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RESEARCH ARTICLE

The Impact of *Chenopodium murale* L. on Blood Sugar, Lipid Profiles, and Hepatic Health in Streptozotocin Induced Diabetic Mice

Monalisa Monwar[1]*, Tripti Rani Paul[2], Miss Nuzhat Tasnim[2], A.K.M. Shafiur Rahman[2], Nazmun Nahar Tamanna[2], Syeda Faizaebnat Maisha[2], Md. Rafiqul Islam Khan[3] and Mir Imam Ibne Wahed[3]

[1]Institute of Biological Sciences, University of Rajshahi, Rajshahi 6205, Bangladesh

[2]Department of Pharmacy, Faculty of Science and Technology, Varendra University, Rajshahi 6204, Bangladesh

[3]Department of Pharmacy, Faculty of Science, University of Rajshahi, Rajshahi 6205, Bangladesh

***Correspondence:**
Email: mona.ibsc@gmail.com

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Abstract

The global prevalence of diabetes is increasing at an alarming rate, making its management a primary clinical concern. While conventional pharmacological treatments are effective, they are often associated with significant side effects. Consequently, research has shifted toward identifying safer, natural alternatives. This study investigates the antidiabetic and antihyperlipidemic potential of *Chenopodium murale* leaf (CML) extract in a mice model. The study utilized an acute toxicity test and an oral glucose tolerance test (OGTT) in healthy mice. Diabetes was induced via an intraperitoneal injection of streptozotocin (STZ). Diabetic mice were then treated with CML extract (200 and 400 mg/kg), metformin (150 mg/kg), or a vehicle control (0.5% CMC) for 15 days. Key parameters measured included blood glucose levels, body weight, and a complete lipid profile (TC, TG, LDL, and HDL). Additionally, liver function was assessed by measuring serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvic transaminase (SGPT) levels. The extract was found to be safe up to a dose of 1000 mg/kg. Treatment with CML extract significantly improved glucose tolerance compared to the untreated diabetic group (both $p<0.001$ at 90 min). Both doses (200 and 400 mg/kg) produced a dose-dependent reduction in blood glucose levels (both $p<0.001$ vs. DC). Furthermore, the extract effectively lowered TC, TG and LDL cholesterol levels (400 mg/kg, $p<0.001$ vs. DC); while slightly increasing HDL cholesterol (400 mg/kg, $p<0.01$ vs. DC). A significant reduction in SGOT (200 & 400 mg/kg; $p<0.01$, $p<0.001$ vs. DC) and SGPT levels (400 mg/kg, $p<0.05$ vs. DC) also indicated potent liver protective effect. The CML extract exhibits significant glucose and lipid lowering efficacy alongside protect liver functions in diabetic mice, considered as a natural alternative for diabetes management.

Keywords: *Chenopodium murale*, Hepatic function, Hyperglycemia, Lipid disorder, Murine model, Streptozotocin.

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Introduction

People have used natural products as their main source of drugs since ancient times (Grover et al. 2002). Today, many still choose medicinal plants as a practical, natural alternative to modern medicines due to less or no side effects (Das et al. 2017). In developing countries as nearly 70-95% of populations meet their basic healthcare needs by using herbal medicine (Singh et al. 2010). More than 8000 types of plants with their various 2000 species have been reported to have medicinal properties (Johnson et al. 2008) and these plants have the ability to reduce severe chronic diseases as it is occupied with phytochemicals, responsible for that therapeutic action (Liu et al. 2003). Owing to having a massive number of bio-active compounds applicable for new drug synthesis, the industrialized countries have replaced their medical research with medicinal plants (Tabuti et al. 2003). Medicinal herbs are safer than some of approved pharmaceutical drugs (Lazarou et al. 1998). All these benefits together with therapeutic activities have increased the synthesis of drugs from medicinal herbs (Jones et al. 2006). Hence medicinal plants are using extensively from the past with the objective of treating several kinds of diseases (Rao et al. 2010, Salahi-Moghaddam et al. 2012) like diabetes, hyperlipidemia, cancer, skin diseases etc.

