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Study of Pharmacodynamic Interaction between a Polyherbal Formulation BSL-150 and Metformin

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Abstract: The limitations of currently available oral antidiabetic agents either in terms of efficacy/safety coupled with the emergence of the disease into global epidemic have encouraged alternative therapy that can manage diabetes more efficiently and safely. Herbal medications are the most commonly domestic medicines accepted as alternative therapy for blood sugar control; however, their safety and efficacy need to be further evaluated by well-designed, controlled clinical studies. Because various non-standardized forms of the herbs have often been the testing material, the results have been difficult to replicate. Therefore, preparation of standardized medicinal herbs is urgently needed in future studies and therapies. Though the herbs used for the treatment of diabetes are thought to have less drawbacks, potential adverse herb-drug interactions should be kept in mind for patients also receiving conventional antidiabetic medications. The present study was designed to investigate the possible herb drug interaction between a proprietary polyherbal formulation BSL-150 and metformin in streptozotocin-nicotinamide (STZ-NIC) induced diabetes induced in mice. Metformin was given orally at a dose of 500 mg/kg and BSL-150 was administered at a dose 250 mg/kg. The effect of single and repeated oral administration of BSL-150 and metformin on blood glucose level, serum and pancreatic insulin, liver glycogen content and glycosylated haemoglobin was evaluated. The significant ($P<0.001$) reduction in the blood glucose, liver glycogen content and glycosylated haemoglobin while no significant change in serum and pancreatic insulin level in combination treated group was observed as compared to the group treated with metformin alone.

Keywords: BSL-150, diabetes, herb-drug interaction, metformin, streptozotocin-nicotinamide.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by a hyperglycemia caused by insulin deficiency, often combined with insulin resistance. Hyperglycemia occurs because of uncontrolled hepatic glucose output and reduced uptake of glucose by skeletal muscle with reduced glycogen synthesis [1]. Diabetes mellitus is classified on the basis of the pathogenic process that leads to the hyperglycemia. The broad categories of DM are designated type 1 and type 2. Type 1A DM results from autoimmune beta cell destruction which leads to insulin deficiency. Individuals with type1B lack immunologic markers indicative of autoimmune destruction process of the beta cell. Type 2 DM is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired glucose secretion, and increase glucose production. Distinct genetic and metabolic defects in insulin action and / or secretion give rise to the phenotype of hyperglycemia in type 2 DM [2]. DM is a serious metabolic disease that has a significant impact on the health, quality of life and life expectancy of patients, as well as on the health care system [3]. As the number of people with diabetes multiply worldwide, the disease takes an ever-increasing proportion of national and international health care budgets. It is projected to become one of the world's main disablers and killers within the next 25 years. Regions with greatest potential are Asia and Africa, where DM rates could rise to two to three-folds than the present rates. It is currently growing at a rapid rate throughout the world, and it is the 16th leading cause of global mortality [4]. Current estimates indicate that 8.3% of the adult population, or 71.4 million people, had diabetes in 2011, 61.3 million of whom were in India. The number of people with diabetes in the region will increase to 120.9 million by 2030, or 10.2% of the adult population. A further 23.8 million people have impaired glucose tolerance (IGT) in 2011, and this will increase to 38.6 million by 2030. The number of people with diabetes in India, Bangladesh and Sri Lanka make up 99% of the total for the region. The estimated increase in regional diabetes prevalence to 10.2% in 2030 is a consequence of increasing life expectancy in India (the proportion of the population over 50 years is expected to increase from 16% to 23% from 2011 to 2030), and of rapid urbanization [5].

