Study On Antihyperglycemic Activities Of Bark of *mimusops elengi* Roxb.

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Abstract

This research paper deals with the investigations of antihyperglycemic activities and nontoxic effects from bark of *Mimusops elengi* Roxb.. The antihyperglycemic effects of two crude extracts (aqueous and 70% ethanol extracts) and isolated organic compound (lupeol)from the bark of *M. elengi* Roxb. were investigated on adrenaline induced hyperglycemic rats. The effective doses for oral administration were observed 2g/kg body weight for each extract and 2mg/kg body weight for isolated compound. From this test, it was observed that the blood glucose levels of aqueous and 70% ethanol extracts were decreased at 1 hr and 2 hr compared with that of control rats. Among two extracts, 70% ethanol extract were observed to possess more pronounced antihyperglycemic activity than aqueous extract. The effective reduction of blood glucose level of isolated compound also showed at 2 hr. In acute toxicity test, toxic or harmful effects of each extract in different dosages (2 g,4 g and 8g/kg body weight) were not observed in *vivo* method using albino mice models. According to these investigations, each extract and isolated compound may be utilized safely as antihyperglycemic agents in medicinal formulations for treatment of diabetic mellitus.

Keywords :*Mimusops elengi* Roxb. , antihyperglycemic activity, acute toxicity

Introduction

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic disorders in which there are high blood sugar (hyperglycemia) levels over a prolonged period (Steven *et al.,* 2001).The new classification system identifies four types of diabetes mellitus: type-1 diabetes (insulin dependents diabetes mellitus–IDDM), type-2diabetes (non-insulin dependents diabetes mellitus–NIDDM), gestational diabetes mellitus and other specific types. The most common forms are type 1 and type 2diabetes. Each type of diabetes mellitus identified extends across a clinical continuum of hyperglycemia and insulin requirements.Other forms of diabetes are associated with other conditions such as pancreas damage, drug or chemical use, infections and other diseases. A number of risk factors are attributed to the incidence of diabetes including family history, age and social group characteristics, as well as lifestyle, psychological, and clinical factors (Edwards *et al.,* 1995). The chronic hyperglycemia of diabetes mellitus is associated with long term damage, dysfunction and failure of various organs especially the eyes, kidneys, nerves, heart and blood vessels. Effective treatment strategies in the management of diabetes include strict diet control, reduction in body weight, modification of life style, regulations of plasma lipids and blood pressure.

Medicinal plants are nature's gift to human beings to make disease free health life and play a vital role to preserve our health (Rakesh S Shivatare*et al*., 2013). They are believed to be much safer and proven elixir in the treatment of various ailments. The present paper involves the determination of antihyperglycemic activities and nontoxic effectsfrom the bark of *Mimusops elengi* Roxb.. *M. elengi* is commonly known as Spanish cherry belonging to Sapotaceae family. Myanmar name is Kha-yay.It is cultivated mainly in North and Peninsular India, and Andaman Islands.Itgrows wild and common in plains and also cultivated in

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Myanmar (KyawSoe *et al.,* 2004). The tree is frequently cultivated in gardens chiefly for its fragrant flowers and ornamental foliate. The bark, fruit and seeds of *M. elengi* possess several medicinal properties such as astringent, tonic and febrifuge. The bark can be used for mental disorders increase, cardiac muscle contractility, promotes digestion, antiseptic, gum and teeth diseases, heart disease, diarrhoea and dysentery.

Materials and Methods

Plant Material Collection

Bark of *M. elengi* was collected from Yangonregion in Myanmar. The plant sample was identified at Departmentof Botany, Yangon University in Myanmar. The sample was cleaned, dried and ground into powder and stored in air-tight container.

Preparation of Crude Extracts for Bioactivities

The dried powdered sample (100 g) was refluxed with distilled water and 70 % ethanol respectively. Complete removal of the solvents under reduced pressure provided water and 70 % ethanol extracts.

Screening of Antihyperglycemic Activities of Crude Extracts

In the present work, two crude extracts (aqueous and 70% ethanol) were used to determine the antihyperglycemic activities by using adrenaline induced hyperglycemic rats.

