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Antidiabetic Activity of *Thalassia Hemprichii* (Seagrass) In Alloxan Induced Diabetic Mice

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Abstract
Objectives: *To study the antidiabetic activity of Thalassiahemprichiiin normal and alloxan-induced diabetic mice.*
Materials and Methods: *Ethanolic extract of fruiting bodies of Thalassiahemprichiiwas tested for their antidiabetic activity. BALB/C mice (25-30gm) were divided into four groups of six animals each normal control mice, diabetic control mice, diabetic mice post-treated with standard drug glibenclamide and diabetic mice treated with Thalassiahemprichiiethanolic extract. Blood glucose level, biochemical parameters such as serum total cholesterol (TC), LDL, HDLVLDL,triglyceride creatinine, urea, and Serum glutamate oxaloacetate transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT) were studied in alloxan induced diabetic mice after 15 days of treatment.*
Results: *Animals treated with the ethanolic extract of Thalassiahemprichii showed a significant decrease in serum glucose level ($P<0.01$) .The post-treatment with Thalassiahemprichiiextract reduced serum cholesterol, triglyceride and LDL-cholesterol.The serum HDL cholesterol was significantly increased in post-treated groups. The serum creatinine, urea levels were significantly reduced in post-treated group, whereas the decrease in the body weight was arrested by administration of Thalassiahemprichiiextract to the animals.*
Discussion and Conclusion: *The consumption of Thalassiahemprichiiproduced a significant hypoglycemic effect in diabetic mice and it is capable of improving hyperlipidemia and the impaired kidney functions in alloxan-induced diabetic mice. Thus, indicating that the ethanolic extract ofThalassiahemprichii could be added in the list of medicinal preparations beneficial in diabetes mellitus.*
Keywords*: Thalassiahemprichii,Glibenclamide, Hyperlipidemia, Hypoglycemic, Alloxan*

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I. Introduction

Diabetes mellitus is a common disorder associated with markedly increased morbidity and mortality rate and characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action or both resulting in impaired function in carbohydrate, lipid and protein metabolism(1).In persons with type 1 diabetes, the beta cells of the pancreas, which are responsible for insulin production, are attacked by the misdirected immune system(2).Hyperglycemia, as a common end point for all types of diabetes mellitus, is followed by micro and macrovascular complications leading to cardiovascular disease (CVD), neuropathy, retinopathy and nephropathy. Vascular complications are the most common reasons of morbidity and mortality in diabetic patients(3)Endothelium acts as an inhibitory regulator of vascular contraction, leukocyte adhesion, vascular smooth muscle cell growth and platelet aggregation, through the production of a number of biologically active molecule(4).Beside hyperglycemia, several other factors including dislipidemia or hyperlipidemia are involved in the development of micro and macrovascular complication of diabetes, which are the causes of morbidity and death(5). As the number of people with diabetes multiply worldwide, the disease takes an ever-increasing proportion of national and international health care budgets. It is projected to become one of the world's main disablers and killers within the next 25 years(6)Present number of diabetics worldwide is 150 million and this is likely to increase to 300 million or more by the year 2025 (7) Reasons for this rise include increase in sedentary lifestyle, consumption of energy rich diet,obesity, higher life span, etc (8).In India, indigenous remedies have been used in the treatment of DM since the time of Charaka and Sushruta (6th century BC)(9).Diabeties mellitus is the most important non infective epidemic to hit the globe in the present

