Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 15 (1), 2024; 90-96

Beneficial effect of *Barleria buxifolia* leaves extract in the treatment of diabetes and associated complications

Research Article

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Abstract

The global occurrence of diabetes has witnessed persistent escalation, starting from 151 million individuals in the year 2000, the time when the IDF Diabetes Atlas was initially introduced, to 285 million in 2009, and further reaching 382 million in 2013. Diabetes mellitus is characterised by elevated levels of glucose in the blood plasma. This condition emerges as a consequence of insufficient insulin secretion, insulin resistance or both, subsequently leading to metabolic abnormalities. The plant known as *Barleria buxifolia (*BB), belonging to the Acanthaceae family, is intended to possess the ability to treat various ailments in the traditional Indian system of medicine. Hence, this research aims to investigate the effects of methanolic and aqueous extracts of BB leaves on diabetic models of rats. STZ (60 mg/kg) was administered to induce diabetes in the rats. Both the extracts of BB were orally administered at doses of 200 and 400 mg/kg, respectively to the normal and diabetic rats, commencing from the 3rd day until the 28th day. Blood samples were examined to determine glucose levels. The STZ-treated rats exhibited a notable rise in blood glucose levels. The aqueous and methanolic extracts of BB reduced the elevated glucose levels significantly ($P > 0.01$) and enhanced the body weight of rats. Thus, it can be inferred that BB possesses considerable potential for the management of diabetes mellitus.

Keywords: *Barleria buxifolia,* Diabetes mellitus, Hypoglycemic activity, Diabetic complications, Blood glucose level.

Introduction

Diabetes mellitus (DM), widely referred to as diabetes, is a group of metabolic disorders characterised by persistently elevated blood sugar in the blood. An estimated 463 million people (8.8% of the world's population) were predicted to have diabetes in 2019, with type 2 diabetes accounting for over 90% of cases. The prevalence of the condition is similar among both genders. In the same year, diabetes was responsible for approximately 4.2 million deaths. Diabetic neuropathy, a well-recognised long-term complication of diabetes, affects approximately half of the diabetic population and is associated with increased morbidity and mortality. This condition can lead to various high-risk complications, ranging from alterations in heart rate to visual disturbances. Diabetic neuropathy may also result in the loss of sensation in the feet, among other possible complications (1). This may lead to a lack of sensation towards lacerations or lesions, potentially resulting in a subsequent infection. If left untreated, infection within a limb may necessitate amputation. Additionally, profound urinary bladder and kidney infections can manifest, provoking various health complications (2). Taking proper care of your feet is essential to avoiding the consequences of Diabetic Peripheral Neuropathy (DPN). According to a large prospective study carried out across 16 European nations approximately 25% of type 1 diabetes patients experienced painful DPN throughout seven years. The symptoms of painful DPN manifest with a symmetrical distribution resembling the pattern of "stocking and gloves," and are often exacerbated at night (3). These symptoms can range from a mild sensation of pins and needles to a severe, persistent, and even unpleasant electric shock-like sensation. The intensity of the pain experienced by individuals with painful DPN can be so severe that it hinders their ability to carry out daily activities, thus negatively impacting their employment and social life (4). The combination of continuous, ongoing pain and social detachment usually ends in the onset of depression. In extreme cases, patients may experience a loss of appetite and significant weight loss (5). "The occurrence of painful DPN has been found to vary, with a prevalence of 11% reported in Rochester. In terms of the identification and development of medicinal plants and related compounds, phytopharmaceutical studies for treating neuropathic pain have gained increasing attention worldwide (6).

Mankind has traditionally employed herbal medicine, and in recent years, its use has grown in favour because it has fewer side effects and lower chance of complications than synthetic medications (7). Several medicinal plants have been identified as potential treatments for neuropathic pain, including *Acorus calamus* Linn, *Artemisia dracunculus* Linn.,

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Butea monosperma Lam., *Phyllanthus amarus* Schumach. & Thonn., *Citrullus colocynthis* (L.) Schrad., *Momordica charantia* M. charantia, *Nigella sativa* L., *Mitragyna speciosa* Korth., *Pterodon pubescens* Benth, *Rubia cordifolia* L., *Ocimum sanctum* L., *Salvia officinalis* L., and *Terminalia arjuna* Roxb (8). The current study focuses on *Barleria buxifolia* (BB) a medicinal plant found in waste areas of India. Pharmacological studies have demonstrated that the leaves of BB possess antioxidant, hepatoprotective, antidepressant, and anti-inflammatory properties in experimental animals, which can be attributed to various phytochemicals such as flavonoids, amino acids, alkaloids, phenols, saponins, terpenoids, and anthraquinones. These findings suggest the potential of BB as a therapeutic option for the treatment and management of diseases (9).

