WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 23, 1013-1031. Research Article ISSN 2277–7105

HYPOGLYCEMIC ACTIVITY OF LYOPHILIZED TULSI LEAF POWDER AND ITS PROTECTIVE ROLE ASSOCIATION WITH THE ANTIOXIDANT AND ANTIDYSLIPIDEMIC PROPERTIES IN ALLOXAN-INDUCED DIABETIC RATS

Umadevi C. Jadaramkunti*

Department of Zoology, Government First Grade College, Hubballi-580032, Karnataka.

Article Received on
07 October 2024,

Revised on 28 Oct. 2024,
Accepted on 17 Nov. 2024

DOI: 10.20959/wjpr202423-34760

***Corresponding Author**
Umadevi C. Jadaramkunti
Department of Zoology, Government First Grade College, Hubballi-580032, Karnataka.

ABSTRACT

This study was aimed to assess the antidiabetic and antihyperlipidaemic potentials of lyophilized tulsi leaf powder (LTLP) and in correlation, with standard drug glibenclamide (2.5 mg/kg BW/day) on alloxan monohydrate induced diabetic male albino rats. Diabetes was induced in Wistar rats by the administration of alloxan monohydrate at 100 mg/kg of body weight. After 48 hrs, rats with fasting blood glucose levels of 200 mg/dL and above were taken into consideration diabetic and used for the study. Wistar albino rats were divided into five groups of 10 animal each viz. Group I rats non-diabetic (normal control), Group II consisted of diabetic control rats that received no treatment, Group III rats diabetic conventional treated (with Glibenclamide) and Groups of IV and V rats diabetic dealt with (supplementation with graded doses of LTLP, 50 and 100mg/ kg, BW/ day, respectively). Blood samples were collected at 28 post-treatment and blood glucose, relative body weight, serum biochemical parameters insulin, glucose, urea, creatinine and glycosylated haemoglobin (HbA1c), protein, albumin, globulin, SGPT, SGOT, ALP AST and ALT, lipid profile (TC, TG, LDL-C, VLDL-C, HDL-C, and phospholipids). In addition, to evaluate the protective potentials of LTLP, the pancreas and liver and tissues were collected, and tissues homogenate were prepared to analyse for the assessment of activities of oxidative stress parameters (total protein, MDA, GSH, GST, SOD and catalase activities) in diabetic induced rats were performed. The results established that graded doses of LTLP supplementation in alloxan induced diabetic rats significantly ($P \leq 0.05$) altered blood glucose levels, HbA1c and other biochemical parameters level were

observed in diabetic rats compared to diabetic control rats. Treatment with LTLP produced significant ($P \leq 0.05$) recovery in the levels of lipid profiles towards the control levels when compared with diabetic control and glibenclamide drug. These findings showed clearly indicates that carbohydrate nature of LTLP has an important antidiabetic, antidyslipidemic, and antioxidant potential.

KEYWORDS: LTLP, Antidiabetic, Antioxidant Antihyperlipidaemic, Alloxan, Glibenclamide, Serum biochemical indices.

INTRODUCTION

Diabetes Mellitus is a metabolic ailment characterized by changes in glucose, lipid, with variation of protein, carbohydrates, and fats metabolism, resulting in hyperglycemia and inadequate insulin production, action, or both.[1] It is likewise an indication of co-morbidities together with obesity, hypertension and hyperlipidemia that are metabolic complications of both scientific and experimental diabetes. This have become extra obvious following World Health Organization recommendations regarding the need to expand and evaluate better pharmacological agents for improving insulin secretion and insulin sensitivity, preventing beta (β) cells destruction, promoting β-cells regeneration or repair interrupting pathways main to diverse complications of diabetes.[2] Hence, irrespective of the presence of those hypoglycemic pharmacological drugs, supplementation of herbal primarily based drugs to treat diabetes is now a promising and novel remedy method due to its safe and non-toxic nature.[3] Many medicinal plants have been reported to be beneficial in diabetes worldwide and their products are suggested and believed to contain chemical substances such as flavonoids, carotenoids, terpenoids, glycosides, alkaloids with potential curative effects and considered an excellent source for alternative medicine to treat diabetes by distinctive feature of their active phytochemical constituent.[4] Though, the study of plant for hypoglycemic, antioxidant and hypolipidemic activities may give new pharmacological approaches in the treatment of diabetes mellitus.[5] The benefits of an herbal medicinal product may be because of a single phytocompound or, more ideally, a synergistic effect of multiple phytochemicals. Although synthetic oral hypoglycemic agents/insulin are a popular diabetes therapy and are efficient in controlling hyperglycemia, they have significant side effects and do not significantly modify the course of diabetic complications.[6]

Ocimum sanctum L. (Tulsi, a Lamiaceae family) is one of the most versatile medicinal plants having a huge spectrum of biological interest and several critiques summarized the various

therapeutics function of tulsi, and extensive progress achieved on pharmacological potential and medicinal application of tulsi within the ultimate five decade.[6-8] Based on traditional healers make use of tulsi leaves to treat diabetes, the leaf extract of tulsi is proven to have anti-hyperglycemic outcomes by increasing the insulin secretion from isolated islets, perfused pancreas and clonal pancreatic β–cells.[9] According to scientific reports indicated tulsi leaves has hypoglycaemic influence to regulate the crucial biochemical parameters and had beneficial outcomes on blood glucose levels and significantly lower HbA1c levels in diabetes patients,[10] and substantial elevation in body weight gain and antidiabetic activity due to the enhancement of glucose metabolism[11-13] in experimentally induced diabetic rats. Oral effective dose of tulsi leaf extract in alloxan-induced diabetic rats normalized the altered levels of blood glucose and serum insulin[13] recommended that it performs a significant role in management of diabetes mellitus.

Preceding experimental studies verified that the chemical nature of LTLP, using Fehling's test, was found to be carbohydrate in nature and acute toxicity study shown no adverse reactions or behavioral changes after every graded dose of LTLP administration endorsed that the oral LD_{50} of dosages of preparations was greater than 1000mg/kg. This study revealed that LTLP as a good natural antioxidant source by way of defensive the liver from CCl_4-induced hepatic injury[14] and appears to be possible unique mechanisms like free radical scavenging as well as immune modulation to bring about the wound healing consequences in rats.[15] Recent study clearly specified that LTLP supplementation in alloxan-induced diabetic rats made over the interest of antioxidant enzymes may be the result of the capability to prevent the inactivation of the antioxidants thereby suggesting that hypoglycaemic result of LTLP may be related and mediated via modulation of cell antioxidant defence device.[16] Therapeutic capacity of all or some of those bioactive compounds may be responsible for hypoglycemic, antidyslipidemic and acknowledged to own remarkable lipid lowering and antioxidant activities.[8] Based on the present obtainable data on the substitute herbal treatment for diabetes, the present experimental studies have, therefore, been supposed to determine antidiabetic, antioxidant and antidyslipidemic roles of LTLP in alloxan advanced hyperglycaemia.

