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Nabam Peter
Research scholar, School Of Medical Science and Technology Indian Institute of Technology, Kharagpur, West Bengal, India

Jayjeet Majumdar
Research scholar, School Of Medical Science and Technology Indian Institute of Technology, Kharagpur, West Bengal, India

Gaurav Biswas
Research scholar, School Of Medical Science and Technology Indian Institute of Technology, Kharagpur, West Bengal, India

Harpreet S Pawar
Research scholar, School Of Medical Science and Technology Indian Institute of Technology, Kharagpur, West Bengal, India

Anirban Mitra
Head of the Department, Department of Computer Science and Engineering, Vignan Institute of Technology and Management, Berhampur, Odisha, India

Analava Mitra
Associate Professor, School of Medical Science and Technology, Indian Institute of Technology, Kharagpur, West Bengal, India

Correspondence
Analava Mitra
Associate Professor, School of Medical Science and Technology, Indian Institute of Technology, Kharagpur, West Bengal, India

Effects of mangiferin isolated from *Mangifera indica* leaves and evaluation of biologic activities of β-cyclodextrin-mangiferin complex particularly its anti-diabetic and hypolipidaemic propertieson Type 1 diabetes rat model

Nabam Peter, Jayjeet Majumdar, Gaurav Biswas, Harpreet S Pawar, Anirban Mitra and Analava Mitra

Abstract
Diabetes both Type 1 and Type 2 is on the increase in Indian subcontinent and both nature and nurture has to blame. In Indian rural populace the initial lower prevalence is increased and comparable to urban leading to effective management protocols (phytotherapy) optimized to rural settings. The present paper deals with the effects of mangiferin obtained from mango leaves, isolated and characterised as per existing scientific protocol (isolated mangiferin and cyclodextrin complex). Evaluation was done in respect to enzyme inhibition studies and animal experimentation models. Our findings shows enzyme inhibition studies shows to statistically significance. Further, animal experimentation studies on Type1 diabetic rat model shows mangiferin thus isolated has promising hypoglycaemic potentials in and also leading to beneficial cholesterol lowering effect, thus leading to further scientific validation in Type 1 diabetics with commercial market explore in entire diabetic scenario both Type 1 and Type 2 (existing in the market)

Keywords: Mangiferin, cyclodextrin, diabetes, hypoglycaemia, hypolipidaemia, phytotherapy.

1. Introduction
Worldwide, a 122% rise is projected in Type 2 diabetes, from the total of 135 to 300 million. The multi-factorial cause mainly points population ageing and growth, as well as obesity, un pattern and improper diets and sedentary lifestyles. Of the different types of diabetes [1] Type 2 is most common, though in present day prevalence of both types of diabetes are on the increase and mainly due to nature and nurture effects. These factors are closely associated with urbanization and industrialization and usually exist from childhood [2]. In the next 25 years, India will not only have the largest number of people with diabetes in the world but also the greatest increase in the disease burden [3]. In India, he pooled estimates of prevalence rates for diabetes mellitus for urban and rural areas were found to be 118.02 per thousand and 38.67 per thousand respectively. The overall prevalence rate of diabetes in urban and rural areas combined was estimated as 62.47 per thousand [4]. It has been reported that diabetes occurs at a younger age in the developing countries. In the developed world, most people with diabetes are over 65 years of age while in developing countries the majority is in the age group 45-64, a trend that will accentuate [5]. Considering the devastation and catastrophe of diabetes in socio-economic scenario in India and gaps in Heath Survey Research an alternative strategy to combat the disease particularly in rural sectors is essential. Herbal medicine having wide cultural and traditional acceptance and being considerably cheap in India needs to be widely explored. Presently ethno-pharmaceutical documents more than 600 plants having anti-diabetic effects in India but all of them are not explicitly studied and evidence based documentation with toxicological profile lacking [6, 7, 8]. The present work is focussed onmangiferin obtained from *Mangifera indica*, available in all areas of India and its effect on Type 1 diabetes.
Though advanced technology is being used for the treatment of diabetes, we are far away in investigating many aspects of this disease. A number of oral anti-diabetic drugs available for the treatment of diabetes have shown side effects and drug-drug interactions. Further, considering the chronic nature of the disease the cumulative cost involvement will be the additional burden. This has been the rationale for the development of new anti-diabetic drugs, which includes herbal agents also. Indian Medicinal plants and their derivatives have been an invaluable source of therapeutic agents to treat various disorders including diabetes.

The reported pharmacological activities of mangiferin include antioxidant, radio protective, antitumor, immunomodulatory, anti-allergic, anti-inflammatory, antidiabetic, lipolytic, anti-boneresorption, monoamine oxidase inhibiting, antiviral, antifungal antibacterial and antiparasitic properties, which may support the numerous traditional uses of the plant [9]. Antioxidant activity of mangiferindoes not necessarily leads to an in vivo biological responsedue to its poor bioavailability and also due to its low solubility in water: 0.111 mg/mL. This problem can be minimized by using cyclodextrins (CDs) that present special ability to complex with a variety of guest molecules and achieve a controlled release of certain constituents, and also increase its antioxidant activity [10].

In this paper we made an attempt to explore the effects of mangiferin obtained from leaves in slow release capsule of chitosan to evaluate its antidiabetic and hypolidiaemic potentials. Our lab has already documented the effects of mangiferin obtained from stem bark of *Mangifera indica* and validated its hypoglycaemic and hypolipidaemic effects in Type 2 diabetes [10]. The active ingredients from leaves of *Mangifera indica*, mangiferin will be validated for itshypoglycaemic and hypolipidaemic effects in Type 1 diabetes.

