International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Antidiabetic effect of *Cleome gynandra* L extract and extract loaded solid lipid nanoparticles against streptozotocin-nicotinamide-induced diabetic rats

Alagiri ABARNADEVIKA[1], Sethuraman NAGALAKSHMI[2*], Ganesan ARIHARASIVAKUMAR[3], Dhakshinamoorthi ANUSHA[4]

[1]Department of Pharmacology, KMCH College of Pharmacy, Coimbatore, Tamil Nadu, India.
[2]Department of Pharmaceutics, Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu, India.*
[3]Department of Pharmacology, KMCH College of Pharmacy, Coimbatore, Tamil Nadu, India.
[4]Department of Pharmacology, Sri Ramachandra Medical College and Research institute, Chennai, Tamil Nadu, India.

Abstract

Diabetes mellitus is a group of metabolic syndromes characterized by fasting hyperglycemia, postprandial hyperglycemia, and hyperlipidemia, resulting from defects in carbohydrate, fat, and protein metabolism. An increasing number of people are switching to using herbs and natural remedies to cure or prevent diabetes. It has been demonstrated that *Cleome gynandra* L ingestion offers therapeutic advantages. Additionally, it has a lot of phenolic chemicals that can treat conditions including cancer, asthma, diabetes, and cardiovascular illnesses. Numerous research has recently concentrated on the encapsulation of plant extracts to increase the prolonged release of active ingredients in the gut for optimum absorption. The goal of the current work was to create *Cleome gynandra* L extract-loaded solid lipid nanoparticles for the treatment of diabetes. After heat homogenization, an ultrasonic process was used to create solid lipid nanoparticles loaded with *Cleome gynandra* L extract. The streptozotocin-nicotinamide-induced diabetic animal model was used for the evaluation of the antidiabetic activity of *Cleome gynandra* L extract-loaded solid lipid nanoparticles. Against streptozotocin-nicotinamide-induced diabetic rats, the *Cleome gynandra* L extract and extract-loaded solid lipid nanoparticles effectively generated antidiabetic activity. The invention of solid lipid nanoparticles may increase the activity of *Cleome gynandra* L extract and allow for dose reduction. It was concluded that the lower dose, the solid lipid nanoparticles may significantly increase *Cleome gynandra* L extract's bioavailability and exhibit considerable antidiabetic efficacy.

Keywords: *Cleome gynandra*; antihyperglycemic; antioxidant; hepatic key enzyme

Full length article **Corresponding Author*, e-mail: nagalakshmi.s@sriramachandra.edu.in

1. Introduction

Diabetes mellitus is a group of metabolic syndromes characterized by fasting hyperglycemia, postprandial hyperglycemia, and hyperlipidemia, resulting from defects in carbohydrate, fat, and protein metabolism [1,2], an estimated 537 million adults aged 20-79 years worldwide (10.5% of all adults in this age group) have diabetes. By 2030, 643 million, and by 2045, 783 million adults aged 20-79 years are projected to be living with diabetes. Thus, while the world's population is estimated to grow by 20% over this period, the number of diabetes is estimated to increase by 46% [3]. Treatment of diabetes without side effects and dyslipidemia is still a challenge to the medical community [4]. As an alternative, a greater number of people are seeking herbs/natural products to prevent or treat diabetes [5]. For thousands of years plants and their derivatives are being used for the treatment of diabetes mellitus [6]. Although, herbal medicines have long been used effectively in treating diseases throughout the world and are frequently considered to be less toxic and free from side effects as compared to synthetic ones [7]. The

phytochemical constituents of the plant are important sources of natural antioxidants and the plant extract's efficacy is more when consumed as a crude extract [8, 9]. Therefore, the management of diabetes mellitus is considered a global problem and successful treatment is yet to be discovered. The Cleomaceae family plant has highly nutritive and contains health-promoting bioactive compounds important in combating malnutrition and reducing human degenerative diseases [10]. Spider plant (*Cleome gynandra* L) is an indigenous leafy vegetable, which has been widely consumed by the majority of the rural population in Zimbabwe. About 90 % of the rural population lives in arid regions and relies on indigenous leafy vegetables for relish in Zimbabwe [10]. Besides being a source of relish, the fresh spider plant is rich in proteins, vitamins, carbohydrates, and minerals, which are lacking in most leafy vegetables. The consumption of *Cleome gynandra* L has been shown to have medicinal benefits. It also contains many phenol compounds that can cure diseases such as cancer, asthma, diabetes, and cardiovascular diseases [13]. However, the major drawback is that the quantity of herbal extract necessary for treatment is higher due to the degradation of different plant constituents such as alkaloids, amides, phenols, steroids, and hydrocinnamic acid in the gastrointestinal tract since they are very sensitive to the acidic pH of the stomach, which promotes their destruction, and loss of the desired effect and a longer duration of treatment is needed due to the poor absorption of these constituents in the intestine [11]. Recently, many studies focused on the encapsulation of plant extracts to augment the sustained release of active constituents in the intestine for maximum absorption [12, 13]. Therefore, the current study aimed to develop solid lipid nanoparticles loaded with *Cleome gynandra* L extract for the treatment of diabetes.

2. Materials and Methods

Streptozotocin, Nicotinamide, and Glibenclamide were purchased from Sigma Aldrich. The fresh aerial parts of the *Cleome gynandra* L were collected from Kalapatti, Coimbatore, Tamil Nadu, India, in May 2019 and authenticated from the Botanical Survey of India, Coimbatore (BSI/SRC/5/23/2019/Tech).

2.1. Extraction of the plant material

About 1 kg of finely powdered powder of *Cleome gynandra* L was defatted with petroleum ether in a Soxhlet apparatus for 48 h, and obtained marc was further extracted with ethanol in Soxhlet apparatus for 48 h [9]. After extraction, the extracts were separately concentrated by distillation and dried at room temperature until a viscous solid mass was obtained. The obtained crude extracts were weighed and stored at 4^0C for further analysis [14].

2.2. Preparation of Cleome gynandra L extract loaded solid lipid nanoparticle

Cleome gynandra L extract loaded solid lipid nanoparticle was prepared by hot homogenization method followed by ultra sonication. In the hot homogenization method, the solid lipid and stearic acid were dissolved in a mixture of chloroform and methanol in the ratio of 1:1, to which ethanolic extract of *Cleome gynandra* L (EECG) was added. Then this mixture was taken into evaporation and all the organic solvents were completely removed. The resulting residue was melted approximately 5-10°C above the melting point of the lipid. A 2% aqueous surfactant solution of 100 mL is prepared and heated to the same temperature as the lipid phase. Then the hot aqueous surfactant solution was added to the lipid phase. Homogenization was carried out at 15000 rpm by using a High-speed homogenizer for 3 h. The temperature was maintained at 5-10°C above the melting point of the lipid to prevent lipid recrystallization. After homogenization is finished, the obtained coarse emulsion is allowed to cool to room temperature. The dispersion is then ultra-sonicated using a probe sonicator processor for 30 min [15, 16].

2.3. Experimental animals

The Wistar rats of 6-8 weeks old and 160-180 g body weight were obtained from KMCH College of Pharmacy, Coimbatore. All rats were housed and maintained under standard conditions of temperature (25±5°C), relative humidity (55±10%), and 12/12 h light/dark cycle. Protocols for the study were approved by the Institutional Animal Ethical Committee (IAEC) for animal care and were in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Government of India (KMCRET/Ph.D/01/2019-20).