millennium. By the year 2025, India shall have the maximum number of diabetics in the world making it the Diabetic capital of the world(10)In certain African countries for instance, up to 90% of the population still relies exclusively on plants as a source of medicines (11).As a consequence, the World Health Organization (WHO) had in one of its charters in Geneva recommended further investigation into this area, particularly as it concerns chronic and debilitating diseases such as diabetes mellitus. Plant drugs are frequently considered to be less toxic and more free from side effects than synthetic ones (12).Typical diabetes mellitus will develop about 24 to 48 hours after the injection of a diabetogenic dose of alloxan when it is administered parenterally, intravenously, intraperitoneally or subcutaneously. Alloxan-treated animals were considered as excellent tools to study the pathogenesis of human diabetes, although in alloxan diabetes, in contrast to type I diabetes in humans, there is no autoimmune component and no insulin resistance as in type II diabetes (13).An insulin-like hypoglycemic protein known as polypeptide-p or p-insulin was isolated from thefruits, seeds and leaves of bitter melon and shown to lower blood glucose levels in gerbils, langurs and humans when injected subcutaneously. Plant phenolics are a large and diverse group of phenolic compounds present in all plants which include cathechins, isoflavones, anthocyanins, phenolic acids, etc.(14)Seagrasses are sometimes found in patches, but these patches can expand to form huge seagrass beds, or meadows. The beds can be made up of one species of seagrass, or multiple species. Seagrasses require lots of light, so the depths at which they occur in the ocean are limited by light availability. Seagrasses are vascular plants and reproduce by flowering and producing seeds. Seagrasses attach to the ocean bottom by thick roots and rhizomes, horizontal stems with shoots pointing upward and roots pointing downward. Their roots help stabilize the ocean bottom. Seagrass meadows are conspicuous and wide spread in the shallow marine environs throughout the world, producing a greater amount of organic matter and serving as a good substratum for a variety of epiphytic algae including diatoms (15) and sessile fauna. As mangrove and coral reef ecosystems are closely associated with the seagrass ecosystem, there is a lot of export of organic matter and nutrients form the latter. Seagrass meadows are highly productive and dynamic ecosystems, which rank among the most productive ecosystems of the oceans (16). It is estimated that seagrasses were abundant in the Asia-pacific region 45 million years ago. Today, India is home to more than fifteen species of seagrasses found in different coastal areas of Eastern, Southern and Western parts of India. Most of the species are found in healthy numbers along the Southeast coast (which is the Gulf of Mannar and the Palk Bay), coast of Tamil Nadu, and the sporadic islands of Lakshadweep and Andaman and Nicobar. *Thalassiahemprichii*is a tropical seagrass species that ranges from the shallow subtidal to below 10 meters in depth (17) belonging to the family Hydrocharitaceae. The common name for *Thalassiahemprichii*is turtle grass (Figure 1). This seagrass has separate male and female plants. The flowers form at the base of the shoot and is hidden by the sheath until they emerge. The male flower is held on a long stalk, maturing into 6 or more parts. The female flower appears similar but has a finer texture. Fruits are oval and prickly, containing up to 9 tiny seeds. The species is common and dominating in many seagrass associations in the western and eastern Indian Ocean (18) as well the western Pacific Ocean(19).

II. Materials AndMethods

COLLECTION AND IDENTIFICATION OF THALASSIA HEMPRICHII

*Thalassiahemprichii*was collected from the Northern side of Tuticorin port area. It was confirmed with help of identification manual prepared by seagrass watch, www.seagrasswatch.org/id_seagrass.html,which is an organization exclusively dedicated to seagrass ecosystem. This sample was also parallely confirmed by Dr.V. Deepak Samuel, Program Specialist, Energy and Environment Unit, United Nation Development Program, Gulf of Mannar, Marine Biosphere Reserve Trust. This seagrass is not included in Wild Life Protection Act (1972). It produces large biomass in the Gulf of Mannar. Tonnes of seagrasses are washed ashore every year.

PREPARATION OF EXTRACT

Dried leaves of samples were made into powder. 25 g of this powder was extracted with 250 ml of ethanol at 70°C for 10 hours using soxhlet apparatus. The residual solvent was removed by evaporation at 50°C for 20 min using a rotary vaccumevaporator. The resulting organic extracts were further evaporated by keeping it in water bath at 45° c and finally the crude extract was obtained. Followed by storage in sterile capped bottles under refrigeration condition (4°C) prior to use for subsequent assays (21).

IDENTIFICATION AND PURIFICATION OF BIOACTIVE COMPOUNDS

Phytochemical Analysis of *Thalassiahemprichii*Extract by High Performance Thin Layer Chromatography (HPTLC)The phytoconstituents present in the organic extracts were determined qualitatively by HPTLC. In HPTLC, the extracts spotted on silica coated plates, were developed using butanol-glacial acetic-water (100: 10:10) as the solvent system. The HPTLC results were further used to validate the presence of alkaloids based on positive reaction (brown coloration) with Dra-gendorff's reagent, steroids based on positive reactions (violet to blue or green) with acetic anhydride and H_2SO_4 and flavanoids with 1% Ethanolic Aluminium chloride reagent followed by terpenoids using Anisaldehyde sulphuric acid reagent (Keller-Killani test) .

GC-MS Analysis of Bioactive Components in Ethanolic Extract of *Thalassiahemprichii*

Gas chromatography-mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. GC-MS can provide meaningful information for components that are volatile, non-ionic and thermally stable and have relatively low molecular weight. Dried extract of *Thalassiahemprichii*were dissolved in 95% v/v methanol and analyzed using GC Clarus 500, PerkinElmer, USA and equipped with Turbo mass gold-pekin Elmer Detector and split injection system. 2µl of sample was injected for analysis. The sample injector temperature was maintained at 250°C throughout the experiment period. The mass spectroscopic analysis was done with 70eV electron energy level, between 45 m/z and 450 m/z for the duration of 45 min. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained (10).

Experimental Animal

Inbred BALB/C (6-8 weeks) mice, weighing 25-30g, was housed under standard condition of temperature, 12 hours light/dark and fed with standard pellet diet and water ad libitum. Animals were acclimatized to laboratory condition at least 24 hours before conducting the experiments. All animal experiments were conducted according to the rules and regulations of Animal Ethics Committee, Government of India.