(i) Preparation of Doses

The amountofeach extractto be administered was calculated based on the body weight of each rat. Two extracts (2 g/kg body weight) were suspended in distilled water to get the required doses respectively.

(ii) Procedure

Winstarstrain rats, male sex (250-300 g) were used in this experiment. Rats were kept without food for 18 hours before the test. However, distilled water was allowed for all animals. The animals were divided into two groups of five animal each. All the rats were made diabetic (hyperglycemia) by injecting them subcutaneously with standard dose 0.2 mg/kg body weight of adrenaline tartrate in distilled water using the method of Gupta *et al*., (1967). The blood glucose levels were determined at 0 hr, 1 hr, 2 hr, 3 hr and 4 hr after administration of adrenaline. After one week, the blood glucose levels of the same groups were again measured and the rats were made diabetic. Then, blood glucose levels of each group were recorded hourly after oral administration of tested doses by using Super Glucocard II blood glucose test meter (GT-1640).

(iii) Statistical Analysis

All the results of the blood glucose levels were expressed as Mean ± Standard Error Mean (S.E.M) from five rats in each group. All the grouped data were statistically evaluated and the significance of various treatments was calculated by using student's t test and SPSS software. Probably level of less than 0.05 was considered significant.

Investigation of Acute Toxicity

Two crude extracts (aqueous and 70% ethanol) were used to determine the acute toxicity by using albino mice models.

(i) Preparation of Doses

The different doses of water and 70% ethanol extracts are 2 g/kg, 4 g/kg and 8 g/kg body weightrespectively.Each extract was dissolved in distilled water to get the required doses.

(ii) Procedure

In the present study, the mice of both sexes (20-35 g) were used. Acute toxicity of different doses of aqueous and 70% ethanol extracts was evaluated by the method of Lorke(1983). The mice were assigned to seven groups with ten animals in each group. The animals were housed in standard cages with food and water at air conditioned room of 20 ± 5ºC temperature with artificial light. Group I was treated with normal food and water and considered as controlled. Group II to IV mice were treated with aqueous extract of different dose and group V to VII mice were treated with 70% ethanol extract of different dose orally. The dosages employed were 2g, 4g and 8 g/kg body weight respectively. Afteroral administration of extracts, each group of rat was housed separately in a cage with free

access to food and water. Observation andsurvivors were also observed for a total of 7 days.

Extraction, Isolation and Purification of Organic Compound

Dried powered bark of *M. elengi* (80 g) was extracted with 70% ethanol by soxhlet extraction method for 15 hr. This procedure was repeated for nine times. The combined solvents were evaporated under reduced pressure by means of a rotary evaporator and obtained ethanol extract. The ethanol extract was suspended in water and partitioned with pet-ether. Removal of the solvent from combined pet-ether layer provided pet-ether extract gel (40 g) using pet-ether and ethyl acetate with increasing polarity ratio as eluent to yield (6.33 g). The whole pet-ether extract was separated by column chromatography over silica 59 fractions. Each fraction obtained by column chromatography was checked by TLC. From the inspection of TLC chromatogram, the fractions of the same R_f values were combined to give 5 fractions.

The fraction-2 (R_f= 0.47, PE :EtOAC – 9:1 V/V) was further purified by pet-ether and ethyl acetate provided colorless needle shape crystals.The structure of isolated compoundwas identified by melting point and modern spectroscopic methods such as UV, FT-IR and ^{1}H NMR.

Screening of Antihyperglycemic Activity of Isolated Compound

Antihyperglycemic activity of isolated compound was also investigated with a dosage of 2 mg/kg body weight according to the procedure mentioned in the previous section.

