PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTI DIABETIC ACTIVITYOF ROOTS *MANGIFERA INDICA* LINN.

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Summary

Mangifera indica Linn (Anacardiaceae) is well known for curing a variety of ailments such as abscesses, broken horn, tumour, snakebite, stings, datura poisoning, heat stroke, anthrax, blisters and wounds in the mouths.
The present study was to evaluate the preliminary phytochemical screening and antidiabetic activity of aqueous & alcoholic extract of *Mangifera indica* Linn root in alloxan-induced diabetic rats. Albino rats were rendered diabetic by alloxan (150 mg/kg, intraperitoneally). The aqueous and ethanolic extracts were orally administered to diabetic rats at 200 and 400 mg/kg doses daily for 15 days to determine antidiabetic activity.
The phytochemical screening of alcoholic and aqueous extracts shows the presence of carbohydrates, glycoside, phenolic compounds, tannin and flavonoids. The fasting blood glucose level decreases by 29.5, 58.9 % (aqueous extract) and 38.8, 48.1 % (ethanolic extract) after 15th day in diabetic rats treated with a different doses of 200 mg and 400 mg/kg body weight respectively.
In conclusion, the present study using biochemical assays pertaining to blood glucose level of different animal models reveals that aqueous and alcoholic extract of root of Mangifera *indica* has moderate hypoglycemic and antidiabetic potential.

Keywords: *Mangifera indica*; antidiabetic activity; alloxan; diabetic rats; Mango

Introduction

Mangifera indica Linn (Anacardiaceae) commonly known as mango, chosa, am, is native to southern Asia, especially Burma and eastern India. It spread early on to Malaya, eastern Asia and eastern Africa. Mangos were introduced to California (Santa Barbara) in 1880. In this day and age, *M. indica* resides in most tropical biotopes in India, Southeast Asia, Malaysia, Himalayan regions; Sri lanka, Africa, America and Australia [1-3].Mangos basically require a frost-free climate. The mango must have warm, dry weather to set fruit. The plant is used in ophthalmia and eruption, hemorrhage of uterus, lungs or intestine. The ripe fruit is laxative, diuretic. The dried mango peel can be used as a fuel for biogas plant. The all parts are used to treat abscesses, broken horn, tumour, snakebite, stings, datura poisoning, heat stroke, anthrax, blisters and wounds in the mouths.

The seed kernel extracts have antibacterial activity against *Bacillus subtilis, Staphylococcus albus and Vibrio cholerae* [4, 5, 6] and antifungal activity [5]. An alcoholic extract of the seed kernel of *Mangifera indica* has anti-inflammatory activity [7]. Mangiferin was found to be effective in controlling herpes simplex virus type 2, in vitro [8, 9]. The induction of interferon release from the macrophages. The mangiferin have Immunomodulatory activity [9, 10, 11]. A 50% ethanolic extract of the leaves has hypoglycemic activity.

The most abundant terpene hydrocarbons in fruits are limonene, β- myrcene and cis and trans- ocimene and most abundant oxygenated compound include methyl butanoate, ethyl 2 methyl butanoate, α- terpineol. The fruit pulp contains vitamins A and C, β–carotene and xanthophylls. An unusual fatty acid cis 9, cis 15- octadecadienoic acid (mangiferic acid, 5.4% of total acyl groups) is present in the pulp lipids of mango fruit from philipines, whereas a common octadecadienoic acid, linoleic acid [12], is in minor quantity. The leaves contain a petacyclin triterpene alcohol, indicenol[13], besides taraxone, taraxerol, fridelin, lupeol and β- sitosterol[14]. Mango leaves contain several sugars and amino acids. Some esters of benzophenone glycosides and kinic and shikmic acids has also been reported from the leaves [15]. The leaf and flower yield an essential oil containing humulene, elemene, ocimene, linalool [16], camphene [17], nerol.

The stem bark contains the mangiferin [18] and triterpens mangophanol (nopan-28-al-mangoleanone (olcanan-3-one) and mangiferolic acid, dihydro mangiferolic acid, mangiferonic acid, 5α stigmastane-3β-6α-diol. Indicoside A and B, manghopanal, mangoleanone, taraxerol, friedelin, cycloaratan-3 beta-30-diol and derivatives, mangsterol, manlupenone,mangocoumarin,n-tetacosane,n-heneicosane,n-triacontane and mangiferolic acid methyl ester and Mangostin, 29-hydroxymangiferonic acid and mangiferin have been isolated from the stem bark of *mangifera indica*. The flowers yielded alkyl gallates such as gallic acid, ethyl gallate, methyl gallate, n-propylgallate, n-pentyl gallate, n-octyl gallate, 4-phenyl-nbutylgallate, 6-phenyl-n-hexyl gallate and dihydrogallic acid. The roots contains the chromones, 3–hydroxy-2-(4'-methylbenzoyl)-chromone and 3-methoxy-2-(4'-methylbenzoyl)-chromone**.**

