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Anti-Diabetic Activity of Leaf Methanolicextract of *Eurya Japonica* Thunb. Instreptozotocin Induced Diabetes in Mice and Isolation of Fraction (EJ-1) from the Extract

[1]Thingbaijam Rosalind, [1]Biman Kumar Dutta and [2]SatyaBhusan Paul

[1]Microbial and Agricultural Ecology and Biodiversity Conservation Laboratory,
Department of Ecology and Environmental Science,
Assam University, Silchar-788011, India
[2]Department of Chemistry, Assam University, Silchar-788011, India

Abstract: To evaluate the anti-diabetic activityinstreptozotocin (STZ) induced diabetes in mice and isolation of fraction (EJ-1) from the methanol leaf extract of *Eurya japonica* Thunb. Diabetes was induced in adult Swiss albino mice (20-30g) by intraperitoneal administration of streptozotocin (70 mg/kg, b.w). Mice were divided into 5 groups (*n = 6*): Normal, diabetic control, methanol leaf extract (300mg/kg b.w. and 600mg/kg b.w) and standard glibenclamide (10mg/kg b.w). Fasting blood sample were collected from tail for glucose estimation on 0^{th},5^{th},10^{th} and 15^{th} day. The animals were sacrificed after blood collection on the 15^{th} day and the serawere separated fordetermination of lipid profile. Histopathological studies were carried out on Pancreas and Liver. *Eurya japonica*Thunb.extract at 300mg/kg b.w. and 600mg/kg b.welicited significant reduction (P <0.001) in blood glucose level,56.56% and 60.80% respectively which was comparable to that of glibenclamide treatment (10mg/kg b.w) 60.82%, after 15days of treatment. Also lipid profile showed marked improvement due to the treatment which *Eurya japonica*Thunb. leaf extract at the end of the treatment period.However, the histopathological studies that the extract can ameliorate the STZ-induced histological damage of islets of langerhands in pancreas and hepatocytes in liver. The fraction (EJ-1) isolated from the leaf was found to be 6-(2-hydroxybenzyloxy)-3,4,5-trihydroxy-tetrahydro-2H-pyran-2-yl)methoxy)-3-methylpent-4-enal.The present resultssuggested that the plant extract has anti-diabetic property and further work may be initiated to study the antidiabteic activity of the isolated fraction.

Key words: Anti-diabetic · *Eurya japonica* Thunb. Histopathological · Streptozotocin (STZ).

INTRODUCTION

Diabetes mellitus (DM) is a clinical syndrome characterized by inappropriate hyperglycemia caused by a relative or absolute deficiency of insulin or by a resistance to the action of insulin at the cellular level [1]. This disorder occurs worldwide and its occurrence is increasing lately in most of the countries [2]. Diabetes mellitus is the sixth leading cause of death globally [3].Conventionally, type I diabetes is managed with exogenous insulin and type 2 with oral hypoglycemic agents (sulphonylureas, biguanidesetc), but they have side effects associated with their uses [4].

*Eurya japonica*Thunb. (Family: Theacae)is commonly known as *Japanese eurya* in English and in Manipuri language it is known as *Uyanggallaba.* The fruits and leaves of *Eurya japonica* Thunb. (Theaceae) are used in the Chinese traditional medicine "Lingmu" for the treatment of rheumatoid arthritis, tympanites, hemostasis of injuries, etc [5]. The components of the leaves [6] and berries [7] of this plant include halleridone, cornoside and flavonoids. A number of flavonoids (anthocyanins) [8,7], flavones and flavonol glycosides [9], as well as a few isoprenoids (betulinic acid and β-sitosterol)[10], have been identified from the fruit, leaf and bark of *Eurya japonica* Thunb.

Corresponding Author: Thingbaijam Rosalind, Microbial and Agricultural Ecology and Biodiversity Conservation Laboratory, Department of Ecology and Environmental Science, Assam University, Silchar-788011, India.

Several tribal groups in North Eastern India especially in Manipur, used this plant for the treatment of diabetes. The present study was aimed to investigate the antidiabetic activity of the leaf methanolic extract of *Eurya japonica*Thunb.onstreptozotocin (STZ) induced diabetes in mice to ascertain their ethnobotanical uses. The study was extended to isolate of a fraction (EJ-1) from this extract.

MATERIALS AND METHODS

Plant Materials: The leavesof *Eurya japonica* Thunb. plant were collected randomly from the Phayeng village of Imphal West district, Manipur, India. The leaves were shade dried at room temperature and the dried leaves were grounded into powderby using grinder. The powdered sample (100g) subjected to soxhlation with methanol for 72 hrs. The excess solvents were distilled off at lower temperature under reduced pressure in the rotary evaporator. The obtained crude extract was stored in airtight container in a refrigerator for further studies.

Experimental Animals: Adult Swiss albino mice (both male and female) weighing about 20-30 g were used in the present investigation. All the mice were given a period of acclimatization for 15 days before starting the experiment. They were fed with standard food pellet and water (*ad. libitum*). Animals were housed under the temperature (25°±1°C), humidity controlled room and a 12 hours light- dark cycle. All the experimental procedures were done as per the animal research guidelines of the care and use of laboratory animals and were approved by the Ethical Committee of the Assam University, Silchar (IEC/AUS/2013-014/dt 20/03/2013).