Results and Discussion

For the screening of antihyperglycemic activities, the tested groups were administered 2 g/kg body weight for each extract. From this experiment, it was found that the blood glucose levels of aqueous extract weresignificantly decreased at 1 hr (19.94%, $p < 0.01$) and 2 hr(21.61 %, $p < 0.01$)compared with that of control rats(Table-1and Figure-1). 70% ethanol extract was decreased the blood glucose levels at 1 hr(30.11%, $p < 0.01$) and2 hr (35.25%, $p < 0.01$) (Table-2 and Figure-2). The percent inhibitions of blood glucose levels of aqueous and 70% ethanol extracts were 19.94%, 21.61%, 15.30%, 6.02% and 30.11%, 35.25%, 6.69%, 4.84% at 1 hr time interval (Figure-3).From these result, it may be deduced that 70% ethanol extract exhibits more antihyperglycemic effect than aqueous extract. By studying antihyperglycemic activity of isolated compound , the blood glucose level was significantly reduced after 2 hr oral administration of 2 mg/kg body weightwhere $P<0.01$ and 19.44% reduction. The percent reductions of blood glucose levels were 16.18%, 19.44%, 14.16% and 11.74% (Table-3 and Figure-4).From this data, it may be inferred that the isolated compound from bark of selected plant possesses the antihyperglycemic potency.

The acute toxicity screening for plant extracts wasdone with the dosage of 2g, 4g and 8 g/kg body weight in mice. The condition of mice was denoted after one week treatment. All the animals remained alive and did not show any visible symptoms of toxicity like restlessness, respiratory, disorders, convulsion, aggressive activities, coma and death at the tested dosage. So, the median lethal dose (LD_{50}) was more than 8 g/kg body weight. From these results, it was found that both plant extracts were free from acute toxic or harmful effects under experimental condition (Table-4).

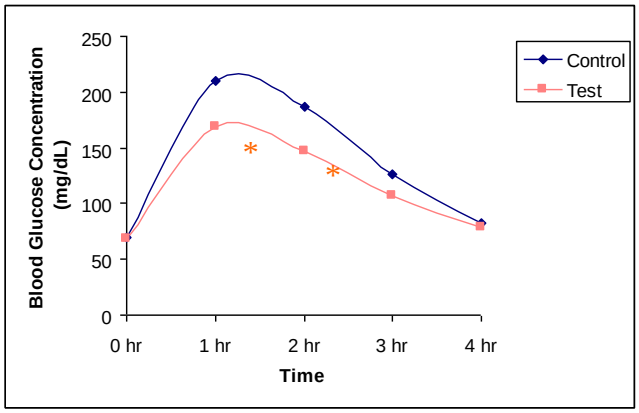
Table- 1 Mean Blood Glucose Levels (±SEM) in Adrenaline Induced Hyperglycemic Rats with Aqueous Extract

Test Group	Blood Glucose Concentration (mg/dL)				
	0 hr	1 hr	2 hr	3 hr	4 hr
Group I (Control) (n=5)	70.0 ± 2.0	210.67 ± 18.5	186.67 ± 11.7	126.33± 4.9	83.0 ± 13.9
Group I (Aqueous Extract) (n=5)	68.0 ± 2.6	168.67 ± 6.0 (19.94%R)*	146.33 ± 4.7 (21.61%R)*	107.0 ± 10.6	78.0 ± 10.1

* = P < 0.01 R = Reduction in hyperglycemia

Table- 2 Mean Blood Glucose Levels (± SEM) in Adrenaline Induced Hyperglycemic Rats with 70% Ethanol Extract

Test Group	Blood Glucose Concentration (mg/dL)				
	0 hr	1 hr	2 hr	3 hr	4 hr
Group II (Control) (n=5)	72.0± 1.0	189.33± 12.6	179.67 ± 0.6	99.67 ± 5.5	82.67 ± 14.6
Group II (70% Ethanol Extract) (n=5)	70.0 ± 2.0	132.33 ± 22.7 (30.11%R)*	116.33 ± 13.7 (35.25%R)*	93.00 ± 7.2	78.67 ± 12.2



* = p < 0.01

Figure-1 Time course of effect of aqueous extract on adrenaline induced hyperglycemic rats

