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Original Article

Effect of Some Naturally Occurring Monoterpenes viz d-Limonene, p-Cymene and Terpinolene on the Glycemic and Hepatic Function in a Rat Model of Type 2 Diabetes Mellitus

Sheeba Shakeel, Nahida Tabassum*

ABSTRACT

Background: Diabetes mellitus is an increasingly serious health problem in society with type 2 diabetes the most common type of diabetes. **Objectives:** Our main aim was to test the efficacy of some naturally occurring pure monoterpenes viz d-limonene, *p*-cymene, terpinolene in modulating glycemic and hepatic function. **Materials and Methods:** Rats were given soybean oil (as source of fat) for 28 days once daily per os and a single dose of 35mg/kg streptozotocin i.p. at the end for diabetes induction. Post diabetes detection, animals were treated with d-limonene 300mg/kg, *p*-cymene (150mg/kg and 200mg/kg), terpinolene (12.5mg/kg and 25 mg/kg) and standard anti-diabetic drug glibenclamide (5mg/kg) once daily orally for a period of another 28 days. At the end of the experimental period, blood glucose, serum levels of insulin, HbA_{1c}, ALP, ALT, AST, GGT, total protein and albumin were determined. Liver was isolated for histopathology. **Results:** Biochemical profile revealed that d-limonene, *p*-cymene, and terpinolene significantly restored blood glucose, serum insulin, glycated hemoglobin, ALP, ALT, AST, GGT, albumin and total protein. However, d-limonene (300mg/kg) and terpinolene (25mg/kg) exhibited more pronounced activity than p-cymene (150mg/kg). Histopathology of diabetic group revealed binucleated cells, degeneration of parenchyma, clear cell foci, granular cytoplasm, prominent nucleoli and darkly stained nucleolus while d-limonene, p-cymene and terpinolene treated groups were successful in slowing down the progression of pathology associated with hepatic architecture. **Conclusion:** D-limonene, p-cymene and terpinolene have the potential to control parameters related to glycemia. Because of the fact that type 2 diabetics are at risk of several liver pathologies, d-limonene, p-cymene and terpinolene exhibited hepatoprotective activity also.

Keywords: Diabetes, Streptozotocin, Rats, Liver, Histopathology.

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INTRODUCTION`

Diabetes mellitus is a group of metabolic disorders distinguished by a chronic hyperglycemic state exacerbated by defects in insulin secretion, insulin action, or both. Diabetes mellitus is classified into two types: i) Type 1 diabetes, also known as insulin-dependent diabetes mellitus (IDDM), is caused by a lack of insulin secretion by pancreatic beta cells. ii) Type 2 diabetes, also known as non-insulin dependent diabetes mellitus, is caused by a decrease in insulin sensitivity in target tissues.[1]

Whereas, incidence of beta cell autoantibodies is the recognition of type 1 diabetes, a blend of maladjusted insulin secretion by pancreatic beta cells and peripheral insulin resistance is the characteristic of type 2 diabetes. Obesity is one of the chief factors behind the silent development of type 2 diabetes. Advancement of beta cell loss in type 2 diabetes has been attributed to increased build-up of lipid intermediates in nonadipose tissue, increased glucotoxicity, stress initiated by the endoplasmic reticulum, and finally cell death. The conventional risk components for cardiovascular complications, insulin resistance, and type 2 diabetes are visceral adiposity, obesity, and dyslipidemia.[2]

Among the many established animal models of type 2 diabetes induction, a new type 2 diabetes mellitus model has gained popularity in the recent years. The model aims to produce a slight beta-cell dysfunction without total cessation of insulin secretion. This type 2 diabetes mellitus model is stemmed by feeding high-fat diet (HFD) to rats for a certain period of time to tempt insulin resistance and thereon injecting low dose streptozotocin (STZ). Therefore, the natural progression of type diabetes mellitus (from insulin resistance to β-cell impairment) as well as the metabolic characteristics involved with it are very meticulously mimicked. HFD / STZ has been widely investigated for the pathological mechanisms associated with the development of type 2 diabetes mellitus and is exploring promising candidates that have therapeutic potential.[3]

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Nature has bestowed mankind with an abundant reservoir of components that besides their attractive gustation do good to the body in innumerable ways. One of such families of monoterpenes is very widely distributed in the natural flora and finds use in cooking. Reports of monoterpenes acting as antifungal, antibacterial, antioxidant, anticancer, antiarrhythmic, anti-aggregating, local anesthetic, antinociceptive, anti-inflammatory, antihistaminic, antidiabetic and anti-spasmodic have been confirmed by various research groups.[4]

D-limonene belongs to the class of monocyclic monoterpenes. It finds use as a flavoring agent and is present in orange peel, citrus oils, e.g. mandarin, lime, lemon, grapefruit.[5] Its pharmacological activities are abundant which include antioxidant,[6] anti-inflammatory,[7] anti-cancer,[8] anti-diabetic and anti-glycating,[9] gastroprotective effect,[10] anti-atherogenic and cardioprotective,[11] immunomodulatory effect,[12] anti-genotoxic,[13] hepatoprotective,[14] anti-stress,[15] role in gall stone dissolution.[5] In the Federal Regulation Code, d-limonene is generally recognized as safe (GRAS) as a flavoring agent.[5]

p-cymene is also a monoterpene and exists in about 100 plant species which have been over the time utilized for food and medicinal purposes. Its medicinal properties include mainly antinociceptive, anti-inflammatory, antioxidant, anticancer, anxiolytic and antimicrobial effects.[16] Being a flavoring agent, it is used in the manufacture of pesticides and fungicides.[17] It is used medicinally to prevent cough and exclusion of phlegm.[18] It has been reported to possess properties related to inhibition of protein glycation and thus prevention of complication of diabetes.[18] It is deemed to be "generally recognized as safe" (GRAS) by the U.S. Food and Drug Administration.[19]

