Available online on www.ijppr.com

International Journal of Pharmacognosy and Phytochemical Research 2023; 15(2); 50-71

eISSN: 0975-4873, pISSN: 2961-6069

Research Article

Beneficial Effects of Lyophilized Tulsi Leaf Powder (LTLP) on Antioxidant Defense System in Alloxan-Induced Diabetic Male Albino Rats

Umadevi C. Jadaramkunti[1], MukhtarAhmed G. Ghodesawar[2], Ravindranath H. Aladakatti[3]

[1]Department of Zoology, Government First Grade College, Hubballi-580032, Karnataka
[2]Department of Zoology, Anjuman Arts, Sciences and Commerce College, Vijayapura-586101, Karnataka
[3]Central Animal Facility, Indian Institute of Science, Bengaluru-560012, Karnataka

Received: 30th March 23; Revised: 20th April 23; Accepted:24th May 23; Available Online:25th June 23

ABSTRACT

This study was aimed to evaluate the antidiabetic and antioxidant potentials of dose dependent carbohydrate nature of lyophilized tulsi leaf powder (LTLP) and in comparison, with standard drug glibenclamide (2.5 mg/kg BW/day) on alloxan monohydrate induced diabetic male albino rats. Diabetes was induced in male Wistar rats by the administration of alloxan monohydrate at 100 mg/kg of body weight. After 48 h, rats with fasting blood glucose levels of 200 mg/dL and above were considered 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 (treated with Glibenclamide) and Groups of IV and V rats diabetic treated (supplementation with graded doses of LTLP, 50 and 100mg/ kg, BW/ day, respectively). Blood samples were collected at days 14 and 28 post-treatment for haematological and serum insulin parameters. At the end of the experimental period (28 days), the liver and skeletal muscle tissues were collected for the assessment of activities of carbohydrate metabolic enzymes and glycogen content. The intestine was collected, and intestinal homogenate was prepared to analyse the intestinal enzymatic and non-enzymatic antioxidants activities. To assess the protective potentials of LTLP, the histopathological studies on liver and pancreas 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, hematological and serum chemistry profiles, glycogen content and carbohydrate metabolic enzyme activities in liver and skeletal muscle towards the control levels. Treatment with LTLP produced significant ($P \leq 0.05$) recovery in the levels of enzymic and non-enzymic antioxidant markers of intestinal homogenate when compared with diabetic control and Glibenclamide drug. Histopathological investigations revealed that microscopic architecture of liver and pancreatic improvised in LTLP treated diabetic rats with respect to diabetic control. These findings showed clearly indicates that carbohydrate nature of LTLP may be utilized as important source of natural antioxidants with antidiabetic and antihyperlipidemic potential and can be used as an herbal medicine for diabetes.

Keywords: Antidiabetic, Antioxidant, LTLP, Alloxan, Glibenclamide, Haemostatic indices, Biochemical indices

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disease characterized by alterations in glucose, lipid, with variation of protein, carbohydrates, and fat metabolism, resulting in hyperglycemia and inadequate insulin production, action, or both[1]. Effective treatment of DM is an important task for the medical community because most of the currently available drugs have various adverse effects. This became more apparent following World Health Organization recommendations regarding the need to develop and evaluate better pharmacological agents for improving insulin secretion, enhancing insulin sensitivity, preventing beta (β) cells destruction, promoting β-cells regeneration or repair interrupting pathways leading to various complications of diabetes[2]. Hence, regardless of the presence of these hypoglycemic pharmacological drugs, supplementation of herbal based drugs to treat

**Author for Correspondence: drumasuresh@gmail.com*

diabetes is now a promising and novel treatment strategy due to its safe and non-toxic nature[3]. Many treatments that include the use of medicinal plants 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 virtue of their active phytochemical constituents[4]. The benefits of a natural medicinal product may be due to a single phytocompound or, more preferably, a synergistic effect of multiple phytochemicals. Although synthetic oral hypoglycemic agents/insulin are a popular diabetes therapy and are effective in controlling hyperglycemia, they have significant side effects and do not significantly modify the course of diabetic complications[5].

Ocimum sanctum L. (Tulsi, a Lamiaceae family) is one of the most versatile medicinal plants having a wide spectrum of biological activity and several reviews summarized the various therapeutics role of tulsi and considerable progress achieved on pharmacological potential and medicinal application of tulsi in the last five decades[5-7]. Based on traditional healers make use of tulsi leaves to treat diabetes, the leaf extracts of tulsi have been shown to have anti-hyperglycaemic effects by increasing the insulin secretion from isolated islets, perfused pancreas and clonal pancreatic β–cells[8-9]. According to scientific reports indicated tulsi leaves has hypoglycaemic influence to regulate the essential biochemical parameters and had beneficial effects on blood glucose levels and significantly lower HbA1c levels in diabetes patients[10-11], and significant elevation in body weight gain and antidiabetic activity due to the enhancement of glucose metabolism[12-14] 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[14-15]suggested that it plays a significant role in management of DM. Preceding experimental studies demonstrated that LTLP revealed as a good natural antioxidant source by means of protecting the liver from CCl_4-induced hepatic damage[16] and appears to be possible different mechanisms like free radical scavenging as well as immune modulation to bring about the wound healing effects in rats[17]. It is suggested that such responses could be due to 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[6]. Therapeutic potential of all or some of these bioactive compounds may be responsible for hypoglycemic, antidyslipidemic and known to possess potent lipid lowering and antioxidant activities[7]. Despite the introduction of hypoglycaemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical problem. Based on the current available data on the alternative herbal treatment for diabetes, the present experimental studies have, therefore, been intended to determine antidiabetic and antioxidant activities of LTLP supplementation in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Preparation of lyophilized tulsi leaf powder (LTLP)

Plant material 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. Tulsi leaves were collected and then lyophilized following extraction procedure published earlier[18]. 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, 100, 250, 500 and 1000mg /ml) were freshly prepared before use.

Chemical nature ingredients of LTLP

The chemical nature of ingredients of LTLP was studied for the presence of lipid, protein, and carbohydrate. Preparation of Fehling's solution for the carbohydrate estimation was performed as described elsewhere[18-19]. Fehling's solution was prepared by mixing copper sulfate (34.65g in 500 ml) solution and alkaline tartrate (125g of potassium hydroxide and 173g Rochelle salt in 500 ml) solution. To prevent deterioration, these solutions were preserved separately in a rubber-stoppered bottle and mixed in equal volumes when needed for use. For carbohydrate estimation, to the warm Fehling's solution, LTLP was added, and the mixture was heated after each addition.

The production of yellow or brownish-red cuprous oxide indicates that reduction has taken place. The differences in colour of the cuprous oxide precipitates under different conditions are apparently due to difference in the size of the particles, the more finely divided precipitates having a yellow colour, while the coarser ones are red[20].

Animals

Wistar male 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 Purpose of Control and Supervision on Experiments on Animals (CPCSEA) and necessary approval from the Institutional Animal Ethics Committee (IAEC) was obtained before undertaking animal experimentation.

Acute Toxicity

Studies Acute oral toxicity study was performed as per OECD-404 guidelines[21]. 10 male albino rats/group were used for the study. Group 1 was control group and other groups were that of LTLP at different doses (250, 500 and 1000mg/kg BW, respectively). Single dose of the LTLP was separately administrated orally to each animal. The animals were fasted overnight prior dosing. Food was withheld for a further 3 to 4 hours after dosing. Observations were done on mortality and behavioral changes of the rats following treatment for 24 hours. The acute toxicity LD_{50} was calculated at the statistic mean of the dose that resulted in 100% lethality and that cause no lethality at all.

Experimental protocol

Antidiabetic analysis

Diabetes was induced in male albino rats by a single intraperitoneal (I.P.) injection of freshly prepared solution of alloxan monohydrate (120 mg/kg) in normal saline. 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. 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 weekly, and blood was collected for haematology and serum for biochemical assays on days 14 and 28 post-treatment.

Measurement of fasting blood glucose and body weight

Fasting blood glucose was determined at intervals of 7 days during the 28-day experimental period using one touch Accuchek active Glucometer (Roche, USA). Body weight of animals was also determined at intervals of 7 days using a weighing balance.

Hematological analysis and Serum preparation

Blood was collected for haematological evaluation on days 14 and 28 post-treatment. Under mild ether anesthesia, from each rat, 5 ml of fresh whole blood was collected through the retro-orbital venous plexus. Of the 5 ml of blood, 2 ml was used for haematological analysis. Blood was collected immediately into tubes containing EDTA for analysis of hematological parameters viz. hemoglobin concentration (Hb), total red blood cells (RBC), packed cell volume, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total white blood cells (WBC), neutrophils, lymphocytes, eosinophils, monocytes, basophiles, and total platelet count using automated hematology analyzer (Sysmex KX-21, Japan). The remaining 3 ml of blood was also collected into sterile tubes and left for about 30 min to clot. The clotted blood was thereafter centrifuged at 4000 rpm for 10 min. Serum was harvested into sample bottles and stored at -20°C until the time of analysis for insulin assay and protein concentrations in the serum.

Preparation of tissues homogenate

After collection of blood from each of the animals, all the rats were sacrificed under light ether anesthesia after the 28^{th}day of the treatment and a portion of the relevant organs like liver, skeletal muscle and intestine were dissected out and stored at -20^0C until all the organs have been collected from all animals. Liver and skeletal muscle were used for biochemical analysis of glycogen content and the activities of carbohydrate metabolic enzymes in the respective tissue sample. A portion of the intestine was harvested on ice, rinsed, and homogenised in aqueous potassium buffer (0.1 M, pH 7.4), the homogenate was centrifuged at 10000 rpm (4°C) for 10 min to obtain the post-mitochondrial fraction (PMF) and used for determination of antioxidant enzymes and non-enzymatic antioxidants.

Biochemical assays

Serum chemistry profile

A portion of each blood sample was collected (on days 14 and 28 post-treatment) into plain bottles and thereafter centrifuged to obtain serum which was used to estimate the lipid profiles such as total cholesterol, triglycerides (TGs) and high-density lipoproteins (HDL) and low-density lipoproteins (LDL), using commercial kits (Excel Diagnostics Pvt. Ltd) and following standard

procedures as outlined by the producer. 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.

Glycogen content in liver and skeletal muscles

Glycogen content in liver and skeletal muscle were measured according to the standard method[22]. Liver and skeletal tissues were homogenised separately in hot 80% ethanol at the tissue concentration of 100 mg/ml and then centrifuged at 8000×g for 20 min. The residue was collected, dried over a water bath, and then extracted at 0°C for 20 min by adding a mixture of 5 ml water and 6 ml of 52% perchloric acid. The collected material was centrifuged at 8000×g for 15min and the supernatant was separated. From the recovered supernatant 0.2 ml was transferred in graduated test tube and made to 1 ml volume by the addition of distilled water. Graded standards were prepared using 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of a working standard solution and volume of all these standards were made up to 1 ml using distilled water. Anthrone reagent (4ml) was added to all test tubes and the tubes were then heated in a boiling water bath for 8 min. After these tubes were allowed to cool at room temperature and the intensity of the green to dark green colour of the solution recorded at 630 nm. Glycogen content of the tissue samples was determined from a standard curve prepared with standard glucose solution. The amount of glycogen in tissue sample was expressed in microgram of glucose/ milligram of tissue.

