Journal of Advanced Scientific Research

Available online through https://sciensage.info

ISSN
0976-9595
Research Article

Evaluation of Acute Toxicity and Antidiabetic Potential of Combined Metformin and Blueberry Extract in STZ-Induced Type 2 Diabetes Mellitus in Albino Rats

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Received: 10-05-2025; Accepted: 06-06-2025; Published: 28-06-2025

 https://doi.org/10.55218/JASR.2025160604

ABSTRACT

A combination of blueberry extract (BBE) and metformin was evaluated according to OECD NO. 423TG for its oral toxicity and antidiabetic activity in STZ-induced Type 2 diabetes mellitus (T2DM) in albino rats.

For an acute oral toxicity study, albino rats (n=6) were divided into six experimental groups. A single oral dose of BBE (2000 mg/kg) and metformin (200 mg/kg), as well as a combination of metformin and BBE in the doses 60 + 200 mg/kg, 80 + 200 mg/kg and 100 + 200 mg/kg, were given. Signs, symptoms, and mortality in various groups of animals were noted for 14 days. Body weight, organ weight, and histology of the liver, heart, and kidney were performed. The above-mentioned combinations in the rats were found to be either non-significant or non-toxic in this investigation. No mortality was found and observed with a single dose of 2000 mg/kg in experimental rats. The acute oral toxicity study of metformin in doses of 60, 80, 100, and 200 mg/kg was performed for 14 days. Parameters such as body weight, vital organs weight, blood glucose, MDA, GSH, SOD and protein levels were observed. No toxicity and mortality were observed in any combination dose group. STZ (20 mg/kg; i.p) was given to induce T2DM in the rats. Treatment with varied combinations and different doses of BBE and Metformin showed reduction in the blood glucose level and body weight significantly suggesting a better and safer therapeutic approach for the treatment of T2DM.

Keywords: Blueberry extract, Metformin, Streptozotocin, High Fat diet, Type 2 DM, Acute oral toxicity, Antidiabetic.

INTRODUCTION

Diabetes mellitus (DM) is one of the most challenging global public health problems of this century [1]. The prevalence rate of diabetes is increasing exponentially and the World Health Organization (WHO) predicts that just fewer than half a billion people are currently suffering from diabetes worldwide. This number is projected to increase by 25% in 2030 and 51% in 2045 [2]. The medicinal herbs tend to lower blood sugar levels by decreasing insulin resistance, improving β-cell function, maintaining GLP-1 homeostasis, and glucose reabsorption [3]. Type 2 DM may contribute to myocardial infarction, cardiovascular disease and stroke, which may seriously endanger human health and impose a heavy burden on society [4].

Blueberries are categorized as being part of the cyanococcus portion of vaccinium Ericaceae family. Cranberries, bilberries, and Madeira blueberries are also components of vaccinium [5]. Blueberries are one of the most well-known dietary fruits, having potential health advantages. Several health-promoting properties of blueberries are due to the presence of flavonoids and anthocyanins [6]. Dietary flavonoids have a beneficial effect on glucose homeostasis [7]. Flavonoids were shown to regulate carbohydrate digestion, insulin secretion, and insulin signaling and glucose uptake in insulin-sensitive tissues through various intracellular signaling pathways [8]. Anthocyanins and polyphenolics are secondary metabolites produced by the plant [9], which are responsible for their high antioxidant capacity [10]. Higher consumption of anthocyanins, particularly from blueberries, has been consistently associated with a lower risk of T2DM [11].

Combination therapy may be a novel and highly efficacious therapeutic approach for the control of hyperglycemia. Combination of commercial drugs with phytochemicals may reduce the side effects caused by these synthetic drugs [12]. Medicinal plants have beneficial multiple activities including manipulating carbohydrate metabolism by various mechanisms, preventing and restoring the integrity and functioning of cells, controlling insulin release, improving glucose uptake and utilization, and antioxidant properties [13]. Blueberry extract (BBE) has been used in several diseases along with other drugs. BBE and oxaliplatin have been used for colon cancer as a combination therapy [14]. The effect of BBE and lithium on oxidative

stress, and acetyl cholinesterase (AchE) and Na^+K^+-ATPase activity has been observed in an experimental ketamine-induced model of mania [15]. The combination therapy of blueberry and etanercept has been studied for Juvenile idiopathic arthritis [16], blueberry and verapamil in the medical management of Peyronie's disease [17], while the combination of blueberry with 5-fluorouracil (5-FU) has been studied for Colorectal cancer [18].

