GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Ameliorative potential of freeze-dried methanolic leaf extract of *Moringa oleifera* Lam. on hyperglycemia, hyperlipidemia, anti-oxidative enzymes and hepatopathy in Streptozotocin-induced diabetic mice

Ankita Dey [1,*], Antonia R Lyngdoh [1], Sanchayeeta Roy [2], Ridashisha Rymbai [1] and Surya Bhan [1]

[1] *Department of Biochemistry, North-Eastern Hill University, Shillong, Meghalaya, India-793022.*
[2] *Department of Zoology, North-Eastern Hill University, Shillong, Meghalaya, India-793022.*

GSC Biological and Pharmaceutical Sciences, 2025, 30(03), 286-297

Publication history: Received on 03 February 2025; revised on 15 March 2025; accepted on 17 March 2025

Article DOI: https://doi.org/10.30574/gscbps.2025.30.3.0107

Abstract

The leaves of *Moringa oleifera* commonly known as Drumstick or 'Sajina' were collected from Dibrugarh, Assam and the methanolic leaf extract (MLE) was prepared using a lyophilyzer. Phytochemical screening reveals that phenols and flavonoids were strongly present in the MLE. The total phenolic and flavanoid contents were 72.43±0.031 and 43.44±0.010 (µg/mg) respectively. Toxicology study reveals that the LD50 value of MLE is higher than 2000 mg/kg b.w. The anti-hyperglycemic study shows that all the doses of MLE (150- 550 mg/kg b.w.) were effective against FBG levels in STZ- induced diabetic mice but the dose 350 mg/kg b.w. was observed as the optimum dose. The lipid profile shows that elevated levels of total cholesterol, triglyceride, LDL-C and VLDL-C levels and reduced levels of HDL-C during diabetic conditions were restored to near normal levels upon treatment with MLE. The hepatic marker enzyme activity also revealed that the elevated levels of serum ALP, SGPT and SGOT under diabetic conditions were reduced significantly when treated with MLE. Similarly, liver antioxidant enzyme activity shows that the enzymes Superoxide dismutase (CuZnSOD and MnSOD), Catalase and Glutathione reductase were restored to near normal levels upon treatment with MLE. The histological study also shows that the MLE exerted hepatoprotective effects and could reverse and suppress the injuries caused by diabetes in the liver. Hence from the findings of the present study we can conclude that the MLE of the plant *M. oleifera* possess anti-hyperglycemic, anti-hyperlipidemic, anti-oxidative and anti-hepatopathy properties in STZ-induced diabetic mice models.

Keywords: *Moringa oleifera*; Diabetes Mellitus; Anti-Hyperglycemia; Anti-Hyperlipidemia; Anti-Oxidant and Anti-Hepatopathy

1. Introduction

Diabetes mellitus (DM) is a group of chronic metabolic diseases characterized by prolonged hyperglycemia that results from altered insulin secretion or function or both [1]. Currently, many countries are on the verge of a global diabetes "epidemic", which is rapidly spreading across the globe [2]. Chronic hyperglycemia is often accompanied by damages, dysfunctions and failure of various organs and tissues that ultimately leads to the development of hepatopathy, nephropathy, neuropathy, retinopathy and cardiovascular complications [3]. According to the WHO classification (1999, with amendments), there are two types of DM which are distinguished as type 1 DM (DM1) resulting from destruction of pancreatic β-cells which are immune-mediated leading to absolute insulin insufficiency and type 2 (DM2) mainly related to insulin resistance, relative insulin insufficiency or mainly due to decreased insulin secretion with or without insulin resistances [4]. Diabetes mellitus is also associated with hyperlipidemia, hypertension and are often linked with increased free radicals leading to compromised defence system [5, 6]. Since the therapeutic management of DM without any side effects still remains a challenge, however in response, there is a growing interest in exploring

*Corresponding author: Ankita Dey

various herbal remedies, which are seen to have minimal to no side effects [7]. After some primary epidemiological investigations the plant which is found suitable for the study is *Moringa oleifera*, belonging to the Moringaceae family also locally known as Drumstick or '*Sajina'*. The plant is indigenous to the Indian subcontinent and is widely used in the treating various health issues including diabetes. Although the mechanisms by which *M. oleifera* decreases hyperglycemia remain uncertain [7] but the assessment of its hypoglycemic, anti-hyperlipidemic and anti-oxidant properties is of utmost importance to formulate a standard alternative natural and efficient method for the treatment and management of diabetes [8]. Therefore, the present study is aimed to evaluate and reveal the anti-hyperglycemic, anti-hyperlipidemic, anti-oxidative and anti-hepatopathy properties of the methanolic leaf extract of *M. oleifera* in STZ-induced diabetic mice models.

2. Materials and methodology

2.1. Chemicals

All chemicals used are of analytical grade. Methanol, Folin Ciocalteu, Alumunium chloride was purchased from Sisco Research Laboratories Pvt. Ltd. India. Streptozotocin (STZ), Pyrogallol, Ascorbic acid, diethylenetriaminepenta-acetic acid (DETAPAC) was purchased from Sigma-Aldrich Co. USA. Total Cholesterol, Triglycerides, HDL-C, ALP, SGOT, SGPT Test kits were purchased from Coral Bio-systems, India. All other chemicals were purchased from Sisco Research Laboratories Pvt. Ltd. India and Hi-Media Laboratories, India.

2.2. Sample Collection and Identification

The leaves of *M. oleifera* were collected from various areas of Dibrugarh district located in upper Assam in the month of April 2023. The specimen was submitted to Botanical survey of India (BSI) for identification and accession number. The plant was identified/authenticated as Voucher No. 99985 by Dr. N. Odyuo, Scientist-E and Ho.O, Botanical survey of India (BSI), Shillong, Meghalaya.

2.3. Preparation of Methanolic Extract

The leaves of *M. oleifera* were collected in bulk, separated and washed thoroughly under running water and then air dried in shade at room temperature. The air-dried leaves were powdered in a grinder, weighed accordingly and was dissolved in 10x volume of methanol and water in 4:1 ratio. The prepared solution was kept overnight at room temperature with continuous stirring and then filtered using Whatman filter paper no.1. The filtrate obtained was then freezed at -80°C overnight and then lyophilyzed to dryness using Scanvac cool safe freeze dryer. The dried methanolic extract was weighed and kept in an air tight container and stored at -20°C for further experiments [9].

2.4. Preliminary Phytochemical screening

The methanolic leaf extract (MLE) of *M. oleifera* was used for qualitative phytochemical analysis for phenols, alkaloids, flavonoids, tannins, and saponins as per the standard protocol [10].

