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Effect of methanolic extract of *Ventilago maderaspatana* leaves on liver function markers and histopathology of liver tissue in streptozotocin-induced diabetic rats

Research Article

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Abstract

Aim: The liver is particularly vulnerable to oxidative stress brought on by hyperglycemia since it is the main tissue. In order to better understand how *Ventilago maderaspatana* leaf methanolic extract (MEVML) affects liver function markers and the histology of liver tissue in STZ-induced diabetic rats, this study looked into the hepatoprotective potential of MEVML. Method: A single STZ dose of 50 mg/kg body weight was given to the animals to cause diabetes. All of the experimental groups' serum was tested for levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), and urea content. Results: The results revealed a marked increase in the serum levels of urea, ALT, AST, ALP, and GGT activity. The results of MEVML treatment, however, were equivalent to those of glibenclamide treatment in that they led to a decline in the activity of ALT, ALP, AST, GGT, and urea content in the diabetic group of rats. Furthermore, diabetic rats' liver tissue showed considerable histological alterations. However, treatment with *Ventilago maderaspatana* methanolic leaf extract and glibenclamide considerably reduced these histological alterations. Conclusion: Overall, the findings of our study show that MEVML reduces liver function biomarkers and restores the architecture of hepatic tissue to reduce hepatic tissue damage under diabetes stress conditions.

Keywords: *STZ, Diabetes, Liver functioning markers, Urea, Ventilago maderaspatana*.

Introduction

Diabetes currently affects more than 230 million people worldwide, and it is predicted to affect 700 million people by the year 2049 (1). According to the IDF Diabetic Atlas, the country with the second-highest number of cases of diabetes after China is India, with 69.1 million individuals affected (2). The prevalence of DM ranges from 5–17% in India, with higher rates seen in the south and in metropolitan areas (3). Current epidemiological data indicate an accelerating DM incidence and occurrence in middle class and working poor urban India's (4). Historically, DM was a disease of the wealthy. A chronic disease called type 2 diabetes alters how the body metabolises glucose, leading to hyperglycemia, or elevated blood sugar levels. This can happen if the pancreas is unable to produce enough insulin or if the body grows resistant to insulin. As demonstrated by a more frequent familial pattern of occurrence, Type 2 Diabetes, also known as Noninsulin Dependent Diabetes Mellitus (NIDDM), has a genetic base that appears to be stronger than in IDDM. Weight gain results from consuming more calories, and obesity is likely a significant contributor to the development of the disease (5).

The primary organ for carrying out a number of crucial metabolic bodily processes, including the synthesis, storage, and secretion of numerous substances, is the liver. Both the regulation of lipid and glucose metabolism and its interruption are caused by non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes, respectively (6). The liver is an organ that insulin targets. The liver is the main insulin-sensitive tissue and is more susceptible to oxidative damage brought on by hyperglycemia (7). The pathogenic condition of diabetes mellitus (DM) is worse by both inflammatory reactions and oxidative stress (8). In rare instances, DM leads to an excessive accumulation of fat cells in the liver, which develops a fatty liver and ultimately NAFLD. Consequently, hepatic necrosis,

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inflammation, and fibrosis—symptoms of a disorder known as Non-Alcoholic- Steato-Hepatitis (NASH)—appear in 2-3% of NAFLD patients (8). Hepatocellular Carcinoma (HCC) will develop in cirrhotic form in a capacitated or fibrotic liver, and the liver will finally suffer a liver catastrophe (9).

Due to the long-term use of hypoglycemic drugs and their adverse effects, there is a big market for effective, low-risk, and cost-effective diabetic treatments (10,11). Traditional medicine has employed a number of medicinal plants to treat a variety of chronic conditions, diabetes mellitus being the most significant of these. Over 1500 of the more than 2500 plants that are used to treat diabetes are available in India, according to estimates from the WHO. 800 of them are plants that are utilized as diabetes treatments. *Ventilago maderaspatana* is one of those plants

An annual herb known as *Ventilago maderaspatana* can be found in several locations in India, Sri Lanka, Bangladesh, and Tropical Africa. This plant is a member of the "Rhamnaceae" family and is commonly referred to as Red Creeper. This plant has historically been used extensively to cure a number of illnesses, including diabetes (12), stomach issues (13), hepatitis, etc. Additionally, this plant contains diuretic (14), wound-healing, analgesic, anti-ulcer, antioxidant (15), anti-inflammatory, and antibacterial activities (16). It was crucial to extract, standardise, and characterise the active components from herbal sources for anti-diabetic activity using knowledge of Ayurveda complemented by modern science. The goal of the current investigation was to determine if the methanolic extract of *Ventilago maderaspatana* leaves (MEVML) had hepatoprotective effects on liver function markers and the histology of liver tissue in STZ-induced diabetic rats.

Materials and Methods

Animals

Thirty male albino Wistar strain rats weighing 150± 30 grammes each were chosen and purchased from Sri Venkateswara Enterprises in Bangalore, Karnataka. The Institutional Animal Ethics Committee accepted the use of animals in its resolution No:09/(i)/a/ CPCSCA/ IAEC/ SVU/ ZOOL/KSR/08. The rats were then acclimated for 7 days in an animal house in propylene cages with top stainless-steel grills, preserved under standard temperature (24-25^{0}c), fed with a standard pellet diet (Hindustan Lever Ltd., Mumbai), and given access to unlimited amounts of water.

Procurement of chemicals

The following scientific businesses provided the chemicals for the current study: Sigma (St. Louis, MO, USA), Fisher (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), and Qualigens (Mumbai, India). All of the chemicals were AnalaR Grade.

