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A. Rajeswari and M. Shabana Begum

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Protective Effect of *Phyllanthus maderaspatensis* Ethanol Extract against Hepatic Tissue Injury in Streptozotocin Induced Diabetic Rats

A. Rajeswari*[1] and M. Shabana Begum[2]

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A B S T R A C T

In Diabetes Mellitus (DM) induced complications, oxidative stress plays a key role. In this research, protective impact against diabetes-induced liver oxidative stress and dysfunction of ethanolic leaf extract of *Phyllanthus maderaspatensis* (*Pm*L-Et) was assessed. Rats were split randomly into six groups. An intravenous injection of any freshly prepared STZ solution at 45mg/kg bw in acidified saline solution made a group of diabetic rats (0.9 %; pH 4.5) Group I was treated as a typical control, receiving distilled water orally. After 72h, when the DM situation stabilized, the animals with blood glucose levels over 200mg/dl were chosen for the research. The ultimate aim of the research is to determine how the ethanolic leaf extract of *Phyllanthus maderaspatensis* impacts oxidative stress in hepatic tissue of diabetic rats. This was accomplished via the administration of the streptozotocin (STZ). All of the groups were tested for biochemical variables such as protein, urea, uric acid, and creatinine, liver enzymes (AST, ALT, and ALP), enzyme and non-enzyme antioxidants. Treatment with *Pm*L-Et resulted in reduced levels of these enzymes in STZ-induced diabetic mice. In the liver of STZ induced diabetic rats treated with *Pm*L-Et, antioxidant enzyme activities such as SOD, CAT, GPx, GST, and Vit C and Vit E levels were found to be decreased.

***Key words*:** Diabetes, *Phyllanthus maderaspatensis*, Streptozotocin, Glibenclamide, Oxidative stress

Diabetes mellitus (DM) is an insulin shortage or malfunction metabolic disease, characteristic for its presence in blood of excessive amounts of glucose [1]. In general, individuals with diabetes face an incalculable amount of suffering and severe consequences, which may result in morbidity and death [2]. According to the International Diabetes Federation's most current estimates, diabetes was responsible for 5 million deaths worldwide [3]. More than 2.5 percent of the world's overall population has also been found to be DM suffering earlier [4]. This rise in the prevalence rate coupled with weakening problems guarantees that efficient treatment methods must be sought urgently.

Diabetes mellitus is a set of etiological diseases caused by insulin secretion defects, insulin action, or both [5]. A persistent hyperglycemia, resulting in carbohydrate, lipid, and protein disorders is also caused by insulin deficit. Non-insulin dependent (NIDDM) diabetes is called type 2 diabetes, whereas insulin dependent (IDDM) is known as type 1 diabetes. Type 1 DM is often linked with immune-mediated death of the pancreatic β cells [6]. Type 2 diabetes progresses slowly as insulin resistance and/or reduced insulin production cause glucose intolerance and insulin resistance [7]. In addition to being prone to the long-term consequences of hyperglycemia, individuals with sustained hyperglycemia face an increase in microvascular and macrovascular issues, which causes additional morbidity and death [8].

Presently, there are over 150 million diabetics on the planet, and this figure is expected to climb to 300 million by 2025 [9]. This forecasted increase in the population with diabetes can be attributed to an increase in sedentary lifestyle, intake of energy-dense food, and obesity [10]. Diabetes is one of the most persistent causes of death in affluent countries. It is predicted that by 2025, there would be 84–224 million diabetes in India, and the highest number of sufferers will be found in urban populations.

A substantial number of research investigations have established a connection between diabetes and oxidative stress, resulting in an increased production of ROS, including O_2-, H_2O_2, and OH− [11]. Non-enzymatic protein glycosylation, auto-oxidation of glucose, deficient antioxidant enzyme, and creation of peroxides all link to oxidative stress and the development of diabetes. Oxidative stress is characterized by the presence of lipid peroxidation

* A. Rajeswari
✉ lekshmigangadhar.nano@gmail.com
[1-2] Department of Biochemistry, Muthayammal College of Arts and Science, Rasipuram Taluk, Namakkal District, Tamil Nadu, India

(LPO). Oxidative deterioration of polyunsaturated fatty acids leads in severe membrane damage and malfunction because of free radicals (which are produced as a by-product of oxidation) [12].