Metformin, an insulin-sensitizing biguanide used to treat T2DM, has been shown to be as effective as insulin [19]. Patients with type 2 diabetes who are obese may benefit especially from metformin. Its major mode of action is to reduce hepatic glucose production, which is increased at least twofold in patients with type-2 diabetes [20]. Metformin is a successful oral antihyperglycemic agent by virtue of its reduced hepatic glucose output (primarily through inhibition of gluconeogenesis and, to a lesser extent, glycogenolysis) and increased insulin-stimulated glucose uptake in skeletal muscle and adipocytes [21].

Dose evaluation and establishment of a safe dose for metformin and BBE combination is unique for the therapeutic evaluation of Type 2 DM in albino rats. Combination therapy produces enhanced therapeutic effects due to additive or synergism while reduced doses of metformin have been observed with lesser damage to physiological organs. The effect of BBE *per se* as well as its combination with metformin, has not been evaluated for hypoglycemic effects in non diabetic subjects. Hence, Metformin and BBE combination were evaluated for their toxicity level and for their antihyperglycemic potential.

MATERIALS AND METHODS

Chemicals and reagents

Streptozotocin (STZ) was procured from Sigma Aldrich, US, while metformin (API grade) was a generous gift from Cipla Ltd. The chemicals and reagents utilized in the research were of high-grade chemical and purchased from companies like R K Worldlnfocom, CDH India, and others.

Plant materials

Standardized Blueberry extract powder was procured from Arkure Health Care, Haryana, India.

Protocol for experimental animals

The research protocol was approved by the Institutional Animal Ethics Committee of Lloyd Institute of Management and Technology, Greater Noida (India), having approval number 1206//PO/Re/S/08/CPCSEA/08/2022. Albino Wistar rats weighing 200 to 250 g were collected from the animal house facility of Lloyd Institute of Management & Technology, Greater Noida (U.P). The rats were housed in clean polypropylene cages maintained at a temperature of 22 ± 2°C, an atmospheric moisture of 55 ± 10% and a 12-hour light/dark cycle. Regular rat feed and clean RO-purified water were provided to the animals *ad libitum*. Prior to the treatment, the animals were acclimatized to the laboratory surroundings for a week.

Acute Oral toxicity

- *Acute Oral Toxicity of B.B Extract*

BBE was assessed for acute oral toxicity in albino rats in compliance with OECD test (OECD, 2001) guidelines (OECD guideline 423). Healthy male albino rats (200–250 g and 8–12 weeks old) were selected and housed under normal conditions for five days. Rats were left without food for 3 to 4 hours with access to water *ad libitum* before receiving BBE (2000 mg/kg; po). The animals were closely observed for the first 30 minutes, followed by the next 4 hours. After 1-2 hours of dosing, animals were provided with a standard pellet diet. The vehicle-treated group rats received 1% carboxymethyl cellulose (CMC) in an equivalent amount to the treated group. Any form of toxic effect due to drugs is detected within 6 hours, although the animals were continuously observed for 2 weeks for differences in the pattern of the skin, hair, eye, mucus membrane, usual behaviors, tremor, salivation, convulsion, diarrhea, sleep, lethargy, coma and mortality. The rats were weighed twice before dosing and after completion of 2 weeks. The blood samples were collected through direct cardiac puncture under isoflurane anesthesia. Serum was separated for the assessment of biochemical and hematological parameters. Rats were sacrificed by cervical dislocation and the weight of vital organs (liver, heart, and kidney) was noted and preserved in 10% formalin solution for histopathology.

- *Acute oral toxicity of metformin and BBE combination*

The oral acute toxicity analysis of combined metformin (60 mg/kg; po, 80 mg/kg; po, 100 mg/kg; po and 200 mg/kg; po) and BBE (Selected Dose after Acute oral toxicity) was assessed in compliance with OECD test (OECD, 2001) guidelines (OECD guideline 423). The combined dosage was administered according to body weight for each rat, and closely observed for the first 30 minutes, followed by the next 4 hours. After 1-2 hours of dosing, the standard pellet diet was given to rats. The vehicle-treated group (n = 6) received 1% carboxymethyl cellulose (CMC) in an equivalent amount to the treated group. Any form of toxic effect was observed for 6 hours for both groups and later for 2 weeks. The rats were weighed twice before dosing and after completion of 2 weeks. The blood samples were collected through direct cardiac puncture under isoflurane anesthesia. Serum was separated for the assessment of biochemical and hematological parameters. Rats were sacrificed by cervical dislocation and the weight of vital organs (liver, heart, and kidney) was noted and preserved in 10% formalin solution for histopathology. The tissue was trimmed to a thickness of around 5µm using a rotatory microtome, and the tissue sections were stained with hematoxylin & eosin dye (H&E) for observation under 100X object lens resolution.