2.5. Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) of the MLE of *M. oleifera* was measured by Folin-ciocalteu method, using gallic acid as the standard. The reagent Folin-ciocalteu was used to oxidise the phenolic compounds present in the MLE which resulted in a deep blue coloured reaction mixture which was measured spectrophotometrically at λ 765 nm, against an appropriate blank. The results were expressed as µg of gallic acid per mg of extract [11, 12].

2.6. Determination of Total Flavonoid Content (TFC)

The total flavanoid content (TFC) of the MLE of *M. oleifera* was determined using aluminium chloride method, using quercetin as the standard. The $AlCl_3$ reacts with the keto or hydroxyl groups of flavones or flavanols present in the MLE and forms a yellow acid-stable complex which was measured spectrophotometrically at λ 420 nm, against an appropriate blank. The results were expressed as µg of quercetin per mg of extract [13].

2.7. Experimental Animal Model

Healthy female and male swiss albino (Balb/C strain) mice with 23-29 gm body weight were used for further study. Mice were kept in an animal house under favourable conditions. Mice were kept in polypropylene cages under a maintained temperature of 25 ± 2°C with a 12 hrs day-night cycle. They were provided a standard laboratory feed obtained from Amrut Laboratory, Pune, India and water *ad libitum*. All the experiments were approved by Institutional

ethics committee (Animal model) North-Eastern Hill University, Shillong, India (Date: 31st May, 2023) and were carried out in accordance with the same.

2.8. Acute toxicology study

An acute toxicology test was performed on healthy female swiss albino (Balb/C strain) mice to determine the median lethal dose i.e. LD50 for the MLE of the plant *Moringa oleifera*. The test was carried out in accordance with the Organisation for Economic Cooperation and Development Guidelines 425 (OECD) guidelines [14]. The study was performed on five different test groups containing six healthy female mice in each group. An ascending doses of 400, 800, 1200, 1600 and 2000 mg/kg body weight (b.w.) of the mice were intraperitoneally administered while following the limit test procedure. During the study mice were fed well and were provided with water. The mice in all the test groups were observed for 24 hrs [15]. The LD50 value was determined by the following formula [15]:

$$LD50 = LD100 - (\text{Product A} \times \text{B/No. of animals in each group}),$$

Where A = Dose difference (Dose in given interval − Dose in upper interval) and B = Mean mortality (No. of dead mice in given interval + No. of dead mice in upper interval)/2.

2.9. Induction of Diabetes Mellitus

Healthy male mice were fasted overnight (only provided with water *ad libitum*) and the next day they were administered with a single high dose of Streptozotocin (STZ) which is 150 mg/kg b.w. interperitonially (i.p.). STZ was prepared in ice cold 0.1M citrate buffer with pH 4.5. After administration the mice had free access to food along with that 5% of glucose solution was also provided to counter the hypoglycemic shock. The fasting blood glucose (FBG) levels of the mice were checked after 72 hrs with SD check glucometer (SD Biosensor Inc. Korea). Mice with FBG levels above 200 mg/dL were selected for further experiments [16].

2.10. Anti-hyperglycemic Study

An anti-hyperglycemic study was performed in six different groups of overnight fasted, STZ-induced diabetic mice consisting of six mice in each group. Various doses of the MLE 150, 250, 350, 450 and 550 mg/kg b.w. were intraperitoneally administered every alternate day for a total period of 21 days to the respective groups. The FBG levels were measured, recorded and compared against the FBG levels of diabetic control group (DC) on the 1st, 7th, 14th and 21st day respectively [17].

2.11. Anti-hyperlipidemic Study

An anti-hyperlipidemic study was performed in four different mice groups, normal control (NC), diabetic control (DC), diabetic mice treated with 50 mg/kg b.w. of ascorbic acid (D+ASC) and diabetic mice treated with 350 mg/kg b.w. of methanolic leaf extract (D+MLE). Doses of ascorbic acid and MLE were intraperitoneally administered on every alternate day for a total period of 21 days [16]. After the 21st day, mice were fasted overnight and blood was collected from their retro-orbital sinus plexus under anesthesia. The serum was isolated from the whole blood samples according to the protocol described in Gatsing et al; 2005 [18] to carry out lipid profile tests. In lipid profile test, total cholesterol, triglyceride and high-density lipoprotein-cholesterol (HDL-C) levels were estimated by using the Coral diagnostic kits such as: CHOD/PAP kit, GPO/PAP kit and PEG/CHOD-PAP kit respectively. The low-density lipoprotein-cholesterol (LDL-C) and very high-density lipoprotein-cholesterol (VLDL-C) levels were also calculated using the Friedewald's formula [19]:

$$VLDL\text{-}C = Triglycerides/5,$$

$$LDL\text{-}C = Total\ Cholesterol - (HDL\text{-}C + VLDL\text{-}C).$$

All the values obtained and calculated were expressed as micrograms/decilitre (mg/dL).

2.12. Hepatic Marker Enzyme Activity

The hepatic marker enzyme activity was performed in four different mice groups, normal control (NC), diabetic control (DC), diabetic mice treated with 50 mg/kg b.w. of ascorbic acid (D+ASC) and diabetic mice treated with 350 mg/kg b.w. of methanolic leaf extract (D+MLE). Doses of ascorbic acid and MLE were intraperitoneally administered on every alternate day for a total period of 21 days. After the 21st day of the study, mice were fasted overnight and blood was collected from their retro-orbital sinus plexus under anesthesia. The serum was isolated from the whole blood samples

according to the protocol described in Gatsing et al; 2005 [18] to carry out the hepatic marker enzyme activities such as Alkaline phosphatase (ALP), Serum glutamate oxalate - transaminase (SGOT) and Serum glutamate pyruvate - transaminase (SGPT). The values obtained were compared against normal control group (NC) and diabetic control group (DC) values. Activities were determined by using modified International Federation of Clinical Chemistry (IFCC), using Coral diagnostic test kits Coral Clinical Systems, Goa, India and their mean activities were expressed as units/litre (U/L) [20].

2.13. *In-vivo* Anti-oxidative Assays

The *in-vivo* anti-oxidative assays were performed in four different mice groups, normal control (NC), diabetic control (DC), diabetic mice treated with 50 mg/kg b.w. of ascorbic acid (D+ASC) and diabetic mice treated with 350 mg/kg b.w. of methanolic leaf extract (D+MLE). Doses of ascorbic acid and MLE were intraperitoneally administered on every alternate day for a total period of 21 days.

2.14. Preparation of cytosolic and mitochondrial fractions of tissues homogenate

After the 21st day the liver tissue from all the experimental groups were excised after sacrifice by cervical dislocation. The tissue homogenate was prepared according to the protocol described by Bora et al; 2018 [17].

