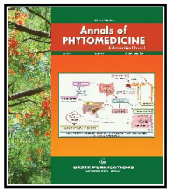
Annals of Phytomedicine 13(1): 589-601, 2024

DOI: http://dx.doi.org/10.54085/ap.2024.13.1.61

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Antidiabetic efficacy of leaf extracts of *Piper betle* L. in streptozotocin-nicotinamide induced diabetic rats: An *in vivo* model

Venkatesh Rajendran*,*, Hima Bindu Kaipa**◆, Subban Kamalraj*, Ramakrishnan Ramanathan**, Chelliah Jayabaskaran* and Gejjenahalli Puttanaik Mohankumar ****

**Department of Biochemistry, Indian Institute of Science, Bengaluru-560012, Karnataka, India*

*** Division of Flower and Medicinal Crops, Indian Institute of Horticultural Research, Hesaraghata Lake Post, Bengaluru-560089, Karnataka, India*

**** Department of Biochemistry (PG) and Research, Kongunadu Arts and Science College (Autonomous), G.N. Mills Post, Coimbatore-641029, Tamil Nadu, India*

Article Info

Article history
Received 4 March 2024
Revised 24 April 2024
Accepted 25 April 2024
Published Online 30 June 2024

Keywords
Piper betle L.
Leaf extract
Antidiabetic activity
Streptozotocin
In vivo

Abstract

Piper betle L. is widely used in traditional medicine to treat upper respiratory diseases, bad breath, stomachalgia, and rheumatism. The present study focused on the antidiabetic potential of the methanolic leaf extracts of betel vine cultivars, using the streptozotocin-induced diabetic rats model. Methanolic leaf extract of Swarna Kapoori (MESK) and Halisahar Sanchi (MEHS) with a dosage of 200 mg/kg b.w was administered to the streptozotocin-nicotinamide induced diabetic Wister albino rats, for twenty-eight days. *In vivo* analyses on changes in the hypoglycemic effects, fluctuations in body weight, lipid profile, hematological indicators, biochemical markers, hormonal modulations, and histopathological observations were carried out. Evaluation of the antidiabetic potential of the methanolic leaf extract of Swarna Kapoori (MESK) and methanolic leaf extract of Halisahar Sanchi (MEHS) revealed marked alterations such as a significant decrease in the levels of blood glucose, total cholesterol (TC), triglycerides (TGL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and significant increase was noted in the serum high-density lipoprotein (HDL), counts of hemoglobin, PCV, RBC, WBC, platelets and other blood indices. Examination of both leaf extracts indicated a remarkable change in diabetic attributes such as a decrease in the level of liver enzymes, renal functional markers, insulin, and cortisol. Moreover, histopathological observations on the pancreas, hepatocytes, and renal cells revealed, a significant retrieval from an impairment, endorsing the favorable evidence obtained through biochemical findings. The investigation concluded that the methanolic leaf extracts of both Swarna Kapoori and Halisahar Sanchi cultivars exhibited antidiabetic properties, with MESK proving to be the most effective.

1. Introduction

The incidence of type 2 diabetes mellitus (T2D) has been increasing and, it is prevalent Worldwide. Many researchers are faced with the challenge of developing natural remedies for the treatment of type 2 diabetes (Jayaprasad *et al*., 2016; Garg *et al*., 2023). Diabetes is a chronic metabolic deficiency, characterized by fasting hyperglycemia, which affects a large number of human communities of all social conditions. A significant number of diabetic cases were observed in India, China, the U.S., the Russian Federation, and Brazil. Among them, India has been ranked first, with the highest number of patients reported with type 2 diabetes (T2D) (Chauhan *et al*., 2016). A convincing report alarmed that Worldwide, about 200 million people are affected by T2D, and 300 million are expected to be susceptible to diabetes by 2025 (Bigoniya *et al*., 2012). Currently, many researchers have been faced with the exploration of indigenous Indian-originated medicinal plants that have been used for remedies for type 2 diabetes after the recommendation of WHO (Jayaprasad *et al*., 2016; Kavipriya, 2013). *P. betle*, a perennial ever-green climber which is valued for its fresh leaves with many medicinal properties has been reported to lower blood sugar and lipid peroxidation ability (Arsalan, *et al*., 2019).

Diabetes causes troubles in the metabolism of carbohydrates, proteins, and lipids due to absolute or relative deficiency of insulin secretion, with or without insulin resistance exhibiting symptoms such as increased thirst, increased urination, increased hunger, burning of vision, weight loss, and feeling tired or drowsy (Edijala *et al*., 2005; Moree *et al*., 2013; Chellammal, 2022). These complex multiple disorders of metabolic changes often lead to functional impairment and damage of various tissues (Momose *et al*., 2002), and the associated disturbances are usually characterized by hyperglycemia, hypercholesterolemia combined with a low level of insulin, c-peptide, and high-density lipoprotein cholesterol (Valcheva-Kuzmanova *et al*., 2007; Tunali and Yanardag, 2006; Rani *et al*., 2023).

Betel vine (*Piper betle* L.) belongs to the Piperaceae family and is a popular plant with numerous therapeutic properties asexually propagated and commercially cultivated as a cash crop for its varied importance (Patra *et al*., 2011). Leaves of *P. betle* contain numerous effective ingredients, reportedly involving, vital biological activities, and are used widely in Traditional Medicinal Systems (Arambewela *et al*., 2004) for curing diabetes-related risks (Kaleem *et al*., 2005). It is estimated that betel leaves are used by 14 million people regularly every day as a pan (Ekta *et al*., 2016).

Corresponding author: Dr. Hima Bindu Kaipa
Principal Scientist, Division of Flower and Medicinal Crops, Indian Institute of Horticultural Research, Hesaraghata Lake Post, Bengaluru-560089, Karnataka
E-mail: himabindu.k@icar.gov.in
Tel.: +91-9449040463

Several investigations described, valuable scientific information on its therapeutic efficacy and use, against various diseases such as cancer, diabetes, allergies, malaria, and antibacterial, antifungal, insecticidal, antioxidant, gastroprotective, cytotoxic, antiplatelet, wound healing activity, chlorophyllase activity, oral hygiene, antiasthmatic properties (Rekha *et al*., 2014; Row and Ho, 2009; Arawwala *et al*., 2014; Duhan *et al*., 2022; Sruthy and Balasubramaniam, 2023). Over, 80 cultivars cover this family and these landraces are classified into different groups, *i.e.,* Bangla Desawari, Kapoori, Meetha, and Sanchi majority of the literature demonstrated the bioactivity-associated events of betel vine; however, no report specified, on the cultivar used. The present study highlighted the antidiabetic properties of methanolic leaf extracts of two cultivars, Swarna Kapoori (MESK) and Halisahar Sanchi (MEHS), using authentic plant materials collected from Field Gene Bank, maintained at ICAR-IIHR. The investigation was designed to explore the effects of methanolic extract of both selected cultivars, on the antidiabetic indicators, using streptozotocin stimulated T2D Wistar rats, animal model.

2. Materials and Methods

2.1 Collection of plant materials

Fresh mature leaves of Swarna Kapoori (IIHR-BV 47) and Halisahar Sanchi (IIHR-BV 24) (Figure 1 a, b) with indicated accession numbers of cultivars of *P. betle*, were collected from the ICAR-IIHR (ICAR-Indian Institute of Horticultural Research) field located in Hirehalli, Bengaluru, Karnataka, India, and brought to the laboratory. The specimen plant was identified and authenticated from the Centre for Conservation of Natural Resources, Foundation for Revitalisation of Local Health Traditions (FRLHT), Bengaluru, Karnataka, India. The Accession numbers are: Halisahar Sanchi (FRLHT-124379) and Swarna Kapoori (FRLHT-124380). The leaves were washed with clean running tap water, followed by treatment with 0.1 per cent mercuric chloride solution and subsequent rinsing with sterile double-distilled water. Afterwards, fresh leaves were individually sliced into small fragments and ground into powder using liquid nitrogen. The leaf powder was collected and preserved at -80^{o}C for further analytical uses.

(a) (b)

Figure 1: (a) Swarna Kapoori (IIHR-BV 47) and (b) Halisahar Sanchi (IIHR-BV 24).

2.2 Preparation of the extract

The powdered leaves (100 g) were soaked with methanol (300 ml) and kept for 24 h at room temperature (37^{o}C.) under shaking conditions vigorously. Leaf powder extract was filtered, using Whatmann No.1 filter paper (pore size 25 ìm). The extract was dried using a rotary vacuum evaporator and stored for future analysis. The methanol leaf extract was dissolved in 10% DMSO and used for *in vivo* diabetic intervention.

2.3 Chemicals

Streptozotocin (Analytical grade) used in the studies was procured from Sigma Aldrich. Additionally, other high-purity reagents were utilized in the experiments.

2.4 Animal ethical committee approval

Animal Ethical Committee approval was obtained from the animal ethical committee of the institute (IISc), before conducting the research experiments and also explaining all the parameters involved in the study. Subsequently, the Institutional Animal Ethical Committee approval number was accorded (CAF/Ethics/554/2017 dated 06 February 2017).

2.5 Experimental animals

Adult male albino Wistar rats with a mean body weight (b.w.) above 300 g at 10 to 12 weeks, old from conception, were acquired from Central Animal Facility, Indian Institute of Science, Bengaluru, India. The rats were fed with a standard, commercial rat pellet diet, and water *ad libitum*. The experiment was performed in the Central Animal Facility, Indian Institute of Science, Bengaluru, India, with due permission from the Institutional Animal Ethics Committee (R.No. 48/GO/ReBi/SL/1999/CPCSEA).

2.6 Selection of therapeutic doses

Based on toxicity studies, different doses of methanolic leaf extracts of Swarna Kapoori (MESK) and Halisahar Sanchi (MEHS), ranging from 100 mg, 200 mg, 400 mg, and 500 mg/kg body weight were used to treat rats for 14 days to determine the effective dose of the leaf extract without any toxic effects. Finally, the effective dose of both the varieties (200 mg/kg/body weight) was fixed based on the hematological, biochemical, and histopathological studies.

2.7 Experimental design

A total number of 42 Wister albino rats were bifurcated into seven groups, with six animals in each group.

Group I (normal rats) received an equal volume of vehicles (10% DMSO).