2. Methodology

2.1 Reagents

Chemicals and reagents Standard mangiferin, alloxan, starch azure, porcine pancreatic amylase, alpha glucosidase from yeast Saccharomyces cerevisiae, para-nitrophenylgluco-pyanoside, Tris-HCl buffer and melting point capillary tubes were procured from Sigma Chemicals, USA. Dimethyl sulfoxide, acetic acid, calcium chloride, petroleum ether, acetone, ethanol, methanol, potassium bromide, potassium dihydrogen phosphate, dipotassium hydrogen phosphate and sodium carbonate were purchased from Merck, India. The thin layer chromatography plates were obtained from Merck (silica gel 60 F254 grade, Germany). DMSO-d6 was obtained from Cambridge Isotope Laboratories Inc, USA. Two types of β-cyclodextrin (β-CD) [CAS Number 7585-39-9 (for DSC studies) and CAS Number 68168-23-0 were purchased from Sigma–Aldrich.

2.2 Extraction, identification and authentication of leading bioactive molecules from leaves of *Mangifera indica* [11, 12]

Conventional extraction methods presents some drawbacks, among them low selectivity and/or low extraction yields, time consuming processes, use of large volumes of toxic organic solvents, and extended concentration steps that results possibly in loss or degradation of antioxidants. High pressure extraction techniques, such as supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE), have been used in recent years so result interesting alternatives to overcome the above mentioned drawbacks. In lab set up we followed conventional method [13].

Soxhlet extraction was used as reference method, which yielded 57 mg/g in 5 h. Under optimal conditions such as microwave irradiation time 5 min, ammonium sulphate concentration 40% w/v, power 272 W, solute to solvent ratio 1:20, slurry to *t*-butanol ratio 1:1, soaking time 5 min and duty cycle 50%, the mangiferin yield obtained was 54 mg/g by microwave assisted three phase partitioning extraction (MTPP) [12].

Mangifera indica leaves (2 kg) were collected from the upper Assam region and Arunachal Pradeshin the month of December 2015. The leaves were authenticated by Prof. S. Dey, Professor Biotechnology, Indian Institute of Technology, Kharagpur. A careful inspection of the leaves was doneto isolate healthy leaves, collected and stored hygienically for a brief period of 72 hours to observe any desired effects due to contaminants. *Mangifera indica* leaves after collection were dried aseptically in lab conditions; the dried materials were then grounded to fine powder using electric grinder (Bajaj appliances, Mumbai). The dried powdered leaves of *Mangifera indica* thus collected (2 kg) was serially extracted with boiling petroleum ether (2 x 2 L; 6hr each and every time) to remove fatty matter, cold acetone (4 x 2 L; 24 hreach and every time) to remove tannins and with 70% ethanolic solvent (4 x 1 L; 6 hreach and every time). The ethanolic fraction was concentrated under reduced pressure using rotary evaporator (Buchi Rotavapor R- 210). Mangiferin separated as a dry yellow colour powder (2.4 gm). Confirmative test for mangiferin thus isolated was performed. Mangiferin isolated was dissolved in aqueous ethanol showing deep yellow colour solution. Alcoholic solution of isolated mangiferinon being treated with ferric chloride gave deep green colour while with ferric chloride and hydrochloric acid it showed greenish yellow precipitate [14].

HPTLC analysis [10] was made using silica gel 60F254 plates. Known amounts (0.3 mg) of isolated and standard mangiferin solutions were applied to the layers as band 6mm wide, 10mm from the bottom of the plate using Camag Linomat 5 automated TLC applicator with nitrogen flow. Delivery was made by syringe and was 10µl/sample. Plates developed at room temperature with ethyl acetate-methanol-water-formic acid (10:1:1:0.5) as solvent system in a Camag twin-trough glass chamber saturated for 15 min with mobile phase vapour. The plate developed was dried at 105ºC for 15 min. Peak areas for the isolated were recorded and compared with standard mangiferinat 270 nm using Camag Model-3 TLC Scanner (CAMAG, Switzerland) Win CATS 4 software. Small amount of mangiferin was taken in a melting point capillary tube (sigma) and placed in the melting point apparatus (SUNBIM). The temperature was increasedgradually. At certain temperature range (271 ºC-274 ºC) the mangiferin melted and this temperature noted as melting point of mangiferin. FTIR spectra of the isolated mangiferin were recorded and compared with standard mangiferinin an FTIR spectrometer (Nexus-870) using potassium bromide pellet. Molecular weight of isolated mangiferin was determined using ESI-MS spectrometer (Waters, UK). ESI-MS spectrometer was operated in positive ionization mode. Structural identification of isolated mangiferin was performed by 13C NMR ((DMSO-d6; 50 MHZ) and 1H NMR (DMSO-d6; 200MHZ)). DMSO-d6 used as a solvent. All the spectra were on a Bruker 200 NMR spectrometer using Topspin software.

2.3 Preparation of the complexes of Mangiferinand β cyclodextrin (β –CD)

The physical mixture of mangiferin andβ cyclodextrin was prepared in an equimolar ratio (1:1), and mixed thoroughly by use of an agate mortar, until homogenization of mixturesoccurs. Co-evaporation leads to the complex (1:1) thus obtained. Mangiferin and β cyclodextrinin the equimolar ratio (1:1) were added to an aqueous solution of 5 mL ethanol/100 mL water. The solution was kept in dark and shaken in amechanical shaker at 170 rpm at 25 °C (Tarson) for 24 h, ethanol from the reaction mixture was evaporated, the uncomplexed mangiferin was removed by filtration. After that water was evaporated under reduced pressure in a Büchi Rotavapor (Büchi, Germany) and dried in vacuum, giving the mangiferin: β cyclodextrincomplex [15].