There are varieties of glucose-lowering agents available for the treatment of DM 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 glu-

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cose control had increased weight gain, increased risk of hypoglycemia and increased risk of mortality during the study [6]. The limitation of currently available oral antidiabetic agents either in terms of efficacy/safety coupled with the emergence of the disease into global epidemic has encouraged alternative therapy that can manage diabetes more efficiently and safely. Physical interventions such as Yoga, Acupuncture, Massage, use of medicinal herbs and dietary supplement are the some alternative therapies reported to be useful in glucose lowering [7]. The Indian prehistoric literature reports more than 800 plant species with antidiabetic properties. While ethno pharmacological surveys indicate that more than 1,200 plant species can be used for hypoglycemic activity [8]. Herbs are also known to provide symptomatic relief and aid in the prevention of the secondary complications of the disease including cholesterol lowering action. Some of these herbs have also been proven to help in the regeneration of β-cells and in overcoming insulin resistance [9].

The alternative therapies with antihyperglycemic effects are increasingly sought by patients with diabetes. Herbal medications are the most commonly used alternative therapy for blood sugar control; however, their safety and efficacy need to be further evaluated by well-designed, controlled clinical studies. Because various non-standardized forms of the herbs have often been the testing material, the results have been difficult to replicate. Therefore, preparation of standardized medicinal herbs is urgently needed in future studies and therapies. Although herbs used for diabetes are less likely to have the drawbacks of conventional drugs, potential adverse herb-drug interactions should be kept in mind for patients also receiving conventional antidiabetic medications [10].

BSL-150 is a polyherbomineral formulation, a proprietary medicine manufactured by Indu Pharma, Jejuri. As shown in Table **1**, it consists of *Syzygium cumini, Gymnema sylvester, 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. Though the formulation BSL-150 is utilized by the patients with diabetes as alternative therapy for diabetes, its interaction with widely used antidiabetic drugs is not yet evaluated. So in the present investigation the formulation BSL-150 is evaluated for interaction with metformin in STZ-NIC induced diabetes in mice.

MATERIALS AND METHODS

Chemicals

The BSL-150 was obtained as gift sample from Indu Pharma, Jejuri. Metformin was obtained as gift sample from industries. The chemicals required were purchased from local supplier Research laboratories, Hadapsar, Pune. The composition of BSL-150 is shown in Table **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 were provided *ad libitum*. The experimental protocol was approved by the IAEC (SGRS/IAEC/10/20010-11).

Preparation and Administration of Test Drugs

The test herbal drug was triturated and suspended in 5% carboxymethyl cellulose (CMC). Metformin was dissolved in water. The accurately measured dose was administered to animals [11].

Induction of Diabetes with Streptozotocin–Nicotinamide (STZ-NIC)

In overnight fasted mice, streptozotocin (STZ) was injected (150 mg/kg, i.p.) 15 min after Nicotinamide (NIC)

Table 1. Composition of BSL-150.

Sr.No.	Common Name	Quantity	Botanical Name
1	Jambhulbeej	150 mg	*Syzygium cumini*
2	Madhunashini	100 mg	*Gymnema sylvester*
3	Amalaki	100 mg	*Embillica officinalis*
4	Guduchi Ghana	50 mg	*Tinospora cardiofolia*
5	ShuddhaShilajit	40 mg	Mineral
6	AbhrakBhasma	10 mg	Bhasma of mica
7	NaagBhasma	10 mg	Bhasma of Lead
8	JasadBhasma	10 mg	Zinc Oxide
9	KantlohaBhasma	10 mg	Iron
10	Vangbhasma	10mg	Bhasma of Tin
11	SuvarnamakshikBhsama	10 mg	Bhasma of Gold

injection (110 mg/kg) in all the groups except for group I which was non-diabetic. Animals were fed with glucose solution (5%) for 12 hrs to avoid hypoglycemia. Hyperglycemia was confirmed after 3 days. Mice having serum glucose >250 mg/dl were selected for the study [12].

Interaction Study

The mice were divided in five groups (each consisting 6 mice) and treated with test drugs as following (Table **2**).

Table 2. The experiment design for the induction of diabetes with streptozotocin Nicotinamide (STZ-NIC).