Group II (diabetic control) diabetic induced rats.

Group III (diabetic rats) received MESK 200 mg/kg b.w. (dissolved in 10% DMSO) by oral administration daily for 28 days.

Group IV (diabetic rats) received MEHS 200 mg/kg b.w. (dissolved in 10% DMSO) by oral administration daily for 28 days.

Group V (normal rats) received MESK 200 mg/kg b.w. (dissolved in 10% DMSO) by oral administration daily for 28 days.

Group VI (normal rats) received MEHS 200 mg/kg b.w. (dissolved in 10% DMSO) by oral administration daily for 28 days.

Group VII (diabetic rats) received the standard drug glibenclamide10 mg/kg b.w. (dissolved in 10% DMSO) by oral administration daily for 28 days.

2.8 Induction of T2D

Type 2 diabetes was induced by overnight fasted experimental animal groups by adopting, a standard procedure (Masiello *et al*., 1998). Type 2 diabetes was induced by a single intraperitoneal (i.p.) injection of fresh streptozotocin (STZ) (Sigma-Aldrich, St. Louis, MO, USA) (65 mg/kg b.w.) dissolved in 0.1 M citrate buffer (pH 4.5), 15 min after the i.p. administration of nicotinamide (NA) (110 mg/kg b.w.), dissolved in normal saline. NA partially protects the β cells of the pancreas against the STZ-mediated cytotoxic impairment. NA was found to preserve the intracellular pool of nicotinamide adenine dinucleotide (oxidized form) (NAD) either by acting as a precursor of NAD or by inhibiting the activity of poly (ADP ribose) synthetase which is a NAD-consuming enzyme, activated by STZ. To break in the hypoglycemia during the first day after the STZ administration, diabetic rats were given a 5% oral glucose solution. Seven days after the injection, the blood glucose level was measured, using a portable, advanced glucometer (Accu-Chek Roche) in the blood, collected from the tail veins, and fixed, the animals with blood glucose levels above 250 mg/dl, considered to be the limit of diabetic (Aminu *et al*., 2015). In all experiments, rats were fasted for 16 h before STZ injection.

2.9 Collection of tissues

After the treatment exposure, the animals were sacrificed under mild diethyl ether anesthesia. Blood was collected in EDTA and centrifugal tubes by heart puncturing, and serum was separated by centrifugation at 1000 rpm for 10 min and utilized for various biochemical assays. The organs such as the pancreas, liver, and kidney were removed, immediately and thoroughly washed with ice-cold physiological saline and blotted dry. A part of the tissues from the pancreas, liver, and kidney was detached and fixed in 10% formalin for histopathological examinations.

2.10 Body weight and blood glucose evaluation

Body weight was measured, once a week, for 4 weeks of experimental tenure. The level of blood glucose concentration was observed, during the entire experimental period, using a portable glucometer (Accu-Chek Roche). Blood was collected from the tail veins of the rats; the instrument measuring capacity limit is a maximum of 600 mg/dl.

2.11 Haematological examination

The blood sample was analyzed, to detect, hematological indices such as count of hemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and platelets, and those were performed, using SYSMEX Kx-21(Eraba, Transasia) automatic hematology analyzer.

2.12 Biochemical scrutiny

The serum was examined for various biochemical estimations, such as total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, VLDL, alanine transaminases (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, direct bilirubin, indirect bilirubin, total protein, albumin, globulin, urea, creatinine, and uric acid, using acceptable commercial assay kits Erba, Mannheim, Germany. The quantitative measures were carried out, on the levels of hormones such as insulin, cortisol, and c-peptide by employing an electro chemiluminescence immunoassay assay (ECLIA).

2.13 Histopathological observation

The pancreas, liver, and kidney tissues from the specified animal groups were subjected to histopathological examinations. They were cut into small pieces measuring approximately 1 mm × 1 mm × 1 mm and preserved in 10% normal saline for 48 h. Subsequently, they were dehydrated using a series of ethyl alcohol-water mixtures (50%, 80%, and 95%) and incubated in alcohol, followed by cleaning in xylene and embedding in paraffin. Samples were then sliced into ultra-thin sections using an ultra-microtome, stained with hematoxylin and eosin dye, and mounted in neutral deparaffinization xylene (DPX) medium for microscopic analysis to identify necrosis, fat changes, hyaline degenerations, and ballooning degeneration. Micrographs were captured using a Canon 10.1-megapixel digital camera attached to an Axiostar plus microscope (Zeiss-Germany).

2.14 Statistical analysis

Statistical analyses were executed by using GraphPad Prism version 5 software. Experimental findings were expressed as mean ± SD. One-way analysis of variance (ANOVA), followed by Post hoc Dennett's test was applied to determine statistical significance. The values were considered statistically significant when $p<0.05$, and $p<0.01$.

3. Results

3.1 Body weight

The body weight of all treated animals was calculated, for prior and post-tenure of the trial. The initial body weight of control and treated groups of rats appeared to be around similar. However, at the end of the experiments, the body weight of the diabetic-induced rats was found to be significantly ($p<0.05$, $p<0.01$) decreased compared with treatment groups (III, IV, and VII). The control (I), MESK, and MEHS (V, VI-200 mg/kg b.w.) treated rats exhibited no alteration in the body weight (Figure 2).

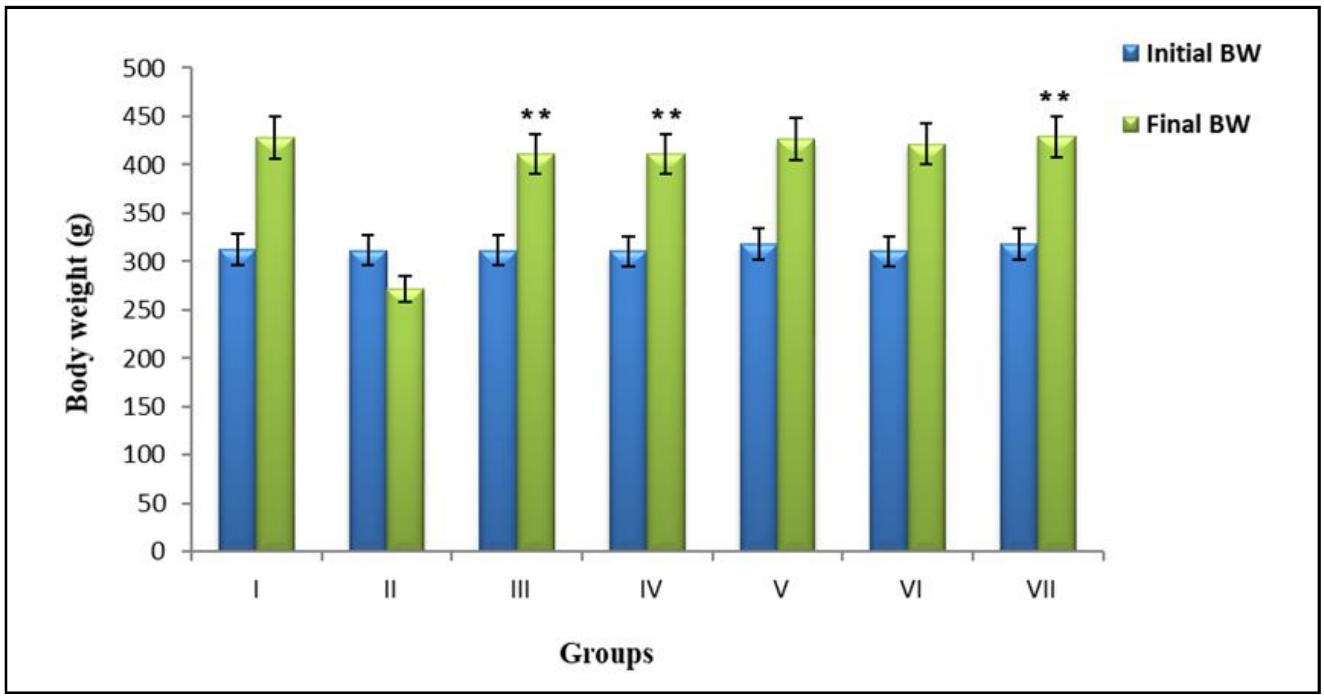


Figure 2: Effect of MESK and MEHS on body weight of control and experimental groups of rats. The data are presented as mean ± standard deviation (n = 6).*significant at $p<0.05$ in comparison to control diabetic group, ** significant at $p<0.01$. # significant at $p<0.05$ in comparison to the control normal group, and ## significant at $p<0.01$.

3.2 Haematological determination

Varied hematological indices were observed, in control and experimental animals, and were depicted in Table 1. A decrease in counts on Hb, PCV, RBC, WBC, platelets, and other determinants was observed in Group II diabetic rats. The values were gradually elevated, close to a normal level, while on treatment with MESK and MEHS at 200 mg/kg and standard drug glibenclamide (10 mg/kg) exhibited, a significant increase ($p<0.05$). Among them, MESK showed an optimum increase while, compared to glibenclamide (10 mg/kg).

Table 1: Effect of MESK and MEHS on haematological indicators of control and treated groups of rats

Grouping of animals	Hb (g/dl)	PCV (%)	RBC (millions / mm^3)	WBC (Thousands / mm^3)	Platelets (lakhs / mm^3)	MCV (fL)	MCH (Pg)	MCHC (g/dl)
Control	15.11 ± 0.21	51.90 ± 0.40	9.57 ± 0.17	11.75 ± 0.90	13.58 ± 0.70	53.75 ± 0.39	15.60 ± 0.16	28.66 ± 0.36
Diabetic control	14.46 ± 0.32	50.30 ± 1.19	9.14 ± 0.09	5.71 ± 0.14	9.61 ± 2.78	52.88 ± 1.41	15.81 ± 0.19	26.16 ± 0.10
Diabetic + MESK (200 mg/kg b.w.)	15.98 ± 0.49**	55.38 ± 2.75**	10.20 ± 0.66**	8.21 ± 0.86**	11.15 ± 0.70	53.83 ± 1.58	16.03 ± 0.89	29.96 ± 0.87
Diabetic + MEHS (200 mg/kg b.w.)	15.58 ± 0.14**	52.61 ± 0.44*	9.45 ± 0.15	8.46 ± 0.08**	10.66 ± 2.08	53.05 ± 0.78	16.16 ± 0.25	29.8 ± 0.26
Control + MESK (200 mg/kg b.w.)	15.38 ± 0.19	51.33 ± 0.85	8.85 ± 0.42	6.76 ±0.15#	16.00 ± 0.33#	54.05 ± 1.37	16.50 ± 0.16	29.75 ± 0.83
Control + MEHS (200 mg/kg b.w.)	15.41 ± 0.20#	52.45 ± 0.21#	9.61 ± 0.05	9.76 ± 2.83#	13.13 ± 1.77	54.68 ± 0.19	16.21 ± 0.15	29.5 ± 0.12
Diabetic + Glibenclamide (10 mg/kg b.w.)	15.21 ± 0.15**	51.75 ± 0.25	9.48 ± 0.07	8.21 ± 0.06**	10.00 ± 0.73	54.90 ± 0.70	16.06 ± 0.39	29.91 ± 0.72

Each value is expressed as the mean ± standard deviation (n = 6).

