

International Journal of Applied Sciences and Biotechnology

A Rapid Publishing Journal

ISSN 2091-2609



Available online at:

<http://www.ijasbt.org>

&

<http://www.nepjol.info/index.php/IJASBT/index>

Indexing and Abstracting

CrossRef, Google Scholar, Global Impact Factor, Genamics, Index Copernicus, Directory of Open Access Journals, WorldCat, Electronic Journals Library (EZB), Universitätsbibliothek Leipzig, Hamburg University, UTS (University of Technology, Sydney): Library, International Society of Universal Research in Sciences (EyeSource), Journal Seeker, WZB, Socolar, BioRes, Indian Science, Jadoun Science, Jour-Informatics, Journal Directory, JournalTOCs, Academic Journals Database, Journal Quality Evaluation Report, PDOAJ, Science Central, Journal Impact Factor, NewJour, Open Science Directory, Directory of Research Journals Indexing, Open Access Library, International Impact Factor Services, SciSeek, Cabell's Directories, Scientific Indexing Services, CiteFactor, UniSA Library, InfoBase Index, Infomine, Getinfo, Open Academic Journals Index, HINARI, etc.

CODEN (Chemical Abstract Services, USA): IJASKD

Vol-2(4) December, 2014



Impact factor*: **1.422**

Scientific Journal Impact factor#: **3.419**

Index Copernicus Value: **6.02**

*Impact factor is issued by Universal Impact Factor. Kindly note that this is not the IF of Journal Citation Report (JCR).

#Impact factor is issued by SJIF INNO SPACE.

For any type of query and/or feedback don't hesitate to email us at: editor.ijasbt@gmail.com



Research Article

INVIVO ASSESSMENT OF ANTIOXIDANTS AND ANTIHYPERGLYCEMIC EFFECT OF *BARLERIA CRISTATA* LEAVES IN STREPTOZOTOCIN- INDUCED DIABETIC RATS

Narmadha Rajasekaran¹, Gomathi Duraisamy², Kalaiselvi Manokaran³ and Devaki Kanakasabapathi^{4*}

¹Acharya Institute of Health Sciences, Bangalore- 560032, Karnataka, India

²Tamilnadu Agricultural University, Coimbatore- 641003 Tamil Nadu, India

³Kongu Nadu Arts and Science College, Coimbatore- 641029 Tamilnadu – India

⁴Assistant Professor in Biochemistry, Karpagam University, Coimbatore-641 021 Tamilnadu, India

*Corresponding author's email: dr.devaki.ku@gmail.com

Abstract

Objective: Many new bioactive drugs isolated from plants having hypoglycaemic effects showed anti diabetic activity equal and sometimes even more potent than known oral hypoglycaemic agents. In this present study, designed to evaluate antihyperglycemic and antioxidants effect on ethanolic leaf extracts *Barleria cristata* (EtBc) in streptozotocin-induced diabetic rats at dose level 400mg/kg body weight for the treatment of 45 days. **Method and materials:** The experimental rats were randomly divided into five groups as a control, streptozotocin induced with diabetes (45mg/kg bw) without any treatment, treated with standard drug glibenclamide (1.25 mg/kg bw), EtBc (400 mg/kg bw) in diabetic induced rats and treated with EtBc alone without diabetic rats. At the end of 45th day animals were sacrificed, collect the serum, liver, kidney and pancreas for estimate the glucose, insulin, C-peptide, glycosylated hemoglobin, hemoglobin in serum, protein, enzymatic and non-enzymatic antioxidants and lipid peroxidation in tissues. **Results:** After the administration of EtBc, blood glucose levels were showed significantly reduction (P<0.05) in diabetic rats and it has been observed alternation occurred in body and organ weight and it was also normalized the serum level of glycemic profile like insulin, C-peptide, total hemoglobin and glycosylated hemoglobin levels similar to that of control rats. Antioxidants enzymes were return to back their levels as control in different tissues when compared to diabetic rats and also observed no significance difference between control and EtBc alone group rats at the end of 45th day. Therefore it was suggested that *Barleria cristata* may act by potentiation of pancreatic secretion of insulin or increasing glucose uptake by muscle cells. **Conclusion:** In this study, suggested the efficacy of *Barleria cristata* proved the maintenance of glucose homeostasis and may be used as a therapeutic agent in the management of diabetes mellitus.

Key words: *Barleria cristata*; Leaf extract; Antioxidants; Glycemic profile; Lipid Peroxidation.

Introduction

Diabetes mellitus (DM) is syndrome, initially characterized by a loss of glucose homeostasis resulting from defects in insulin secretion or insulin action. It is the most prevalent disease in the world affecting 25% of population of people and expected to touch 300 million marks by 2025. It is a chronic disorder caused by partial or complete insulin deficiency, resulting in hyperglycemia leading to acute and chronic complications. DM also gives rise to various secondary problems such as retinopathy, peripheral vascular insufficiencies and neuropathy. These secondary problems take place due to the oxidative stress and DNA damage caused by the generation of free radicals in the cells (Ghate *et al.*, 2014).

Insulin is a major anabolic hormone in the body. Pancreatic insulin reserve is an important parameter of status islet function with tight coupling between insulin secretion and production being necessary for adequate of pancreatic beta

cells. Streptozotocin (STZ) (2-deoxy-2-({methyl (nitroso) amino} carbonyl} amino)-β-D-glucopyranose) is a naturally occurring compound. The diabetogenic action of STZ is the direct result of irreversible damage to the pancreatic beta cells resulting in degranulation and loss of capacity to secrete insulin (Hmza *et al.*, 2013).