Table 1: Composition of high fat diet preparation

Ingredients	*Diet (g/kg)*
Powdered of new product development	365
Oil	310
Yogurt	250
Dythol	10
Vitamin and mineral mix	60
DL-Methionine	03
Fermentation agent	01
NaCl	01

Table 2: Behavioral parameter of BBE+ Metformin combination-treated groups

Observations of vehicle control and Blueberry Extract+ Metformin combinations treated groups

Parameters	30 MIN		4 HRS		24 HRS		48 HRS		7 DAYS		14 DAYS	
	Control Group	Test Group	Control Group	Test Group	Control Group	Test Group	Control Group	Test Group	Control Group	Test Group	Control Group	Test Group
Skin and Fur	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Mucous membrane	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Sleep	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Lethargy	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found
Convulsion	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found
Coma	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found
Tremors	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found
Diarrhea	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found
Morbidity	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found
Mortality	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found

Experimental Design

- *Model for the induction of Type 2 DM in albino rats*

High Fat Diet followed by Streptozotocin (STZ) Induced Type 2 DM in Rats: High Fat Diet Preparation: HFD (58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal) *ad libitum* respectively, for the initial period of 2 weeks [22] followed by low dose STZ (20 mg kg^{-1}; i.p) [23]. The composition (Table no: 1) and preparation of HFD were described elsewhere [24].

Intra-peritoneal injection of STZ (20 mg/Kg) was given to 12-hour fasted rats which have been on a high-fat diet for two weeks. STZ was dissolved into the desired volume of citrate buffer at pH 4.5. After 21 days of dietary modification (i.e., post 7 days of STZ injection), diabetic rats treated with HFD plus STZ and having a fasting blood glucose level of at least 200 mg/dl were randomly assigned to various groups to ensure that their mean blood glucose levels did not differ from one another. Six groups (n = 6) of experimental rats were assigned to different treatments for five weeks. Each treatment was administered at a dose of 2 mL/100g body weight. Body weight was monitored once weekly. Biochemical estimation was done using a commercial diagnostic kit.

Statistical Analysis

Results are expressed as mean ± SEM and were analyzed using one-way ANOVA followed by Dunnett's test of multiple comparisons. The mean values of the treatment groups were compared with the mean values of the control group. The criteria of statistical significance were ****p <0.0001; ***p <0.001; **p <0.01; *p <0.05; ns: non-significance.

RESULTS

Results are expressed as mean ± SEM and were analyzed using one-way ANOVA followed by Dunnett's test of multiple comparisons. The mean values of the treatment groups were compared with the mean values of the control group. The criteria of statistical significance were ****p <0.0001; ***p <0.001; **p <0.01; *p <0.05; NS: non- significance.

Acute Oral Toxicity of BBE alone and combined with Metformin and BBE in varying doses

The BBE treatment group and the vehicle control group of 3 rats each were regularly observed for 14 days. The signs and symptoms, such as differences in the pattern of the skin, hair, eye, mucus membrane, usual behavior, tremor, salivation, convulsion, diarrhea, sleep, lethargy, coma, and mortality, were observed.

Behavioral and clinical observation

No mortality was found and noticed with a single dose of 2000 mg/Kg in experimental rats. No toxicity was seen and noticed with the combined treatment of BBE and metformin at various doses.

effect on body weight of rats

Body weight was measured on the first, third, seventh, and fourteenth day for each group. There was no significant change in body weight found on each day of the study. All the data of each group were compared with the control group as shown in Table 3.

Effect on blood glucose of rats

Blood glucose was measured on the first, third, seventh, and fourteenth day for each group. There was non-significant change of blood glucose observed during these days in the treatment groups when compared to the control group as shown in Table 4.

Effect of the combination treatment of BBE+metformin on relative organ weight

No significant changes were observed in the organ weight of the liver and kidneys in the different groups of the study. It was found that all the combination treatments have no significant changes in other vital organs like the liver and kidney as shown in Table 5.