* $p<0.05$ significant from diabetic control, while ** significant at $p<0.01$.

significant at $p<0.05$ from the normal control, and ## significance at $p<0.01$.

3.3 Hyperglycemia scrutiny

Blood glucose level was measured in control and experimental groups of rats on the days 0, 5, 15, and 28 of treatment (Figure 3). In STZ-induced diabetic rats (II), an elevated level of blood glucose was detected, However, after treatment with MESK and MEHS (200 mg/kg), blood glucose level was significantly ($p<0.05$, $p<0.01$) declined when compared to standard drug treatment.

3.4 Glucose and serum lipid profile

Blood glucose and serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) levels of experimental groups were illustrated in Figures 4, 5. There was a significant increase in the level of blood glucose, TC, TG, LDL, and VLDL of untreated diabetic rats in comparison to normal rats. Treatments with MESK and MEHS showed a significant ($p<0.05$, $p<0.01$) decrease and reverted to the normal level of serum lipids, and blood glucose. Furthermore, a significant increase in HDL content was observed in all the examined treatment groups, over untreated. However, control rats treated with MHSK and MEHS showed a significant decrease in the lipid profile, excluding, HDL cholesterol.

3.5 Protein profile

Changes in the level of protein profile, such as total protein, albumin, and globulin were displayed in Figure 6. Results elucidated that there was a significant ($p<0.05$, $p<0.01$) increase in the levels of total protein in treated groups. No significant changes were observed in the level of albumin and globulin in the treated groups. The administration of MESK and MEHS exhibited, no effects, on protein profile, when compared to the control rats.

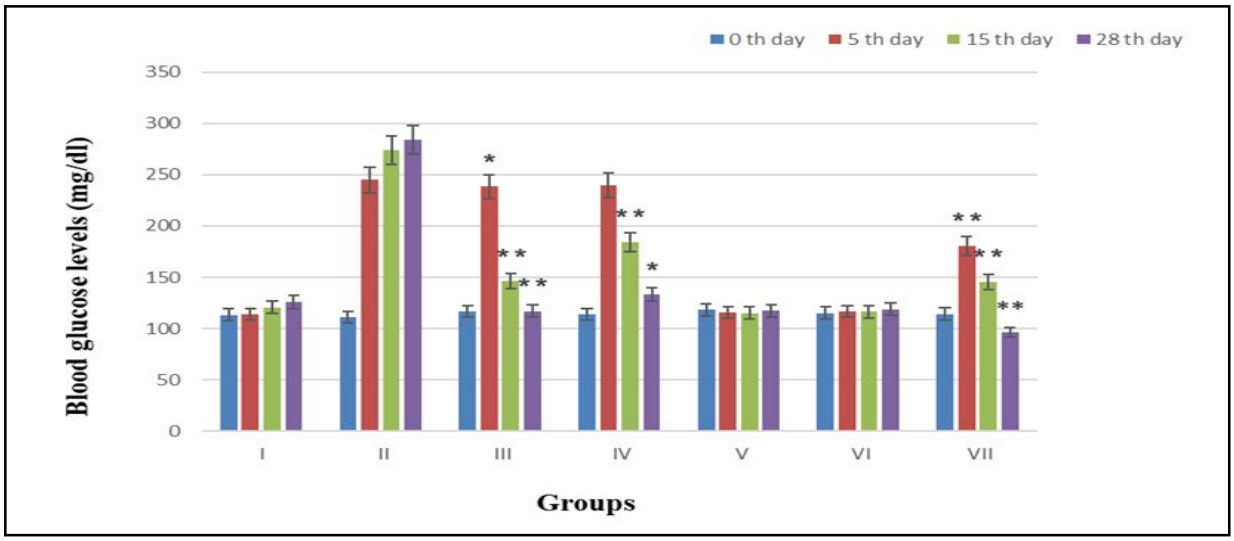


Figure 3: Effect of MESK and MEHS on blood glucose levels of control and experimental groups of rats. Mean data are depicted as mean ± standard deviation (n = 6). * significant at $p<0.05$ from control diabetic group, while ** significant at $p<0.01$. # significant at $p<0.05$ from the control normal group, and ## significant at $p<0.01$.

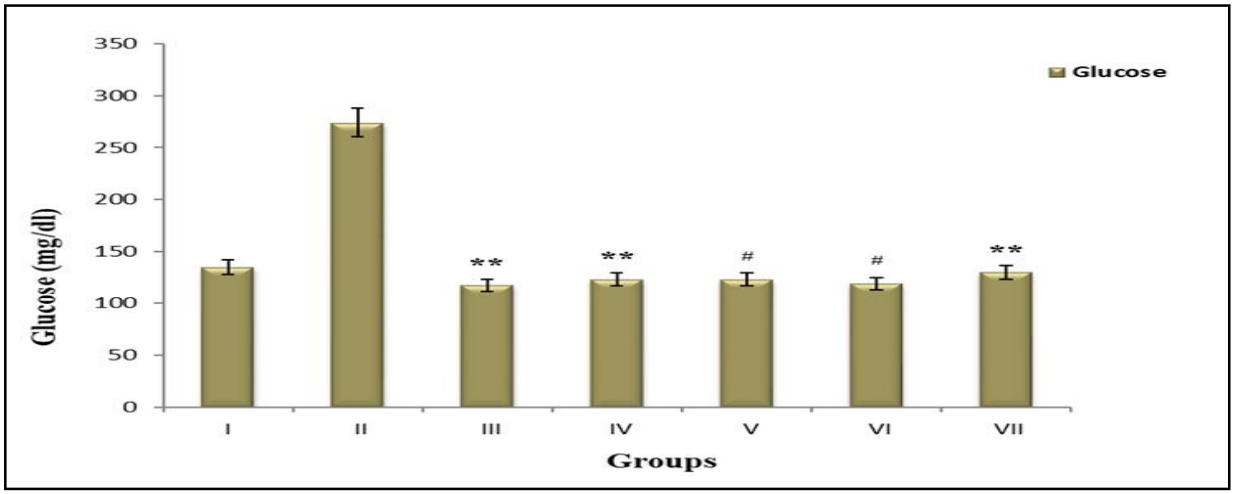


Figure 4: Effect of MESK and MEHS on blood glucose of control and experimental groups of rats. The data are presented as mean ± standard deviation (n = 6). * Statistical significance at $p<0.05$ compared to the diabetic control group, while ** indicates significance at $p<0.01$. # indicates significance at $p<0.05$ compared to the normal control group, and ## indicates significance at $p<0.01$.

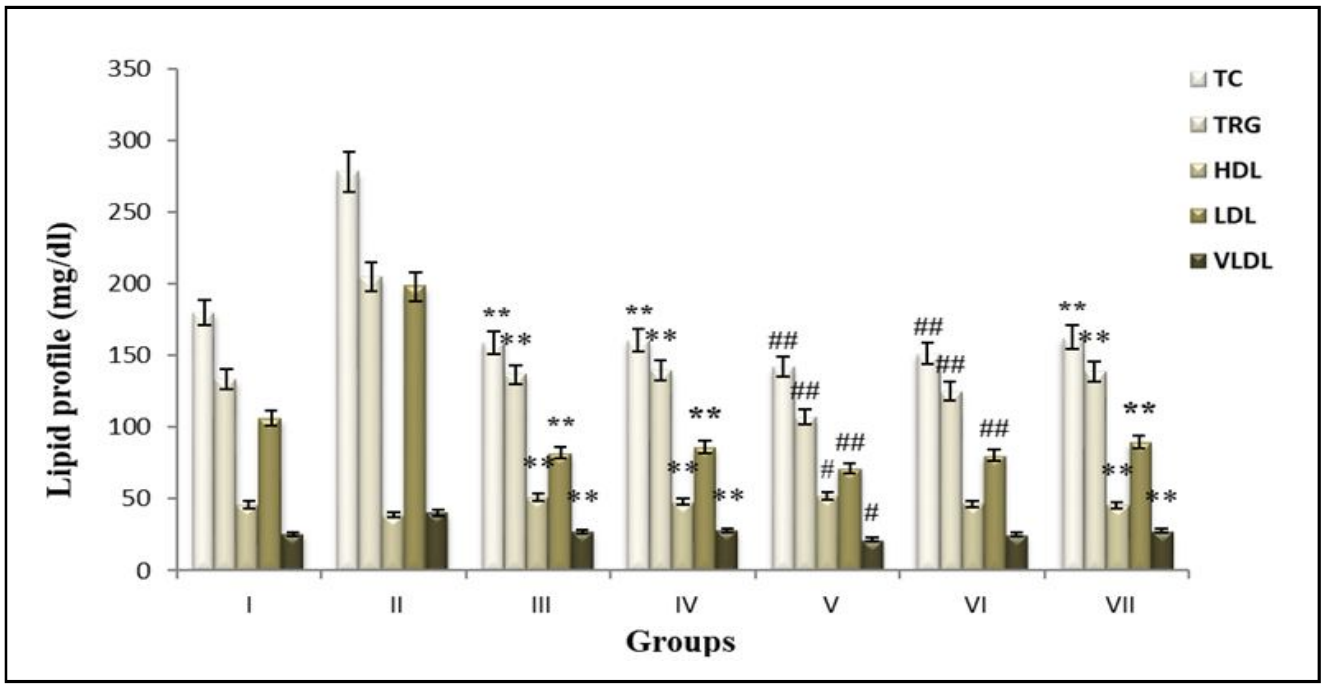


Figure 5: Effect MESK and MEHS on serum lipid profile of control and experimental groups of rats. The data are presented as mean ± standard deviation (n = 6). * statistical significance at $p<0.05$ from diabetic control group, ** significance at $p<0.01$. # significance at $p<0.05$ from the normal control group, and ## significance at $p<0.01$.