Carbohydrate metabolic enzymes activities in liver and skeletal muscles

Glucose-6-phosphatase (G-6-Pase) activity

G-6-Pase activity of liver and skeletal muscle was measured according to the standard protocol[23]. Tissues were homogenised separately in ice cold of 0.1M phosphate buffer saline (pH 7.4) at the tissue concentration of 50 mg/ml. In a calibrated centrifuge tube, 0.1 ml of 0.1M G-6-Pasesolution and 0.3 ml of 0.05M maleic acid buffer (pH 6.5) were taken and brought to 37^0C in water bath for 15min. The reaction was stopped with 1ml of 10% trichloroacetic acid (TCA) followed by chilling in ice and centrifuge at 3000 × g for 10min. The optical density was taken at 340nm. The enzyme activities from each tissue were expressed as milligram of inorganic phosphate liberated/ gram of tissue.

Glucose-6-phosphate dehydrogenase (G-6-PDase) activity

G-6-PDase activity of liver and skeletal muscle was measured according to the standard protocol[24]. Tissues were homogenised separately in ice cold, 0.1M phosphate buffer saline (pH 7.4) at the tissue concentration of 50 mg/ml. In a spectophotometric cuvette, 0.3 ml of 1M Tris–chloride buffer (pH 7.5), 0.3 ml of 2.5×10^{-2}M G-6-Pase, 0.1 ml of 2×10^{-3} M NADP and 0.3 ml of 0.2M $MgCl_2$ and 0.3 ml of ice-cold tissue homogenate were taken. The rate of change of absorbency at 340nm was recorded. One unit of enzyme activity define as that quantity which catalyses the reduction of 1μM of NADP/ minute.

Hexokinase (HK) activity

HK activity in liver and skeletal muscles tissues was determined spectrophotometrically[25]. Briefly, the assay mixture contained 3.7mM glucose, 7.5mM $MgCl_2$, 11mM thioglycerol, and 45mM HEPES (4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid) buffer; 0.9mL of this mixture and 0.03mL of 0.22M adenosine triphosphate was mixed well in a cuvette; 0.1mL of the tissue supernatant was then added into the cuvette and absorbance noted. The enzyme activity was determined based on reduction of NADPH coupled with HK which was measured spectrophotometrically at 340nm.

Determination of intestinal enzymatic and non-enzymatic antioxidants activities

Hydrogen peroxide (H_2O_2) generation

H_2O_2 generation was determined according to Woff[26]. To 2.5ml of 0.1M potassium phosphate buffer (pH 7.4), 0.250ml of ammonium ferrous sulphate (AFS), 0.1ml of sorbitol, 0.1ml of xylenol orange (XO), 0.025ml of H_2SO_4 and 0.050ml of intestinal PMF was added. The mixture was mixed thoroughly by vertexing until it foamed, and a light pink colour of the reaction mixture was observed. The reaction mixture was subsequently incubated at room temperature for 30 min. The absorbance was assessed at 560nm, using distilled water as blank. The H_2O_2 generated was extrapolated from the H_2O_2 standard curve.

Thiobarbituric acid reactive substances

Thiobarbituric acid reactive substance was quantified as malondialdehyde (MDA) in the intestinal PMF. MDA was determined by spectrophotometry of the pink coloured product of thiobarbituric acid (TBA) reactive substances complex. Briefly, 0.4ml of the intestinal PMF was mixed with 0.5 ml of 30% TCA and 0.5 ml of 0.75% TBA prepared in 0.2 M HCl were added to 1.6 ml of Tris-KCl. The reaction mixture was incubated in the water bath at 80°C for 45 min, cooled on ice and centrifuged at 4000 rpm for 15 min. The absorbance of the resulting pink solution was measured against a reference blank of distilled water at 532nm. The intestinal PMF was calibrated using the MDA as a standard and the results was expressed as the amount of free MDA produced. The MDA level was

determined according to the method of Varshney and Kale[27]. Lipid peroxidation in units/mg protein was calculated with a molar extinction coefficient of 1.56 × 10^5 m/cm.

Reduced glutathione concentration

The intestinal reduced glutathione (GSH) was estimated by the method of Jollow et al[28]. The assay is based on the oxidation of GSH by 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB). DTNB and glutathione (GSH) react to generate 2-nitro-5-thiobenzoic acid (TNB) which has yellow colour. Therefore, GSH concentration can be determined by measuring absorbance at 412nm. Briefly, 0.5 ml of 4% sulphosalicylic acid (precipitating agent) was added to 0.5ml of intestinal PMF and centrifuged at 4000 rpm for 5 min. To 0.5ml of the resulting supernatant, 4.5ml of Ellman's reagent (0.04 g of DTNB in 100 ml of 0.1M phosphate buffer, pH 7.4) was added. The absorbance was read at 412nm against distilled water as blank prepared under the same conditions.

Total cellular thiols concentrations

These were measured as protein and non-protein thiols concentrations by the method of Sedlak and Lindsay[29]. Briefly, an aliquot of the intestinal PMF was treated with 6% trichloroacetic acid. Following centrifugation, the supernatant and the pellet dissolved in 2 ml of 0.4 M Tris–HCl buffer containing 10% SDS, were reacted with DTNB and the absorbance was measured at 412nm. A calibration curve was obtained using reduced glutathione (GSH) as standard and the protein and non-protein thiol contents were expressed as µmol/mg protein.

Superoxide dismutase (SOD) activity

The level of SOD activity was determined by the method of Misra and Fridovich[30]. Briefly, 100mg of epinephrine was dissolved in 100ml distilled water and acidified with 0.5ml concentrated hydrochloric acid. This involves inhibition of epinephrine autoxidation, in an alkaline medium at 480nm in a UV vial spectrophotometer. Approximately 0.01ml of intestinal PMF was added to 2.5ml of 0.05M carbonate buffer (pH 10.2), followed by the addition of 0.3ml of 0.3 mM adrenaline. For the determination of specific activity of SOD in intestinal PMF, the rate of autoxidation of epinephrine was noted at 30 seconds intervals in all groups. The enzyme activity was expressed in arbitrary units considering inhibition of autoxidation, as one unit of SOD specific activity.

$$\text{SOD activity (U/mg protein)} = \frac{\text{SOD activity (U/ml}}{\text{Concentration protein (mg/ml)}}$$

Glutathione peroxidase (GPx) activity

The intestinal GPx activity was also measured according to Beutler et al[31]. The reaction mixtures contained 0.5 ml of potassium phosphate buffer (pH 7.4), 0.1 ml of sodium azide, 0.2 mL of GSH solution, 0.1ml of hydrogen peroxide (H_2O_2), 0.5 ml of PMF and 0.6 ml of distilled water. The mixture was incubated in the water bath at 37°C for 5 min and 0.5 ml of TCA was added and centrifuged at 4000 rpm for 5 min. 1 ml of the supernatant was taken and 2 ml of K_2PHO_4 and 1 ml of Ellman's reagent were added. The absorbance was read at 412 nm using distilled water as blank. The activities were expressed as µg of GSH consumed/ min/mg protein.

Glutathione-S-transferase (GST) activity

For quantification of GST activity, spectrophotometric method of Haque et al[32] was used. The reaction assay consisted of 0.1mL tissue homogenate, 1.67mL of 0.1M sodium phosphate buffer pH6.5, 0.2mL of 1mM GSH and 0.025mL of 1mM 1-chloro-2,4-dinitrobenzene (CDNB). Change in absorbance was spectrophotometrically measured at 340nm and activity of GST was expressed as nmoles of CDNB conjugates formed/ min/mg protein using molar extinction coefficient of 9.6 x $0^3M^{-1}cm^{-1}$.

Histopathology of liver and pancreas

Animals were sacrificed and small pieces of liver and pancreas were collected in 10% formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Sections of 5µm in thickness were made, stained with haematoxylin and eosin and the sections were observed under light microscope for histopathological changes.

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

Chemical nature of LTLP

Using Fehling's test, the nature of the tulsi leaves extract was found to be carbohydrate in nature. Carbohydrates having a free or potentially free aldehyde or ketone group have the assets of readily reducing the ions of certain metals such as copper, bismuth, mercury, iron, and silver. The best extensively used tests for sugar are based on this property. For instance, when blue cupric hydroxide suspended in an alkaline medium is heated, it is converted into insoluble black cupric oxide. Though, in the presence of reducing agents such as certain sugars, the cupric hydroxide is reduced to insoluble

yellow or red cuprous oxide[20]. Based on the above property, the chemical nature of tulsi leaves extract was studied using Fehling's test. The production of yellow or brownish-red cuprous oxide stated that reduction has taken place. The variance in colour of cuprous oxide precipitate under different settings was seemingly because of differences in the particles size, the more finely divided precipitates having yellow colour while the coarser ones were red. This test was found to be positive, representing that the tulsi leaves extract is carbohydrate in nature.

Acute toxicity study

In acute toxicity study, no adverse reactions or behavioral changes were observed after each graded doses of LTLP administration during the entire period of experimentation. No substantial changes in overall feed and water consumption rates suggesting that this LTLP had no effect on normal growth of rats. The outcomes suggested that the oral LD_{50} of dosages of LTLP preparations was greater than 1000mg/kg.

Effect of LTLP on blood glucose and body weight of alloxan-induced diabetic rats

Alloxan monohydrate induces hyperglycaemia in rats. The LTLP caused a significant ($P \leq 0.05$) decrease in the fasting blood glucose of treated rats (Figure 1A) 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 (Figure 1B).

Effect of LTLP on haematological parameters in alloxan-induced diabetic rats

The induction of diabetes caused statistically significant ($P \leq 0.05$) reductions in the PCV and Hb of diabetic rats compared with the normal control. However, treatment with graded doses of LTLP resulted in a significant increase in the values of these parameters when compared with the diabetic untreated group (Figure 2A and B). The RBC values of LTLP-treated rats showed a nonsignificant increase when compared with the diabetic control group (Figure 2C). Concerning haematometric indices, a significant increase in the MCHC in graded doses of LTLP-treated rats when compared with the diabetic control and glibenclamide-treated groups (Figure 2F). However, there were no significant differences in the values of MCV and MCH (Figure 2D and E). Platelet count of LTLP-treated diabetic rats exhibited significantly ($P \leq 0.05$) decreased when compared with the diabetic control and glibenclamide-treated rats and was comparable with that of the normal control (Figure 3A). Total WBC and differentials were increased in diabetic rats when compared with normal control. Administration of LTLP resulted in a decrease in the total white blood cells (TWBC) and lymphocyte counts (Figure 3B and C). The neutrophil count in untreated diabetic group showed significant increase when compared with the normal control and the diabetic groups with graded doses of LTLP-treated rats (Figure 3D). Eosinophil count was significantly reduced in the untreated diabetic rats when compared with the normal control and the LTLP-treated groups (Figure 3F). There were no significant changes in the monocyte counts (Figure 3E).

Effect of LTLP on serum insulin and lipid profile of alloxan-induced diabetic rats

Alloxan induced diabetic animal displayed a significant elevation ($P \leq 0.05$) in the levels of serum insulin (Figure 4A), the lipid profiles such as TGs, cholesterol, and decrease ($P \leq 0.05$) in high density lipoprotein (HDLs) levels in comparison to the control group. 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 (Figure 4B-D). There were no significant changes in the levels of low-density lipoproteins (LDLs) when compared with the normal control and the diabetic groups with graded doses of LTLP-treated rats (Figure 4E).