Oxidative stress may constitute the key and common events in the pathogenesis of secondary diabetic complications. Free radicals are continuously produced in the body as a result of normal metabolic process and interaction which environmental stimuli. This results from an imbalance between radical-generating and radical scavenging systems that has increased free radical production or reduced activity of antioxidant defenses or both. Implication of oxidative stress in the pathogenesis of diabetes mellitus is suggested not only by oxygen free radicals generation, but also due to non-enzymatic protein glycosylation, auto oxidation of glucose, impaired glutathione metabolism (Kuldeep *et al.*, 2014).

In diabetic condition, oxidative stress seems to be caused by increased production of ROS, a sharp reduction in antioxidants defenses and altered cellular redox status. Antioxidants provide protection to living organism from damage caused by uncontrolled production of ROS concomitant lipid peroxidation, protein damage and DNA strand breaking (Savini *et al.*, 2013).

The management of diabetes without any side effects is still a challenge to the medical system. There is an increasing demand from patients to use the natural products with antidiabetic activity, because insulin and oral hypoglycemic drugs possess undesirable side effects (Duganath *et al.*, 2011). In the indigenous Indian system of medicine (Ayurveda), many herbal medicines have been recommended for the treatment of diabetes or 'madhumeha' and some of them have been experimentally evaluated (Sujatha and Shalin, 2012).

Barleria cristata L. is known as Philippine violet, it belongs to the family of Acanthaceae. It is an erect herb found in the mountains of Western Ghats and Himalaya and also found in subtropical region includes, Sikkim and Southeast Asia. It has been used as a traditional herbal remedy in Thailand and it allegedly acts as a tonic, diuretic and blood purifier (Sriram and Sasikumar, 2012). Different parts of *Barleria cristata* have been used in the treatment of various diseases like anemia, toothache and cough. Root and leaves are used in the treatment of swelling and inflammation (Amutha and Doss, 2012). The present study was aimed to evaluate the antihyperglycemic and antioxidants effect of *Barleria cristata* in STZ-induced in Wistar albino rats.

Materials and Methods

Collection of plant sample

The leaves of *Barleria cristata* used for the investigation were obtained from Coimbatore district, Tamilnadu, India. The plant was authenticated by Dr. G.V.S. Murthy, Botanical Survey of India, TNAU Campus, Coimbatore and the voucher specimen No. is BSI/SRC/5/23/2011-12/Tech.-n62.

Preparation of plant extraction

The *Barleria cristata* leaves were shadow dried. It took about six days for the leaves to dry completely and become crisp and brittle to touch and then it was made to a coarse powder and stored in an air tight container in fridge for future use. The 50 g of powdered sample material was taken and subjected to successive solvent extracted with 250ml of ethanol for 8-10 hours.

Animals and diet

Wistar albino rats of either sex weighing about 150–180 g were procured from the animal house of Karpagam University, Coimbatore, India. The animals were under standard conditions and were housed four per cage in polypropylene cages with a wire mesh top and a hygienic bed of husk in a specific pathogen free animal room under

controlled conditions of 12 hr light and 12 hr dark cycle, with temperature of $24 \pm 2^\circ\text{C}$, relative humidity of $50 \pm 10\%$ and fed with rodent diet and water *ad libitum*. The study was approved by Institutional Animal Ethical Committee constituted for the purpose of CPCSEA, Government of India.

Induction of diabetes

Rats were fasted overnight before inducing diabetes with streptozotocin. The rats were given an intraperitoneal injection of streptozotocin (45mg/kg) freshly prepared in 0.1M sodium citrate buffer. The diabetic state was confirmed 48 hr after streptozotocin injection.

Experimental animals:

The experimental rats, who had fasted overnight, were randomly divided into five groups with six rats in each group. Control group rats were received 1.0 ml of physiological saline.

Group 1: Control, normal healthy rats

Group 2: Rats were induced with diabetes by a single intra peritoneal injection of 45mg/kg bw of streptozotocin and kept without any treatment for 45 days

Group 3: Rats were induced with diabetes as mentioned in group 2 and treated with glibenclamide (1.25 mg/kg bw) orally for a period of 45 days

Group 4: Rats were induced with diabetes as mentioned in group 2 and treated with *Barleria cristata* (400 mg/kg bw) orally for a period of 45 days

Group 5: Rats were treated with *Barleria cristata* alone (400 mg/kg bw) orally for a period of 45 days

Preparation of serum and tissue

After the experimental period of 45 days, the animals were sacrificed by cervical dislocation under mild chloroform anesthesia. Blood was collected by decapitation and serum was separated by centrifugation (for 10 min at 1500 rpm). The liver and kidney were excised immediately and thoroughly washed in ice cold saline and weights and immediately used for estimation. A 10% homogenate of the washed tissues were prepared with 0.1 M Tris-HCl buffer pH 7.4 at 4°C in a potter homogenizer, fitted with a Teflon plunger at 6000 rpm for 15 min. The filtrate was used for further biochemical analysis like, blood glucose, insulin, C-peptide, glycosylated hemoglobin, hemoglobin in serum, protein, enzymatic (SOD, CAT, GR and GPx) and non-enzymatic (Vitamin C, E and GSH) antioxidants and lipid peroxidation in tissues.

Statistical analysis

Results are expressed as the Mean \pm SD. Statistical significance was evaluated by One way analysis of variance (ANOVA) using SPSS version (10.0) and the individual comparisons were obtained by the Duncan multiple range test (DMRT) (Duncan, 1957). A value of $p < 0.05$ was

considered to indicate a significant difference between groups. For body weight the comparison was made by using student “t” test (p <0.05).