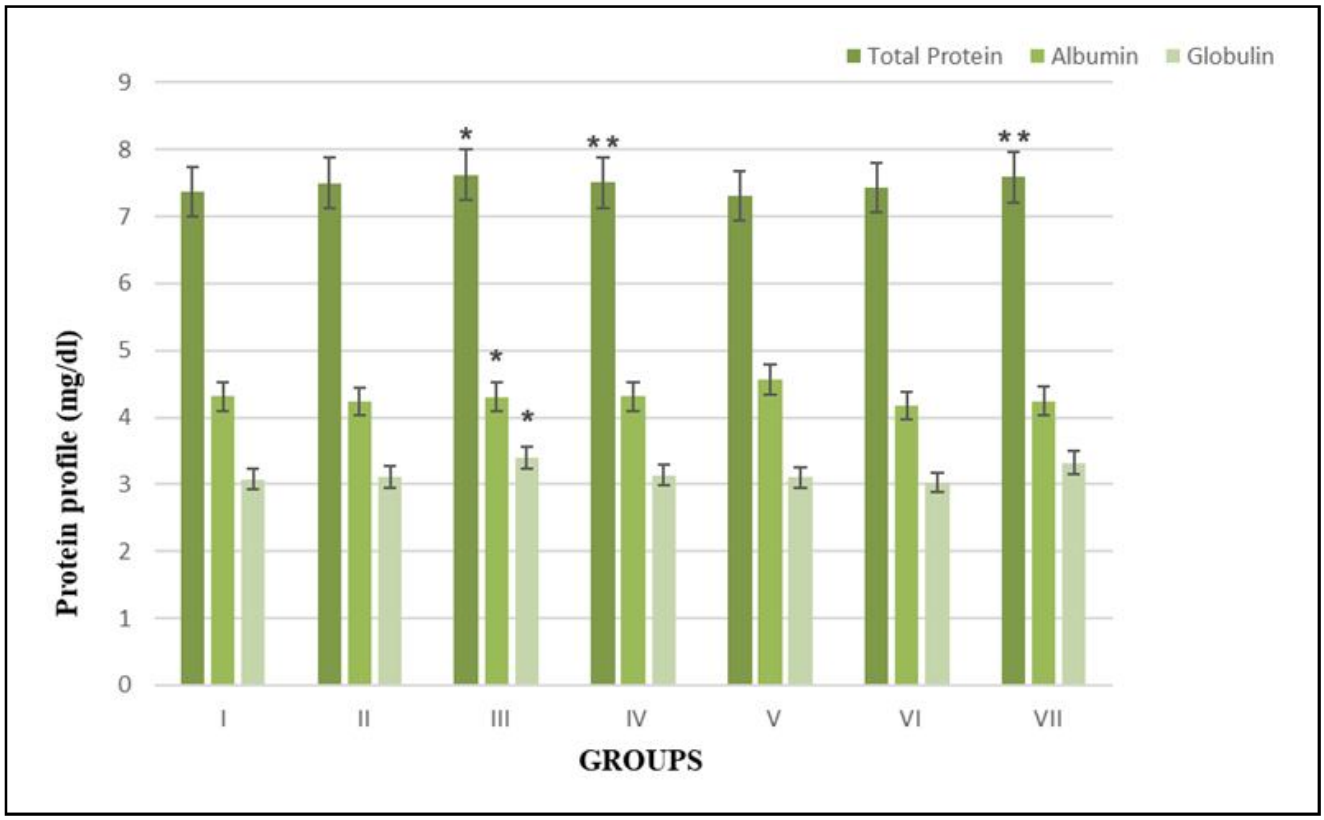


Figure 6: Effect of MESK and MEHS on serum protein profile of control and experimental groups of rats. The data are presented as mean ± standard deviation (n = 6).* statistical significance at $p<0.05$ from the diabetic control group, while ** significance at $p<0.01$. # significance at $p<0.05$ from the normal control group, and ## significance at $p<0.01$.

3.6 Level of liver enzymes and bilirubin

The level of liver enzymes, such as AST, ALT, ALP, total bilirubin, direct bilirubin, and indirect bilirubin was illustrated in Figures 7, 8. The increased level of liver enzymes was observed in diabetic rats compared to control rats, after treatment with betel leaf extracts and standard drugs, a significant decrease in the AST, ALT, and ALP levels was noticed. No significant change was detected in the bilirubin profiles of group IV treated rats.

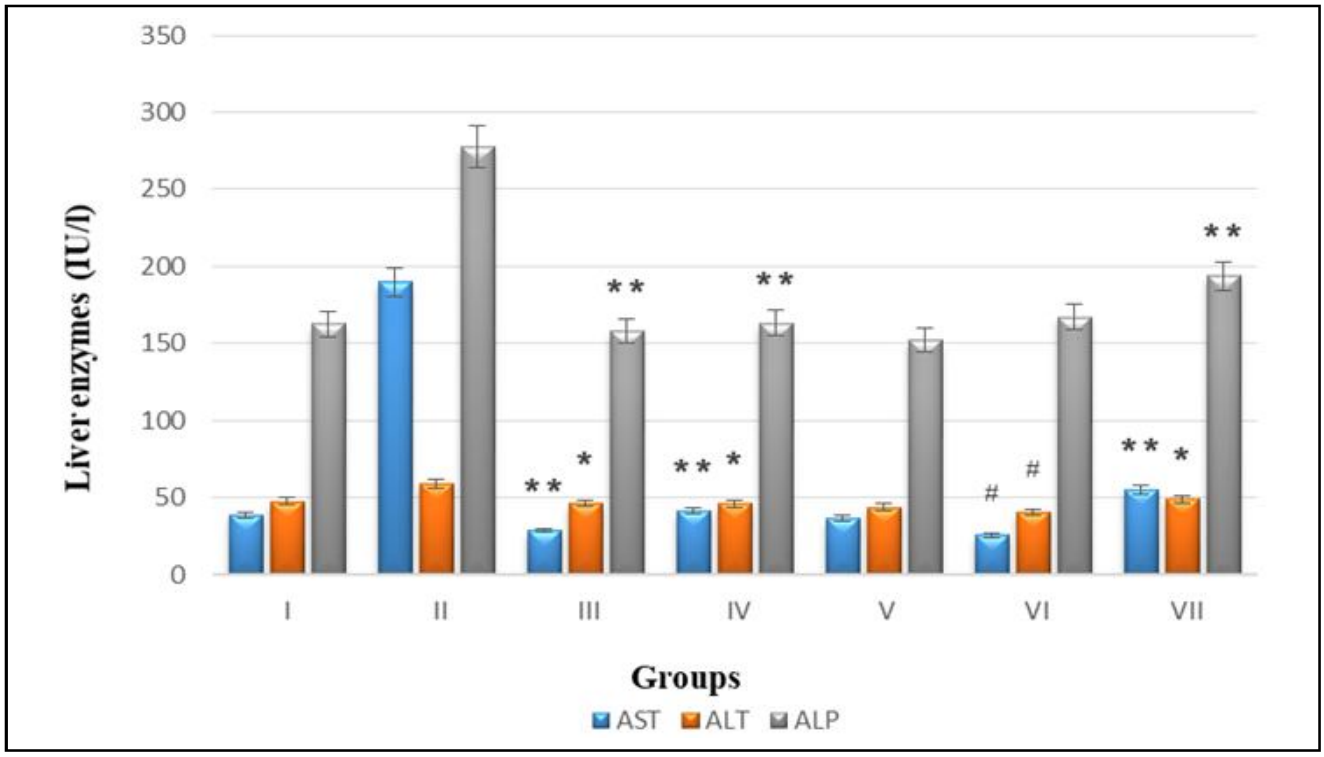


Figure 7: Effect of MESK and MEHS on liver function marker enzymes of control and experimental groups of rats. The data are presented as mean ± standard deviation (n = 6). * statistical significance at $p<0.05$ from the diabetic control group, while ** significance at $p<0.01$. # significance at $p<0.05$ from the normal control group, and ## significance at $p<0.01$.

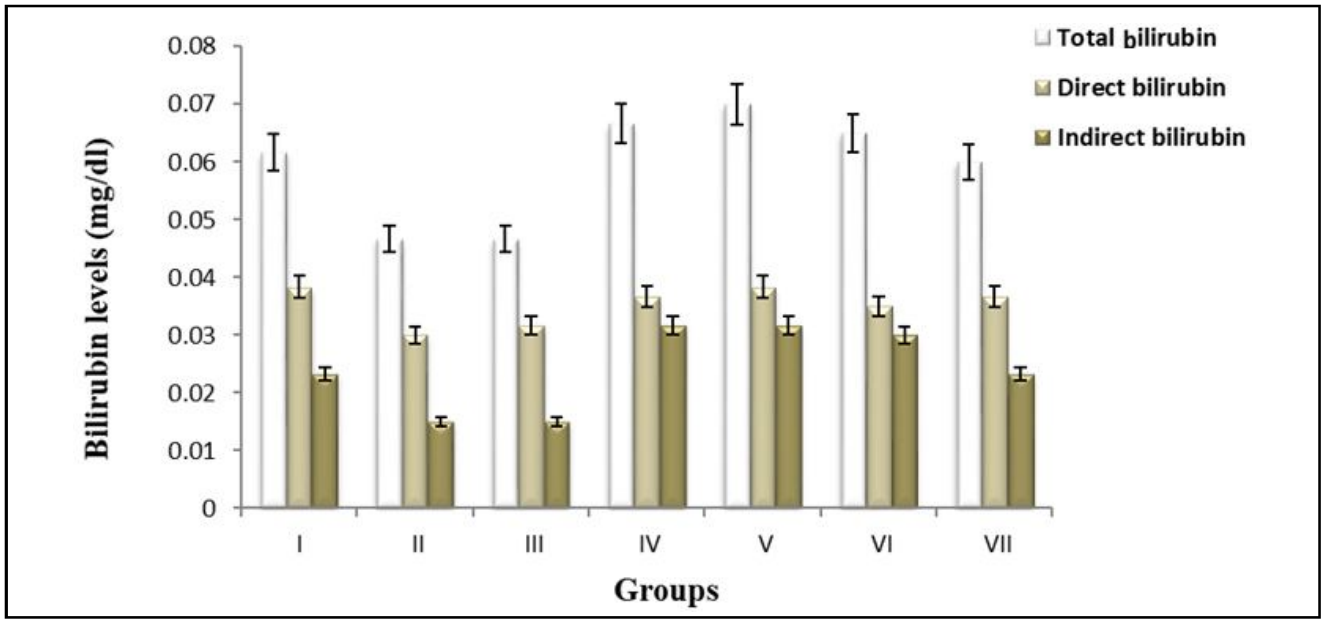


Figure 8: Effect of MESK and MEHS on total bilirubin of control and experimental groups of rats. The data are presented as mean ± standard deviation (n = 6).* statistical significance at $p<0.05$ from the diabetic control group, while ** significance at $p<0.01$. # significance at $p<0.05$ from the normal control group, and ## significance at $p<0.01$.