Terpinolene also belongs to the class of monoterpenes. It is used extensively as a flavoring agent and naturally occurs in different herbs. Being an important component of plant extracts, its therapeutic, pharmacological, and biological properties remain under explored.[20] Scientific reports have classified terpinolene as bioactive compound possessing substantial biological activities of which antifungal,[21] insecticidal,[22] and antioxidant,[23] are more prominent.

The present study focused on the potential of naturally occurring monoterpenes viz d-limonene, *p*-cymene, terpinolene in modulating glycemic and hepatic dysfunction induced by high fat dietary manipulation and single dose of streptozotocin. Our study involved the manual administration of fatty diet into the body of rats with oral gavage rather than free access to it. Therefore, it is first of its kind in terms of mimicking pre-diabetic state and intervention and comparison with novel molecules.

MATERIALS AND METHODS

Experimental Animals and Exposure Conditions

Female Wistar rats, pathogen-free and germ-free, were obtained from the central animal house facility at IIIM Jammu. The animals were housed in clean polypropylene cages with a natural 12 hr/12 hr light-dark cycle and temperature and humidity control (25 ± 5°C). Rats were acclimatized to laboratory conditions for at least a week before the experiments. During this time, all rats had unlimited access to a normal food diet and water. Animal experiments were carried out according to the guidelines of the Committee for the Control and Supervision of Animal Experiments in India and were approved by the institutional Animal Ethics Committee of the Department of Pharmaceutical Sciences (Approval No: F(IAEC-Approval) KU/2019/01).

Drugs and Doses

Soybean oil used to mimic the metabolically impaired pre-diabetic state was obtained from Merck Darmstadt Germany and administered orally in the dose of 4 ml / kg of body weight. Streptozotocin, which is selectively toxic to pancreatic beta cells, was obtained from Merck Darmstadt in Germany and administered as a single intraperitoneal injection at a dose of 35mg/kg body weight. It was freshly prepared in cold citrate buffer (pH 4.5, 0.1 M). Glibenclamide, the standard anti-diabetic drug, was received as a free sample and administered orally at a dose of 5 mg/kg of body weight. Test drugs viz d-limonene, p-cymene and terpinolene each were obtained from Merck, Darmstadt Germany. D-limonene was administered orally at a dose of 300mg/kg body weight. Oral doses of p-cymene of 150 mg/kg body weight and 200mg/kg body weight were used. Terpinolene was also given orally in two doses of 12.5mg/kg and 25mg/kg body weight.

Experimental Design and Induction of Type II Diabetes
Establishment of Type II diabetic model

A total of 77 female Wistar rats weighing 120-150g were used in the experiment. Animals were randomly divided into seven groups, with 10 animals in each group. Only normal control received 7 animals. There were a total of 8 groups in the study. Sparing normal control, all animals in the other groups received 4ml/kg of soybean oil via oral gavage once daily in the morning for four weeks. A similar volume of distilled water was given to the control animals. After a 28-day diet of soybean oil, rats were overnight fasted and injected intraperitoneally with 35mg/kg of freshly prepared streptozotocin in 0.1 M citrate buffer pH 4.5. Control animals were given the same amount of vehicle (0.1M citrate buffer pH 4.5). To prevent death from severe hypoglycemia, all animals treated with streptozotocin received an oral glucose solution of 5% in an oral feeding bottle after two hours. Blood samples were taken from the tail vein seven days after streptozotocin administration, and blood glucose levels were measured using an ACCU CHEK glucometer. Only 58 of the 70 streptozotocin-treated animals presented with blood glucose levels greater than 240mg/dl. Diabetic rats with blood glucose levels greater than 240mg/dl were included in a subsequent therapeutic study with test compounds after two weeks. The remaining 12 animals were not included in the study.

Treatment with test compounds

Female diabetic rats were regrouped at random into seven new groups. The study included eight groups, including a preexisting normal control group. Sequence of grouping of diabetic animals was as follows:

Group I: Preexisting normal control (n = 7).

Group II: HFD + STZ-induced diabetic group (n=6+4)

Group III: Glibenclamide 5mg/kg (n=6+2)

Group IV: D-Limonene 300 mg/kg (n=6+2)

Group V: p-Cymene 150 mg/kg (n=6+2)

Group VI: p-Cymene 200 mg/kg (n=6+2)

Group VII: Terpinolene 12.5 mg/kg (n=6+2)

Group VIII: Terpinolene 25mg/kg (n=6+2).