Effect of LTLP on glycogen content and carbohydrate metabolic enzymes activities in liver and skeletal muscle of alloxan-induced diabetic rats

Glycogen content in hepatic and skeletal muscle were decreased in the alloxan induced diabetic group in comparison with the non-diabetic control group animals. The administration of graded doses of LTLP or glibenclamide to the diabetic animals to diabetic animals shows a significant ($P<0.05$) recovery in the glycogen content towards the control level (Figure 5A). The induced diabetic animal displayed a significant elevation in G-6-Pase activity (Figure 5B) along with a diminution in the activities of G-6-PDaseand HK(Figure 5C and D) in the liver and skeletal muscle in comparison to the control group. From comparative analysis it has been indicated that administration of the graded doses of LTLP or glibenclamide to the diabetic animals resulted in significant protection and the levels of these parameters were resettled towards the control group. There was no significant difference in the levels of these parameters between the LTLP-treated group and the glibenclamide treated group.

Effects of LTLP on intestinal antioxidant enzymes and non-enzymatic antioxidants

Study revealed that induction of diabetes with alloxan monohydrate led to a significant ($P \leq$

0.05) increase in the H_2O_2 and MDA levels of the intestinal tissue. However, treatment with graded doses of LTLP (50 mg/kg and 100 mg/kg) led to a significant ($P \leq 0.05$) reduction in the level of H_2O_2 generated and MDA content in the intestinal tissue (Figure 6A and B). A significant ($P \leq 0.05$) reduction of thiol content and GSH levels were seen in intestinal homogenate of diabetic untreated rats when compared with control, while treatment with LTLP at dose of 100 mg/kg triggered significant ($P \leq 0.05$) elevation in these parameters relative to untreated diabetic rats, the same result was observed for glibenclamide (Figure 6C-E). Further, a significant ($P \leq 0.05$) reduction in the activities of SOD, GPx and GST were observed in the diabetic untreated rats, while treatment with LTLP led to a significant increase ($P \leq 0.05$) in these enzyme activities when compared with the normal control and untreated diabetic rats (Figure 7A-C).

Histopathology of the liver and pancreas

Histopathological changes in liver and pancreases were observed after Hematoxylin and Eosin (H&E) staining in all groups of animals (Figures 8 and 9). In the histopathological investigations, the liver of control rats was found to be divided into the classical hepatic lobules; each formed of cords of hepatocytes radiating from the central vein to the periphery of the lobule. The cell cords were separated by narrow blood sinusoids (Figure 8A). The alloxan induced diabetic rats displayed periportal necrosis of the hepatocytes near the portal areas. The liver also showed dilated and congested portal vessels as well as areas of inflammatory cell infiltration. Histopathological changes including presence of congested central veins, degenerated and irregular-shaped hepatocytes, cloudy swelling, and vacuolization of cytoplasm (Figure 8B). However, in diabetic rats treated with standard glibenclamide and graded doses of LTLP has reduced the severity of these changes. Sections obtained from glibenclamide and graded doses of LTLP groups showed normal hepatocytes with partly recovered normal arrangement hepatocytes and less congestion of central veins indicating a restoration of normal liver architecture (Figure 8C-E).

In pancreas sections of control group rats showed the normal appearance of acinar cells and Islet β-cells. The islets appeared lightly stained than the surrounding acinar cells. The acinar cells are formed of pyramidal cells with basal nuclei and apical acidophilic cytoplasm (Figure 9A). In diabetic control shows pathological changes of both exocrine and endocrine components. The acinar cells were swollen, and small vacuoles were observed in almost all acinar cells. Interlobular ducts were lined with flattened epithelium. Islet β-cells are almost in varying sizes, few and far between and presence of fibrosis and disarrangement of cells (Figure 9B). Diabetic rats that received glibenclamide and graded doses of LTLP (50 and 100mg/ kg, BW/day) showed moderate normal proportion of acinar cells and the Islet β-cells were smaller in volume with mild pathologies (Figure 9C-E). On the other hand, compared with the control group, these treated groups depicted evidence of cellular regeneration among the islets of Langerhans. Marked improvement of the cellular injure as evident from the partial restoration of islet cells, reduced β-cell damage, more symmetrical vacuoles, and an increase in number of islet cells. Atrophic change of the acinar cells was less severe and the border between exocrine and endocrine portions became more distinct. Diabetic rats that received LTLP (100mg/ kg, BW/ day) was considered the group that was improved the most (Figure 9E).

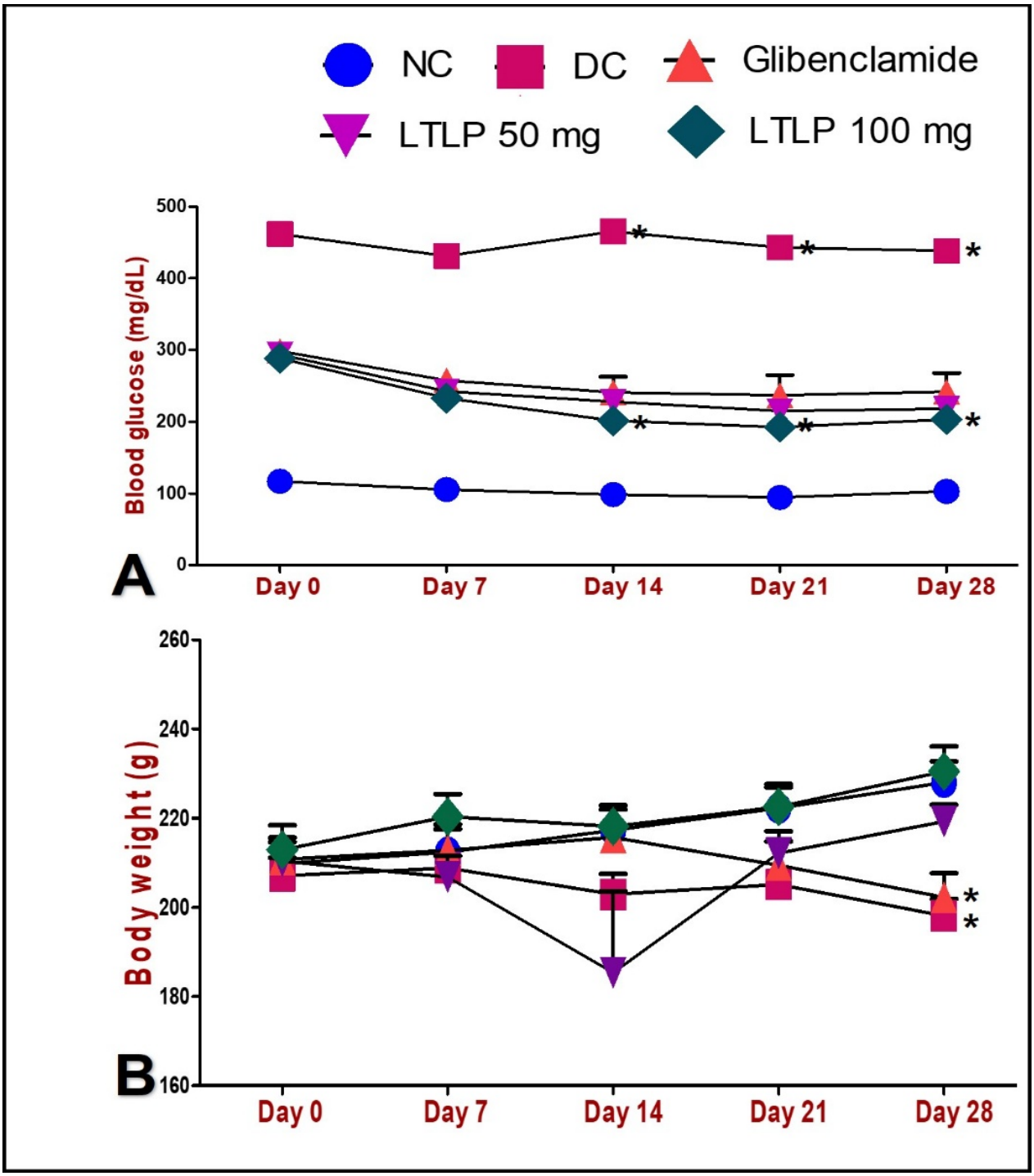

Figure 1: Effect of LTLP on (A) blood glucose and (B) body weight of alloxan-induced diabetic rats. Values are the mean ± SEM, N=10 for each group. * $P \leq 0.05$ compared with the experimental and control rats

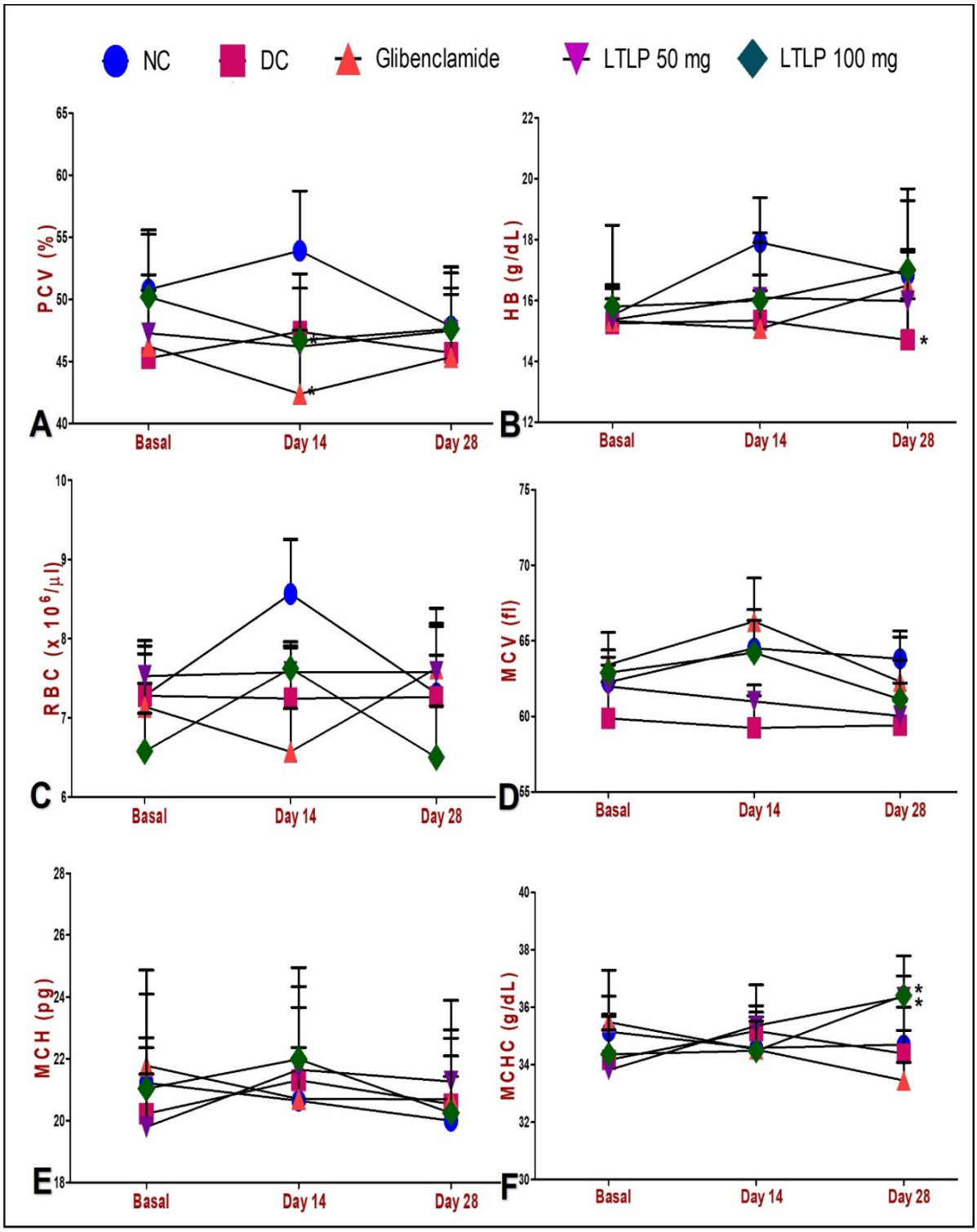

Figure 2: Effect of LTLP on (A) PCV (B) HB Concentration, (C) RBC, D) MCV, (E) MCH And (F) MCHC of Alloxan-Induced Diabetic Rats. Values are the Mean ± SEM, N=10 For Each Group. * $P \leq 0.05$ Compared with the Experimental and Control Rats