2.4. Acute toxicity study

According to OECD-423 recommendations, research on acute oral toxicity was conducted. The rats were divided into eight groups of three animals each, fasted overnight, and given access to free extra water before receiving oral doses of EECG and EECG-loaded solid lipid nanoparticles (EECG-SLN) of 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg body weight. They were observed for mortality and toxic symptoms periodically for 30 min during the first 24 h and specific attention was given during the first 4 h daily for a total period of 14 days [17].

2.5. Evaluation of the antidiabetic activity of EECG and EECG-SLN in STZ-NIC-induced type 2 diabetic rats

The animals were divided into seven groups of six animals each. The grouping of animals was listed in Table 1. The rats were kept fasting for the whole night, and the rat tail vein was used to examine the animals' initial fasting blood glucose levels. Nicotinamide (NIC) was dissolved in sterile saline, and streptozotocin (STZ) was dissolved in citrate buffer (pH 4.5). A single intraperitoneal dose of 60 mg/kg STZ was administered to overnight-fasted rats 15 minutes after 120 mg/kg NIC was administered. This caused the rats to develop non-insulin-dependent diabetic mellitus [18]. Diabetes was confirmed by the elevated levels of blood glucose determined at 72 h. The animals with blood glucose concentrations more than 200 mg/dL were used for the study. The vehicle (saline), standard Glibenclamide, EECG, and EECG-SLN were administered to the respective group animals for 28 days. Throughout the study period

Glibenclamide, EECG, and EECG-SLN were freshly dispersed in normal saline before the administration. The fasting animal's body weight, and blood glucose level were estimated on 0, 7th, 14th, 21st, and 28th day from the tip of the rat tail vein [19, 20, 21].

2.6. Estimation of serum biochemical parameters

The blood samples were taken from the retro-orbital sinus using centrifugation tubes at the conclusion of the 21-day treatment period. Using the centrifugation technique, the serum was separated from the blood. Additionally, using common enzymatic kits, serum SGOT, SGPT, HDL, Triglycerides, Total Cholesterol, Urea, and Creatinine levels were calculated [22,23,24].

2.7. Analysis of antioxidant and hepatic key enzyme levels in liver

After treatment with EECG and EECG-SLN, the rats were euthanized by ketamine HCl, the liver was isolated and washed with normal saline and stored for 12 h for *in vivo* antioxidant studies. Using a Teflon-covered homogenizer, 10% of liver homogenate was prepared with 0.1 M Tris-HCl buffer (pH 7.4). The supernatant was used for the study of hexokinase, glucose 6-phosphatase, catalase (CAT), reduced glutathione (GSH), Superoxide Dismutase (SOD), and lipid peroxidation (LPO) levels. The standard procedure was followed as per the literature [25,26].

2.8. Statistical analysis

Statistical calculations [mean ± standard deviation (SD)] were analyzed by one-way analysis of variance (ANOVA), and significant differences were determined by Dunnett's post hoc test using GraphPad Instat version 3.06 computer software.

3. Results and discussion

The formulated EECG-SLN was milky white in appearance and odorless. It was stable and did not show sedimentation even after centrifugation (2000 rpm for 30 min). The EECG did not cause any drug-related toxicity or mortality in the Wistar rats up to 2000 mg/kg. However, EECG-SLN caused mortality at the dose of 2000 mg/kg and did not show any mortality up to the dose of 300 mg/kg. So, 200 and 400 mg/kg doses were selected for EECG and 30 and 60 mg/kg doses were selected for EECG-SLN in further antidiabetic evaluation in experimental animals. Diabetes in rats was manifested via intraperitoneal administration of STZ-NIC. The STZ administration alone might bring about hyperglycemia via fatal devastation of Langerhans of β-cells [27]. Adjacent to this in a current experimental model combination of STZ with NIC exhibits hyperglycemia and reduce glucose resistance may cause DNA strand breaks which may lead to the progression of type-II diabetes [28]. However, β-cells are still able to release insulin in the presence of sugar, which is likely to be the same as type II Diabetes [29]. The effect of EECG and EECG-SLN on blood glucose levels of STZ-NIC-induced diabetic rats are shown in Figure 1, oral administration of EECG and EECG-SLN for 28 days showed a significant reduction in blood glucose level. The possible mechanism by which the EECG reduced the blood glucose level in diabetic rats might be due to the stimulation of surviving β-cells leading to an increase in insulin secretion. The effect of EECG and EECG-SLN on the body weights of STZ-NIC-induced diabetic rats is shown in Figure 2. A decrease in body weight in STZ-NIC-induced type 2 diabetic rats was observed during the study. This notable decrease in body weight in diabetic rats might be because of protein wasting or degradation of structural proteins [30]. The loss of protein is probably due to the unavailability of carbohydrates for energy metabolism [31]. Diabetic rats treated with EECG and EECG-SLN showed an improvement in body weight as compared to the diabetic control rats which may be due to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis [32]. The effect of EECG and EECG-SLN on serum lipid levels of STZ-NIC-induced diabetic rats is shown in Figure 3. In the lipid profile, diabetic rats exhibited marked elevation of serum triglycerides and total cholesterol and reduction of HDL levels. The treatment of STZ-NIC-induced diabetic rats with EECG and EECG-SLN produced significant improvement in these altered serum lipid variables [33]. The effect of EECG and EECG-SLN on kidney functional parameter levels of STZ-NIC-induced diabetic rats is shown in Figure 4. Serum urea and creatinine levels demonstrate the role of EECG and EECG-SLN in renal dysfunction. An augmented level of urea and creatinine cause osmotic diuresis and depletion of extracellular fluid volume in the diabetic condition in the serum of STZ-NIC-induced diabetic rats [34]. The EECG (200 and 400 mg/kg) and EECG-SLN (30 and 60 mg/kg) treated groups showed a declined urea and creatinine levels.
The effect of EECG and EECG-SLN on liver functional parameter levels of STZ-NIC-induced diabetic rats is shown in Figure 5. Increased activities of SGOT and SGPT are common signs of liver disease [35]. In STZ-NIC-induced type 2 diabetic rats increase in serum SGOT and SGPT levels may be due to the leakage of these enzymes from the liver cytosol into the bloodstream as a result of the hepatotoxic effect of STZ. Treatment with EECG and EECG-SLN decreased the levels of SGOT and SGPT in diabetic animals, which indicates that the EECG and EECG-SLN tend to prevent liver damage in diabetes by maintaining the integrity of plasma membrane, thereby suppressing the leakage of enzymes through the membrane [36, 37, 38]. In experimental diabetes, enzymes of glucose metabolism are markedly altered. One of the key enzymes in the catabolism of glucose is hexokinase, which phosphorylates glucose and converts it into glucose-6-phosphate [39, 40]. The effect of EECG and EECG-SLN on carbohydrate metabolic enzyme levels of STZ-NIC-induced diabetic rats are shown in Figure 6. Hexokinase insufficiency in STZ-NIC-induced diabetic rats can cause decreased glycolysis and decreased utilization of glucose for energy production [41]. Administration of EECG, EECG-SLN, and Glibenclamide to STZ-NIC-induced diabetic rats resulted in a significant reversal in the activity of hexokinase. The increased decreased glucose in diabetic rats given EECG and EECG-SLN may also be a result of increased hepatic hexokinase activity, thereby increasing glycolysis.