Because the study lasted 4 weeks, each treatment group received +2 animals as attrition. And due to the increased probability of death due to progressive loss of beta cells in the HFD+STZ group, +4 animals were kept as attrition numbers. Deaths were reported in the HFD+STZ groups, as expected. Furthermore, deaths were reported in the groups treated with p-cymene. To alleviate suffering, animals which appeared ill and unfit for treatment were sacrificed using the proper protocol by cervical dislocation. All of the compounds were administered orally once daily in the morning, and the test compound treatment lasted 28 days.

Analysis of biochemical markers

Blood was collected from all groups through the retro orbital venous plexus for metabolic marker analysis. Blood glucose levels were measured using an ACCU CHEK glucometer for serum separation, samples were

centrifuged at 3000 rpm for 20 min. Serum levels of insulin, HbA_{1c}, alkaline phosphatase, alanine aminotransferase, gamma glutamyl transferase, aspartate aminotransferase, total protein and albumin were measured using the BA 400 random access analyzer (BIOSYSTEMS) and were measured using diagnostic kits from DiaSys Diagnostic India Private Limited, Navi Mumbai.

Histopathology

All the rats were sacrificed by cervical dislocation. The liver tissues were removed, washed with normal saline and preserved in 10% buffered formalin. The histopathological procedures were carried out at Pathology department, SKUAST-Kashmir. All the processes involved in the histopathological procedures from micro sectioning to staining were carried out at SKUAST-Kashmir. Paraffin embedding technique was followed for micro sectioning of isolated liver. Rotary type microtome was used for section cutting and sections were stained with hematoxylin and eosin (H&E). Examination of slides was carried by a pathologist.

Statistical analysis

Graph pad prism software was used for statistical analysis. Data from individual groups are presented as the mean ± standard error of the mean (SEM). Differences between groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test and the minimum criterion for statistical significance was set at $P< 0.05$ for all comparisons.

RESULTS

Blood Glucose Levels

Blood glucose levels were extremely significantly increased ($^{###}P<0.001$) in group II (HFD/STZ) as compared to normal control. Compared to group II rats (HFD / STZ), an extremely significant ($^{***}P<0.001$) restore in blood glucose levels was observed in groups treated with D limonene (300mg/kg), p-cymene (200mg/kg) terpinolene (12.5mg/kg and 25mg/kg) and Glibenclamide (5mg/ kg). P-cymene (150mg/kg) exhibited a nonsignificant effect (ns) on blood glucose levels Figure 1.

Glycated hemoglobin levels

Hba_{1c} % levels increased significantly increased ($^{###}P<0.001$) in group II (HFD / STZ) compared to normal control. Compared to group II rats (HFD / STZ), an extremely significant ($^{***}P<0.001$) restore in Hba_{1c} % levels was observed in the treated groups with limonene (300mg/kg), p-cymene (150mg/kg and 200mg/kg) terpinolene (12.5mg/kg and 25mg/kg) and Glibenclamide (5 mg / kg) Figure 2.

Serum Insulin levels

Serum Insulin levels were extremely significantly decreased ($^{###}P<0.001$) in group II (HFD/STZ) as compared to normal control. Compared to group II rats (HFD / STZ), an extremely significant ($^{***}P<0.001$) restore in serum insulin levels (*** P <0.001) was observed in the treated groups with limonene (300mg/kg), p-cymene (150mg/kg and 200mg/kg) terpinolene (12.5mg/kg and 25mg/kg) and Glibenclamide (5 mg / kg). Figure 3

Effects on liver function tests

Liver function tests

Effect on Alkaline Phosphatase Levels

The alkaline phosphatase levels in the HFD/STZ group were extremely significantly increased ($^{###}P<0.001$) as compared to normal control. D-limonene (300mg/kg), p-cymene (150mg/kg and 200mg/kg), terpinolene (12.5mg/kg and 25mg/kg) and glibenclamide (5mg/kg) decreased the levels extremely significantly ($^{***}P<0.001$) Figure 4.

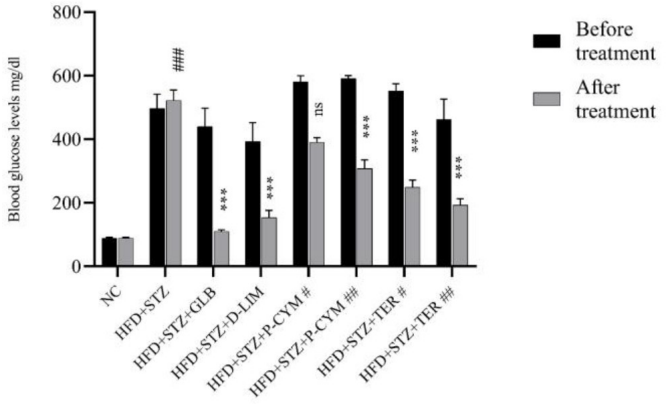

Figure 1: Effect of 4 weeks treatment with d-limonene 300mg/kg, p-cymene # 150mg/kg, p-cymene ## 200mg/kg, terpinolene # 12.5mg/kg, terpinolene ## 25mg/kg on blood glucose levels. Data are expressed as mean+SEM (n=6) $^{***}p<0.001$ is extremely significant. ### represents the negative control and all other treatment groups are compared with it. Differences between groups were analyzed by using One way ANOVA test followed by Tukey's test for multiple comparisons.