2.4 Sample preparations for evaluating the activities
The magniferin and mangiferin: β-Cyclodextrin complex were prepared with 5 mL ethanol/100 mL water. The solution of the Mangiferin: β-Cyclodextrin (1:1) complex was prepared at concentrations of 50 μmol L^{-1}. The solutions were stirred (170 rpm) for 24 h at 25 ℃. Toxicity profile shows it is nontoxic orally even to a dose of 1g/kg b.w. of mice [16, 17].

2.5 Animals
Sprague-Dawley rats, the body weight of which was 350 to 415 g were used for the investigation. The animal experiment was carried out vide 473/01/a/CPCSEA dated 5/9/2013 of IRM, Kolkata maintain the guidelines. Animals had been reared in barrier rooms and tested frequently for multiple pathogens. They had been found to be test negative for certain parasites, including mites and pinworms.

2.6 Induction of Type 1 Diabetes
Alloxan was dissolved in 0.9% Nacl solution (200mg/kg bw of rat) and was injected intraperitoneally (i.p). We test blood glucose concentration frequently (at least twice per day) for the first 48 h after alloxan administration and allow animals unlimited access to a tasty oral sugar-containing fluid for the first week after alloxan treatment. Type-1 DM is characterized by marked, unpredictable swings in glycaemia, whereas the hyperglycaemia of Type 2 DM is more stable. If blood ketone concentration is ≥ 1.5 mM, the rat is considered to have Type 1 DM. We measure venous ketone concentration in the conscious animal by puncture of the saphenous vein. Beginning 4 h after alloxan is given, we measure blood glucose concentration approximately every 8 h for the first 2 days. If the glucose value is > 200 mg/dl, we assay blood ketone concentration. If ketone values are >1.5 mM, we initiate insulin [18]. Ultimately, if DM remains consistently well controlled, experiment is started usuallyafter 2 days fasting blood sugar level is more than 225mg/dl and remains stable.

2.7 Experimental Design for Type 1 Diabetes
Group-I served as normal control
Group-II served as diabetic control
Group III received 100 mg/kg of mangiferin: β-Cyclodextrin complexorally daily
Group IV received 200 mg/kg of mangiferin:β-Cyclodextrin complex orally daily
Group V received 40 IU/ml Insulin (Positive control)
Oral administration ofmangiferin: β-Cyclodextrin complex was carried out daily for 30 days. The blood samples were collected from each rat by retro-orbital venipuncture. Biochemical parameters were estimated in both Type 1 diabetic rats at the beginning and after 30 days of experiment.

2.8 Biochemical parameters
Biochemical parameters particularly fasting blood sugar (FBS), total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and high-density lipoprotein (HDL) level in blood serum were measured (Semi-Autoanalyzer, Microlab 300, Merck) by using reagents and diagnostic kits obtained from Merck, India.

2.9*In vitro* alpha amylase inhibitory assay
The assay was carried out following the method laid down by [10].Starch azure (2 mg) was added in a tube containing 0.2ml of 0.5 M Tris-HCl buffer (pH 6.9). Another tube was containing 0.01 M calcium chloride (substrate).The tube was then boiled for 5 min and then incubated at 37º C for 5 min. Required quantities (1mg)of mangiferin:β-Cyclodextrin complex (1:1) dissolved with 1ml of 0.1% of dimethyl sulfoxide in order to obtain concentrations of 10, 20, 40, 60, 80 and 100 µg/ml. 0.2 ml of mangiferin:β-Cyclodextrin complex of a particular concentration was added in the tube containing the substrate solution. 0.1 ml of porcine pancreatic amylase in Tris-HCl buffer (2units/ml) was then added to the tube containing the mangiferin and substrate solution and the reaction was allowed at 37º C for 10 min. The reaction was stopped by addition of 0.5 ml of 50% acetic acid. The reaction mixture was centrifuged (Eppendorf -5804 R) at 3000 rpm for 5 min at 4º C. The absorbance of resulting supernatant was measured at 595nm using spectrophotometer (Perkin Elmer Lambda 25 UV-VIS). The assay was repeated thrice. The concentration of the mangiferin: β-Cyclodextrin complex required to inhibit 50% of alpha amylase activity was the IC_{50} value**.** The α-amylase inhibitory activity was calculated as follows:
The α-amylase inhibitory activity = (Ac+) – (Ac-) – (As-Ab) / (Ac+) – (Ac-) × 100
Where, Ac+, Ac-, As, Ab are defined as the absorbance of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme), a test sample (with enzyme) and a blank (a test sample without enzyme) respectively.

2.10 *In vitro* alpha glucosidase inhibitory assay
The *in vitro* alpha glucosidase inhibitory assay was performed with standard protocol [19] Alpha glucosidase (2U/ml) was mixed with 20 µl of mangiferin:β-Cyclodextrin complex at various concentrations (10, 20, 40, 60, 80 and 100 µg/ml) and was then incubated for 5 min at 37ºC. 1mM para-nitrophenylgluco-pyanoside (pNPG) (20 µl) in 50mM of phosphate buffer (pH 6.8) was added to initiate the reaction. The mixture was incubated at 37ºC for 20 min. The reaction was stopped by addition of 50µl of 1M sodium carbonate and the final volume was made up to 150µl. Alpha glucosidase activity was determined spectrophotometrically at 405nm on a BioradMicroplate reader by measuring the quantity of paranitrophenol released. The assay was done thrice. The concentration of the mangiferin:β-Cyclodextrin complex required to inhibit 50% of alpha glucosidase activity was the IC_{50} value.