Biochemical Estimation

Estimation of MDA: MDA levels in the kidney tissue of rats in all the treatment groups were observed and analyzed and were found to be non-significant when compared to the control group. The results are shown in Table 6.

- *Estimation of GSH*

GSH levels in the kidney tissue of rats in all the treatment groups were observed and compared with the control group and were found to be non-significant. The results are shown in Table 7.

- *Estimation of SOD*

SOD level in kidney tissue of rats in all the treatment groups was observed to be comparable to the control group and the results of all treatment groups were found to be non-significant when compared to the control group. The results are shown in Table 8.

- *Estimation of Protein*

Protein level in kidney tissue of rats in all the treatment groups was observed to be comparable to the control group and the results of all treatment groups were found to be non-significant when compared to the control group. The results are shown in Table 9.

Table 3: Effect on body weight of rats

Treatment Groups	Body weight (g)			
	1st day	3rd day	7th day	14th day
Vehicle Control Group	349.33 ± 0.88	351.33 ± 0.33	352.33 ± 0.66	352.33 ± 0.66
BBE (2000 mg/Kg; po)	351.33 ± 0.66^{ns}	352.33 ± 0.66^{ns}	353.33 ± 0.33^{ns}	354.33 ± 0.33^{ns}
Metformin (200 mg/Kg; po)	351.33 ± 0.33^{ns}	353.33 ± 0.88^{ns}	357.33 ± 0.66^{ns}	358.33 ± 0.33^{ns}
Metformin (60 mg/Kg; po) + BBE 200 mg/Kg; po)	349.33 ± 0.66^{ns}	352.33 ± 0.66^{ns}	354.33 ± 0.33^{ns}	356.33 ± 0.88^{ns}
Metformin (80 mg/Kg; po) + BBE mg/Kg; po)	351.33 ± 0.66^{ns}	354.33 ± 0.88^{ns}	355.33 ± 0.66^{ns}	355.66 ± 0.66^{ns}
Metformin (100 mg/Kg; po) +BBE (200 mg/Kg; po)	352.33 ± 0.88^{ns}	353.33 ± 0.33^{ns}	357.33 ± 0.33^{ns}	358.66 ± 0.66^{ns}

Table 4: Effect on blood glucose in rats

Treatment Groups	*Blood glucose (mg/dl)*			
	1st day	*3rd day*	*7th day*	*14th day*
Vehicle Control Group	123.33 ± 0.66	124.33 ± 0.88	124.33 ± 0.66	125.33 ± 0.66
BBE (2000 mg/Kg; po)	124.33 ± 0.88^{ns}	124.33 ± 0.88^{ns}	125.33 ± 0.88^{ns}	125.33 ± 0.88^{ns}
Metformin (200 mg/Kg;po)	124.33 ± 0.88^{ns}	125.33 ± 0.66^{ns}	122.33 ± 0.33^{ns}	122.33 ± 0.33^{ns}
Metformin (60 mg/Kg; po) + BBE 200 mg/Kg; po)	122.33 ± 0.33^{ns}	123.33 ± 0.57^{ns}	123.33 ± 0.57^{ns}	124.33 ± 0.57^{ns}
Metformin (80 mg/Kg; po) + BBE mg/Kg; po)	123.33 ± 0.66^{ns}	124.33 ± 0.66^{ns}	124.33 ± 0.66^{ns}	125.33 ± 0.88^{ns}
Metformin (100 mg/Kg; po) +BBE (200 mg/Kg; po)	123.33 ± 0.88^{ns}	125.33 ± 0.88^{ns}	125.33 ± 0.88^{ns}	126.33 ± 0.88^{ns}

Table 5: Effect of the combination drug on relative organ weight

Treatment groups	*Relative weight of organs (g)*		
	Liver	*Kidney (Right)*	*Kidney (Left)*
Vehicle control group	16.185 ± 0.115	2.235 ± 0.169	2.535 ± 0.117
BBE (2000 mg/Kg; po)	15.895 ± 0.103^{ns}	2.181 ± 0.116^{ns}	2.303 ± 0.114^{ns}
Metformin (200 mg/Kg; po)	16.982 ± 0.109^{ns}	2.231 ± 0.171^{ns}	2.532 ± 0.115^{ns}
Metformin (60 mg/Kg; po) + BBE 200 mg/Kg; po)	15.811 ± 0.112^{ns}	2.122 ± 0.058^{ns}	2.112 ± 0.054^{ns}
Metformin (80 mg/Kg; po) + BBE 200 mg/Kg; po)	15.888 ± 0.109^{ns}	2.071 ± 0.061^{ns}	2.097 ± 0.045^{ns}
Metformin (100 mg/Kg; po) + BBE 200 mg/Kg; po)	16.333 ± 0.115^{ns}	2.168 ± 0.114^{ns}	2.221 ± 0.101^{ns}