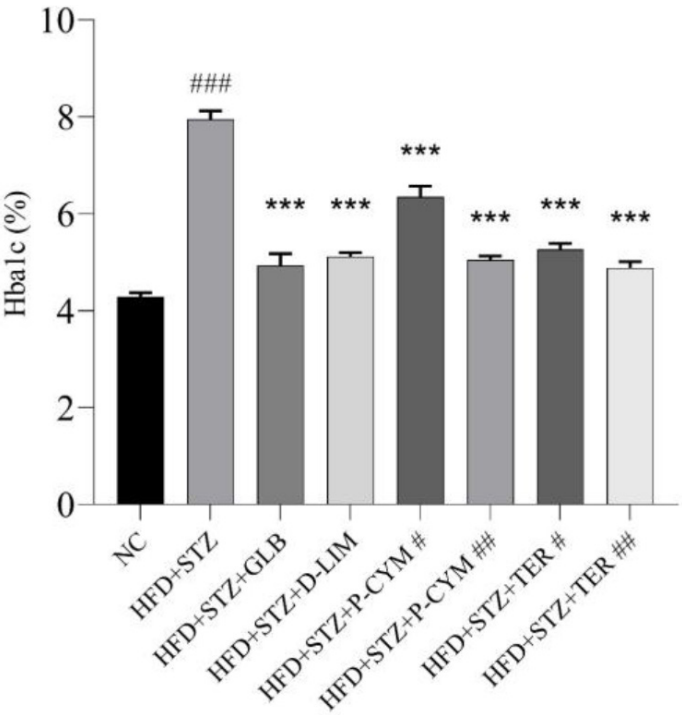

Figure 2: Effect of 4 weeks treatment with d-limonene 300mg/kg, p-cymene # 150mg/kg, p-cymene ## 200mg/kg, terpinolene # 12.5mg/kg, terpinolene ## 25mg/kg on glycated hemoglobin levels. Data are expressed as mean+SEM (n=6) where $^{***}p<0.001$ is extremely significant. ### represents the negative control and all other treatment groups are compared with it. Differences between groups were analyzed by using One way ANOVA test followed by Tukey's test for multiple comparisons.

Effect on Alanine Aminotransferase Levels

The alanine aminotransferase levels in the HFD/STZ group were extremely significantly increased ($^{###}P<0.001$) as compared to normal control. D-limonene (300mg/kg) and terpinolene (12.5mg/kg and 25mg/kg) and glibenclamide (5mg/kg) decreased the levels back to normal extremely significantly ($^{***}P<0.001$). P-cymene (150mg/kg and

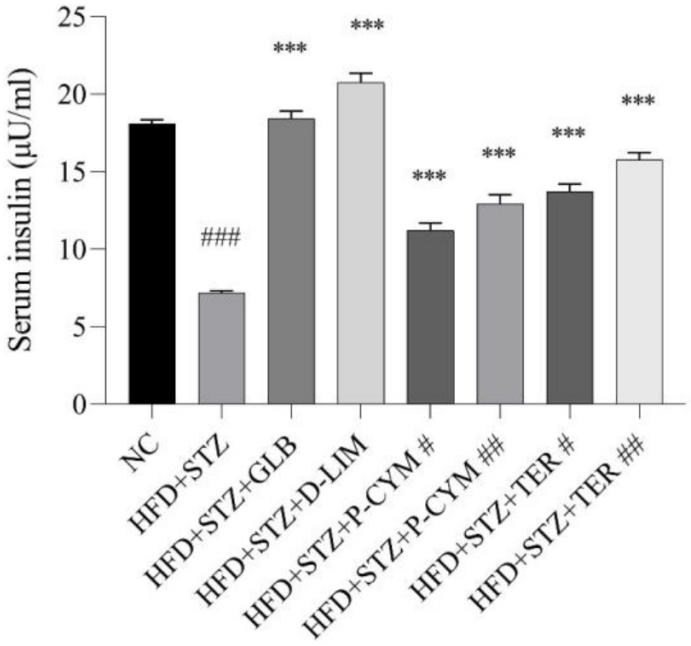

Figure 3: Effect of 4 weeks treatment with d-limonene 300mg/kg, p-cymene # 150mg/kg, p-cymene ## 200mg/kg, terpinolene # 12.5mg/kg, terpinolene ## 25mg/kg on serum insulin. Data are expressed as mean+SEM (*n*=6) where ****p*<0.001 is extremely significant. ### represents the negative control and all other treatment groups are compared with it. Differences between groups were analyzed by using One way ANOVA test followed by Tukey's test for multiple comparisons.

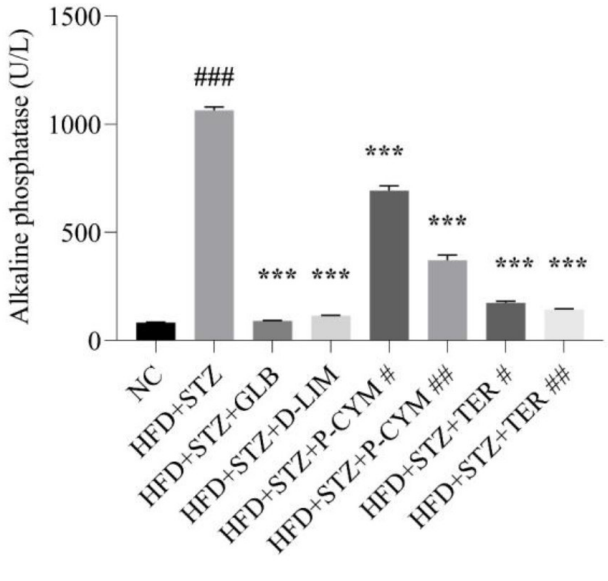

Figure 4: Effect of 4 weeks treatment with d-limonene 300mg/kg, p-cymene # 150mg/kg, p-cymene ## 200mg/kg, terpinolene # 12.5mg/kg, terpinolene ## 25mg/kg on alkaline phosphatase. Data are expressed as mean+SEM (*n*=6) where ****p*<0.001 is extremely significant. ### represents the negative control and all other treatment groups are compared with it. Differences between groups were analyzed by using One way ANOVA test followed by Tukey's test for multiple comparisons.