MATERIALS AND METHODS

Preparation of lyophilized tulsi leaf powder (LTLP)

Tulsi leaves were collected from local and was authenticated by department of Botany, Government First Grade College, Hubballi. The voucher specimen no (GFGC/2011/47) was deposited at the herbarium of the Botanical department. An aqueous extract was prepared from tulsi leaves and then lyophilized following extraction procedure published earlier.[14] Briefly, tulsi leaves were ground in the presence of distilled water and then filtered. The filtrate was then centrifuged at 5000 rpm for 10 min at 4°C and supernatant was collected. The filtrate was washed with chloroform in (1:1; v/v) proportion, centrifuged at 3000 rpm for 20 min, to remove fat-soluble ingredients. The aqueous phase (upper phase) was collected, lyophilized, and kept at -20°C until use. Working concentrations of LTLP (50 and 100mg/ ml) were freshly prepared before use.

Animals

Wistar albino rats weighing 200-220g were obtained from the rat colony maintained in the department and were acclimatized for 10 days under standard housing conditions (26°±2°C; 45-55% RH with 12:12 h light/dark cycle). The animals were maintained on a standard diet and water was given ad libitum and habituated to laboratory conditions for 48 h prior to the experimental protocol to minimize any non-specific stress. The animals were maintained under standard conditions in the animal house approved by Committee for the Control and Supervision on Experiments on Animals (CCSEA) and necessary approval from the Institutional Animal Ethics Committee (Ref:IAEC/Ethics/558/2017) was obtained before undertaking animal experimentation.

Experimental protocol

Induction of diabetes in experimental animal

Diabetes was induced in albino rats by a single intraperitoneal (I.P.) injection of freshly prepared solution of alloxan monohydrate (120 mg/kg) in normal saline. Before alloxan administration, the rats were subjected to sixteen hours of fasting. Forty-eight hours after induction, fasting blood glucose level was assessed using one touch Accuchek active Glucometer (Roche, USA) and rats with fasting blood glucose higher than 200 mg/dL were selected for the antidiabetic study.

Experimental design

In this study, a total of 50 rats were randomly allotted to five groups of 10 animals each. Group I animals were not diabetic and received vehicle + normal saline and served as control, Group II animals were diabetic rats and did not receive any treatment, Group III comprised diabetic rats that received glibenclamide at 2.5 mg/kg and Groups IV and V received the graded doses of LTLP (50 and 100mg/ kg, BW/ day), respectively. All treatments were done daily via the oral route and lasted for 28 days. Blood glucose level and weight of rats were measured on day 28 post-treatment. Blood samples were collected and centrifuged at 3000 rpm for 15 min to obtain the serum and plasma, respectively. Serum was harvested into sample bottles and stored at -20°C until the time of study for lipid profile, and oxidative stress parameters. The rats were anesthetized using IP ketamine (75 mg/kg) and xylazine (10 mg/kg), their liver and pancreas were excised. Tissues were homogenized in 10 volume of 100 mM KH_2PO_4 buffer containing 1 mM EDTA (pH 7.4) and centrifuged at 12,000 g for 30 min at 4°C. The supernatant was collected and used for enzymatic studies.

Measurement of fasting blood glucose and body weight

On day 28 post-treatment, fasting blood glucose was determined using one touch Accuchek active Glucometer (Roche, USA) and body weight of animals was also determined using a weighing balance.

Serum biochemical analyses

Serum glucose was measured by the o-toluidine method.**[17]** The levels of serum insulin were determined using an ELISA kit specific for rat insulin (Invitrogen Insulin Rat ELISA Kit) according to the manufacturer's instructions. The level of insulin in serum was expressed in µIU/ml.[18] Urea estimation was carried out by the method of Varley;[19] serum creatinine was estimated by the method of Owen et al.[20] The glycated haemoglobin was estimated according to the method of Nayak and Pattabiraman.[21]

Serum protein[22] and serum albumins were determined by quantitative colorimetrically method by using bromocresol green. The total protein minus the albumin gives the globulin, serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT) aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured spectrophotometrically by utilizing the method of Reitman and Frankel.[23] Serum alkaline phosphatase (ALP) activity by the method of Kind and King.[24]

Estimation of lipid profiles

Aliquots of each blood sample were placed in plain vials and centrifuged to get serum using the commercial kit (Excel Diagnostics Pvt. Ltd.). The sera were then used to assess lipid profiles including total cholesterol,[25] total triglycerides,[26] low density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C),[27] high density lipoprotein cholesterol (HDL-C)[28] and phospholipids[29] were analysed according to the manufacturer's recommended standard working procedures.

Assessment of tissue oxidative stress markers

The following were determined in the post-mitochondria fractions of the liver and pancreas of the rats: total protein (TP),[22] lipid peroxidation (MDA),[30] reduced glutathione (GSH),[31] glutathione-s-transferase (GST),[32] superoxide dismutase activity (SOD),[33] and catalase activity).[34]

Statistical analysis

Data were analyzed using one way analysis of variance (ANOVA) using the Graph Pad Prism software method, followed by either Dunnet test or Turkey's multiple comparison tests by comparing all treated groups against controls. Values represented are mean ± SEM (n=10). $P \leq 0.05$ is considered to indicate a significant difference between experimental and controls.

RESULTS

Effect of LTLP on body weight and fasting blood glucose

Alloxan monohydrate induces hyperglycaemia in rats. The LTLP caused a significant ($P \leq 0.05$) decrease in the fasting blood glucose of treated rats (Fig.1) when compared with the diabetic control and the glibenclamide-treated group. This decrease was comparable with that of the normal control. Also, graded doses of LTLP-treated groups showed statistically significant ($P \leq 0.05$) increases in weight gain at the end of 28 days when compared with diabetic control (Fig.2A and B).

Effect of LTLP on blood glucose level and other parameters

There was a significant ($P \leq 0.05$) decreased in serum insulin level (Fig.3A) and elevation ($P \leq 0.05$) in the levels of blood glucose (Fig.3B), urea (Fig.3C), creatinine (Fig.3D) and HbA1c (Fig.3E) were observed in alloxan induced diabetic rats when compared to control rats. Administration of LTLP (100mg/kg) and glibenclamide to diabetic rats for 28 days tended to bring these parameters significantly ($P \leq 0.05$) towards the normal.