Result and discussion

The fundamental defect in diabetes mellitus is an absolute absence of biologically active insulin, which results in the impairment of uptake and storage of glucose and also reduced glucose utilization for energy purpose. The underlying goal of all diabetes treatment and management is to maintain an adequate blood glucose concentration. Progress in understanding the metabolic staging of diabetes over the past few years has led to significant advances in regimen for the treatment of this devastating disease (Manohar *et al.*, 2012)

Effect of EtBc on blood glucose level

Fig.1 depicts the blood glucose levels of each group estimated at initial, 5th, 15th, 30th and 45th days after the administration of EtBc in experimental rats. Significant increase in the blood glucose level upto 45th day was shown in diabetic control rats. This is due to the destruction of beta cell of pancreas which has caused severe insulin deficiency leading to elevated blood glucose level. Diabetic rats treated with EtBc showed significant decrease (P<0.05) decrease in blood glucose level on 15th day and the level was restored to near normal on 30th day and this was also continued till 45th day.

Effect of EtBc on body weight

The changes in body weight of control and experimental group of rats were estimated and the results are depicted in Fig. 2. It describes the body weight of each group estimated on initial and final days of treatment. Diabetic control rats showed a significant loss in body weight and the reduction in bodyweight was observed till the end of the study. This is due to increased muscle wasting and excessive breakdown of tissue protein in diabetic rats (Haldar *et al.*, 2010).

Therefore, EtBc treated rats produced a positive effect for diabetes mellitus and this effect might be due to the stimulation of the β cells of pancreas which increases the insulin secretion or increased peripheral glucose utilization (Babu *et al.*, 2012).

After the treatment with standard drug and EtBc, it was observed that the body weights of the diabetic rats were significantly increased and the results were similiar to that of control rats. Administration of EtBc improved the body weight of diabetic rats, that indicates control the over muscle wasting and protein sparing effect. This result suggests that EtBc is involved in the maintainance of glucose homeostasis by altering the metabolic activity which was supported by Rakesh *et al.*, 2012.

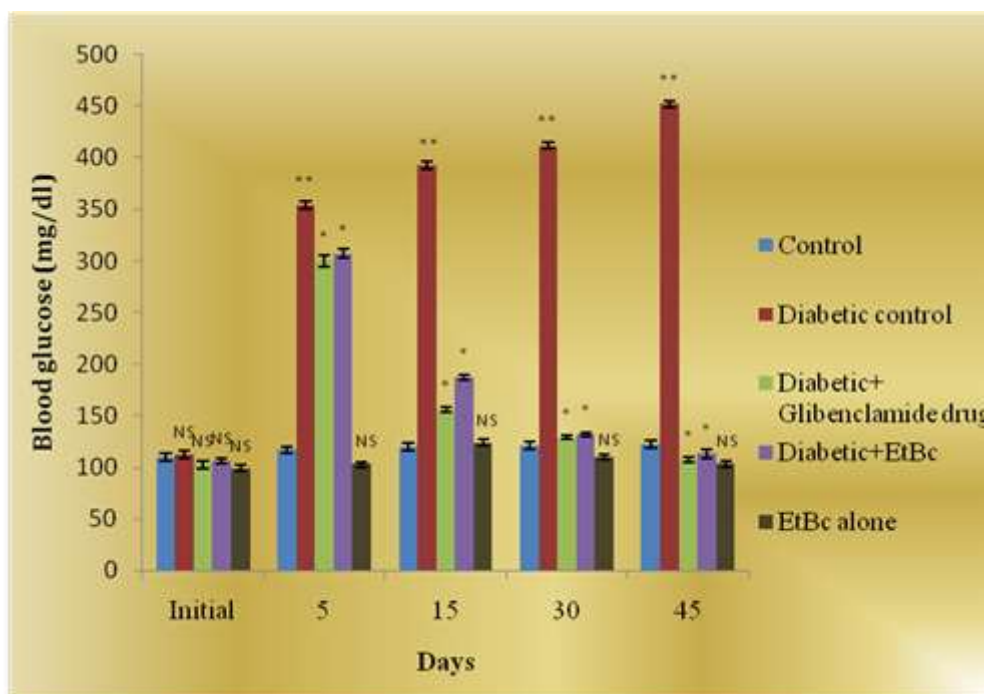


Fig 1: Changes of blood glucose level of experimental rats

- a. 5th day compared with initial day;
- b. 15th day compared with initial day
- c. 30th day compared with initial day
- d. 45th day compared with initial day

** : Significance at 95% level (P<0.05%) * : Significance at 99% level (P<0.05%) NS: Non-Significant at 95% level (P<0.05%)

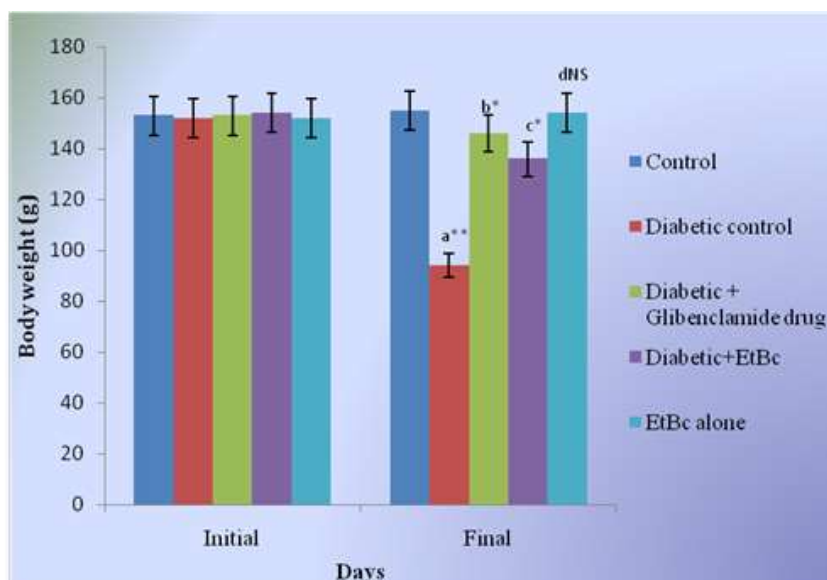


Fig 2: Changes in body weight of experimental rats

- a. Group I compared with Group II
- b. Group I compared with Group III
- c. Group I compared with Group IV
- d. Group I compared with Group V