Induction of diabetes

Following the Rakieten *et al*. (17) protocol, a single intraperitoneal dose of 50 mg/kg body weight of STZ was administered to the animals in the current study. Just before injection, streptozotocin was prepared in 0.1M cold sodium citrate buffer (pH 4.5). To treat the drug-induced hypoglycemia, 10% glucose solution was made available to the animals for immediate consumption. Following a 72-hour Streptozotocin injection, each animal's plasma blood glucose levels were then assessed using an Accu-Chek Sensor comfort glucometer (manufacturer: Roche Germany). The presence of diabetic rats was confirmed by fasting blood glucose levels greater than 300 mg/dl, and they were employed for therapy.

Preparation of the plant extract

The plant *Ventilago maderaspatana* was harvested in Andhra Pradesh's Seshachalam Hills, Tirumala, and Chittoor (dt). An herbarium expert from the Department of Botany at Sri Venkateswara University in Tirupati, Andhra Pradesh, India, correctly recognized and verified the plant. The *Ventilago maderaspatana* leaves were collected, carefully washed with tap water three times, shade dried at room temperature, and then powdered using a scientific pulverizer. The powder was filtered with What's Man No. 1 filter paper after being washed in methanol for 72 hours in a dark area. The rotary evaporator was used to concentrate the methanol extract. The methanolic extract was subsequently shade dried and kept in the refrigerator for later research.

Experimental design

Six rats each made comprised each of the five groups into which the rats were separated.

- **Group I: Normal control (NC):** For 28 days, an orogastric tube filled with 0.9% sodium chloride per kilogramme of body weight was given to six rats.
- **Group II: Diabetic control (DC):** Six rats were used as controls for diabetes. After fasting, STZ was administered intraperitoneally (50 mg/kg body weight) to induce diabetes.
- **Group III: Normal rats + MEVML (PC):** For 28 days, MEVML 200mg/kg body weight was given to six healthy rats.
- **Group IV: Diabetic rats + Glibenclamide (Di+GLB):** Glibenclamide 20 mg/kg body weight was administered to six diabetic rats for 28 days.
- **Group V: Diabetic rats + MEVML (Di+ Pt):** Six diabetic rats were given a 28-day MEVML treatment at a dose of 200 mg/kg body weight.

Analytical procedures

The 28-day treatment period was completed, and the rats were then sacrificed by cervical dislocation. After performing a myocardial puncture with syringes and needles, blood was drawn into vials that were coated with EDTA. The blood was then centrifuged for 15 minutes at 4^{0}C and 2000 rpm. For biochemical analysis, serum was taken and preserved. At 4^{0}C, the liver was removed. The histopathological examinations employed a portion of the tissue

Biochemical analysis

Determination of liver biomarkers in serum

Estimation of Aspartate transaminase (AST) activity in serum

AST activity was determined in serum using the Recon Diagnostics kit and the procedure indicated in IFCC (18). In a nutshell, 0.05 µL of sample was mixed with 1 mL of the working reagent and heated to 37°C for 60s. With a 30s interval, the absorbance was measured at 340 nm for two minutes. U/L was used to express the values.

Estimation of Alanine transaminase (ALT) activity in serum

Using a Recon Diagnostics kit, the method specified (18) was used to quantify the ALT activity in serum. 50 µL of sample and 1 mL of the working reagent were combined, and they were incubated at 37^0C for 60s. For two minutes, the absorbance was measured continuously at 340 nm at 30-second intervals. U/L was used to express the value.

Estimation of Alkaline Phosphatase (ALP)

With the aid of a Recon Diagnostics kit, the method modified by Wright et al., (19) was used to evaluate the ALP activity in serum. In a nutshell, 10 µL of serum received 500 µL of reagent. At 405 nm, the absorbance was measured. The final values were presented in U/L while taking into account the mean absorbance per minute.

Estimation of γ–Glutamyl transferase (GGT) activity in serum

The Szasz (20) approach was used to measure GGT activity in serum using a Recon Diagnostics kit. In a nutshell, 0.1 mL of serum was added to L-γ-glutamyl-p-nitroanilide, which was made in an ammediol-HCL buffer with a pH of 8.2. At 405 nm, a UV/VIS spectrophotometer read the absorbance.

Determination of liver biomarkers in liver

Alanine aminotransferase & Aspartate amino transferase

Alanine aminotransferase & Aspartate amino transferase activity in liver tissue was assessed in the current investigation using the Reitman and Frankel (21) approach.

Estimation of Urea

Utilising auto analyser kits, urea was calculated. In a nutshell, 0.1 ml of serum was introduced to a test tube marked "T," 1 ml of STD urea was added to another test tube marked "S," and 1 ml of water was added to a third test tube marked "B." Then, 3 ml of acid reagent was added, along with 1 ml each of amicarbalide and diacetyl monoxime, to the test tubes labelled "B," "S," and "T," and everything was thoroughly mixed. The third test tube was then held in a pot of boiling water for 15 minutes before being cooled. Using distilled water as a blank, a spectrophotometer was used to measure the absorbance at 540 nm.

Histopathology

After separating the liver tissues from the sacrificed animal groups, they were immediately fixed in 10% formalin for 24 hours. The tissues underwent additional processing, including washing and dehydration with the aid of an increased alcohol content. The desiccated tissues were coated in paraffin wax and divided into slices using a microtome that were about 5 µm thick. On glass slides, every section was deparaffinized in xylene and hydrated using the opposite method of alcohol treatment. For tissues mounted on slides, hematoxylin was used as the primary stain and eosin as the counterstain.

Statistical Analysis

The results were presented as mean ± SD, and one-way analysis of variance (ANOVA) with Duncan multiple range tests were used to determine whether there was a difference between the groups and within the groups. The SPSS software (16.0) was used to perform statistical analysis on the data. Statistics were judged significant at $p \leq 0.05$.