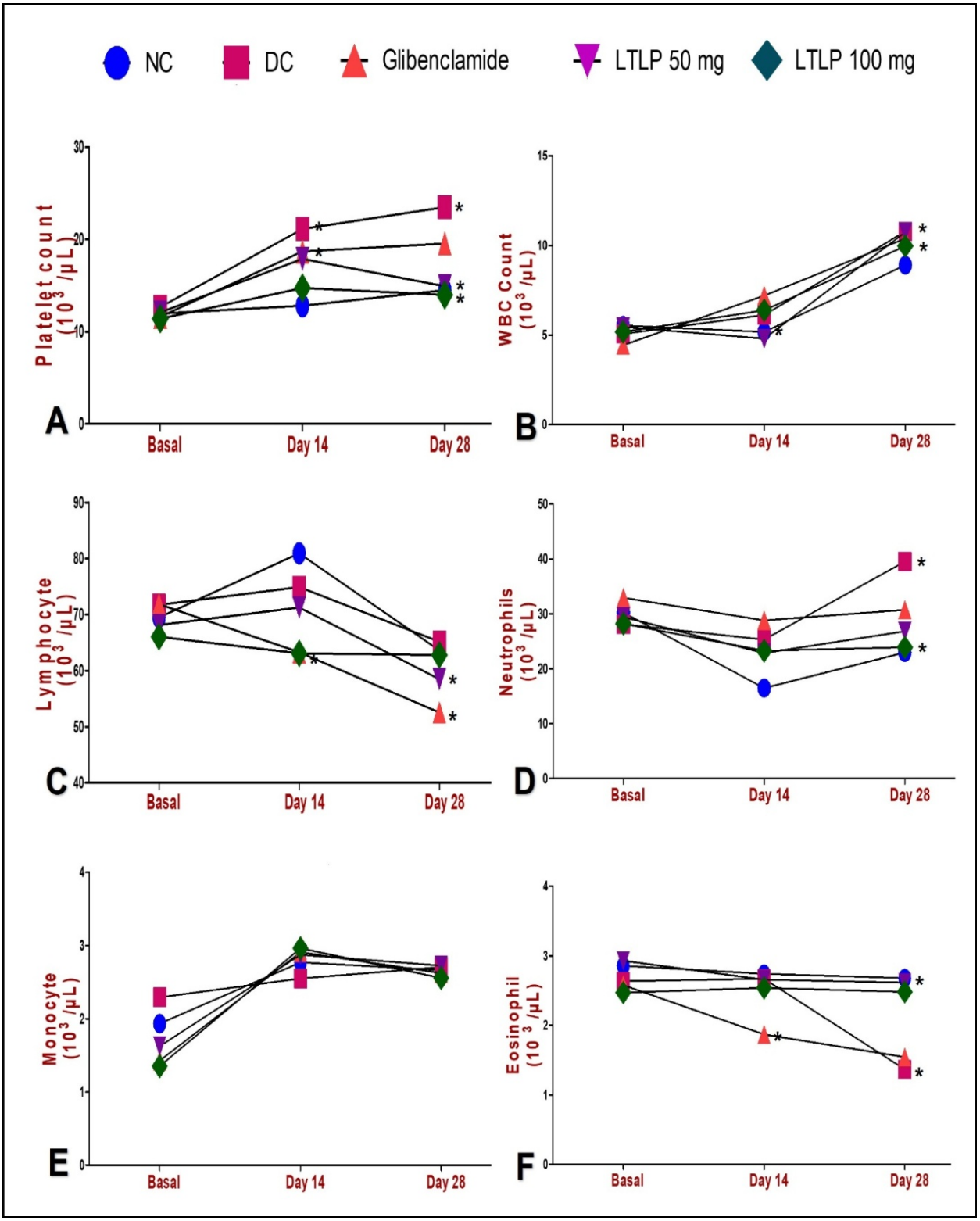

Figure 3: Effect of LTLP on (A) Platelet Count, (B) WBC Count And (C-F) White Blood Cell Differentials Count (Lymphocyte, Neutrophil, Monocyte, Eosinophil) Of Alloxan-Induced Diabetic Rats. Values are the Mean ± Sem, N=10 For Each Group. * $P \leq 0.05$ Compared with the Experimental and Control Rats

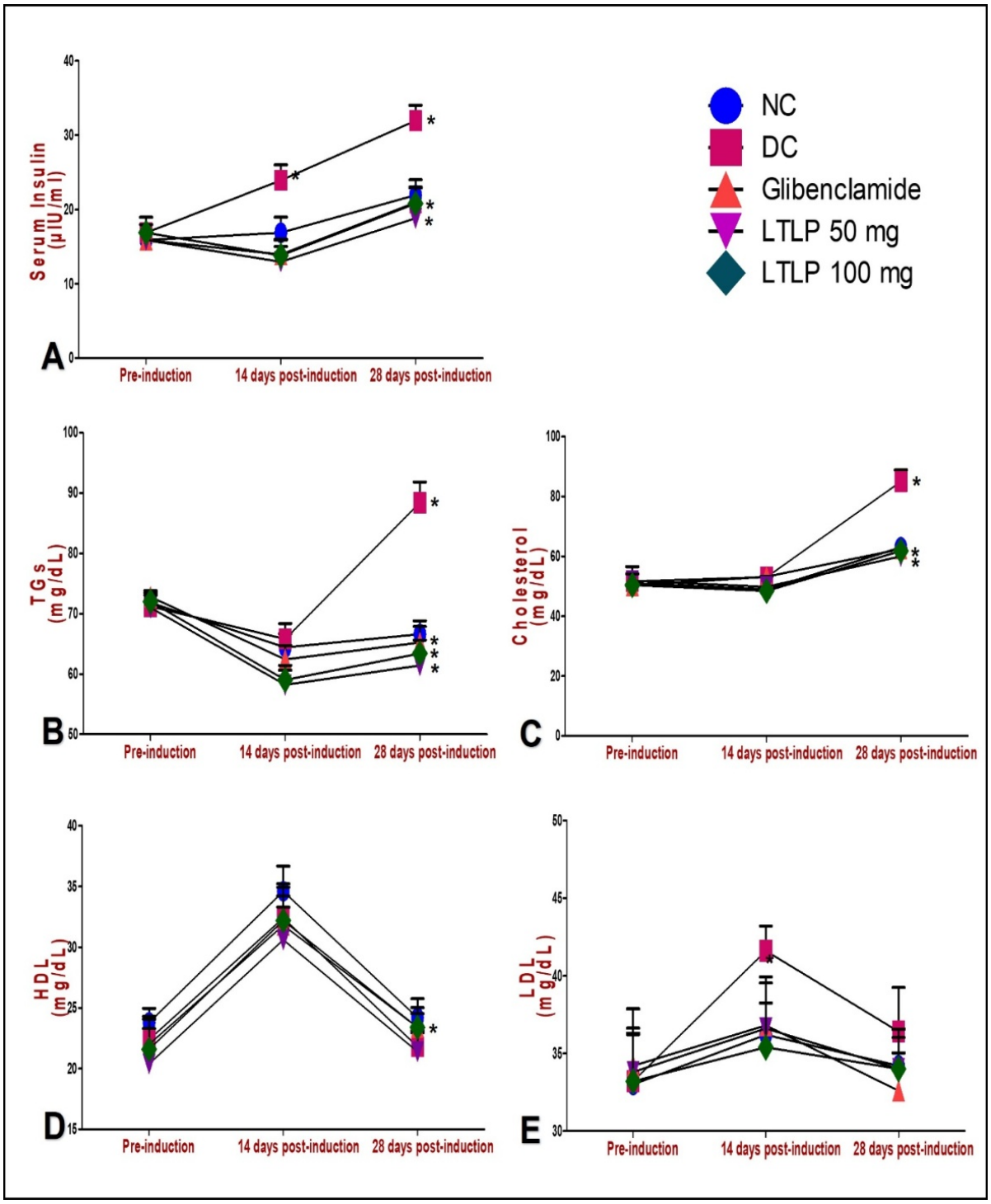

Figure 4: Effect of LTLP on (A) Serum Insulin And (B-E) Lipid Profiles (TGs, Cholesterol, HDL, And LDL) of Alloxan-Induced Diabetic Rats. Values are the Mean ± SEM, N=10 For Each Group. * $P \leq 0.05$ Compared with the Experimental and Control Rats