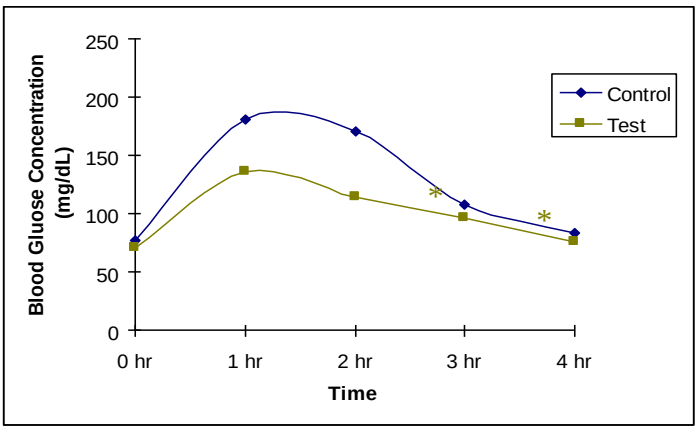


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Figure- 2 Time course of effect of 70% ethanol extract on adrenaline induced hyperglycemic rats

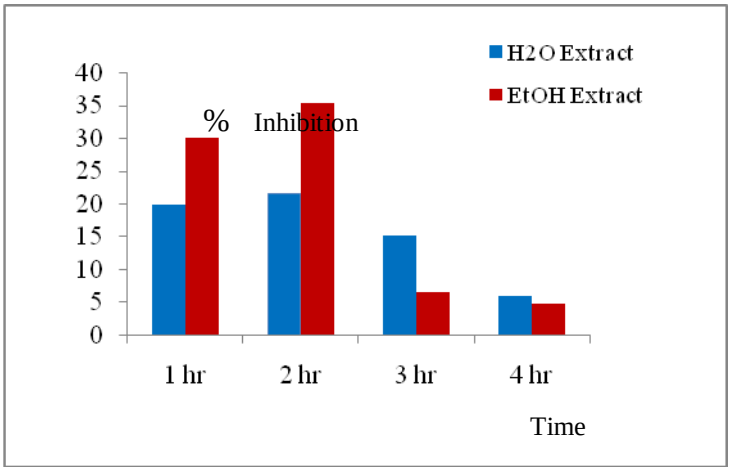
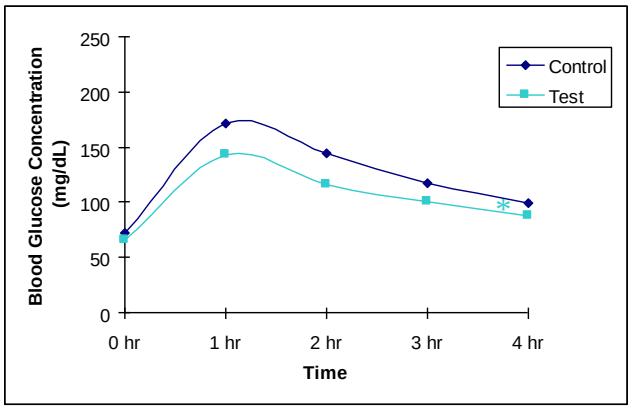


Figure- 3 Time course of % inhibitionof two extracts on hyperglycemia

Table- 3 Mean Blood Glucose Levels (± SEM) in Adrenaline Induced Hyperglycemic Rats with Isolated Compound

Test Group	Blood Glucose Concentration (mg/dL)				
	0 hr	1 hr	2 hr	3 hr	4 hr
Group III (Control) (n=5)	72.67 ± 6.5	171.00 ± 22.6	144.00 ± 14.1	117.67 ± 3.5	99.33 ± 4.2
Group III (Compound) (n=5)	66.33 ± 5.1	143.33 ± 9.1	116.00 ± 6.2 (19.44%R)*	101.00 ± 4.4	87.67 ± 4.2



* = p < 0.01

Figure-4 Time course of effect of isolated compound on adrenaline induced hyperglycemic rats

Table- 4 Results of Acute Toxicity Test of Aqueous and 70% Ethanol Extracts

Group No.	Doses(g/kg)	Ratio of Dead & Tested Mice No.	Observed % Dead	Expected % Dead	Observed -Expected
I(Control)	0	0:10	0	0	0
II & V (Aqueous and Ethanol)	2	0 :10	0	0	0
III & VI (AqueousandEthanol)	4	0 :10	0	0	0
IV & VII (Aqueous and Ethanol)	8	0 :10	0	0	0