200mg/kg) also decreased the levels highly significantly (***P*<0.01). Figure 5

Effect on Aspartate Aminotransferase Levels

The Aspartate Aminotransferase levels in HFD/STZ group were extremely significantly increased (###*P*<0.001) as compared to normal control. D-limonene (300mg/kg), terpinolene (12.5mg/kg and 25mg/kg) and glibenclamide (5mg/kg), decreased the levels of aspartate aminotransferase in an extremely significantly (****P*<0.001). The results exhibited by p-cymene (150mg/kg and 200mg/kg) were statistically non-significant (ns) Figure 6.

Effect on Gamma Glutamyl Transferase Levels

The Gamma glutamyl transferase levels in the HFD/STZ group were increased extremely significantly (###*P*<0.001) as compared to normal control group. Terpinolene (25mg/kg) and glibenclamide (5mg/kg) decreased the levels extremely significantly (****P*<0.001). While d-limonene (300 mg/kg) and terpinolene (12.5 mg/kg) decreased the levels highly significantly (***P*<0.01), p-cymene (150mg/kg and 200 mg/kg) exhibited non-significant results (ns) Figure 7.

Effect on Total Protein Levels

The total protein levels of HFD/STZ group were decreased extremely significantly (###*P*<0.001) as compared to normal control. D-limonene (300mg/kg), terpinolene (25mg/kg) and standard drug glibenclamide (5mg/kg) restored the levels extremely significantly (****P*<0.001). Terpinolene (12.5mg/kg) increased the total protein levels highly significantly (***P*<0.01). P-cymene (200mg/kg) increased the total protein levels significantly (**P*<0.05) but p-cymene (150mg/kg) exhibited non-significant results (ns) Figure 8.

Effect on Albumin levels

The serum albumin levels in the HFD/STZ were extremely significantly decreased (###*P*<0.001) as compared to normal control. D-limonene (300mg/kg), terpinolene (25mg/kg) and glibenclamide increased levels

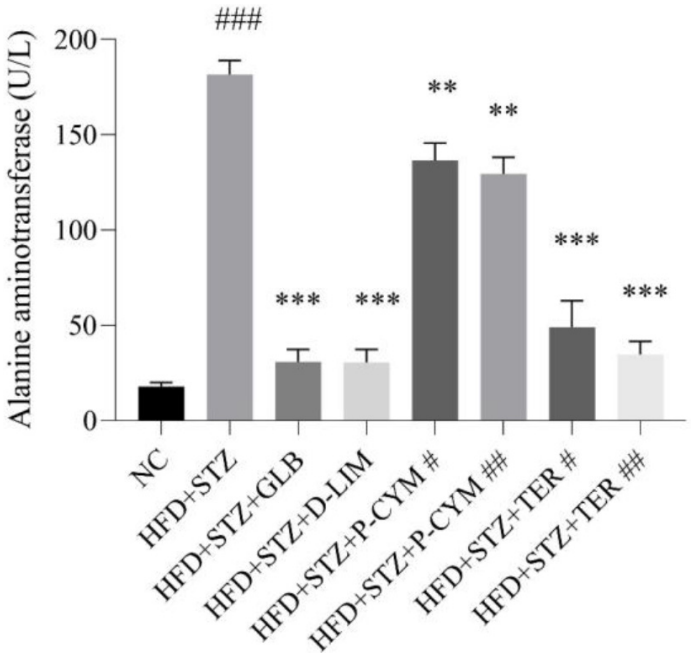

Figure 5: Effect of 4 weeks treatment with d-limonene 300mg/kg, p-cymene # 150mg/kg, p-cymene ## 200mg/kg, terpinolene # 12.5mg/kg, terpinolene ## 25mg/kg on alanine aminotransferase. Data are expressed as mean+SEM (*n*=6) where ***p*<0.01 is highly significant and ****p*<0.001 is extremely significant. ### represents the negative control and all other treatment groups are compared with it. Differences between groups were analyzed by using One way ANOVA test followed by Tukey's test for multiple comparisons.