Acute Toxicity Study: Acute oral toxicity test was performed as per OECD-423 guidelines [11]. All the animals (both male and female mice) were randomly distributed into one control group (A) and three treated groups (Group B, C and D), containing five animals in each group. Groups B, C and D were orally administered 1000mg/kg, 2000mg/kg and 3000 mg/kg body weight methanol extract of *Eurya japonica*Thunb. The control group received distilled water. The animals were observed continuously for first 24 hours and 7 days for any signs of behavioural changes, toxicity, mortality and body weight.

The number of mice died within 24 hours and up to 7 days was noted and their LD_{50} of the extract was calculated using the arithmetic method of Karber as modified by Aliu and Nwude[12] as follow:

LD_{50} = Maximum dose - Sum of (Dose difference X Mean dose)/Number of animals.

Antidiabetic Activity: Induction of diabetes: Streptozotocin (STZ) was freshly dissolved in 0.1 M citrate buffer (pH = 4.5) at the dose of 70 mg/kg body weight and injected intraperitoneally within 15 min of dissolution in a vehicle volume of 0.3 ml. Diabetes was confirmed by the determination of fasting glucose concentration on the third day post administration of streptozotocin. A rest period of two days is allowed for the blood glucose level to stabilize. During this period the animals used to have free access to both water and food. The hyperglycemic mice (blood glucose level > 200 mg/dl) were separated and divided into different groups comprising of 6 mice in each group for the anti-diabetic study [13].

Glibenclamide: Single dose of glibenclamide provokes a brisk release of insulin from pancreas. It acts on β-cell membrane leading to enhance calcium flux across it, hence degranulation. After chronic administration the insulinemic action of glibenclamide declined, but improvement in glucose tolerance is maintained. Thus it is an oral antidiabetic preparation with an efficient hypoglycemic action [14]. Daonil (glibenclamide) manufactured by Aventis Pharma Ltd. Goa, India was collected from the market and used for the experiment.

Experimental Design: Mice in these groupswere divided into the following groups (10% and 20% of LD_{50}).

Group I: Consisted of 6 mice which served as normal control and were treated with distilled water.

Group II: Consisted of 6 STZ induced diabetic mice and served as diabetic control and were treated with distilled water.

Group III: Consisted of 6 STZ induced diabetic mice and were treated orally withglibenclamide at the dose of 10 mg/kg body weight for 15 days, once a day.

Group IV: Consisted of 6 STZ induced diabetic mice and were treated orally with methanol extract of*Eurya japonica*Thunb. (EJ) leaves at the dose of 300 mg/kg body weight for 15 days, once a day.

Group V: Consisted of 6 STZ induced diabetic mice and were treated orally with methanol extract of *Eurya japonica*Thunb. (EJ) leaves at the dose of 600 mg/kg body weight for 15 days, once a day.

After 15 days of treatment with glibenclamide and the leaf extract, the experiment was terminated and observations were made. Body weight were estimated on 0^{th} and 15^{th} day of the treatment. Fasting blood samples were collected from the tail for glucose estimation just before sample administration on the first day and 1 h after sample administration on days 5^{th}, 10^{th} and 15^{th} day. The animals were sacrificed after blood collection under chloroform anaesthesia on the 15^{th} day and pancreas as well as liver were removed for histopathological studies. Blood samples were centrifuged at 3000 RPM to separate sera for estimation of serum protein and lipid profile. Serum total cholesterol, HDL- cholesterol, LDL-cholesterol, VLDL-cholesterol and triglycerides were analysed.

Biochemical Analysis: Blood glucose was estimated by the Glucocard 01-mini blood glucose monitoring kit. Serum protein was estimated by Lowry method. Total cholesterol (TC), triglycerides (TG) and HDL were estimated by Crest Biosystem cholesterol kit (CHOD/PAP method), Crest Biosystem triglycerides kit (GPO/PAP method) and Crest Biosystem HDL cholesterol kit (PEG precipitation method). For determination of VLDL and LDL, Friedewald's formula was used [15]. LDL cholesterol was calculated using the formula given below,

$$\text{LDL cholesterol} = \text{Total cholestrol} - \text{HDL} + \left[\frac{\text{TG}}{5}\right]$$

and VLDL cholestrol was calculated using the formula $\left[\frac{\text{TG}}{5}\right]$

Histopathologicalstudy: Pancreas and liver were isolated and preserved in 10% neutral formalin fixative solution for histopathological examination. After fixation the tissues were processed and embedded in paraffin,then the solid sections were cut into 5ìm stained with haematoxylin and eosin [16].

Method of Isolation: The powdered extract of *Eurya japonica*Thunb.leaveswas subjected to defatting with petroleum ether (24 h), followed by methanol (48 h) extraction.

Column chromatography of methanolicextracts of *Eurya japonica* Thunb. leaveswas conducted using silica gel (Mesh 60-120) that was packed by wet packing method using petroleum ether [17]. The column was then eluted using petroleum ether, petroleum ether: ethyl acetate mixture, with the gradual increase in polarity. The fractions were collected and marked. The marked fractions were subjected to thin layer chromatography to check homogeneity of various fractions. Similar fractions were pooled together. Fractions were further purified using preparative TLC. Spots were identified and recovered by scraping the adsorbent off the plate and the substance was extracted from the adsorbent using chloroform, then Centrifuge at 3000 RPM, room temperature for 5 minutes for 2/3 times. The solvent is filtered and dried. The dried fraction was collected for spectral analysisnamely IR, GC-MS and^{1}H and^{13}C NMR for structural elucidation [18-21].