Materials and Methods

Procurement and authentication of plant materials

Barleria buxifolia plants have been collected from Thiruvalla, Kerala. The plant has been identified and authenticated by Mrs. A. M. Gaharwar, Assistant Professor of Vasantrao Naik College of Agricultural Biotechnology, Yavatmal (Ref No. VNCABT/Ytl/Hort/1266/2022).

Extraction of *Barleria buxifolia* leaves

The leaves of *Barleria buxifolia* plant have been collected, dried in shade, and coarsely powdered. The powdered leaves have been subjected to maceration with the use of methanol and thereafter water to get methanolic and aqueous extracts respectively (10). The aqueous and methanolic extract of BB were investigated for presence of various phytochemicals (11).

Drug and chemical substances

Streptozotocin (STZ) is used for the induction of diabetes, and glibenclamide is used as a standard drug (12). All distinctive chemical substances and reagents have been used which are of analytical grade.

Experimental animals

Male Sprague Dawley rats, in good health, were obtained from NIN Hyderabad, India. The rats were approximately 8 weeks old and weighed approximately 200-210 grams. They were bought specifically to carry out pharmacological screening. The animals were kept in polypropylene cages equipped with cord-mesh tops and filled with husk bedding. The rats were maintained in controlled environmental settings, which included a temperature range of 22 to 20°C, relative humidity between 55% and 60%, and a 12-hour cycle of light and darkness. They were provided with a standard pellet diet and had access to water at all times (13). All animal studies conducted in this research were conducted as per approved protocols by the Institutional Animal Ethical Committee (IAEC) at P. Wadhwani College of Pharmacy, Yavatmal, under research protocol number 650/PO/Re/S-2002/2022/CPCSEA/23.

Methodology

Experimental Design

For this study, animals were categorised into 7 groups, each group having 6 rats (n=6). Group I animals were treated with a normal saline solution (normal control). Group II rats were administered STZ (60 mg/kg) to induce diabetes (negative control). After confirmation of diabetes in 72 hours, rats in group V were administered a dosage of 400 mg/kg of methanolic extract of BB. Rats in group IV were given a dosage of 200 mg/kg of methanolic extract of BB. Group VII diabetic rats were given 400 mg/kg of aqueous extract of BB. Group VI diabetic rats were given a dose of 200 mg/kg of aqueous extract of BB. Group III diabetic rats were administered the anti-diabetic drug glibenclamide at a dose of 5 mg/kg.

Induction of diabetes

Diabetes was induced in rats by administering intraperitoneal injection of STZ at a single dose of 60 mg/kg. The injection was prepared using a freshly made cold citrate buffer with a pH of 4.5. To prevent the occurrence of potentially fatal hypoglycaemia caused by excessive release of pancreatic insulin, the rats were provided with 5% dextrose glucose solution for 24 hours. Rats with a blood glucose level of 200 mg/dl after 72 hours were identified as diabetic and selected for the study. Throughout the experimental period, the diabetic rats were housed under standard laboratory conditions (14).

Biochemical Parameter

Blood glucose level: The blood of rats was procured retro-orbitally to ascertain the levels of glucose in the bloodstream. After centrifugation (3000 revolutions per minute at a temperature of 4°C for 20 minutes), the plasma was acquired. 10µl of sample was added to 100µl of enzyme reagent, thoroughly mixed, then kept at a temperature of 37°C for 2 minutes. The absorbance of the standard and test samples was measured against a reagent blank at 505 nm (15).

Estimation of body weight: The body mass of every rat in every group was assessed utilising a weighing balance. The body mass of each rat was determined to be within the range of 200-210 grams (16).

Serum urea level: 0.05 ml of serum sample mixed with 0.5 ml of naphthylethylenediamine (NED) reagent and 0.5 ml colour reagent incubate for 10 min at 100°C. The absorbance of sample and standard was read after cooling at 540 nm against blank (17).