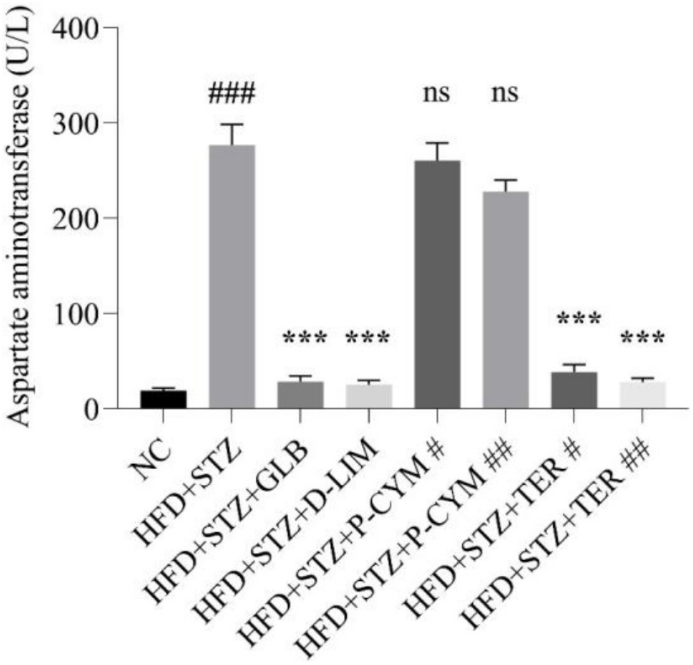

Figure 6: Effect of 4 weeks treatment with d-limonene 300mg/kg, p-cymene # 150mg/kg, p-cymene ## 200mg/kg, terpinolene # 12.5mg/kg, terpinolene ## 25mg/kg on aspartate aminotransferase. Data are expressed as mean+SEM (n=6) where ***p<0.001 is extremely significant and ns is non-significant. ### represents the negative control and all other treatment groups are compared with it. Differences between groups were analyzed by using One way ANOVA test followed by Tukey's test for multiple comparisons.

extremely significantly (***P<0.001). Terpinolene (12.5mg/kg) increased the serum albumin levels highly significantly (**P<0.01). P-cymene (150mg/kg and 200mg/kg exhibited non-significant (ns) restore in serum albumin levels Figure 9.

Histopathology

Histopathological analysis of liver of high fat diet and streptozotocin treated rats revealed degeneration of parenchyma, binucleated cells, clear cell foci and prominent nucleoli in comparison to rats of normal group. D-limonene, p-cymene and terpinolene to a good extent were successful in curbing the progression of pathologies those observed in high fat and streptozotocin treated rats Figure 10.

DISCUSSION

The leading cause of morbidity and mortality in human populations, type 2 diabetes mellitus is the most prevalent metabolic disorder in the world. Under diabetic conditions, consuming too much dietary fat blunts the effects of insulin on glucose uptake and encourages insulin resistance.[24] Additionally, rats receiving HFD and a low dose of STZ (40 mg/kg b.w.) present with mild necrosis, which results in insulin deficiency.[25] The combination of HFD-fed and low-dose STZ-treated rats serves as an alternative animal model simulating the natural disease progression and metabolic characteristics of type 2 diabetes, according to studies by Srinivasan *et al*.[26] This model is also best suited for assessing the therapeutic potential of substances with effects that sensitize and secrete insulin. Chronic hyperglycemia alters metabolism, which triggers the onset of diabetic complications.

Soybean finds its extensive use in salad dressings, margarines, processed foods and is first line choice in majority of fast-food establishments and eateries.[27] Its involvement in obesity has received very little attention

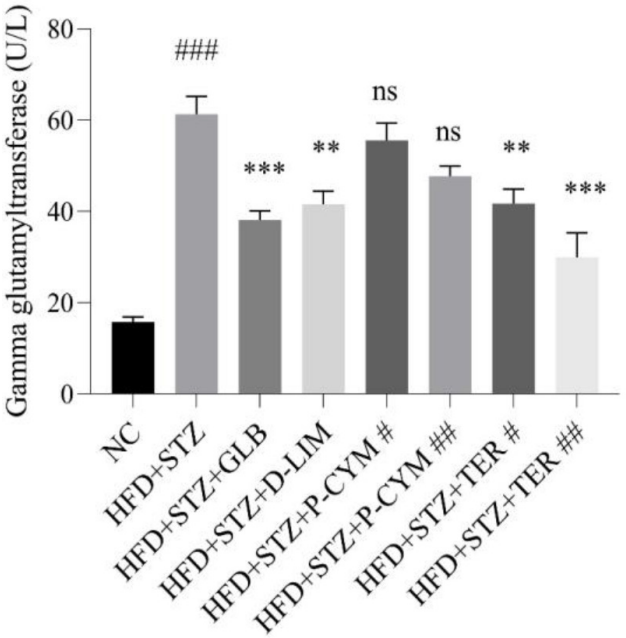

Figure 7: Effect of 4 weeks treatment with d-limonene 300mg/kg, p-cymene # 150mg/kg, p-cymene ## 200mg/kg, terpinolene # 12.5mg/kg, terpinolene ## 25mg/kg on gamma glutamyltransferase. Data are expressed as mean+SEM (n=6) where **p<0.01 is highly significant and ***p<0.001 is extremely significant and ns is non-significant. ### represents the negative control and all other treatment groups are compared with it. Differences between groups were analyzed by using One way ANOVA test followed by Tukey's test for multiple comparisons.