** : Significance at 95% level (P<0.05%) NS: Non-Significant at 95% level. (P<0.05%)

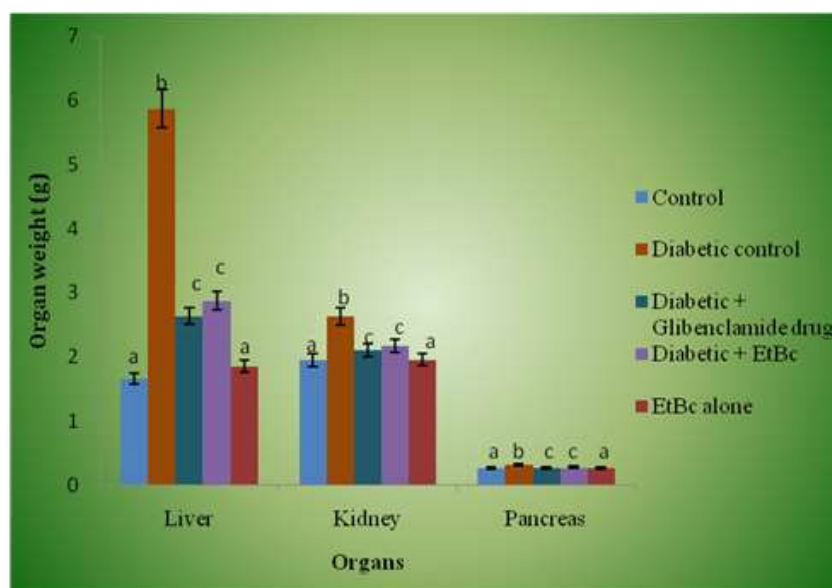


Fig 3: Changes in organ weight of experimental rats

Values are expressed as Mean ± SD for six animals in each group.

Values not sharing common superscript letters (a-d) differ significantly at p<0.05(DMRT).

Effect of EtBc on organ weight

The organ (liver, kidney and pancreas) weights of the normal and diabetic rats are depicted in Fig. 3 There is a significant increase (p<0.05) in the weight of liver, kidney and pancreas in streptozotocin induced diabetic rats when compared with the control group. Our results are in agreement with (El-Shobaki *et al.*, 2010). who found that the weights of the organs were increased in diabetic rats when compared with control rats. It reported that increased kidney weight may indicate the progression of renal failure worsened by oxidative stress (Feng *et al.*, 2013). However, diabetic rats fed with EtBc shown significantly decreased organ weights when compared with diabetic rats. No

significant difference was found between control and Barleria cristata alone treated group. This results indicated that the Barleria cristata leaves control diabetes mellitus and afford protection against the oxidative stress deveopled during diabetes mellitus.

Effect of EtBc on glycemic profile in serum

Table 1 shows the status of insulin, C-peptide, total hemoglobin and glycosylated hemoglobin in control and experimental group rats. C-peptide and insulin levels were significantly decreased in STZ-induced diabetic rats due to the destruction of β-cells of pancreas thereby inhibiting the insulin release. Oral administration of EtBc significantly increased the levels of insulin and C-peptide in

STZ-induced diabetic rat when compared with diabetic control rats. This explains that *Barleria cristata* may act by potentiation of pancreatic secretion of insulin or increasing glucose uptake by muscle cells (Malini *et al.*, 2011).

The decreased level of total haemoglobin in diabetic rats is mainly due to the increased formation of HbA_{1c}. HbA_{1c} was found to be increase in patients with diabetes mellitus and the amount of increase is directly propotional to the fasting blood glucose level. During diabetes mellitus, the excess glucose present in the blood reacts with haemoglobin to form HbA_{1c} (Kasar, 2011). HbA_{1c} is used as a marker for estimating the degree of protein glycation in diabetes mellitus. Administration of EtBc to diabetic rats reduced the glycosylation of haemoglobin by virtue of its normoglycaemic activity and thus decreases the levels of glycosylated haemoglobin in diabetic rats. This normalisation of glycosylated haemoglobin indicates decreased glycation of protein in diabetic rats. There is no significance difference was observed between control and *Barleria cristata* alone group rats. This suggests the efficacy of *Barleria cristata* in the maintenance of glucose homeostasis and may be used as a therapeutic agent in the management of diabetes mellitus.

Effect of EtBc on protein levels of tissue

In streptozotocin diabetes rats, body cells are unable to utilize glucose as a source of energy due to which proteins are spared as energy source. The amino acids released from the proteins are used for gluconeogenesis (Chaurasia *et al.*, 2010). This leads to decrease in protein storage which in turn reduces body weight (Bandawane *et al.*, 2011). Table 2 shows that protein levels were significantly decreased in diabetic rats of liver, kidney and pancreas when compared to the normal control rats due to insulin deficiency that causes excessive catabolism of protein. Oral administration

of EtBc significantly improved the protein levels in diabetic rats when compared to diabetic control rats suggests that the EtBc may improve insulin secretion and thereby make the cells to utilize glucose instead of protein. No significant difference was found between control and *Barleria cristata* alone treated groups. This indicates that a *Barleria cristata* leaves is not interfering with the metabolism of the above mentioned organs.