Serum creatinine level: 0.10 ml of the serum sample was combined with 1 ml of alkaline picric acid and thoroughly mixed. The absorbance of both the standard and test samples was measured at 520 nm. (18).

Statistical Analysis: The data underwent statistical analysis using the one-way analysis of variance (ANOVA) method, followed by Dunnett's comparison test, in which equal sample sizes were utilised.

Additionally, the student t-test was employed to compare unpaired groups. A notable disparity was deemed significant when the p-value was less than 0.01. All numerical values were presented as the mean plus the standard deviation (S.D.).

Results

The preliminary phytochemical screening of plant leaf extract was carried out by suitable tests (10,11) as given in Table 1.

Table 1: Results of phytochemical screening of methanolic and aqueous extracts of *Barleria buxifolia*

Sr. no	Plant constituents	Methods for screening	BB methanolic extract	BB aqueous extract
1	Test for alkaloids	Hager's Reagent	+	+
		Dragondorrf's test	+	+
2	Test for carbohydrates	Molisch Test	+	+
		Fehling's test	-	-
		Benedict's test	+	+
3	Test for glycosides	Killer Killani Test	-	-
4	Test for amino acid	Ninhydrin test	-	-
5	Test for tannins	Gelatine test	+	+
		Lead acetate test	+	+
6	Test for steroids	Salkowski test	+	+
7	Test for flavonoids	Ferric chloride test	+	+
		Lead acetate test	+	-
8	Test for terpenoids	Salkowski test	+	+
9	Test for saponins	Foam test	+	+

The presence or absence of phytochemicals is denoted as + and – respectively.

Table 2 and Figure 1 illustrates the impact of STZ on the blood glucose levels of the rats on days 0, 3, 14, and 28. There was a notable and statistically significant rise ($p<0.01$) in the blood glucose level observed in negative control group as compared to the normal control group of rats. In contrast, groups IV, V, VI and VII exhibited a significant reduction ($p<0.01$) in the blood glucose level in comparison to negative control group.

Figure 1. Effect of *Barleria buxifolia* on blood serum glucose level in STZ-induced diabetic rats at 0, 3, 14, and 28 days

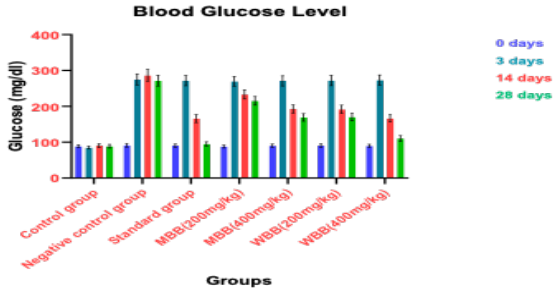

Table 2. Effect of *Barleria buxifolia* on blood serum glucose level in STZ induced diabetic rats at 0, 3, 14 and 28 days

Sr. No.	Groups	Glucose (mg/dl)			
		0 days	3 days	14 days	28 days
1	Group I: Control	88.7± 4.28	85.2±3.96	90.5±5.25	88.9±4.95
2	Group II: Negative control	91.4±5.20	274.8±15.6@	286.6±16.4@	271.6±15.2@
3	Group III: Standard group	90.4±4.55	271.8±14.2	165.85±11.4**	95.2±6.10**
4	Group IV: MBB (200mg/kg)	87.96±4.20	269.7±13.8	233.24±12.8*	215.5±12.10**
5	Group V: MBB (400mg/kg)	90.17±4.74	271.1±14.20	192.68±11.5**	169.10±10.70**
6	Group VI: WBB (200mg/kg)	90.45±4.65	272.2±14.3	191.70±11.7**	170.2±10.4**
7	Group VII: WBB(400mg/kg)	89.85±4.45	273.24±14.35	166.50±10.10**	110.5±7.87**

Results are expressed as mean ± SD in mg/dl. (n=6) @$p<0.01$ Compared with corresponding normal control group, **$p<0.01$ Compared with diabetic control group, *$p<0.05$ compared with diabetic control group.