Materials and methods:

Collection and authentication of plant

The root of *Mangifera indica* Linn was collected freshly from Sonipat (Haryana) in the month of December 2008 depending upon its easy availability. It was authenticated by Dr. H.B. Singh, at National Institute of Science Communication and Information Resources (NISCAIR), New Delhi (letter no. NISCAIR/RHMD/Conslt/2008-09/1121/152). The root of *Mangifera indica* was subjected to shed drying and further crushed to powder, and then the powder was passed through the mesh 40.

Drugs and chemicals

Metformin (Ranbaxy Pharma. Ltd. India) obtained as a gift sample, alloxan monohydrate (Central Drug House Pvt. Ltd., Delhi), glucose estimation kit (Merck Specialities Pvt. Ltd., India), Ethanol (Merck, India) and d-glucose (S.D. Fine- Chem. Ltd., India), were purchased from respective vendors.

Preparation of aqueous & ethanolic extract of stem of Mangifera indica

Weighed quantity (500 g) of air-dried powder of the root of *Mangifera indica* was successively macerated with petroleum ether (60:80), alcohol and water at room temperature for 72 hours and filtered. The filtrate was concentrated using rotary evaporator. The alcoholic and aqueous extract was dissolved in distilled water to prepare the drug solution of different

concentration of 200 mg/kg and 400 mg/kg body weight of the animals and used for pharmacological studies.

Preliminary phytochemical screening

The preliminary phytochemical analysis for alcoholic and aqueous extract was carried out for the alkaloid (Mayer's, Hager's, Dragendorff's and Wagner's test), carbohydrates (Molisch's test), flavonoids (Shinoda test), sterols (Salkowski, Liberman-Burchard test, Libermann's test), phenolic compounds & tannins, glycosides, saponins and free amino acids.

Experimental animals and research protocol approval

Swiss albino rats (150-200 g) of either sex were purchased from National Agriculture University, Hissar, India. Animals were maintained at a temperature of 23 ± 2°C and relative humidity of 45–55% under 12-h light: 12-h dark cycle. The animals had free access to food pellets (Raju Oil Mills, Sonepat, India) and water was available *ad* libitum. The Study was conducted after obtaining ethical committee clearance from the Institutional Animal Ethics Committee of Hindu College of Pharmacy, Sonepat. No: 585/02/C/CPCSEA in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA), India.

Induction of experimental diabetes and determination of serum glucose level

The selected animals, weighing between 150-200 g were fasted overnight were administered with alloxan 150 mg/kg i.p route. Fasting blood sugar levels were determined on 5^{th} day after administering alloxan to confirm stable hyperglycemia.

The diabetic rats after confirmation of stable hyperglycemia were divided into different groups of 6 rats each. That day was considered as the 0^{th} day. Drug and doses were administered as mentioned as below.

Experimental procedure

The rats were divided into following groups of six animals each:

Group I: Normal control (untreated rats)
Group II: Diabetic control rats
Group III: Metformin (50 mg/ml) treated diabetic rats
Group IV: Aqueous extract (200 mg/kg) treated diabetic rats
Group V: Aqueous extract (400 mg/kg) treated diabetic rats
Group VI: Alcoholic extract (200 mg/kg) treated diabetic rats
Group VII: Alcoholic extract (400 mg/kg) treated diabetic rats

The drugs, dissolved in 1 ml of distilled water, were administered orally via a standard orogastric cannula. Antihyperglycemic activity in diabetic rats was assessed by fall in Fasting Blood Glucose. Blood samples were collected from overnight fasted rats at 0 day, 7^{th} day and 15^{th} day by retro-orbital venepuncture technique using microcapillary after given the drugs to determine blood glucose levels. Blood samples were collected in anticoagulant (sodium fluoride and potassium oxalate) vials for plasma. The blood was then centrifuged at 2500 rpm for 10 min to obtain clear serum. Blood glucose in plasma was estimated by glucose oxidase method.

Statistical analysis

Results were expressed as mean ± S.E.M. Statistical analysis was done by using repeated measure analysis of variance (ANOVA) followed by Dunnett's test and two-way ANOVA followed by Tukey's test at 5%. The data were analyzed with Graph Pad Prism 4.0 v for Windows (Graph Pad Software, San Diego, CA, USA). The difference showing a *P* level of 0.05 or lower was considered to be statistically significant.