Effect of EtBc on enzymatic antioxidants in tissue

Tables 3 and Table 4 showed the activities of SOD, CAT, GPx and GR in the liver and kidney of normal and experimental rats. During diabetes there was a significant reduction in the activities of SOD, CAT, GPx and GR in tissues, such as liver and kidney. Oxidative stress in diabetes coexists with a reduction in the antioxidant capacity, which can increase the deleterious effects of free radicals. The reduced activities of SOD and CAT were observed in the liver and kidney during diabetes may be result of accumulation of superoxide radicals and hydrogen peroxides. SOD scavenges the superoxide radical by converting it to H₂O₂ and molecular oxygen (Assady *et al.*, 2011). The observed decrease in SOD activity in diabetic rats could result from inactivation of SOD by H₂O₂ or by glycation of the enzyme, which have been reported to occur in diabetes. CAT is a hemeprotein, which catalyzes the reduction of hydrogen peroxide and protect the tissues from highly reactive hydroxyl radicals. The decrease in CAT activity during diabetes could result from inactivation of the enzyme by glycation. Further, SOD protects the CAT enzyme inactivation by superoxide radical (Dodamani *et al.*, 2012). Thus, the increase in SOD activity may indirectly play an important protective role in preserving the activity of CAT.

Table 1: Changes of serum insulin, C-peptide, total hemoglobin and glycosylated hemoglobin level of experimental rats

Particulars	Control	Diabetic control	Diabetic + Glibenclamide	Diabetic + EtBc	<i>Barleria cristata</i> alone treated
Insulin (μU/ml)	17.56±0.24 ^a	6.55±0.22 ^b	15.06±0.36 ^c	14.4±0.27 ^c	16.61±0.23 ^d
C-peptide (ng/ml)	6.21±0.14 ^a	3.13±0.13 ^b	5.18±0.16 ^c	4.3±0.13 ^d	5.65±0.24 ^d
Glycosylated Hb (%HbA _{1c})	5.97±0.04 ^a	14.99±0.43 ^b	6.27±0.22 ^{cd}	6.50±0.19 ^d	5.95±0.23 ^a
Hb (g/ml)	13.82±0.16 ^a	6.85±0.21 ^b	13.27±0.53 ^c	12.74±0.29 ^c	13.83±0.29 ^a

Values are expressed as Mean ± SD for six animals in each group.

Table 2: Changes of protein levels in tissues of experimental rats

Particulars (mg/g)	Control	Diabetic control	Diabetic + Glibenclamide	Diabetic + EtBc	<i>Barleria cristata</i> alone treated
Liver	2.44±0.05 ^a	1.12± 0.02 ^b	2.01±0.06 ^c	1.81± 0.04 ^d	2.45±0.03 ^a
Kidney	2.14±0.04 ^a	0.99±0.05 ^b	2.17±0.04 ^c	1.78±0.06 ^d	2.07±0.06 ^a
Pancreas	2.01±0.03 ^a	0.73±0.02 ^b	1.85±0.08 ^c	1.76±0.06 ^d	1.91±0.03 ^a

Values are expressed as mean ± SD for six animals in each group.

Values not sharing common superscript letters (^{a-d}) differ significantly at p<0.05 (DMRT).

Table 3: Changes of enzymatic antioxidant enzymes in liver of experimental rats

Particulars	Control	Diabetic control	Diabetic Glibenclamide +	Diabetic EtBc +	<i>Barleria cristata</i> alone treated
Superoxide dismutase*	3.75±0.49 ^a	1.33±0.20 ^b	3.18±0.49 ^c	3.07±0.46 ^c	3.59±0.074 ^a
Catalase **	0.96±0.02 ^a	0.20±0.18 ^b	0.86±0.02 ^c	0.81±0.14 ^d	0.90±0.06 ^a
Glutathione reductase #	3.27±0.33 ^a	1.91±0.56 ^b	2.90±0.27 ^c	2.43±0.43 ^c	3.22±0.21 ^a
Glutathione peroxidase #*	2.24±0.10 ^a	0.96±0.14 ^b	1.91±0.28 ^c	1.65±0.22 ^c	2.27±0.05 ^a

Values are expressed as mean ± SD for six animals in each group.

Values not sharing common superscript letters ^(a-e) differ significantly at p<0.05 (DMRT).

Units:

*: Enzyme required for 50% inhibition of NBT reduction/min/mg protein

** : μmoles of H₂O₂ utilized/min/mg/protein;

: n moles of NADPH oxidized/min/mg protein

#* : μmoles of GSH utilized/min/mg/protein

Table 4: Changes of enzymatic antioxidant enzymes in kidney of experimental rats

Particulars	Control	Diabetic control	Diabetic Glibenclamide +	Diabetic EtBc +	<i>Barleria cristata</i> alone treated
Superoxide dismutase *	3.31±0.33 ^a	1.10±0.64 ^b	2.98±0.43 ^c	2.92±0.33 ^d	3.10±0.23 ^e
Catalase **	0.72±0.06 ^a	0.30±0.30 ^b	0.66±0.03 ^c	0.63±0.054 ^c	0.71±0.05 ^a
Glutathione reductase #	3.18±0.73 ^a	1.96±0.88 ^b	3.02±0.89 ^c	2.92±0.95 ^c	3.11±0.72 ^a
Glutathione peroxidase #*	1.96±0.08 ^a	0.76±0.21 ^b	1.51±0.10 ^c	1.31±0.39 ^d	1.12±0.08 ^c

Values are expressed as mean ± SD for six animals in each group.