Table 3 illustrates the impact of STZ on the rat's body weight on days 3, 14, and 28. A significant reduction ($p<0.01$) in body weight was observed in the negative control group in comparison to the normal control group on the 3rd, 14th, and 28th days. Groups IV, V, VI and VII exhibited a substantial increase ($p<0.01$) in body weight in contrast to the negative control group.

Table 3: Effect of STZ on body weight of rats in grams on 3rd, 14th, and 28th days

Group	Day 3	Day 14	Day 28
Group I: Control	204.67 ± 6.4	206.17 ± 4.53	203.17 ± 3.18
Group II: Negative control	197.5 ± 2.73ns	160.33 ± 3.32**	137.33 ± 1.75**
Group III: Standard group	202.67 ± 6.28ns	203.5 ± 1.87**	201.5 ± 1.87**
Group IV: MBB (400mg/kg)	203.5 ± 6.47ns	198.5 ± 3.08**	202.17 ± 2.48**

Group V: MBB (200mg/kg)	201.67 ± 6.71ns	195.67 ± 1.75**	200.5 ± 1.87**
Group VI: WBB (400mg/kg)	200.67 ± 6.71ns	197.17 ± 1.94**	199.17 ± 1.47**
Group VII:WBB (200mg/kg)	199.17 ± 1.72ns	194.83 ± 3.76**	198.67 ± 1.86**

The results are expressed as mean ± SD (n = 6), ns p>0.05, ** p<0.01 when compared to STZ-treated negative control group.

Table 4 and Figure 2 depicts serum urea level in the control and experimental groups of rats. The urea level was increased in diabetic control. After the administration of MBB and WBB at both dosages the serum urea level were significantly reduced (p<0.01).

Table 4. Effect of *Barleria buxifolia* on serum Urea level in STZ induced diabetic rats at 0, 3, 14 and 28 days

Sr. No.	Groups	Urea			
		0 days	3 days	14 days	28 days
1	Group I: Control	15.35± 0.43	15.51 ± 0.47	15.63 ± 0.36	15.68 ± 0.26
2	Group II: Negative control	15.41 ± 0.42	15.55± 0.35 ns	15.70 ± 0.25ns	19.20 ±0.46@
3	Group III: Standard group	15.67± 0.41ns	15.45± 0.41ns	16.05 ± 0.12ns	15.35± 0.41**
4	Group IV: MBB (200mg/kg)	15.46± 0.40 ns	15.57+0.39ns	15.38+ 0.41ns	16.08±0.17**
5	Group V: MBB (400mg/kg)	15.54+ 0.42 ns	15.47± 0.38ns	15.41+ 0.43ns	15.37±0.25**
6	Group VI: WBB (200mg/kg)	15.35± 0.40 ns	15.55+ 0.41ns	15.51+ 0.44ns	16.03± 0.21**
7	Group VII: WBB (400mg/kg)	15.52+ 0.40ns	15.39+ 0.40ns	15.37± 0.39ns	15.14±0.33**

Results are expressed as mean ± SD in mg/dl. (n=6), @p<0.01 Compared with corresponding normal control group, **p<0.01 Compared with diabetic control group, ns p>0.05 compared with diabetic control group.

Table 5 and Figure 3 represents the serum creatinine level in all groups of rats. It shows a significant reduction (p<0.01) in serum creatinine level in comparison to diabetic control after the administration of 200 and 400 mg/kg of MBB and WBB.

Figure 2. Effect of *Barleria buxifolia* on serum urea level in STZ-induced diabetic rats at 0, 3, 14, and 28 days

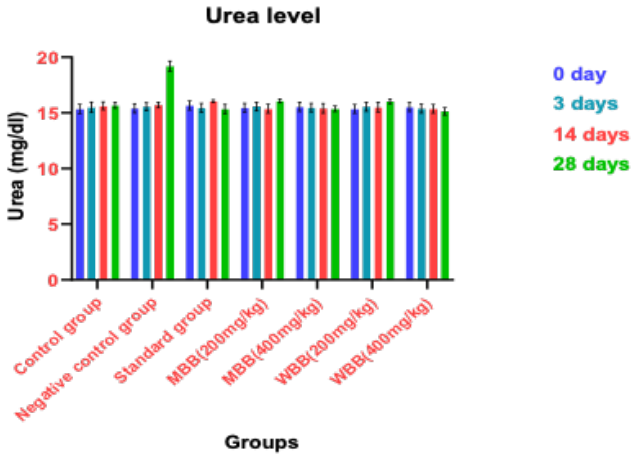

Figure 3. Effect of *Barleria buxifolia* on serum creatinine level in STZ-induced diabetic rats at 0, 3, 14 and 28 days