Statistical Analysis: The data were expressed as mean ± SEM. Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Post hoc multiple comparisons test (Tukey Test). The results were considered statistically significant if the P values were 0.001 or less andP values were 0.05 or less.

RESULTS AND DISCUSSION

Acute Toxicity: Experimental screening method is imperative in order to establish the safety and efficacy of traditional and herbal products and also to set up the active components of the herbal products [22]. In the acute toxicity test of the methanol leaf extract of *Eurya japonica*Thunb. there was no mortality or any sign of behavioural changes or toxicity observed at the dosage of 1000, 2000 and 3000mg/kg b.w in mice. The calculated LD_{50} was found to be 3000 mg/kg b.w (Table 1). It was therefore concluded that the methanol leaf extract of *Eurya japonica*Thunb. was found to be orally nontoxic according to the acute toxicity studies in mice.

Blood Glucose Level: A significant reduction ($p<0.001$) in blood glucose level was observed after treatment with the methanol extractleaf of *Eurya japonica*Thunb.(300and 600mg/kg b.w.)in the STZ- induced diabetic mice on 0^{th}, 5^{th}, 10^{th} and 15^{th} days of treatment. In STZ- induced diabetic control group, the blood glucose level increased from 255 to 362mg/dl during the experimental observation period. The blood glucose level in STZ- induced diabetic mice treated with glibenclamide (10mg/kg b.w.)decreased from 249.4mg/dl to 97.75mg/dl and *Eurya japonica* Thunb. (300mg/kg b.w. and 600mg/kg b.w) decreased from, 276.75mg/dl to 120.25mg/dl and 266.0mg/dl to104.25mg/dl

Table 1: LD_{50} Calculated by arithmetic method of Karber Groups.

Groups	No. of Mice	Dose of extract(mg/kg)	Number of dead Mice	Dose Difference	Mean death(Md)	Dose difference X Md
I	5	Control	0	0	0	0
II	5	1000	0	1000	0	0
III	5	2000	0	1000	0	0
IV	5	3000	0	1000	0	0

LD_{50} = Maximum dose - Sum of (Dose difference X Mean dose)/ Number of animals.

LD_{50} = 3000mg/kg.

Table 2: Effects of *Eurya japonica*Thunb. methanol leaf extract on the body weight and blood glucose level in normal and STZ-induced diabetic mice.

				Blood glucose concentration (mg/dl)				
Group	Treatment	Initial body weight (0^{th} day)	Final body weight (15^{th} day)	0^{th} day	5^{th} day	10^{th} day	15^{th} day	% reduction
Group I	Normal	20.55±0.08	23.81±1.0	83± 5.1	75.75± 8.7	95.25± 4.4	89± 3.1	-7.22
Group II	Diabetic control	26.57±0.8	24.07±2.0	255±10.8	306.75±12.7	337.5±15.8	362±26.5	-41.96
Group III	Glibenclamide standard	22.85±0.2	24.14±1.0	249.5±4.1	210.5±13.6[a]	142.75±2.6[a]	97.75±2.5[a]	60.82
Group IV	Methanolic extract (EJ)							
	300mg/kg body weight	25.72±1.5	23.90±0.7	276.75±6.0	153.25±11.7[a]	122.25±0.2[a]	120.25±0.2[a]	56.56
Group V	Methanolic extract (EJ)							
	600mg/kg body weight	24.63±1.9	24.43±1.6	266±18.1	175.5±11.9[a]	135.75±2.4[a]	104.25±2.0[a]	60.80

Values are mean ± SEM, n= 6, [a] P values<0.001 and [b] P values <0.05 when compared to diabetic control. % Reduction in blood glucose level as compared to 0th day.

Table 3: Antihyperlipidemic effects of *Eurya japonica* Thunb.methanol leaf extract in normal and STZ-induced diabetic mice.

		Changes in mg/dl					
Group	Treatment (mg/kg body weight)	Serum protein (g/dl)	Serum cholesterol	Serum triglycerides	Serum HDL	Serum LDL	Serum VLDL
Group I	Normal	6.27±0.4[a]	102.88±3.4	75.33±2.9[a]	31.62±2.0[a]	58.55±1.6[a]	15±0.5[b]
Group II	Diabetic control	3.98±0.3	281.17±53.0	168.20±30.3	16.49±2.2	185.35±34.6	33.63±6.1
Group III	Glibenclamide standard	5.27±0.2[b]	147.00±17.5	93.42±4.8[b]	62.66±1.3	90.41±6.2[a]	18.68±0.9[b]
Group IV	Methanolic extract (EJ)						
	300mg/kg body weight	5.08±0.2	141.91±12.0	105.29±3.6[b]	34.94±3.6[b]	91.41±4.3[b]	21.05±0.7[b]
Group V	Methanolic extract (EJ)						
	600mg/kg body weight	5.71±0.2[b]	155.32±10.1	110±2.0	43.48±3.3[a]	88.52±4.5[b]	22±0.4[b]

Values are mean ± SEM, n= 6, [a] P values<0.001 and [b] P values <0.05 when compared to diabetic control.

respectively from 0^{th} day to 15^{th} days treatment. The methanol leaf extract showed a dose dependent reduction in blood glucose level and this antihyperglycemic effect was comparable with that of standard oral antidiabetic agent, glibenclamide. The antihyperglycemic effect of *Eurya japonica* Thunb.at 600mg/kg b.w (60.80% fall) was found to be closely comparable with that of glibenclamide treatment at 10mg/kg b.w(60.82%) (Table 2). From our results, it can be concluded that the methanol leaf extract of *Eurya japonica* Thunb. atdosage of 300mg/kg b.w. and 600 mg/kgb.w. possesses significant antihyperglycemic activity on treatment in STZ-induced diabetic mice.