Diabetes is a life-threatening metabolic chronic disease that reduces the life expansion and is becoming an alarming issue for the time being (Heald et al. 2020). According to World Diabetes Federation (WDF), currently, 536.6 million people are a victim of diabetes and by 2045 it can attain 783.2 million over the world (Sun et al. 2022). Diabetes mellitus (DM) is a condition when the body's metabolic system exhibits abnormalities since the insulin produced by the pancreas isn't sufficient to run the metabolism or the insulin fails to meet the peripheral target tissue to produce a response and resulting in hyperglycemia (Endrich et al. 2015). Based on insulin requirements and resistance, DM have been categorized Type 1 DM (T1DM) and type 2 DM (T2DM) and also familiar as insulin-dependent and non-insulin-dependent DM respectively (Olokoba et al. 2012). T1DM is comparatively rare as among the total cases, more than 90-95% of the people are living with T2DM (Kleefstra et al. 2007) and the suffering patients can acquire other complications like heart failure, renal failure, lack of vision, stroke, or another kind of cardiovascular diseases (Alva et al. 2014). Besides that, another metabolic disorder hyperlipidemia may associate with T2DM because the deficiency of insulin increases the breakdown of lipids in adipose tissue. Hyperlipidemia is familiar as the key risk factor of cardiovascular diseases manifested by the elevation of plasma concentrations of the various bad lipoproteins (Reiner et al. 2006). The secondary type of hyperlipidemia is more likely due to DM, which indicates significantly treating the original disease rather than hyperlipidemia (Suzuki et al. 2006). Medicines are available to treat diabetes but apart from reducing blood glucose levels the available market preparations of anti-diabetic drugs possess a variety of adverse reactions including cutaneous, gastrointestinal, hypoglycemic coma, and malfunction of kidney & liver (Gandhi et al. 2011) which may introduce nausea, vomiting, weight gain, hyponatremia, obstructive jaundice, hematological and dermatological reactions (UKPDS 1998). Thus, for reducing the side effects, toxicity, and hassles in consumption the desire moves towards medicinal plants other than synthetic drugs (Campbell-Tofte et al. 2012). Previous studies have shown that the phytochemical terpenoids, coumarins, flavonoids, glutamic acid, arginine, and several secondary metabolites can possess anti-diabetic effects. Therefore, there is a high probability to unlock diabetic complications through herbal remedies (Nammi et al. 2003).

Because *Chenopodium murale* contains diverse bioactive compounds like flavonoids and phenolic acids, coumarins, glycosides, terpenes and steroidal glycosides (Abbas et al. 2012, Saleem et al. 2014, Rehman and Rao 2017) its leaf methanolic extract which might be responsible for therapeutic activities against diabetes and its associated complications (Ahmed et al. 2003). Hence, the effects of *Chenopodium murale* leaves methanolic extracts were investigated on blood glucose, lipid profiles and liver functions in diabetic mice.

Material and Methods

Drugs and chemicals

To ensure purity and accuracy, analytical grade chemicals were used. Metformin Hydrochloride was a generous gift from Teams Pharmaceutical Ltd., Rajshahi, Bangladesh, Streptozotocin (STZ) was purchased from Sisco Research Laboratories (Mumbai India). Blood glucose was measured using a Glucosuria glucometer (Origin, Taiwan) and a commercial kit. Serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and serum glutamate oxaloacetate transaminase (SGOT), and serum glutamate pyruvic transaminase (SGPT) were determined using a commercial kit (Human, Germany).

Collection and preparation of plant material

Chenopodium murale was collected from Meherpur, Bangladesh in February 2022. The plant was recognized by an expert taxonomist from the Department of Botany, University of Rajshahi (Voucher specimen No. 36, 10/02/2022). The fresh leaves were separated and the roots were removed from the plant, then washed through distilled water, cut into small pieces, and allowed to sun dry. For better grinding, sun-dried plant materials were again allowed to dry in the oven at a considerably low temperature (40˚C). Finally, by using the high-capacity grinding machine the dried plant material was crushed into a coarse powder.

Extraction of the plant material

For preparing the methanolic extract of the plant about 250 gm of *C. murale* leaves (CML) powdered material were soaked in 1.0 liter of methanol in a separate clean round amber glass flask (5 liters) and sealed with a cotton plug and aluminum foil. The container was kept for a period of 14 days by maintaining occasional shaking and stirring at systematic intervals. After 14 days the extract was then filtered. The collected filtrate from the straining was then placed in a rotary evaporator till dryness to form a solid residue by evaporating the liquid.