Table 1. Grouping of animals for STZ-NIC-induced diabetic model

Groups	Sample size	Group specification
Group I	6	Only normal saline
Group II	6	STZ 60 mg/kg/b.w. (i.p) +NIC 120 mg/kg (i.p)
Group III	6	STZ (60 mg/kg) NIC 120 mg/kg (i.p) rats treated with Glibenclamide 20 mg/kg (p.o)
Group IV	6	STZ (60 mg/kg) +NIC 120 mg/kg (i.p) rats treated with EECG 200 mg/kg (p.o)
Group V	6	STZ (60 mg/kg) +NIC 120 mg/kg (i.p) rats treated with EECG 400 mg/kg (p.o)
Group VI	6	STZ (60 mg/kg) +NIC 120 mg/kg (i.p) rats treated with EECG-SLN 30 mg/kg (p.o)
Group VII	6	STZ (60 mg/kg) + NIC 120 mg/kg (i.p) rats treated with EECG-SLN 60 mg/kg (p.o)

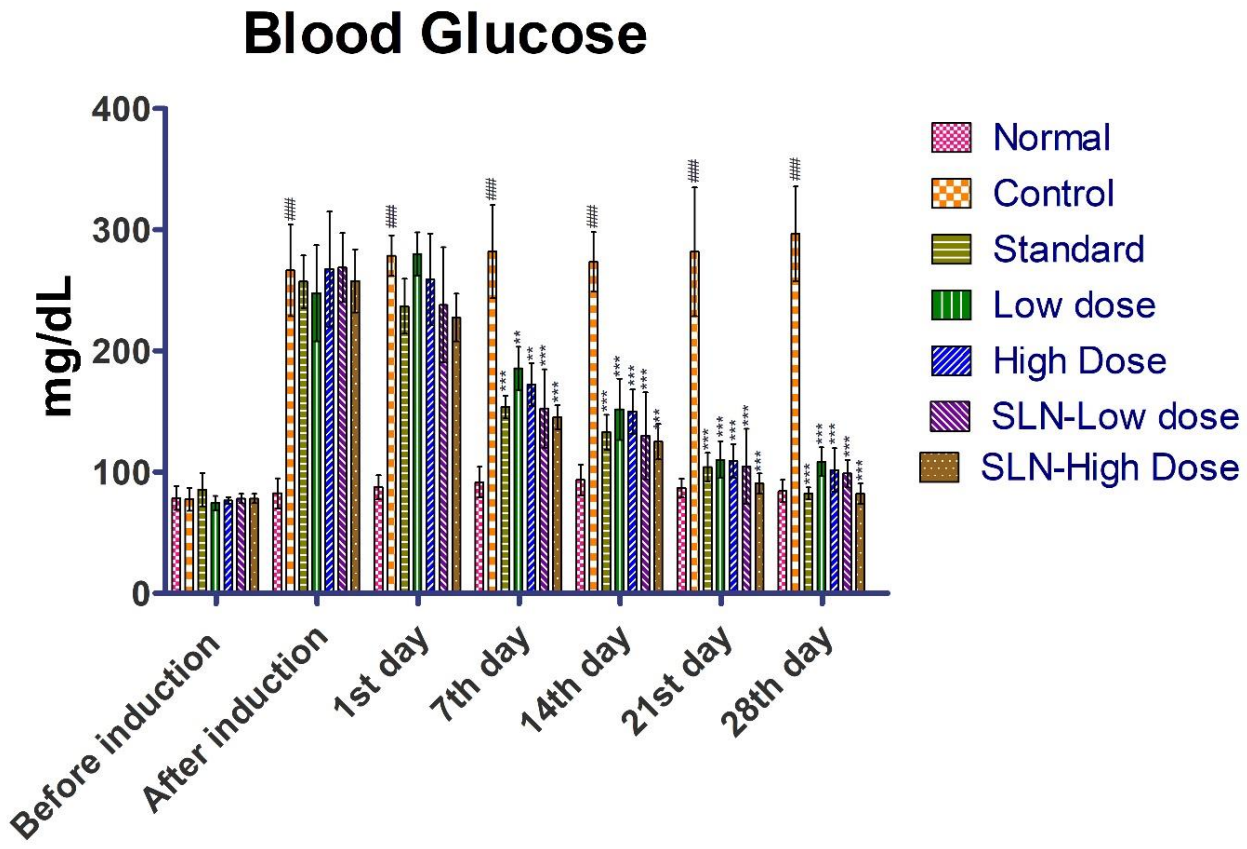

Figure 1. Effect of EECG and EECG-SLN on blood glucose levels of streptozotocin-nicotinamide-induced diabetic rats.

(Group I is the normal group, Group II is disease control, Group III is the standard Glibenclamide treated group, Group IV and V are treated with EECG at the dose of 200 and 400 mg/kg, Group VI and VII are treated with EECG-SLN at the dose of 30 and 60 mg/kg. Data is expressed as mean ± SD; n=6, One way ANOVA followed by Dunnett's test; ns = non-significant; Compared with normal control; # $p<0.05$, ## $p<0.01$, ### $p<0.001$ Compared with disease control; * $p<0.05$, ** $p<0.01$, *** $p<0.001$).

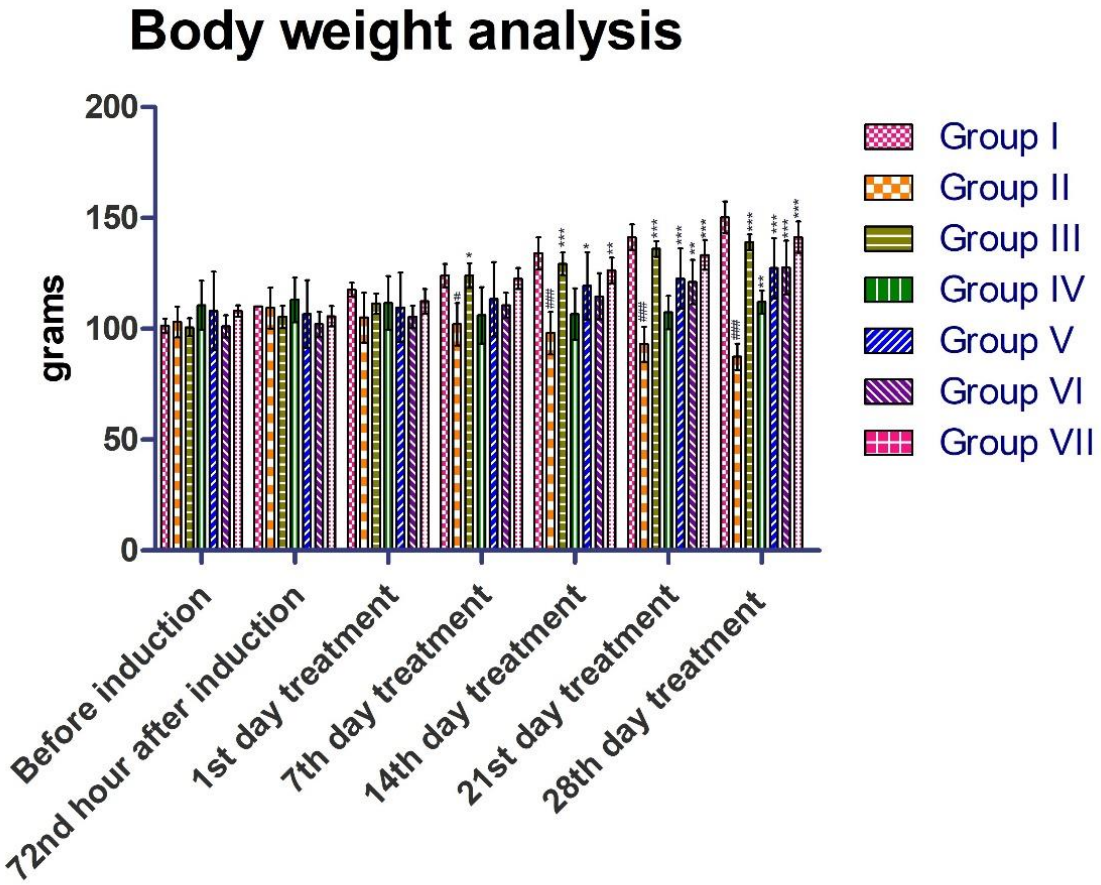

Figure 2. Effect of EECG and EECG-SLN on body weights of streptozotocin-nicotinamide-induced diabetic rats.