Results & Discussion:

Preliminary phytochemical screening

The pharmaceutically important groups are the organic constituents which forms the basis of the pharmacological actions. The phytochemical screening of alcoholic and aqueous extracts shows the presence of carbohydrates, glycoside, phenolic compounds, tannin and flavonoids. The results are shown in Table 1.

Table 1 – Qualitative chemical examination of alcoholic and aqueous extracts

Plant constituents Test/ Reagent used	**Alcoholic extract**	**Aqueous extract**
Alkaloids		
Dragendroff's test	-	-
Mayer's reagent test	-	-
Hager's reagent test	-	-
Wagner's reagent test	-	-
Carbohydrates & Glycosides		
Molisch's test	+	+
Fehling's test	+	+
Benedict test	+	+
Borntrager's test	-	-
Keller-Killiani test	+	+
Sterols		
Salkowski reaction	+	-
Liebermann- Burchard's reaction	+	-
Hersch-Sohn's Reaction	+	-
Saponins		
Foam test	-	-
Sodium bicarbonate test	-	-
Phenolic compounds & Tannins		
Ferric chloride test	+	+
Lead acetate test	+	+
Amino acids		
Ninhydrin reagent test	+	+
Proteins		
Millon's test	+	-

Biuret test	+	-
Flavonoids		
Shinoda/Pew test	+	+
Ammonia test	+	+

Blood glucose and body weight

Blood samples from the experimental mice were collected by retro-orbital plexus technique using heparinised capillary glass tubes. The collected blood samples were placed in fluoride tube (1.5ml). The serum was separated by centrifugation using Eppendorf's Cryocentrifuge (model no. 5810, Germany), maintained at 4^0C and run at speed of 7000 rpm for 15 min. 10 micro liters of serum and 1ml of working reagent (GOD/POD) were mixed and incubated for 15 min at 37^0C. The absorbance of sample (*A*s) and standard (*A*std) (CDH New Delhi, India) solutions were measured against blank at 505 nm using UV–Vis Spectrophotometer (Shimadzu 1700 IPC).

Glucose level was estimated by using the formula:

Glucose (mg/dl) = As/Astd × 100;

Whereas *A*s = sample reading; *A*std = standard reading

Treatment with the alcoholic and aqueous extracts exhibited remarkable glycemic control in diabetic rats as evident by significant decrease ($p < 0.001$) in the levels of Fasting Blood Glucose. The Aqueous extract and the alcoholic extracts of *Mangifera indica* roots reduce the blood glucose level moderately in diabetic rats. Metformin was used as reference drug in diabetic models for positive control. However, the higher concentration of the extract was used against the reference drug because there may be a small amount of active substance present in the extract.

The Fasting Blood Glucose levels decreases by 29.5, 58.9% (aqueous extract) and 38.8, 48.1 % (alcoholic extract) after 15^{th} day in diabetic rats treated with a different doses of 200 mg and 400 mg/kg body weight respectively (Table 2 & Figure 1).

Table 2: The effects of 2-week treatment with aqueous and alcoholic extracts of *Mangifera indica* on blood glucose levels (mg/dl) after alloxan (150 mg/kg i.p.) induced diabetes in rats

Group No.	**Treatment**	**Blood glucose level (mg/dl in days)**		
		0 day	**7^{th} day**	**15^{th} day**
I	Control	80.5 ± 2.12	82.5 ± 2.12	84.5 ± 2.12
II	Diabetic control	231.66 ± 1.4	244.3 ± 1.14	265.16 ± 1.53
III	Metformin (50 mg/kg)	262.66 ± 5.8	161.8 ± 1.07	133.3 ± 1.14
IV	Aqueous extract (200 mg/kg)	251±1.06	225.66±1.47	213.3±1.05
V	Aqueous extract (400mg/kg)	300± 1.4	265.5±1.1	224.3± 1.40**
VI	Alcoholic extract (200 mg/kg)	274.8±1.19	242.5±0.76	224.6±1.11
VII	Alcoholic extract (400mg/kg)	314±1.18	275.5± 0.76	252.8±0 .44**