Phytochemical screening tests

The phytochemical analysis was done by the study of standard established validated methods to identify the presence of major chemical groups in CML extract (Pollock and Stevens 1965, Plummer 1987).

Animals

Healthy Swiss albino male mice were chosen and acquired from the zoology department's animal home University of Rajshahi in Rajshahi, Bangladesh. The six-week-old mice weighed between 30 and 40 gm. Under ambient circumstances, animals were kept on a 12-hour light cycle followed by a 12-hour dark cycle. The mice were fed specially prepared mouse food and water for seven days before the trial. The Varendra University Animal Ethics Committee in Rajshahi, Bangladesh, accepted the experimental protocol (Ref. VU/ ERC/2021-2022/004).

Acute toxicity studies

The investigation of acute oral toxicity was conducted in accordance with OECD recommendations. Five groups of animals (n = 3) were created, and they remained fasted for the following days. The animals in all groups received varying doses of CML extract in increasing dose levels of 100, 250, 500, 1000, and 2000 mg/kg body weight. Individual animals were observed for the first two hours, then at regular intervals for the first 24 hours, and then every day over the following fourteen days (OECD 2002).

Oral glucose tolerance test (OGTT)

OGTT was performed on starved (18 hrs) normal Swiss albino mice. The mice were randomly divided into five groups of five mice in each (n = 5). Different doses of extract based on body weight (BW), CML 200 and 400 mg/kg, metformin 150 mg/kg, vehicle, 0.5% carboxy methyl cellulose (CMC), were administered to the mice and after 30 min, 2g/kg glucose was fed. The glucose loading and the blood glucose levels were estimated by using a glucometer at 0 min before and after 30, 60, 90, and 120 min withdrawal from the tail vein (Bonner-Weir 1988).

Experimental induction of diabetes and protocol

STZ (45 mg/kg) was used as a diabetogenic agent, and dissolved into 0.1 M citrate buffer (pH = 4.5). Diabetes was induced by a single intraperitoneal injection (i.p). Normal control (NC) mice only received citrate buffer as a vehicle. Mice treated with STZ were not allowed to eat overnight and the initial hypoglycemia induced by the drug was overcome by allowing the mice to drink 10% glucose solution for 24 h. After three days (96 h) of STZ injection, blood was drawn to assess the blood sugar levels. Animals showed fasting plasma glucose levels greater than 10.5 mmol/L, indicating the presence of diabetes (Arunachalam and Parimelazhagan 2013). Animals were separated into five groups (n = 5) in each group and subjected to oral ingestion of CML extract, 200 mg/kg and 400 mg/kg BW, disease standard (DS), metformin 150 mg/kg BW, disease control (DC), and NC, 0.5% CMC once daily for fifteen days.

Measurement of BW and determination of blood glucose level

Before treatment beginning and post-treatment on the 5^{th}, 10^{th}, and 15^{th} day of the treatment, mice's BW were recorded. To estimate the degree of diabetes in all the experimental animals, the initial fasting blood glucose levels were measured. The standard medication was then diluted in water, the extracts were suspended in a vehicle (0.5 % CMC), and mice were given this solution for 15 days. On the 0th, 5th, 10th, and 15th days, blood samples were taken from the tail vein and the glucose levels were assessed using a glucometer (Ahmed et al. 2012, Bergmeyer et al. 2012).

Estimation of lipid profile

Diabetic mice received CML extract once daily for 15 days, and Metformin. Blood was taken 15 days after the end of the drug rehabilitation program. The serum was kept at -80°C for biochemical analysis after being separated from the blood samples by centrifuging at 4000 rpm for 20 minutes. Commercial kits were used for the spectrophotometric analysis of plasma concentrations of TG, TC, and HDL (Human, Germany). LDL = TC- (HDL + VLDL) and VLDL = TG/5 were used to calculate the amounts of LDL and VLDL (Reddy et al. 2012). Calculations were done to determine the LDL/HDL cholesterol ratio.