Results and Discussion

In the current investigation, the blood levels of all experimental groups, including Normal Control (NC), Diabetic Control (DC), Normal + MEVML (PC), Diabetes + Glibenclamide (Di+GLB), and Diabetes + MEVML (Di+Pt), were assessed for the activities of ALT, AST, ALP, GGT, and urea.

Fig: 1

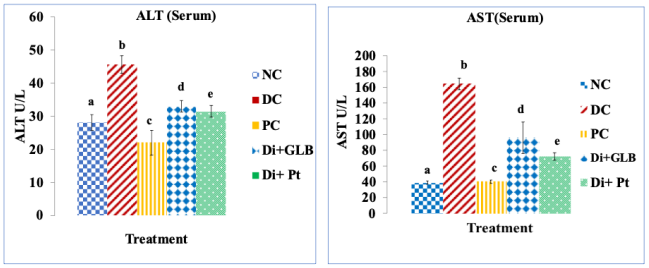

Fig-1: Changes in ALT & AST activity in Serum of the Normal Control (NC), Diabetic Control (DC), Normal + MEVML (PC), Diabetes + Glibenclamide (Di+ GLB), Diabetes + MEVML (Di+Pt) treated rats. Data mean ± SD values (n=6). The groups that do not have same letters are significant (P<0.05abcde) to the control.

The activities of ALT and AST in the serum and liver tissue were significantly increased during diabetes. However, as shown in Fig. 1, treatment with MEVML and Glibenclamide reduced the activity of AST and ALT in the diabetic group of rats. We observed that the normal rats treated with MEVML displayed no appreciable variations in ALT and AST activity when compared to the normal control rats.

Fig: 2

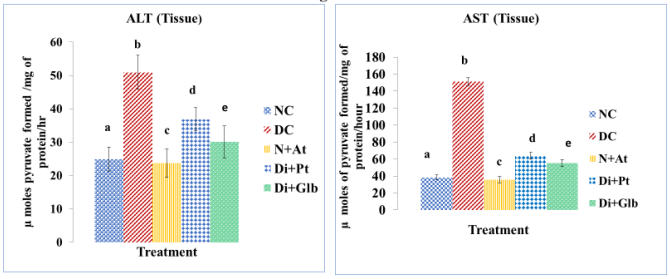

Fig:2: Changes in ALT & AST activity in Liver tissue of the Normal Control (NC), Diabetic Control (DC), Normal + MEVML (N+At), Diabetes+ MEVML (Di+Pt), Diabetes + Glibenclamide (Di+GLB) treated rats. Data mean ± SD values (n=6). The groups that do not share same letters are significant (P<0.05abcde) to the control

All experimental groups in the current investigation, including Normal Control (NC), Diabetic Control (DC), Normal + MEVML (PC), Diabetes + MEVML (Di+Pt), and Diabetes+Glibenclamide (Di+GLB), had their ALT and AST activities in the liver tissue measured. The activities of ALT and AST in the liver tissue were significantly increased during diabetes. However, as shown in Fig. 2, treatment with MEVML and Glibenclamide reduced the activities of AST and ALT in the diabetic group of rats. It was found that the normal rats treated with MEVML did not show any appreciable variations in ALT and AST activity when the findings were compared to the normal control rats.

Fig: 3

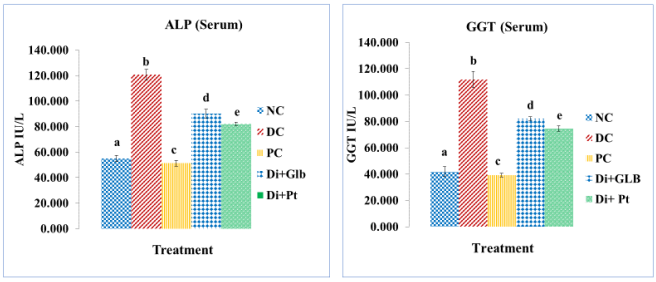

Fig.3: Changes in ALP & GGT activity in Serum of the Normal Control (NC), Diabetic Control (DC), Normal + MEVML (PC), Diabetes+ Glibenclamide (Di+GLB), Diabetes + MEVML (Di+ Pt) treated rats. Data mean ± SD values (n=6). The groups that do not have same letters are significant (P<0.05abcde) to the control

The activity of ALP and GGT in the serum significantly increased in the diabetes state. However, as shown in Fig. 3, MEVML administration resulted in a reduction in the activity of ALP and GGT in the diabetic group of rats. This drop-in activity was equivalent to what was seen after taking glibenclamide. ALP & GGT activity in normal rats treated with MEVML and normal control rats did not differ significantly.

Fig: 4

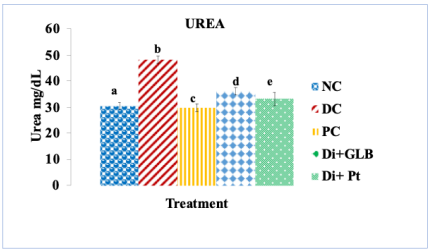

Fig-4: Changes in Urea content in Serum of Normal Control (NC), Diabetic Control (DC), Normal + MEVML (PC), Diabetes + Glibenclamide (Di+ GLB), Diabetes + MEVML (Di+ Pt) treated rats. Data mean ± SD values (n=6). The groups that do not have same letters are significant (P<0.05abcde) to the control.

All experimental groups had their serum urea levels checked. According to the experimental groups, diabetic control rats' serum urea levels were greater than those of normal control rats, as shown in Fig. 4. To manage the serum urea levels, however, and bring them closer to the urea values seen in the glibenclamide-treated rats, MEVML was given to the diabetic rats during the whole experiment. When normal rats treated with MEVML were compared to normal control rats, we saw that there were no discernible differences in the levels of urea in either group.