Acute Toxicity Studies

Acute toxicity study was carried out according to OECD guideline (423).Ethanolic extract of *Thalassiahemprichii*at a dose range of 50 mg–500 mg/kg were administered intraperitoneally to different group of animals (six mice in each group). The animals were observed continuously for 2h,for any symptoms of toxicity (behavior pattern,tremors,sleep,coma) and or death.They were under observation for a period of further 2 weeks (11,12).

Experimental Design:

Four groups of mice, six in each received the following treatment schedule.

Group I: Normal control (saline).
Group II: Alloxan treated control (150 mg/kg.ip)
Group III: Alloxan (150 mg/kg.ip) + Standard drug, Glibenclamide (10mg/kg.ip)
Group IV: Alloxan (150 mg/kg.ip) + *Thalassiahemprichii*(250 mg/kg.ip)

Induction of Diabetes in Experimental Animals:

Freshly prepared solutions of alloxan monohydrate (Sigma Aldrich Chemicals,Pvt.,Ltd.,Bangalore) dissolved in sterile normal saline at a dose of 150mg/kg body weight were injected into the overnight fasted mice. After 72 hours of alloxan injection,the mice with serum glucose levels of >250 mg/dl were included in the study. Treatment with *Thalassiahemprichii*extract was started 72 h after alloxan injection.

Collection of Blood Samples:

Blood samples were drawn from tail tip of mice at weekly intervals till the end of study (i.e., 15 days). Blood glucose estimation was done on day 1^{st}, 7^{th}, and 15^{th} of the study and body weight measurements were done on 1 and 15^{th} of the study. Blood glucose estimation can be done by o-toludine method. On day 15, blood was collected from heart under mild ether anesthesia from overnight fasted mice and serum was isolated (14).Serum was analyzed for total cholesterol (15),triglyceride (17), HDL (16),LDL (15),creatinine (18), urea (19), and SGOT and SGPT (20) were estimated. The whole liver from each animal was removed after sacrificing the animal and was kept in 10% formaline solution, and immediately processed by the paraffin technique. Sections of 5μ thickness were cut and stained by haematoxylin and eosin (H & E) for histopathological examination (21).

Statistical Analysis

All the grouped data were statistically evaluated via the statistical package (SPSS statistical software). Data were analyzed statistically by one-way ANOVA. Values are expressed as mean ± SEM (Standard Error of Mean) for six animals in each group. Unpaired student t-test is used for statistical comparison between the four different groups. Changes are considered to be statistically significant if the P-value was less than 0.05.The analysis was carried out using GraphPad Prism, Version 4.01. (GraphPad Software, San Diego, California, USA). $*p < 0.05$, $**p < 0.01$ was considered statistically significant.

III. Results

Diabetes mellitus is probably the single most important metabolic disease and is widely recognized as one of the leading causes of death and disability. It affects every cell in the body and the body's essential biochemical processes, and it is a major public health problem in developing countries(31) Decreased physical activity,increasing obesity, stress and changes in food consumption have been implicated in this increasing prevalence in the past two decades. Diabetes mellitus of long duration is associated with several complications such as atherosclerosis, myocardial infarction, nephropathy etc. These complications have been assumed to be related to chronically elevated glucose level in blood (32).

Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes, there is an increased demand by patients to use natural products with antidiabetic activity.The currently available drug regimens for management of diabetes mellitus have certain drawbacks and therefore, there is a need to find safer and more effective antidiabetic drugs (33).

Phytochemical Analysis of *Thalassiahemprichii*Extract by HPTLC

The phytochemical screening of crude extracts of *Thalassiahemprichii* were carried out to determine the presence of active secondary metabolites (Fig.1). The *Thalassiahemprichii*extracts were screened for the presence of alkaloid, steroid, flavonoidsand terpenoids according to established procedures (Table.1).

GC-MS analysis of bioactive components in ethanolic extract of *Thalassiahemprichii*

Gas chromatography/mass spectrometry (GC-MS) is the synergistic combination of two powerful analytic techniques. The gas chromatography separates the components of a mixture in time. The mass spectrometer provides information that aids in the structural identification of each component (Fig 2). Analysis by GC-MS is essential for identification of natural fatty acids, sterols and alkanes isolated from biological samples. The *Thalassiahemprichii*were found to contain numerous middle-chain aliphatic alcohols, aldehydes and ketones, which are believed to be the degradation products of fatty acids (Table 2).

The effect of *Thalassiahemprichii*on the body weight, fasting blood glucose level,serum total cholesterol, HDL-cholesterol, LDL-cholesterol, serum triglycerides, serum urea, serum creatinine, SGOT and SGPT levels were investigated in the normal healthy control, alloxan –induced diabetic control,negative control and treated mice with *Thalassiahemprichii*ethanolic extract.