Group	Name of the Group	Treatment
Group-I	Normal Control	vehicle 5% CMC p.o.
Group-II	Diabetic Control	NIC (110 mg/kg, i.p) + STZ (150 mg/kg, i.p.) + vehicle 5% CMC p.o.
Group-III	BSL	NIC (110 mg/kg, i.p) + STZ (150 mg/kg, i.p.)+ BSL-150 (250 mg/kg p.o.)
Group IV	M	NIC (110 mg/kg, i.p.)+ STZ (150 mg/kg, i.p.)+ Metformin (500 mg/kg p.o.)
Group V	M+BSL	NIC (110 mg/kg, i.p.)+ STZ (150 mg/kg, i.p.)+ Metformin (500 mg/kg p.o.) + BSL-150 (250mg/kg p.o.)

The study was carried out in three ways:

OGTT: The mice were fasted for 12-14 hrs. 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 0, 30, 60, 90, and 120 min was measured [13, 14].

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, 0.5, 1, 2, 4, 6, and 24 hrs was evaluated [14, 15].

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 on 0, 7^{th}, 14^{th}, 21^{st} and 28^{th} day [14, 15].

Determination of Serum insulin content: On day 28, blood was collected by retro orbital puncture method in Eppendorff's tube. Serum was separated by centrifugation of blood at 7,000 RPM for 20 min by using Remi's cold centrifuge.Serum insulin was determined by using SIEMENS ADVIA Centaur immunoassay system [16].

Determination of pancreatic insulin content: Pancreata from euthanized mice was homogenized in an ice cold concentrated hydrochloric acid: ethanol (1:4, v/v) and centrifuged at 4 ºC at 7,000 RPM The supernatant obtained after centrifugation was pooled and stored in amber colour vials at -20 ºC until assayed [17]. Pancreatic insulin was assayed by using SIEMENS ADVIA Centaur immunoassay system [16].

Determination of liver glycogen content: Tissue glycogen was extracted and estimated by the method of Morales *et al.* [18].The alkali extract of the tissue was prepared by digesting 50 mg of fresh tissue for 15 min with 3 ml of 30% potassium hydroxide solution in a boiling water bath. The tubes were cooled and mixed with 5 ml of absolute alcohol and a drop of 1 M ammonium acetate was added to precipitate glycogen and then placed in the freezer overnight for complete precipitation. Glycogen was collected by centrifugation for 20 min at 2,000 rpm. The final precipitate was dissolved in saturated ammonium chloride solution; 4 ml of Anthrone reagent was added by cooling the tubes in an ice bath. The tubes were shaken well, covered with marble caps, and heated for 20 min in a boiling water bath. After cooling, the absorbance was read at 640 nm against a reagent blank treated in a similar manner. A standard glucose solution was also treated similarly. The glycogen content was calculated from the amount of glucose present in the sample and expressed as mg/g tissue.

Determination of glycosylated haemoglobin content: The glycosylated content was measured by using ErbaChem assay kit [19].

Statistical Analysis

The data are presented as the mean ± 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 Co-administration of BSL-150 with Metformin on OGTT in STZ-NIC Induced Diabetic Mice

There was a significant ($p<0.001$) decrease in glucose clearance observed in group-II (DC) when compared with group-I (NC). In group-III (BSL), group-IV (M) and V (BSL+M) significant ($p<0.001$) decrease in blood glucose level at 60, 90 & 120 min was observed as compared to group-II (DC). Similarly in group V (BSL+M) treated with a combination of BSL-150 (250 mg/kg, p.o) and Metformin (500 mg/kg, p.o) significant ($p<0.001$) decrease in blood glucose level at 90 min and at 120 min was observed as compared to group-IV treated with Metformin (500 mg/kg, p.o). At 90 and 120 min, significant ($p<0.001$) increase in glucose clearance has been observed in all the groups, group-III (BSL), group IV (M), group -V (M+BSL) when compared to group-II (DC) as seen in Table **3**.