Values not sharing common superscript letters ^(a-e) differ significantly at p<0.05 (DMRT).

Units:

* : Enzyme required for 50% inhibition of NBT reduction/min/mg protein

** : μmoles of H₂O₂ utilized/min/mg/protein;

: n moles of NADPH oxidized/min/mg protein

#* : μmoles of GSH utilized/min/mg/protein

Table 5: Changes of non-enzymatic antioxidant in the liver of experimental rats

Particulars	Control	Diabetic control	Diabetic Glibenclamide +	Diabetic + EtBc	<i>Barleria cristata</i> alone treated
Vitamin E (μg /mg ptn)	3.54±0.08 ^a	1.31±0.51 ^b	2.83±0.15 ^c	2.73±0.33 ^d	3.38±0.09 ^c
Vitamin C (μg /mg ptn)	1.92±0.13 ^a	0.92±0.19 ^b	1.61±0.11 ^c	1.15±0.20 ^d	1.92±0.11 ^a
Total reduced glutathione (μg/mg ptn)	28.7±0.44 ^a	15.56±2.25 ^b	26.45±0.99 ^c	27.23±0.85 ^c	27.99±0.51 ^a

Values are expressed as mean ± SD for six animals in each group.

Values not sharing common superscript letters ^(a-e) differ significantly at p<0.05 (DMRT)

Table 6: Changes of non-enzymatic antioxidant in the kidney of experimental rats

Particulars	Control (Group I)	Diabetic control (Group II)	Diabetic + Glibenclamide (Group III)	Diabetic + EtBc (Group IV)	<i>Barleria cristata</i> alone treated (Group V)
Vitamin E (μg /mg ptn)	3.02±0.27 ^a	1.63±0.62 ^b	2.95±0.17 ^c	2.76±0.13 ^d	3.18±0.15 ^d
Vitamin C (μg /mg ptn)	2.97±0.10 ^a	1.11±0.052 ^b	2.35±0.69 ^c	2.09±0.15 ^c	2.93±0.10 ^a
Total reduced glutathione (μg /mg ptn)	25.54±1.17 ^a	13.82±2.05 ^b	21.83±5.43 ^c	24.62±0.48 ^c	5.39±0.80 ^a

Values are expressed as mean ± SD for six animals in each group.

Values not sharing common superscript letters ^(a-e) differ significantly at p<0.05 (DMRT)

GPx and GST work together with glutathione in the decomposition of H₂O₂ and other organic hydroperoxides to non-toxic products at the expense of the GSH. Reduced activities of GPx may result from radical-induced inactivation and glycation of the enzyme. Decreased the activity of GR result in the involvement the deleterious oxidative changes and also insufficient availability of reduced glutathione. In this context, other researchers also

reported a decrease in the activity of these antioxidant enzymes (SOD, CAT, GPx and GR) in the liver and kidney of diabetic rats (Wang *et al.*, 2012). After the administration of EtBc and glibenclamide, the treated rats showed significant increase the activity of SOD, CAT, GPx and GR than diabetic rats. This result indicates the efficacy of the EtBc in preventing the oxidative stress. There is no significant difference was found between control and EtBc

alone treated groups. Therefore, *Barleria cristata* afford protection against free radicals induced oxidative stress by enhancing the cellular antioxidant defense.

Effect of EtBc on non-enzymatic antioxidants in tissue

The levels of non-enzymic antioxidants in the streptozotocin-induced diabetic rats of liver and kidney tissues were illustrated in Table 5 and 6. In the present study, the streptozotocin induced diabetic rats had shown decreased activities of GSH, vitamin C and E in both liver and kidney tissues. Treatment with EtBc and glibenclamide showed reversal of all these parameters to near normal when compared to diabetic control rats.

Glutathione is a tripeptide normally present at high concentrations in intracellular compartment and constitutes for the major reducing capacity of the cytoplasm. Glutathione is known to protect the cellular system against toxic effects of lipid peroxidation (Cameron and Pakrasi, 2010). Decreased level of GSH in the liver and kidney during diabetes represents its increased utilization due to oxidative stress. In the present study, a significant elevation of GSH level was observed in the extract treated diabetic control rats. This indicates that the extract can either increase the biosynthesis of GSH or reduce the oxidative stress leading to less degradation of GSH or have both effects.

Ascorbic acid (Vitamin C) and α -tocopherol (Vitamin E) are interrelated by recycling process. Recycling of tocopheroxyl radicals to tocopherol is achieved with ascorbic acid (Lobo *et al.*, 2010). In our study, ascorbic acid was decreased in diabetic rats as reported earlier (Iwata *et al.*, 2010). Reduction in liver and kidney ascorbic acid levels may occur due to excessive oxidation and lack of regeneration from their radical form to reduced form. Vitamin E is a hydrophobic antioxidant found in

lipoproteins and provides secondary stage protection against free radicals. It is the most efficient scavenger of lipid peroxy radicals. In diabetic rats, it was significantly decreased in liver and kidney when compared to the control rats. Impaired regeneration of these non-enzymatic antioxidants can contribute to oxidative stress in diabetic condition (Brouwers *et al.*, 2010). After the administration of EtBc and glibenclamide drug treatments they were brought back to the normal levels than that of diabetic control rats. Therefore, this study indicates that *Barleria cristata* had an excellent antioxidant activity.