Effect of LTLP on biochemical parameters

Significant ($P \leq 0.05$) reductions in serum protein (Fig.4A), albumin (Fig.3B) and globulin (Fig.4C) were observed in alloxan induced diabetic rats when compared to control rats. On administration of LTLP to the diabetic rats, these parameters levels were found to be restored in normal. Also, the SGPT (Fig.4D), SGOT (Fig.4E) and ALP (Fig.4F), ALT (Fig.4G) and AST (Fig.4H) levels were elevated significantly ($P \leq 0.05$) in alloxan induced diabetic rats compared to control rats. Both the doses of LTLP and glibenclamide treatment significantly reduced above parameters compared to diabetic control rats.

Effect of LTLP on lipid profiles

Alloxan induced diabetic animal displayed a significant elevation ($P \leq 0.05$) in the serum levels of lipid profiles such as total cholesterol (Fig.5A), total triglycerides (Fig.5B), LDL-C (Fig.5D), VLDL-C (Fig.5E) phospholipid (Fig.5E) except HDL-C (Fig.5C) when compared with normal rats. However, treatment of diabetic groups with glibenclamide and LTLP (50mg/kg and 100 mg/kg) respectively, significantly ($P \leq 0.05$) reversed in these parameter levels towards normal.

Tissue oxidative stress markers

Study revealed that LTLP effectively reversed ($p < 0.05$) increased the levels of total protein and malondialdehyde caused by alloxan in the liver and pancreas of the rats (Fig .6A and B). However, treatment with graded doses of LTLP (50 mg/kg and 100 mg/kg) led to a significant recovering ($p < 0.05$) reduced glutathione concentration in the liver and pancreas of hyperglycaemic rats (Fig. C and D). Further, a significant ($P \leq 0.05$) reduction in the activities of SOD (Fig.6E) and GST (Fig.6F) were observed in the alloxan diabetic rats; meanwhile, LTLP reverses the effect by increasing the activity in a manner comparable to glibenclamide, though treatment with LTLP and glibenclamide does not normalize GST activity in alloxan diabetic rats (Fig.6F).

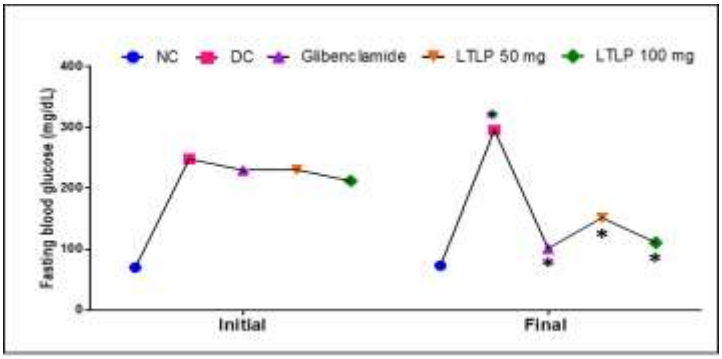

Figure 1: Effect of LTLP on fasting blood glucose of alloxan-induced diabetic rats. Values are the mean ± SEM, N=10 for each group. * P ≤ 0.05 compared with the experimental and control rats.

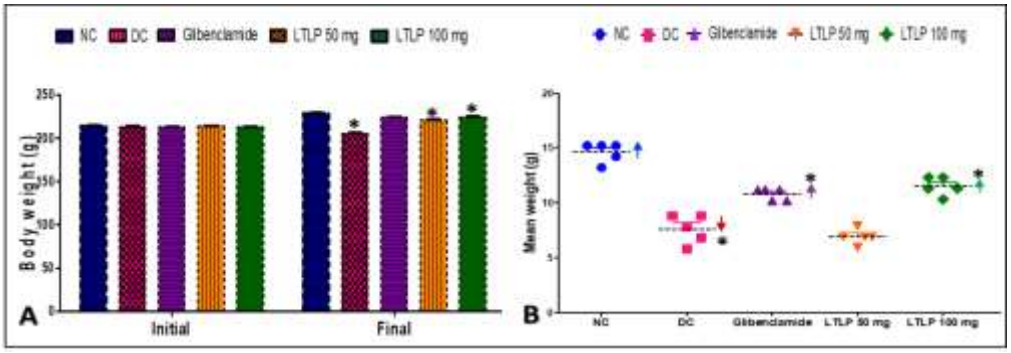

Figure 2: Effect of LTLP on body weight (A) and mean weight (B) gain (↑)/ loss (↓) of alloxan-induced diabetic rats. Values are the mean ± SEM, N=10 for each group. * P ≤ 0.05 compared with the experimental and control rats.

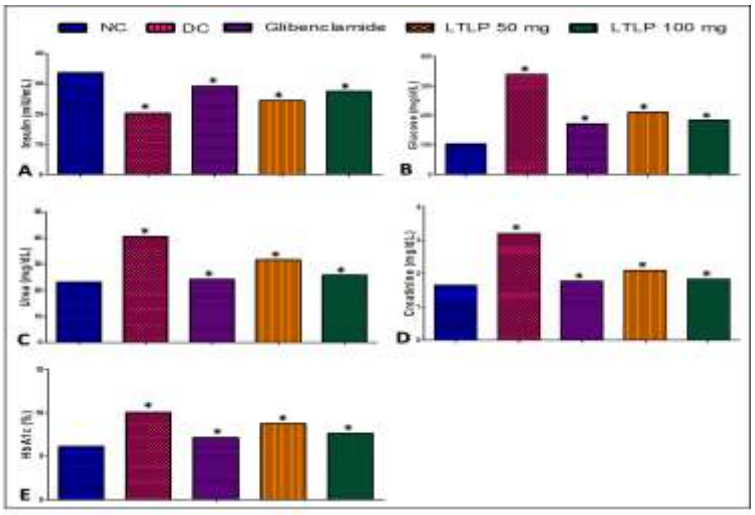

Figure 3: Effect of LTLP on serum insulin (A), glucose (B), urea (C), creatinine (D) and HbA1c (E) levels of alloxan-induced diabetic rats. Values are the mean ± SEM, N=10 for each group. * P ≤ 0.05 compared with the experimental and control rats.