(Group I is the normal group, Group II is disease control, Group III is the standard Glibenclamide treated group, Group IV and V are treated with EECG at the dose of 200 and 400 mg/kg, Group VI and VII are treated with EECG-SLN at the dose of 30 and 60 mg/kg. Data is expressed as mean ± SD; n=6, One way ANOVA followed by Dunnett's test; ns = non-significant; Compared with normal control; # $p<0.05$, ## $p<0.01$, ### $p<0.001$ Compared with disease control; * $p<0.05$, ** $p<0.01$, *** $p<0.001$).

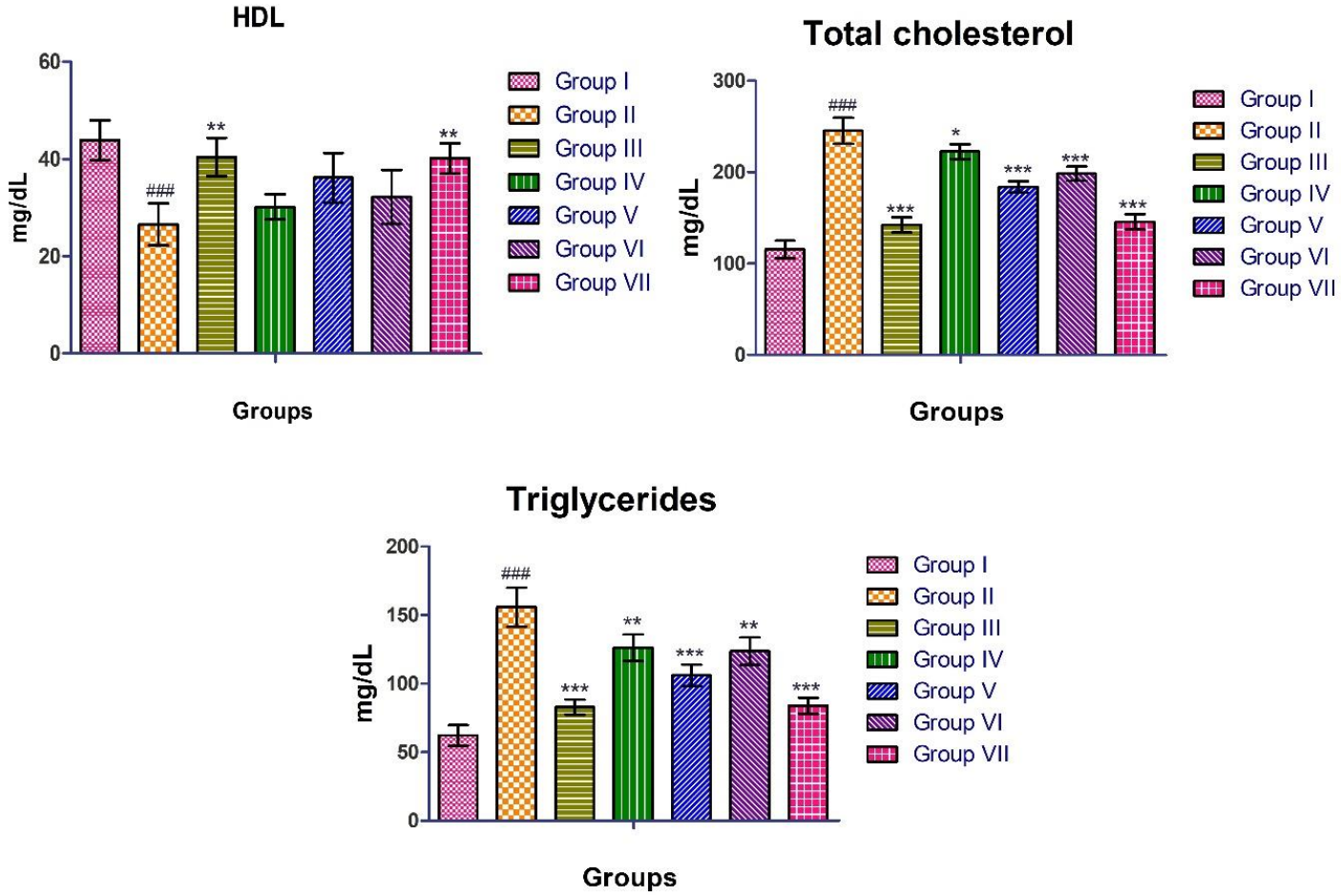

Figure 3. Effect of EECG and EECG-SLN on serum lipid levels of streptozotocin-nicotinamide-induced diabetic rats.

(Group I is the normal group, Group II is disease control, Group III is the standard Glibenclamide treated group, Group IV and V are treated with EECG at the dose of 200 and 400 mg/kg, Group VI and VII are treated with EECG-SLN at the dose of 30 and 60 mg/kg. Data is expressed as mean ± SD; n=6, One way ANOVA followed by Dunnett's test; ns = non-significant; Compared with normal control; # $p<0.05$, ## $p<0.01$, ### $p<0.001$ Compared with disease control; * $p<0.05$, ** $p<0.01$, *** $p<0.001$).