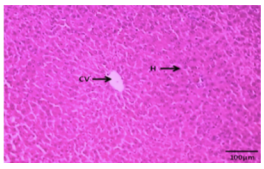	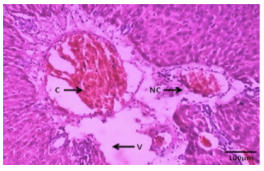	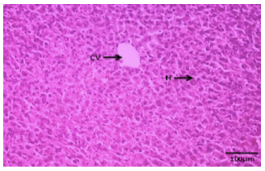	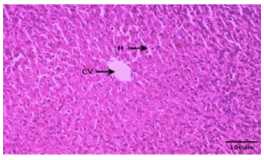	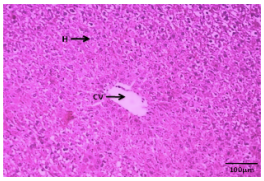
Plate: 1: Group:1: Normal control (NC) rat liver tissue photomicrograph showing (H&E, 10X)	**Plate: 2: Group:2: Diabetic liver tissue photomicrograph showing ((H&E, 10X)**	**Plate: 3: Group: 3: Methanolic leaf extract of *Ventilago maderaspatana* treated liver tissue photo**	**Plate: 4: Group: 4: Methanolic leaf extract of *Ventilago maderaspatana* treated diabetic liver tissue**	**Plate: 5: Group: 5: Glibenclamide treated diabetic liver tissue photo micrograph showing (H&E, 10X)**

The liver tissue of the experimental groups Normal Control (NC), Diabetic Control (DC), Normal Rats Treated with MEVML (PC), Diabetic Rats Treated with Glibenclamide (Di+GLB), and Diabetic Rats Treated with MEVML showed the histological alterations. The diabetic group of rats experienced these notable changes. The histological abnormalities that took place in the liver tissue of diabetic rats, however, were dramatically reversed by treatment with Glibenclamide and methanolic leaf extract of *Ventilago maderaspatana*.

Plate 1, 2, 3, 4, and 5 display the results of light microscope histological tests of liver tissue.

Atmaca *et al*. (22), Prasath and Subramanian (23), and others have revealed that STZ intoxication can increase free radicals through a protein glycation process that causes tissue damage in diabetes conditions and increases the ALT and AST in serum. Additionally, these enzymes' activity has been employed as tissue damage markers in experimental diabetes. According to earlier studies, elevated ALT and AST levels in serum and tissue are indicative of the health and proper operation of the liver and heart (24). Serum ALT and AST levels that are elevated have been linked to cardiovascular disorders, tissue necrosis, and cellular damage (25).

Similar outcomes were seen in our investigation. Rats with diabetes induced by STZ had higher levels of ALT and AST in their serum and liver tissue. It has been demonstrated that STZ toxicity causes diabetes in experimental rats due to its capacity to cause insulin deficiency by the selective destruction of pancreatic beta cells and hyperglycemia make strikingly high levels of ROS, which react with cell membrane proteins and lipids leading to histological variations as well as functional alterations (26). It is well recognised that

elevated levels of ALT, AST, and ALP in serum and plasma result in hepatocellular damage due to cellular leakage and a loss of the functional integrity of the hepatic cell membranes (27). In diabetes condition, an imbalance between radical generation and radical scavenging mechanisms boosts the oxidative stress. Lipid peroxidation is one of the additional traits of chronic diabetes; as a result of an increase in free radicals that may interact with polyunsaturated fatty acids in cell membranes, lipid peroxidation causes tissue damage (28).

The activities of ALT and AST in serum and liver tissue decreased in diabetic rats treated with *Ventilago maderaspatana* leaf extract in methanol. According to Xie *et al*. (29) and Abolfathi *et al*. (30), *Ventilago maderaspatana* and its components have a cytoprotective effect against oxidative stress caused by diabetes. The liver tissue is harmed by the ALT and AST enzymes when they are discharged into the bloodstream and induce necrosis or membrane damage. As a result, the level of these enzyme activity in serum largely determines the liver's state of health. High levels of AST and ALT cause symptoms such cellular leakage and loss of cell functional integrity (31). Similar effects were seen in our study when STZ-induced diabetic rats were treated with methanolic leaf extraction of *Ventilago maderaspatana* (MEVML). This suggests that MEVML has hepatoprotective activity in diabetic conditions.

Alkaline phosphatase, a hydroxylase enzyme, is present in all bodily tissues but is notably concentrated in the liver, kidney, bile duct, bone, and placenta. It removes the phosphate groups from a variety of substances, including proteins and nucleotides. ALP-L is a tissue-nonspecific isoform found in the liver, bones, and kidneys; ALP-P is an isozyme found in the placenta; and ALP-I is an isoform found in the intestine (32). As a result, ALP was released when the liver was disturbed, most commonly in diabetes disease (33).

In our investigation, diabetic rats' serum ALP activity was substantially higher than that of the normal control rats. ALP is a glycoprotein membrane-bound enzyme with high concentrations in the periportal, central, and sinusoidal veins, but low amounts in the biliary canaliculated. According to earlier studies (34), diseased liver and renal conditions resulted in elevated serum ALP levels. Furthermore, it has been noted in other earlier studies that diabetic rats' elevated serum ALT levels were not accompanied by any changes in the histoarchitecture of the liver, suggesting that the damaging effects of hyperglycemia may also affect other organs besides the liver, such as the kidney and skeletal muscle. However, elevated ALP and -GT levels in diabetic rats were linked to biliary obstruction (35).