2.11 Statistical analysis
All values were expressed mean ± standard deviation. All the data were analyzed by one way analysis of variance followed by multiple comparison test (Tukey's test) at the 5% level of significance. $P < 0.05$ was considered statistically significant. *In vitro* inhibitory assay statistical difference and linear regression analysis were performed using Graphpad prism 5 statistical software.

3. Results
Mangiferin (Figure 1), a natural glucosylxanthone [14]. The HPTLC analysis showed that retention factor (R_f)of standard and isolated mangiferin compound were found to be ≈ 0.50 and ≈ 0.51 respectively (Figure 2). Mangiferin is a yellow powder with structure C_{19} H_{18} O_{11}, melting point: 271ºC-274ºC.
FTIR spectrum (Figure 3) results of isolated mangiferin compound showed that peaks at 3316 cm-1 indicated presence of secondary OH- bond, peak at 2942 cm-1 showed presence of C-H anti-symmetric stretching, peak at 2830 cm-1, 1655 cm-1, 1450 cm-1 and 1115 cm-1 indicated presence of C-H

symmetric stretching, C-O stretching, CH-CH bending and C-O bond. Peak at 1023 cm-1 showed presence of C-C stretching in the mangiferin structure.

^{1}H NMR and ^{13}C NMR (DMSO-d_6 solvent)-^{1}H NMR (DMSO-d_6): δ 13.78 (1H, 1-OH), 10.57 (2H, 6,7-OH), 9.85 (1H, 3-OH), 4.90 (2H, 3',4'-OH), 4.58 (1H, 6'-OH), 3.73 (1H, 2'-OH), 7.39 (1H, 8-H), 6.88 (1H, 5-H), 6.39 (1H, 4-H). ^{13}C NMR (DMSO-d_6): (Ring A): δ 162.26 (1-C), 108.56 (2-C), 164.31 (3-C), 93.74 (4-C), 156.70 (4a-C), 101.79 (8b-C). (Ring B): 151.25 (4b-C), 103.10 (5-C), 154.50 (6-C), 144.21 (7-C), 108.11 (8-C), 112.21 (8a-C), 179.57 (C=0). Additional signals due to glucopyranosyl carbons: δ 82.06 (5'-C), 79.48 (3'C), 73.58 (1'-C), 71.14 (4'-C), 70.73 (2'-C), 62.00 (6'-C). MS (m/z,% intensity): m/z 423 $[M+H]^{+}$

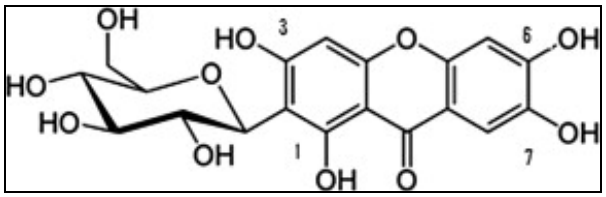

Fig 1: Structure of Mangiferin

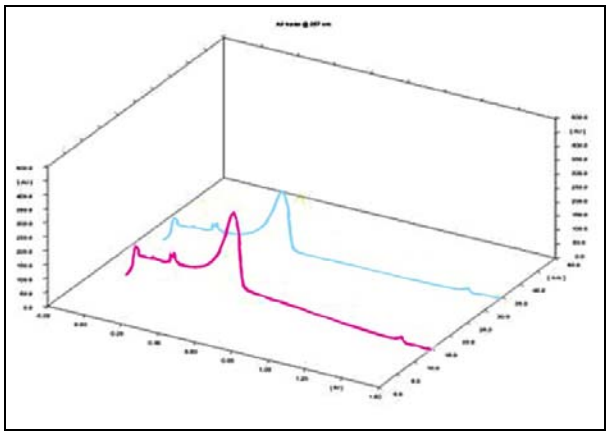

Fig 2: HPTLC Analysis of Standard and Isolated Mangiferin.

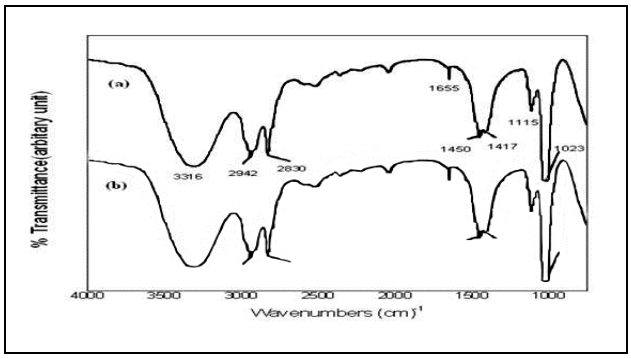

Fig 3: (a) FTIR Spectrum of Isolated Mangiferin
(b) FTIR Spectrum of Standard Mangiferin

3.1 Physicochemical characterization of Mangiferin:β-Cyclodextrin complex

Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared spectroscopy (FTIR) DSC analysis was carried out for mangiferin, β-Cyclodextrin and magniferin:β-cyclodextrin complexwith a DSC-60 calorimeter (range 25–500 °C) (Shimadzu, Kyoto, Japan). The temperature was calibrated using α-alumina powder. Samples (5.0–10.0 mg) were placed in standard aluminum pans and measurements were being performed at a heating rate of 5 °C min^{-1} from 25 to 400 °C in a dynamic nitrogen atmosphere (flow rate = 20 mL/min) (Figure 4).The FTIR spectra of mangiferin, β-Cyclodextrin and magniferin:β-cyclodextrin complex were recorded at room temperature in a spectral region between 4000 and 500 cm^{-1}. Samples were prepared as small pellets by mixing each of them with KBrin a mortar (1:100) and then the resultant mixture is pressed. A blank KBr disc was also used as a background (Figure 5).