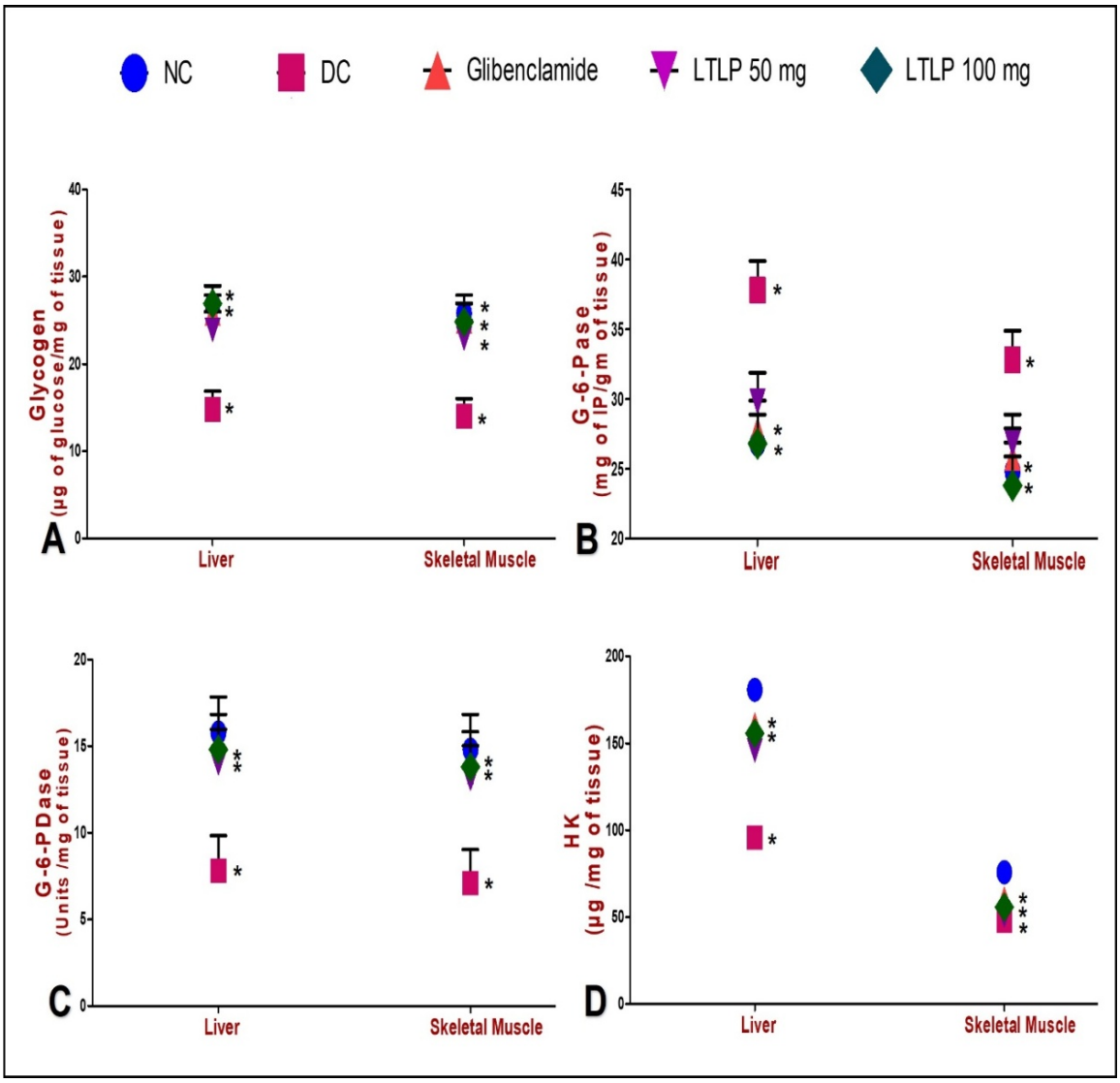

Figure 5: Effect of LTLP on (A) Glycogen Level And (B-D) Carbohydrate Metabolic Enzymes (G-6-Pase, G-6-PDase and HK) Activities in Liver and Skeletal Muscles of Alloxan-Induced Diabetic Rats. Values are the Mean ± SEM, N=10 For Each Group. * $P \leq 0.05$ Compared with the Experimental and Control Rats

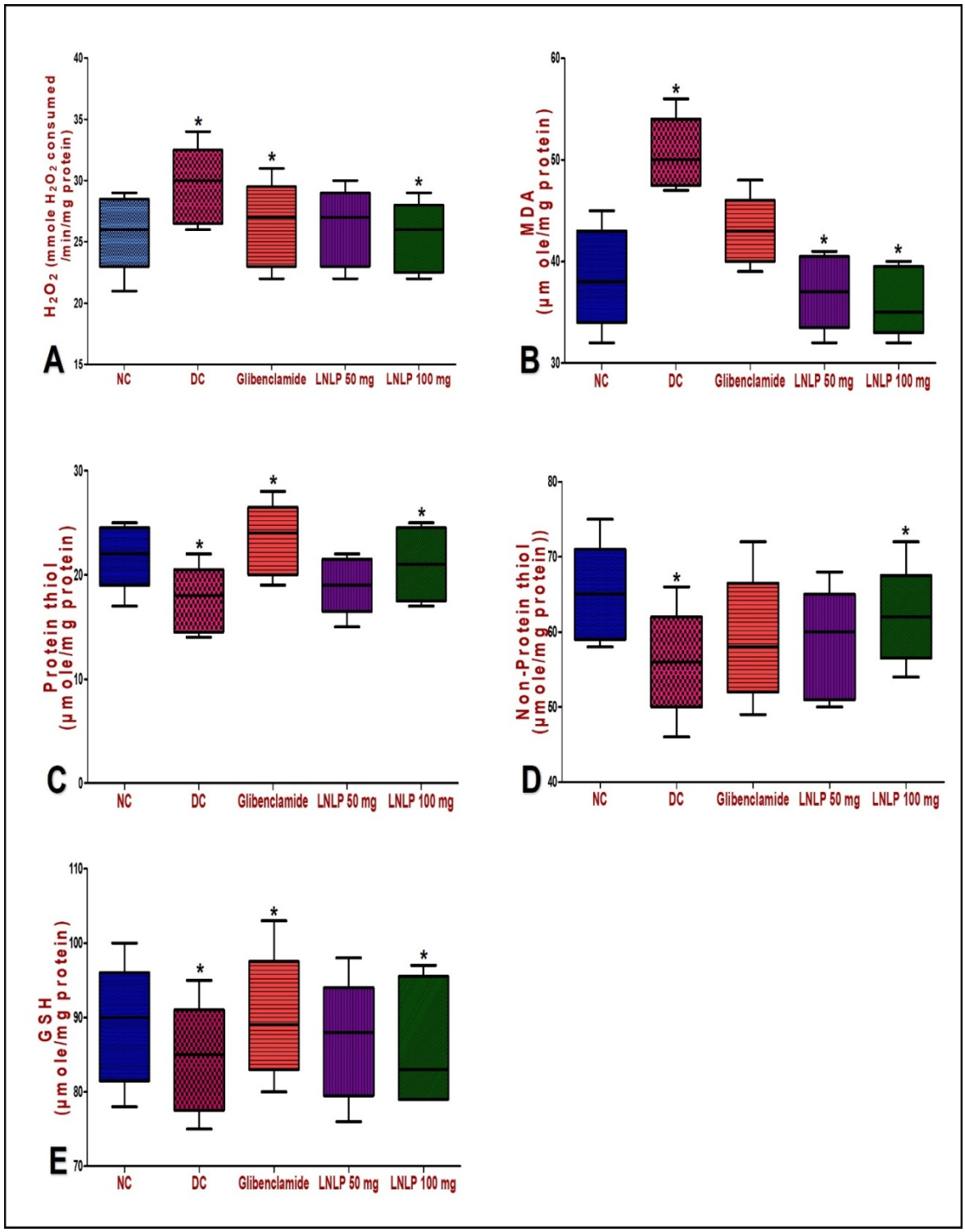

Figure 6: Effect of LTLP on (A) H_2O_2 Levels, (B) MDA Content, (C) Protein Thiol, (D) Non-Protein Thiol And (E) GSH Concentration in Intestinal Tissue of Alloxan-Induced Diabetic Rats. Values are the Mean ± SEM, N=10 For Each Group. * $P \leq 0.05$ Compared with the Experimental and Control Rats

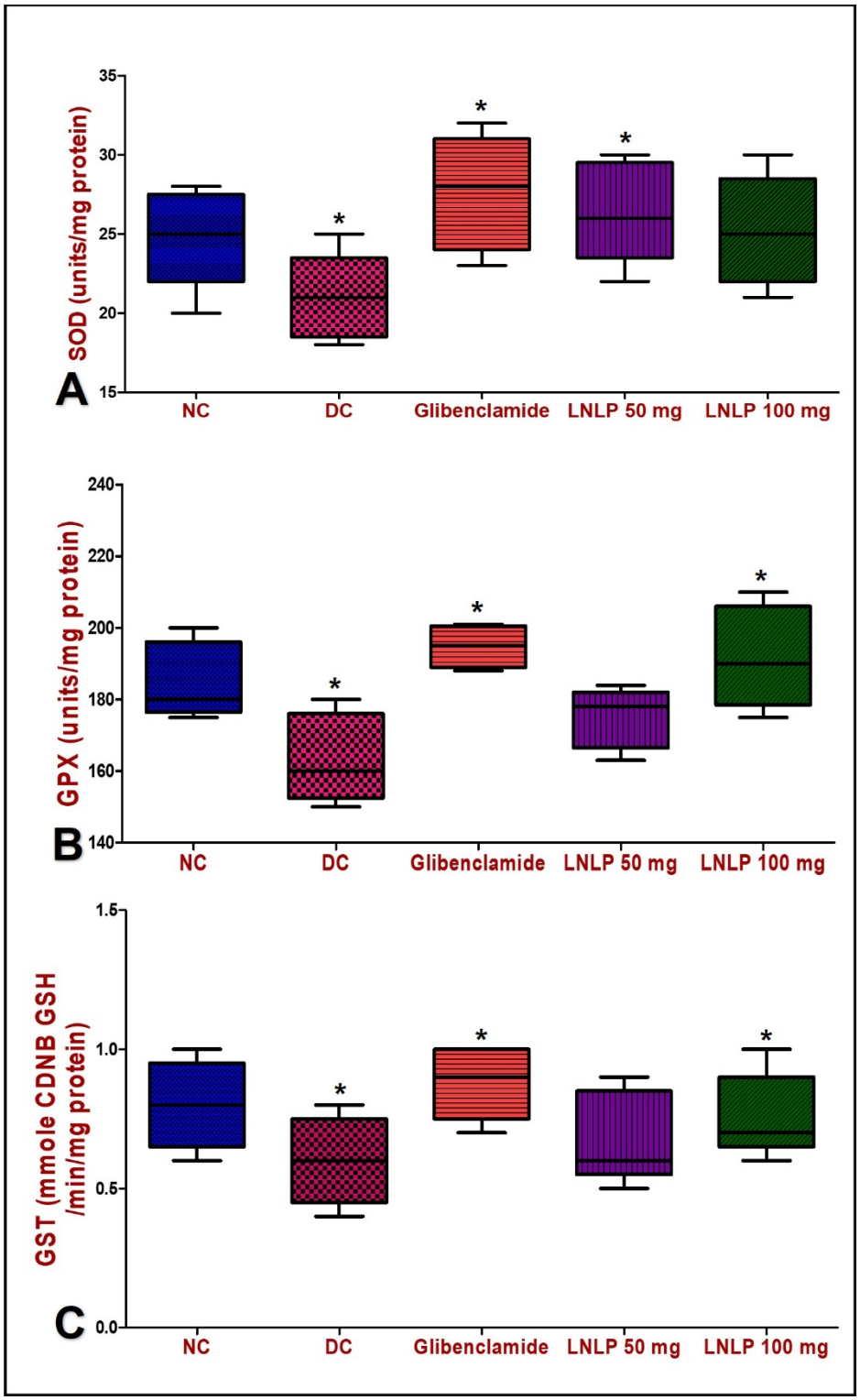

Figure 7: Effect of LTLP on (A) SOD, (B) GPx and (C) GST Activities in Intestinal Tissue of Alloxan-Induced Diabetic Rats. Values are the Mean ± SEM, N=10 For Each Group. * $P \leq 0.05$ Compared with the Experimental and Control Rats

