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ACUTE AND SUBACUTE TOXICITY STUDIES OF THE ANTIDIABETIC POLYHERBOMINERAL FORMULATION BSL-150 IN EXPERIMENTAL ANIMAL MODELS

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ABSTRACT

A polyherbomineral formulation BSL-150 is an anitidabetic formulation manufactured by Indu Pharma and consists of combination of herbs and various bhasmas. It is used for the treatment of diabetes mellitus. The purpose of this study is to investigate the formulation for its safety and toxicity effects after oral administration in Swiss albino mice. Each tablet of BSL-150 weighing 500 mg contains *Gymnema sylvestere, Tinospora cardiofolia, Embellica officinalis* and various bhasmas. In an acute toxicity study the BSL 150 suspended in 5% CMC was administered orally at the dose of 2000 mg/kg and were observed closely for 72 hrs with special attention for first 4 hrs. No any mortality was observed at the dose of 2000 mg /kg. In sub acute toxicity studies BSL 15o was administered orally at the dose of 250, 500, and 1000 mg /kg to animals for 28 days. Then the animals were sacrificed and various serum parameters were measured. The histopathological changes in liver, kidney, and pancreas were studied. The results of the 28 day sub acute toxicity study shows no any change in the hematological, serum parameters and histological studies.

It is concluded that the BSL 150 is safe at the dose of 2000 mg/kg of oral dose per day. The results indicate the safety of the formulation in the long term treatment with the BSL 150.

Key Words: Diabetes mellitus, BSL-150, acute and sub acute toxicity.

1. INTRODUCTION

Diabetes mellitus is an endocrine disorder that is characterized by hyperglycemia.[1] It is well known that the incidence of diabetes mellitus is high all over the world, especially in Asia. Various types of Oral Hypoglycemic agents such as biguanides, sulphonylureas and insulin for the treatment of diabetes mellitus[2] but are associated with the side effects. [3] There is growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relative low cost. Numbers of investigations have been conducted to evaluate hypoglycemic potential of plants used in traditional medicines and many plants have been found with good activity. [4][5]The World Health Organization (WHO) also has recommended the evaluation of plants efficacy. [6] The pharmacological effects of many plants have been studied in various laboratories whereas there are many limitations regarding the safety and efficacy of these preparations. [7] 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 sylvester, Embillica officinalis, Tinospora cardiofolia* and bhasmas of tin, gold, iron, lead and mica along with shilajjet. The herbs and bhasmas used in the formulation are used in traditional medicine to treat diabetes mellitus. But as the safety and efficacy of the formulation is not evaluated pre-clinically the formulation BSL150 was evaluated for acute and sub acute toxicity in the present study. .

2. MATERIAL & METHODS

2.1 Chemicals and Drugs

The BSL 150 was obtained as gift sample from Indu Pharma, Jejuri. The composition of BSL 150 is as follows.

2.2 Heavy Metal Analysis

Heavy metal analysis was done at Food Hygiene and Health Laboratory, Pune (Invoice No. 447).

2.3 Animals

Swiss albino mice and Wistar albino rats 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 experimental protocol was approved by the IAEC (SGRS/IAEC/20/2011)

Table No. 1: Composition of BSL-150

Sr.No.	Common Name	Quantity	Botaniacal Name
1	Jambhul beej	150 mg	*Syzygium cumini* [8]
2	Madhunashini	100 mg	*Gymnema sylvester* [9]
3	Amalaki	100 mg	*Embillica officinalis* [10]
4	Guduchi ghana	50 mg	*Tinospora cardiofolia* [11]
5	Shuddha Shilajit	40 mg	Mineral [11]
6	Abhrak Bhasma	10 mg	Bhasma of mica [13]
7	Naag Bhasma	10 mg	Bhasma of Lead [13]
8	Jasad Bhasma	10 mg	Zinc Oxide [13]
9	Kantloha Bhasma	10 mg	Iron [13]
10	Vang bhasma		Bhasma of Tin [13]
11	Suvarna makshik Bhsama	10 mg	Gold[13]

The powder of this formulation was suspended in the 5 % Carboxy Methyl Cellulose and administered to the animals by oral feeding needle.