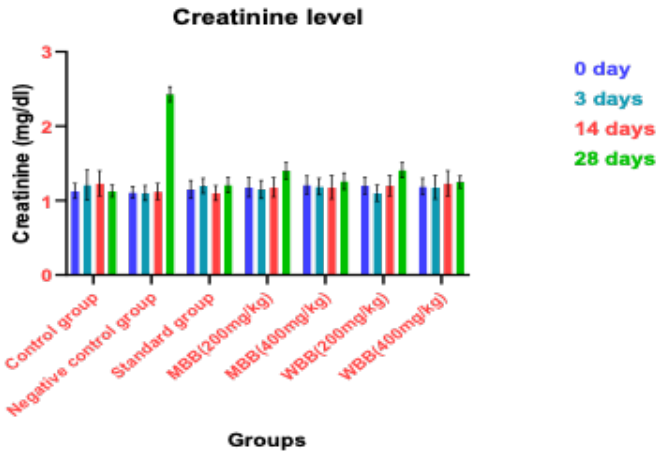

Table 5. Effect of *Barleria buxifolia* on serum creatinine level in STZ induced diabetic rats at 0, 3, 14 and 28 days

Sr. No.	Groups	Creatinine			
		0 days	3 days	14 days	28 days
1	Group I: Control	1.13 ± 0.28	1.21± 0.13	1.23±0.17	1.13±0.22
2	Group II: Negative control	1.11±0.08ns	1.1± 0.1ns	1.12±0.11ns	2.43±0.35@
3	Group III: Standard group	1.15± 0.12ns	1.2± 0.1ns	1.1±0.1ns	1.21±0.13**
4	Group IV: MBB (200mg/kg)	1.18± 0.13ns	1.15±0.12ns	1.18± 0.13ns	1.4±0.11**
5	Group V: MBB (400mg/kg)	1.21± 0.12ns	1.19± 0.11ns	1.18± 0.16ns	1.26±0.11**
6	Group VI: WBB (200mg/kg)	1.2± 0.11ns	1.1±0.11ns	1.2±0.14ns	1.41±0.1**
7	Group VII: WBB (400mg/kg)	1.19± 0.11	1.18±0.16ns	1.23± 0.17	1.25±0.08**

Results are expressed as mean ± SD in mg/dl. (n=6) @p<0.01 Compared with corresponding normal control group, **p<0.01 Compared with diabetic control group, ns p>0.05 compared with diabetic control group.

Table 6 and Figure 4 illustrates the significant reduction (p<0.01) in serum SGOT level when compared to negative control group.

Table 6: Effect of *Barleria buxifolia* on serum SGOT level in STZ induced diabetic rats at 0, 3, 14 and 28 days

Sr. No.	Groups	SGOT			
		0 days	3 days	14 days	28 days
1	Group I: Control	27.23+0.81	27.27+ 0.45	27.50 + 0.53	27.33 + 0.52
2	Group II: Negative control	26.92+0.75^{ns}	26.43+0.55^{ns}	27.26 +0.47^{ns}	32.00 +1.77@
3	Group III: Standard group	26.26 + .75^{ns}	26.55+0.65^{ns}	26.46 +0.62^{ns}	27.66+ 0.63**
4	Group IV: MBB (200mg/kg)	26.21± 0.52^{ns}	26.25± 0.45^{ns}	27.16± 0.40 ^{ns}	29.40 ± 0.51**
5	Group V: MBB (400mg/kg)	26.45± 0.61^{ns}	26.40± 0.66^{ns}	26.97± 0.55^{ns}	27.80 ± 0.68**
6	Group VI: WBB (200mg/kg)	26.34± 0.49^{ns}	26.50± 0.45^{ns}	26.81± 0.41^{ns}	28.70± 0.67**
7	Group VII: WBB (400mg/kg)	26.40 ±0.51^{ns}	26.20± 0.65^{ns}	26.57± 0.42^{ns}	28.30± 0.62**

Results are expressed as mean ± SD in mg/dl. (n=6) @p<0.01 Compared with corresponding normal control group, **p<0.01 Compared with diabetic control group, ns p>0.05 compared with diabetic control group.