Phyllanthus maderaspatensis (Euphorbiaceae) is a widely distributed medicinal plant with a long and illustrious history of use in the treatment of liver problems. It is a member of the Phyllanthus family [13-14]. Traditional healers in India utilise whole plant extracts of the herb *Phyllanthus maderaspatensis* to treat liver diseases, according to the plant's scientific name. A 200mg/kg dose of *P. maderaspatensis* is demonstrated to be an effective hepatoprotective agent against acetaminophen, CCl_4, and thioacetamide-induced acute hepatocellular injury in rats in previous investigations, according to the researchers [15-16]. Furthermore, the herb possesses exceptional antioxidant properties [17]. There is little information available in the existing literature about the effect of *P. maderaspatensis* on STZ-induced diabetes and other activities that are abnormally altered as a result of diabetes mellitus. The ongoing study examines the effects of *P. maderaspatensis* on STZ-induced diabetic rats and comparing those towards the effects of glibenclamide, which will be supposed to be a standard medicine. It has antibacterial, antimicrobial, anti-cataleptic, and other therapeutic properties such as deobstruent, stomachic, astringent, febrifugal diuretic and antiseptic potentials [18]. Also, *P. maderaspatensis* has expectorant and diaphoretic characteristics that help with strangury and sweating [15].

This research was thus performed to assess the protective impact of medicinal plants on streptozotocin-induced rats. Biochemical parameters such as urea, uric acid, total protein and creatinine, enzyme and non-enzyme antioxidants were examined in diabetic and *Pm*L-Et -treated rats. According to this study. STZ-induced diabetic rats exhibited low levels of superoxide dismutase (SOD), catalase (CAT), non-enzymatic antioxidant vitamin C, E and lowered glutathione and the GSH. 45 days of oral treatment of plant extracts, reduced lipid peroxidation, enzyme and non-enzyme antioxidants in diabetic rats to almost normal. Current results show that the therapy of *Pm*L-Et treated has a protection effect on diabetes by lowering oxidative stress caused by hyperglycemia and increasing antioxidant activity. The *Pm*L-Et may be utilized for diabetes therapy as a dietary component. The increased incidence and serious consequences of this illness emphasize the need for effective treatment methods. In addition to the grave effects, this increased incidence emphasizes the urgent need for better treatment approaches.

MATERIALS AND METHODS

Collection and authentication of plant material

The clean and developed leaves of *P. maderaspatensis* were collected in the Kolli Hills, Namakkal district, Tamil Nadu, India. The leaves were collected, properly cleaned with running tap water, and then dried in the shade. The plant was authenticated by Dr. A. Balasubramanian, ABS Herbal Gardens in Salem, Tamil Nadu, India.

Drugs and chemicals

Streptozotocin was purchased from Himedia in Bangalore, India, while glibenclamide was procured from Strides Arcolab Ltd. in Bangalore, India. The rest of the compounds were of analytical grade.

Animals

Male Wistar rats weighing 150-180g were acquired and housed in the Department of Biochemistry at Muthayammal College of Arts and Science in Rasipuram, India. They were fed a normal pellet diet (Amrut, Pune, India) and were given clean drinking water. The Institutional ethics committee of Muthayammal College of Arts and Science (1416/PO/a/11/CPCSEA&7 MARCH 2011) validated the research's methodologies.

Preparation of the ethanol extracts

Plants were cleaned with fresh water, shade dried and pulverized at room temperature. The powder was extracted with ethanol (10g/400ml) using a Soxhlet extraction method. Until the solvent left no residue when evaporated on a clean glass plate, the extraction was continuous. Using a rotary evaporator, the solvent was extracted from the extract at reduced pressure (VV2000, Heidolph, and Schwabach, Germany). The extract was kept in deep freezers for further usage.

Study of PmL-Et in experimental rats

Adult male Wistar albino rats (150-180g) were given an intraperitoneal injection of STZ (45mg/kg bw) dissolved in citrate buffer to induce diabetes (0.1M, pH 4.5). Within a few days of being injected with STZ, animals developed severe glycosuria and hyperglycemia. Diabetes was confirmed in STZ-induced rats by measuring fasting blood sugar levels 72 hours after STZ injection. Albino rats having a blood glucose level more than 250mg/dL were treated as diabetic individuals and used in the experiment.