2.15. Liver anti-oxidant enzyme activity

Liver anti-oxidant enzyme activity of superoxide dismutase (SOD) both MnSOD and CuZnSOD were performed according to the method described by Marklund and Marklund; 1974 [21] and absorbance of the reaction was read at λ 470 nm and the values were expressed as units/milligram protein (units/mg protein). Catalase (CAT) activity was performed according the method described by Hugo Aebi; 1984 [22] and the absorbance of the reaction was read at λ 240 nm and the values were expressed as units/milligram protein (units/mg protein). Glutathione reductase (GR) activity was performed according to the method described by Carlberg & Mannervik; 1985 [23] and the absorbance of the reaction was read at λ 340 nm and the values were expressed as units/milligram protein (units/mg protein).

2.16. Histological examination

The histological examination study was performed in four different mice groups, normal control (NC), diabetic control (DC), diabetic mice treated with 50 mg/kg b.w. of ascorbic acid (D+ASC) and diabetic mice treated with 350 mg/kg b.w. of methanolic leaf extract (D+MLE). Doses of ascorbic acid and MLE were intraperitoneally administered on every alternate day for a total period of 21 days. After the 21st day the liver tissue from all the experimental groups were excised after sacrifice by cervical dislocation and the histological study was performed using hematoxylin-eosin (HE) staining protocols [24] with some modifications and the images were viewed using Olympus BX51 light microscope.

2.17. Statistical Analysis

Results are expressed as mean ± Standard error mean for six mice in each group. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was performed to compare differences between experimental groups using the SPSS software. Statistical significance was set at $p<0.05$.

3. Results

3.1. Preliminary Phytochemical Screening

Phenols and flavonoids were strongly present in the MLE whereas alkaloids, tannins, terpenes and saponins were moderately present.

3.2. Quantitative Phytochemical Assay

The total phenolic content was calculated to be 72.43 ±0.031 (µg Gallic acid /mg of extract) and the total flavanoid content was calculated to be 43.44 ±0.010 (µg Quercetin/mg of extract).

3.3. Acute Toxicity Study

It is known that smaller the LD50 value, the more toxic the compound is and vice-versa. However from the experiment it was found that none of the MLE doses (400-2000 mg/kg b.w.) has caused any mortality and hence it indicates that the LD50 value of MLE of *Moringa oleifera* is higher than 2000 mg/kg b.w. which implies that even at higher doses *Moringa oleifera* is safe and non-toxic to mice.

3.4. Anti-Hyperglycemic Study

It was observed that the MLE administered to various groups of diabetic mice showed prominent and prolonged anti-hyperglycemic potential in time versus dose-dependent manner as shown in Figure: 1. The anti-hyperglycemic effect was found to be maximum at the 21st day for all the doses used. At 150 mg/kg b.w. dose there was a minimum reduction of 18.07% of FBG level at 21st day. At 250 mg/kg b.w. dose there was a significant reduction of 26.17%, 35.18% and 56.38% at 7th, 14th and 21st day respectively. Also at 350 mg/kg b.w. dose there was a significant reduction of 28.11%, 38.19% and 60.51% at 7th, 14th and 21st day respectively. At 450 mg/kg b.w. dose there was a drastic reduction of 32.62%, 44.12% and 69.64% at 7th, 14th and 21st day respectively. At 550 mg/kg b.w. dose there was a drastic reduction of 36.33%, 54.32% and 75.54% at 7th, 14th and 21st day respectively. Therefore the dose 350 mg/kg b.w. was chosen as the optimum dose instead of 550 mg/kg b.w. dose because of its ability to reduce FBG at the same significance level as 550 mg/kg b.w. dose on the 21st day and thus the dose 350 mg/kg b.w. was used to carry out further experiments.

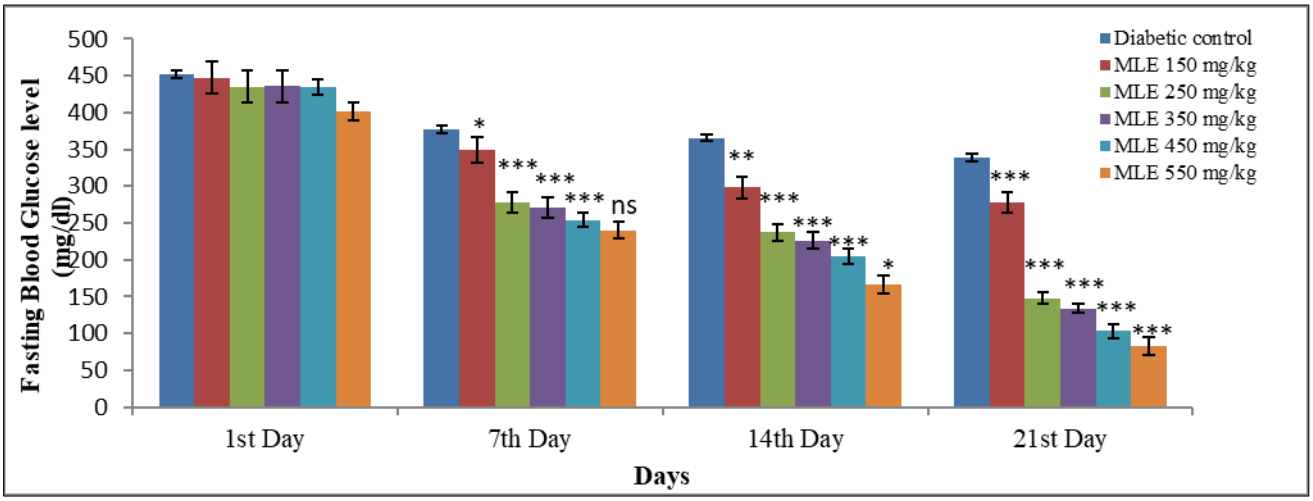


Figure 1 The graph elaborates variations of the FBG levels upto a period of 21 days in response to the various effective doses of MLE in diabetic mice. Values are expressed as the mean ± SEM; where n=6. Significant differences: *p<0.05, **p<0.01 and ***p<0.001 against DC

3.5. Anti-hyperlipidemic Study

It is observed that the Total cholesterol, Triglyceride, LDL-C and VLDL-C levels increased significantly and HDL-C levels decrease significantly in the diabetic control group (DC) than the normal control (NC). However, the ascorbic treated group (D+ASC) and the methanolic leaf extract treated group (D+MLE) exhibited a significant decrease in the total cholesterol, triglyceride, LDL-C and VLDL-C levels and increase in HDL-C levels when compared against the diabetic control group (DC) as shown in Table 1.

