limit.

Figures 1, 2, 3 and 4 show the effect of treatment with BSL-150 on serum insulin, pancreatic insulin, liver glycogen and Glycosylated haemoglobin respectively. Insulin is the main regulator for glycogenesis in liver. The decrease of liver glycogen observed in this study may be due to lack of insulin in diabetic state (Grover JK *et al.*, 2002) or oxidative stress by diabetes may inactivate the glycogen synthetase. In study conducted also similar decrease in the serum insulin, pancreatic insulin and liver glycogen was observed in diabetic animal. After 28 days treatment with BSL-150 the liver glycogen level was significantly elevated but the serum and pancreatic insulin were found to be unchanged as compared to diabetic control group. In the view of glycogen level, possible way of antidiabetic action of BSL 150 may be by preventing the inactivation of the glycogen synthetase and by synthesize the glycogen synthetase (Selvan VT *et al.*, 2008).

In uncontrolled or poorly controlled diabetes, there is an increased glycosylation of number of proteins including haemoglobin. (Pari L & Amarnath SM, 2004) Figure 4 shows the effect of treatment with Glycosylated haemoglobin. Statistically significant decrease ($p < 0.001$) in the Glycosylated haemoglobin was observed while the untreated diabetic controlled group showed the increase in Glycosylated haemoglobin. This could be due to the result of improved glycemic control produced by components of BSL-150.

A decrease on the number of cytoplasmic secretory granules has been reported in beta cells of STZ induced diabetic rats (Hong EV *et al.*, 2002; Decermenci I

et al., 2005). As observed in figure 5 the histopathological studies of pancreas shows the marked decrease in beta cell mass and cytoplasmic granules in Diabetic control group as compared to normal control group. In the groups treated with BSL-150 and glipizide showed less damage to beta cell mass and reduced loss of cytoplasmic granules indicating protective role of components of BSL 150. The results of this study are in accordance with the previous report.

CONCLUSION

The present finding suggests that BSL 150, a

combination of traditionally used herbs and bhasmas has a potent hypoglycemic action in STZ-Nicotinamide induced diabetic mice. The formulation is significantly reducing the glycemic parameters, thus it can be an alternative as well as supportive medication in case of type 2 diabetic individuals.

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