Group I was treated as a typical control, receiving distilled water orally. Groups II–VI were made diabetic intravenously using a 45mg/kg bw STZ solution in acidified saline solution (0.9%; pH 4.5). In this case, the group II animals only received the acidified saline solution. After 72h, when the DM condition maintained, the animals with BG levels over 200mg/dl were chosen for this study. *Pm*L-Et was administered orally to groups III to V at dosages of 100, 200, and 300mg/kg, respectively. As a reference, glibenclamide was administered to animals in group VI at a dosage of 600g/kg per kg of body weight. All of the rats were sacrificed by cervical dislocation one day following the final dose (day 61). Blood was drawn to determine the following factors:

Analysis of common biochemical factors

The main and common biochemical variables such as plasma protein [19], urea [20], uric acid [21] and creatinine [22] were evaluated using established techniques, and the significance of changes from abnormal to normal levels was compared in order to determine the therapeutic potency of *Pm*L-Et extract.

Measurement of liver enzymes

Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) were determined by method described by Reitman and Frankel [23] and Kind and Kings method [24].

Measurement of nonenzymatic antioxidants

Changes in the levels of the most essential nonenzymatic antioxidants, including vitamin C [25],

vitamin E [26], and glutathione (GSH) [27], were measured and their importance compared using established techniques.

Detection of enzymatic antioxidants

The tremendous differences in the levels of enzymatic antioxidants such as super oxide dismutase [28], catalase [29], glutathione peroxidase [30] and glutathione-s-transferase [31] were observed and statistically compared.

Statistical analysis: All data are expressed as mean ± S.E.M. One-way analysis of variance (ANOVA) was performed followed by Tukey's test to compare the differences between treatments. Differences were considered statistically significant for $p < 0.05$.

RESULTS AND DISCUSSION

Effect of PmL-Et on the serum Total protein, urea, uric acid and creatinine in control and experimental rats

The result showed that the level of blood urea, serum uric acid, serum creatinine levels were significantly increased in the STZ induced diabetic rats compared to normal rats. Treatment of *Pm*L-Et at 300 mg/kg doses significantly ($p<0.001$) reduced the creatinine, urea and uric acid levels, but there was a significant decrease in total protein in STZ induced diabetic rats when compared with control rats. Administration of *Pm*L-Et and glibenclamide tended to bring serum protein, urea, uric acid and creatinine towards near normal range (Table 1).

Table 1 Effect of ethanol extract of leaves of *P. maderaspatensis* (*Pm*L-Et) on the serum biochemical constituents in control and experimental rats

Groups	Total protein (g/dl)	Blood urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Control	8.16±0.14	29.11±0.65	0.46±0.05	2.17±0.05
STZ induced	4.2±0.1[a*]	51.63±0.91[a*]	1.13±0.07[a*]	5.22±0.11[a*]
STZ induced+100mg/kg bw of *Pm*L-Et	6.75±0.14[b*]	18.43±0.5[b*]	0.61±0.05[b*]	2.75±0.06[b*]
STZ induced+200mg/kg bw of *Pm*L-Et	6.92±0.14[b*]	20.43±0.5[b*]	0.55±0.03[b*]	2.62±0.04[b*]
STZ induced+300mg/kg bw of *Pm*L-Et	7.04±0.1[b*]	24.74±0.54[b*]	0.51±0.03[b*]	2.48±0.02[b*]
STZ induced+600µg/kg bw of Glibenclamide	7.23±0.14[b*]	25.16±0.29[b*]	0.55±0.12[b*]	2.65±0.38[b*]

All values are mean ± S.E.M for six animals;
[a]values deviate significantly from diabetic control group by comparison with normal rats (P≤0.05);
[b]values deviate very significantly from diabetic control groups

Effect of ethanol extract of leaves of P. maderaspatensis (PmL-Et) on the liver enzymes in control and experimental rats

The levels of liver function marker enzymes alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) in the tissue of experimental animals were dramatically raised. Interestingly, treatment with an ethanol extract of *P. maderaspatensis* (*Pm*L-Et) resulted in a significant reduction in ALT, AST, and ALP levels in the tissue of diabetic-induced experimental rats. The ethanol extract of *P. maderaspatensis* (*Pm*L-Et) treated rats had a substantial ($p < 0.05$) drop in the liver function marker enzymes ALT, AST, and ALP in their tissue (Table 2). Similarly, animals treated with the standard drug Glibenclamide exhibited a considerable drop in the liver function marker enzymes.