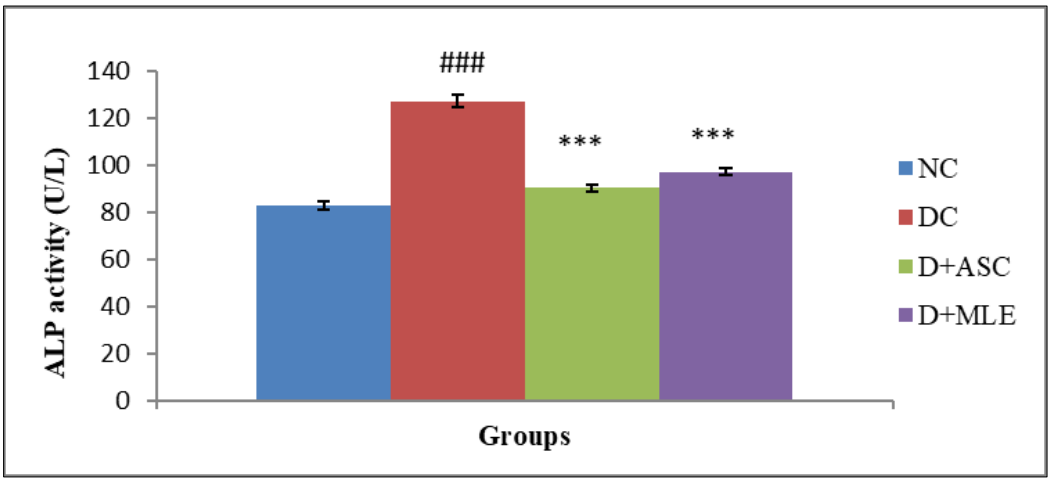
Table 1 The levels of lipid profile markers (Total cholesterol, Triglycerides, HDL-C, LDL-C and VLDL-C) in the serum of different groups of mice

Groups	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
NC	102.33 ±0.65	91.73 ±1.33	76.02±0.80	7.96 ±1.07.	18.34±0.27
DC	165.19 ±3.78##	153.03 ±1.35##	25.31±1.06#	109.3 ±5.14##	30.6 ±0.26###
D+ASC	126.57±2.06**	102.57±0.77***	69.19±0.64**	36.87±2.86***	20.51±0.15***
D+MLE	133.91 ±0.84*	113.7 ±2.63**	64.65±1.41**	46.52±1.16**	22.73±0.52**

Values are expressed as the mean ± SEM; where n=6. Significant differences: #p<0.05, ##p<0.01 and ###p<0.001 when compared against NC and *p<0.05, **p<0.01 and ***p<0.001 when compared against DC.

3.6. Hepatic marker enzyme activity

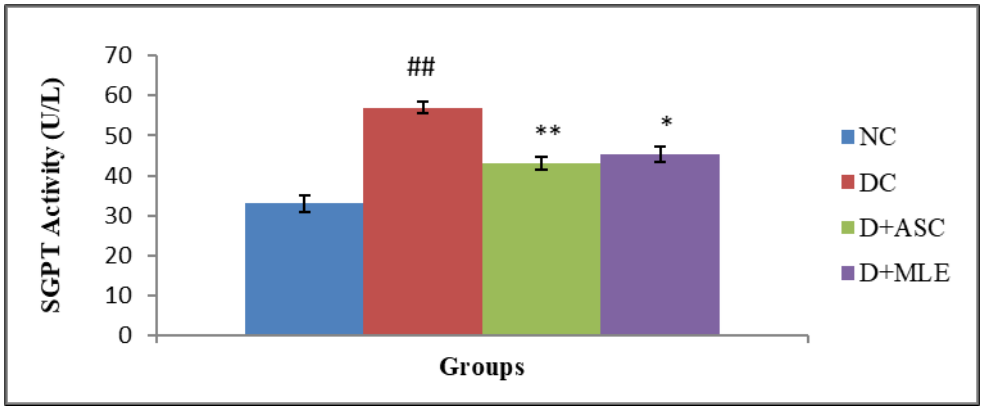
Alkaline phosphatase activity (ALP): It was observed (Figure: 2) that the ALP activity significantly increased upto 53.64% (127±2.51) in the diabetic control group (DC) than the normal control (NC) (82.66±1.76). However, the ascorbic acid treated group (D+ASC) and methanolic leaf extract treated group (D+MLE) exhibited a significant decrease of 28.87% (90.33±1.45) and 23.62% (97±1.52) respectively when compared against diabetic control group (DC).



Values are expressed as the mean ± SEM; where n=6. Significant difference: ### p<0.001 against NC, ***p<0.001 against DC.

Figure 2 The graph shows the activity of ALP in Normal control (NC), Diabetic control (DC), Ascorbic acid treated group (D+ASC) and Methanolic leaf extract treated group (D+MLE)

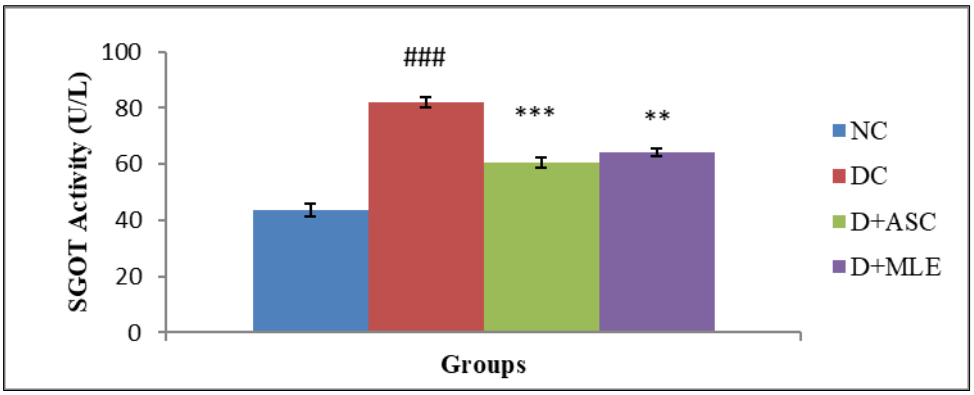
Alanine transaminase or Serum glutamic pyruvic transaminase activity (ALT or SGPT): It was observed (Figure: 3) that the SGPT activity significantly increased upto 72% (57±1.52) in the diabetic control group (DC) than the normal control (NC) (33±2). However, the ascorbic acid treated group (D+ASC) and methanolic leaf extract treated group (D+MLE) exhibited a significant decrease of 24% (43±1.52) and 20.47% (45.33±2.02) respectively when compared against diabetic control group (DC).



Values are expressed as the mean ± SEM; where n=6. Significant difference: ##p<0.01 against NC, **p<0.01 and *p<0.05 against DC.