Estimation of hepatic enzymes

To assess the liver enzymes, serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvic transaminase (SGPT), wet reagent diagnostic kits (Human, Germany) were used in accordance with the manufacturer's guidelines (Barnet et al. 1973, Schumann et al. 2002).

Statistical analysis

The mean ± standard error of the mean (SEM) was used to express the results. One-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test, was used to compare the data statistically. Values were evaluated statistically significant when the P value was $p<0.05$.

Results

Phytochemicals of *C. murale* leaves extract

Phytochemicals of *C. murale* leaves extract showed the presence of the following bioactive compounds.

Table 1: Phytochemicals of *C. murale* leaves extract.

Extract	Steroid	Alkaloid	Glycoside	Tannin	Triterpene	Saponin	Flavonoids
C. murale leaves	-	+	+	+	+	+	+

+ indicates present, and − indicates absent.

Acute toxicity study

Different doses of methanolic CML extract at an increasing dose level of 150, 250, 500, 1000, and 2000 mg/kg BW were administered to animals of all groups. Animals were individually observed for any change in behavior and mortality for the first 2 h, then periodically during the first 24 h and daily thereafter for a total of 14 days. After one week, it has been observed that taking CML extract 2000 mg/kg, 20% mice died. Therefore, we considered carrying out our investigations with one-fifth and one-tenth of the harmful dose of CML extract which was 200 mg/kg and 400 mg/kg.

Oral glucose tolerance test (OGTT)

After 30 minutes of glucose loading, mice from all groups exhibited high blood glucose levels which were decreased by the acute action of CML extract pretreated mice, resulting in a substantial reduction in the rising blood glucose levels (Fig. 1). Blood glucose levels in the DS group peaked after 30 minutes and then gradually decreased during the following 90 minutes. When compared to NC mice with mice treated with CML 200, and CML 400, exhibited significantly lower blood glucose levels at 60, 90, and 120 minutes. However, the glucose tolerance of the CML 400 showed better control (Fig. 1).

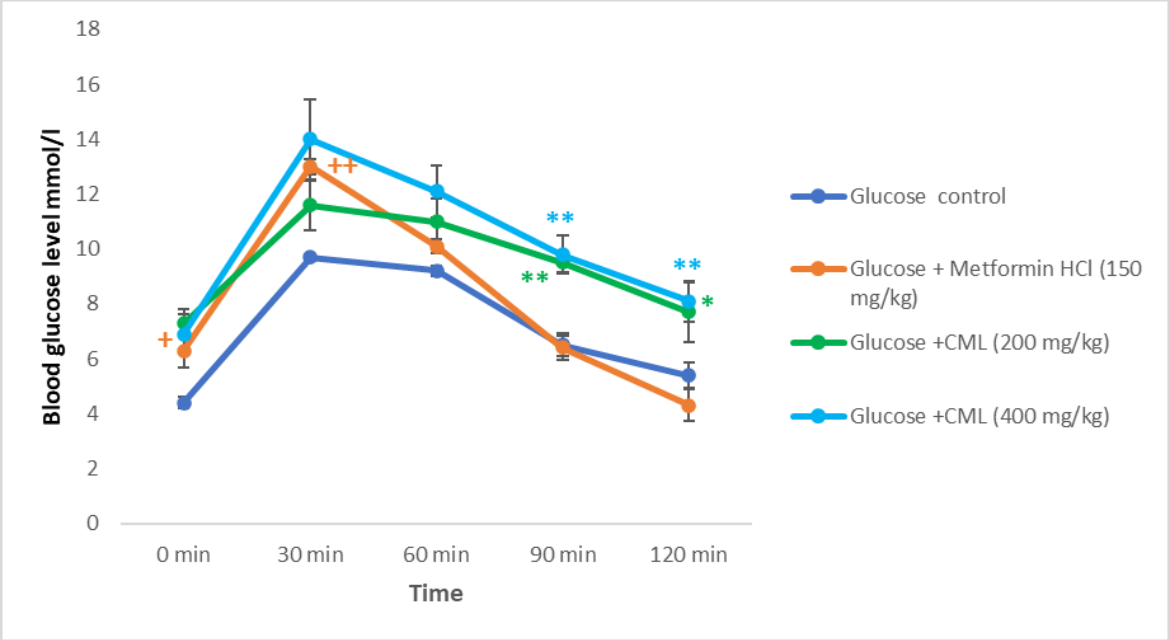

Fig. 1: Effect of CML extract on oral glucose tolerance. $^{**}p<0.01$, $^{*}p<0.05$ vs. standard; $^{++}p<0.01$, $^{+}p<0.05$ vs. glucose control

Clinical trajectory

Neither of the study mice passed away during the treatment period. As a result, all the mice in the treatment group remain alive.