3.7 Renal function markers

The renal function markers such as urea, creatinine, and uric acid were tabulated in Table 2. A significant decrease in the level of urea was observed in the treated group with MESK, MEHS, and glibenclamide, compared to the untreated rats. No significant change in uric acid and creatinine content was observed, in treated groups compared to untreated.

Table 2: Effect of MESK and MEHS on serum renal function biomarkers of control and treated groups of rats

Grouping of animals	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Control	29.96 ± 0.65	0.43 ± 0.08	1.08 ± 0.11
Diabetic control	58.00 ± 2.60	0.53 ± 0.02	3.11 ± 0.44
Diabetic + MESK (200 mg/kg b.w.)	45.58 ± 0.62**	0.47 ± 0.07	2.71 ± 0.15
Diabetic + MEHS (200 mg/kg b.w.)	39.28 ± 2.51**	0.55 ± 0.05	2.81 ± 0.35
Control + MESK (200 mg/kg b.w.)	30.51 ± 4.93	0.66 ± 0.12	3.68 ± 0.60#
Control + MEHS (200 mg/kg b.w.)	34.33 ± 2.37	0.80 ± 0.08	4.01 ± 1.00#
Diabetic + Glibenclamide (10 mg/kg b.w.)	26.74 ± 1.29**	0.74 ± 0.16	3.35 ± 0.07

Each value is expressed as the mean ± standard deviation (n = 6).
* denotes statistical significance at $p<0.05$ compared to the diabetic control, while ** indicates significance at $p<0.01$.
# indicates significance at $p<0.05$ compared to the normal control, and ## signifies significance at $p<0.01$.

3.8 Serum insulin, cortisol, and c-peptide determination

The concentration levels of serum insulin, cortisol, and C-peptide are displayed in Table 3. The level of insulin and C-peptide was found to be, significantly increased when compared to the control, after treatment with the MESK, MEHS, and glibenclamide; however, a gradual lowering ($p<0.05$, $p<0.01$) was also detected. No significant variation was observed in cortisol after treatment with MEHS and glibenclamide.

Table 3: Effect of MESK and MEHS on serum insulin, cortisol and C-peptide of control and treated groups of rats

Grouping of animals	Insulin (µIU/ml)	Cortisol (µg/dl)	C-peptide (ng/ml)
Control	4.66 ± 0.13	9.18 ± 0.36	0.88 ± 0.02
Diabetic control	66.61 ± 1.89	15.37 ± 0.15	2.96 ± 0.18
Diabetic + MESK (200 mg/kg b.w.)	21.76 ± 1.25**	18.83 ± 0.73**	1.79 ± 0.07**
Diabetic + MEHS (200 mg/kg b.w.)	18.60 ± 0.62**	15.61 ± 0.11	1.33 ± 0.29**
Control + MESK (200 mg/kg b.w.)	14.13 ± 1.32##	11.55 ± 2.30#	1.15 ± 0.29#
Control + MEHS (200 mg/kg b.w.)	13.55 ± 1.48##	13.86 ± 1.51##	1.18 ± 0.07#
Diabetic + Glibenclamide (10 mg/kg b.w.)	13.53 ± 2.41**	14.30 ± 1.31	0.80 ± 0.08**

Each value is expressed as the mean ± standard deviation (n = 6).
*statistical significance at $p<0.05$ from the diabetic control group, while ** significance at $p<0.01$.
#significance at $p<0.05$ from the normal control group, and ## significance at $p<0.01$.

3.9 Histopathological examinations of the pancreas, liver, and kidney

In the histopathological investigation, the pancreas of control rats showed larger-sized islets with numerous numbers of β-cells, while the untreated diabetic rats found to be, reduced islets cells (atrophy) and injury to the β-cell were noticed (Figure 9). There was an abnormality found, alleviated by the treatment with MHSK, MEHS, and glibenclamide in a dose-dependent manner. In streptozotocin-induced diabetic rats, structural adjustments in the kidney tissues, and interstitial inflammation were also observed. In the diabetic rats administered with betel vine leaf extracts, the kidney architecture appears to be more or less similar to the control. The histopathological examination of the liver of diabetic rats showed necrosis of hepatic cells with massive changes in fat and loss of liver parenchymal cells was also detected (Figures 10, 11). Diabetic rats treated with MESK and MEHS (200 mg/kg body weight) exhibited regeneration, which was absent in the control and glibenclamide groups. The histopathological investigation of the pancreas, kidney, and liver tissues of control rats treated with MESK and MEHS, unveiled, no remarkable deviations.

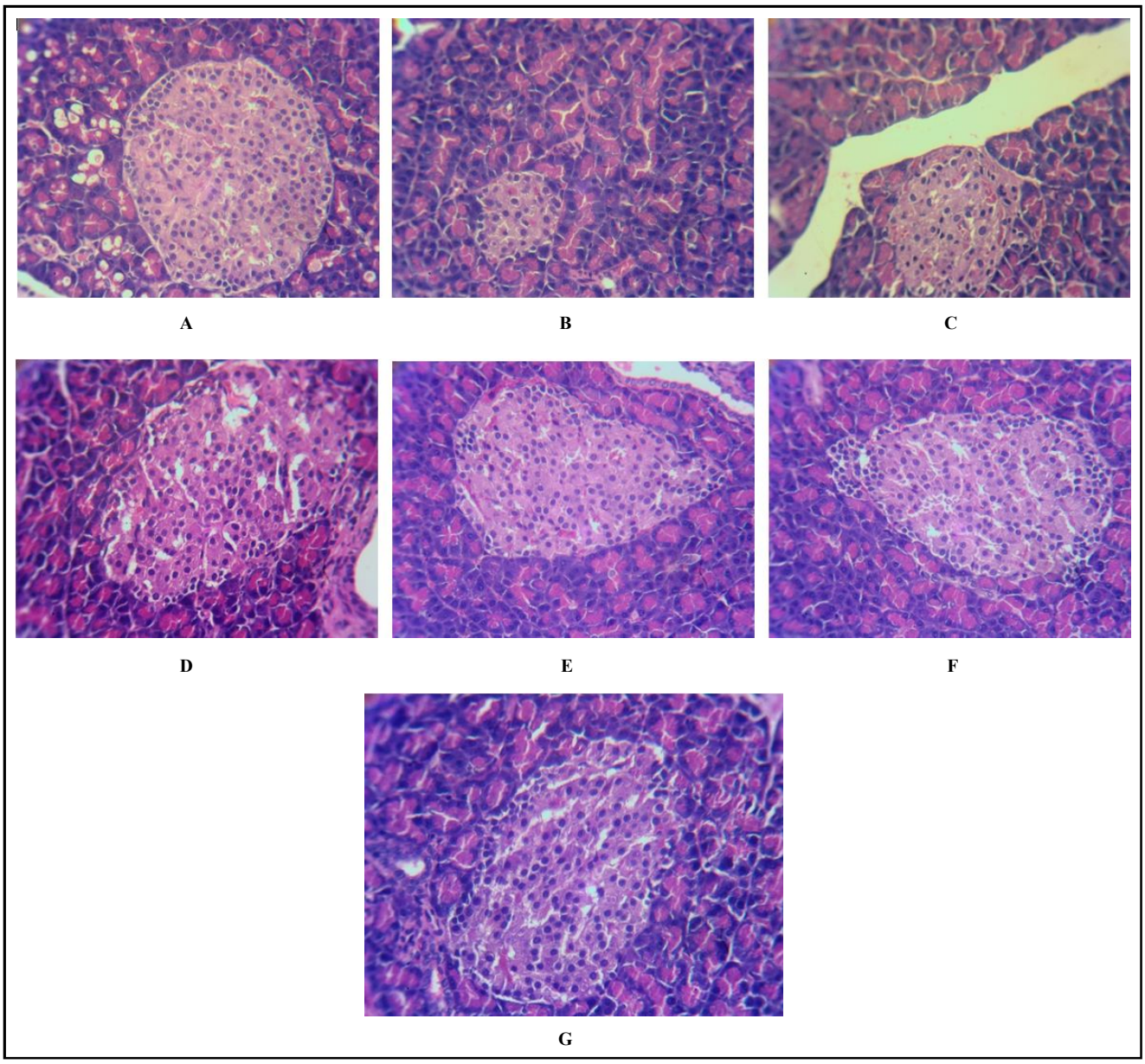
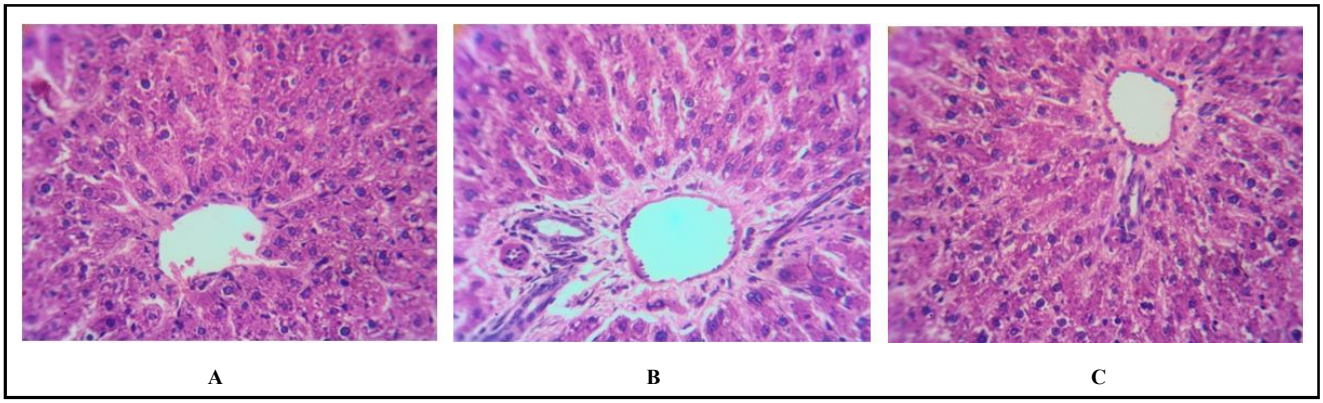


Figure 9: Histopathological changes in pancreas of normal and treated rats. Optic microscopy (H&E x 400): (A) normal, (B) diabetic control, (C) diabetic + MESK (200 mg/kg b.w.), (D) diabetic + MEHS (200 mg/kg b.w.), (E) control + MESK (200 mg/kg b.w.), (F) control + MEHS (200 mg/kg b.w.), (G) diabetic + glibenclamide (10 mg/kg b.w.).