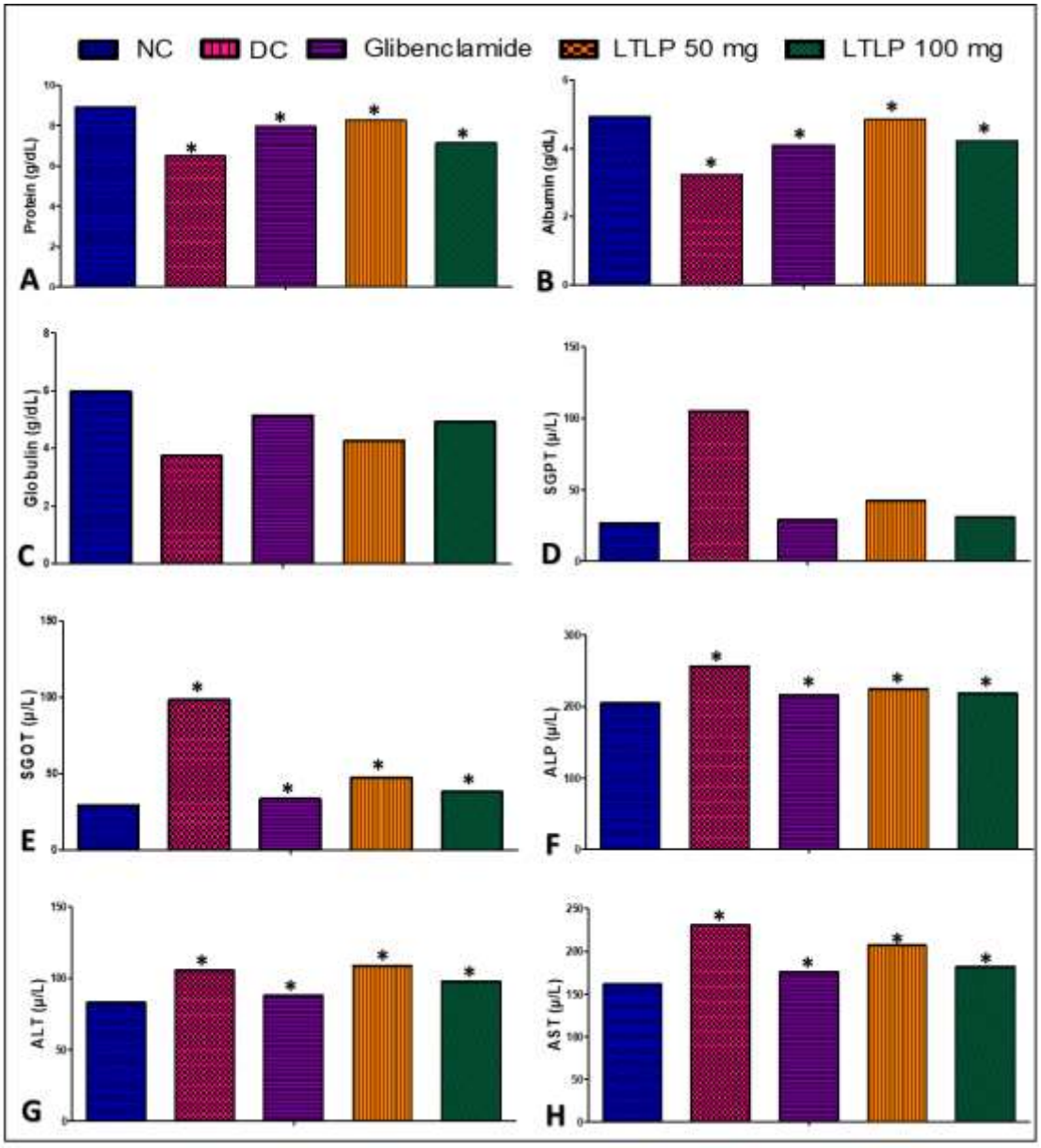

Figure 4: Effect of LTLP on serum protein (A), albumin (B), globulin (C), SGPT (D), SGOT (E), ALP (F), ALT (G) and AST (H) levels of alloxan-induced diabetic rats. Values are the mean ± SEM, N=10 for each group. * P ≤ 0.05 compared with the experimental and control rats.