Effect of CML extract on fasting blood glucose (FBG) levels in diabetic mice

Fig. 2 displays the time course of the increase in blood sugar levels. Before treatment, on day 0, the blood glucose levels in the DC mice were significantly higher than those in the NC group. On days 5, 10, and 15, oral administration of CML extract of 200 mg/kg and 400 mg/kg, significantly reduced fasting blood sugar levels by degrees that were comparable to group DS. After 15 days of extract administration, fasting blood sugar levels were noticeably reduced. The most notable effect was observed in the CML 400 and it was dose-dependent.

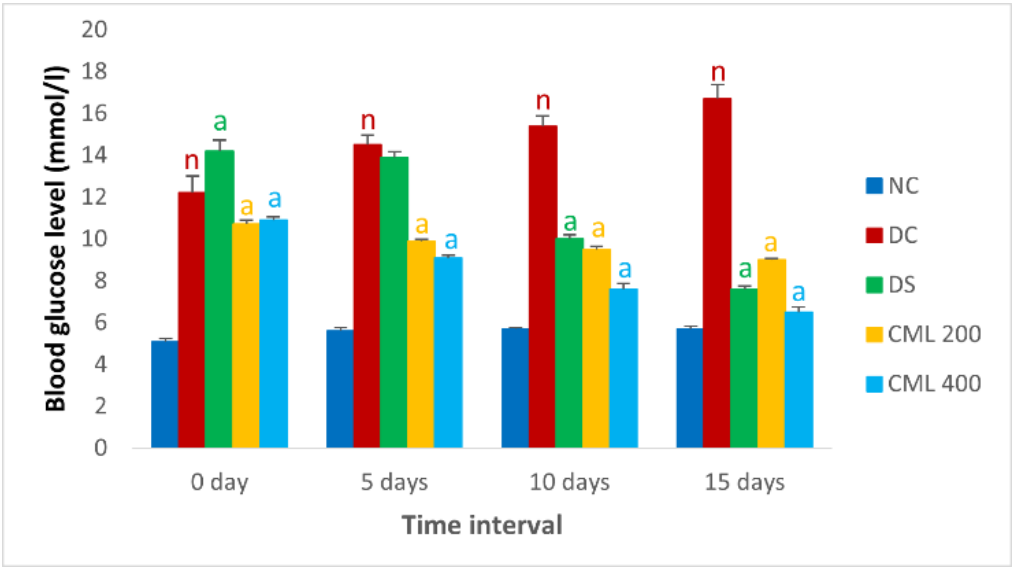

Fig. 2: Effect of *C. murale* on blood glucose level. $^{n}p<0.001$ vs. NC; $^{a}p<0.001$, $^{b}p<0.01$, $^{c}p<0.05$ vs. DC.