Table 2 Effect of ethanol extract of leaves of *P. maderaspatensis* (*Pm*L-Et) on the liver enzymes in control and experimental rats

Groups	ALP[a]	AST[b]	ALT[b]
Control	161.67±5.74	52.23±1.98	43.41±1.68
STZ induced	223.68±6.60[a*]	95.60±1.69[a*]	77.95±2.59[a*]
STZ induced+100 mg/kg bw of *Pm*L-Et	171.35±1.84[b*]	63.50±1.77[b*]	55.12±2.16[b*]
STZ induced+200 mg/kg bw of *Pm*L-Et	160.44±4.34[b*]	51.76±2.02[b*]	43.79±2.80[b*]
STZ induced+300 mg/kg bw of *Pm*L-Et	173.99±4.58[b*]	61.23±1.32[b*]	53.46±4.34[b*]
STZ induced+600 µg/kg bw of Glibenclamide	187.54±1.09[b*]	65.21±2.19[b*]	59.06±3.61[b*]

[a*] values deviate significantly from diabetic control group by comparison with normal rats (P≤0.05);
[b*] values deviate very significantly from diabetic control groups.
a nmoles of phenol liberated/min/mg protein
b nmoles of pyruvate liberated/min/mg protein

Table 3 Effect of ethanol extract of leaves of *P. maderaspatensis* (*Pm*L-Et) on the non-enzymic antioxidants in liver of control and experimental rats

Groups	Vitamin C (µM/mg of tissue)	Vitamin E (µM/mg of tissue)	GSH (mg/100 g of tissue)
Control	3.62±0.11	1.65±0.3	31.42±1.6
STZ induced	1.97±0.43[a*]	0.71±0.09[a*]	18.62±2.3[a*]
STZ induced+100 mg/kg bw of *Pm*L-Et	2.67±0.34[b*]	1.22±0.15[b*]	26.81±2.33[b*]
STZ induced+200 mg/kg bw of *Pm*L-Et	2.76±0.41[b*]	1.31±0.14[b*]	27.53±2.92[b*]
STZ induced+300 mg/kg bw of *Pm*L-Et	2.89±0.25[b*]	1.43±0.16[b*]	28.32±2.88[b*]
STZ induced+600 µg/kg bw of Glibenclamide	3.21±0.65[b*]	1.51±0.32[b*]	29.59±0.09[b*]

[a*] values deviate significantly from diabetic control group by comparison with normal rats (P≤0.05);
[b*] values deviate very significantly from diabetic control groups

Effect of ethanol extract of leaves of P. maderaspatensis (PmL-Et) on the non-enzymic antioxidants in liver of control and experimental rats

The levels of non-enzymatic antioxidants (Vitamin C, Vitamin E and GSH) in liver of normal and experimental rats are shown in the (Table 3). The Vitamin C, Vitamin E and GSH levels were significantly decreased in diabetic rats as compared to normal animals. Administration of *P. maderaspatensis* (*Pm*L-Et) increased the levels of Vitamin C, Vitamin E and GSH in liver respectively when compared to diabetic control. The standard drug, glibenclamide treated group significantly showed the levels of Vitamin C, Vitamin E and GSH level in liver to be near normal when compared with diabetic control.

Table 4 Effect of ethanol extract of leaves of *P. maderaspatensis* (*Pm*L-Et) on the enzymic antioxidants in liver of control and experimental rats

Groups	SOD (U/mg protein)	Catalase [mol of H_2O_2 consumed min^{-1} $(mg\ protein)^{-1}$]	GPx [glutathione consumed min^{-1} $(mg\ protein)^{-1}$]	GST [mol of CDNB–GSH conjugate formed min^{-1} $(mg\ protein)^{-1}$]
Control	5.27±0.24	10.24±0.39	3.72±0.16	47.91±1.87
STZ induced	2.86±0.18^{a*}	7.14±0.16^{a*}	1.63±0.10^{a*}	23.44±1.43^{a*}
STZ induced+100 mg/kg bw of *Pm*L-Et	5.03±0.50^{b*}	8.52±0.14^{b*}	3.02±0.15^{b*}	40.33±1.90^{b*}
STZ induced+200 mg/kg bw of *Pm*L-Et	5.18±0.44^{b*}	9.22±0.46^{b*}	3.79±0.14^{b*}	42.13±2.98^{b*}
STZ induced+300 mg/kg bw of *Pm*L-Et	5.19±0.43^{b*}	10.12±0.52^{b*}	3.9±0.05^{b*}	47.02±1.19^{b*}
STZ induced+600 µg/kg bw of Glibenclamide	4.49±0.43^{b*}	9.52±0.52^{b*}	3.17±0.05^{b*}	39.80±1.19^{b*}