Table 6: Effect of MDA level in kidney tissue treated with BBE+ Metformin

Treatment groups	*MDA (μ mols/gm)*
Vehicle Control Group	0.427 ± 0.10
BBE (2000 mg/Kg; po)	0.512 ± 0.09^{ns}
Metformin (200 mg/Kg; po)	0.462 ± 0.12^{ns}
Metformin (60 mg/Kg; po) + BBE 200 mg/Kg; po)	0.503 ± 0.10^{ns}
Metformin (80 mg/Kg; po) + BBE 200 mg/Kg; po)	0.489 ± 0.11^{ns}
Metformin (100 mg/Kg; po) + BBE 200 mg/Kg; po)	0.465 ± 0.12^{ns}

Table 7: Effect of GSH Level in kidney tissue treated with BBE + Metformin

Treatment groups	*GSH (μ mols/gm)*
Vehicle control group	1.767 ± 0.118
BBE (2000 mg/Kg; po)	1.653 ± 0.117^{ns}
Metformin (200 mg/Kg; po)	1.518 ± 0.113^{ns}
Metformin (60 mg/Kg; po) + BBE 200 mg/Kg; po)	1.456 ± 0.101^{ns}
Metformin (80 mg/Kg; po) + BBE 200 mg/Kg; po)	1.498 ± 0.102^{ns}
Metformin (100 mg/Kg; po) + BBE 200 mg/Kg; po)	1.502 ± 0.103^{ns}

Table 8: Effect of SOD Level in kidney tissue treated with BBE + Metformin

Treatment Groups	*SOD (μ mols/gm)*
Vehicle Control Group	2.121 ± 0.041
BBE (2000 mg/Kg; po)	2.021 ± 0.016^{ns}
Metformin (200 mg/Kg; po)	2.009 ± 0.008^{ns}
Metformin (60 mg/Kg; po) + BBE 200 mg/Kg; po)	1.987 ± 0.049^{ns}
Metformin (80 mg/Kg; po) + BBE 200 mg/Kg; po)	1.991 ± 0.055^{ns}
Metformin (100 mg/Kg; po) + BBE 200 mg/Kg; po)	2.012 ± 0.010^{ns}

Table 9: Effect of protein level in kidney tissue treated with BBE + Metformin

Treatment Group	*Protein (μ mol/gm)*
Vehicle control group	1.702 ± 0.057
BBE (2000 mg/Kg; po)	1.681 ± 0.068ns
Metformin (200 mg/Kg; po)	1.602 ± 0.057ns
Metformin (60 mg/Kg; po) + BBE 200 mg/Kg; po)	1.568 ± 0.020ns
Metformin (80 mg/Kg; po) + BBE 200 mg/Kg; po)	1.591 ± 0.042ns
Metformin (100 mg/Kg; po) + BBE 200 mg/Kg; po)	1.621 ± 0.056ns

Histopathological Evaluation

- *Histopathology of Liver*

The result showed that there are no changes in the basic cells and in the liver portal triad and central vein of treated animals when compared to the vehicle control group, and the given doses are considered safe when compared to the control group (Fig. 1).

- *Histopathology of Kidney*

The result was found as safe and non-degenerative due to the effect of various doses of treatment drugs when compared to the control group (Fig. 2).

3.2 High Fat Diet plus STZ-Induced T2 DM and antidiabetic activity of the evaluated safe combinational therapy of (Metformin and BBE).