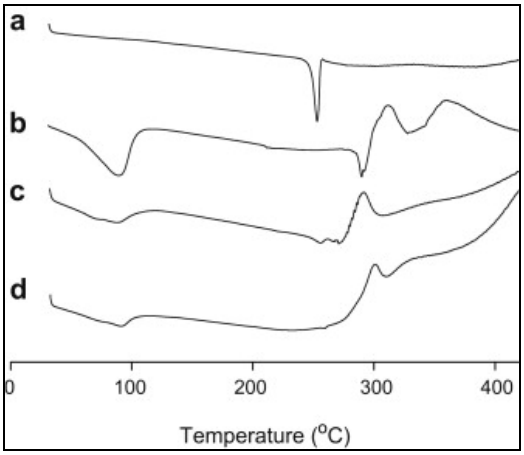

Fig 4: DSC thermograms of: a) mangiferin; b) β-Cyclodextrin; c) mangiferin and β-Cyclodextrin physical mixture in a molar ration 1:1 and d) mangiferin: β-Cyclodextrin (1:1) complex obtained by co-evaporation method.

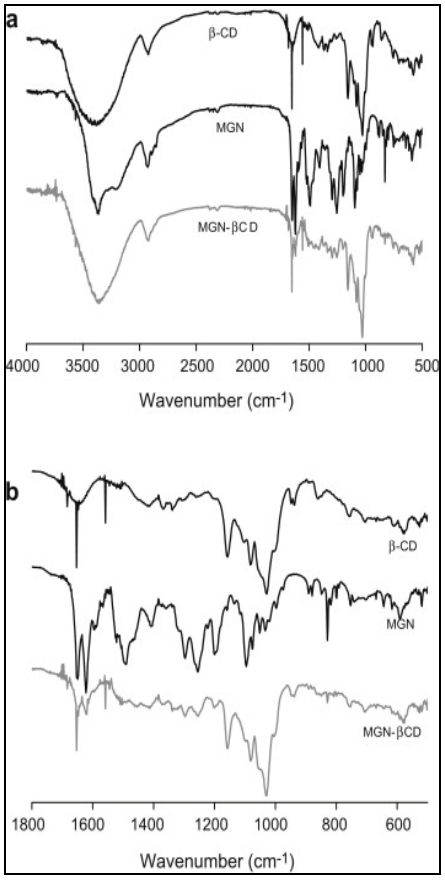

Fig 5: (a) FTIR spectra of mangiferin, β-Cyclodextrin and mangiferin:β-cyclodextrin complex obtained by co-evaporation method;
(b) Amplification of FTIR spectra at 1800–500 cm^{-1} region mangiferin, β-Cyclodextrin and.magniferin:β-cyclodextrin complex

3.2 Alpha amylase and alpha glucosidase in hibitory effects of mangiferin: β-Cyclodextrin complex

In addition, comparing alpha amylase and alpha glucosidaseinhibitoryeffects of mangiferin:β-Cyclodextrin complex(Figure 6), it was observed that mangiferin exhibited appreciable effect alpha amylase inhibitory effects (IC_{50} value 66.35±1.4µg/ml) and alpha glucosidase inhibitory effects (IC5037.88±1.5µg/ml) when compared with acarbose (IC_{50} 83.33±1.2µg/ml).

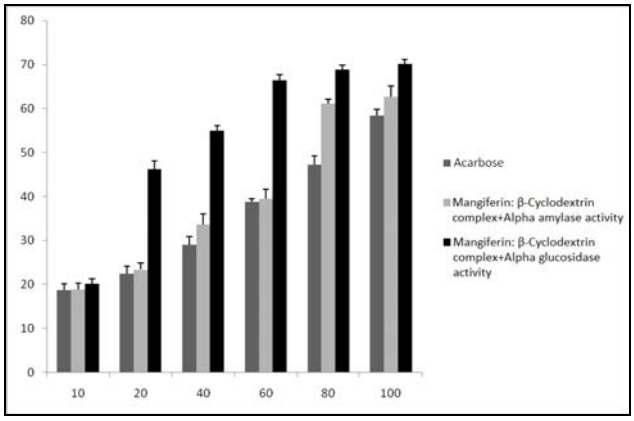

Fig 6: Alpha amylase and alpha glucosidaseinhibtory effects of mangiferin:β-Cyclodextrin complex

In Type 1 diabetic model, the body weight was slightly increased in normal control rats compared to initial body weight whereas alloxan-induced diabetic rats showed loss of body weight (192±1.72g) after 30 days as compared with initially weight of diabetic rats (201.8±2.69g). Body weight of diabetic rats was restored significantly (P<0.05) by treating with insulin (40 IU/ml) in Type 1 diabetic model respectively. In Type 1 diabetic model, body weight of diabetic rats was restored by treating with mangiferin: β-Cyclodextrin complex (at a dose 100 and 200 mg/kg, orally) daily for 30 days when compared with diabetic control (Table 1).