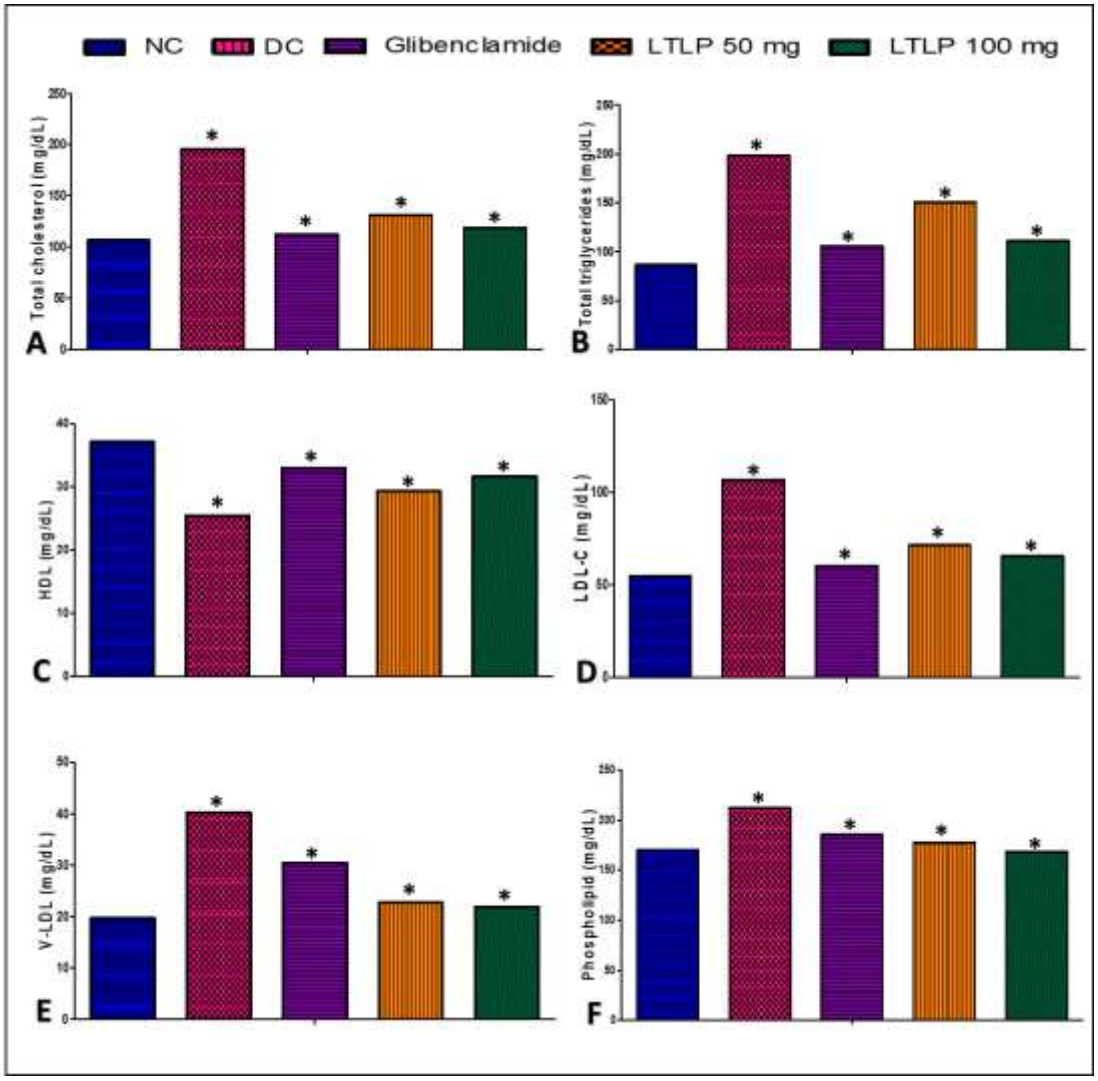

Figure 5: Effect of LTLP on serum lipid profile of total cholesterol (A), total triglycerides (B), high density lipoprotein cholesterol (HDL, C), low density lipoprotein cholesterol (LDL-C, D), very low-density lipoprotein cholesterol (VLDL- C, E) and phospholipids (F) of alloxan-induced diabetic rats. Values are the mean ± SEM, N=10 for each group. * P ≤ 0.05 compared with the experimental and control rats.

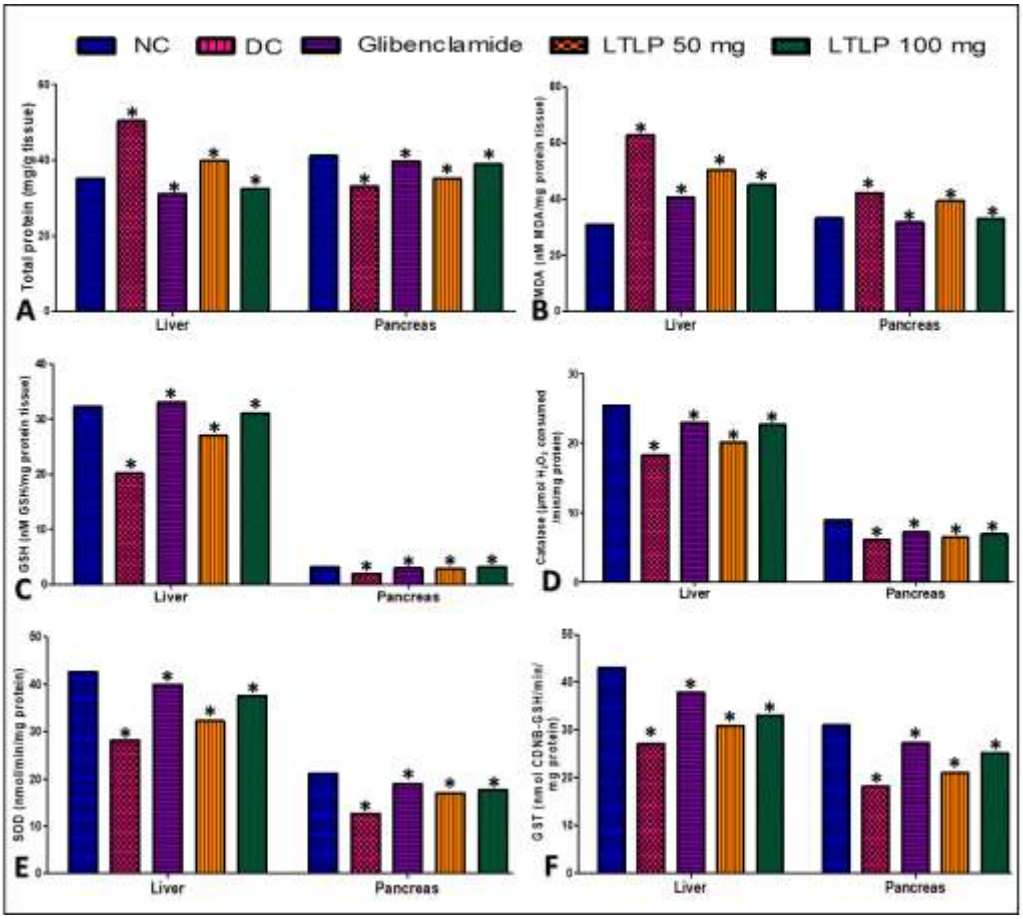

Figure 6: Effect of LTLP on tissue antioxidant marker of total protein (A), malondialdehyde (MDA, B) and reduced glutathione (GSH, C) and antioxidant enzyme activities of catalase (D), SOD (E)and GST(F) of alloxan-induced diabetic rats. Values are the mean ± SEM, N=10 for each group. * P ≤ 0.05 compared with the experimental and control rats.

DISCUSSION

Studies confirmed that Ocimum species are without any toxic consequences and feature the potential to normalize blood glucose levels and lipid profile inhibit lipase activity, ameliorate chemical-induced hepatotoxicity, and are suggested to be beneficial in handling diabetic complications.[35-36] However, further mechanism of action and improvement of standardized formulations are certainly required to expand these species as drugs for managing metabolic disorders and related comorbidities.[8] This study evaluated the antihyperglycemic, antidyslipidemic, and antioxidant potentials of LTLP in the alloxan diabetic model. Alloxan is one of the common diabetogenic agents often used to assess the antidiabetic potential and has been elucidated that insulin deficiency which caused a drastic elevation in glucose levels because of excessive production of endogenous changes in body weight.[37] In the present

study, induction of diabetes by alloxan produced increase in blood glucose level, decrease in body weight and polyuria. The reduction in blood glucose and body weight gain impact observed in diabetic rats dealt with LTLP can be attributed to the enhancement of glucose metabolism and an indication of its hypoglycaemic properties.[12] In assessment, non-toxic nature as evident, oral administration of graded doses of LTLP confirmed significant increase in body weight gain from day 28 suggesting that LTLP substantially improved their general health status and metabolic mechanisms through effective glycaemic control or a reversal of gluconeogenesis.[11, 13]