Figure 4. Effect of *Barleria buxifolia* on serum SGOT level in STZ-induced diabetic rats at 0, 3, 14 and 28 days

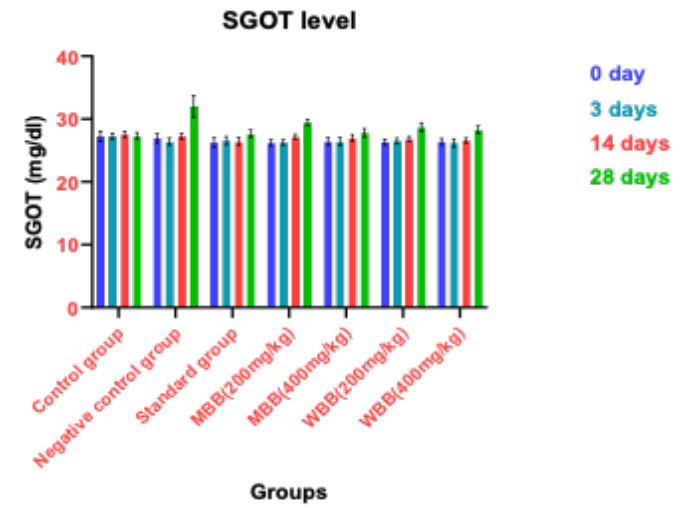

Figure 5. Effect of *Barleria buxifolia* on SGPT level in STZ-induced diabetic rats at 0, 3, 14 and 28 days

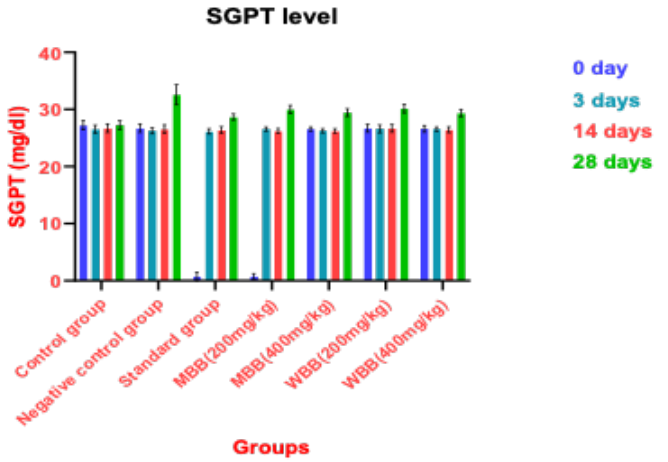

Table 7 and Figure 5 illustrates the significant reduction (p<0.01) in serum SGPT level when compared to negative control group.

Table 7. Effect of *Barleria buxifolia* on SGPT level in STZ induced diabetic rats at 0, 3, 14 and 28 days

Sr. No.	Groups	SGPT			
		0 days	3 days	14 days	28 days
1	Group I: Control	27.20± 0.80	26.54± 0.71	26.73±0.74^{ns}	27.25± 0.77
2	Group II: Negative control	26.73 ± 0.74^{ns}	26.27±0.51^{ns}	26.56±0.74^{ns}	32.59± 1.77@
3	Group III: Standard group	26.56 ± 0.74^{ns}	26.16±0.44^{ns}	26.37±0.60^{ns}	28.67± 0.53**
4	Group IV: MBB (200mg/kg)	26.38 ± 0.51^{ns}	26.57 ± 0.35^{ns}	26.23± 0.41^{ns}	30.00 ± 0.73**
5	Group V: MBB (400mg/kg)	26.57±0.35^{ns}	26.27± 0.35^{ns}	26.21± 0.40^{ns}	29.44 ± 0.66**
6	GroupVI: WBB (200mg/kg)	26.72 ± 0.70^{ns}	26.61 ± 0.68^{ns}	26.69± 0.67^{ns}	30.15 ± 0.74**
7	GroupVII: WBB (400mg/kg)	26.60 ± 0.58^{ns}	26.57± 0.36	26.45± 0.51^{ns}	29.37 ± 0.64**

Results are expressed as mean ± SD in mg/dl. (n=6) @p<0.01 Compared with corresponding normal control group, **p<0.01 Compared with diabetic control group, ns p>0.05 compared with diabetic control group.