Effect of EtBc on lipid peroxidation in tissue

Lipid peroxidation was induced by glucose through activation of lipoxygenase enzymes (Kaimal *et al.*, 2010). Free radical induced lipid peroxidation has gained much importance because of its involvement in several pathologies such as ageing, wound healing, oxygen toxicity, liver disorders, inflammation, etc., (Gupta and Sharma, 2010).

This study has demonstrated the involvement of free radicals in the genesis of diabetes mellitus and their role in the induction of lipid peroxidation during diabetes (Nadro and Onoagbe, 2012). From the Fig. 4, it is showed that the lipidperoxidation levels were significantly higher in diabetic rats when compared to the control rats. This increased concentration of malondialdehyde (aldehydic products of lipidperoxidation) is a biomarker for intensified lipidperoxidation and it is an indirect evidence for the high free radical production in diabetes. After administration of EtBc, a significant reduction in LPO was observed normal to that of glibenclamide treated rats. Rats treated with EtBc alone showed no significant difference when compared to control rats. Since, *Barleria cristata* could interfere with the activity of the natural antioxidative defense system and thus inhibit the *de novo* generation of free radicals.

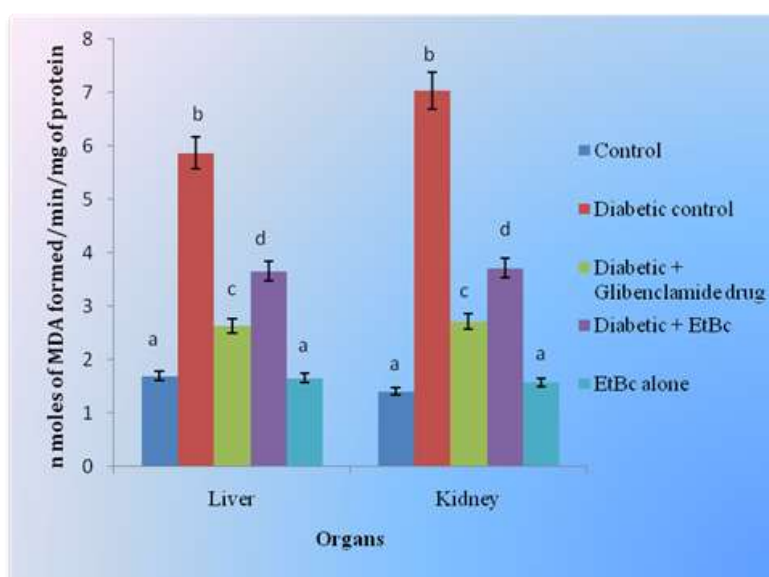


Fig. 4: Changes of lipid peroxidation in liver and kidney of experimental rats

Values are expressed as mean \pm SD for six animals in each group.

Values not sharing common superscript letters (a-c) differ significantly at $p < 0.05$ (DMRT).

Conclusion

This study indicates that ethanolic leaf extract of *Barleria cristata* had investigation revealed that, it has significant and consistent hypoglycemic effects by which it alters the changes that occur in the diabetic condition. This extract maintains the antioxidant levels, decrease the lipid peroxidation and more occurs it also secrete the insulin level. Therefore, *Barleria cristata* could be considered for further studies on verifying the isolation and identification of phytochemical responsible for antihyperglycemic activity.

Acknowledgement

The authors are grateful to the Chancellor, Vice-Chancellor and Registrar of Karpagam University, Coimbatore for providing facilities to carry out this research work.