Effect of BSL- 150 with Metformin on Blood Glucose Level in STZ-NIC Induced Diabetic Mice (Acute Study)

As observed in Table **4**, significant ($p<0.001$) increase in blood glucose was observed in group-II (DC) when compared with group-I (NC). At 24 hrs of single dose administration of test drugs significant ($p<0.001$) decrease in the blood glucose level was observed in the groups, III (BSL), group-IV (M) and group-V (BSL+M) as compared to group-II (DC). In group V (BSL+M) treated with a combination of

Table 3. Effect of co-administration BSL-150 with metformin on OGTT in STZ-NIC induced diabetic mice.

Sr. No.	Treatment Groups (mg/kg)	Blood Glucose Level (mg/dl)				
		0 min	30 min	60 min	90 min	120 min
Group I	Normal Control	95.3± 1.44	131± 1.93	156.6± 4.60	127± 2.27	94.6± 1.89
Group-II	Diabetic Control	348.5± 4.91	545.33± 4.92^{***}	540.5± 4.18^{***}	525± 2.47^{***}	487.5± 3.16^{***}
Group-III	BSL	305.6± 11.36	354.5± 13.01^{**}	311.5± 11.16^{**}	250.6± 6.58^{**}	184.5± 3.94^{**}
Group-IV	M	353.83± 17.83	495.33± 19.79^{NS}	407.16± 23.17^{***}	359.83± 19.27^{***}	328± 32.81^{***}
Group-V	BSL+ M	357.45± 14.35	460± 18.60^{NS}	392.16± $13.39^{***,NS}$	281.33± $21.65^{***,\#\#}$	246.5± $14.96^{***,\#}$

Notes: All values are expressed as mean ± SEM. n = 6. All data are subjected to One Way ANOVA followed by Bonferroni's multiple comparison test. BSL: BSL-150, M: Metformin. Group II is compared with group I. Group III, IV, V, are compared with group II. *p<0.05, **p<0.01, ***p<0.001, NS: Not Significant. Multiple Comparison: Group V is compared with group IV. # p<0.05, ## p<0.01, ### p<0.001, NS: Not Significant.

Table 4. Effect of co-administration BSL-150 with metformin on blood glucose level in STZ-NIC induced diabetic mice (Acute study).

Sr. No.	Treatment Groups (mg/kg)	Blood Glucose Level (mg/dl)						
		0 hr	0.5 hr	1 hr	2 hr	4 hr	6 hr	24 hr
Group-I	Normal Control	90.83± 2.40	91.33± 2.34	90.67± 2.40	90.83± 2.41	91.67± 1.54	90.83± 2.02	91.5± 2.17
Group-II	Diabetic Control	383.16± 5.28	400.33± 26.77^{***}	385± 19.76^{***}	375.33± 26.80^{***}	388± 28.65^{***}	398.16± 14.53^{***}	398.16± 13.45^{***}
Group-III	BSL	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^{**}
Group-IV	M	403.33± 19.41	383.50± 21.82^{NS}	348± 25.18^{NS}	316.66± 22.29^{NS}	293.83± 23.43^{NS}	250.55± 26.42^{***}	227.45± 18.75^{***}
Group-V	BSL+ M	389± 15.12	335.50± $16.66^{NS,NS}$	266.± $13.07^{***,NS}$	210.66± $9.16^{***,\#\#}$	149.50± $8.58^{***,\#\#\#}$	114± $6.27^{***,\#\#\#}$	106.33± $4.26^{***,\#\#\#}$

Notes: All values are expressed as mean ± SEM. n = 6. All data are subjected to One Way ANOVA followed by Bonferroni's multiple comparison test. BSL: BSL-150, M: Metformin. Group II is compared with group I. Group III, IV, V are compared with group II. *p<0.05, **p<0.01, ***p<0.001, NS: Not Significant. Multiple Comparison: Group V is compared with group IV. # p<0.05, ## p<0.01, ### p<0.001, NS: Not Significant.