2.4 Acute Toxicity Study

Acute toxicity study was carried out as per the OECD 425 guidelines.

Swiss albino mice of either sex weighing 18-22 gm were administered with the dose of 2000 mg/kg orally of BSL 150 and observed closely for first 4 hours for behavioral and neurological and neurological symptoms and then for 72 hours for mortality. [14][15]

2.5 Sub Acute Toxicity Studies

The Swiss albino rat weighing 200-225 gm were divided in four groups. Group I received vehicle for 28 days and the Group II, Group III and Group IV received the test drug in the dose of 250 mg/kg, 500 mg/kg and 1000 mg/kg p.o. once daily for 28 days respectively. Body weight, food intake and water intake were monitored. The blood was collected from treated animals by retro-orbital puncture method under anesthesia. The serum was separated and analyzed for hematological parameters such as heamoglobin, RBC, [16] WBC, [17] hematocrit etc and biochemical parameters such as Aspartate amino transferase (AST), [18] alanine aminotransferase (ALP), alkaline phsphatase, [19]serum Creatinine, [20] blood urea

nitrogen. [21] The parameters were analyzed on autoanalyzer by using Erba Chem diagnostic kits. The liver and kidney were isolated for histopathological studies.

2.6. 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

The heavy metal analysis done as shown in Table no. 2 reports the presence of heavy metals lead, mercury and arsenic within the limits as per the USFDA as herbal formulation is concerned.

Table No.2: Report on Heavy Metal Analysis

Sr. No.	Heavy Metal	PPM present in the test	Limit as per USFDA as herbal Formulation
1	Lead	<0.5	20 ppm
2	Mercury	<0.005	0.5 ppm
3	Arsenic	<0.005	5 ppm

As seen in the Fig. 1 in the sub acute toxicity study the groups treated with BSL 150 did not show any significant changes in body weight as compared to control group.

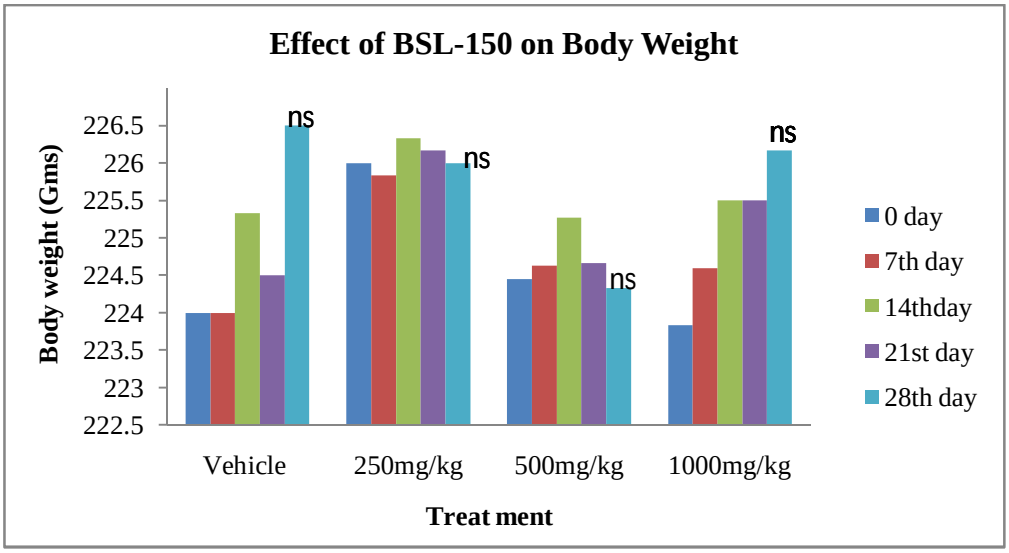


Fig.1: Effect of BSL-150 on Body weight

All the data is expressed as Mean ±SEM (n= 6) analyzed by One Way ANOVA followed by Turkey's multiple comparision test . Significance is compared with control at p<0.05

Table No. 3shows the the hematological parameters such as Hemoglobin, RBC, WBC Count and differential leukocyte count, heamtocrit and platelet count measured on 29th day

Table No.3: Effect of BSL150 on Hematological Parameters.