However, *Ventilago maderaspatana* (MEVML) leaf extract significantly reduced the ALP activity in diabetic rat serum. By inhibiting lipid peroxidation and boosting the activity of antioxidant enzymes, *Ventilago maderaspatana* (MEVML) leaf extracts can scavenge free radicals and shield cells from oxidative stress.

According to recommendations made by Bahadora *et al*. (36), plant-derived components from the *V. maderaspatana* are natural antioxidants linked to the protection of many pathological illnesses and diseases, such as diabetes, malaria, cardiac dysfunction, etc. ALP is often produced in high concentrations by cells lining bile canaliculi in reaction to cholestasis, which raises biliary pressure. By extracting the leaves of *V. maderaspatana*, it is possible to effectively control the activity of alkaline phosphatase towards a strengthened secretory mechanism of the hepatic cells.

GGT is a key player in the synthesis, xenobiotics, and drug detoxification processes as well as the transfer of g-glutamyl functional groups and glutathione formation and degradation. Additionally, it also functions as a pro-oxidant and has demonstrated regulatory effects on cellular pathophysiology and signal transduction at many levels (37). Gamma-Glutamyl Transferase (GGT) was found in a variety of tissues, but the liver stood out above the others. However, it was related with ALP as a marker of cholestasis and was a highly sensitive but unreliable indicator of liver illness (38). Recent studies have employed serum gamma glutamyl transferase as a measure for oxidative stress. According to Ghosh *et al*. (39), there is a connection between pre-gravid liver enzyme level and risk of gestational diabetes.

There is mounting evidence that the independent factor serum Gamma Glutamyl Transferase (GGT) is a Type 2 Diabetes interpreter and that it interacts with liver illness. Additionally, serum GGT may be a good indicator of hepatic steatosis, which can arise from visceral and hepatic fat deposition and eventually lead to T2DM. According to Atmaca *et al*. (22), serum GGT activity has a possible relationship with total triglycerides, cholesterol, HDL, and LDL in people with T2DM. Numerous research on humans and animal models have suggested a connection between GGT and diabetes and have found that people with diabetes have significantly higher serum GGT activity (40). According to Sridhar *et al*. (41), the transfer of gamma-glutamyl groups from glutathione to other acceptors by GGT was used as a biomarker for the liver's healthy operation. According to Ahn *et al*. (42) and Ryoo *et al*. (43), oxidative stress and circulating insulin levels are biologically related to both diabetes and the GGT level.

In the current investigation, methanolic leaf extraction of Ventilago maderaspatana (MEVML) significantly reduced GGT activity in diabetic rats. Hepatic functional enzymes like AST, ALP, and ALT were shown to be elevated in diabetic rats, which is a sign of hepatic tissue destruction. Ketogenesis and gluconeogenesis were boosted by transaminase activity that was prominent (44).

According to Pundir *et al*. (45), urea, a non-toxic, nitrogenous organic end product of protein breakdown, helps the body eliminate 80–90% of nitrogen. Improved liver function and protein-energy deficiency can both cause lower blood urea levels, while increased blood urea levels are indicative of compromised renal function (46). Therefore, poor liver outcomes are also associated with high urea levels. Recent experimental studies have shown that hepatic fibrosis is associated with decreased urea production, even in early CLD (47, 48&40).

Additionally, disruption of the urea cycle aids in the development of cancer (50). As a result, CLD patients with low urea levels may have an advanced form of the condition and be at risk for illness progression.

A symptom of a diabetic problem involving the kidney and liver functions is an abnormally high level of urea in a diabetic condition. In the current study, diabetic rats' serum urea levels considerably increased as compared to normal rats, indicating aberrant renal tissue function in diabetic rats. Urea levels may rise as a result of aberrant renal function, which may come from the glycosylation of proteins that hyperglycemia causes (51). The MEVML therapy dramatically decreased the aforementioned measures when compared to diabetic control rats, and it may also have a protective impact on the kidneys.

In diabetic experimental rats, blood urea levels rise and plasma protein levels fall, which causes a negative nitrogen balance and accelerated degradation of plasma and tissue proteins. Due to abnormal protein metabolism brought on by the supraphysiological quantity of glucose in diabetes conditions, a negative nitrogen balance occurs. According to Salek *et al*. (52), these higher urea levels in turn serve as biochemical diagnostic markers for determining renal impairment and drug-induced toxicity.

In our work, methanolic extraction of Ventilago significantly reduced the urea levels in diabetic rats. It has strong anti-oxidant properties and can reduce the amount of free radicals created when a person has diabetes (53). By releasing ROS, the concerned oxidative stress is reflected and causes the diabetes problem. A symptom of a diabetic problem involving the kidney and liver functions is an abnormally high level of urea in a diabetic condition. As a result of its antidiabetic effect, the results of the current investigation demonstrated lowered urea levels in diabetic conditions.

When compared to a standard medication like glibenclamide, the current study has shown that MEVML may be able to regulate urea in serum by their antioxidant and antidiabetic activity under diabetic conditions. This is because MEVML is enriched with bioactive constituents that may be able to control diabetic complications.

The liver of the control rat has a typical histology with a central vein (CV), hepatocellular architecture, central solitary nucleus (N), and normal hepatocytes (NH) devoid of inclusions. Hepatocytes are organised in sheets and have a polygonal original architecture. There are no haemorrhages, fibrosis, or dilated hepatic sinusoids (HS) present.

Degenerative alterations in the liver tissue, including Degenerative Hepatocytes (DGH), Hepatic Sinusoids Dilatation (DHS), Necrotic alterations, and Loss of Architecture of Hepatic Cards, were seen in diabetic (STZ-induced) rats. Central vein vacuolization and congestion were found. The rate of drug administration, the length of fasting prior to drug administration, the rate of drug infusion, age, species, bodyweight of the experimented animals, and the animals' level of hydration are all important factors that affect the toxicity of STZ, according to Lucchesi et al. (54). The first two weeks following the introduction of diabetes are usually when systemic harmful effects of STZ become apparent.