BSL-150 (250 mg/kg, p.o) and Metformin (500 mg/kg, p.o), significant (p<0.001) decrease in blood glucose level at 4, 6 and 24 hrs was observed as compared to group-IV treated with Metformin (500 mg/kg, p.o). The results suggest the potentiation of effect of metformin by BSL-150 at 4, 6 and 24 hrs of treatment.

Effect of BSL- 150 with Metformin on Reduction in Blood Glucose Level in STZ-NIC Induced Diabetic Mice (Acute Study)

As observed in Fig. (**1**) the percentage reduction in the blood glucose level is calculated from the data obtained in acute study. The percentage reduction was found to be 42.43± 1.12 in group-III (BSL), 45.04± 2.55 in group-IV (M), while 72.60± 0.86 in group-V (BSL+M). Significant (p<0.001) decrease in percentage blood glucose level in group V (BSL+M) was observed as compared to group-IV (M) indicating potentiation of antihyperglycemic effect of metformin by BSL-150.

Effect of BSL- 150 with Metformin on Blood Glucose Level in STZ-NIC Induced Diabetic Mice (Sub-acute Study)

In sub acute study conducted in STZ-NIC induced diabetic mice, significant (p<0.001) increase in blood glucose level in group-II (DC) was observed as compared to group I (NC). At the end of 28 day of treatment schedule, significant (p<0.001) decrease in the blood glucose level was observed in the groups, III (BSL), IV (M) and V (BSL+M) as compared to group-II (Diabetic Control). In group V (BSL+M) treated with combination of BSL-150 (250 mg/kg, p.o) and Metformin (500 mg/kg, p.o), significant (p<0.001) decrease

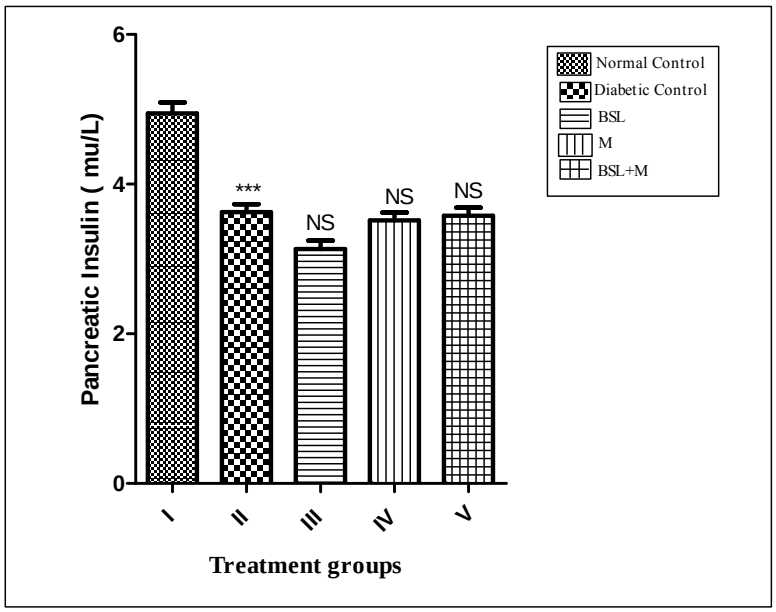

Fig. (4). Effect of coadministration of BSL-150 with metformin on pancreatic insulin in STZ-NIC induced diabetic mice. All values are expressed as mean ± SEM. n = 6. All data are subjected to One Way ANOVA followed by Bonferroni's multiple comparison test. BSL: BSL-150, M: Metformin. Group II is compared with group I. Group III, IV, V are compared with group II. *p<0.05, **p<0.01, ***p<0.001, NS: Not Significant. Multiple Comparison: Group V is compared with group IV.# p<0.05, ## p<0.01, ### p<0.001, NS: Not Significant.