Effect of*Thalassiahemprichii*Extract on Body Weight and Organ Weight of Alloxan Induced Diabetic Mice

Weight loss which is one of the clinical features of diabetes mellitus may be due to the degeneration of the adipocytes and muscle tissues to make up for the energy lost from the body due to frequent urination and over conversion of glycogen to glucose (Table 3).Organ indices for liver and kidney were measured as tissue weight per 10 gm body weight (Table 3).Hyperglycemia is the main characteristics of diabetes mellitus. Increase in the level of blood glucose in the diabetic control mice is statistically significant when compared to the normal healthy control animals (Table 3).The effect of diabetes mellitus on lipid metabolism is well established. The association of hyperglycemia with an alteration of lipid parameters presents a major risk for cardiovascular complications in diabetes. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic mice.In this study,a marked increase in total cholesterol,LDL-cholesterol,VLDL-cholesterol and triglycerides were observed in diabetic control mice,where there was a decline in HDL-cholesterol level.The administration of *Thalassiahemprichii*extract significantly reduced total cholesterol,LDL-Cholesterol,VLDL-cholesterol,Serum triglycerides and significantly increased HDL-cholesterol level.

The level of serum cholesterol was lower in normal healthy control mice and the elevation was found in diabetic control (Table 3).The present results showed that the level of serum total cholesterol was significantly elevated in the diabetic control group as compared to the normal healthy control .After supplementation with *Thalassiahemprichii*extract,the alteration in lipid meatabolism was partially attenuated as evidenced by decreased serum total cholesterol level(Table 3).Low HDL-cholesterol concentration is one of the distinctive features observed in diabetic dyslipidemia. The level of HDL-cholesterol was significantly reduced in untreated diabetic mice and these lowered levels of HDL cholesterol were enhanced significantly in*Thalassiahemprichii*extract treated animals (Table 3).

It is well known that LDL and VLDL plays an important role in artherosclerosis and hypercholesterolemia which are associated with a defect relating to the lack of LDL receptors The level of Glycemic control is the major determinant of serum level of triglycerides. As shown in the Table 3, triglyceride levels were increased significantly in the diabetic group of animals.Administration of *Thalassiahemprichii* extract to diabetic mice brought down the level of triglycerides and thus reduced remarkably in the treated group(Table 3).

Effect of Effect of *Thalassiahemprichii*extract on SGPT and SGOT of alloxan induced diabetic mice

The effect of *Thalassiahemprichii*extract on serum GPT of alloxan induced mice is presented in Table 4. In diabetic mice,the level of SGPT was elevated significantly and the enzyme level was resulted to normal level after the intraperitoneal administration of *Thalassiahemprichii*extract.

The level of SGOT was raised significantly in diabetic control mice. Following the intraperitoneal administration of *Thalassiahemprichii*extract, SGOT level was reduced significantly as shown in Table 4.

Effect of *Pleurotusostreatus*extract on serum Urea and Creatinine level of alloxan induced diabetic mice

The effect of *Thalassiahemprichii* extract on serum urea of alloxan induced mice is presented in Table 4. The increased levels of serum urea in diabetic mice was significantly ($p<0.01$) restored to near normal levels in the *Thalassiahemprichii*extract treated mice. An increase in the level of serum urea in diabetic mice were found to be slightly decreased in the treated mice.Table 4 depicts serum creatinine levels in the healthy control,diabeticcontrol,positive control and treated groups. In the diabetic mice, the increased serum creatinine was noticed and it was found to be reduced significantly in the treated mice.

Microscopic examination of the liver of alloxan induced diabetic mice revealed remarkable changes versus the control mice.These changes included periportal fatty infilteration with focal necrosis of hepatocytes (Figure 4).The normal healthy control liver tissue sections showed liver parenchyma with hepatocytes which appear normal (Figure 3).Diabetic mice treated with *Thalassiahemprichii*extract(Figure 5) revealed a remarkable improvement of hepatic tissues where reduced necrosis was observed and the cellular arrangement of hepatocytes were found to be normal.This was represented by intact blood sinusoids and hepatocytes.

IV. Discussion

Decreased physical activity, increasing obesity, stress and changes in food consumption have been implicated in this increasing prevalence in the past two decades. Diabetes mellitus of long duration is associated with several complications such as atherosclerosis, myocardial infarction, nephropathy etc. These complications have been assumed to be related to chronically elevated glucose level in blood (32)

Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes, there is an increased demand by patients to use natural products with antidiabetic activity (39) The currently available drug regimens for management of diabetes mellitus have certain drawbacks and therefore, there is a need to find safer .Weight loss which is one of the clinical features of diabetes mellitus may be due to the degeneration of the adipocytes and muscle tissues to make up for the energy lost from the body due to frequent urination and over conversion of glycogen to glucose.