Administration of LTLP significantly decreased blood glucose level in diabetic rats which represents reversal of insulin resistance or increasing insulin secretion possibly by regeneration of damaged pancreatic β-cells in alloxan-induced diabetic rats.[16] However, LTLP administration decreased the levels blood glucose to normal range probably by improving insulin sensitivity in agreement with above reference studies shown that increase in blood glucose level and the same has been brought back to control level in diabetic induced rats suggesting that LTLP has antidiabetic potential. Nevertheless, the actual mechanism of action that brings up on the action of hypoglycaemia is not understood but these actions are supposed to be exhibited due to cumulative impact of various phytoconstituents present in the tulsi leaves extracts as reported in review of literature.[6-8] In diabetes, elevated levels of serum urea and creatinine are observed which may be due to renal damage caused by abnormal glucose regulation or elevated glucose and glycosylated protein tissue levels.[38] The significant increase in serum urea and creatinine levels were observed in diabetic rats compared to normal control rats which indicate impaired renal function in diabetic rats. The treatment with LTLP lowered the above parameters significantly compared to diabetic control rats and it showed protective effect of LTLP on the kidneys. HbA1c is taken into consideration as a diagnostic marker and helps to know about degree of protein glycation, long term blood sugar level and correlation of diabetes associated complications.[39] In present study, alloxan induced diabetic rats confirmed a significant increase in HbA1c level compared with normal rats meanwhile, LTLP dealt with rats showed a noteworthy decrease in the content of glycosylated haemoglobin that could be due to an improvement in glycemic status.

In diabetic situation, incidence of reduction in protein and albumin may be due to proteinuria, albuminuria or increased protein catabolism, which are clinical markers in diabetic

nephropathy.[40] The protein and albumin level were decreased after the induction of diabetes and treatment of LTLP improved both levels considerably in diabetic rats towards normal level. This action possibly is through increase in the insulin mediated amino acid uptake, enhancement of protein synthesis and/ or inhibition of protein degradation.[41] Also, increased serum enzymes levels were reported in diabetes, and it may be due to liver dysfunction.[42] In this study, increased level of SGPT, SGOT and ALP, ALT and AST levels were observed in alloxan-induced diabetic rats which may have occurred by leakage of enzymes from the liver cytosol into the blood stream; it represents the toxicity of alloxan on liver. Diabetic rats treated with LTLP considerably decreased both enzyme levels which represents its defensive action of in diabetic condition.

Hyperglycaemia is followed with dyslipidemia which is characterized through increased levels of TGs, TCs, LDL and decreased level of HDL is an important biochemical abnormality of diabetes mellitus.[43] The abnormal excessive concentrations of serum lipids in diabetic animals are especially because of an increased mobilization of free fatty acids from peripheral fat depots.[44] In the present study, notably elevated levels of serum TC, TG, V-LDL and LDL as well as marked reduction in serum HDL level following induction of diabetes with alloxan confirming that the dyslipidaemia associated with diabetes mellitus. The altered serum lipid profile was, however, reversed significantly following treatment with the LTLP further corroborate the ability to attenuate deranged lipid metabolism normally associated with diabetes mellitus. The above action could be useful in preventing diabetic complications such as coronary heart diseases and atherosclerosis in diabetic situation. This agrees with the studies performed on alloxan or streptozotocin-induced diabetes rats following treatment with aqueous or other extracts of tulsi leaves.[12, 45-46]

Administration of LTLP to alloxan diabetic rats moderated tissue oxidative stress markers in the designated tissues. Oxidative stress in the designated tissues, as seen in alloxan diabetes rats, occurs while the endogenous antioxidant system (superoxide dismutase, catalase, and glutathione) which effectively break down hydrogen peroxide and hydroperoxides to harmless molecules are compromised because of overproduction of reactive species.[47] It has been stated in numerous studies that alloxan caused an increase in markers of oxidative stress in the liver, kidney, and pancreas of rats.[48-49] Also, diabetes has been reported to have a detrimental effect on the activities of endogenous antioxidant enzymes, and concentrations of antioxidant substances;[50] those effects are often attributed to uprising reactive oxygen

species that in addition cause organ impairment. In fact, one of the mechanisms via which alloxan pathophysiology occurs in the pancreatic β-cell is selective inhibition of thiol groups which serve as a glucose sensor for triggering glucose-induced insulin secretion.[51]

Diabetes represents a state of excessive oxidative stress because of hyperglycaemia induced ROS generation, which in turn causes injury to the cells resulting secondary complications in diabetes mellitus.[52] Free radicals or nonradical species react with lipids producing peroxidation, leading to the release of products such as MDA, H_2O_2 and OH.[53] In this study, there could be oxidative damage in the tissues of diabetic rats evidenced by the significant decrease in activities of the antioxidant enzyme systems (SOD and GST) in the diabetic control rats. Reduction in SOD activity could result from inactivation by H_2O_2 or via glycation of the enzyme, which has been reported to occur in diabetes[54] because of depletion owing to excessive use of these enzymes to mop up the hyperglycaemia-induced free radical generation. Also, these enzymes are targets of glycation that may result in inhibition of their enzymatic activity.[55] The reduced activity of GST observed in diabetic state may be because of the inactivation caused by ROS.[56] Studies revealed that *Ocimum sanctum* species capable of neutralizing free radicals effectively decrease Ca^{2+} levels attributed to its antioxidant consequences and decrease the severity of diabetic complications.[57] In this study, the increased the level of reduced glutathione, activities of SOD, catalase and GST and reduced the tissue concentration of malondialdehyde representing that LTLP may be a good source of exogenous antioxidants for rat tissues. In addition, LTLP may help in the process required in the reduction and/or scavenging of free radicals. In this study, the content of these levels and activities of the antioxidant enzyme systems, however, reversed considerably in LTLP treated rats when compared with the diabetic control further substantiate the ability to attenuate disturbed lipid metabolism normally associated with diabetes mellitus. The result of this study supports the other diverse set of studies on tulsi extracts aimed to assess the antioxidant effect and/or to test boost the body's natural defenses.[12-13, 36]

CONCLUSION

In this study, the administration of LTLP to alloxan induced hyperglycemic rats demonstrated prominent reduction in blood sugar level, normalization of serum biochemical profile including lipid content, as compared to alloxan control rats. Such responses might be because of the presence of various phytoconstituents as aqueous extracts of tulsi leaves (dried or fresh) identified chemical compositions containing indicated ursolic acid flavonoids such as

apigenin, polyphenols, anthocyanins and luteolin, eugenol, thymol, sesquiterpenes and monoterpenes, glycosides, steroids, sterols. It is suggested that the presence of various phytoconstituents may be the cause of these reactions. The therapeutic potential of all or some of these bioactive compounds may be due to their hypoglycemic, antidyslipidemic, and antioxidant activities as well as their well-known lipid-lowering and antioxidant properties. The beneficial result of effective antioxidative and antidiabetic activity was comparable with standard drug glibenclamide in our study indicating LTLP is a better drug as a natural product to regress dyslipidemia and oxidative stress in diabetes. The LTLP exhibited worthy antihyperglycemic, antidyslipidemic, and antioxidant efficacies when administered in alloxan diabetic rats and these consequences may be attributed to either single or synergistic action of the above phytoconstituents and could be an excellent source of antidiabetic agents as it controls the hyperglycemic index and other associated biochemical indices. Before stabilizing LTLP as a therapeutically effective hypoglycemic agent, further studies are needed to isolate the active compound(s) from LTLP which are responsible for generating antihyperglycaemic and antioxidant activities and their exact mechanism(s) of action.