In our work, we found that diabetes affects not just the lipid metabolic profile but also the way that glucose is metabolised. Diabetes-related dyslipidemia was mostly caused by the lipase enzyme being active when there is an insulin shortage, which causes adipose tissue to produce a lot of free fatty acids. The liver processed the large amount of free fatty acids into phospholipids and cholesterol. Triglycerides (TGs) are created when cholesterol and phospholipids combine, and they are then released into the bloodstream as lipoproteins. Furthermore, the STZ-induced disruption of the hepatic architecture arises from liver lipid membrane degradation brought on by an excess of free radicals that produce liver biomarkers.

The conspicuous nucleus (N), normal hepatocytes (NH), and normal central vein (CV) were seen in *Ventilago maderaspatana*-treated normal rats, as well as the enlargement of the hepatic sinusoids (DHS).

Diabetic rats treated with *Ventilago maderaspatana* leaf extract demonstrated regenerative changes in liver tissue that were comparable to normal tissue. The current research, however, showed that oral administration of *Ventilago maderaspatana* methanolic leaf extract treatment had significant protective effects on diabetic liver, which was supported by decreased liver biomarkers in diabetic rats when treated with *Ventilago maderaspatana* methanolic leaf extract.

Conclusions

The findings of our investigation also showed that liver damage brought on by STZ is responsible for the rise of intracellular hepatic functioning enzymes. The oral administration of *Ventilago maderaspatana* MEVML significantly decreased transaminases and restored the liver damage in diabetic rats. In diabetes, free radicals produced by protein glycation and glucose autoxidation, which in turn catalyse lipid peroxidation, cause tissue damage.

Additionally, diabetes causes the glutathione metabolism and antioxidant defence system to change, which raises the amounts of transaminase enzymes. *Ventilago maderaspatana* leaf extract (MEVML) supplementation improved liver function and reversed signs of cellular infiltration and functional liver cell membrane change in diabetes. Because of its antioxidant and hypoglycemic properties, *Ventilago maderaspatana* leaf extract (MEVML) likely protected the liver in diabetes conditions.

Additionally, MEVML has improved liver tissue damage caused by diabetes stress by lowering liver function biomarkers and by restoring the architecture of the liver. As a result, the diabetes condition's hepatic tissue damage is lessened with MEVML treatment.

As a result, we state that additional research is necessary to ascertain MEVML's precise makeup and mode of operation in order to demonstrate that it is a hepatoprotective agent during diabetes.