Parameter	control	BSL 250mg	BSL 500 mg	BSL 1000mg
Hb	10.9± 0.14	10.5±0.07	11.1±0.09	10.0±0.06
RBC	5.81±0.09	6.1±0.071	7.17± 0.03	8.67±0.20
Total WBC	7300± 91.87	9400±39.62	5400±32.20	8100± 54.37
Neutrophil	64±0.30	65± 0.33	70±0.42	76±0.68
Lymphocytes	28± 0.30	29± 0.54	20± 0.33	19±0.77
Eosinophills	04± 0.22	02± 0.22	05± 0.16	03±0.30
Monocytes	04± 0.30	04± 0.34	05± 0.25	02±0.16
Basophils	00	00	00	00
HCV	37.3± 0.16	36.6±0.07	40.5±0.57	35.6±0.10
MCV	98.1± 1.28	87.0±0.11	78.5±0.11	82.9±0.05
MCH	28.6± 0.10	27.9±0.07	30.4±0.07	30.6± 0.04
MCHC	29.2± 0.07	28.6±0.18	27.4±0.21	28.0±0.11

Figure 2 indicates the biochemical parameters evaluated to assess liver function test and kidney function test respectively.

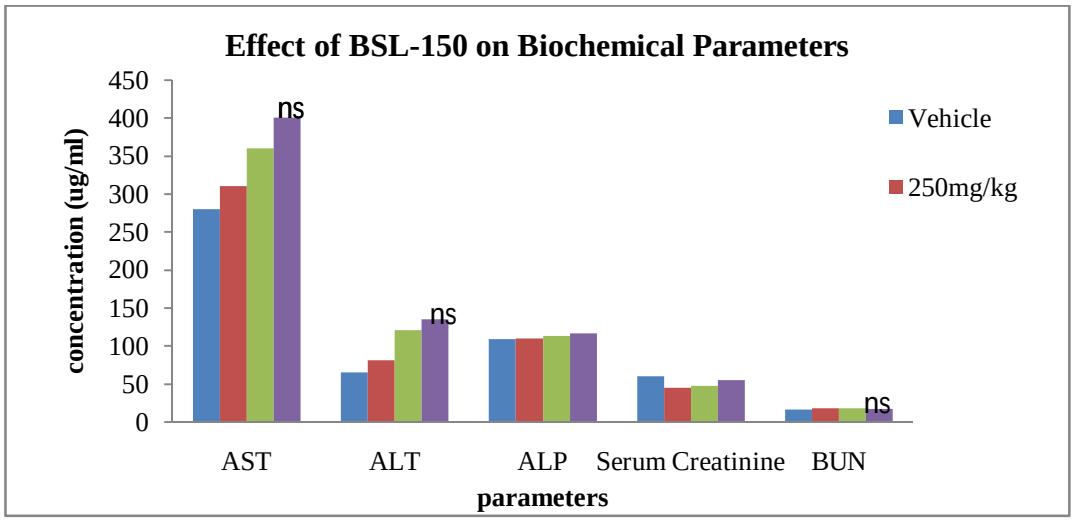
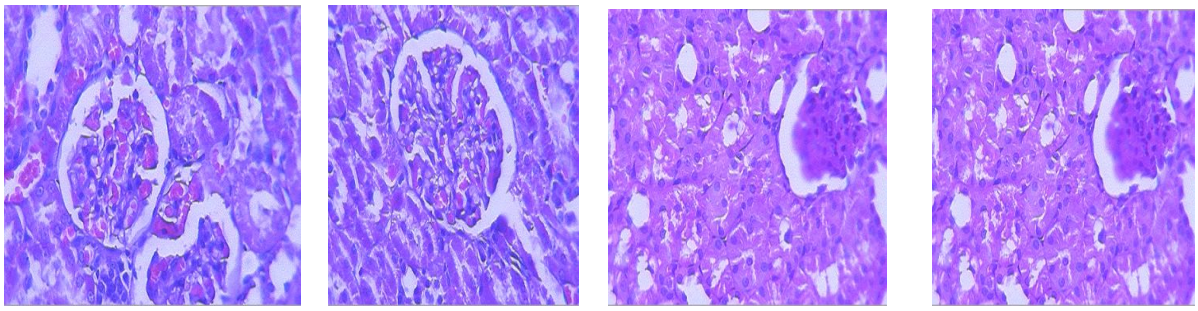