Normal control animals were found to be stable in their body weight (26.4 ± 2.28, $p<0.01$) but the alloxan induced diabetic mice showed significant reduction (23.37 ± 0.956, $p<0.01$) in their body weight during the period of the study (Table 5). Alloxan mediated body weight reduction was significantly reversed by the administration of *Thalassiahemprichii* extract. Intraperitoneal administration of the extract resulted in a notable increase in body weight (33.77 ± 1.404, $p<0.01$).

All animal in diabetic control group revealed a significant loss (23.37 ± 0.956, $p<0.01$) in body weight which was observed till the end of the study period. Daily treatment of *Thalassiahemprichii* extract for a period of 15 days treatment led to a dose dependent fall in their percentage mean body weights (33.77 ± 1.404, $p<0.01$). Mice treated with the extract showed progressive increase in their mean body weight.The decrease in body weight could be due to excess breakdown of tissue proteins or may be due to the dearrangement of metabolic pathways (35).Mice treated with the extract showed progressive increase in their percentage mean body weights. This could be explained by protein sparing action ie. gluconeogenesis from the muscle protein would result in decrease in total protein (27).

Organ indices for liver and kidney were measured as tissue weight per 10g body weight. The mean body liver and kidney indices were significantly different ($p<0.01$) in the diabetic control compared to the normal healthy control (Table 3). When the diabetic control group was compared to the normal control group, liver indices were significantly lower and kidney indices were higher. *Thalassiahemprichii*extract treated mice showed a trend towards greater liver weight gain. Table 6 revealed that there are no significant differences in kidney index seen between the diabetic control and the treated group because the kidney index showed a decreasing trend as the treatment proceeds. This indicated that the extract treatment increased the liver index and decreased the kidney index of mice with alloxan-induced diabetes.

Hyperglycemiais the main characteristic of diabetes mellitus.(36) reported that animals which received a single alloxan injection developed type I diabetes demonstrated by high blood glucose level. Increase in the level of blood glucose in the diabetic control mice is statistically significant when compared to the normal healthy control animals. The reduction in blood glucose level is due to normal metabolism of glucose and feedback mechanism(37).Theincrease may be also due to the result of the destruction of the beta cells of pancreas by alloxan which was brought to normal in the diabetic mice treated with *Thalassiahemprichii*extract.

The effect of diabetes mellitus on lipid metabolism is well established. The association of hyperglycemia with an alteration of lipid parameters presents a major risk for cardiovascular complication in diabetes. In this study, a marked increase in total cholesterol, LDL-cholestrol, VLDL-cholestrol and triglyceride were obsereved in diabetic control mice, whereas there was a decline in HDL-cholestrol. The administration of *Thalassiahemprichii* extract significantly reduced total cholesterol, LDL-cholestrol, VLDL-cholestrol, serum triglycerides and significantly increased HDL-cholestrol level.

Diabetes mellitus is usually complicated with hyperlipoproteinemia. The level of serum cholesterol was lower in normal healthy control mice and the elevation was found in diabetic control (Table 8). The present results showed that the level of serum total cholesterol (153±40.33mg/dl, $p<0.01$) was significantly elevated in the diabetic control group as compared to the normal healthy control (34.6 ±12.8mg/dl, $p<0.01$). After supplementation with *Thalassiahemprichii* extract, the alteration in lipid metabolism was partially attenuated as evidenced by decreased serum total cholesterol level (109±0.7mg/dl, $p<0.01$). Benkhayal revealed that diabetic rats showed a decline in the total cholesterol level.

The level of HDL-cholesterol was significantly reduced in untreated diabetic mice (37.4±5.2mg/dl) and these lowered levels of HDL-cholestrol were enhanced significantly to (65.2±1.92mg/dl, $p<0.01$) in *Thalassiahemprichii*extract treated animal (Table 4).

LDL plays an important role in artherosclerosis and hypercholestrolemia which are associated with a defect relating to the lack of LDL receptors. Compared to normal control group, the levels of LDL cholesterol (98.19±38.4 mg/dl) and VLDL cholesterol (30.46±0.19mg/dl) levels were significantly ($p<0.01$) elevated in the diabetic control group (Table 10 and 11). *Thalassiahemprichii*extract in the treated group i.e both LDL and VLDL cholesterol levels were decreased to near normal level 33.79±0.82mg/dl and 26.37±0.48mg/dl.

Administration of *Thalassiahemprichii*extract to diabetic mice brought down the level of triglycerides and thus reduced remarkably (114±0.44mg/dl) in the treated group. The diabetes induced hyperlipidemia might be due to excess mobilization of fat from the adipose tissue because of underutilization of glucose. *Thalassiahemprichii*extract normalized all the lipid profile parameters and thus this extract could attribute to antihyperlipidemic activity.Deficiency of lipoprotein lipase activity may contribute significantly to the elevation of triglycerides in diabetes (22).The effect of *Thalassiahemprichii*extract on serum GPT of alloxan induced mice is presented in (Table 4). In diabetic mice,the level of SGPT was elevated significantly from 0.78±0.001IU/L to 0.98±0.001IU/L and the enzyme level was reached to normal level 0.80±0.002IU/L after the intraperitoneal administration of *Thalassiahemprichii*extract.