Effect of ethanol extract of leaves of P. maderaspatensis (PmL-Et) on the enzymic antioxidants in liver of control and experimental rats

Data in (Tables 4) show the levels of antioxidant enzymes catalase (CAT), glutathione s- transferase (GST), superoxide dismutase (SOD) and glutathione peroxidase (GPx) in liver in normal and experimental animals. CAT, GST, SOD and GPx activities were significantly decreased in STZ-induced diabetic rats when compared to normal rats. Treatment of STZ-induced diabetic rats with *P. maderaspatensis* (*Pm*L-Et) resulted in a marked increase in CAT, GST, SOD and GPx activities in liver when compared to the activities of STZ-induced diabetic rats. Normal rats treated with *P. maderaspatensis* (*Pm*L-Et) showed little or no significant difference in the activities of enzymatic antioxidants.

Protein metabolism produces urea. Amino acid deamination occurs in the liver, where ammonia is transformed to urea and expelled through urine [31]. Muscles create and release creatinine into the blood. It is also a by-product of creatinine metabolism. It is produced in the liver and absorbed almost entirely by skeletal muscle. The rate at which creatinine is excreted in the blood is a standard rate of renal function. Its blood retention indicates kidney disease. In this investigation, STZ-induced diabetic rats (Group II) had significantly higher levels of urea and creatinine than normal control rats (Group I). *P. maderaspatensis* (*Pm*L-Et) and glibenclamide restored urea and creatinine levels in STZ-diabetic rats. Increased activity of mitochondrial urea metabolising enzymes in STZ-induced diabetic rats resulted in urea production and excretion [32]. STZ can also cause abnormal glomerular function, as seen by a decrease in glomerular filtration rates and an increase in serum creatinine, indicating acute renal failure. The levels of serum protein significantly decreased in STZ-induced diabetic rats when compared to control group. On the other hand, oral administration of ethanolic extracts of leaf of *P. maderaspatensis* (*Pm*L-Et) treated diabetic rats never deviated protein metabolism from normal range.

Streptozotocin inhibits the action of liver enzymes. STZ-treated rats experienced liver damage, as evidenced by increased enzyme activity [33]. When rats were treated to STZ, the activity of liver marker enzymes were considerably higher than in the control group. The ethanolic extracts of leaf of *P. maderaspatensis* (*Pm*L-Et), and glibenclamide lowered the levels of SGOT, SGPT and ALP in the serum of STZ-induced diabetic rats. The higher levels of these enzymes in diabetic rats may raise glucose precursor concentrations. Thus, the gluconeogenic action of SGOT and SGPT provides fresh glucose supplies from other sources, such as amino acids. Diabetes causes enhanced gluconeogenesis and ketogenesis due to insulin deficiency and amino acid availability [34]. Insulin resistance may cause hepatic dysfunction. Diabetic individuals' livers were necrotized [35]. Because of this, an increase in the activity of the liver enzymes SGOT, SGPT and ALP in serum [36] may be attributable to their leakage from the liver cytosol into the blood stream [37]. However, treatment of diabetic rats with plant ethanol extracts greatly improved liver enzyme levels.

Vitamin C is a powerful non-enzymatic antioxidant that reacts with oxygen free radicals and interacts with vitamin E [38]. In our study, diabetic rats had higher levels of vitamin E, which could be attributed to greater membrane damage caused by ROS. Treatment with ethanolic extracts of leaf of *P. maderaspatensis* (*Pm*L-Et) restored vitamin E levels to near normal levels, possibly due to decreased membrane damage as shown by lower lipid peroxidation. Vitamins C and E are linked by the recycling process. Vitamin C facilitates the recycling of tocopheroxyl radicals to tocopherol. In our study, diabetic rats had lower levels of vitamin C. This could be attributed to increased use of vitamin C as an antioxidant defence against increasing ROS or a decrease in GSH levels, as GSH is required for vitamin C recycling. Treatment with ethanolic extracts of leaf of *P. maderaspatensis* (*Pm*L-Et) restored vitamin C levels to near normal.