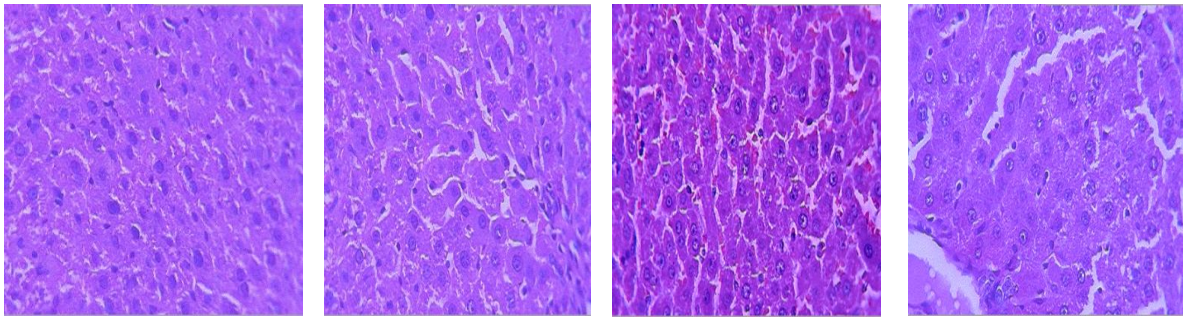
Fig 2: Effect of BSL-150 on Biochemical Parameters.

Figure 3 and Figure 4 indicates the histopathological changes in the kidney and respectively. No any significant tissue damage was observed in both the organs.



(A) (B) (C) (D)

Fig 3: Histopathological sections of kidney: A: Normal Control, B: Treated with 250mg.kg of test drug, C: Treated with 500mg.kg of test drug and D: Treated with 100mg.kg of test drug



(A) (B) (C) (D)

Fig 4: Histopathological sections of liver: A: Normal Control, B: Treated with 250mg.kg of test drug, C: Treated with 500mg.kg of test drug and D: Treated with 100mg.kg of test drug

DISCUSSION

In acute toxicity studies the polyherbomineral formulation did not exhibit any lethality or toxic symptoms at the dose of 2000 mg/kg. According to OECD guidelines 425 the dose of 2000mg /kg and above is categorized as unclassified and hence the drug is found to be safe. As the dose of 2000 mg/kg was well tolerated by the animals treated further studies were 250 mg, 500 mg and 1000 mg of body weight.

As seen in the Fig. 1 in the sub acute toxicity study the groups treated with BSL 150 did not show any significant changes in body weight as compared to control group. This indicates that it did not have any adverse effect on the body weight which is used to assess the response to the therapy of drugs [22] and adverse effects of the drug. [23] The hematological and

biochemical parameters did not show any significant changes in the BSL treated groups as compared to the control groups. As observed in the Table No. 3 there were no any significant changes in the hematological parameters such as Hemoglobin, RBC, WBC Count and differential leukocyte count. This suggests that the test drug is not toxic to the circulating blood cells and hematopoeisis. As seen in the Fig. No. 2 no any significant changes in the liver function parameters AST, ALT and ALP was also observed as compared to control group indicating that BSL 150 is not toxic to the liver which was further confirmed by histopathological studies. [16] The normal levels of blood urea and serum Creatinine indicate that the test drug did not interfere in the renal function and histopathological studies indicate that even the renal integrity is maintained.

CONCLUSION

The present finding suggests that BSL 150 is non toxic since no any marked changes in the hematological, biochemical and histopathological parameters were observed at all the dose levels studied. Thus at normal therapeutic doses it is considered to be safe for long term treatment in diabetic conditions.

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