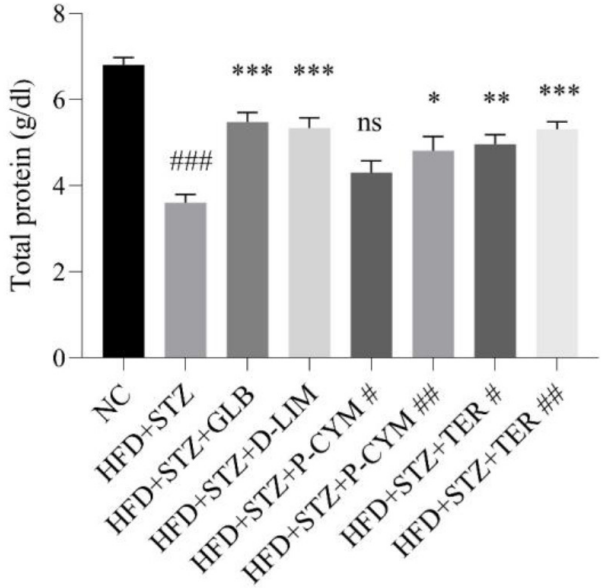

Figure 8: Effect of 4 weeks treatment with d-limonene 300mg/kg, p-cymene # 150mg/kg, p-cymene ## 200mg/kg, terpinolene # 12.5mg/kg, terpinolene ## 25mg/kg total protein. Data are expressed as mean+SEM (n=6) where *p<0.5 is significant, **p<0.01 is highly significant, ***p<0.001 is extremely significant and ns is non-significant. ### represents the negative control and all other treatment groups are compared with it. Differences between groups were analyzed by using One way ANOVA test followed by Tukey's test for multiple comparisons.

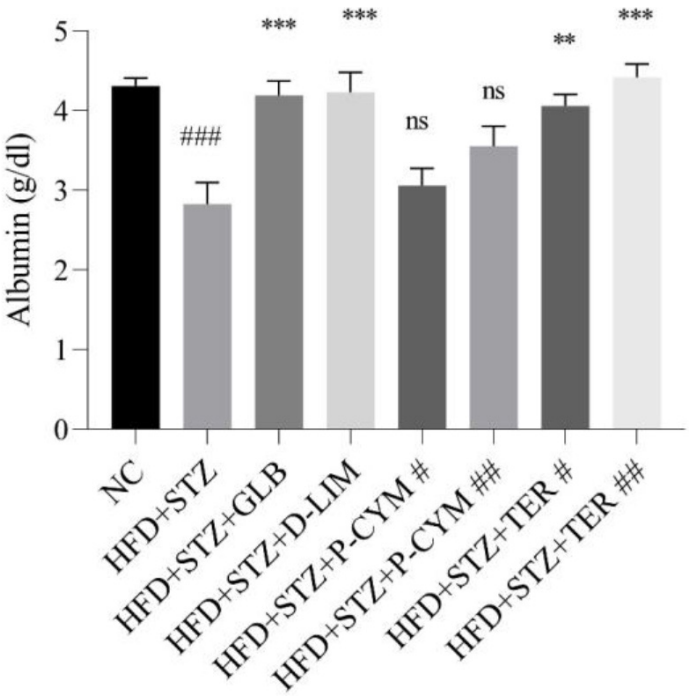

Figure 9: Effect of 4 weeks treatment with d-limonene 300mg/kg, p-cymene # 150mg/kg, p-cymene ## 200mg/kg, terpinolene # 12.5mg/kg, terpinolene ## 25mg/kg on albumin. Data are expressed as mean+SEM (n=6) where **p<0.01 is highly significant and ***p<0.001 is extremely significant and ns is non-significant. ### represents the negative control and all other treatment groups are compared with it. Differences between groups were analyzed by using One way ANOVA test followed by Tukey's test for multiple comparisons.

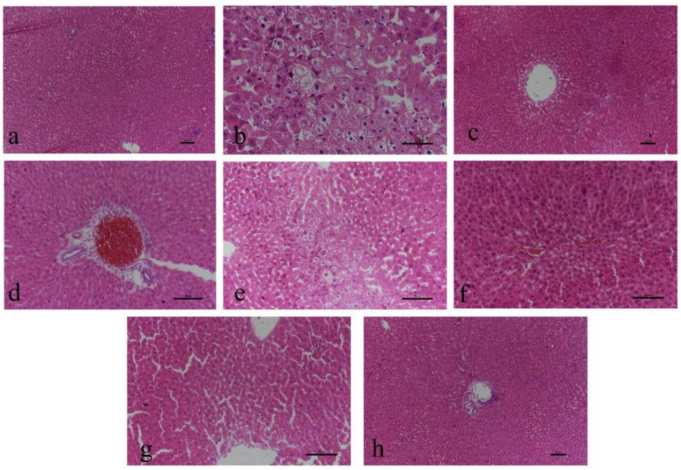

Figure 10: a) Normal control: Normal architecture b) High fat diet and streptozotocin treated: Binucleated cells, degeneration of parenchyma, clear cell foci, granular cytoplasm, prominent nucleoli, darkly stained nucleolus c) Glibenclamide treated: Intact hepatic architecture d) D-Limonene treated: mild dilatation and congestion of central vein e) p-cymene 150mg/kg: Mild necrosis of hepatocytes f) p-cymene 200mg/kg: Mild sinusoidal congestion g) terpinolene 12.5mg/kg: fatty infiltration h) terpinolene 25mg/kg: mild infiltration around central vein.

compared to other dietary components, especially saturated fatty acids. Although most research studies use lard, which is high in saturated fats, soybean oil is primarily high in polyunsaturated fatty acids. Results contradictory to expectation, point to the finding that soybean oil rich in polyunsaturated fatty acids is more obesogenic and diabetogenic as compared to coconut oil comprising chiefly of saturated fat.[28] Research studies have revealed that wistar rats treated with soybean oil exhibited insulin resistance and impaired secretion of insulin from islets.[29] Our study involved manual administration of soybean oil orally so as effective pre-conditioning for metabolic syndrome is attained.