GSH is a significant non-protein thiol in living organisms, and it is important in coordinating the body's antioxidant defence process. GSH is the body's first line of defence against oxidative stress [39]. GSH deficiency protects cellular proteins from oxidation via the glutathione redox cycle and also directly detoxifies ROS. STZ produces oxygen free radicals, which cause lipid peroxidation. GSH levels in diabetic rats' liver and kidney were found to be lower. GSH depletion exacerbates the cellular damage

produced by oxidative stress. GSH depletion in diabetic rats shows that it is being used more effectively against reactive oxygen species [40]. The administration of ethanolic extracts of leaf of *P. maderaspatensis* (*Pm*L-Et) orally raised the level of GSH in diabetic rats' liver.

Enzymatic antioxidants such as SOD, CAT, GPx and GST play a crucial role in avoiding oxidative damage to cells [41]. High glucose merely inactivates antioxidant enzymes SOD, CAT, GPx, and others de diabetes mellitus by glycating these proteins, and hyperglycemia generates oxidative stress, which causes lipid peroxidation [42]. A decrease in the activity of these antioxidants can result in an excess of superoxide anion O_2– and hydrogen peroxide in biological systems, leading in the generation of hydroxyl radicals and the onset and spread of lipid peroxidation [43]. The elevated activities of SOD and CAT in diabetic rats treated with ethanolic extracts of leaf of *P. maderaspatensis* (*Pm*L-Et) revealed that this chemical exhibited free radical scavenging activity, which could benefit against pathological modifications produced by the presence of O_2• and OH•. This revealed that sitosterol can help scavenge O_2–•, prevent the production of •OH, and minimise lipid peroxidation [44]. This activity could be mediated by processes similar to the antioxidant effect of ethanolic extracts of leaf of *P. maderaspatensis* (*Pm*L-Et) on type 2 diabetes. Depletion of GSH, GPx, and GST enhances the production of ROS and oxidative stress, resulting in a cascade of effects that damage both the functional and structural integrity of cell and organelle membranes [45-46]. The treatment of ethanolic extracts of leaf of *P. maderaspatensis* (*Pm*L-Et) boosted the activities of GPx and GST in diabetic rats' liver.