Lipid Profile: Beneficial effects of *Eurya japonica*Thunb.on serum lipids, one of the major cardiovascular risk factors in type 2 diabetes mellitus, can be observed from lipid-related data (Table 3). Compared with the STZ-induced diabetic control group, *Eurya japonica*Thunb. (300mg/kg b.w. or 600 mg/kgb.w.)treated groups showed significant reduction (P<0.05) in the serum levels of triglycerides, total cholesterol, LDL and VLDL, in concomitant improvement in serum protein and HDL levels, on the period of experimentation for 15^{th} days treatment. Glibenclamide also showed reduction in the levels of triglycerides, total cholesterol, LDL and VLDL, accompanied with improvement in serum protein and HDL level, on the period of experimentation for 15^{th}days treatment. There was an increase in body weight of the mice in the standaring and extract treated groups when compared with the diabetic control. The level of total protein in serum decreased in diabetic control group when compared to those of normal control, while there was an increase in STZ-induced diabetic mice treated with *Eurya japonica*Thunb. The higher lipid levels seen in STZ-induced diabetic mice may be due to increased mobilization of free fatty acids from peripheral depots and also due to lipolysis caused by hormones [23,24]. It is well

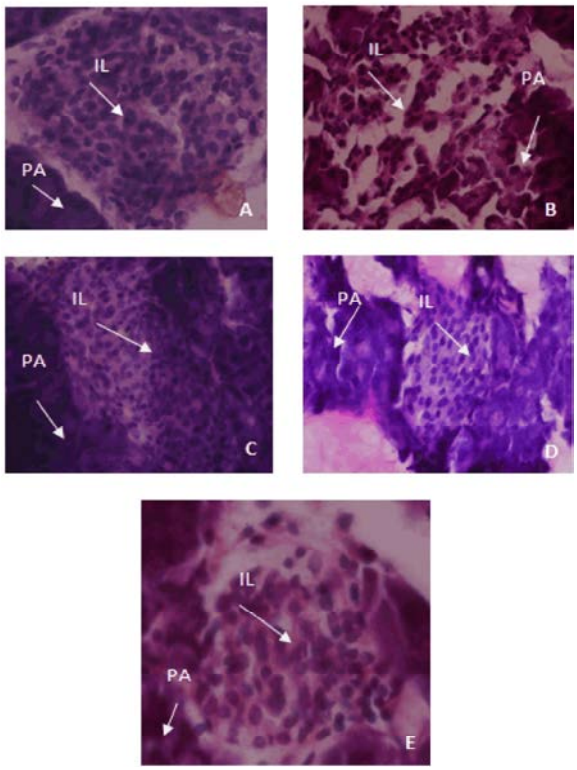


Fig. 1: Photomicrog ragh

A) Sectionof the pancreas showing normal appearence of the pancearic acini (PA) and islet of langerhans (IL) in the pancreas of the normal control group.

B) Sectionof the pancreas showing marked degeneration of the pancearic acini (PA) and islet of langerhans (IL) in the pancreas of the diabetic control group.

C) Sectionof the pancreas showing marked regeneration of the normal cellular architecture of pancreatic acini (PA) and islet of langerhans (IL) in the pancreas of the group treated with glibenclamide.

D & E) pancreas section showing marked regeneration of the cellurar architecture of the pancreatic acini (PA) and islet of langerhans (IL) in the pancreas of the group treated with methamol leaf extract of Eurya japonica thunb. (300mg/kg b.w & 600mg/kg b.w) respectively.

known that LDL plays an important role in arteriosclerosis and that hypercholesterolemia is associated with a defect relating to the lack of LDL receptors [25]. Preliminary phytochemical screening of the plant leaf extract of *Eurya japonica* Thunb.revealed the presence of alkaloids, glycosides, flavonoids, terpenoids, saponins, tannins and reducing sugar [26]. Flavonoids, sterols/triterpenoids, alkaloids and phenolics are known to be bioactive and having antidiabetic principles [27-30] and therefore it is suggested that extracts exhibited antihyperglycemic effects through increased insulin secretion due to the presence of phenolic and flavonoids compounds. Studies have shown that *Eurya japonica* Thunb. hasantioxidant activity [31]. The antidiabetic effect of the leaf extract may be due to the presence of more than one antihyperglycemic principles and their synergistic properties. In this study, the antihyperglycemic activity caused by glibenclamide in STZ-induced diabetic mice is an indication of the presence of some β cells, as glibenclamide is known to stimulate insulin secretion from β cells [32]. This study suggested that *Eurya japonica* Thunb. leaf extracts may act through the mechanisms of glibenclamide by reversing the abnormalities in the pancreatic islets. Based upon the results it can be hypothesized that the plant leaf extract probably act by releasing insulin from the pancreatic β-cells by stimulating the insulin secretion.