In Type 1 diabetic model, insulin showed significant anti-diabetic and hypolipidemic effects. But the mangiferin (at a dose 100 and 200 mg/kg, orally) showed anti-diabetic and hypolipidemic effects in Type 1 diabetic model on being compared with insulin (Table 2).

Table 1: Body weights of streptozotocin-induced Type 1 diabetic rats (g) after treatment with mangiferin: β-Cyclodextrin complex

Group	Initial body weight	Final body weight
Normal control	402±5.72	405.8 ± 7.87
Diabetic control	352.2±2.42	272.6±4.33
100 mg/kg of mangiferin	382.3± 4.33	370.4±3.32
200 mg/kg of mangiferin	388.4±3.42	366.2±2.54*
40 IU/ml Insulin	380.4±3.45	377.7±4.53*

*Significant (P<0.05) compared with treated diabetic groups Vs Diabetic control. Values are expressed as mean± S.D.

Table 2: Effect of mangiferin: β-Cyclodextrin on FBS, TC, TG, HDL, LDL and VLDL in normal and Type 1 diabetic model rats for one month

	Biochemical Parameters					
Groups	**FBS**	**TC**	**TG**	**HDL**	**LDL**	**VLDL**
Group I						
0 Day	92.4±3.62	158.6±2.47	93.4±2.64	36.7±1.72	100.6±5.61	22.7±0.75
30 Days	95.6±2.67	160.4±2.60	95.6±3.42	38.2± 1.64	98.4±4.76	25.5±0.71
Group II						
0 Day	258.2±1.40†	286.3±0.61†	176.1±5.46†	37.4±1.61†	212.6±6.76†	36.3±0.75†
30 Days	273.1±1.65†	268.4±1.67†	188.4±4.40†	36.2±1.66†	196.9±6.95†	37.2±0.69†
Group III						
0 Days	272.4±2.40	268.0±0.58	185.6±3.82	39.6±1.88	196.6±8.38	34.2± 0.57
30 Days	258.6±1.86	250.3±1.57	170.4±3.37	40.1±1.76	177.6±7.82	30.9±1.18
Group IV						
0 Days	286.1± 4.32	262.0± 0.55	185.6±366	44.2±1.65	179.3±7.66	36.6± 2.40
30 Days	245.4±3.46*	244.5±1.50*	158.3±4.42*	46.2±1.99*	165.8±8.76*	31.5±2.20*
Group V						
0 Days	280.6±2.44	246.4±1.70	173.6±4.82	44.4±1.57	166.1±7.60	34.3±2.54
30 Days	183.6±2.46*	215.7±1.14*	172.6±3.20*	46.6± 1.43*	138.4±8.15*	34.6±3.12*

Values are expressed as mean ± S.D. n=6/group. Group 1: normal control, Group 2: (Diabetic control), Group 3: (Diabetic+10mg/kg mangiferin: β-Cyclodextrin), Group 5: (Diabetic+20mg/kg mangiferin: β-Cyclodextrin), Group 5: positive control (Diabetic+40 IU/ml Insulin).

† P<0.05 Group1 Vs Group 2. * P<0.05 treated diabetic groups Vs diabetic control group

4. Discussion

The present study was done to investigate the anti-diabetic and hypolipidemic effects of mangiferin, β-Cyclodextrin complex in Type 1 diabetic rats model. Mangiferin(1,3,6,7-tetrahydroxy-2-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]xanthen-9-one) is poorly absorbable in water and absorption is erratic in oral route. By complexing with β-Cyclodextrin the effects were observed in Type 1 diabetic rats, the complex being given orally. *Mangifera indica* is a freely available species in India; its stem bark is documented containing mangiferin. Here an attempt is made from easy available source, i.e. leaves. When compared with the FTIR spectra, Electron-Spray Ionization (ESI) - Mass spectrum, 1H and 13C NMR spectrum of standard mangiferin, the sample (isolated mangiferin) showed significant similarity.

Insulin was the drug for Type 1 diabetic model rats. It is well established that insulin activate glucose uptake in various tissues including adipocytes and muscles, nerve cells etc. In the present study indicated that daily administration of mangiferin: β-Cyclodextrincomplex (at a dose of 200 mg/kg, orally) up to 30 days showed anti-diabetic and hypolipidemic effects in Type 1 diabetic rats. This is an interesting, as the continuous use of mangiferin in the Type 1 diabetic rats will not resultin hypoglycemic episodes.

Our group [10] had earlier documented in Type 2 diabetic model rats, an increase in blood sugar levels in diabetic rats were prevented by use of mangiferin (at a dose 10 and 20 mg/kg, i.p). The effects were comparable to the standard drug

glibenclamide. This effect is due to potentiating the effect of insulin and more of its release from the bound form.

Diabetes is usually accompanied with dyslipidaemia particularly hyperlipidaemia. Therefore ideal treatment of diabetesis selection of an agent preferably natural in origin yet cheap and acceptable which will have a favorable effect on lipid profiles. High level of TC and LDL are major coronary risk factors [20]. TG itself is independently related to coronary heart disease [21, 22]. Dyslipidaemia may play an important role in occurrence of premature and severe atherosclerosis, which affects patients with diabetes [23].

In this study, mangiferin: β-Cyclodextrin complex (at a dose 200 mg/kg orally) showed significant reduction in TC, TG, LDL, VLDL levels and increased level of HDL in Type 1 diabetic model rats and thus directs further researches to be used as hypoglycaemic and hypolipidaemic agent in Type 1 diabetes.