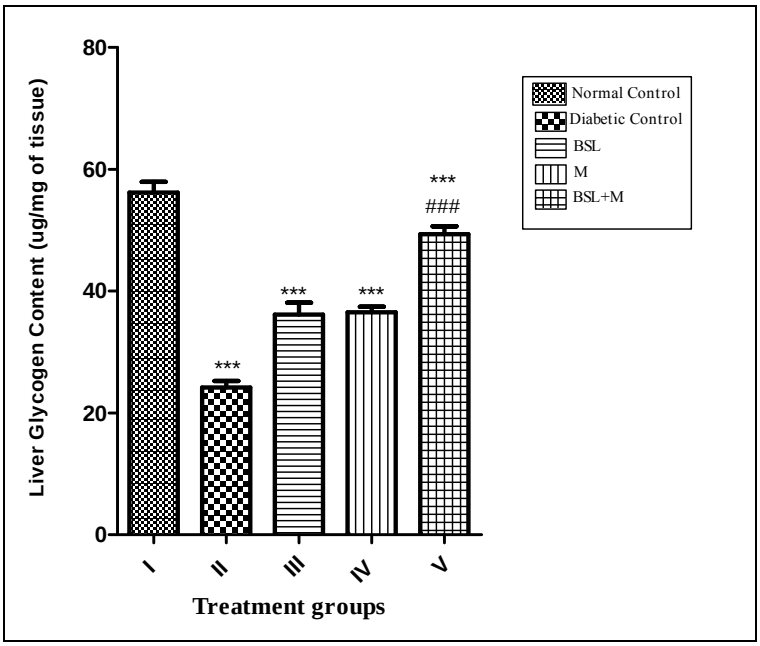

Fig. (5). Effect of coadministration of BSL-150 with metformin on liver glycogen content in STZ-NIC induced diabetic mice. All values are expressed as mean ± SEM. n = 6. All data are subjected to One Way ANOVA followed by Bonferroni's multiple comparison test. BSL: BSL-150, M: Metformin. Group II is compared with group I. Groups III, IV, V are compared with group II. *p<0.05, **p<0.01, ***p<0.001, NS: Not Significant. Multiple Comparison: Group V is compared with group IV. # p<0.05, ## p<0.01, ### p<0.001, NS: Not Significant.

dangerous side effects and / or reduced benefits from the medication. Currently, there is very little information published on herb-drug interactions whilst the use of herbs is progressively growing across the world. As there is large belief that herbal medicines are safe to use, it needs to be understood that depending on the amount and potency of the pharmacologic principles contained in the herbal preparation, potential exists for herb-drug interaction to occur when the herbal product is consumed with the modern day medicine [20].