ACKNOWLEDGEMENTS

The author is thankful to research facilities from Department of Zoology, Government First Grade College, Hubballi, Karnataka, India.

REFERENCES

1. Zimmet P, Alberti K.G, Magliano D.J, Bennett P.H. Diabetes mellitus statistics on prevalence and mortality: facts and fallacies. Nat. Rev. Endocrinol, 2016; 12: 616–622.
2. World Health Organisation. WHO Study Group Report on Prevention of Diabetes Mellitus. WHO, Geneva, 1994; pp: 1- 92.
3. Choudhury H, Pandey M, Hua C. K, Mun C. S, Jing, J.K, Kong L, Ern L.Y, Ashraf N. A, et al. An update on natural compounds in the remedy of diabetes mellitus: A systematic review. J. Tradit. Compl. Med, 2018; 8: 361–376.
4. Verma S, Gupta M, Popli H, Aggarwal G. Diabetes mellitus treatment using herbal drugs. Int. J. Phytomed, 2018; 10(1): 1-10
5. Sharma N, Bora K. S. Role of medicinal plants in the management of diabetes mellitus: A Review. J. Pharm. Res. Int, 2021; 33: 2196–2207.
6. Walia S, Dua J. S, Prasad D.N. Herbal drugs with anti-diabetic potential. J. Drug Del. Therapeut, 2021; 11(6): 248-256.

7. Singh D, Chaudhuri P. K. A review on phytochemical and pharmacological properties of holy basil (*Ocimum sanctum* L.). Ind. Crops Prod, 2018; 118: 367–382.
8. Garg N, Singh T. G, khan H, Arora S, Kaur A, Mannan A. Mechanistic interventions of selected Ocimum Species in management of diabetes, obesity and liver disorders: Transformative developments from preclinical to clinical approaches. Biointer. Res Appl. Chem, 2022; 12(1): 1304 -1323.
9. Hannan J. M, Marenah L, Ali L, Rokeya B, Flatt P.R, Abdel-Wahab Y. H. *Ocimum sanctum* leaf extracts stimulate insulin secretion from perfused pancreas, isolated islets and clonal pancreatic beta-cells. J. Endocrinol, 2006; 189: 127-136.
10. Somasundaram G, Manimekalai K, Salwe, K J, Pandiamunian J. Evaluation of the antidiabetic effect of *Ocimum sanctum* in type 2 diabetic patients. Int. J. Life Sci. Pharma. Res, 2012; 5: 75-81.
11. Shittu STT, Oyeyemi W. A, Lasisi T. J, Shittu S. A, Lawal T. T, Olujobi S. T. Aqueous leaf extract of *Ocimum gratissimum* improves hematological parameters in alloxan-induced diabetic rats via its antioxidant properties. Int. J. App. Basic. Med. Res, 2016; 6: 96-100.
12. Ramzan T, Aslam B, Muhammad F, Faisal M. N, Hussain A. Influence of *Ocimum sanctum* (L.) extract on the activity of gliclazide in alloxan-induced diabetes in rats. Rev. Chim, 2020; 71(10): 101-110.
13. Jayant S. K, Sharma S, Srivastava N. Effect of *Ocimum sanctum* and *Allium sativum* on lipid peroxidation and antioxidant enzymes in alloxan induced diabetic rats. Biosc. Biotech. Res. Comm, 2020; 13(2): 882-888.
14. Aladakatti R. H, Samuel R. J, Kadiyala, A. Hepatoprotective evaluation of aqueous Tulsi leaf powder (*Ocimum sanctum*) against carbon tetrachloride induced liver toxicity in rats. Int. J. Adv. Sci. Eng. Technol, 2020; 8(3): 9-15.
15. Jadaramkunti U. C, Ghodesawar M.G, Aladakatti R.H. Wound healing potentials of *Ocimum sanctum* leaves extracts in Wistar albino rats. Int. J. Sci. Adv. Res. Technol, 2020; 6(12): 217-224.
16. Jadaramkunti U. C, Ghodesawar G, Aladakatti R.H. Beneficial effects of lyophilized tulsi leaf powder (LTLP) on antioxidant defense system in alloxan-induced diabetic male albino rats. Int. J.Pharmacog. Phytochem. Res, 2023; 15(2): 50-71.
17. Sasaki T, Masty S, Sonae A. Effect of acetic acid concentration on the colour reaction in the o-toluidine boric acid method for blood glucose estimation. Rinsho Kagaku, 1972; 1: 346-353.

18. Anderson L, Dinesen B, Jørgonsen P. N, Poulsen F, Røder M. E. Enzyme immune assay for intact human insulin in serum or plasma. Clin Chem, 1993; 39: 578-582.
19. Varley H. Practical clinical biochemistry. Chennai: Arnold Heinemann Publication Pvt. Ltd, 1976; p: 452.
20. Owen J. A, Iggo J. B, Scangrett F. J, Steward I. P. The determination of creatinine in plasma or serum, and in urine - a critical examination. Biochem J., 1954; 58: 426-437.
21. Nayak S. S and Pattabiraman T.N. A new colorimetric method for the estimation of glycosylated hemoglobin. Clinica. Chimica. Acta, 1981; 109(3): 267–274.
22. Lowry O. H, Rosenbrough N. J, Farr A. L, Randall R. J, Protein measurement with the Folin phenol reagent. J. Biol. Chem, 1951; 193: 265–275.
23. Reitman S, Frankel S. A. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol, 1957; 28: 56-63.
24. Kind P.R.N, King E. J. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine". J. Clin. Pathol, 1954; 7: 322-326.
25. Parekh A. C, Jung D.H. Cholesterol determination with ferric acetate, uranium acetate and sulphuric acid, ferrous sulphate reagent. Anal. Chem, 1970; 42: 1423-1427.
26. Rice E. W. Triglycerides in serum. In: Roderick MP, editor. Standard methods of clinical chemistry. 9th ed. New York: Academic Press, 1970; p: 215-222.
27. Friedwald W. T, Levy R. I, Fredrickson D. S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultra centrifuge. Clin. Chem, 1972; 18: 499-502.
28. Warnick G. R, Nguyan T, Albers A. A. Comparison of improved precipitation methods for quantification of high-density lipoprotein cholesterol. Clin. Chem, 1985; 31: 217-222.
29. Takayama M, Itoh S, Nagasaki T, Tanimizu I. A new enzymatic method for determination of serum phospholipids. Clin. Chem. Acta, 1977; 79: 93-98.
30. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by the thiobarbituric acid reaction. Anal. Biochem, 1979; 95(2): 351–358.
31. Ellman G. L. Tissue sulfhydryl groups. Arch. Biochem. Biophys, 1959; 82: 70–77.
32. Habig W. H, Pabst M. J, Jokoby W. B, (1974). Glutathione transferase: a first enzymatic step in mercapturic acid III formation. J. Biol. Chem, 1959; 249: 7130-7139.
33. Beyer WE, Fridovich I. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal Biochem, 1987; 161: 559–566.
34. Beers R. F, Sizer J. W. Spectrophotometric method for measuring the breakdown of hydrogen peroxide catalase. J. Biol. Chem, 1952; 195: 133-140.