Histopathological Studies: Light photomicrographs of pancreas (Fig. 1) in the normal mice, showed normal histological structure of β-cells at the central zone in the islet of Langerhans in the endocrine portion and the normal histological structure of the acini in the exocrine portion were recorded. In the STZ-induced diabetic control mice, atropy and degeneration were observed mostly in the β-cells of the central zone at the islet of Langerhans in the endocrine portion. Necrosis of the pancreatic tissues of the acini in the exocrine portion was also recorded. In the treatment of STZ- induced diabetic mice withglibenclamide and methanol leaf extracts (300 and 600mg/kg b.w.) of *Eurya japonica* Thunb. the restoration of normal cellular architecture of the affected β-cells of the central zone at the islet of Langerhans in the endocrine portion and pancreatic tissues of the acini in the exocrine portion were recorded. Results are comparable to that of the standard glibenclamide. This may explain both antihyperglycemic and antihyperlipidemic actions of the plant.

Light photomicrographs of liver (Fig. 2) of normal mice showed a normal appearance of hepatic lobules which are roughly hexagonal or pentagonal in shape, with portal triads at the vertices and a central vein (CV) in the middle. Liver of the diabetic untreated mice showed degenerative changes in the hepatocytes and some necrotic regions. The hepatic lobules were observed to have many vacuoles giving them foamy appearance and some of them showed pyknotic nuclei. Liver of STZ- induced diabetic mice treated with glibenclamide and plant extracts (300mg/kg b.w.and 600mg/kg b.w.) showed

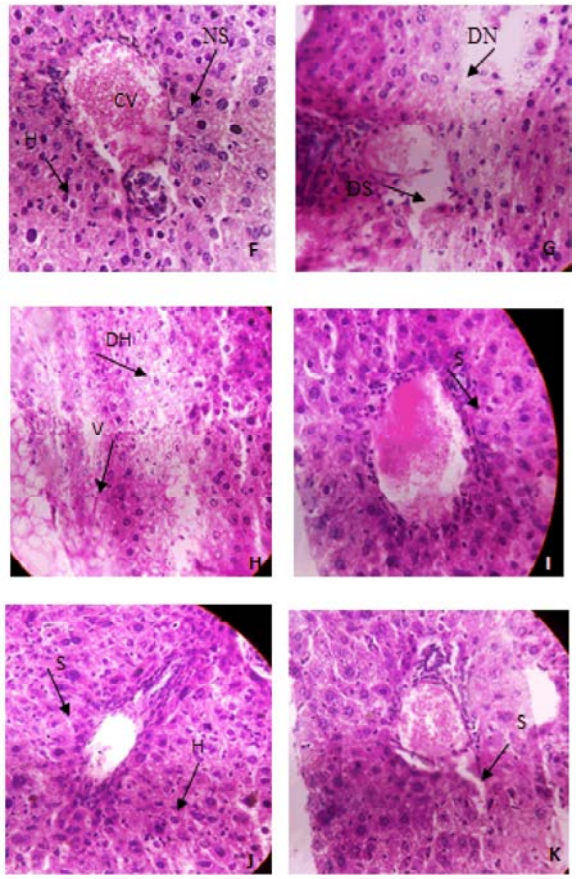


Fig. 2: Photomicrog ragh

F) Sectionof the liver showing normal appearence of the lobules with portal traints at the vertices, central vein (CV) in the middle, normal sinusoid (NS) and hepatocytes (H) in the liver of normal control group.

G & H) Liver section showing dilated sinusoid (DS), dilated necrosis (DN) degrenerartive hepatocytex (DH) vanusold (V) in the diabetic control group.

I) Liver section showing regenerative effect of hepatocytes and sinusold (S) in glnbenclaimide treated group.

J &K) Liver section showing regenerative effect of hepatocytes (H) and sinusold (S) in treated with methamol leaf extract of Eurya japonica thunb. (300mg/kg b.w & 600mg/kg b.w) respectively.

regenerative effect of hepatocytes and decreased number of vacuolized cells and degree of vacuolisation. This suggests that the consumption of experimental plant extract could reverse most of the histopathological and biochemical changes in the liver of the diabetic groups of mice comparable to the glibenclamide standard. These findings suggest that treatment by *Eurya japonica* Thunb. methanol leaf extract may be able to restored the detected deformities in STZ induced diabetic mice to a certain extend.

Table 4: Column chromatography fractions of leaf extract of the plant:

Eluent	Fraction	Remark
Eurya japonica Thunb.		
P.E : E.A (9:1)	1 (EJ-1)	Dark green colour

Table 5:

Wave number	Assignment of peaks	Bond
3194 cm $^{-1}$	γ_{O-H}Stretch	-O-H
2955 and 2928 cm $^{-1}$	γ_{C-H}Stretch of Methyl and methylene moiety	-C-H
1666 cm $^{-1}$	$\gamma_{C=O}$Stretch of Ketonic group	-C=O
2721 cm $^{-1}$	γ_{C-H}Stretch of Aldehydic group	

On the basis of the current investigation, it can be concluded that the methanol leaf extract of *Eurya japonica*Thunb. acted in a similar fashion to glibenclamide (Standard drug) and it could be suggested that these results provide pharmacological evidence for its folklore claim as an anti-diabetic agent.

Isolation of EJ-1: Column chromatography of the methanolic leaf extracts of *Eurya japonica* Thunb has collected some fractions. The fractions were subjected to Preparative TLC (Table 4).