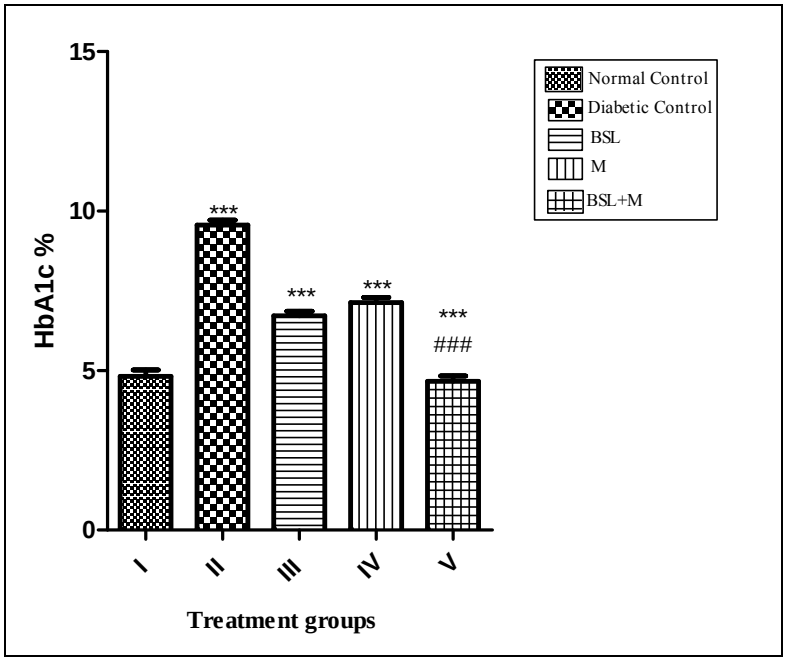

Fig. (6). Effect of coadministration of BSL-150 with OHAs on glycosylated heamoglobin in STZ-NIC induced diabetic mice. All values are expressed as mean ± SEM. n = 6. All data are subjected to One Way ANOVA followed by Bonferroni's multiple comparison test. BSL: BSL-150, M: Metformin. Group II is compared with group I. Groups III, IV, V are compared with group II. *p<0.05, **p<0.01, ***p<0.001, NS: Not Significant. Multiple Comparison: Group V is compared with group IV.# p<0.05, ## p<0.01, ### p<0.001, NS: Not Significant.

Today, the understanding of the interactions between drugs and herbs and between drugs and food is still in its infancy. Much research is still required in herbal therapy to examine individual plant constituents and to determine how plants interact with drugs and food. Some researchers suggest that herb-drug interactions occur less often than predicted. If an interaction between herb and a drug does occur, conventional drugs are usually the culprits because they are more pharmacologically active [21].

BSL-150, a polyherbomineral proprietary medicine manufactured by Indu Pharma, Jejuri. consists of *Syzygium cumini, Gymnema sylvester, 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 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 [22]. *Gymnema sylvester* has been reported for the treatment of diabetes since 2,000 years [23, 24]. *Embillica officinalis* has its beneficial role in cancer, diabetes, liver treatment, heart trouble, ulcer, anemia and various other diseases [25]. While *Tinospora cardiofolia* 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 [26].

The probable mechanism of action of BSL-150 may be through antihyperglycemic activity as the components of BSL-150 have been reported for antihyperglycemic effect in previous studies [23, 27, 28].

As the formulation BSL-150 is used clinically as antidiabetic formulation but its interaction with currently used oral hypoglycemic agents such as biguanides, sulphonylureas etc. is not yet evaluated. The present study was aimed at the evaluation of pharmacodynamic interaction between metformin and BSL-150.

STZ 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 hyperglycemia mainly by its direct cytotoxic action on the pancreatic beta cells. Evidences are accumulating on the mechanisms associated with diabetogenecity 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 diabetogenecity. 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 have been reported [29].

Recently, a new animal model of type 2 diabetes has been produced by the combination of STZ and NIC administration in adult rats. The rats administered NIC (230 mg/kg, ip) 15 min before STZ (65 mg/kg, iv) showed to develop moderate and stable non-fasting hyperglycemia without any significant change in plasma insulin level. As NIC is an antioxidant, it 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 has found to be an advantageous tool for investigation of insulinotropic agents in the treat-

ment of type 2 Diabetes [30]. Recently, moderate insulin deficiency and type 2 diabetes were developed in a nonrodent model of Gottingen pig by the combination of STZ and NIC, which provide good opportunity to investigate diabetes in much closely similar pathophysiological situation as in human [31].

The interaction studies were carried out by co-administration of BSL-150 with metformin agents with the aim to evaluate the pharmacodynamic interaction between them. The prime aim in the treatment of diabetes mellitus is to decrease the elevated blood glucose to normal physiological level so as to prevent further micro and macro vascular complications. Metformin is recognized as a first-line antidiabetic agent for the management of type 2 diabetes [32]. It is suitable irrespective of age, body weight, severity of hyperglycemia and provides a convenient pharmacological base for combined therapy with other antidiabetic agents [33]. Metformin has lower mortality and cardiovascular risk as compared with most insulin secreting agents such as glimepiride, glibenclamide, glipizide and tolbutamide in patients with type 2 diabetes mellitus [34]. Another benefit of metformin is that it does not produce hypoglycemia because it does not stimulate insulin secretion when it is given alone in patients with type diabetes mellitus [35].