Figure 3 The graph shows the activity of SGPT in Normal control (NC), Diabetic control (DC), Ascorbic acid treated group (D+ASC) and Methanolic leaf extract treated group (D+MLE)

Aspartate transaminase or Serum glutamic oxaloacetic transaminase activity (AST or SGOT): It was observed (Figure: 4) that the SGOT activity significantly increased upto 87.81% (82±1.73) in the diabetic control group (DC) than the normal control (NC) (43.66±2.33). However, the ascorbic acid treated group (D+ASC) and methanolic leaf extract treated group (D+MLE) exhibited a significant decrease of 26.02% (60.66±1.76) and 21.54% (64.33±1.33) respectively when compared against diabetic control group (DC).



Values are expressed as the mean ± SEM; where n=6. Significant difference: ###p<0.001 against NC, ***p<0.001 and **p<0.01 against DC.

Figure 4 The graph shows the activity of SGOT in Normal control (NC), Diabetic control (DC), Ascorbic acid treated group (D+ASC) and Methanolic leaf extract treated group (D+MLE)

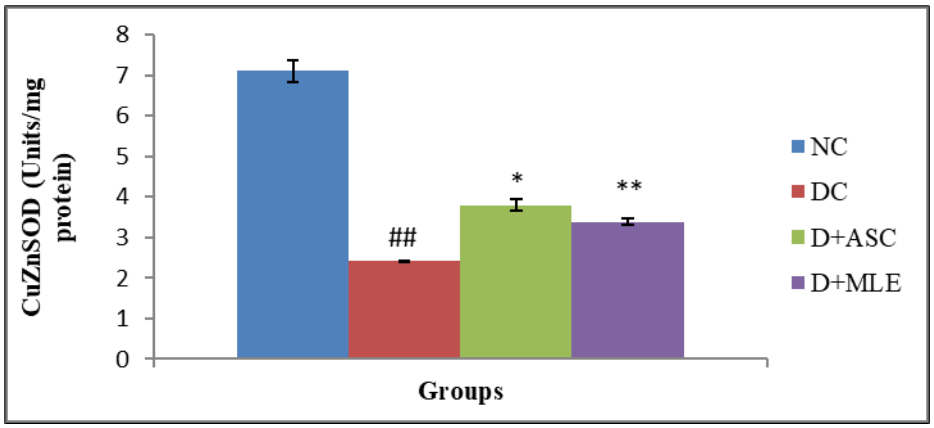
3.7. Liver anti-oxidant enzyme activity

3.7.1. Antioxidant enzyme assays

The Superoxide dismutase (CuZn-SOD and Mn-SOD), Catalase (CAT) and Glutathione reductase (GR) activities in liver tissue of the normal control mice (NC), diabetic control mice (DC), diabetic mice treated with ascorbic acid (D+ASC) and diabetic mice treated with methanolic leaf extract (D+MLE) are shown in Figure: 5, 6, 7 and 8 respectively.

3.7.2. Superoxide dismutase activity (CuZn-SOD and Mn-SOD)

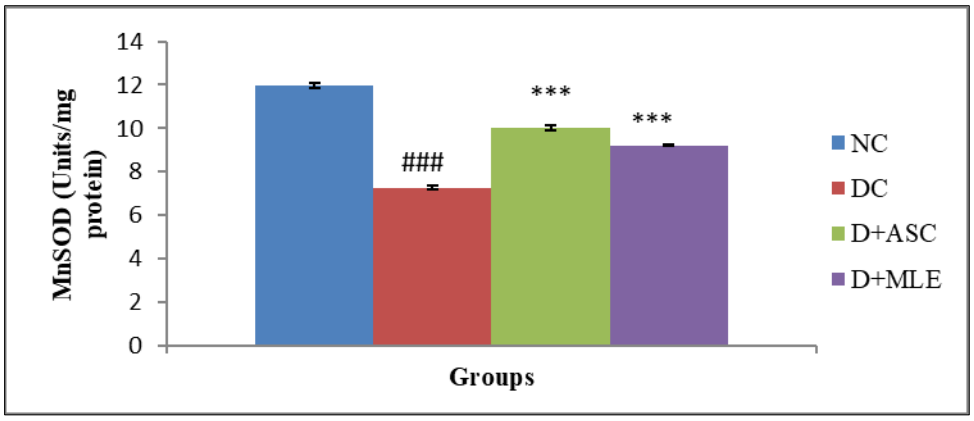
CuZnSOD activity: It was observed that the CuZnSOD activity significantly decreased upto 66.05% (2.41±0.01) in the diabetic control group (DC) than the normal control (NC) (7.1±0.28). However, the ascorbic acid treated group (D+ASC) exhibited a significant increase of 57.67% (3.8±0.15) when compared against DC. Further the methanolic leaf extract treated group (D+MLE) also showed a significant increase of 40.24% (3.38±0.08) against DC (Figure: 5).



Values are expressed as the mean ± SEM; where n=6. Significant difference: ## p<0.01 against NC, *p<0.05 and **p<0.01 against DC.

Figure 5 The graph shows the CuZnSOD activity in Normal control (NC), Diabetic control (DC), Ascorbic acid treated group (D+ASC) and Methanolic leaf extract treated group (D+MLE)

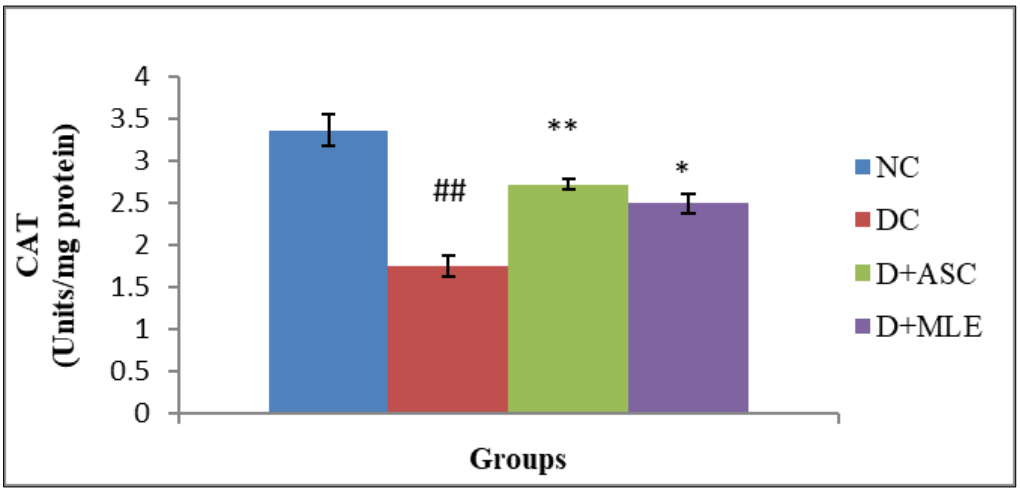
MnSOD activity: It was observed that the MnSOD activity significantly decreased upto 64.78 % (7.27±0.07) in the diabetic control group (DC) than the normal control (NC) (11.98±0.13). However, the ascorbic acid treated group (D+ASC) exhibited a significant increase of 37.68% (10.01±0.13) when compared against DC. Further the methanolic leaf extract treated group (D+MLE) also showed a significant increase of 26.54% (9.2±0.03) against the DC (Figure: 6).