35. Singh P, Jayaramaiah R. H, Agawane S. B, Vannuruswamy G, Korwar A. M, Anand A. et al. Potential dual role of eugenol in inhibiting advanced glycation end products in diabetes: Proteomic and mechanistic insights. Sci. Rep, 2016; 6: 18798.
36. Ezeani C, Ezenyi I, Okoye T, Okoli C. *Ocimum basilicum* extract exhibits antidiabetic effects via inhibition of hepatic glucose mobilization and carbohydrate metabolizing enzymes J. Intercul. Ethnopharmacol, 2017; 6(1): 22-28.
37. Ramachandran A and Snehalatha C. Diabetes prevention programs. Med Clinics, 2011; 95(2): 353–372.
38. Biri, SRK, Sankeerthi SLV, Sandhya Rani T, Gundu R, Vadlakonda, A. A study on evaluating blood urea and serum creatinine in diabetes mellitus patients International Journal of Clinical Biochemistry and Research, 2021; 8(4): 285–288.
39. Hussain N. Implications of using HBA1C as a diagnostic marker for diabetes. Diabetol Int, 2016 Mar; 7(1): 18–24.
40. Selby N. M, Taal M. W. An updated overview of diabetic nephropathy: Diagnosis, prognosis, treatment goals and latest guidelines. Diabetes Obes Metab, 2020; 22 (Suppl. 1): 3–15.
41. Petersen M. C, Shulman G. I. Mechanisms of insulin action and insulin resistance. Physiol Rev, 2018; 98: 2133-2223.
42. Chilay A, Mehra N, Misra M, Jatale R, Ramchandran S. Liver function test and diabetes mellitus: Correlation from a laboratory perspective. Ind. J. Med. Biochem, 2023; 27(2): 40-44.
43. Schofield J. D, Liu Y, Balakrishna P. R, Malik R. A, Soran H. Diabetes dyslipidemia. Diabet. Ther, 2016; 7(2): 203–219.
44. Azizi R, Goodarzi T. G, Salemi Z. Effect of biochanin A on derum visfatin level of streptozocin-induced diabetic rats. Iran. Red. Crescent. Med. J., 2014; 16(9): e15424.
45. Enyievi P. B, Mgbeje B. IA, Nja G M E, Edu B. C, Ejemot-Nwadiaro R. I. Effect of *Ocimum gratissimum* leaf-extract on hematological indices and lipid profile of streptozotocin-induced diabetic wistar rats. Pak. J. Biol. Sci, 2020; 23(12): 1523-1529.
46. Gupta L, Arora M, Tomar R, Pathak A, Gupta V, Garg A, Sahoo J, Grover P. Synergistic effect of *Ocimum sanctum* and *Andrographis paniculata* against diabetic complications. J. Pharmaceut. Res. Int, 2021; 33(33A): 151-163.
47. Ighodaro O, Akinloye O First line defense antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defense grid. Alex. J. Med, 2018; 54(4): 287–293.

48. Abdullah K. M, Abul Qais F, Hasan H, Naseem I, Anti-diabetic study of vitamin B6 on hyperglycaemia induced protein carbonylation, DNA damage and ROS production in alloxan induced diabetic rats. Toxicol. Res, 2019; 8(4): 568–579.
49. Ulla A, Alam M A, Rahman M, Isha Olive Khan D M, Sikder B, Islam M, Rahman T, et al. Supplementation of Citrus maxima fruits peel powder improves glucose intolerance and prevents oxidative stress in liver of alloxan-induced diabetic rats. Mediterranean Journal of Nutrition and Metabolism, 2019; 12(1): 33–44.
50. Jia G, Whaley-Connell A, Sowers J. R. Diabetic cardiomyopathy: a hyperglycemia-and insulin-resistance-induced heart disease. Diabetol, 2018; 61(1): 21–28.
51. Lenzen S. Chemistry and biology of reactive species with special reference to the antioxidative defence status in pancreatic beta-cells. Biochim. Biophys. Acta. Gen. Sub, 2017; 1861: 1929–1942.
52. Mahmoud M. S. Protective and therapeutic effectiveness of taurine in diabetes mellitus: A rationale for antioxidant supplementation. Diabetes Metab. Syndr. Clin. Res. Rev, 2015; (9): 55–64.
53. Moldovan L, Moldovan N I. Oxygen free radicals and redox biology of organelles. Histochem. Cell. Biol, 2004; 122(4): 395–412.
54. Sozmen E. Y, Sözmen B, Delen Y, Onat T. Catalase/superoxide dismutase (SOD) and catalase/paraoxonase (PON) ratios may implicate poor glycemic control. Arch. Med. Res, 2001; 32(4): 283-287.
55. Jung, H. K. Modification and inactivation of human Cu, Zn-superoxide dismutase by methylglyoxal. Mol. Cells, 2003; 15(2): 194-199.
56. Andallu, B. & Varadacharyulu, N.C. Antioxidant role of mulberry leaves in streptozotocin-diabetic rats. Clinica. Chimica. Acta, 2003; 338: 3–10.
57. Wang Z. Li. H; Wang J, Chen Z, Chen G, Wen D, Chan A. Gu. Z. Transdermal colorimetric patch for hyperglycemia sensing in diabetic mice. Biomater, 2020; 237, 119782.