The level of SGOT was raised significantly from 0.072±0.001 IU/L to 0.083±0.001 IU/L in diabetic control mice. Following the intraperitoneal administration of *Thalassiahemprichii*extract, SGOT 0.040±0.004IU/L level was reduced significantly as shown in (Table 14).

Experimentally it was found that fruits of *Cocciniagrandis* exhibited hepatoprotective activity by lowering the levels of SGPT and SGOT and this may be due to antioxidant property exerted by flavanoids present in the fruit extract (20)The effect of *Thalassiahemprichii*extract on serum urea of alloxan induced mice is presented in (Table 4). The increased levels of serum urea in diabetic mice was significantly ($p<0.01$) restored to near normal levels in the *Thalassiahemprichii* extract on serum urea of alloxan induced mice. An increase in the level of serum urea in diabetic mice (7.07±1.38mg/dl) were found to be slightly decreased in the treated mice (5.73± 0.42mg/dl).(Table 4) depicts serum creatinine levels in the healthy control, diabetic control, positive control and treated groups. In the diabetic mice, the increased serum creatinine was noticed (13.12±1.9mg/dl, $p<0.01$) and it was found to be reduced significantly (6.93±0.7md/dl, $p<0.01$) in the treated mice.Administration of *Thalassiahemprichii*extract decreased the serum urea and creatinine levels by enhancing the renal function that is generally impaired in diabetic mice. The aqueous extract of *Clitoriaternatea* (CTL) and flowers (CTF) increased the total protein and lowered the serum urea and creatinine levels by enhancing the renal function that is generally impaired in diabetic rats(40)

Microscopic examination of the liver of alloxan induced diabetic mice revealed remarkable changes versus the control mice. These changes included periportal fatty infilteration with focal necrosis of hepatocytes (Figure 3). The normal healthy control liver tissue sections showed liver parenchyma with hepatocytes which appear normal (Figure 4). Sinusoids were seen. Results of the cellular architecture and integrity of the hepatocytes examined in the diabetic mice treated with *Thalassiahemprichii*extract (Figure 5) revealed a remarkable improvement of hepatic tissues where reduced necrosis was observed and the cellular arrangement of hepatocytes were found to be normal. This was represented by intact blood sinusoids and hepatocytes.

The main histopathological changes found in diabetic mice were periportal infilteration. Treatment with *Thalassiahemprichii* extract brought back the cellular arrangement to near normal. The effect of *T.arjuna* stem bark extract on histopathology of liver in alloxan-induced diabetic rats where reported that the normal liver tissue section shows sinusoidal cards of hepatocytes with central vein and portal tracts and the diabetic rat liver tissue section shows distortion in arrangement of cells around the central vein, periportal fatty infilteration with focal necrosis of hepatocytes(41)

V. Conclusion

The current diabetes epidemic is placing quite a burden on global health and the global economy. Diabetes mellitus,a chronic metabolic disorder, has now become an epidemic,with a worldwide incidence of 5% in the general population.The present study is being conducted to identify the bioactive components present in the ethanolic extract of *Thalassiahemprichii*. Also the present work is an attempt to evaluate the anti-diabetic properties of *Thalassiahemprichii* in experimental animal models of diabetes mellitus induced by alloxan.

Diabetes affects both glucose and lipid metabolism. A significant increase in blood glucose was observed in alloxan-induced diabetic mice. Treatment of alloxan induced diabetic animals with the *Thalassiahemprichii*extract for 15 days significantly normalized the lipid profile parameters towards near normal range.Increased level of serum GPT and GOT in hyperglycemic mice indicates liver failure. This study showed that the*Thalassiahemprichii*extract may also decrease the risk of liver failure associated with diabetes by significantly reducing the elevated levels of SGOT and SGPT in the alloxan induced diabetic mice.The diabetic hyperglycemia induces elevation of serum levels of urea and creatinine which are considered as significant markers of renal dysfunction. After treatment of alloxan-induced diabetic mice with *Thalassiahemprichii*extract, the elevation of serum urea and creatinine levels caused by diabetes were declined significantly. The present investigation suggests that the *Thalassiahemprichii*exhibit significant antihyperglycemic as well as antihyperlipidemic effect. Histopathological studies of liver in diabetic and extract treated groups substantiate the cytoprotective action of the extract. Thus it can be a good natural source to develop a new drug to treat diabetes mellitus. Further investigation is needed to elucidate the active principles and the exact mechanism of action of *Thalassiahemprichii*extract.