Values are expressed as the mean ± SEM; where n=6. Significant difference: ###p<0.001 against NC, ***p<0.01 against DC.

Figure 6 The graph shows the MnSOD activity in Normal control (NC), Diabetic control (DC), Ascorbic acid treated group (D+ASC) and Methanolic leaf extract treated group (D+MLE)

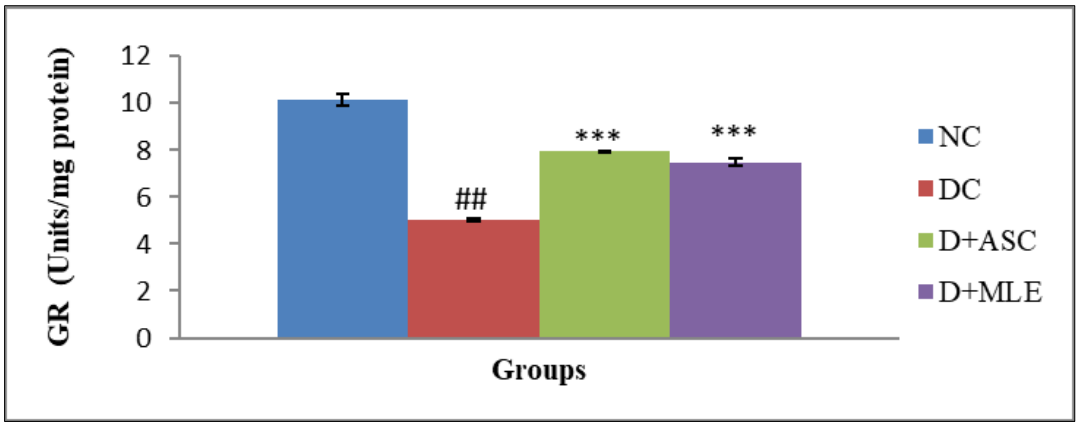
Catalase activity: It was observed that the catalase activity significantly decreased upto 47.91% (1.75±0.13) in the diabetic control group (DC) than the normal control (NC) (3.36±0.19). However, the ascorbic acid treated group (D+ASC) exhibited a significant increase of 55.42% (2.72±0.06) when compared against DC. Further the methanolic leaf extract treated group (D+MLE) also showed a significant increase of 42.28% (2.49±0.11) against DC (Figure: 7).



Values are expressed as the mean ± SEM; where n=6. Significant difference: ## p<0.01 against NC, **p<0.01 and *p<0.05 against DC.

Figure 7 The graph shows the catalase activity in Normal control (NC), Diabetic control (DC), Ascorbic acid treated group (D+ASC) and Methanolic leaf extract treated group (D+MLE)

Glutathione reductase (GR) activity: It was observed that the GR activity significantly decreased upto 50.59% (5.013±0.07) in the diabetic control group (DC) than the normal control (NC) (10.14±0.26). However, the ascorbic acid treated group (D+ASC) exhibited a significant increase of 58.36% (7.939±0.02) when compared against DC. Further the methanolic leaf extract treated group (D+MLE) also showed a significant increase of 49.33% (7.486±0.14) against the DC (Figure: 8).



Values are expressed as the mean ± SEM; where n=6. Significant difference: ## p<0.01 against NC, ***p<0.001 against DC.

Figure 8 The graph shows the catalase activity in Normal control (NC), Diabetic control (DC), Ascorbic acid treated group (D+ASC) and Methanolic leaf extract treated group (D+MLE)

3.8. Histology studies

The normal morphological features of the liver tissues of normal mice (NC) consist of central vein, portal vein, hepatic sinusoids and hepatocytes. There was a loss in the normal histological structure in the liver of diabetic mice (DC) with enlarged and distorted central and portal vein, sinusoidal dilation with red blood cells congestion was seen inside central and portal vein when compared with normal mice. However, diabetic mice treated with ascorbic acid (D+ASC) and methanolic leaf extract (D+MLE) showed almost normal histological structure with no central or portal vein enlargement or distortion. Moreover, sinusoidal dilation and red blood cells congestion around central or portal vein were also not seen in the images (Figure: 9)**.**

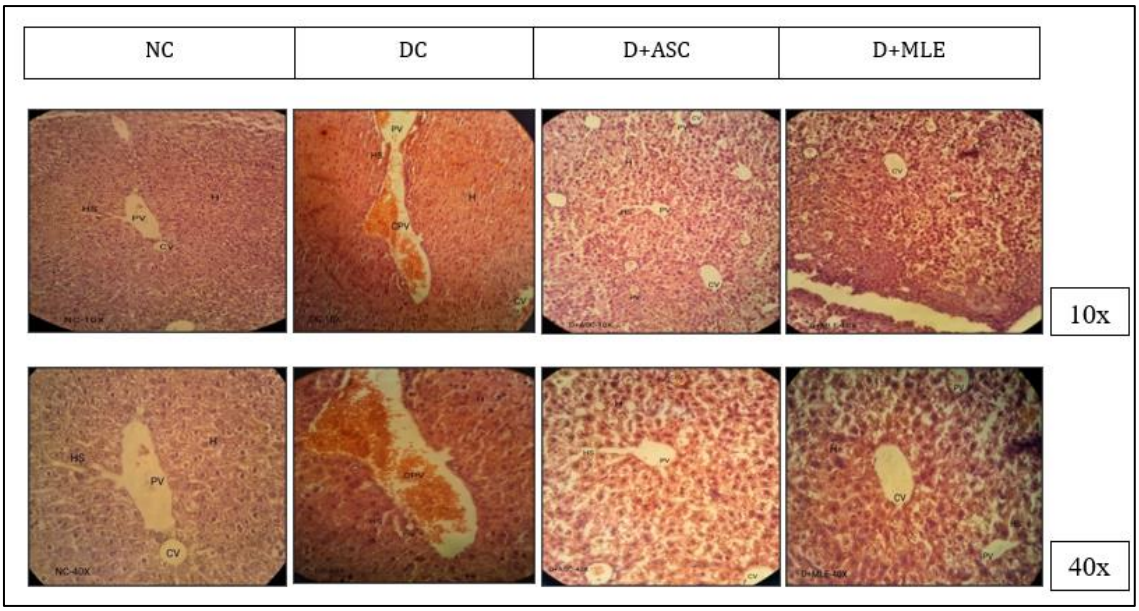


Figure 9 The representative photographs shows the morphological features of the liver tissues of a normal control mice (NC), diabetic control mice (DC), diabetic mice treated with ascorbic acid (D+ASC) and diabetic mice treated with methanolic leaf extract (D+MLE). H: Hepatocytes, CV: Cental vein, PV: Portal vein, HS: Hepatic sinusoids. The images shown are taken in 10X and 40X magnifications respectively