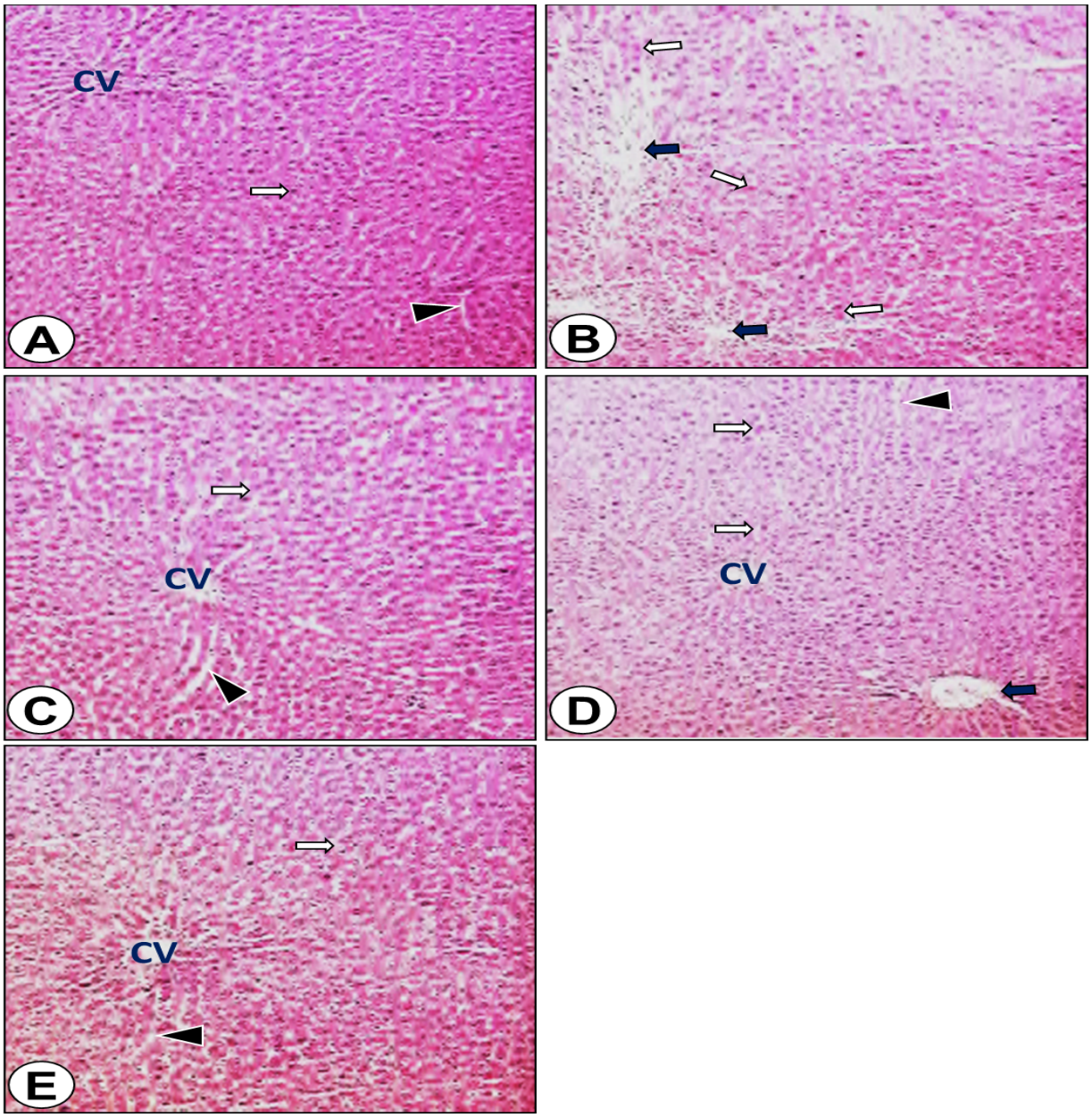

Figure 8: Photomicrograph of The Liver Section of Alloxan-Induced Diabetic Rats. (A) Normal Control Rat Liver Showing Normal Hepatocytes (Arrow) Surrounding the Central Vein (CV), and Hepatic Sinusoid (Black Arrowhead) with Normal Radial Arrangements Around Hepatic Cords. (B) Diabetic Control Rat Liver Showing Increase Vacuolation in The Cytoplasm Of Hepatocytes and Hepatic Sinusoid (Black Arrowhead) Appeared As Indistinct Clear Vacuoles (Arrows) With Cloudy Swelling, Spotty Necrosis (Black Arrow) With The Accumulation of Lipids And Congested CV. (C) Diabetic Rats Treated With Glibenclamide Demonstrating Restoration Of The Normal Liver Structure With Normal Hepatocytes (Arrows) Arranged in Normal Sheets or Cord Around CV. (D) And (E) Diabetic Rats Treated With 50mg And 100mg/Kg LTLP Presented With Less Pathological Changes And Improved Liver Architecture With Partly Recovered Normal Arrangement Hepatocytes (Arrows). Moderate Effects in The Liver Include Congested CV, Hydropic Degeneration and Degranulated Cytoplasm of Hepatocytes. Hematoxylin And Eosin (H&E) Staining (200x).

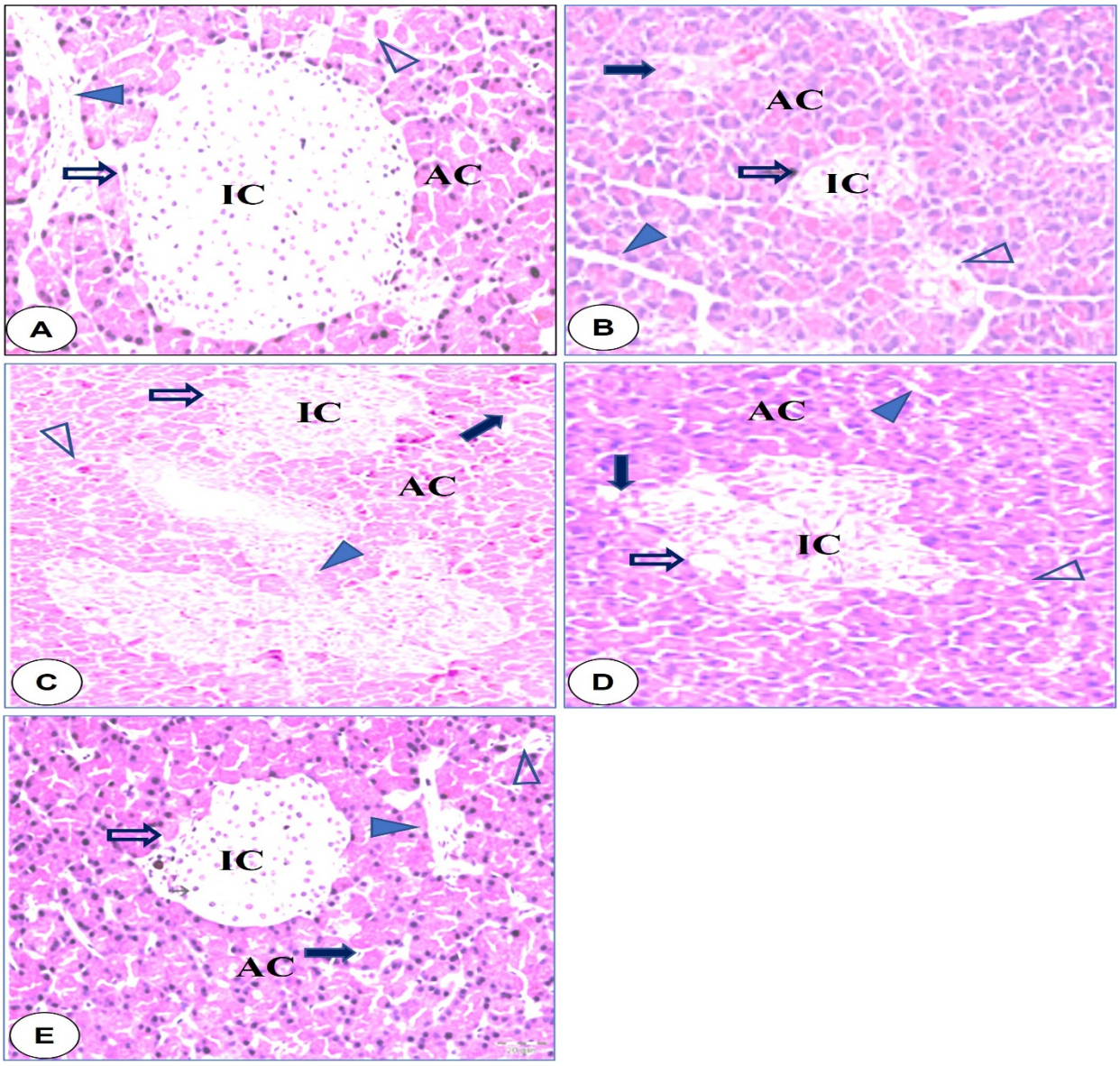

Figure 9: Photomicrograph of The Pancreas Section of Alloxan-Induced Diabetic Rats. (A) Normal Control Shows Normal Exocrine Acini (Hollow Arrows); The Intralobular and Interlobular Ducts Are Essentially Normal and Contain Pancreatic Secretion. The Acinar Cells (Acs), Which Stained Strongly, Were Arranged in Lobules with Prominent Nuclei. The Islet Cells (IC) Were Seen Embedded Within the Acs and Surrounded by A Fine Capsule. (B) Diabetic Control Shows Islets in Varying Sizes, Few and Far Between. The Acinar Cells Were Swollen, And Small Vacuoles (Black Arrows) Were Observed in Almost All Acinar Cells. Interlobular Ducts Were Lined with Flattened Epithelium (Black Arrowhead). Islet β -Cells Are Almost in Varying Sizes, Few and Far Between and Presence of Fibrosis and Disarrangement of Cells. (C) Glibenclamide-Treated Group Shows Islets in Varying Sizes, Few and Far Between (Hollow Arrows). The Islet Cells Are Seen Interspersed Between the Acinar Cells. The Islets Appeared Lightly Stained Than the Surrounding Acinar Cells. Wider Interlobular (Black Arrowhead) And Intralobular (Hollow Arrowhead) Duct Were Observed. Diabetic Rats Treated With 50mg/Kg LTLP (D) And 100mg/Kg LTLP (E) Displaying Nearly Normal Structure of Islets of Langerhans. Atrophic Change of The Acinar Cells Was Less Severe and The Border Between Exocrine and Endocrine Portions Became More Distinct. Islets Show Hyalinisation in The Centre While the Remnant Islet Cells Are Limited to The Periphery; These Are Few and Far Between (Hollow Arrows). Hematoxylin And Eosin Staining (200x)

DISCUSSION

Studies showed that Ocimum species are devoid of any toxic effects and have the potential to normalize blood glucose levels and lipid profile inhibit lipase activity, ameliorate chemical-induced hepatotoxicity, and are reported to be beneficial in managing diabetic complications[33-34]. However, further mechanism of action and development of standardized formulations are certainly required to develop these species as drugs for managing metabolic disorders and related comorbidities[7]. Previous studies based on rat model reported that LTLP or different tulsi leaves extracts did not show any toxicity even at 1000mg /kg BW or 5g/kg/BW[16-17], respectively. In this study the non-toxic nature of LTLP is evident by the absence of mortality for a period of 14 days even when maintained on limit dose indicating this LTLP could be safe up to 1000 µg/kg BW. Since substances possessing LD_{50} higher than 50 mg/kg are non-toxic[35], the tested extracts were considered safe.

The major aim of this study was to assess the antidiabetic and antioxidant roles of LTLP using alloxan developed hyperglycaemia. 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[36].In this study, the reduction in blood glucose and body weight gain effect observed in diabetic rats treated with LTLP may be attributed to the enhancement of glucose metabolism and an indication of its hypoglycaemic properties[8,13]. In contrast, non-toxic nature as evident, oral administration of graded doses of LTLP showed significant increase in body weight gain from day 21 suggesting that LTLP substantially improved their general health status and metabolic mechanisms by effective glycaemic control or a reversal of gluconeogenesis[12,14].However, LTLP administration decreased the levels blood glucose to normal range probably by improving insulin sensitivity in agreement with above reference studies revealed 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 effect of various phytoconstituents present in the tulsi leaves extracts as reported in review of literature[5-7].