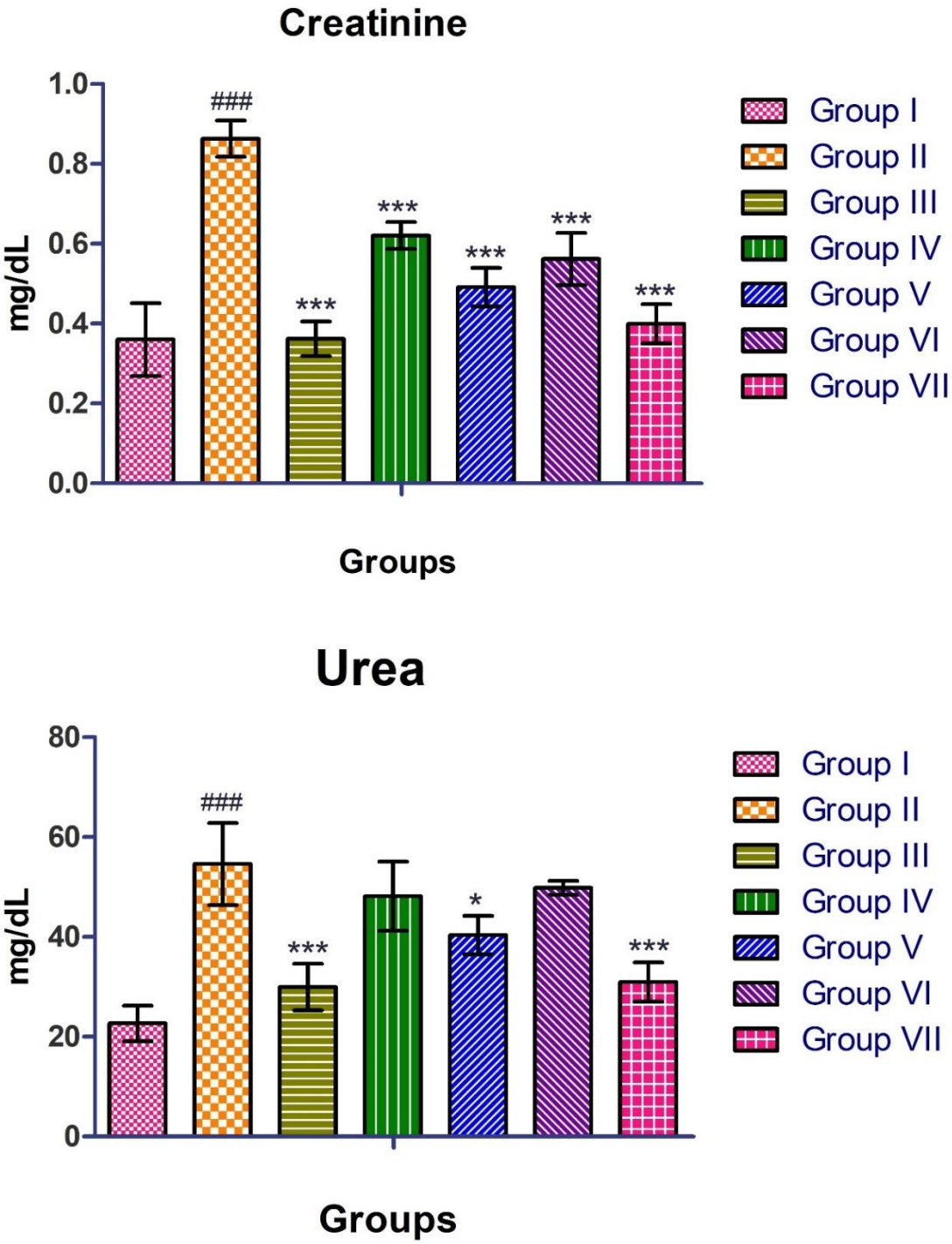

Figure 4. Effect of EECG and EECG-SLN on kidney functional parameter levels of streptozotocin-nicotinamide-induced diabetic rats.

(Group I is the normal group, Group II is disease control, Group III is the standard Glibenclamide treated group, Group IV and V are treated with EECG at the dose of 200 and 400 mg/kg, Group VI and VII are treated with EECG-SLN at the dose of 30 and 60 mg/kg. Data is expressed as mean ± SD; n=6, One way ANOVA followed by Dunnett's test; ns = non-significant; Compared with normal control; # $p<0.05$, ## $p<0.01$, ### $p<0.001$ Compared with disease control; * $p<0.05$, ** $p<0.01$, *** $p<0.001$).

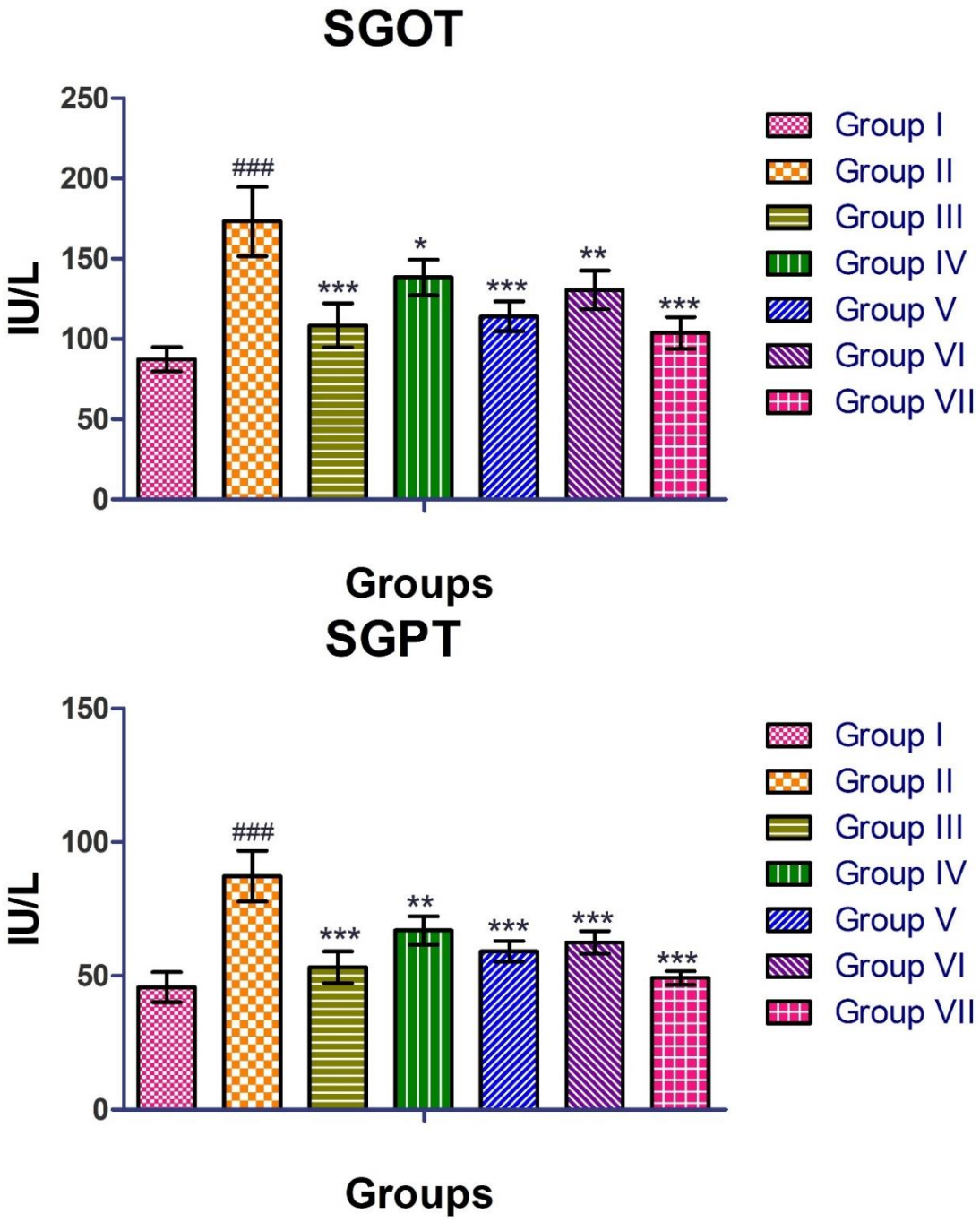

Figure 5. Effect of EECG and EECG-SLN on liver functional parameter levels of streptozotocin-nicotinamide-induced diabetic rats.

(Group I is the normal group, Group II is disease control, Group III is the standard Glibenclamide treated group, Group IV and V are treated with EECG at the dose of 200 and 400 mg/kg, Group VI and VII are treated with EECG-SLN at the dose of 30 and 60 mg/kg. Data is expressed as mean ± SD; n=6, One way ANOVA followed by Dunnett's test; ns = non-significant; Compared with normal control; # $p<0.05$, ## $p<0.01$, ### $p<0.001$ Compared with disease control; * $p<0.05$, ** $p<0.01$, *** $p<0.001$).