4. Discussion

Various studies have shown that the plant *M. oleifera* possesses a wide range of pharmacological properties, including anti-oxidative, anti-inflammatory and anti-hyperlipidemic effects [8]. Hence, this has led to a significant research interest in further examining and evaluating the anti-oxidative, anti-diabetic and hepato-protective potential of *M.*

oleifera in the prevention and management of diabetes [8]. Therefore this study was conducted to show the presence of various phytochemicals in the methanolic leaf extract of *M. oleifera* through preliminary phytochemical screening tests along with the quantitative phytochemical assay (total phenolics and total flavanoids). The phytochemicals possess natural anti-oxidative properties and have shown significant benefit in various plants medicinal sector [25, 26]. In this study an acute toxicology test was conducted to determine the median lethal dose i.e. LD50 for the MLE of the plant *Moringa oleifera*. From the experiment it was found that none of the MLE doses (400-2000 mg/kg b.w.) has caused any mortality which indicates that the LD50 value of MLE of *Moringa oleifera* is higher than 2000 mg/kg b.w. Thus, it implies that intraperitoneal administration of *Moringa oleifera* even at higher doses is safe, non-toxic and had non-lethal effects on the mice. Since it has been well documented that Streptozotocin (STZ) or N-(methyl nitro carbamoyl)–D-glucosamine is one of the β-cytotoxic drug commonly used to cause destruction of the pancreatic beta-cells (ß-cells) and insulin deficiency leading to increase in the production of glucose by the liver and decrease in utilization of glucose by the peripheral tissues which in turn leads to hyperglycemia or diabetes mellitus. Hence, STZ was used and administered to cause a significant increase in blood glucose level in the experimental mice model for the present study [27]. Thus a long term sub-acute anti-hyperglycemic study was conducted using various doses of the MLE ranging from 150-550 mg/kg body weight (of the mice). After intraperitoneal administration of various doses of MLE it was observed that the FBG level in the mice was reduced in a dose- dependent manner (Figure: 1). Although all the doses of MLE (150-550 mg/kg b.w.) were quite effective in reducing the blood glucose level in diabetic mice but however the dose 350 mg/kg b.w. was considered as the optimum dose because of its ability to reduce FBG at the same significance level as 550 mg/kg b.w. dose on the 21st day thus proving that it exerts a potent anti-hyperglycemic action in STZ-induced diabetic mice models and hence this particular dose was used for the further study. The lipid profile assay showed that the total cholesterol, triglyceride, LDL-C and VLDL-C levels were increased with decreased HDL-C levels during diabetic conditions. However upon treatment with MLE for 21 days there was a remarkable reduction of total cholesterol, triglyceride, LDL-C and VLDL-C followed by an increase in HDL-C levels which indicated that MLE has potent anti-hyperlipidemic activity [28]. The presence of phenols and flavanoids in the MLE is mainly associated with the lipid-reduction [29]. Some preclinical studies have also suggested that flavonoids affect the function of reverse cholesterol transport (RCT) and HDL beyond the simple HDL cholesterol concentration. Flavanoids can also inhibit HMG-CoA reductase which in turn affects the production of cholesterol [30], thus imparting a potent hypolipidemic effect on STZ-induced diabetic mice models. The evaluation of liver damage was gauged by measuring the serum levels of ALP, SGPT and SGOT, which are popularly recognized as prevalent indicators of liver damage. It's certain that when the integrity of the cellular membrane of the liver is compromised, several enzymes that basically reside inside the cellular cytosol are released into the bloodstream. The concentration of these enzymes in the blood serum provides a valuable quantitative estimate of the magnitude and nature of hepatocellular injury [31] from the present study it was observed that the aminotransferases and acid phosphatases levels of the serum were elevated under diabetic conditions when the liver was damaged due to hyperglycemia induced oxidative stress in diabetic mice. However it was also observed that the MLE-treated diabetic mice group showed significantly reduced levels of serum ALP, SGPT and SGOT against the untreated diabetic mice group (Fig: 2, 3 and 4). This perhaps is due to the protective action of MLE of *M. oleifera* on liver tissues against the damages caused due to oxidative stress [26]. Superoxide dismutase or SOD (Mn-SOD and CuZn-SOD) are antioxidant enzymes present in the mitochondria and cytosol and acts as a defence mechanism against reactive oxygen species (ROS). They aid in conversion of superoxide anion to hydrogen peroxide [15]. Catalase or CAT enzyme is also an antioxidant enzyme that helps to neutralize hydrogen peroxide to water and oxygen [15]. Glutathione reductase or GR is another anti-oxidative enzyme found in the cytoplasm which also facilitates in converting oxidized glutathione to its reduce form GSH and helps in striking a balanced intracellular environment [17]. In this study, it was found that the activities of the enzymes SOD (CuZnSOD and MnSOD), CAT, and GR was decreased significantly in diabetic mice as compared to normal mice. But further in the study it has been observed that MLE treated groups exhibited elevated activities of the anti-oxidative enzymes as compared to the diabetic group (Figure: 5, 6, 7 and 8). Therefore, it could be said that MLE of *M. oleifera* protects the liver tissue from further oxidative stress under diabetic condition. Moreover, the histological study conducted also shows that the MLE of *M. oleifera* was able to protect and reverse the injuries caused due to diabetes and also confirms that the extract was capable to suppress tissue damages caused due to oxidative stress and enhance the anti-oxidative enzyme activity in the liver, thus allowing the tissue to recover and restore from further damage (Figure: 9).

5. Conclusion

Hence from the findings of the present study we can conclude that the methanolic leaf extract of the plant *Moringa oleifera* has anti-hyperglycemic, anti-hyperlipidemic, anti-oxidative and anti-hepatopathy properties against STZ-induced diabetic mice. However, the exact mechanism is yet to be elucidated and further ongoing investigations are in progress.

Compliance with ethical standards

Acknowledgments

The authors would like to thank NEHU, Shillong, India for providing NON-NET fellowship and would also like to thank DRS programme, for providing infrastructural support to the Department of Biochemistry, NEHU, Shillong.