In Type 1 diabetic model, body weight of diabetic rats was partially restored (p=0.046) by treating with mangiferin: β-Cyclodextrin complex (at a dose 20 mg/kg, orally) daily for 30 days in comparison to with insulin (40IU/ml) in Type 1 diabetic model. Several studies reported that *Mangifera indica* and its polyphenol compound mangiferin possess anti-diabetic activity. Oral administration of aqueous extract (1g/kg) of *Mangifera indica* showed hypoglycemic activity in diabetic mice [17, 24]. Stem bark extract of *Mangifera indica* showed potent antioxidant effects both in vitro and in vivo [25]. However the researches are mainly concentrated on Type 2 diabetic animals and the intraperitoneal mode of application of stem bark yield mangiferin [26]. Plant bio active effect depends on many factors and of the agronomic conditions soil is most important. Here the leaves are collected from upper altitudes of upper Assam and Arunachal where particular soil may have some potentiating effects which need to be further explored. Cyclodextrin has a special ability to complex with a variety of molecules and thus has effects on their stability, bioavailability, solubility, protection to light-induced decomposition and also to suppress unpleasant odors or tastes and achieve a controlled release of certain constituents), but still increase the antioxidant activity of many compounds.The exact cause of its effects on Type 1 diabetes is unknown. It may be due to its anti inflammatory effectswith respect to NFκB, PPARγ and the immune system,effects on angiogenesis, oxidative stress through Nrf2/ARE signalling, ROS elimination and catalase activity [27].Mangiferin is protective to diabetic cardiac neuropathy and nephropathy. In diabetic mice it hinders the hypoglycaemic and atherogenic changes. The accumulating evidences suggest that both pancreatic and extra pancreatic mechanisms might be involved in its anti-diabetic or anti-hyperglycemic action [28, 29]. The reduction of triglycerides following administration with mangiferin would also facilitate the glucose oxidation and utilization and subsequently the reduction of hyperglycemia [30]. Mangiferin showed anti-diabetic activity in KK-Ay mice [31].

Components of stem bark extract of *Mangiferaindica* such as terpenoids, cathechin, fatty acids and microelements exhibited antioxidant properties [32, 33]. Aqueous extract from the bark of *Mangiferaindica* was used as a food supplement in Cuba showed potent in vitro and in vivo antioxidant activities [34].Mangiferin improves serum lipid profiles in overweight patients with hyperlipidaemia [35].

The plant based alpha amylase and alpha glucosidase inhibitor offers a prospective therapeutic approach for the management of post-prandial hyperglycemia [36]. In the present study, mangiferin exhibited appreciable alpha amylase inhibitory and alpha glucosidase inhibitory effects when compared with acarbose. Therefore, mangiferin could be useful in management of post-prandial hyperglycemia due to inhibition of both alpha amylase and alpha glucosidase enzymes.

5. References

1. American Diabetes Association (ADA).Diagnosis and classification of diabetes mellitus. Diabetes Care, 2006; 29(1):S43:48.
2. Ramachandran A, Snehalatha C, Satyavani K, Sivasankari S, Vijay V. Type 2 diabetes in Asian-Indian urban children. Diabetes care, 2003; 26(4):1022-5.
3. Meisinger C, Thorand B, Schneider A, Stieber J, Döring A, Löwel H. Sex differences in risk factors for incident type 2 diabetes mellitus: the MONICA Augsburg cohort study. Archives of internal medicine, 2002; 162(1):82-9.
4. Mohan V, Sandeep S, Deepa R, Shah B, Varghese C. Epidemiology of type 2 diabetes: Indian scenario. Indian Journal of medical Research, 2007; 125(3):217.
5. Mitra A, Bhattacharya D, Roy S. Dietary influence on TYPE 2 Diabetes (NIDDM).J Hum Ecol, 2007; 21:139-47.
6. Gupta SS. Prospects and perspectives of natural plant products in medicine. Indian J Pharmacol, 1994; 26(1):1-2.
7. Alarcon-Aguilara FJ, Roman-Ramos R, Perez-Gutierrez S, Aguilar-Contreras A, Contreras-Weber CC, Flores-Saenz JL. *et al.* Study of the anti-hyperglycemic effect of plants used as antidiabetics. Journal of Ethnopharmacology, 1998; 61(2):101-10.
8. Subramoniam A. The problems and prospects of plant drug research in India: Pharmacological evaluation of ecotypes in herbal drug development. Indian J Pharmacol, 2001; 33:145-146.
9. Wauthoz N, Balde A, Balde ES, Van Damme M, Duez P. Ethnopharmacology of *Mangifera indica* L. bark and pharmacological studies of its main C-glucosylxanthone, mangiferin. International Journal of Biomedical and Pharmaceutical Sciences, 2007; 1(2):112-9.
10. Dineshkumar B, Mitra A, Manjunatha M. Studies on the anti-diabetic and hypolipidemic potentials of mangiferin (xanthone glucoside) in streptozotocin-induced Type 1 and Type 2 diabetic model rats. International Journal of Advances in Pharmaceutical Sciences, 2010; 1(1).
11. Fernández-Ponce MT, Casas L, Mantell C, Rodríguez M, de la Ossa EM. Extraction of antioxidant compounds from different varieties of *Mangifera indica* leaves using green technologies. The Journal of Supercritical Fluids, 2012; 72:168-75.
12. Kulkarni VM, Rathod VK. A novel method to augment extraction of mangiferin by application of microwave on three phase partitioning. Biotechnology Reports, 2015; 6:8-12.
13. Mohan CG, Deepak M, Viswanatha GL, Savinay G, Hanumantharaju V, Rajendra CE, *et al.* Anti-oxidant and anti-inflammatory activity of leaf extracts and fractions of *Mangifera indica*. Asian Pacific journal of tropical medicine, 2013; 6(4):311-4.
14. Bhatia VK, Ramanathan JD, Seshadri TR. Constitution of mangiferin. Tetrahedron, 1967; 23(3):1363-8.
15. da Rocha Ferreira F, Valentim IB, Ramones EL, Trevisan MT, Olea-Azar C, Perez-Cruz F, *et al.* Antioxidant activity of the mangiferin inclusion complex with β-cyclodextrin. LWT-Food Science and Technology, 2013; 51(1):129-34.
16. Organization for Economic Cooperation and