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) [36].

As seen in Table **2**, in group V (BSL+M) treated with a combination of BSL-150 (250 mg/kg, p.o) and Metformin (500 mg/kg, p.o), significant ($p<0.001$) decrease in blood glucose level at 90 min and at 120 min was observed as compared to group-IV treated with Metformin (500 mg/kg, p.o). The results indicate the BSL -150 in combination with metformin increases the clearance of the glucose from blood which might be due to enhanced uptake of glucose from the cells which is regulated by insulin in the presence of metformin.

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-NIC induced diabetic mice as shown in Table **3**. In group-V (BSL+M) treated with a combination of BSL-150 (250 mg/kg, p.o) and Metformin (500 mg/kg, p.o), significant ($p<0.001$) decrease in blood glucose level at 4, 6 and 24 hr was observed as compared to group-IV treated with Metformin (500 mg/kg, p.o). The results suggest the potentiation of effect of metformin by BSL-150 at 4, 6 and 24 hrs of treatment. The percentage reduction in blood glucose level is calculated from the data obtained in study. The significant ($p<0.001$) decrease in percentage blood glucose level in group V (BSL+M) was observed as compared to group-IV (M) indicating potentiation of antihyperglycemic effect of metformin by BSL-150.

As shown in Table **4**, in the subacute study conducted in STZ-NIC induced diabetic mice, the consistent decrease in the blood glucose level was observed in the groups treated with metformin, BSL-150 and a combination of metformin and BSL-150. In group-V (BSL+M) treated with a combination of BSL-150 (250 mg/kg, p.o) and Metformin (500 mg/kg, p.o), significant ($p<0.001$) decrease in blood glucose level at 28 day was observed as compared to group-IV treated with Metformin (500 mg/kg, p.o). Similarly, significant ($p<0.01$) reduction in percentage blood glucose was observed in group-V (BSL+M) as compared to group IV (M) indicating potentiation of antihyperglycemic effect of metformin by BSL-150.

Figs. (**3**, **4**, **5**, and **6**) show the effect of treatment with BSL-150 on serum insulin, pancreatic insulin, liver glycogen and glycosylated heamoglobin respectively. Insulin is the main regulator for glycogenesis in liver. The decrease of liver glycogen observed in this study that may be due to lack of insulin in diabetic state or oxidative stress by diabetes may have inactivated the glycogen synthetase [37]. In the study conducted also similar decrease in the serum insulin, pancreatic insulin and liver glycogen was observed in the diabetic animal. In the groups treated with combination, no significant increase in serum insulin, pancreatic Insulin and liver glycogen was observed in group-V (BSL+M) as compared to group-IV (M). While Significant increase in liver glycogen and Glycosylated haemoglobin ($p<0.001$) was observed in group-V (BSL+M) as compared to group-IV (M).

CONCLUSION

The present finding suggests that BSL-150, a polyherbal formulation, potentiates the effect of metformin. The chemical constituents from major components of formulation i.e. *Syzegium cumuni, Tinospora cardiofolia, Embellica officinalis and Gymnema sylvestre* may be responsible for this potentiation. The results obtained in the study suggest possible use of the BSL-150 in a diabetic patient having intolerance to metformin and contraindication to other oral hypoglycemic agents. The patient can be placed on a reduced dose of metformin (which also implies lower adverse effect) while being encouraged to consume BSL-150.

ABBREVIATIONS

ACCORD	=	Action to Control Cardiovascular Risk in Diabetes
BSL-150	=	Proprietary name of Polyherbal formulation by Indu Pharma, Jejuri
DM	=	Diabetes mellitus
NIC	=	Nicotinamide
STZ	=	Streptozotocin
i.p.	=	Intra peritoneal
p.o.	=	Per oral
s.c.	=	Subcutaneous
OGTT	=	Oral glucose tolerance test
mg	=	Milligram
kg	=	Kiligram

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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PATIENT'S CONSENT

Declared none.

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