Disclosure of conflict of interest

There authors declare no conflicts of interest.

Statement of ethical approval

All the experiments were approved by Institutional ethics committee (Animal model) North-Eastern Hill University, Shillong, India (Date: 31st May, 2023) and were carried out in accordance with the same.

References

[1] Darenskaya MA, Kolesnikova LI, Kolesnikov SI. Oxidative Stress: Pathogenetic Role in Diabetes Mellitus and Its Complications and Therapeutic Approaches to Correction. Bulletin of Experimental Biology and Medicine. 2021; 171(2):179-89.

[2] Selvin E, Juraschek SP. Diabetes epidemiology in the COVID-19 pandemic. Diabetes Care. 2020; 43(8):1690-94.

[3] Harding JL, Pavkov ME, Magliano DJ, Shaw JE, Gregg EW. Global trends in diabetes complications: a review of current evidence. Diabetologia. 2019; 62(1):3-16.

[4] WHO: Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia. Geneva: WHO. 2006; P:1-50.

[5] Saxena AK, Srivastava P, Kale RK, Baquer NZ. Impaired antioxidant status in diabetic rat liver: Effect of Vanadate. Biochemical Pharmacology. 1993; 45(3):539-42.

[6] Taylor SI, Accili D, Imai Y. Insulin resistance or insulin deficiency: which is the primary cause of NIDDM?. urnal of Diabetes. 1994; 43(6):735-40.

[7] Gupta R, Mathur M, Bajaj VK, Katariya P, Yadav S, Kamal R, Gupta RS. Evaluation of antidiabetic and antioxidant activity of Moringa oleifera in experimental diabetes. Journal of Diabetes. 2012; 4(2):164-71.

[8] Aljazzaf B, Regeai S, Elghmasi S, Alghazir N, et al. Evaluation of Antidiabetic Effect of Combined Leaf and Seed Extracts of Moringa oleifera (Moringaceae) on Alloxan-Induced Diabetes in Mice: A Biochemical and Histological Study. Oxidative Medicine and Cellular Longevity. 2023; 2023:9136217.

[9] Harborne J. Phytochemical methods. A guide to modern techniques of plant analysis. 3rd ed. Germany: pringer science and business media; 1998.

[10] Obiamine AW, Uche FI. The phytochemical screening and effects of methanolic extract of Phyllanthus amarus leaf on the biochemical parameters of male Guinea pigs. The Journal of Applied Sciences & Environmental Management. 2008; 12(4):73-77.

[11] Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagent. American Journal of Enology and Viticulture. 1965; 16(3):144-58.

[12] Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods in Enzymology. 1999; 299:152-78.

[13] Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Science & Technology. 1995; 28:25-30.

[14] OECD Guidelines for Testing of Chemicals-425. Acute Oral Toxicity Up-and-Down Procedure (adopted on 17th December 2001).

[15] Sahariah P, Bora J, Patar AK, Syiem D, Bhan S. Evaluation Of Antihyperglycemic And Antioxidative Effects Of Dillenia Indica L. Using In-Vivo Approaches. International Journal of Pharmaceutical Sciences and Research. 2018; 9(11):4551-61.

[16] Patar A, Bhan S, Syiem D. Effect of chlorophyllin, an semi-synthetic chlorophyll molecule on hyperglycemia and hyperlipidemia in streptozotocin induced diabetic mice. International Journal of Pharmacy and Pharmaceutical Sciences. 2016; 8:293-96.

[17] Bora J, Sahariah P, Patar AK, Syiem D, Bhan, S. Attenuation of diabetic hepatopathy in alloxan-induced diabetic mice by methanolic flower extract of Phlogacanthus thyrsiflorus Nees. Journal of Applied Pharmaceutical Science. 2018; 8(7)114-20.

[18] Gatsing D, Aliyu R, Kuiate JR, Garba IH, Jaryum KH. et al. Toxicological evaluation of the aqueous extract of Allium sativum bulbs on laboratory mice and rats. Cameroon Journal of Experimental Biology. 2005; 1(1):39-45.

[19] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein in plasma without the use of preparative ultracentrifuge. Clinical Chemistry. 1972; 18(6):499-502.

[20] Younis N, Khan MI, Zahoor T, Faisal MN. Phytochemical and antioxidant screening of Moringa oleifera for its utilization in the management of hepatic injury. Frontiers in Nutrition. 2022; 9:1078896.

[21] Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for SOD. European Journal of Biochemistry. 1974; 47(3):469-74.

[22] Aebi H. Catalase in-vitro. Methods in Enzymology. 1984; 105:121-26.

[23] Carlsberg I, Mannervik B. Glutathione reductase. Methods in Enzymology. 1985; 113:484-90.

[24] Kiernan JA. Histological and histochemical methods: theory and practice. 3rd ed. United Kingdom: Butterworth–Heinemann Publishers; 1999.

[25] Bhalla N, Ingle N, Patri SV, Haranath D. Phytochemical analysis of Moringa Oleifera leaves extracts by GC-MS and free radical scavenging potency for industrial applications. Saudi Journal of Biological Sciences. 2021; 28(12):6915-28.

[26] Nurhayati T, Ridho MF, Santoso PTR, Setiawan S, Goenawan H, Tarawan VM. Effects of Moringa oleifera Leaf Extract on Liver Histopathology: A Systematic Review. Journal of Nutrition and Metabolism. 2024; 2024:6815993.

[27] Rakietan N, Rakietan ML, Nadkarni MV. Studies on the diabetogenic action of streptozotocin (NSC-37917). Cancer Chemotherapy Reports. 1963; 29:91-98.

[28] Lacorte LH, Robles JH, Panganiban C, Cajano JV, Santos JB, Ortiz CK, Gumban MT, Sancho JE, Nas JS. Effects of Moringa oleifera leaf extracts on lipid profile of rats: a meta-analysis and systematic review. Asian Journal of Biological and Life Sciences. 2021; 549-58.

[29] Zeni AL, Moreira TD, Dalmagro AP, Camargo A, Bini LA, Simionatto EL, Scharf DR. Evaluation of phenolic compounds and lipid-lowering effect of Morus nigra leaves extract. Annals of the Brazilian Academy of Sciences. 2017; 7;89(04):2805-15.

[30] Vergara-Jimenez M, Almatrafi MM, Fernandez ML. Bioactive components in Moringa oleifera leaves protect against chronic disease. Antioxidants. 2017; 6(4):91.

[31] Eugenio-Pérez D, Montes de Oca-Solano HA, Pedraza-Chaverri J. Role of food-derived antioxidant agents against acetaminophen-induced hepatotoxicity. Pharmaceutical Biology. 2016; 54(10):2340-52.