In this study, LTLP supplementation in the diabetic rats showed significant increase in the values of PCV, Hb and MCHC in the diabetic rats gives an indication that LTLP contains bioactive principles that can stimulate the formation or secretion of erythropoietin, a substance in the blood that stimulates RBCs production[37]. Studies demonstrated that the stimulation of erythropoietin enhances synthesis of RBC, which is supported by the improved level of MCH and MCHC in alloxan or streptozotocin-induced diabetes rats[12,38]. Further, these parameters are used precisely to define the concentration of haemoglobin of RBCs and to suggest the restoration of oxygen-carrying capacity of the blood[39].Sterner et al[40]shown the elevated platelet levels as well as platelet dysfunction could be injurious to microcirculation regularly seen in diabetics with a long duration of disease and suggested that the possibility that elevated platelet count could be used as a prognostic indicator of future diabetic complications. The observation of elevated platelet counts in diabetic rats and decline in these values after treatment could be the result of the ability of the LTLP to increase the sensitivity of the platelets to insulin, thereby causing a reduction in platelet aggregation. Report shown there is positive correlation between high platelet and TWBC count and raised blood sugar level among the diabetic subjects studied[41]. Further, LTLP supplementation in the diabetic rats showed decrease in the TWBC and lymphocyte counts could be the result of the antiinflammatory property of the tulsi leaf powder which leads to amelioration of the low-grade inflammatory state seen in diabetes because of insulin resistance[42]. The result of this study supports the finding of other studies on hematological parameters and implied a reduction in the process of advance glycation and oxidative stress within the blood cells[12,38]. In diabetes, hyperglycaemia is accompanied with dyslipidemia which is characterized by increased levels of TGs, TCs, LDL and decreased level of HDL is an important biochemical abnormality of DM[43]. In this study, the levels of TC and total TGs were markedly increased following induction of diabetes with alloxan confirming that the dyslipidaemia associated with DM. 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 DM. This agrees with the studies achieved on alloxan or streptozotocin-induced diabetes rats following treatment with aqueous or other extracts of tulsi leaves[13,15,38,44].

Glycogen is the primary intracellular storable form of glucose and its levels in various tissues is a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Selective destruction of β-

cells of islets of Langerhans resulting in marked decrease in insulin levels, it is rational that glycogen levels in tissues such as liver, kidney, and skeletal muscle as they depend on insulin for entry of glucose[45]. In this study, supplementation of LTLP or glibenclamide to diabetic rats significantly increased glycogen content of both hepatic and skeletal muscles by stimulating glycogen synthase and inhibiting glycogen phosphorylase which could be due to increased insulin levels[46] or such beneficial effects which may partly relate to several mechanisms such as insulin sensitizing, glycogen synthesizing, inhibition of carbohydrate metabolizing enzymes, and antioxidant activities[34]. Hence, the current study was focused on assessing the activities of carbohydrate metabolism key enzymes of both hepatic and skeletal muscles in alloxan-induced diabetic rats. G-6-Pase is gluconeogenic enzyme which is involved in the homeostatic regulation of blood glucose concentration mainly in the liver and kidney and critical in providing glucose to other organs during diabetes, prolonged fasting, or starvation[47]. The increased activities of G-6-Pase in these tissues of diabetic induced rats may be due to insulin inadequacy and dwindled the activity of G-6-Pase on supplementation with LTLP or glibenclamide indicates improved insulin secretion which is responsible for the repression of the gluconeogenic key enzymes[48]. HK, an insulin-dependent enzyme, the activity of this enzyme in diabetic rats is almost entirely inhibited or inactivated resulting a marked decline in the rate of glucose oxidation via glycolysis which ultimately leads to hyperglycemia[49]. Decreased enzymatic activity of HK has been reported in diabetic animals, resulting in depletion of liver and muscle glycogen in diabetic animals[50]. Upon supplementation with LTLP or glibenclamide to diabetic rats resulted in a notable reversal in the activity of HK in liver and skeletal muscle suggesting that the hypoglycemic action seen is the result of an increased glucose utilisation at the level of skeletal muscle as well as liver[46]. G-6-PDase, this enzyme is related to the balance between anabolism and catabolism of carbohydrates and the conversion between pyruvic and lactic acids[51]. The activity of the enzyme G-6-PDase is usually found to be decreased in diabetic conditions and administration of LTLP to diabetic rats restored the activity of G-6-PDase to near normalcy suggests that the administration of LTLP, carbohydrates are facilitated more along the hexose monophosphate pathway[52].

Diabetes represents a state of high oxidative stress because of hyperglycaemia induced ROS generation, which in turn causes damage to the cells resulting secondary complications in DM[53]. Research data indicated that alloxan administration initiates the production of ROS, including superoxide radical (O_2), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2), which damage, and later destroy, the cells[54]. Free radicals or nonradical species react with lipids causing peroxidation, leading to the release of products such as MDA, H_2O_2 and OH[55]. The excessive production of glucose derived free radicals in diabetes causes damage to cellular proteins, lipids and eventually cell death resulting in damages to the gastric mucosa[56]. In this study, there could be oxidative damage in the intestine of diabetic rats evidenced by the increase in H_2O_2 levels and MDA content, significant decrease in GSH, protein thiol and non-protein thiol levels and activities of the antioxidant enzyme systems (SOD, GPx and GST) in the diabetic control rats. Reduction in SOD activity could result from inactivation by H_2O_2or by glycation of the enzyme, which has been reported to occur in diabetes[57] 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 which can lead to inhibition of their enzymatic activity[58]. GPx and GST work together with glutathione in the decomposition of $H_2O_2$and other organic hydroperoxides to non-toxic products at the expense of the GSH[59]. The decreased activity of GST observed in diabetic state may be because of the inactivation caused by ROS[60]. Studies revealed that *Ocimum sanctum* species capable of neutralizing free radicals effectively decrease Ca^{2+} levels attributed to its antioxidant effects and reduce the severity of diabetic complications[61]. In this study, the content of these levels and activities of the antioxidant enzyme systems, though, reversed significantly when compared with the diabetic control further substantiate the ability to attenuate disturbed lipid metabolism normally associated with DM. Perceived low levels of non-enzymatic antioxidants (GSH, protein thiol and non-protein thiol) in intestinal tissues of untreated diabetic rats might be due to its utilization to alleviate the oxidative stress and levels of these antioxidants were elevated in in the intestinal homogenates of LTLP-treated rats, this implied that the LTLP possesses antioxidant properties, thus protecting the pancreas from free radicals. 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[13-15,34]. Review article revealed that oxidative stress acts on signal transduction and, via nuclear factor kappa light chain enhancer of activated B cells, affects gene expression of antioxidant enzymes, thereby reducing the expression of antioxidant enzymes[62]. Based on this, revamped the activity of antioxidant enzymes SOD,

GPx and GST in the intestinal homogenates of LTLP-treated rats could be the result of the ability to prevent the inactivation of the antioxidants thereby suggesting that hypoglycemic consequence of LTLP may be linked and mediated through modulation of cellular antioxidant defence system.

In this study, the improvement of histological recovery of both pancreatic and liver tissues provided additional support to the antidiabetic potencies of LTLP. Increased vasculature in pancreatic islets to levels reaching near those seen in normal control rat could justify the improved β-cell number and hence better insulin secretion and better glycemic control. The recovery of islets of Langerhans and cells within the islets of diabetic rat pancreas indicating hypoglycaemic activity of LTLP may be due to antioxidant activities of its glycosides, terpenoids and flavonoids which play a potentiating role in insulin secretion[8-9], results in reducing in β-cell cytotoxicity and restoration of β-cell function that may underlie LTLP stated anti-diabetic action[63]. Besides, hyperglycemia induced liver toxicity confirmed by histological studies; the hepatic toxicity leads to problem in metabolism observed by a vacuolation of hepatic cells with lymphocytic infiltration in liver. LTLP administration showed moderate inhibitory effects against alloxan induced diabetic rats, which basically supported the alterations observed in biochemical assays. Another view for the regenerative activities of pancreatic β-cells of LTLP treated diabetic induced rats is the improvement of carbohydrate metabolic enzymes activities as well as glycogen levels in liver and skeletal muscle[64]. As these biomarkers are under the positive control of insulin[65] so, it may be predicted that the LTLP may progress the pancreatic insulin synthesis or secretion through β-cell regeneration. Hence, presented regenerative effects with supplementation with LTLP can be considered as an expression of the functional improvement of hepatocytes and recovery towards normalization of liver function marker enzymes implicating its antioxidant role by stabilizing action at the membrane level towards normal liver cell function[13,16]. The restoration of gross architectural changes in liver histology may be a consequence of the re-established glucose control and not the direct effect of the LTLP on the liver cells themselves. Literature indicates that medicinal herbs that possess both antidiabetic and antioxidant activity mostly contain high concentrations of polyphenols such as tannins and flavonoids, steroid glycosides, and terpenoids[66]. The antidiabetic and antioxidant properties of LTLP 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 hyperglycaemic index and other associated biochemical indices.

CONCLUSION

The study thus implies that LTLP possesses antidiabetic properties and can prevent or protect diabetics from complications and can make for an efficient and effective alternate complementary medicine in management of DM. Increased insulin secretion after treatment with LTLP positively altered the deranged carbohydrate metabolism in the diabetic rats by decreasing gluconeogenesis and increasing glycolysis, ultimately decreasing hyperglycemia. The beneficial effect 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. 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 antihyperglycemic and antioxidant activities and their exact mechanism(s) of action.

ACKNOWLEDGMENT

The authors are thankful to research facilities from Department of Zoology, Government First Grade College, Hubballi, Karnataka, India and wish to duly acknowledgement to Central Animal Facility, IISc for their technical support.