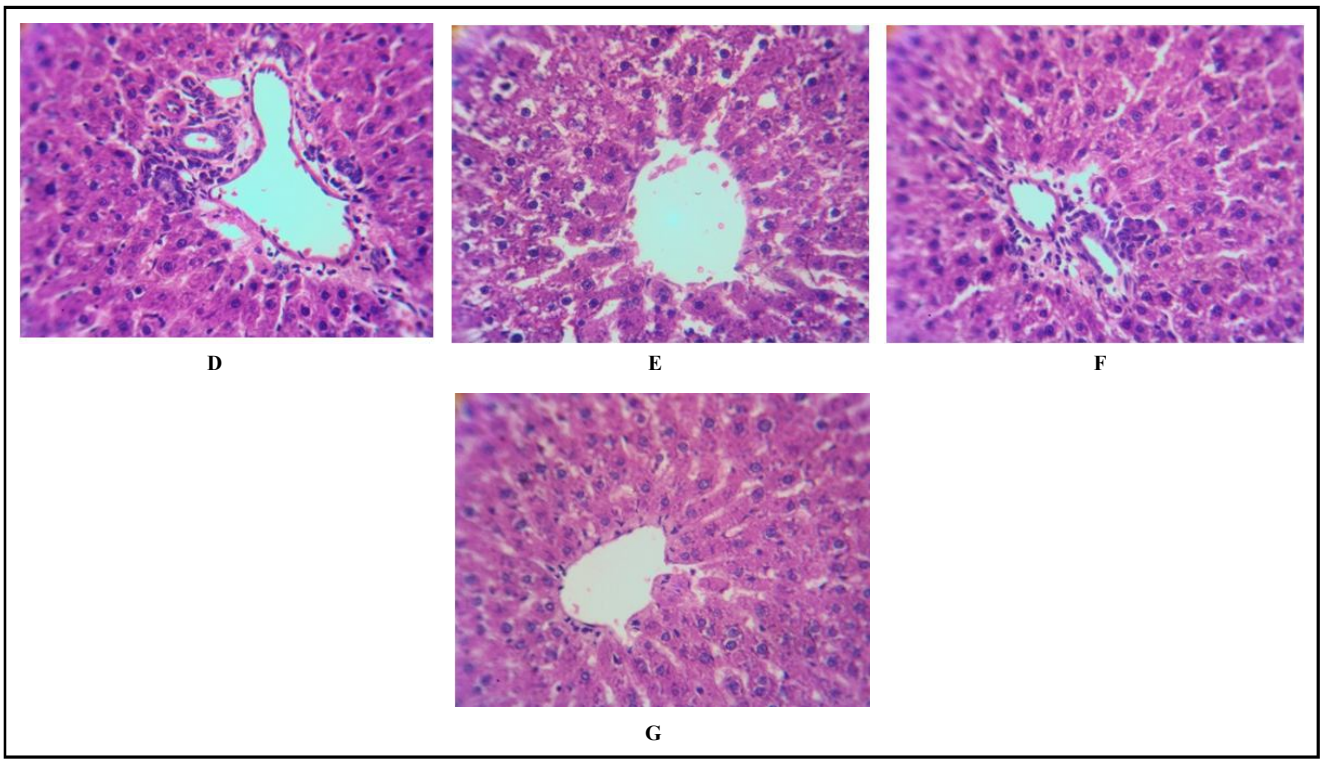
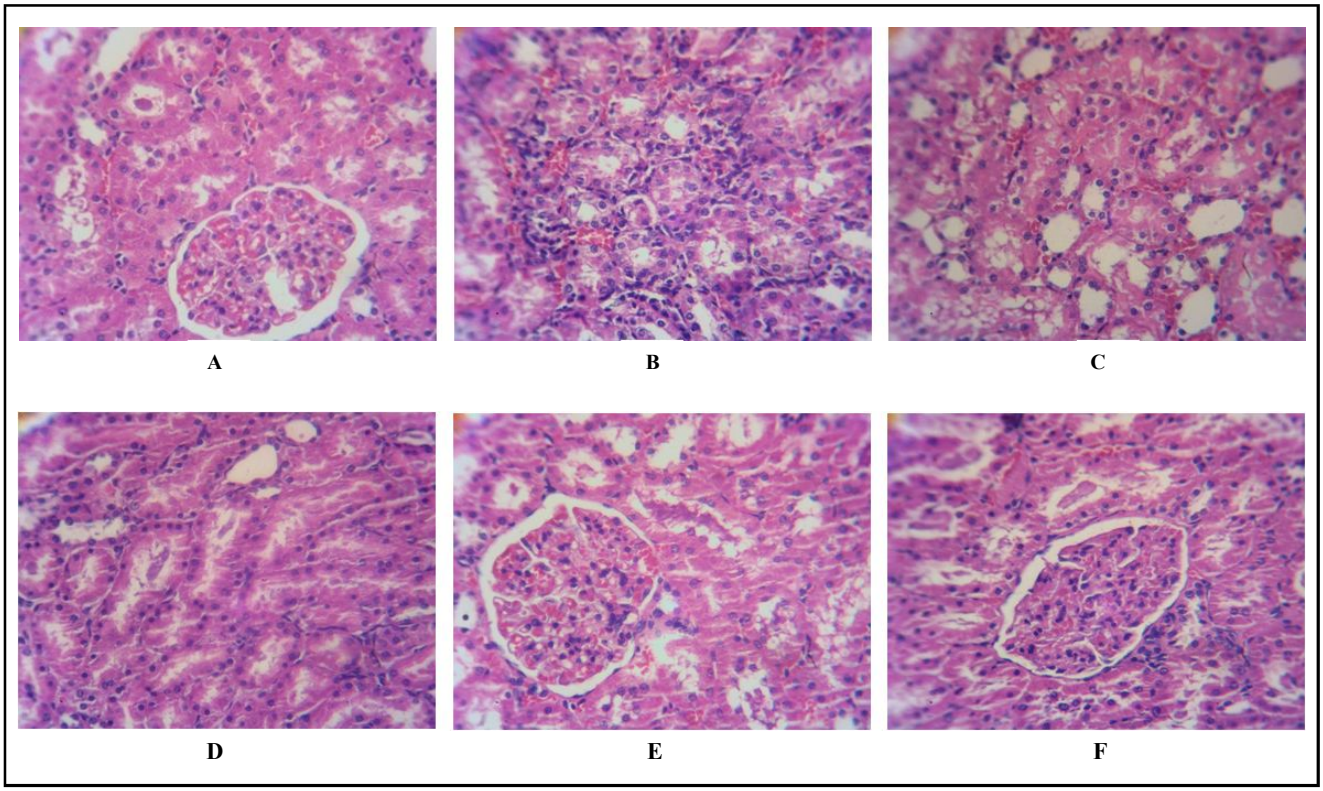
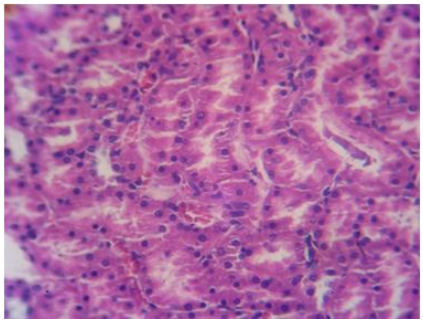


Figure 10: Histopathological changes in liver of normal and treated rats. Optic microscopy (H&E x 400): (A) normal, (B) diabetic control, (C) diabetic + MESK (200 mg/kg b.w.), (D) diabetic + MEHS (200 mg/kg b.w.), (E) control + MESK (200 mg/kg b.w.), (F) control + MEHS (200 mg/kg b.w.), (G) diabetic + glibenclamide (10 mg/kg b.w.). While comparing with D and G displayed retrieval or reversion occurred in hepatocytes to the normal architecture well exhibited in D were as treatment of MESK in diabetic rat illustrated exhibited recovery of hepatocytes and shrinking of centre vein and formation of vacuoles were observed.





G

Figure 11: Histopathological changes in kidney of normal and treated rats. Optic microscopy (H&E x 400): (A) normal, (B) diabetic control, (C) diabetic + MESK (200 mg/kg b.w.), (D) diabetic + MEHS (200 mg/kg b.w.), (E) control + MESK (200 mg/kg b.w.), (F) control + MEHS (200 mg/kg b.w.), (G) diabetic + glibenclamide (10 mg/kg b.w.).