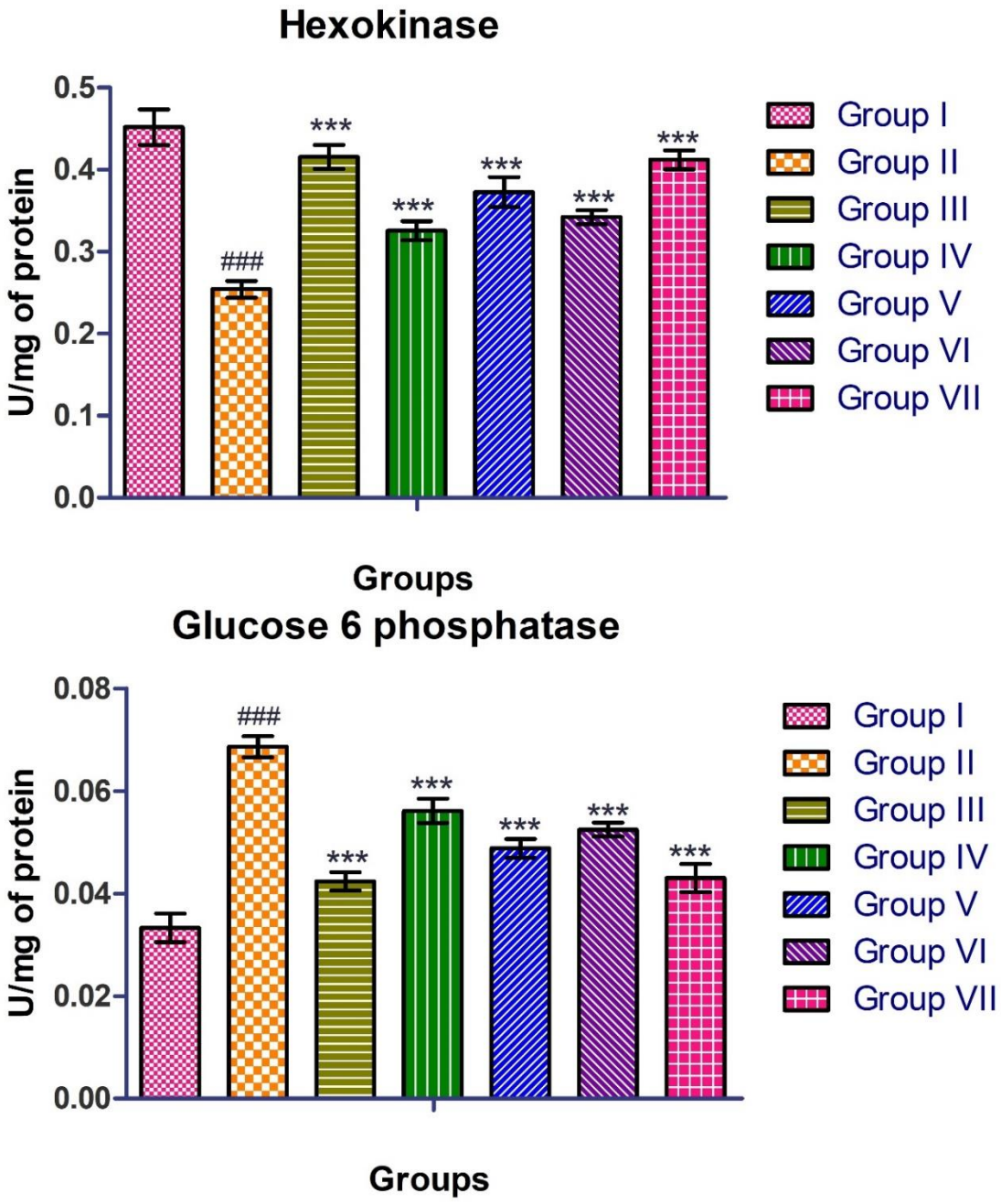

Figure 6. Effect of EECG and EECG-SLN on carbohydrate metabolic enzyme levels of streptozotocin-nicotinamide-induced diabetic rats.

(Group I is the normal group, Group II is disease control, Group III is the standard Glibenclamide treated group, Group IV and V are treated with EECG at the dose of 200 and 400 mg/kg, Group VI and VII are treated with EECG-SLN at the dose of 30 and 60 mg/kg. Data is expressed as mean ± SD; n=6, One way ANOVA followed by Dunnett's test; ns = non-significant; Compared with normal control; # $p<0.05$, ## $p<0.01$, ### $p<0.001$ Compared with disease control; * $p<0.05$, ** $p<0.01$, *** $p<0.001$).

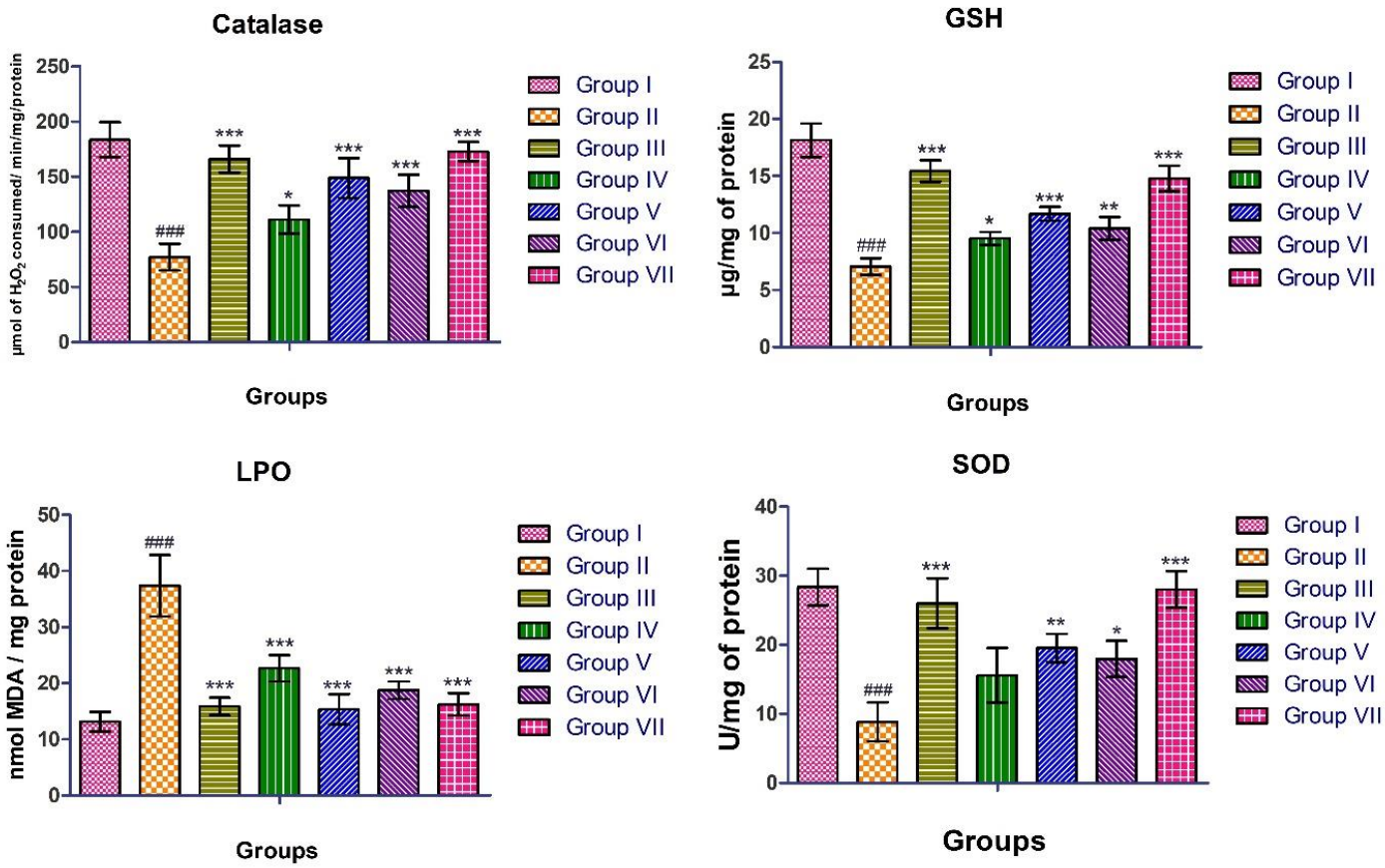

Figure 7. Effect of EECG and EECG-SLN on *in vivo* antioxidant levels of streptozotocin-nicotinamide-induced diabetic rats.