REFERENCES

1. Zimmet P, Alberti KG, Magliano DJ, Bennett PH. Diabetes mellitus statistics on prevalence and mortality: facts and fallacies. Nature Reviews Endocrinology 2016; 12: 616–622.
2. World Health Organisation. WHO Study Group Report on Prevention of Diabetes Mellitus. WHO, Geneva. 1994; 1- 92.
3. Choudhury H, Pandey M, Hua CK, Mun CS, Jing JK, Kong L, Ern LY, Ashraf NA, Kit SW, Yee TS et al. An update on natural compounds in the remedy of diabetes mellitus: A systematic review. Journal of Traditional and Complementary Medicine 2018; 8: 361–376.
4. Verma S, Gupta M, Popli H, Aggarwal G. Diabetes Mellitus Treatment Using Herbal Drugs. International Journal of Phytomedicine 2018;10(1):1-10.
5. Walia S, Dua JS, Prasad DN. Herbal Drugs with Anti-Diabetic Potential. Journal of Drug Delivery and Therapeutics 2021; 11(6):248-256.
6. Singh D, Chaudhuri PK. A review on phytochemical and pharmacological properties of Holy basil (*Ocimum sanctum* L.). Industrial Crops and Products 2018; 118:367–382.
7. Garg N, Singh TG, 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 2022;12(1):1304- 1323.
8. Chattopadhyay RR. Hypoglycemic effects of *Ocimum Sanctum* leaf extract in normal and STZ diabetic rats. Indian Journal of Experimental Biology 1993; 3(11): 891-893.
9. Hannan JM, Marenah L, Ali L, Rokeya B, Flatt PR, Abdel-Wahab YH. *Ocimum sanctum* leaf extracts stimulate insulin secretion from perfused pancreas, isolated islets, and clonal pancreatic beta-cells. Journal of Endocrinology 2006; 189: 127–136.
10. Devra DK, Mathur KC, Agrawal RP, Bhadu I, Goyal S, Agarwal V. Effect of tulsi (*Ocimum sanctum* Linn) on clinical and biochemical parameters of metabolic syndrome. Journal of Natural Remedies 2012; 12(1): 63–67.
11. Somasundaram G, Manimekalai K, Salwe KJ, Pandiamunian J. Evaluation of the antidiabetic effect of *Ocimum sanctum* in type 2 diabetic patients. International Journal of Life Science and Pharma Research 2012; 5: 75–81.
12. Shittu STT, Oyeyemi WA, Lasisi TJ, Shittu SA, Lawal TT, Olujobi ST. Aqueous leaf extract of *Ocimum gratissimum* improves hematological parameters in alloxan-induced diabetic rats via its antioxidant properties. International Journal of Applied and Basic Medical Research 2016; 6: 96-100.
13. Ramzan T, Aslam B, Muhammad F, Faisal MN, Hussain A. Influence of *Ocimum sanctum* (L.) extract on the activity of Gliclazide in alloxan-induced diabetes in rats. Revista de Chimie (Rev. Chim) 2020; 71 (10):101-110.
14. Jayant SK, Sharma S, Srivastava N. Effect of *Ocimum sanctum* and *Allium sativum* on lipid peroxidation and antioxidant enzymes in alloxan induced diabetic rats. Bioscience Biotechnology Research Communications 2020;13 (2): 882-888.
15. Suanarunsawat T, Anantasomboon G, Piewbang C. Antidiabetic and anti-oxidative activity of fixed oil extracted from *Ocimum sanctum* L. leaves in diabetic rats. Experimental and Therapeutic Medicine 2016; 11: 832-840.
16. Aladakatti RH, Samuel RJ, Kadiyala A. Hepatoprotective evaluation of aqueous Tulsi leaf powder (*Ocimum sanctum*) against carbon tetrachloride induced liver toxicity in rats. International Journal of Advances in Science, Engineering and Technology 2020; 8(3): 9-15.
17. Jadaramkunti UC, Ghodesawar MG, Aladakatti RH. Wound healing potentials of *Ocimum sanctum* leaves extracts in Wistar albino rats. International Journal for Science and Advance Research in Technology 2020; 6(12), 217-224.
18. Khillare B, Shrivastv TG. Spermicidal activity of *Azadirachta indica* neem leaf extract. Contraception 2003;68: 225–229.
19. Hawk PB. Hawk's physiological chemistry. New York: McGraw-Hill Book Company. 1965; 83–1322.
20. Prout CK, Carruthers JR, Rossotti FJC. Structure and stability of carboxylate complexes. Part VII. Crystal and molecular structures of copper (II)meso-tartrate trihydrate and copper (II)d-tartrate trihydrate. Journal of the Chemical Society A: Inorganic Physical Theoretical 1971; 3336.
21. OECD-404 guidelines. Acute oral toxicity method. In: OECD guideline for testing of chemicals, No. 404. Organization for Economic Cooperation and Development, Paris, France 1987.
22. Sadasivam S, Manickam A. Carbohydrates, Sadasivam S, Manickam A Edition, Methods in Biochemistry, New Age International Pvt. Ltd, New Delhi 1996; 11–12.
23. Swanson MA. Glucose-6-phosphatase from liver, Colowick, S.P. Kaplan, N.O. Edition, Methods in Enzymol. Academic Press, Vol. II, New York 1955; 541-543.
24. Langdon RG (1966). Glucose-6-phosphate dehydrogenase from erythrocytes, Wood, W.A. Edition, Methods in Enzymol, Academic Press, New York 1966; 9: 126-131.
25. Chou AC, Wilson JE. Carbohydrate metabolism. In: Wood, W.A. Edition, Methods in Enzymology, Vol. XIII, Academic Press, New York. 1975; 20-21.
26. Woff SF. Ferrous ion oxidation in the presence of ferric ion indicator, xylenol orange for measurement of hydrogen peroxides. Methods in Enzymology 1994; 233: 182–189.
27. Varshney R, Kale RK. Effect of calmodulin antagonists on radiation induced lipid peroxidation in microsomes. International Journal of Biology 1990; 158: 733-741.
28. Jollow DJ, Mitchell JR, Zampaglione N Gillette JR. Bromobenzene induced liver necrosis. Protective role of glutathione and evidence for 3,4–bromobenzene oxide as the hepatotoxic metabolite. Pharmacology 1974; 11(3):151-169.
29. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Analytical Biochemistry 1968; 25: 192-205.
30. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a

simple assay for superoxide dismutasem. Journal of Biological Chemistry 1972; 217: 3170–3175.
31. Beutler EO, Duron B, Kelly M. Improved method for the determination of blood glutathione. Journal of Laboratory and Clinical Medicine 1963; 1: 882–888.
32. Haque R, Bin-Hafeez B, Parvez S, Pandey S, Sayeed I, Ali M, Raisuddin S. Aqueous extract of walnut (*Juglans regia* L.) protects mice against cyclophosphamide-induced biochemical toxicity. Human and Experimental Toxicology 2003; 22: 473–480.
33. Singh P, Jayaramaiah RH, Agawane SB, Vannuruswamy G, Korwar AM, Anand A. et al. Potential dual role of eugenol in inhibiting advanced glycation end products in diabetes: Proteomic and Mechanistic Insights. Scientific Reports 2016; 6:18798. DOI:10.1038/srep18798
34. Ezeani C, Ezenyi I, Okoye T, Okoli C. *Ocimum basilicum* extract exhibits antidiabetic effects via inhibition of hepatic glucose mobilization and carbohydrate metabolizing enzymes. Journal of Intercultural Ethnopharmacology 2017; 6(1): 22-28.
35. Buck W, Osweiter G, Van Gelder A. Clinical and Diagnostic Veterinary Toxicology. Kendall/ Hunt publishing Co, Iowa, 1976; 5211.
36. Ramachandran A, Snehalatha C. Diabetes prevention programs. Med Clin North Am 2011; 95 (2): 353–372.
37. Ohlsson A, Aher SM. Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Cochrane Database of Systematic Reviews 2012; 9: CD004863.
38. Enyievi PB, Mgbeje BIA, Nja GME, Edu BC, Ejemot-Nwadiaro RI. Effect of *Ocimum gratissimum* leaf-extract on hematological indices and lipid profile of streptozotocin-induced diabetic Wistar rats. Pakistan Journal of Biological Sciences 2020; 23(12):1523-1529.
39. Wintrobe, MM. Principles of hematologic examination. In: Wintrobe MM, Edition, Clinical hematology, 8th ed. Philadelphia: Lea and Febiger, 1981;7-19.
40. Sterner G, Carlson J, Ekberg G. Raised platelet levels in diabetes mellitus complicated with nephropathy. Journal of Internal Medicine 1998; 244: 437–441.
41. Uko EK, Erhabor O, Isaac IZ, Abdulrahaman Y, Adias TC, Sani Y. et al. Some haematological parameters in patients with type-I diabetes in Sokoto, North-western Nigeria. Journal of Blood and Lymph 2013; 3(1):2165–7831.
42. Vozarova B, Weyer C, Lindsay RS, Pratley RE, Bogardus C, Tataranni PA. High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. Diabetes 2002; 51: 455–461.
43. Schofield JD, Liu Y, Balakrishna PR, Malik RA, Soran H. Diabetes Dyslipidemia. Diabetes Therapy 2016; 7(2): 203–219.
44. 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. Journal of Pharmaceutical Research International 2021; 33(33A): 151-163.
45. Golden S, Wals PA, Okajima F, Katz J. Glycogen synthesis by hepatocytes from diabetic rats. Biochemical Journal 1979;182: 727-734.
46. Vats V, Yadav SP, Grover JK. Ethanolic extract of *Ocimum sanctum* leaves partially attenuates streptozotocin-induced alterations in glycogen content and carbohydrate metabolism in rats. Journal of Ethnopharmacology 2004; 90: 155–160.
47. Bouché C, Serdy S, Kahn CR, Goldfine AB. The cellular fate of glucose and its relevance in type 2 diabetes. Endocrine Reviews 2004; 25:807–830.
48. Petersen MC, Shulman GI. Mechanisms of Insulin Action and Insulin Resistance. Physiological Reviews 2018; 98: 2133–2223.
49. Gupta D, Raju J, Prakash J, Baquer NZ Change in the lipid profile, lipogenic and related enzymes in the livers of experimental diabetic rats. Effect of insulin and vanadate. Diabetes Research and Clinical Practice 1999; 46:1-7.
50. Murray RK, Granner DK, Mayes PA. Harper's Biochemistry. 25th Edition, Appleton and Lange, Stanford, Connecticut, 2000; 610-617.
51. Rodwell VW, Kennelly PJ. Enzymes: general properties. Harper's Biochemistry 1993; 68.
52. Srinivasulu K, Sivakumar E, Changamma C. The effective role of ursolic acid and *Ociumum sanctum* (Linn.) leaf extract on some marker carbohydrate metabolites in male albino rats. International Journal of Pharmaceutical Sciences and Research 2017; Vol. 8(11): 4624-4636.
53. Mahmoud MS. Protective and therapeutic effectiveness of taurine in diabetes mellitus: A rationale for antioxidant supplementation. Diabetes and Metabolic Syndrome: Clinical Research and Reviews 2015; 9: 55-64.
54. Lenzen S, Munday R. Thiol group reactivity, hydrophilicity and stability of alloxan, its reduction products and its N-methyl derivatives and a comparison with ninhydrin. Biochemical Pharmacology 1991; 42: 1385–1391.

55. Moldovan L, Moldovan NI. Oxygen free radicals and redox biology of organelles. Histochemistry and Cell Biology 2004;122(4): 395–412.
56. Chandrasekharan M, Anitha R, Blatt N, Shahnavaz D, Kooby C, Staley S. et al. Colonic motor dysfunction in human diabetes is associated with enteric neuronal loss and increased oxidative stress. Neurogastroenterology and Motility 2011; 23: 131–136.
57. Sozmen EY, Sözmen B, Delen Y, Onat T. Catalase/superoxide dismutase (SOD) and catalase/paraoxonase (PON) ratios may implicate poor glycemic control. Archives of medical research 2001; 32(4):283-287.
58. Jung HK. Modification and inactivation of human Cu,Zn-superoxide dismutase by methylglyoxal. Molecules and Cells2003;15(2): 194-199.
59. Bruce A, Freeman D, James C. Biology of disease free radicals and tissue injury. Laboratory Investigation1982; 47: 412–426.
60. Andallu B, Varadacharyulu NC. Antioxidant role of mulberry leaves in streptozotocin-diabetic rats. Clinica Chimica Acta 2003; 338: 3-10.
61. 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. Biomaterials 2020; 237: 119782.
62. Wiernsperger NF. Oxidative stress as a therapeutic target in diabetes: Revisiting the controversy. Diabetes and Metabolism 2003; 29: 579–582.
63. Sudhakar K, Mishra V, Hemani V, Verma A, Jain A, Jain S, Charyulu RN. Reverse pharmacology of phytoconstituents of food and plant in the management of diabetes: Current status and perspectives. Trends in Food Science and Technology 2021; 110: 594-610.
64. Barthel A, Schmoll D. Novel concepts in insulin regulation of hepatic gluconeogenesis. American Journal of Physiology-Endocrinology and Metabolism 2003; 285:685–692.
65. Barrett EJ. Insulin's on glucose production: Direct or indirect? Journal of Clinical Investigation 2003; 111:434-435.
66. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. Medicines 2018; 5: 93. doi:10.3390/medicines5030093