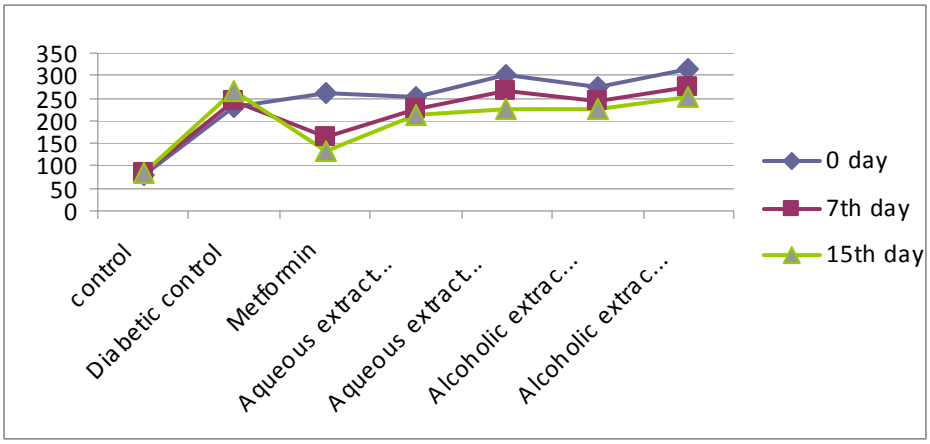

Fig. 1: Effect of Alcoholic and Aqueous extract on serum glucose level in alloxan induced diabetic rats

Values are mean ± S.E.M. n = 6 in each group; statistical analysis by one-way ANOVA; p value <0.05 followed by Dunnett's multiple comparison test; p value **<0.

Table 3: The effect of 2-week treatment with aqueous and alcoholic extracts of *Mangifera indica* on body weight (g) after alloxan (150 mg/kg i.p.) induced diabetes in rats

Group No.	Treatment	Average body weight (g)		
		0^{th} day	7^{th} day	15^{th} day
I	Control	160.2 ± 1.9	175.5 ± 3.96	181.4 ± 3.2
II	Diabetic control	175 ± 2.1	170.2 ± 2.4	161.4 ± 3.1
III	Metformin (50 mg/kg)	180.3 ± 4.2	176.5 ± 3.6	189.4 ± 3.4
IV	Aqueous extract (200 mg/kg)	136.6±15.2	135±13.2	138.3±14.4
V	Aqueous extract (400mg/kg)	146.6±15.2	155±15	160±17.3
VI	Alcoholic extract (200 mg/kg)	125±21.7	121.6±12.5	123.3±15.2
VII	Alcoholic extract (400 mg/kg)	120±26.4	123.3±20.8	125±26.4

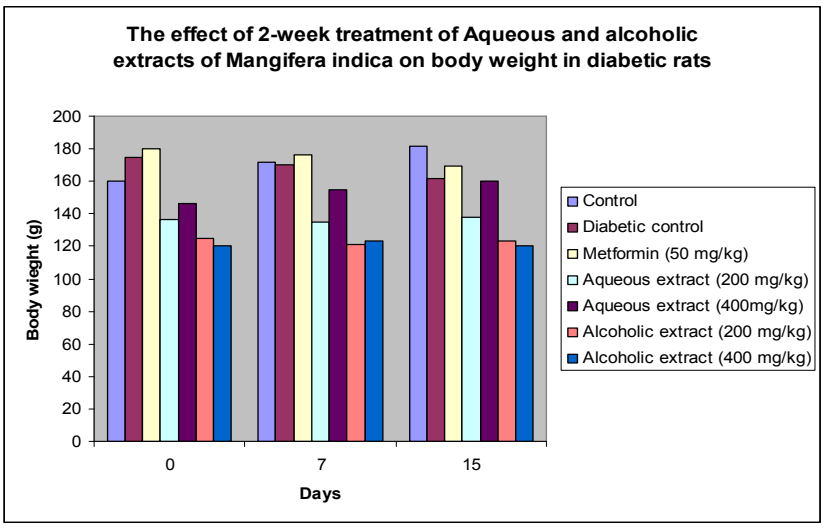

Fig. 2: Effect of alcoholic and aqueous extract on body weight in alloxan induced diabetic rats.

A significant increase ($p < 0.001$) in body weight was also observed in diabetic rats (Table 3 & Figure 2). The weights of diabetic rats increase significantly with the increase in dose of the extracts and with increase in time.

Conclusion

The present study using biochemical assays pertaining to Blood Glucose Levels of different animal models reveals that the aqueous and alcoholic extract (in the dose 400 mg/kg body weight) of roots of *Mangifera indica* was found to have the moderate antidiabetic activity. However, longer duration studies of *Mangifera indica* and its isolated compounds on chronic models are necessary to develop a potent antidiabetic drug.

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