Declaration of Competing Interest

The authors declare no conflict of interest

References

1. English E, Lenters-Westra E. HbA1c method performance: the great success story of global standardization. Crit Rev Clin Lab Sci. September, 2018;55(6);408-419. doi:10.1080/10408363. 2018.1480591.
2. International Diabetes Federation, IDF Diabetic Atlas. 7th ed; 2016.
3. Little M, Humphries S, Patel K, Dodd W, Dewey C. Factors associated with glucose tolerance, pre-diabetes, and type 2 diabetes in a rural community of south India: A cross-sectional study. Diabetol Metab Syndr. 2016; 8:21. doi:10.1186/s13098- 016-0135-7.
4. Tripathy J.P, Thakur J.S, Jeet G, Chawla S, Jain S, Pal A, Prasad R, Saran R. Prevalence and risk factors of diabetes in a large community-based study in North India: results from a STEPS survey in Punjab, India. Diabetol Metab Syndr. 2017;9:8. doi:10.1186/s13098-017-0207-3.
5. American Diabetes Association. 2009. All about diabetes. *Jsp*.
6. Mohamed J, Nazratun Nafizah A.H, Zariyantey A.H, Budin S.B. Mechanisms of Diabetes-Induced Liver Damage: the role of oxidative stress and inflammation. Sultan Qaboos Univ Med J(2). 2016;16(2);e132-e141. doi:10.18295/squmj.2016. 16.02.002.
7. Manna P, Das J, Ghosh J, Sil P.C. Contribution of type 1 diabetes to rat liver dysfunction and cellular damage via activation of NOS, PARP, IkappaBalpha/NF-kappaB, MAPKs, and mitochondria-dependent pathways: prophylactic role of arjunolic acid. Free Radic Biol Med. 2010;48(11);1465-1484. doi:10.1016/j.freerad biomed. 2010.02.025.
8. Romagnoli M, Gomez-Cabrera M.C, Perrelli M.G, Biasi F, Pallardó F.V, Sastre J. Xanthine oxidase-induced oxidative stress causes activation of NF-kappaB and inflammation in the liver of type I diabetic rats. Free Radic Biol Med. 2010;49;171-177. doi:10.1016/j.
9. Cohen J.C, Horton J.D, Hobbs H.H. Human fatty liver disease: old questions and new insights. Science. 2011;332(6037) ;1519-1523. doi:10.1126/ science.1204265.
10. Kesavadev J, Saboo B, Sadikot S. Unproven therapies for diabetes and their implications. Adv Ther. 2017;34(1);60.e77.
11. Bing P, Jing G, Linhua Z. Retrospective study of Traditional Chinese Medicine treatment of type 2 diabetes mellitus. J Trad Chin Med. 2016;36(3); 307.e313.
12. Warrier P.K, Nambiar V.P, Ramankutty C. Indian Medicinal Plants*:* A Compendium of 500 Species*,* Orient Blackswan Publisher; 1996; P. 352.
13. Nandkarni A.K. Materia Medica. 2nd ed; vol 1. Tarun Enterprises; 2000.
14. Hanumantaiah T. Synthesized ventilone-c from the roots of Ventilago maderaspatana Gaertn. Tetrahedron. 1985;45;635-642.
15. Charles B. Synthesized and confirmed that structures of the Ventiloquinones E, G and J with the compounds isolated from the root bark of *Ventilago maderaspatana*. J Chem Soc Perkin Trans 1. 1991:2743-2748.
16. Sudhakar Reddy C, Reddy K.N, Murthy E.N, Raju V.S. Traditional medicinal plants in Seshachalam hills, Andhra Pradesh, India. J Med Plants Res. May 2009;3(5); 408-412.
17. Rakieten N, Rakieten M.L, Nadkarni M.V. Cancer Chemother Rep, 29. 1963. Studies on the diabetogenic action of streptozotocin (NSC-37917); 91-98.
18. IFCC (International Federation of Clinical Chemistry). Physicochemical quantities and units in clinical chemistry. J Clin Chem Clin Biochem. 1980;18;829-854.
19. Wright P.J, Leatherwood P.D, Plummer D.T. Enzymes in rats: alkaline phosphatase. Enzymologia. 1972; 42;317-327.
20. Szasz G. Kinetic photometric method for serum γ-glutamyl transpeptidase. Clin Chem. 1969; 15(2);124-136. doi:10.1093/clinchem/15.2. 124.
21. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957, July, 28; (1); 56-63. doi:10.1093/ajcp/28.1.56.
22. Atmaca M, Ucler R, Kartal M, Seven I, Alay M, Bayram I, Olmez S. Glycogenic hepatopathy in type 1 diabetes mellitus. Case Rep Hepatol. 2015;236143. doi:10.1155/2015/236143.
23. Prasath GS, Subramanian S.P. Fisetin. a tetra hydroxy flavone recuperates antioxidant status and protects hepatocellular ultrastructure from hyperglycemia mediated oxidative stress in streptozotocin induced experimental diabetes in rats. Food Chem Toxicol. 2013;59; 249-255. doi:10.1016/j.fct.2013.05.062.
24. Singh J, Kakkar P. Modulation of liver function, antioxidant responses, insulin resistance and glucose transport by Oroxylum indicum stem bark in STZ induced diabetic rats. Food Chem Toxicol. 2013; 62; 722-731.
25. Hassan S.K, El-Sammad N.M, Mousa A.M, Mohammed M.H, Abd el Razik Hussein Farrag., Amani Nassir Eldin Hashim., Victoria Werner., Ulrike Lindequist., Mahmoud Abd. & El-Moein Nawwar. Hypoglycemic and antioxidant activities of Caesalpinia ferrea Martius leaf extract in streptozotocin-induced diabetic rats. Asian Pac J Trop Biomed. 2015; 5(6); 462–471.
26. Sathya A, Siddhuraju P. Protective effect of bark and empty pod extracts from Acacia auriculiformis against paracetamol intoxicated liver injury and alloxan induced type II diabetes. Food Chem Toxicol. 2013; 56; 162-170. doi:10.1016/j. fct. 2013.02.031.
27. Rashid K, Das J, Sil P.C. Taurine ameliorate alloxan induced oxidative stress and intrinsic apoptotic

pathway in the hepatic tissue of diabetic rats. Food Chem Toxicol. 2013; 51;317-329. doi:10.1016/j.fct.2012.10.007.