The Preparative TLC of the fraction (Fig.3) of *Eurya japonica*Thunb. collected 1 distinct spot that were isolated and collected separately in a tube and marked as EJ-1 which were further used for spectral studies.

Spectral Analysis and Structural Elucidation of the Isolated Fraction (EJ-1) from the Methanol Leaf Extract of *Eurya Japonica*thunb.: Assignment of functional group corresponding to the peaks obtained from FT-IR of the isolated fraction (EJ-1) from the methanol leaf extract of *Eurya japonica*Thunb.was shown in (Fig.4) (Table5). The analytical GC spectrum of thefraction (Fig.5) exhibited one major peak at 28.51 min. when eluted with helium as carrier gas. Trace of methanol solvent accompanying the extract was eluted off with the carrier gas during the initial phase of GC (before 12 minitues) of the experimental analysis. The ^{1}H NMR spectrum of the fraction (Fig.6a and b) exhibited characteristic peak for the presence of sugar moiety in δ 3-5 ppm. The doublet at δ 9.49ppm is due to the aldehydic proton present in the fraction. The oliphenic proton (H-7) appears as a singlet at δ 5.1 ppm. The peak from δ 6-9 ppm corresponds to the proton present over the substituted aromatic system.

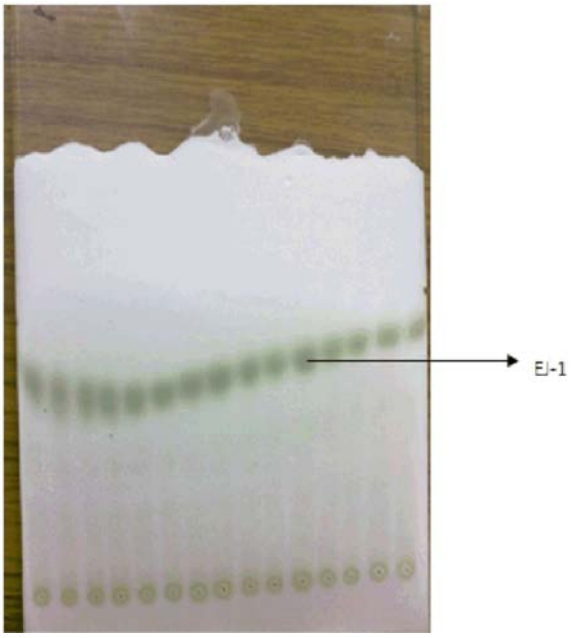


Fig. 3: Preparative TLC of the methanol leaf extract fraction (EJ-1) of *Eurya Thunb.*

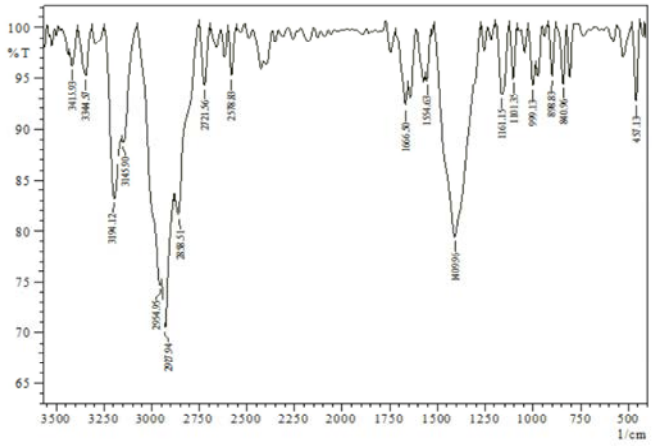
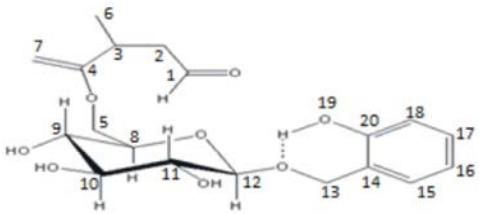


Fig. 4: Assignment of functional group corresponding to the peaks obtained from FT-IR spectrum of the isolated fraction (EJ-1) from the methanol leaf extract of Eurya japonicaThunb.:

The two doublets of doublets at δ 6.25 ppm and δ 6.26 ppm corresponds to the proton (H-16, H-17). The peak at δ 7.25 ppm and δ 8.55 ppm attributed to H-15 and H-18 respectively. The H- bonded phenolic-OH (H-19) resonated at δ9.35 ppm. The presence of strong H-bonding forming a stable six member ring like structure is responsible for the down field resonance of this proton. The methyl proton (H-6) appears at δ 0.87 ppm whereas the proton H-2,H-3 and H-5 resonated as multiplate between δ 1.2 to δ 2.5 ppm.



Assignment of protons corresponding to the peaks obtained from ^{1}H NMR of isolated fraction obtained from the methanol leaf extract of *Eurya japonica* Thunb.