Development (OECD). OECD Guidelines for testing of Chemicals. Acute Oral Toxicity: Up–and–Down Procedure. [Adopted 2001; 17, 2–26.
17. Aderibigbe AO, Emudianughe TS, Lawal BA. Antihyperglycaemic effect of *Mangifera indica* in rat. Phytotherapy Research, 1999; 13(6):504-7.
18. Federiuk IF, Casey HM, Quinn MJ, Wood MD, Ward KW. Induction of type-1 diabetes mellitus in laboratory rats by use of alloxan: route of administration, pitfalls, and insulin treatment. Comparative medicine, 2004; 54(3):252-7.
19. Pistia-Brueggeman G, Hollingsworth RI. A preparation and screening strategy for glycosidase inhibitors. Tetrahedron, 2001; 57(42):8773-8.
20. Temme Eh, Vaqn HPG, Schouten EG, Kesteloot H. Effect of plant sterol-enriched spread on serum lipids and lipoproteins in middlyhypercholesterolaemic subjects.Acta Cardiology, 2002; 57:111-115.
21. Bainton D, Miller NE, Bolton CH, Yarnell JW, Sweetnam PM, Baker IA, *et al.* triglyceride and high density lipoprotein cholesterol as predictors of ischaemic heart disease in British men The Caerphilly and Speedwell Collaborative Heart Disease Studies. British heart journal, 1992; 68(7):60-6.
22. El-Hazmi MA, Warsy AS. Evaluation of Serum Cholesterol and Triglyceride Levels in 1–6-year-old Saudi Children. Journal of Tropical Pediatrics, 2001; 47(3):181-5.
23. Ravi K, Rajasekaran S, Subramanian S. Antihyperlipidemia effect of Eugenia Jambolana seed kernel on streptozotocin induced diabetes in rats. Food chemToxicol, 2005; 43:1433-1439.
24. Aderibigbe AO, Emudianughe TS, Lawal BAS. Evaluation of anti-diabetic action of Mangiferaindica in mice. Phytother Res, 2001; 15:456-458.
25. Martinez G, Giuliani A, Leon OS, Perez G, Nunez-Selles AJ. Effects of Mangiferaindica L extract (QF808) on protein and hepatic lipoperoxidation.Phytother Res, 2001;15:581–585.
26. Singh AK, Sharma VK, Kumar YA, Kumar SS, Sinha SK. Phytochemical and pharmacological investigations on mangiferin. Herba Polonica, 2009; 55(1):126-39.
27. Gold-Smith F, Fernandez A, Bishop K. Mangiferin and Cancer: Mechanisms of Action. Nutrients, 2016; 8(7):396.
28. Muruganandan S, Gupta S, Kataria M, Lal J, Gupta PK. Mangiferin protects the streptozotocin-induced oxidative damage to cardiac and renal tissues in rats. Toxicology, 2002; 176(3):165-73.
29. Muruganandan S, Srinivasan K, Gupta S, Gupta PK, Lal J. Effect of mangiferin on hyperglycemia and atherogenicity in streptozotocin in diabetic rats. J Ethnopharmacol, 2005; 97:497-501.
30. Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose-Fatty acid cycle, its role of in insulin sensitivity and metabolic disturbances in diabetic mellitus. Lancet 1963; 1:785-789.
31. Ichiki H, Miura T, Kubo M, Ishihara E, Komatsu Y, Tanigawa K, *et al.* New antidiabetic compounds, mangiferin and its glucoside. Biol Pharm Bull, 1998; 21: 1389-1390.
32. Beltz LA, Bayer DK, Moss AL, Simet IM. Mechanisms of cancer prevention by green and black tea polyphenols, Anticancer Agents Med Chem, 2006; 6:389–406.
33. Cholbi MR, Paya M, Alcaraz MJ. Inhibitory effect of phenolic compounds on CCL_4-induced microsomal lipid peroxidation. Experientia, 1991; 47:195–199.
34. Sanchez GM, Re L, Giuliani A, Nunez-Selles AJ, Davison GP, Leon-Hernandez OS. Protective effects of *Mangiferaindica* L. extract, mangiferin and selected antioxidants against TPA-induced biomolecules oxidation and peritoneal macrophage activation in mice. Pharmacol Res, 2000; 42:565–573.
35. Na L, Zhang Q, Jiang S, Du S, Zhang W, Li Y, *et al.* Mangiferin supplementation improves serum lipid profiles in overweight patients with hyperlipidemia: a double-blind randomized controlled trial. Scientific reports, 2015; 5:10344.
36. McCue P, Vattem D, Shetty K. Inhibitory effect of clonal oregano extracts against porcine pancreatic amylase *in vitro*. Asia Pac J ClinNutr, 2004; 13:401-408.