LITERATURE CITED

1. Lopaschuk GD. 2016. Fatty acid oxidation and its relation with insulin resistance and associated disorders. *Ann. Nutr. Metab*. 68 (Suppl 3): 15-20.
2. Priya MAV, Neelakantan A. 2020. Prevalence, distribution, and knowledge-attitude-practices, of type 2 diabetes mellitus patients amongst Urban school-going Adolescents - a review. *Jr. Evid. Based Med. Healthc*. 7(45): 2662-2668.
3. Bhattacharyya M. 2012. Diabetes, hypertension and cardiovascular disease- An unsolved enigma. *In*: Phytotherapy in the Management of Diabetes and Hypertension. (Eds) Mohamed Eddouks. Publisher: Bentham Science Publishers. pp 97-130.
4. Ojuade FI, Olorundare OE, Akanbi OB, Afolabi SO, Njan AA. 2021. Antidiabetic and antihyperlipidemic effects of aqueous extract of *Parquetina nigrescens* in streptozotocin-nicotinamide induced type 2 diabetic rats. *Heliyon* 7(6): e07363.
5. Abd Rashed A, Rathi DG. 2021. Bioactive components of *Salvia* and their potential antidiabetic properties: A review. *Molecules* 26(10): 3042.
6. Mechchate H, Es-Safi I, Bourhia M, Kyrylchuk A, El Moussaoui A, Conte R, Ullah R, Ezzeldin E, Mostafa GA, Grafov A, Bekkari H, Bousta D. 2020. *In vivo* antidiabetic activity and *in silico* mode of action of lc/ms-ms identified flavonoids in oleaster leaves. *Molecules* 25(21): 5073.
7. Kumar P, Deepika PP. 2017. Herbal drugs used in the treatment of diabetes: an overview. *World Journal of Pharmacy and Pharmaceutical Sciences* 6: 697-708.
8. Melesie TG, Bule M, Alemayehu GD, Teka F, Abula T. 2020. In vivo antidiabetic activity evaluation of aqueous and 80% methanolic extracts of leaves of *Thymus schimperi* (Lamiaceae) in alloxan-induced diabetic mice. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy* 13: 3205-3212.
9. Sami W, Ansari T, Butt NS, Hamid M. 2017. Effect of diet on type 2 diabetes mellitus: A review. *International Journal of Health Sciences* 11(2): 65-71.
10. Awad SF, O'Flaherty M, El-Nahas KG. 2019. Preventing type 2 diabetes mellitus in Qatar by reducing obesity, smoking, and physical inactivity: Mathematical modeling analyses. *Popul. Health Metrics* 17: 20.
11. Fernandes SM, Cordeiro PM, Watanabe M, Fonseca CD, Vattimo MF. 2016. The role of oxidative stress in streptozotocin-induced diabetic nephropathy in rats. *Arch Endocrinol Metab*. 60(5): 443-449.
12. Al-Nahdi AMT, John A, Raza H. 2018. Cytoprotective effects of n-acetylcysteine on streptozotocin- induced oxidative stress and apoptosis in RIN-5F pancreatic β-cells. *Cell Physiol. Biochemistry* 51: 201-216.
13. Dissassa KD, Ali M, Hussain M, Al-Hamid A. 2013. Effect of stem cells injection in adult male albino rats with streptozotocin-induced diabetic nephropathy. *Suez Canal University Medical Journal* 16(1): 52-61.
14. Schmelzer GH, Gurib-Fakim A, Arroo R, Bosch CH, de Ruijter A, Simmonds MSJ, Lemmens RHMJ, Oyen LPA. 2008. *Plant Resources of Tropical Africa 11(1): Medicinal plants 1*. (Plant Resources of Tropical Africa; No. 11(1)). PROTA Foundation [etc.]. https://edepot.wur.nl/417238
15. Asha VV, Sheeba MS, Suresh V, Wills PJ. 2007. Hepatoprotection of *Phyllanthus maderaspatensis* against experimentally induced liver injury in rats. *Fitoterapia* 78: 134-141.
16. Abdelhafez OH, Fawzy MA, Fahim JR, Desoukey SY, Krischke M, Mueller MJ. 2018. Hepatoprotective potential of *Malvaviscus arboreus* against carbon tetrachloride-induced liver injury in rats. *PLoS One* 13(8): e0202362. https://doi.org/10.1371/journal.pone.0202362
17. Bommu P, Nanjan CM, Joghee NM, Nataraj SM, Bhojraj S. 2008. *Phyllanthus maderaspatensis*, a dietary supplement for the amelioration of adriamycin-induced toxicity and oxidative stress in mice. *Jr. Nat. Med*. 62 :149-154.
18. Dissanayake D, Nilakarawasam, Nayanakanthi, Somaratne S, Weerakoon, Shyama, Ranasinghe, Chandani. 2019. Effect of *Phyllanthus maderaspatensis* L. crude methanolic extract on diet induced hypercholesterolemia in Wistar albino rats (*Mus norvegicus* albinus). *Ceylon Journal of Science* 48: 285.
19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* 193: 265-267.
20. Fawcett JK, Scott JE. 1960. A rapid and precise method for the determination of urea. *Journal of Clinical Pathology* 13: 156.