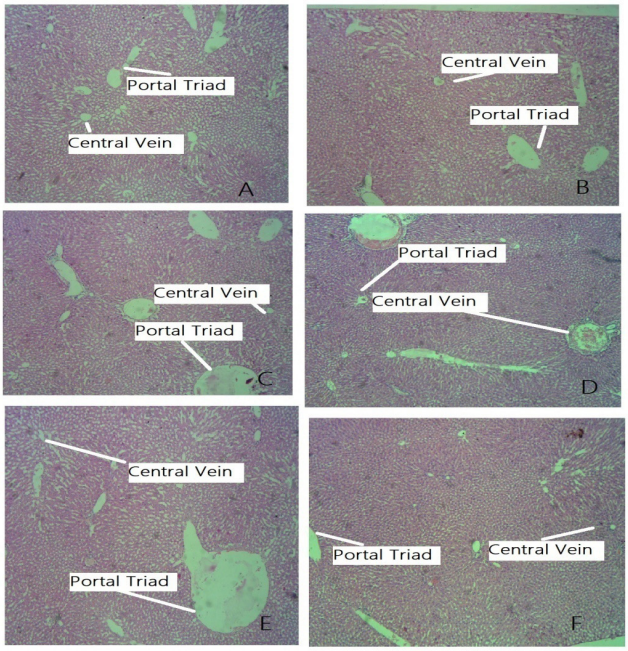

Fig. 1: Histopathological slides of liver tissues (A) Vehicle Control Group (B) BBE Extract (2000 mg/Kg; p.o.) (C) Metformin (Standard) (200 mg/Kg p.o.) (D) Metformin (60 mg/Kg p.o.)+BBE (200 mg/Kg, p.o.) (E) Metformin (80 mg/Kg p.o.)+BBE (200 mg/Kg, p.o.) (F) Metformin (100 mg/Kg p.o.)+BBE (200 mg/Kg, p.o.)

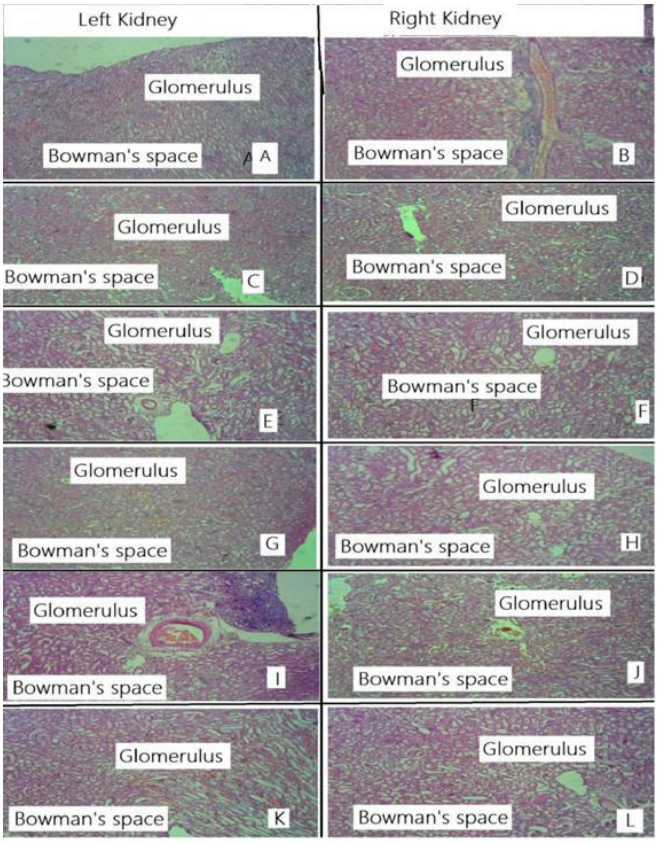

Fig. 2: Histopathological slides of left and right kidney (A) and (B) Vehicle control group; (C) and (D) BBE extract (2000 mg/Kg; p.o.); (E) and (F) Metformin (Standard) (200 mg/Kg p.o.); (G) and (H) Metformin (60 mg/Kg p.o.)+BBE (200 mg/Kg, p.o.); (I) and (J) Metformin (80 mg/Kg p.o.)+BBE (200 mg/Kg, p.o.); (K) and (L) Metformin (100 mg/Kg p.o.)+BBE (200 mg/Kg, p.o.).

Effect on Body weight

The body weight of different groups was measured at 1st, 2nd, 3rd, 4th and 5th week. STZ+HFD (Disease control) treated group showed a significant increase in body weight from 1st week to 5th week, suggesting induction of T2DM. Statistical analysis revealed that in the 1st week, animals treated with different doses of metformin and BBE showed no significant change in body weight compared to the disease control group as shown in Table 10. The findings indicate that Metformin+ BBE (100+200 mg/Kg; p.o.) was more appropriate in treating type 2 diabetes mellitus compared to other treatment groups.