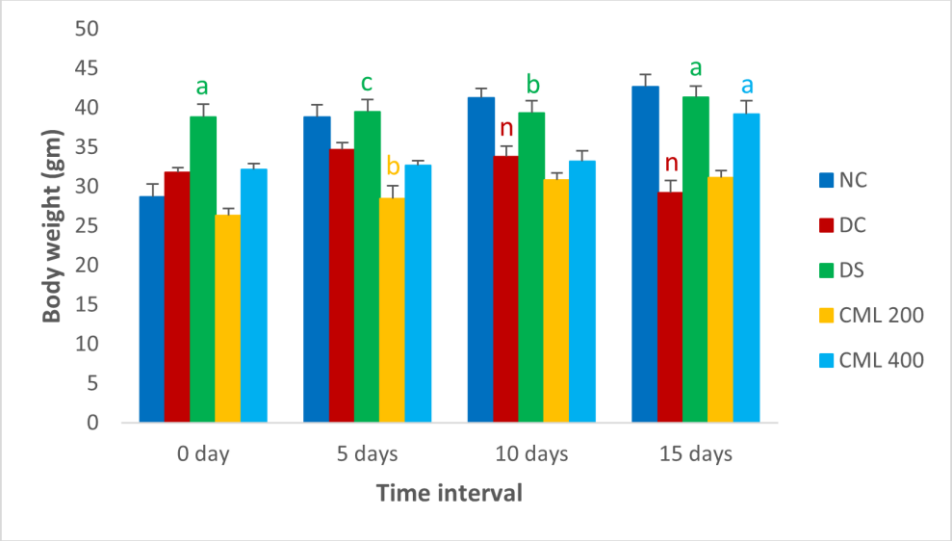

Fig. 3: Effect of *C. murale* leaves extract on body weight. $^{n}p<0.001$ vs. NC; $^{a}p<0.001$, $^{b}p<0.01$, $^{c}p<0.05$ vs. DC.

Effect of CML extract on body weight (BW)

As shown in Fig. 3, STZ-induced diabetic mice in the current study significantly reduced their BW when compared to normal mice. BW of DC mice, NC mice, and treated mice were taken at the conclusion of the 15-day treatment period and between 5 and 10 days earlier. CML extract was administered over the treatment course of 15 days, and all treatment groups displayed an increase in BW. However, the dose of 400 mg/kg showed statistically significant ($p<0.001$) results and was comparable to those of the DC group.

Effect of CML extract on lipid profile in STZ-induced diabetic mice

Table 2: Effect of CML extract on plasma lipid profile.

Groups	TC	TG	LDL	HDL
NC	174.33 ± 1.33	153 ± 1.73	111 ± 1.15	81± 1
DC	220 ± 2.89 $^{+++}$	192 ± 3.06^{+++}	133 ± 1.14^{+++}	43 ± 2.65^{+++}
DS	179 ± 1.15***	161.67 ± 1.20***	107.66 ± 1.33***	51 ± 1.15***
CML 200	217.33 ± 1.45	160 ± 0.58***	140± 0.57**	33 ± 1.15***
CML 400	164 ± 2.08***	156.67 ± 0.88***	114.33 ± 1.76***	35.33 ± 0.88**

Data were expressed SEM, $^{+++}p<0.001$ considered significant compared to the NC. $^{***}p<0.001$, $^{**}p<0.01$ compared to DC.

STZ-induced diabetic mice showed higher TC, TG, and LDL cholesterol levels while decreasing HDL value in mice. In our investigation, it was seen that after 15 days, STZ-induced diabetic mice had raised TC levels of 220 mg/dl, TG levels of 192 mg/dl, LDL levels of 133 mg/dl, and decreased HDL levels of 43 mg/dl in contrast to NC mice. However, mice treated with CML 400 showed a significant decrease ($p<0.001$) in TC, TG, and LDL cholesterol and a somewhat increase ($p<0.01$) in HDL cholesterol (Table 2), which corresponded to the DS group.

Effect of CML extracts on SGOT and SGPT

Table 3 displays the effects of CML extract on SGPT and SGOT levels in different doses. Both enzyme levels were noticeably higher in the DC group. Oral administration of CML extract therapy decreased SGPT and SGOT levels in all the treated groups, and the reductions were equivalent to those seen in the DC and NC groups. As a result, the extract protected the liver, which can partly be attributed to the levels of SGPT and SGOT being reduced. However, CML 400 produced the most significant ($p<0.001$) changes, which were comparable to DS.

Table 3: Effect of CML extract on changes in liver enzymes.

Groups	SGPT (U/L)	SGOT (U/L)
NC	16 ± 1.15	14 ± 1.73
DC	$41 \pm 2.08^{+++}$	$35 \pm 1.53^{+++}$
DS	$25 \pm 1.52^{***}$	$19 \pm 1.15^{***}$
CML 200	41.33 ± 0.88	$27 \pm 2.08^{**}$
CML 400	$36 \pm 2.52^{*}$	$22 \pm 1.53^{***}$

Data were expressed in SEM, $^{+++}p<0.001$ considered significant compared to NC; $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ compared to DC.