21. Caraway WT. 1955. Determination of uric acid in serum by a carbonate method. *American Journal of Clinical Pathology* 25: 840-845.
22. Jaffe MZ. 1886. Methods determining creatinine. *Physiological Chemistry* 10: 39-40.
23. Reitman S, Frankel S. 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology* 28: 56-63.
24. Kind PRN, King EJ. 1954. Estimation of plasma phosphatases by determination of hydrolyzed phenol with amino antipyrine. *Journal of Clinical Pathology* 7: 330-332.
25. Omaye ST, Skala JH, Jacob RA. 1986. Plasma ascorbic acid in adult males: Effects of depletion and supplementation. *Am. Jr. Clin. Nutrition* 44: 257-264.
26. Desai ID. 1984. Vitamin E analysis methods for animal tissues. *Methods Enzymology* 105: 138-147.
27. Ellman GL. 1959. Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics* 82: 70-77.
28. Kakkar D, Das B, Viswanathan PN. 1984. A modified spectro-photometric assay of superoxide dismutase. *Indian Journal of Biochemistry and Biophysics* 21: 130-132.
29. Sinha KA. 1972. Colorimetric assay of catalase. *Analytical Biochemistry* 47: 389-394.
30. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. 1984. Selenium: Biochemical roles as a component of glutathione peroxidase. *Science* 179: 588-590.
31. Habig WJ, Pabst M, Jakoby WB. 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *Jr. Biol. Chemistry* 249: 7130-7139.
32. Kifle ZD, Yesuf JS, Atnafie SA. 2020. Evaluation of *in vitro* and *in vivo* anti-diabetic, anti-hyperlipidemic and anti-oxidant activity of flower crude extract and solvent fractions of *Hagenia abyssinica* (Rosaceae). *Journal of Experimental Pharmacology* 12: 151-167.
33. Dabe NE, Kefale AT. 2017. Antidiabetic effects of artemisia species: A systematic review. *Ancient Science of Life* 36(4): 175-181.
34. Wusa M, Wilson OH, Buraimoh AA, Dibal NI, Obaje SG. 2018. Therapeutic effects of balanitoside in streptozotocin-induced diabetic rats. *Journal of Taibah University Medical Sciences* 13(4): 402-406.
35. Safhi MM, Alam MF, Sivakumar SM, Anwer T. 2019. Hepatoprotective potential of *Sargassum muticum* against STZ-induced diabetic liver damage in Wistar rats by inhibiting cytokines and the apoptosis pathway. *Analytical Cellular Pathology*, 2019 Feb 27; 2019: 7958701. doi: 10.1155/2019/7958701. eCollection 2019.
36. Chao PC, Li Y, Chang CH, Shieh JP, Cheng JT, Cheng KC. 2018. Investigation of insulin resistance in the popularly used four rat models of type-2 diabetes. *Biomedicine and Pharmacotherapy* 101: 155-161.
37. Aldahmash BA, El-Nagar DM, Ibrahim KE. 2016. Attenuation of hepatotoxicity and oxidative stress in diabetes STZ-induced type 1 by biotin in Swiss albino mice. *Saudi Journal of Biological Sciences* 23(2): 311-317.
38. Garg MC, Bansal DD. 2000. Protective antioxidant effect of vitamins C and E in streptozotocin induced diabetic rats. *Indian Jr. Exp. Biol*. 38(2): 101-104.
39. Ramachandran S, Rajasekaran A, Manisenthilkumar KT. 2012. Investigation of hypoglycemic, hypolipidemic and antioxidant activities of aqueous extract of *Terminalia paniculata* bark in diabetic rats. *Asian Pacific Journal of Tropical Biomedicine* 2(4): 262-268.
40. Yogesha M, Grace NJ, Narendhirakannan RT. 2013. Antidiabetic and antioxidant properties of *Triticum aestivum* in streptozotocin-induced diabetic rats. *Advances in Pharmacological Sciences* 2013, Article ID 716073, 9 pages. http://dx.doi.org/10.1155/2013/716073
41. Saravanan G, Ponmurugan P. 2013. S-allylcysteine improves streptozotocin-induced alterations of blood glucose, liver cytochrome P450 2E1, plasma antioxidant system and adipocytes hormones in diabetic rats. *Int. Jr. Endocrinol. Metab*. 11: e10927.
42. Giacco F, Brownlee M. 2010. Oxidative stress and diabetic complications. *Circulation Research* 107(9): 1058-1070.
43. Al-Numair KS, Chandramohan G, Veeramani C, Alsaif MA. 2015. Ameliorative effect of kaempferol, a flavonoid, on oxidative stress in streptozotocin-induced diabetic rats. *Redox Report* 20(5): 198-209.
44. Tangvarasittichai S. 2015. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. *World Journal of Diabetes* 6(3): 456-480.
45. Nita M, Grzybowski A. 2016. The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. *Oxidative Medicine and Cellular Longevity* 2016: 3164734.
46. Kurutas EB. 2015. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutr. Journal* 15: 71.