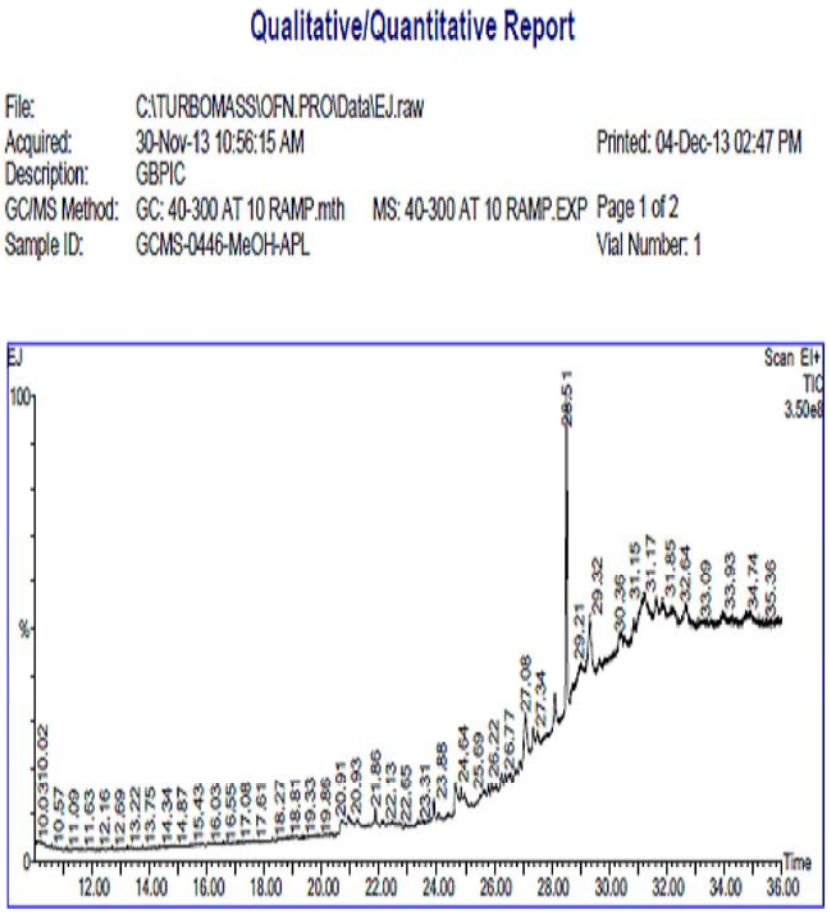


Fig. 5: Mass spectrum of the isolated fraction (EJ-1) from the methanol leaf extract of *Eurya japonica*Thunb.

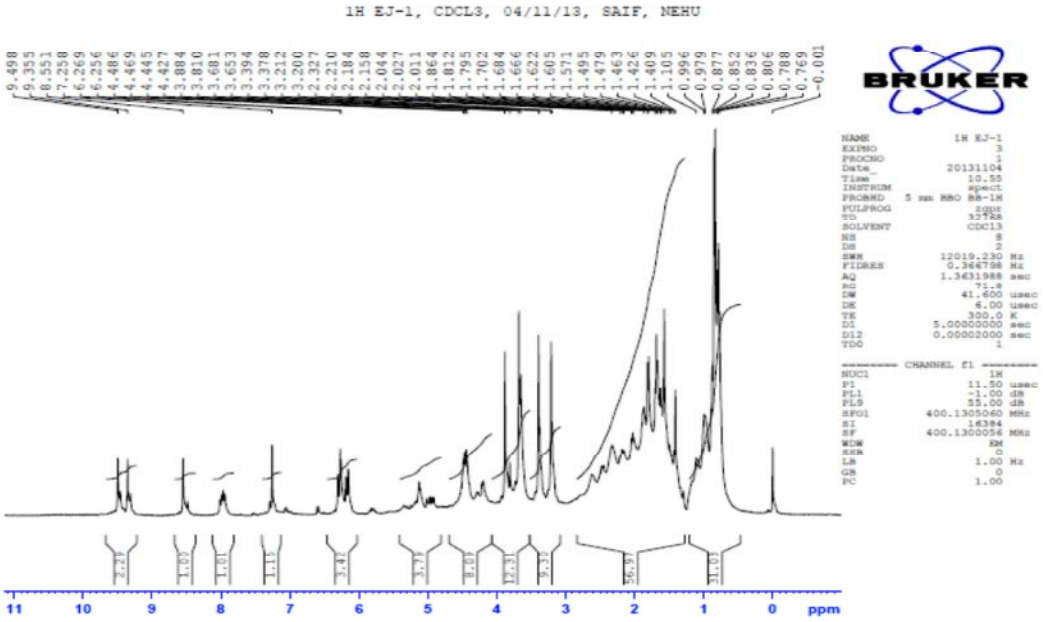


Fig. 6a: [1]H NMR spectra of the isolated fraction (EJ-1) from the leaf extract of *Eurya japonica*Thunb.