28. Bril F, Cusi K. Management of nonalcoholic fatty liver disease in patients with Type 2 diabetes: A call to action. Diabetes Care. 2017;40(3) ; 419-430. doi:10.2337/dc16-1787.
29. Wang P, Xie K, Wang C, Bi J. Oxidative stress induced by lipid peroxidation is related with inflammation of demyelination and neurodegeneration in multiple sclerosis. Eur Neurol. 2014;72(3-4); 249-254. doi:10.1159/000363515.
30. Abolfathi A.A, Mohajeri D, Rezaie A, Nazeri M. Protective effects of green tea extract against hepatic tissue injury in streptozotocin-induced diabetic rats. *Evid Based Complement Alternat Med***.** 2012; 740671. doi:10.1155/2012/740671.
31. Zhang HJ, He J, Pan LL, et al. Effects of moderate and vigorous exercise on nonalcoholic fatty liver disease: A randomized clinical trial. JAMA Intern Med. August, 2016;176(8); 1074-1082. doi:10.1001/jamainternmed.2016.3202.
32. Kim E.E, Wyckoff H.W. Reaction mechanism of alkaline phosphatase based on crystal structures. Two-metal ion catalysis. *J Mol Biol*. 1991;218(2); 449-464. doi:10.1016/0022-2836(91)90724-k.
33. Alvaro D, Benedetti A, Marucci L, Delle Monache M, Monterubbianesi R, Di Cosimo E, Perego, L, Macarri G, Glaser S, Le Sage, & Alpini G. The function of alkaline phosphatase in the liver: regulation of intrahepatic biliary epithelium secretory activities in the rat. Hepatology. 2000;32(2) ;174-184. doi:10.1053/jhep.2000.9078.
34. Kubilay N.Z, Sengel B.E, Wood K.E, Joseph Layon A. Biomarkers in hepatic disease: a review focused on critically ill patients. J Intensive Care Med. 2014; 1-9; 4554897. doi:10.1177/088506661.
35. Justino C.I.L, Duarte A.C, Rocha-Santos T.A.P. Recent progress in biosensors for environmental monitoring: a review. Sensors (Basel). 2017;17(12); 2918. doi:10.3390/s17122918.
36. Bahadoran Z, Mirmiran P, Azizi F. Dietary polyphenols as potential nutraceuticals in management of diabetes: a review. J Diabetes Metab Disord. August, 2013; 12(1); 43. doi:10.1186/2251-6581-12-43.
37. Lee Y.S, Cho Y, Burgess S, Davey Smith, G, Relton C. L, Shin S. Y. & Shin, M. J. Serum gamma-glutamyl transferase and risk of type 2 diabetes in the general Korean population: A Mendelian randomization study. Hum Mol Genet. 2016;25(17); 3877-3886. doi:10.1093/hmg/ddw226.
38. Rajarajeswari D, Krishna T, Naidu M, Naidu J. Serum gamma-glutamyl transferase levels in association with lipids and lipoproteins in type 2 diabetes mellitus. Int J Res Med Sci. 2014;2(3; 838-841. doi:10.5455/2320-6012.ijrms20140819.
39. Ghosh M, Paul D, Islam M.S, Ghosh G. C, Bhuiyan M. M. A, Akter, S, Sultana T, Rahman M. Q, & Ahmed A. N. N. Gamma-glutamyltransferase is an indicator of gestational diabetes mellitus. Bangladesh Med J. September, 2015, 2015; 4 (3).
40. American Diabetic Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2013; 36(1); S67-S74.
41. Sridhar S.B, Xu F, Darbinian J, Quesenberry C.P, Ferrara A, Hedderson M.M. Pregravid liver enzyme levels and risk of gestational diabetes mellitus during a subsequent pregnancy. Diabetes Care. 2014;37(7);1878-1884. doi:10.2337/dc13-2229.
42. Ahn H.R, Shin M.H, Nam H.S, Park K. S, Lee Y. H, Jeong S. K, Choi J. S, & Kweon S. S. The association between liver enzymes and risk of type 2 diabetes: the Namwon study. Diabetol Metab Syndr. 2014;6(1); 14. doi:10.1186/1758-5996-6-14.
43. Ryoo J.H, Oh C.M, Kim H.S, Park S.K, Choi J.M. Clinical association between serum c-glutamyltransferase levels and the development of insulin resistance in Korean men: A 5-year follow-up study. Diabet Med. 2014;31(4); 455-461. doi:10.1111/dme.12315.
44. Li S, Hong M, Tan H.Y, Wang N, Feng Y. Insights into the role and interdependence of oxidative stress and inflammation in liver diseases. Oxid Med Cell Longev. 2016; 2016; 4234061. doi:10.1155/2016/4234061.
45. Pundir C.S, Jakhar S, Narwal V. Determination of urea with special emphasis on biosensors: a review. Biosens Bioelectron. 2019; 123; 36-50. doi:10.1016/j.bios.2018.09.067.
46. Vilstrup H. Synthesis of urea after stimulation with amino acids: relation to liver function. Gut (11). 1980; 21(11); 990-995. doi:10.1136/gut.21.11.990.
47. Thomsen K.L, Grønbæk H, Glavind E, Hebbard L, Jessen N, Clouston A, George J, Vilstrup H. Experimental nonalcoholic steatohepatitis compromises ureagenesis, an essential hepatic metabolic function. Am J Physiol Gastrointest Liver Physiol. 2014; 307(3) ; G295-G301. doi:10.1152/ajpgi.00036.2014.
48. De Chiara F, Heebøll S, Marrone G, Montoliu C, Hamilton-Dutoit S, Ferrandez A, Andreola F, Rombouts K, Grønbæk H, Felipo V, Gracia-Sancho J, Mookerjee R. P, Vilstrup H, Jalan R, Thomsen. Urea cycle dysregulation in non-alcoholic fatty liver disease. J Hepatol. 2018;69(4); 905-915. doi:10.1016/j.jhep.2018.06.023.
49. De Chiara F, Thomsen KL, Habtesion A, Jones H, Davies N, Gracia-Sancho J, Manicardi N, Hall A, Andreola F, Paish H. L, Reed L. H, Watson A. A, Leslie J, Oakley F, Rombouts K, Mookerjee R. P, Mann J, Jalan R. Ammonia scavenging prevents progression of fibrosis in experimental nonalcoholic fatty liver disease. Hepatology. 2020;71(3); 874-892. doi:10.1002/hep.30890.
50. Lee J.S, Adler L, Karathia H, Carmel N, Rabinovich S, Auslander N, Keshet R, Stettner N, Silberman A, Agemy L, Helbling D, Eilam R, Sun Q, Brandis A, Malitsky S, Itkin M, Weiss H, Pinto S, Kalaora S., . . . Erez A. Urea cycle dysregulation generates clinically relevant genomic and

biochemical signatures. Cell. 2018; 174(6); 1559-1570.e22. doi:10.1016/j.cell.2018.07.019.

51. Lal S.S, Sukla Y, Singh A, Andriyas E.A, Lall A.M. Hyperuricemia, high serum urea and hypoproteinemia are the risk factor for diabetes. Asian J Med Sci. 2009; 1; 33-34.
52. Salek J, Byrne J, Box T, Longo N, Sussman N. Recurrent liver failure in a 25-year-old female. Liver Transpl. 2010;16(9); 1049-1053. doi:10.1002/lt.22118.
53. Bais S, Singh G.S, Sharma R. Antiobesity and hypolipidemic Activity of Moringa oleifera Leaves against High Fat Diet-Induced Obesity. In: Rats. 2014. doi:10.1155/162914.
54. Lucchesi A.N, Freitas NTD, Cassettari LL, Marques SFG, Spadella CT. Diabetes mellitus triggers oxidative stress in the liver of alloxan-treated rats: A mechanism for diabetic chronic liver disease. Acta Cir Bras. 2013;28(7); 502-508. doi:10.1590/s0102-86502013000700005.

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