4. Discussion

The undesirable side effects of the modern medicine system for diabetes resulted in a thrust for searching and designing a novel antidiabetic drug (Cherbal *et al*., 2017). Recent literature and folkloric claimed that the beneficial effects of the popular herb, betel vine leaves, could be used for treating diabetics. The present investigation focused on the antidiabetic effects of betel vine cultivars, Swarna Kapoori and Halisahar Sanchi methanolic leaf extract, and changes in various biochemical, histological, and hepatological traits in a streptozotocin-induced diabetic rat model. Betel vine leaves are widely used in traditional medicine to effectively treat, diabetes mellitus and other associated diseases used by local people (Shun *et al*., 2007). A variety of secondary metabolic products, such as flavonoids, saponins, and phenolic compounds were reported in the betel vine leaves (Nur *et al*., 2013; Manoj *et al*., 2011). Moreover, it has been demonstrated in earlier studies, that an oral administration of betel vine leaves exhibited a high potential to decrease blood glucose and cholesterol levels in rats (Shantakumari *et al*., 2006; Thirunavukkarasu *et al*., 2014). As described by Abdul *et al*., (2014), the beneficial effects of betel leaves in lowering the blood sugar level were well determined. Moreover, the presence of essential oils in the leaves was attributed to the flavor and different tastes (Sripardha, 2014).

Our findings elucidated significant antidiabetic activity of MESK and MEHS in biochemical analysis. The present investigations on induction of T2D, showed decline of body weight in the diabetic control group, as illustrated in Figure 2. While, treatment with MESK and MEHS resulted in a significant increase in animal body weight, suggesting a gradual recovery from the diabetic condition. Many studies affirmed that STZ-induced diabetes was characterized by severe loss of body weight in animals, attributed to the loss of strength and muscle weakness (Irudayaraj *et al*., 2012). An increase was observed in the body weight of treated diabetic rats, which might have been due to improvement in glycemic control and upsurge synthesis of structural proteins (Eliza *et al*., 2009). The common and profound symptoms of diabetes mellitus have been classified as, polyphagia, polyuria, polydipsia, and reduction of body weight was already well documented in another study (Unwin *et al*., 2009). Our findings on the loss of weight appeared to be a good agreement, with earlier reports.

The hematological markers of the treated rats also showed a promising potential of the examined extract, to endure those to normal hematological indices (Table 1). A significant ($p<0.05$, $p<0.01$) retrieval, close to the normal level of hemoglobin, packed cell volume, RBC, WBC, and platelets cells indicated, a promising protective role of the betel vine methanolic extract into the tissues and organs, underwent damage, while under chronic diabetic severity. A regular dose of the MESK and MEHS exerted a remarkable decrease in blood glucose level, which that shown to be similar to the standard drug at the end of 28 days (Figure 3). The marked fluctuation in hyperglycemia was detected in an untreated diabetic group throughout the study period, indicating its diabetic condition. On the other hand, the MESK and MEHS-treated diabetic rats responded positively to the treatment and exhibited a significant reduction in diabetes-induced hyperglycemia, close to the normal, after a 28-day experimental period (Figure 4). It has been elucidated that a reduction in blood glucose level was endorsed, while streptozotocin-induced diabetic rats were treated with aqueous and ethanolic *P. betle* leaf extract (Arambewela *et al*., 2005). Results indicate that the treatment with MESK and MEHS with normal rats was found to be ineffective, on the level of blood glucose, indirectly revealing, they exerted antihyperglycemia, and hyperlipidemia impacts (Figures 4, 5).

In the present study, we observed, that streptozotocin caused, an elevation of triglycerides, total cholesterol, LDL-cholesterol, VLDL cholesterol, and decreased HDL-cholesterol. It is important to trigger that hyperglyceridaemia and its associated hypercholesterolemia are believed to be a key determinant for causing T2D and easily accessible to the development of atherosclerosis, and CVD, the factions of T2D, to cause major complications (Ananthan *et al*., 2004).

Dyslipidemias branded by high plasma levels of total cholesterol, LDL cholesterol, and triglycerides, with low plasma levels of HDL cholesterol coupled with scarcity in the secretion of insulin causes cardiovascular diseases (Goldberg, 1981; Bertoni *et al*., 2004). Our findings also envisaged the effects of both extracts which significantly reduced total cholesterol, LDL, and triglycerides, wherein, elevated HDL cholesterol, thus, exhibits, possible therapeutic effects for curing long-term CVD in T2D patients. Similarly, the level of AST, ALT, and ALP fluctuations indicated a protective role of MESK and MEHS (Ohaeri, 2001; Ramesh *et al*., 2010). Similar results were reported in *Aframomum melegueta* by Kokou *et al*. (2013); Nwozo and Oyinloye, (2011) where they found plant extracts, were involved in the hepatoprotection from cytotoxicity.

Significantly lower serum urea in the MESK and MEHS-treated groups compared to the diabetic groups suggested its possible effects on the amelioration of renal function (Table 2). The potentiality of the extracts to uphold the cell's hepatic and kidney to revert to the normal structure was believed to be the curative ability of the extract from external stimuli such as nephropathy as well as hepatopathy. While normal rats treated with MESK and MEHS, with a dose of 200 mg/kg, did not exhibit any significant negative alterations in the studied parameters, during the tenure of the experiment. A significant increase in insulin levels and loss of β-cells of pancreas integrity in the histological architecture was observed in the untreated diabetic groups

when compared to the control group (Table 3, Figure 8). A shorter experimental period might be the reason for, not to reduce; however, to induce, the serum insulin level in the diabetic control group.

Our findings elucidated that, the effects of MESK and MEHS treated group and positive control group animals have shown to be an outstanding reversion, close to the normal architecture of the pancreas was also noticed, attributing to the effects of both extracts. Serum insulin level was an indicator of regenerative events of islet cells, which were recorded in histological examinations. A significant decrease in c peptide might have been due to the potential of a longer half-life, compared to insulin, and it was reflected in the analysis of the level of insulin in circulation (Moree *et al*., 2013).

The degree of damage or protection when examined, it was observed that MESK and MEHS could effectively protect the organs to a great extent, and this is evident in the histological images. The cell architecture of the pancreas liver and kidney in glibenclamide, MESK, and MEHS 200 mg/kg, administered, have shown remarkable protection of the tissues. The curative characteristics of MESK and MEHS were presumed to be due to either some latent antioxidants or active ingredients found in the extract, as reported, which may be the responsible key factors, either, individually or synergistically. Similar results were documented earlier by Yogeswari *et al*. (2020). Betel vine contains several bioactive compounds and its antioxidant properties have already been reported (Rintu *et al*., 2015).

Histopathological examinations of rat liver supported that MESK and MEHS effectively rescued liver steatosis associated with T2D. Moreover, the hypolipidemic activity of MESK and MEHS was recorded, similar to the standard drug glibenclamide. Collectively, obtained observations demonstrated that besides its hypoglycaemic effect, MESK and MEHS also exhibit strong hypolipidemic capacity that has the potential to protect T2D patients from CVD.

5. Conclusion

Results of this study suggest that the antidiabetic activity differed with cultivars showing the presence of intraspecific variability. Methanolic leaf extracts MEHS and MESK at 200 mg/kg b.w. were effective in amending most of the diabetic associated parameters such as body weight, blood content, blood glucose, dyslipidemia, hormone sensitivity, liver enzymes, renal function markers and improving histopathological architecture of pancreas, liver, kidney. The methanolic extract from cultivar Swarna Kapoori appear to be more effective when compared to the extract of cultivar Halisahar Sanchi. It may be due to the various phytochemicals and antioxidants present in the cultivar Swarna Kapoori . Comprehensive studies with more varieties are required to confirm the intraspecific variability for antidiabetic activity among *P. betle* cultivars. Further in depth investigations are essential to isolate and characterise the compounds responsible for antidiabetic activity of *P. betle* leaves.

Acknowledgments

The research described in this study received financial support from the ICAR-Extramural Research Grant, Government of India, New Delhi, India (Scheme code: ICAR0013, 2016). We also acknowledge the DST-FIST, UGC Special Assistance Program, and DBT-IISc Partnership Program for their provision of facilities and financial assistance. The authors express their gratitude to Dr. K. K. Upreti, Principal Scientist (Organic Chemistry), Division of Plant Physiology and Biochemistry, ICAR-IIHR, Bengaluru, Karnataka, India.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Abdul, M. M.; Rupa, B. A.; Azam, N. K.; Ahmed, N. and Hasan, N. (2014). A quick review on antidiabetic plants and action of phytochemicals. International Journal of Advanced Research, **2**(5):227-249.

Aminu, M.; Neil, A. K. and ShahidulIslam, M. (2015). Ethyl acetate fraction of *Aframomum melegueta* fruit ameliorates pancreatic β-cell dysfunction and major diabetes-related parameters in a type 2 diabetes model of rats. Journal of Ethnopharmacology, **175**:518-527.

Ananthan, R.; Latha, M.; Pari, L.; Baskar, C. and Narmatha, V. (2004). Modulatory effects of *Gymnema montanum* leaf extract on alloxan induced oxidative stress in Wistar rats. Nutrition, **20**:280-285.

Arambewela, L. S. R.; Arawwawala, L. D. A. M. and Ratnasooriya, W. D. (2004). Gastro protective activities of Sri Lankan *Piper betle* L. leaf extracts in rats. Sri Lanka Association for the Advancement of Science. 60th Annual Session pp:117.

Arambewela, L. S. R.; Arawwawala, L. D. A. M. and Ratnasooriya, W. D. (2005). Antidiabetic activities of aqueous and ethanolic extracts of *Piper betle* L. leaves in rats. Journal of Ethnopharmacology, **102**:239-245.

Arawwala, L.; Arambewela, L. and Ratnasooriya, W. (2014). Gastro protective effect of *Piper betle* L. leaves grown in Srilanka. Journal of Ayurveda and Integrative Medicine, **5**:38-42.

Arsalan, H. M.; Yasin, M. S.; Amin, Z. S.; Ahmad, S.; Haider, B.; Zahid, M.; Arsalan.; Ahmad, S.; Sohail, H. and Hafiz, M. (2019). Evaluation of lipid peroxidation and biochemical response in ovarian cancer patients: A perspective study from local population of Lahore-Pakistan. Indo American Journal of Pharmaceutical Research, **6**(9):11709-11713.

Bertoni, A. G.; Hundley, W. G.; Massing, M. W.; Bonds, D. E.; Burke, G. L. and David, C. G. (2004). Heart failure prevalence, incidence and mortality in the elderly with diabetes. Diabetes Care, **27**(3):699-703.

Bigoniya, P.; Nishad, R. and Singh, C. S. (2012). Preventive effect of sesame seed cake on hyperglycemia and obesity against high fructose-diet induced type 2 diabetes in rats. Food Chemistry, **133**(4):1355-1361.