(Group I is the normal group, Group II is disease control, Group III is the standard Glibenclamide treated group, Group IV and V are treated with EECG at the dose of 200 and 400 mg/kg, Group VI and VII are treated with EECG-SLN at the dose of 30 and 60 mg/kg. Data is expressed as mean ± SD; n=6, One way ANOVA followed by Dunnett's test; ns = non-significant; Compared with normal control; # $p<0.05$, ## $p<0.01$, ### $p<0.001$ Compared with disease control; * $p<0.05$, ** $p<0.01$, *** $p<0.001$).

The gluconeogenic enzyme glucose-6-phosphatase is a crucial enzyme of glucose homeostasis because it catalyzes the ultimate biochemical reaction of both glycogenolysis and gluconeogenesis [42]. Increased glucose-6- phosphatase activity in STZ-NIC-induced diabetic rats provides hydrogen, which binds with NADP+ in the form of NADPH and enhances the synthesis of fats from carbohydrates (i.e. lipogenesis) [43] and, finally contributes to increased levels of glucose in the blood. The STZ-NIC-induced diabetic rats treated with EECG and EECG-SLN significantly declined the elevated glucose-6-phosphatase levels when compared with STZ-NIC-induced diabetic rats. Antioxidant enzymes form the first line of defense against reactive oxygen species (ROS) in the cells including GSH, CAT, and SOD which play a key role in scavenging the toxic intermediate of incomplete oxidation [44] (Das and Roychoudhury, 2014).

A reduction in the activities of these antioxidant enzymes can result in lead to initiation and propagation of LPO. GSH protects cells against ROS by scavenging free radicals, which damages the membrane and biological structures [45]. SOD can catalyze the dismutation of ($O2^{\circ-}$) into H_2O_2 and then deactivate to H_2O by CAT [46]. The effect of EECG and EECG-SLN on *in vivo* antioxidant levels of streptozotocin-nicotinamide-induced diabetic rats is shown in Figure 7. The STZ-NIC-induced diabetic rats treated with EECG and EECG-SLN significantly reduced LPO levels and significantly increased antioxidant enzymes CAT, GSH, and SOD levels, which confirms that EECG and EECG-SLN can reduce oxidative stress during diabetes [47]. The EECG-SLN at the dose of 60 mg/kg produced a similar effect to the standard Glibenclamide and higher than EECG,

it suggested that the development of solid lipid nanoparticles may improves the activity of EECG and reduced the dose.

4. Conclusions

From the current research work results, we concluded that EECG and EECG-SLN produced significant antidiabetic activity in STZ-NIC-induced diabetic rats. The solid lipid nanoparticles may improve the bioavailability of EECG and produce significant antidiabetic activity at the lower dose.

Conflict of Interest

The author reports no conflicts of interest in this work.

Acknowledgments

None.

Ethical statement

The study was approved by Institutional Animal Ethical Committee (IAEC) in KMCH College of Pharmacy, Coimbatore, Tamilnadu, India for animal care and were in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Government of India (KMCRET/Ph.D/01/2019-20).

Availability of data and material

We declare that the submitted manuscript is our work, which has not been published before and is not currently being considered for publication elsewhere.

Consent to participate

All authors participated in this research study.

Consent for publication

All authors submitted consent to publish this article research in IJCBS.

References

[1] American Diabetes Association. (2010). Diagnosis and classification of diabetes mellitus. Diabetes care. 33 (Supplement_1) S62-S69.

[2] U. Galicia-Garcia, A. Benito-Vicente, S. Jebari, A. Larrea-Sebal, H. Siddiqi, K.B. Uribe, C. Martín. (2020). Pathophysiology of type 2 diabetes mellitus. International journal of molecular sciences. 21 (17) 6275.

[3] D. Atlas. (2015). International diabetes federation. IDF Diabetes Atlas, 7th edn. Brussels, Belgium: International Diabetes Federation. 33 (2).

[4] A. Pandey, P. Tripathi, R. Pandey, R. Srivatava, S. Goswami. (2011). Alternative therapies useful in the management of diabetes: A systematic review. Journal of pharmacy & bioallied sciences. 3 (4) 504.

[5] A. Alqathama, G. Alluhiabi, H. Baghdadi, L. Aljahani, O. Khan, S. Jabal, F. Alhomoud. (2020). Herbal medicine from the perspective of type II diabetic patients and physicians: what is the relationship?. BMC complementary medicine and therapies. 20 (1) 1-9.

[6] N. Tran, B. Pham, L. Le. (2020). Bioactive compounds in anti-diabetic plants: From herbal medicine to modern drug discovery. Biology. 9 (9) 252.

[7] M. Ekor. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Frontiers in pharmacology. 4 177.

[8] D.P. Xu, Y. Li, X. Meng, T. Zhou, Y. Zhou, J. Zheng, H.B. Li. (2017). Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. International journal of molecular sciences. 18 (1) 96.

[9] A. Altemimi, N. Lakhssassi, A. Baharlouei, D.G. Watson, D.A. Lightfoot. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants. 6 (4) 42.

[10] C.V. Mashamaite, A. Manyevere, E. Chakauya. (2022). Cleome gynandra: A wonder climate-smart plant for nutritional security for millions in semi-arid areas. Frontiers in Plant Science. 13 1003080.

[11] S.H. Ansari, F. Islam, M. Sameem. (2012). Influence of nanotechnology on herbal drugs: A Review. Journal of advanced pharmaceutical technology & research. 3 (3) 142.

[12] H.S. Rahman, H.H. Othman, N.I. Hammadi, S.K. Yeap, K.M. Amin, N. Abdul Samad, N.B. Alitheen. (2020). Novel drug delivery systems for loading of natural plant extracts and their biomedical applications. International journal of nanomedicine. 2439-2483.

[13] A. Kyriakoudi, E. Spanidi, I. Mourtzinos, K. Gardikis. (2021). Innovative delivery systems loaded with plant bioactive ingredients: Formulation approaches and applications. Plants. 10 (6) 1238.

[14] A.R. Abubakar, M. Haque. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of pharmacy & bioallied sciences. 12 (1) 1.

[15] N.M. Badawi, M.H. Teaima, K.M. El-Say, D.A. Attia, M.A. El-Nabarawi, M.M. Elmazar. (2018). Pomegranate extract-loaded solid lipid nanoparticles: design, optimization, and in vitro cytotoxicity study. International journal of nanomedicine. 1313-1326.

[16] N. Badawi, K. El-Say, D. Attia, M. El-Nabarawi, M. Elmazar, M. Teaima. (2020). Development of pomegranate extract-loaded solid lipid nanoparticles: quality by design approach to screen the variables affecting the quality attributes and characterization. ACS omega. 5 (34) 21712-21721.

[17] OECD. (1994). OECD Guidelines for the Testing of Chemicals. Oecd.

[18] K. Gopalasatheeskumar, G. Ariharasivakumar, V.K. Kalaichelvan, T. Sengottuvel, V.S. Devan, V. Srividhya. (2020). Antihyperglycemic and antihyperlipidemic activities of wild musk melon (Cucumis melo var. agrestis) in streptozotocin-nicotinamide induced diabetic rats. Chinese Herbal Medicines. 12 (4) 399-405.

[19] R. Rani, S. Dahiya, D. Dhingra, N. Dilbaghi, A. Kaushik, K.H. Kim, S. Kumar. (2019). Antidiabetic activity enhancement in streptozotocin+ nicotinamide–induced diabetic rats through combinational polymeric nanoformulation. International journal of nanomedicine. 4383-4395.