Reference

- Amutha K and Arokia Doss DV (2012). Identification and Antimicrobial Activity of saponin fraction from the leaves of *Barleria cristata* L. *International Journal of pharmaceutical sciences and research* **3**(10): 4040-44.
- Assady M, Farahnak A, Golestani A and Esharghian MR (2011) Superoxide Dismutase (SOD) Enzyme Activity Assay in *Fasciola* spp. Parasites and Liver Tissue Extract. *Iran. J. Parasitol.* **6**:17-22.
- Babu KS, Shaker IA, Kumaraswamy D and Basha SS (2012) Indigenouse effect of *Cynodon dactylon* in experimental induced ulcers and gastric secretions. *International Res. J. Pharm.* **3**: 301-304.
- Bandawane D, Juvekar A and Juvekar M (2011) Antidiabetic and Antihyperlipidemic Effect of *Alstonia scholaris* Linn Bark in Streptozotocin-Induced Diabetic Rats. *Ind. J. Pharm. Edu. Res.* **45**: 114-120.
- Brouwers O, Niessen PM, Haenen G, Miyata T and Brownlee M (2010) Hyperglycaemia-induced impairment of endothelium-dependent vasorelaxation in rat mesenteric arteries is mediated by intracellular methylglyoxal levels in a pathway dependent on oxidative stress. *Diabetologia.* **53**: 989-1000. DOI: DOI: 10.1007/s00125-010-1677-0
- Cameron JC and Pakrasi HB (2010) Essential Role of Glutathione in Acclimation to Environmental and Redox Perturbations in the Cyanobacterium *Synechocystis* sp. *Plant Physiol.* **154**: 1672-1685. DOI: 10.1104/pp.110.162990
- Chaurasia S, Jain R, Saxena RC, Chaurasia ID and Shrivastava R (2010) Hyperglycemic activity of *Eugenia jambolane* in Streptozotocin induced diabetic rats. *J. Chem. Pharm. Res.* **2**: 458-460.
- Dodamani SS, Sanakal RD and Kaliwal BB (2012) Effect of ethanolic leaf extract of *Nymphaea odorata* on biochemical and oxidative stress parameters of liver and pancreas in Alloxan induced diabetic mice. *Res. Opin. Anim. Vet. Sci.* **2**: 151-157.
- Duganath N, RamaKrishna D, Reddy D, Sudheera B, Mallikarjun M and Beesetty P (2011) Evaluation of anti-diabetic activity of *Triumfetta rhomboidea* in alloxan induced Wistar rats. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* **2**(1): 721-726.
- El-Shobaki FA, El-Bahay AM, Esmail RSA, El Megeid AAA and Esmail NS (2010) Effect of fig fruit (*Ficus carica* L.) and its leaves on hyperglycemia in alloxan diabetic rats. *World J. Dairy Food. Sci.* **5**:47-57.
- Feng B, Yan X, Xue J, Xu L and Wang H (2013) The Protective Effects of α -Lipoic Acid on Kidneys in Type 2 Diabetic Goto-Kakisaki Rats via Reducing Oxidative Stress. *Int. J. Mol. Sci.* **14**:6746-6756. DOI: 10.3390/ijms14046746
- Ghate R, Patil VP, Hugar S, Matha NH and Kalyane NV (2014) Antihyperglycemic activity of *Areca Catechu* flowers. *Asian Pacific Journal Tropical Disease* **4**(1):148-152. DOI: 10.1016/S2222-1808(14)60430-8
- Gupta V and Sharma M (2010). Protective effect of *Cinnamomum tejpata* on lipid peroxide formation in isolated rat liver homogenate. *Curr. Res. J. Biol. Sci.* **2**: 246-249.
- Haldar PK, Kar B, Bhattacharya S, Bala A and Kumar RBS (2010) Antidiabetic activity and modulation of antioxidant status by *Sansevieria roxburghiana* rhizome in Streptozotocin-induced diabetic rats. *Diabetologia Croatica* **39**: 115-123.
- Hmza AJA, Osman MT, Adnan A and Omar E (2013) Immunomodulatory Effect of *Nigella Sativa* Oil in the Disease Process of Type 1 Diabetic Rats. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* **4**(1): 980-988.
- Iwata N, Okazaki M, Kamiuchi S and Hibino Y (2010) Protective Effects of Oral Administrated Ascorbic Acid against Oxidative Stress and Neuronal Damage after Cerebral Ischemia/Reperfusion in Diabetic Rats, *Journal of Health Science.* **56** (1) 20-30. DOI: 10.1248/jhs.56.20
- Kaimal S, Sujatha KS and George S (2010). Hypolipidaemic and antioxidant effects of fruits of *Musa AAA* (Chenkadali) in alloxan induced diabetic rats. *Ind. J. Exp. Biol.* **48**:165-173.
- Kasar KAK (2011) Evaluation of changes in levels of serum selenium in diabetic pateints and in diabetic patients with cardiovascular disease, *J. Al-Nahrain University* **14**: 8-66.
- Kuldeep S, Ch RV, Zeashan H and Ri P (2014) Evaluation of In-vivo antioxidant and oral glucose tolerance test of ethanolic extract of *Calotropis gigantea* Linn. against Streptozotocin-induced diabetic rats. *Journal of Pharmacognosy and Phytochemistry* **2**(5): 59-65.
- Lobo V, Patil A, Phatak A and Chandra N (2010) Free radicals and Antioxidants and functional foods: Impact on human health. *Pharmacogn Rev.* **4**(8): 118-126. DOI: 10.4103/0973-7847.70902
- Malini P, Kanchana G and Rajadurai M (2011) Antiperoxidative and antioxidant effect of Ellagic acid on normal and Streptozotocin induced diabetes in albino Wistar rats. *Res. J. Pharm. Biol. Chem. Sci.* **3**: 24-34.
- Manohar VS, Jayasree T, Kishore KK, Rupa LM, Dixit R and Chandrasekhar N (2012) Evaluation of hypoglycemic and antihyperglycemic effect of freshly prepared aqueous

- extract of *Moringa oleifera* leaves in normal and diabetic rabbits. *J. Chem. Pharm. Res.* **4**: 249-253.
- Nadro MS and Onoagbe IO (2012) Effect of aqueous and ethanolic extracts of *Cassia italica* leaves in streptozotocin-induced diabetes in rats. *J. Med. Plants. Res.* **6**: 4675-4681.
- Rakesh P, Sekar S and Senthilkumar KL (2012) A comparative study on the antidiabetic effect of *Nelumbo nucifera* and glimepiride in streptozotocin-induced diabetic rats. *Int. J. Pharma. Bio Sci., Sains Malaysiana.* **41**: 969-975.
- Savini L, Catani MV, Evangelista D, Gasperi V and Avigliano L (2013) Obesity-Associated Oxidative Stress: Strategies Finalized to Improve Redox State. *Int. J. Mol. Sci.* **14**(10): 497-538.
- Sriram S and Sasikumar CG (2012) Pharmacognostic Standardization and Physicochemical analysis of the leaves of *Barleria montana* Wight & Nees. *Asian Pac. J. Trop. Biomed.* **1**: 1-3.
- Sujatha and Shalin J (2012) Complementary Therapeutic Potential: A Focus on Polyherbal Products for Hyperglycemia. *Asian Journal of Scientific Research* **5**: 1-13. DOI: 10.3923/ajsr.2012.1.13
- Wang Y, Han J, Xiong W and Yuan Q (2012) Evaluation of In vivo Antioxidant and Immunity Enhancing Activities of Sodium Aescinate Injection Liquid. *Molecules* **17**:10267-275. DOI: 10.3390/molecules170910267