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Figure 1: TLC plates showing the presence of Alkaloid, Flavonoid, Steroid and Terpenoid after derivatization process

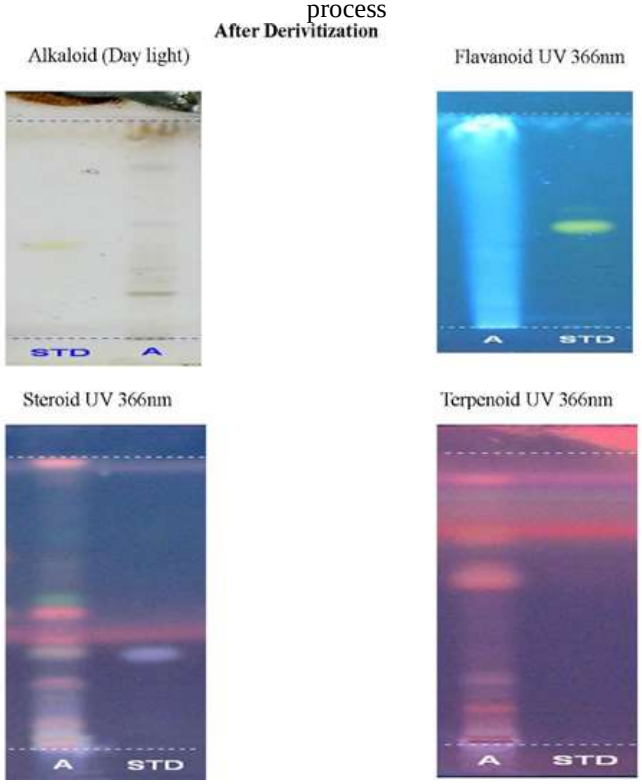

Figure 2: Chromatogram (GC-MS) of ethanolic extract of *Thalassiahemprichii*

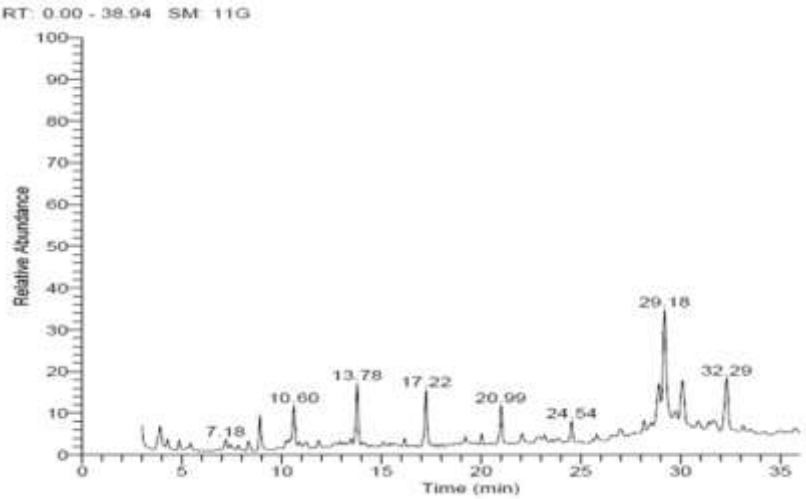

Figure 3: Histopathological changes in liver of normal healthy control mice
Figure 4: Histopathological changes in liver of alloxan induced diabetic mice
Figure 5: Histopathological changes in liver of *Thalassiahemprichii*treated mice