Discussion

Diabetes has been recognized as one of the most primitive diseases suffered by humans and the third leading cause of mortality worldwide (Ahmed 2002, ADM 2010). The intensity and complication of diabetes is rising swiftly instead of taking the initiative and following various strategies prior to controlling or managing the disease (Tiwari et al. 2002). Having interrelated connections complies with the disease breeding other disorder like hyperlipidemia and cardiovascular disease (Reiner and Tedeschi-Reiner 2006, Gomes et al. 2001). Findings of various estimations suggest that a number of plants have the efficacy of preventing diabetes and hyperlipidemia in different diabetic animal models as an alternative and complementary therapy (Patel et al. 2012). The present study aimed to assess the 15 days duration analysis of antihyperglycemic, antihyperlipidemic and hepatostabilizing activity of *C. murale* leaves methanolic extract on diabetic mice. In this study, a gradual increase of blood glucose level is achieved by administration of streptozotocin (STZ) at a low concentration which causes induction of Type 2 Diabetes (T2D) in the mice simultaneously by affecting the insulin receptor (IR) responsible for insulin signal (Ordonez et al. 2007). Metformin, a well-known biguanides derivatives hypoglycemic agent works by moderating the binding probability and function of insulin, was used as a standard (Goth 1978). After the evolution of the 15 days experiment, we found that the mice treated with CML extract showed a significant antidiabetic effect, resembling the standard group ($p<0.001$) in Figure 2, may be due to having α-amylase inhibitory property that had been reported by Khatune et al. (2016). The study also provides evidence of the presence of severe phytochemical and antioxidant properties of *C. murale* L. which can be another cause of showing anti-diabetic properties (Khatun et al. 2020). Previous studies alleged that diabetes, initiated through STZ causes a notable decrease in body weight as STZ further leads to the wasting of muscle tissue and loss of tissue proteins from the body (Chatterjee and Shinde 2002). Unlike that, we also observed that the DC mice showed a gradual loss of body weight during the experimental period may cause significant increase of the body weight remarked by each dose of CML while CML 400 was the most efficacious (Fig. 3), that was comparable to NC mice, it might be due to the presence of protein, fiber, fat, and energy in CML extract (Khatun 2020). Increments of TC, TG, LDL, and a reduction in HDL are linked with hyperglycemia and are assigned to the excessive mobilization of fat from adipose tissue as a result of insufficient peripheral glucose consumption (Krishnakumar et al. 2000). When compared to the healthy control mice, DC mice showed significantly higher plasma levels of TC and TG the effects of C. album leave extract on TG and HDL were found higher than metformin and their effects were comparable to those of metformin. However, treatment with the CML extract stabilized plasma lipid status, probably through regulation of lipid metabolism which is comparable with the standard group, it was in accordance with Chikhi et al. (2014). HDL levels were also improved in DC mice treated with either CML extract or metformin. Being a leading organ involved in glucose and lipid homeostasis, the liver is susceptible to illnesses linked to metabolic abnormalities. SGPT and SGOT are the most common biomarkers that indicate the status of hepatic functioning (Chilay et al. 2024). Positive increment of SGPT and SGOT levels had showed DC mice, by treatment with CML 200 and CML 400 extract significantly reduced both SGOT and SGPT levels in our study.

Conclusion

STZ-induced diabetic mice showed significant attenuation in blood glucose level, and lipid profile management when treated with C. *murale* leaves (CML) extract. Oral treatment with CML extracts significantly reduced SGPT and SGOT levels; and improved body weights in diabetic mice. The CML extract was considered as safe and effective in terms of glucose and lipid lowering efficacy, and might be used to protect liver function in diabetic mice. All of these findings bestow scientific proof in favor of utilizing the plant in conventional remedies to cure diabetes and its complications. However, further phytochemical research is necessary to determine the chemical type of the active component that is responsible for all of these beneficial therapeutic effects.

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Conflict of interest

The authors hereby declare no conflict of interest regarding the publication of this article.

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Data availability: Data generated in the study are reported in the manuscript, and unprocessed data is with the corresponding author and available upon request.

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