Effect on blood glucose

The Blood sugar range of all the groups was measured at 1st, 2nd, 3rd, 4th and 5th week. STZ+HFD (Disease control) treated group significantly elevation in blood glucose level from 1st week to 5th week compared to the control group, indicating induction of severe diabetes. Metformin+ BBE (100+200 mg/Kg; p.o.) treated group showed a significant reduction in the blood glucose level ($p < 0.001$) when compared to the disease control group. Animals treated with Metformin+ BBE (60+200 mg/Kg; p.o.) and Metformin+ BBE (80+200 mg/Kg; p.o.) also exhibited decreased levels of blood glucose (***$p < 0.001$) compared to the disease control group group as shown in Table 11.

DISCUSSION

Traditional medicine plays a significant role in the majority of developing nations' health care systems. The World Health Organization (WHO) estimates that for their primary healthcare requirements, around 80% of developing countries depend upon traditional medicines [25]. Numerous authors have reported the use of herbal medicines and related medicinal plants [26,27]. Diabetes is a chronic disease that impacts a large number of individuals from all areas of life. There are several strategies to decrease the long-term complications and associated problems of diabetes. The antidiabetic drug metformin alone has limited adverse reactions; however, it has the potential to produce side effects like lactic acidosis, which is a further alarming condition and may produce dizziness followed by extreme drowsiness. It also produces pain in the muscles and a feeling of fatigue, shivering and blue skin. Side effects like rapid or difficult breathing, and slow or irregular heartbeat. Incidences of diarrhea, nausea, or vomiting have also been observed. Patients with specific medical conditions are more likely to experience it, such as severe infections, liver or kidney disease, recent surgery, any conditions that result in low blood oxygen levels or poor circulation (such as recent congestive heart failure, recent heart attack), heavy alcohol use, dehydration, and patients who are 80 years or older [28,29]. Herbal formulations are recommended because of their lower cost and fewer side effects. People having chronic T2DM experience macro and micro vascular complications like diabetic nephropathy, diabetic neuropathy and diabetic retinopathy followed by several cardiovascular complications, substantial morbidity and death. No

Table 10: Effect on body weight of combination of metformin and BBE

Treatment Groups (n=6)	*Body weight (g)*				
	1st Week	*2nd Week*	*3rd Week*	*4th Week*	*5th week*
Vehicle Control group	349.33 ± 0.88^{ns}	351.33 ± 0.33***	352.33 ± 0.88***	352.33 ± 0.66***	353.33 ± 0.33***
Disease control (STZ 20 mg/Kg, i.p.+HFD)	351.33 ± 0.66	367.33 ± 0.66	379.33 ± 1.33	387.33 ± 1.20	389.33 ± 0.88
Metformin (200 mg/Kg; p.o.)	352.33 ± 0.66^{ns}	352.33 ± 0.66***	359.33 ± 0.88***	364.33 ± 0.88***	363.33 ± 0.33***
Metformin+ BBE (60+200 mg/Kg; p.o.)	352.33 ± 0.88^{ns}	364.33 ± 0.88*	372.33 ± 0.66*	374.33 ± 1.33**	373.33 ± 0.66***
Metformin+ BBE (80+200 mg/Kg; p.o.)	351.33 ± 0.33^{ns}	360.33 ± 0.33**	368.33 ± 1.20***	369.33 ± 1.33***	369.33 ± 0.88***
Metformin+ BBE (100+200 mg/Kg; p.o.)	350.33 ± 0.33^{ns}	355.33 ± 0.88***	361.33 ± 1.33***	365.33 ± 0.88***	361.33 ± 0.66***

Results are expressed as mean ± SEM and were analyzed using one-way ANOVA followed by Dunnett's test of multiple comparisons. The mean values of the treatment groups were compared with the mean values of the negative control group.