Chauhan, E. S.; Aishwarya, J.; Singh, A. and Tiwari, A. (2016). A Review: Nutraceutical properties of *Piper betle* L. (Paan). American Journal of Phytomedicine and Clinical Therapeutics, **4**(2):028-041.

Chellammal, H. S. J. (2022). Fruits that heal: Biomolecules and novel therapeutic agents. Ann. Phytomed., **11**(1):7-14.

Cherbal, A.; Kebieche, M.; Yilmaz, E. M.; Aydogmus, Z.; Benzaouia, L.; Benguessoum, M.; Benkedidah, M. and Madani, K. (2017). Antidiabetic and hypolipidemic activities of Algerian *Pistachia lentiscus* L. leaves extract in alloxan-induced diabetic rats. South African Journal of Botany, **108**:157-162.

Duhan, J.; Kumari, N.; Avtar, R.; Singh, M.; Sushil.; Nain, S. and Garg, B. (2022). Investigating the potential of underutilized brassica seed meals as a source of natural antioxidants. Ann. Phytomed., **11**(2):1-7.

Edijala, J. K.; Asagba, S. O.; Eriyamremu, G. E. and Atomatofa, U. (2005). Comparative effects of garden egg fruit oat and apple on serum lipid profile in rats fed on a high cholesterol diet. Pakistan Journal of Nutrition, **4**(4):245-249.

Ekta, G. and Goswami, T. M. (2016). Risk factor distribution for cardiovascular diseases among high school boys and girls of urban Dibrugarh, Assam. Journal of Family Medicine and Primary Care, **5**(1):108-113.

Eliza, J.; Daisy, P.; Ignacimuthu, S. and Duraipandiyan, V. (2009). Antidiabetic and antilipidemic effect of eremanthin from *Costus speciosus* (Koen.) Sm., in STZ induced diabetic rats. Chemico-Biological Interactions, **182**(1):67-72.

Garg, B.; Yashveer, S.; Redhu, N. S.; Anita.; Duhan, J. and Sindhu, S. (2023). In silico molecular docking analysis of potential antidiabetic phytochemicals from *Ocimum sanctum* L. against therapeutic targets of type 2 diabetes. Ann. Phytomed., **12**(2):503-515.

Goldberg, R. B. (1981). Lipid disorders in diabetes. Diabetes Care, **4**(5): 561-572.

Irudayaraj, S. S.; Sunil, C.l Duraipandiyan, V. and Ignacimuthu, S. (2012). Antidiabetic and antioxidant activities of *Toddalia asiatica* (L.) Lam. Leaves in streptozotocin induced diabetic rats. Journal of ethnopharmacology, **143**(2):515-523.

Jayaprasad, B.; Sharavanan, P. S. and Sivaraj, R. (2016). Antidiabetic effect of *Chloroxylon swietenia* bark extracts on streptozotocin induced diabetic rats. Beni-suef University Journal of Basic and Applied Sciences, **5**(1):61-69.

Kaleem, M.; Sheema, S. H. and Bano, B. (2005). Protective effects of *Piper nigrum* L. and *Vinca rosea* in alloxan induced diabetic rats. Indian Journal of Physiology and Pharmacology, **49**(1):65-71.

Kavipriya, S.; Tamilselvan, N.; Thirumalai, T. and Arumugam, G. (2013). Antidiabetic effect of methanolic leaf extract of *Pongamia pinnataon* streptozotocin induced diabetic rats. Journal of Coastal Life Medicine, **1**(2):113-117.

Kokou, I.; Damintoti, K. S.; Amegnona, A.; Yao, A. and Messanvi, G. (2013). Effect of *Aframomum melegueta* on carbon tetrachloride induced liver injury. Journal of Applied Pharmaceutical Science, **3**(9):98-102.

Manoj, P. R.; Karadka, R. T.; Princy, L. P.; Prathima, R.; Suresh, R.; Harshith, P. B. and Manjeshwar, S. B. (2011). *Piper betle* L. (Betel vine), the maligned Southeast Asian medicinal plant possesses cancer preventive effects: Time to reconsider the wronged opinion. Asian Pacific Journal of Cancer Prevention, **12**:2149-2156.

Masiello, P.; Broca, C.; Gross, R.; Roye, M.; Manteghetti, M.; Hillaire-Buys, D.; Novelli, M. and Ribes, G. (1998). Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide. Diabetes Care, **47**(2):224-229.

Momose, M.; Abletshauser, C.; Neverve, J.; Nekolla, S. G.; Schnell, O.; Standl, E.; Schwaiger, M. and Bengel, F. M. (2002). Dysregulation of coronary microvascular reactivity in asymptomatic patients with type 2 diabetes mellitus. European Journal of Nuclear Medicine and Molecular Imaging, **29**:1675-1679.

Moree, S. S.; Kavishankar, G. B. and Rajesha. J. (2013). Antidiabetic effect of secoisolariciresinol diglucoside in streptozotocin-induced diabetic rats. Phytomedicine, **20**(3-4):237- 245.

Nur, S. N.; Nalina, T. and Rahim, Z. H. A. (2013). Antioxidant and cyto-protective activities of *Piper betle* L., *Areca catechu*, *Uncaria gambir* and betel quid with and without calcium hydroxide. BMC Complementary and Alternative Medicine, **13**:351.

Nwozo, S. O. and Oyinloye, B. E. (2011). Hepatoprotective effect of aqueous extract of *Aframomum melegueta* on ethanol-induced toxicity in rats. Acta Biochimica Polonica, **58**(3):355-358.

Ohaeri, O. C. (2001). Effect of garlic oil on the levels of various enzymes in the serum and tissue of streptozotocin diabetic rats. Bioscience Reports, **21**:19-24.

Patra, A. P.; Mukherjee, A. K. and Acharya, L. (2011). Comparative study of RAPD and ISSR markers to assess the genetic diversity of betel vine (*Piper betle* L.) in Orissa. American Journal of Biochemistry and Molecular Biology, **1**(2):200-211.

Ramesh, B. K.; Maddirala, D. R.; Vinay, K. K.; Shaik, S. F.; Ethamakula, G. T. K.; Sirasanagandla, S.; Bellamkonda, R. and Chippada, A. R. (2010). Antihyperglycemic and antihyperlipidemic activities of methanol: water (4:1) fraction isolated from aqueous extract of *Syzygium alternifolium* seeds in streptozotocin induced diabetic rats. Food and Chemical Toxicology, **48**(4):1078-1084.

Rani, J.; Kaur, P. and Chuwa, C. (2023). Nutritional benefits of herbs and spices to the human beings. Ann. Phytomed., **12**(1):187-197.

Rekha, V. P. B.; Kollipara, M.; Gupta, B. R. S. S. S.; Bharath, Y. and Pulicherla, K. K. (2014). A review on *Piper betle* L.: Nature's promising medicinal reservoir. American Journal of Ethnomedicine, **1**(5):276-289.

Rintu, D.; Shinjini, M.; Kaustab, M.; Pramathadhip, P.; Umesh, P. S. and Ena, R. B. (2015). Antioxidant and anti-Inflammatory activities of different varieties of betel leaf extracts (*Piper betle* L.). Journal of Nutrition and Food Science, **5**(5):1000415.

Row, L. C. M. and Ho, J. C. (2009). The antimicrobial activity, mosquito larvicidal activity, antioxidant property and tyrosinase inhibition of *Piper betle* L. Journal of Chinese Chemical Society, **56**(3):653-658.

Shantakumari, P.; Prakasam, A. and Pugalendi, K. V. (2006). Antihyperglycemic activity of *P. betle* on streptozotoan induces diabetic rats. Journal of Medicinal Food, **9**(1):108-112.

Shun, C. Y.; Chau, J. W.; Jing, J. L.; Pie, L. P.; Jui, L. H. and Fen, P. C. (2007). Protection effect of *Piper betle* L. leaf extract against carbon tetrachloride induces fever in rats. Archives of Toxicology, **81**(1):45-55.

Sripradha, S. (2014). Betel leaf: The Green Gold. Journal of Pharmaceutical Sciences and Research, **6**(1):36-37.

Sruthy, P. N. and Balasubramaniam, A. (2023). *In vitro* antioxidant, antidiabetic, and in silico docking studies on diabetic wound healing potential of identified compounds of methanolic extract of marine sponge, Agelas clathrodes (Schmidt, 1870) by GC-MS and LC-MS analysis. Ann. Phytomed., **12**(2):537-544.

Thirunavukkarasu, T.; Narayanaswamy, T. and Ernest, D. (2014). Hypolipidemic activity of *Piper betle* L. in high fat diet induced hyperlipidemic rat. Journal of Acute Disease, pp:131-135.

Tunali, S. and Yanardag, R. (2006). Effect of vanadyl sulfate on the status of lipid parameters and on stomach and spleen tissues of streptozotocin-induced diabetic rats. Pharmacological Research, **53**(3):271-277.

Unwin, N.; Whiting, D.; Gan, D.; Jacqmain, O. and Ghyoot, G. (2009). IDF Diabetes Atlas, 4th ed. International Diabetes Federation, Brussels, Belgium.

Valcheva, K. S.; Kuzmanov, K.; Tancheva, S. and Belcheva, A. (2007). Hypoglycemic and hypolipidemic effects of *Aronia melanocarpa* fruit juice in streptozotocin-induced diabetic rats. Methods and Findings in Experimental and Clinical Pharmacology, **29**(2):101-105.

Yogeswari, S.; Bindu, K. H.; Kamalraj, S.; Ashokkumar, V. and Jayabaskaran, C. (2020). Antidiabetic, antithrombin and cytotoxic bioactive compounds in five cultivars of *Piper betle* L. Environmental Technology and Innovation, **20**:101140.

Citation **Venkatesh Rajendran, Hima Bindu Kaipa, Subban Kamalraj, Ramakrishnan Ramanathan, Chelliah Jayabaskaran and Gejjenahalli Puttanaik Mohankumar (2024). Antidiabetic efficacy of leaf extracts of *Piper betle* L. in streptozotocin-nicotinamide induced diabetic rats: An *in vivo* model. Ann. Phytomed., 13(1):589-601. http://dx.doi.org/10.54085/ap.2024.13.1.61.**