[20] R. Barik, S. Jain, D. Qwatra, A. Joshi, G.S. Tripathi, R. Goyal. (2008). Antidiabetic activity of aqueous root extract of Ichnocarpus frutescens in streptozotocin-nicotinamide induced type-II diabetes in rats. Indian Journal of Pharmacology. 40 (1) 19-22.

[21] S. Pottathil, P. Nain, M.A. Morsy, J. Kaur, B.E. Al-Dhubiab, S. Jaiswal, A.B. Nair. (2020). Mechanisms of antidiabetic activity of methanolic extract of punica granatum leaves in nicotinamide/streptozotocin-induced type 2 diabetes in rats. Plants. 9 (11) 1609.

[22] H.B. Yazdi, V. Hojati, A. Shiravi, S. Hosseinian, G. Vaezi. (2019). Liver dysfunction and oxidative stress in streptozotocin-induced diabetic rats: protective role of Artemisia turanica. Journal of pharmacopuncture. 22 (2) 109.

[23] K. Swapna, V.V. Sathibabu Uddandrao, B. Parim, R. Ravindarnaik, P. Suresh, P. Ponnusamy, G. Saravanan. (2019). Effects of asiatic acid, an active constituent in Centella asiatica (L.): restorative perspectives of streptozotocin-nicotinamide induced changes on lipid profile and lipid metabolic enzymes in diabetic rats. Comparative Clinical Pathology. 28 1321-1329.

[24] A. Ahangarpour, H.T. Zamaneh, A. Jabari, H.M. Nia, H. Heidari. (2014). Antidiabetic and hypolipidemic effects of Dorema aucheri hydroalcoholic leave extract in streptozotocin-nicotinamide induced type 2 diabetes in male rats. Iranian journal of basic medical sciences. 17 (10) 808.

[25] U. Sharma, R.K. Sahu, A. Roy, D.K. Golwala. (2010). In vivo antidiabetic antioxidant potential of Stephania hernandifolia in streptozotocin-induced-diabetic rats. Journal of Young Pharmacists. 2 (3) 255-260.

[26] R. Chandran, T. Parimelazhagan, S. Shanmugam, S. Thankarajan. (2016). Antidiabetic activity of Syzygium calophyllifolium in Streptozotocin-Nicotinamide induced Type-2 diabetic rats. Biomedicine & Pharmacotherapy. 82 547-554.

[27] M.L. Graham, J.L. Janecek, J.A. Kittredge, B.J. Hering, H.J. Schuurman. (2011). The streptozotocin-induced diabetic nude mouse model: differences between animals from different sources. Comparative medicine. 61 (4) 356-360.

[28] M.L. Lukic, S. Stošic-Grujicic, A. Shahin. (1998). Effector mechanisms in low-dose streptozotocin-induced diabetes. Developmental immunology. 6 (1-2) 119-128.

[29] M.E. Cerf. (2013). Beta cell dysfunction and insulin resistance. Frontiers in endocrinology. 4 37.

[30] Y. Mohan, G.N. Jesuthankaraj, N. Ramasamy Thangavelu. (2013). Antidiabetic and antioxidant properties of Triticum aestivum in streptozotocin-induced diabetic rats. Advances in Pharmacological and Pharmaceutical Sciences, 2013.

[31] M. Stepien, C. Gaudichon, G. Fromentin, P. Even, D. Tome, D. Azzout-Marniche. (2011). Increasing protein at the expense of carbohydrate in the diet down-regulates glucose utilization as glucose sparing effect in rats. PloS one. 6 (2) e14664.

[32] P. Puigserver, J. Rhee, J. Donovan, C.J. Walkey, J.C. Yoon, F. Oriente, B.M. Spiegelman. (2003). Insulin-regulated hepatic gluconeogenesis through FOXO1–PGC-1α interaction. Nature. 423 (6939) 550-555.

[33] E.O. Adewoye, A.O. Ige. (2016). Lipid profile and electrolyte composition in diabetic rats treated with leaf extract of Musa Sapientum. Journal of dietary supplements. 13 (1) 106-117.

[34] N. Sheela, M.A. Jose, D. Sathyamurthy, B.N. Kumar. (2013). Effect of Silymarin on Streptozotocin-Nicotinamide--induced Type 2 Diabetic Nephropathy in Rats. Iranian journal of kidney diseases. 7 (2).

[35] J.A. Cohen, M.M. Kaplan. (1979). The SGOT/SGPT ratio—an indicator of alcoholic liver disease. Digestive diseases and sciences. 24 835-838.

[36] M. Murillo-Villicaña, R. Noriega-Cisneros, D.J. Peña-Montes, M. Huerta-Cervantes, A. Aguilera-Méndez, C. Cortés-Rojo, A. Saavedra-Molina. (2022). Antilipidemic and hepatoprotective effects of ethanol extract of Justicia spicigera in streptozotocin diabetic rats. Nutrients. 14 (9) 1946.

[37] F.M. El-Demerdash, Y. Talaat, R.A. El-Sayed, W. Kang, N.F. Ghanem. (2022). Hepatoprotective effect of Actinidia deliciosa against streptozotocin-induced oxidative stress, apoptosis, and inflammations in rats. Oxidative Medicine and Cellular Longevity, 2022.

[38] J. Mohamed, A.N. Nafizah, A.H. Zariyantey, S. Budin. (2016). Mechanisms of diabetes-induced liver damage: the role of oxidative stress and inflammation. Sultan qaboos university medical journal. 16 (2) e132.

[39] P.T. Fueger, S. Heikkinen, D.P. Bracy, C.M. Malabanan, R.R. Pencek, M. Laakso, D.H. Wasserman. (2003). Hexokinase II partial knockout impairs exercise-stimulated glucose uptake in oxidative muscles of mice. American Journal of Physiology-Endocrinology and Metabolism. 285 (5) E958-E963.

[40] P.T. Fueger, J. Shearer, T.M. Krueger, K.A. Posey, D.P. Bracy, S. Heikkinen, D.H. Wasserman. (2005). Hexokinase II protein content is a determinant of exercise endurance capacity in the mouse. The Journal of physiology. 566 (2) 533-541.

[41] J.M. Gancedo, C. Gancedo. (1971). Fructose-1, 6-diphosphatase, phosphofructokinase and glucose-6-phosphate dehydrogenase from fermenting and non fermenting yeasts. Archiv für Mikrobiologie. 76 132-138.

[42] G. Mithieux, H. Vidal, C. Zitoun, N. Bruni, N. Daniele, C. Minassian. (1996). Glucose-6-phosphatase mRNA and activity are increased to the same extent in kidney and liver of diabetic rats. Diabetes. 45 (7) 891-896.

[43] K.N. Bopanna, J. Kannan, S. Gadgil, R. Balaraman, S.P. Rathod. (1997). Antidiabetic and antihyperlipaemic effects of neem seed kernel powder on alloxan diabetic rabbits. Indian journal of Pharmacology. 29 (3) 162-167.

[44] K. Das, A. Roychoudhury. (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers in environmental science. 2 53.

[45] P. Arivazhagan, T. Thilakavathy, C. Panneerselvam. (2000). Antioxidant lipoate and tissue antioxidants in aged rats. The Journal of nutritional biochemistry. 11 (3) 122-127.

[46] V.D. Rajput, R.K. Harish, Singh, K.K. Verma, L. Sharma, F.R. Quiroz-Figueroa, S. Mandzhieva. (2021). Recent developments in enzymatic antioxidant defence mechanism in plants with special reference to abiotic stress. Biology. 10 (4) 267.

[47] P. Murugan, L. Pari. (2006). Antioxidant effect of tetrahydrocurcumin in streptozotocin–nicotinamide induced diabetic rats. Life sciences. 79 (18) 1720-1728.