Table 11: Effect on blood glucose

Treatment groups (n=6)	*Blood glucose (mg/dl)*				
	1st Week	***2nd Week***	***3rd Week***	***4th Week***	***5th week***
Vehicle Control group	123.33 ± 1.76***	124.33 ± 2.02***	124.33 ± 2.02*	125.33 ± 2.02**	125.33 ± 1.76***
Disease control (STZ 20 mg/Kg, i.p.+HFD)	243.33 ± 2.33	253.33 ± 1.85	266.33 ± 1.85	268.33 ± 1.76	273.33 ± 1.85
Metformin (200 mg/Kg; p.o.)	220.33 ± 1.66***	199.33 ± 1.76***	147.33 ± 1.85***	135.33 ± 2.02***	126.33 ± 2.02***
Metformin+ BBE (60+200 mg/Kg; p.o.)	229.33 ± 1.85***	225.33 ± 1.45***	209.33 ± 2.40***	199.33 ± 2.33***	195.33 ± 2.02***
Metformin+ BBE (80+200 mg/Kg; p.o.)	225.33 ± 2.40***	209.33 ± 2.40***	188.33 ± 1.76***	173.33 ± 1.85***	166.33 ± 1.85***
Metformin+ BBE (100+200 mg/Kg; p.o.)	222.33 ± 1.85***	201.33 ± 1.85***	175.33 ± 1.85***	153.33 ± 2.40***	133.33 ± 1.85***

Results are expressed as mean ± SEM and were analyzed using one-way ANOVA followed by Dunnett's test of multiple comparison. The mean values of the treatment groups were compared with the mean values of the disease control group.

permanent cure is available despite substantial advancements in T2DM and the development of antidiabetic medications. Very rich sources of medicinal herbs are available for the treatment of T2DM and have been used for decades in complementary and alternative medical systems. Herbal treatments are effective treatment options for T2DM due to their safety and variety of targeted effects. Consequently, combination therapy with metformin and herbal extract has been used to reduce the side effects of metformin and to effectively treat T2DM using metformin and BBE.

In this study toxicity level of the combination by the method of acute oral toxicity testing has been evaluated. For this, acute oral toxicity of BBE (hydro-alcoholic 70%) at a dose of 2000 mg/Kg; p.o. has been performed. The BBE has not shown any mortality and morbidity; thus 1/10th dose of the extract has been chosen for test drug treatments (200 mg/Kg; p.o.) (OECD guideline No. 423)[30]. Furthermore, half dose of metformin (100 mg/Kg; p.o.) of pre-established and reported safe dose of metformin, i.e., 200 mg/Kg; p.o. was taken for the combination formulation with BBE. Lesser doses like 60 mg/Kg; p.o. and 80 mg/Kg; p.o., have also been chosen for other combinations. These combinations were used for the acute oral toxicity study for the safety profile of each combination. All the combinations of metformin (60 mg/Kg; p.o., 80 mg/Kg; p.o., 100 mg/Kg; p.o.) with BBE (200 mg/Kg; p.o.) were found to be safe during the acute oral toxicity study. The same combinations were also evaluated for antidiabetic activity. For this, all the combination groups with standard metformin 200 mg/Kg; p.o. is compared with the negative control group, which is the STZ-induced diabetic group. Treatment with different doses of BBE and Metformin showed significant reduction in the blood sugar level and body weight, suggesting a decrease in blood glucose. Compared to other dose groups, Metformin 100 + BBE 200 mg/Kg; p.o. Showed a more significant reduce in diabetes (***p <0.001).

CONCLUSION

The purpose of this study was to evaluate the antidiabetic effects of B.B. extract and metformin in rats with type 2 diabetes induced by STZ. Firstly, the oral acute toxicity analysis of metformin (60 mg/Kg; p.o., 80 mg/Kg; p.o., 100 mg/Kg; p.o. and 200 mg/Kg; p.o.) and B.B. extract (2000 mg/Kg; p.o.) was performed for 14 days. Parameters such as body weight, vital organ weight, and blood glucose, and MDA, GSH, SOD, and protein level were taken into consideration, which showed that no toxicity was present and that no combination dose group caused any mortality. STZ at a dose of 20 mg/Kg; i.p. leads to the induction of severe type 2 diabetes. Treatment with combinations of different doses of BBE and metformin showed a significantly decrease in the blood sugar level and body weight, suggesting a reduction in diabetes. Compared to other dose groups, metformin 100 + BBE 200 mg/Kg; p.o., showed a more significant decrease in diabetes (***p <0.001). Therefore, it can be suggested that the combination of metformin 100 and BBE 200 mg/Kg; p.o. can be used as an adjuvant therapy for the treatment of type 2 diabetes mellitus.

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HOWTO CITETHIS ARTICLE: Saifi, N., Ali, S.S., Akhtar, Z. Evaluation of Acute Toxicity and Antidiabetic Potential of Combined Metformin and Blueberry Extract in STZ-Induced Type 2 Diabetes Mellitus in Albino Rats. *J Adv Sci Res.* 2025;16(06): 41-48 **DOI:** 10.55218/JASR.2025160604