Discussion

DM and associated diabetic neuropathy, a well-known, long-term problem of diabetes, has an impact on almost half of the population and suggests higher morbidity and mortality. Streptozotocin (STZ) and alloxan (ALX) are the most frequently used drugs, and this model has been useful for the study of multiple factors in the disease (19). Both drugs exert their diabetogenic action an equal time as they'll be administered parenterally. STZ exhibits a longer half-life, lasting for 15 minutes, compared to alloxan, which has a half-life of 1.5 minutes. This enhances the solution's stability both before and following injection into animals. Furthermore, the mechanism by which STZ causes diabetes is not strongly linked to cell destruction, resulting in lower mortality rates in animals (20). Overall, STZ diabetogenicity is more effective and with less variation with animal species. So STZ-induced diabetic model is preferred for the induction of diabetes in rats, which is given intraperitoneally at having dose of 60 mg/kg. In experimental diabetic rats, the

excessively high levels of glucose in the body during diabetes lead to a significant alteration in protein metabolism, resulting in the occurrence of negative nitrogen balance. In addition, there is an increase in the amount of urea in the blood. This is accompanied by a reduction in the amounts of plasma proteins. The reason for this is the accelerated breakdown of proteins in both the plasma and tissues, which leads to a negative nitrogen balance (21). As a result, the levels of urea and creatinine increase, offering biochemical indicators for evaluating kidney damage. The Serum creatinine concentration is often used to evaluate both the decline in renal function and the harmful effects of substances on the kidney in experimental animals (22). Alterations in the levels of SGOT and SGPT are directly correlated with changes in the metabolism of diabetic person, these enzymes play a part in metabolism (23). People with diabetes mellitus (DM) have high levels of transaminases, which work even when insulin isn't present because of amino acids in their blood. This is also what causes gluconeogenesis and ketogenesis to go up (24). SGOT and SGPT levels serve as indicators of liver function; hence, the restoration of these enzyme levels to normal indicates the proper functioning of the liver (25). The current investigation observed changes in the levels of blood glucose, serum urea, creatinine, SGOT, and SGPT in diabetic rats induced by STZ.

Herbal treatment is one of the ancient remedies used by humanity. In recent years, people have been eager to use herbal medicines due to their lower complications and fewer side effects than synthetic drugs. The more medicinal plant that is probably used for the treatment of neuropathic pain are: *Acorus calamus* Linn, *Artemisia dracunculus* L., *Butea monosperma* Lam., *Citrullus colocynthis* L., *Curcuma longa* L., *Crocus sativus* L., *Elaeagnus angustifolia* L., *Ginkgo biloba* L., *Mitragyna speciosa* Korth, *Momordica charantia* M., *and Carthamus tinctorius* L. (26) *Barleria buxifolia* is a plant rich in amino acids, alkaloids, anthraquinones, phenols, saponins, terpenoids, and flavonoids. This plant has been selected for screening in diabetes research. The STZ-induced diabetic rats were treated with aqueous and methanolic extracts of BB at dose of 200 and 400 mg/kg of body weight (group IV to VII) for 28 days. They show a significant reduction ($P<0.01$) in blood glucose level comparison to the negative control group. The, it showed a significant lowering ($P<0.01$) of serum urea, creatinine, SGOT, and SGPT levels in diabetic rats when compared with negative control group. This shows that extract has protected the kidney and liver when exposed to high glucose levels.

It has been observed that BB promotes an improvement in body weight while reducing elevated blood glucose levels. It inferred that the antidiabetic activity of *Barleria buxifolia* L. is due to the presence of phytochemicals such as flavonoids.

Conclusion

This investigation shows that both aqueous and methanolic extracts of *Barleria buxifolia* show a significant reduction in blood glucose, serum urea, creatinine, SGOT, and SGPT as well. Hence it can be used as a potential substitute for the management of diabetes and related complications.

Acknowledgement

The authors are grateful to P. Wadhwani College of Pharmacy, Yavatmal, for providing support to conduct the study.

Conflict of interest

We declare that we have no conflict of interest.

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