Hemoglobin and the crystalline protein in the lens are two examples of the proteins that become glycated non-enzymatically as a result of persistent hyperglycemia. As a reason of persistent hyperglycemia, a non-enzymatic glycation of proteins including hemoglobin and crystalline protein of the lens is resulted. Glycosylation of hemoglobin occurs more quickly in people with uncontrolled diabetes than in healthy people, and the increase is directly proportional to the fasting blood glucose levels.[30] Comparison of blood glucose levels before and after treatment with d-limonene (300mg/kg), p-cymene (200mg/kg) and terpinolene (12.5mg/kg and 25mg/kg) revealed an extremely significant decline in blood glucose levels (***p<0.001) except for p-cymene (200mg/kg) exhibiting non-significant decline (ns). However the HbA_{1c} levels were decreased by all test compounds extremely significantly (***p<0.0001).

Studies have revealed that agents having the capability to scavenge free radicals or are potent antioxidants considerably inhibit protein glycation reactions linked to diabetes mellitus. According to reports, d-limonene reduces the oxidative stress in STZ-induced diabetic rats by decreasing lipid peroxidation and sparing the activities of antioxidant enzymes.[31] In diabetic rats, p-cymene has exhibited its antioxidant activity by restoring the levels of MDA otherwise altered in streptozotocin treated wistar rats.[32] Early *in vitro* studies have shown that terpinolene is a safe and good natural antioxidant and anti-cancer agent.[33] The possible mechanism behind the antihyperglycemic activity of d-limonene, p-cymene and terpinolene may be therefore attributed to their antioxidant properties.

It has been discovered that an excess of free fatty acids during diabetes is directly toxic to hepatocytes.[34] As a result, enzymes such as ALP, AST, and ALT may dissipate from hepatocytes into the circulation, where their levels rise.[35] In the present study the levels of these enzymes were decreased upon treatment with d-limonene, p-cymene, terpinolene like glibenclamide. Studies in the past have confirmed the hepatoprotective activity of d-limonene in rats with metabolic syndrome linked to nonalcoholic fatty liver disease. 2% oral administration of d-limonene for 4 weeks has exhibited hepatomodulatory activity in these animals.[36] A marked decline in the levels of ALP, AST and ALT has also been reported in streptozotocin induced diabetic rats treated with metformin and p-cymene.[37] Almost similar kinds of results have been attained by other research groups.[38-39]

According to the scientific studies, the very first phenomenon to occur in different stress models are the oxidative stress and inflammation. These situations are very comparable to the existence of stress in humans.[40-41] In humans oxidative stress may advance to liver damage, metabolic syndrome, neurodegenerative diseases etc.[42-46] Therefore compounds having the ability to obstruct oxidative stress can prevent onset of diseases related to stress. Because of the fact that d-limonene, p-cymene and terpinolene possess anti-oxidant activities,[31-33] they can prove as potential candidates to accomplish this goal.

CONCLUSION

D-limonene, p-cymene, terpinolene are monoterpenes present in dietary sources and possess anti-diabetic and hepatoprotective activities. In the high fat and streptozotocin rat models, they have the capability to modulate glycemic and liver disease markers. Their anti-diabetic and hepatoprotective activity may be due to their ability to scavenge free radicals. Based on these findings, they can be employed as natural

alternatives to synthetic drugs or can be consumed in regular dietary routine.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

HbA_{1c}: Glycated haemoglobin; **ALP:** Alkaline Phosphatase; **ALT:** Alanine aminotransferase; **AST:** Aspartate aminotransferase; **GGT:** Gamma glutamyltransferase; **HFD:** High fat diet; **STZ:** Streptozotocin; **GRAS:** Generally recognized as safe; **H&E:** Hematoxylin and eosin.

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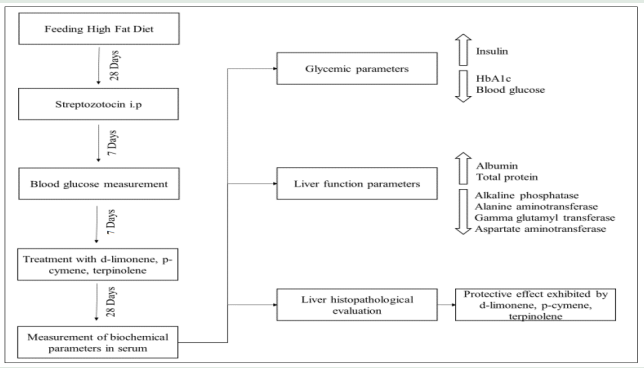
GRAPHICAL ABSTRACT



SUMMARY

D-limonene, p-cymene and terpinolene restored the altered biochemical parameters associated with diabetic affliction and the same was supported by histopathology. This proves their antidiabetic and hepatoprotective potential.

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