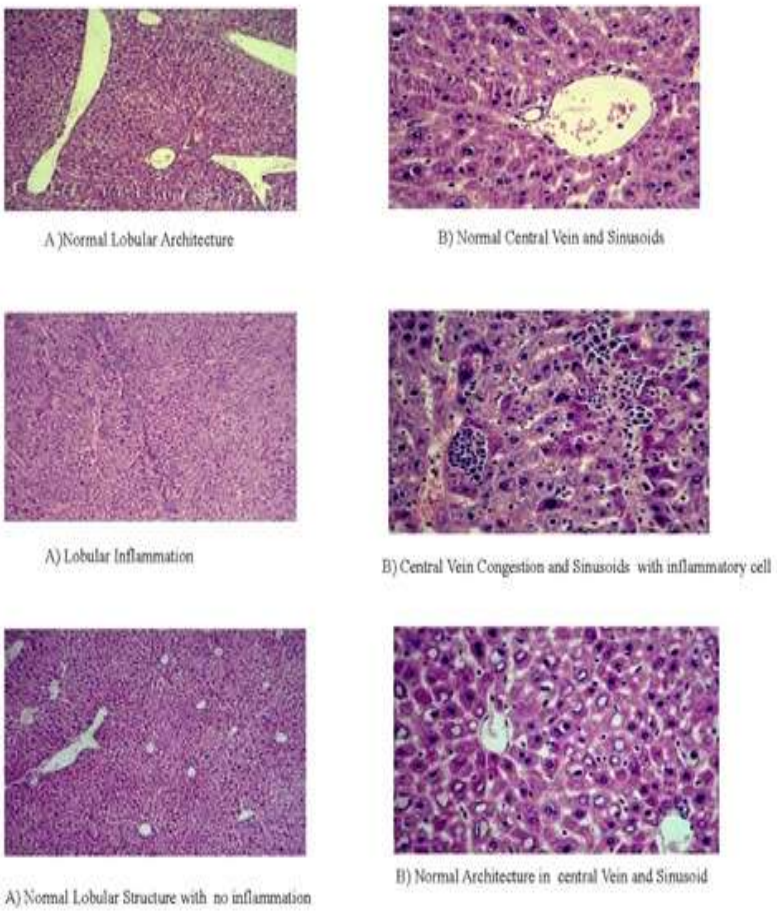

Table: 1 Preliminary Phytochemical Screening of *Pleurotusostreatus*Extract

TEST FOR EXTRACTINFERENCE	
Alkaloid	+
Flavanoid	+
Steroid	+
Terpenoid	+

+ = Presence - = Absence

Table 2: Bioactive components identified in ethanolic extract of Thalassiahemprichii by GC-MS analysis

S.No.	Retention time (RT)	Rf Value	Name of the compound	Molecular formula	Molecular weight
1	17.24	6.00,3	1-Nonadecane	C_{19H38}	266
2	13.78	6.00,3	1-Heptadecene(CAS)	$C_{17}H_{34}$	238
3	21.01	6.00,3	Cyclohexadecane (CAS)	$C_{16}H_{32}$	224
4	10.62	6.00,3	7-Hexadecane, (Z)-(CAS)	$C_{16}H_{32}$	224
5	33.53	6.00,3	Allyl 2,4,6-trimethylphenyl carbonate	$C_{13}H_{16O3}$	220
6	8.91	6.00,3	2-Propenoic acid, 2-ethylhexyl ester (CAS)	$C_{11}H_{20O2}$	184
7	24.54	6.00,3	(cis)-2-nonadecene	$C_{19}H_{38}$	266
8	28.18	6.00,3	1-Hexacosanol (CAS)	$C_{26}H_{54O}$	382
9	7.18	6.00,3	3-Dodecene	C12H24	168

Table: 3 Effect of *Pleurotusostreatus* extract on bodyweight, organ weight and blood Glucose of alloxan induced diabetic mice

Group	Body weight (g)		Organ weight (g)		Blood Glucose (mg/dl)	
	0^{th} Day	15^{th} Day	Liver (g)	Kidney (g)	0^{th} Day	15^{th} Day
Normal Healthy Control	22.025 ± 0.68	26.4 ±2.28	2.213 ± 0.17	0.348 ± 0.01	81.76 ±2.73	93.25 ±3.78
Diabetic Control	25.83 ±0.726	23.37 ±0.956	1.43 ±0.13	0.52 ±0.04	267.12 ±10.355	274±6.45
Positive Control	21.94 ±10.65	29.94 ±2.42	1.54 ±0.06	0.293 ±0.076	269.17 ±10.01	235.19 ±4.32
Treated Group	29.12 ± 1.677**	33.77 ± 1.404**	2.01 ± 0.16**	0.311 ±0.003**	265.54 ±15.96**	204.68 ±3.76**

Values are expressed as Mean ± SD. *p<0.05; **p<0.01 compared with Diabetic Control

Table: 4 Effect of *Pleurotusostreatus* extract on total cholesterol , HDL, LDL, VLDL, triglyceride, SGOT, SGPT, urea and creatinine of alloxan induced diabetic mice

Parameters	Normal Healthy Control	Diabetic Control	Positive Control	Treated Group
Total Cholesterol(mg/dl)	34.6 ±12.8	153±40.33	132±18.18	109±0.7**
HDL Cholesterol (mg/dl)	44.2 ±3.12	37.4±5.2	46.2±1.13	65.2±1.92**
LDL Cholesterol (mg/dl)	30.28 ±2.6	98.19±0.84	28.73±10.6	33.79±0.82**
VLDL Cholesterol (mg/dl)	22.92 ±0.18	30.46±0.19	28.9±0.16	23.39±0.27**
Triglyceride (mg/dl)	100±0.01	148±1.12	119±2.6	114±0.044**
SGOT (IU/L)	0.072±0.001	0.083±0.001	0.070±0.001	0.040±0.004**
SGPT (IU/L)	0.78±0.001	0.98±0.001	0.94±0.003	0.80±0.001**
Urea (mg/dl)	4.01±0.36	7.07±0.18	6.18±0.12	5.03± 0.13**
Creatinine (mg/dl)	5.01±0.22	13.12±1.9	9.18±2.1	6.93±0.7**

Values are expressed as Mean ± SD. *p<0.05; **p<0.01 compared with Diabetic Control

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