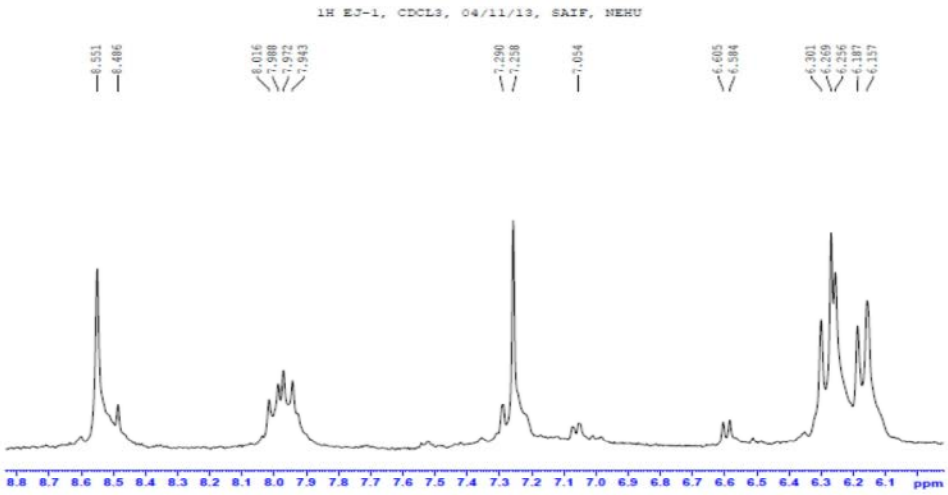


Fig. 6b: [1]H NMR spectra of the isolatedfraction (EJ-1) from the leaf extract of *Eurya japonica*Thunb.

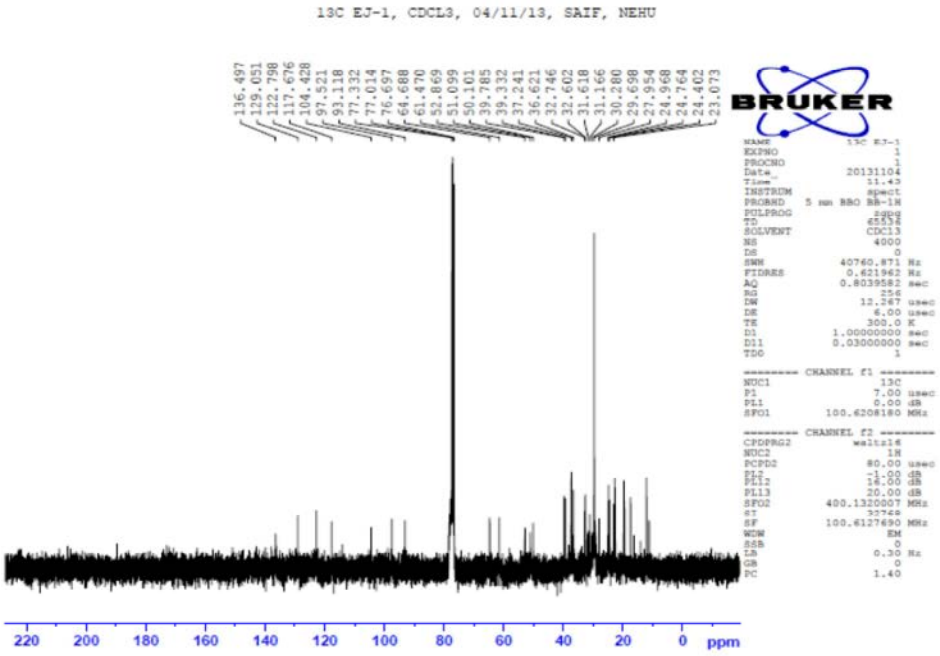


Fig. 6c: [13]C NMR spectra of the isolated fraction (EJ-1) from the leaf extract of *Eurya japonica*Thunb.

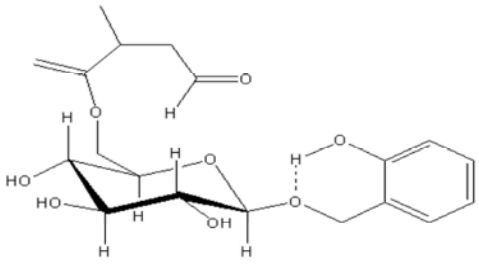


Fig. 7: IUPAC name of EJ-1:
6-(2-hydroxybenzyloxy)-3,4,5-trihydroxy-tetrahydro-2H-pyran-2-yl)methoxy)-3-methylpent-4-enal.

The ^{13}C NMR spectra (Fig. 6c) provided the evidence for the presence of carbonyl group. The aldehyde carbon C-1 resonated at δ 190 ppm. The peak at δ 136 ppm corresponds to C-4 which is an oliphenic carbon. Similarly the peak at δ 112 ppm is attributable at C-7. The aromatic carbon resonated at δ 93.11 ppm (C-16), δ 97.52 ppm (C-17), δ 104.42 ppm (C-15), δ 117 ppm (C-18), δ 122.79 ppm (C-14) and δ 129.05 ppm (C-20) respectively. The five peaks from δ 50- 70 ppm is attributable to the five carbon of the sugar unit. The carbon C-2, C-3, C-6, C-13 and C-5 appears at δ24.96 ppm, δ29.69 ppm, δ23.07 ppm, δ36.62 ppm and δ 31.61 ppm respectively.

Keeping these values in concern (IR, GC-MS and ^{1}H and^{13}C MNR) the tentative structure of the fraction is elucidated to be as follows:

CONCLUSION

In the light of the present results, it could be concluded that the antidiabetic effect of *Eurya japonica* Thunb.was promising.From the above spectral analysis, the fraction isolated from the leaf extract of *Eurya japonica*Thunb. was found to be 6-(2-hydroxybenzyloxy)-3,4,5-trihydroxy-tetrahydro-2H-pyran-2-yl)methoxy)-3-methylpent-4-enal.

For optimization of bioactivity of the isolated fraction (EJ-1) and to know the potency as antidiabetic property further pharmacological and biochemical investigations will clearly elucidate the mechanism of action and will be helpful in projecting this plant as a therapeutic target in diabetes research.

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