The structure of isolated compound (26.26mg, 0.415% yield, m.p. 215-217 °C) was identified by modern spectroscopic methods. It was UV inactive and the functional groups present in compound were also studied by FT-IR (Figure-5). In the FT-IR spectrum, it was found that –OH, C=CH_2, C-O and C $(CH_3)_2$ groups were present in compound. The bands at 1037 cm^{-1} and 998cm^{-1} confirmed that C-O in cyclic compound and –CH out of plane wagging for CH_2 = C. From the observation of ^{1}H NMR spectrum (Figure-6), it was found that isolated compound has 50 protons involving methylene protons appeared at δ 5.11 ppm and δ 5.52

ppm. The hydrogen on the ring carbon directly bounded to hydroxyl group can also be seen at δ 3.21 ppm. So, all of these information suggested the isolated compound to be a lupeol ($C_{30}H_{50}O$). FT-IR (KBr) ν_{max} (cm^{-1}): 3454 (O-H), 3070 ($C=CH_2$),2936,2869 (C-H),1642,1633 (C=C), 1461 (C-H), 1382 (CH_3), 1037 (CH-OH), 998 (C-H) : ^{1}HNMR (400 MHz,$CDCl_3$)(δ_H/ppm): 0.75-2.2 (47H,m,CH_3,=CH_2,-CH), 3.21 (1H,dd,-CH-OH), 5.11 (1H,d,=CH_2),5.52(1H,d,=CH_2)

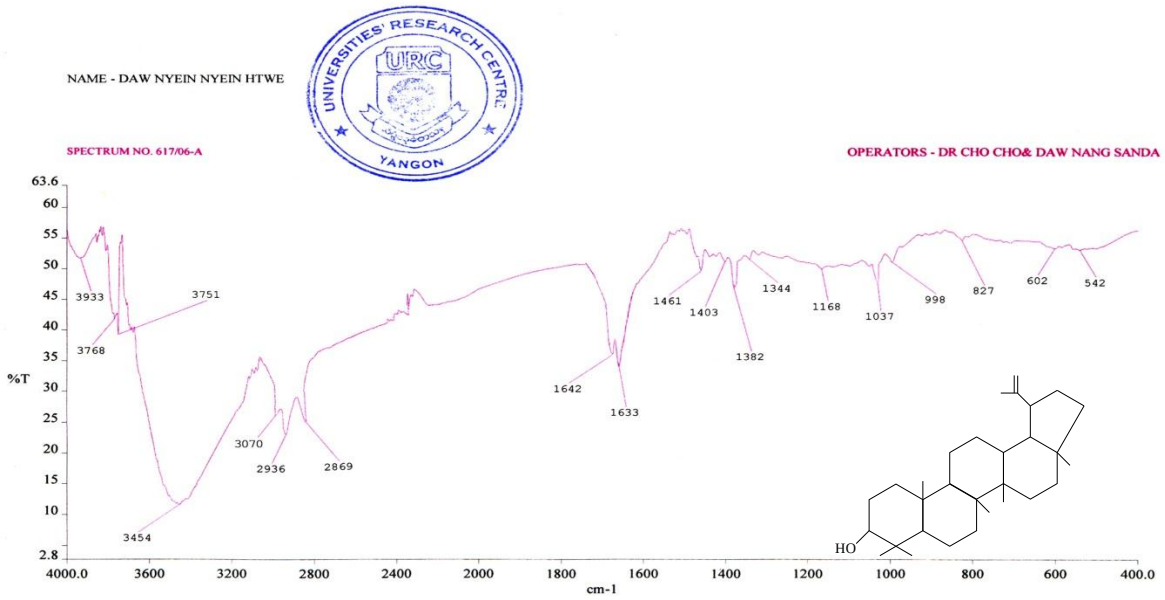


Figure- 5 FT-IR spectrum of isolated compound (KBr)

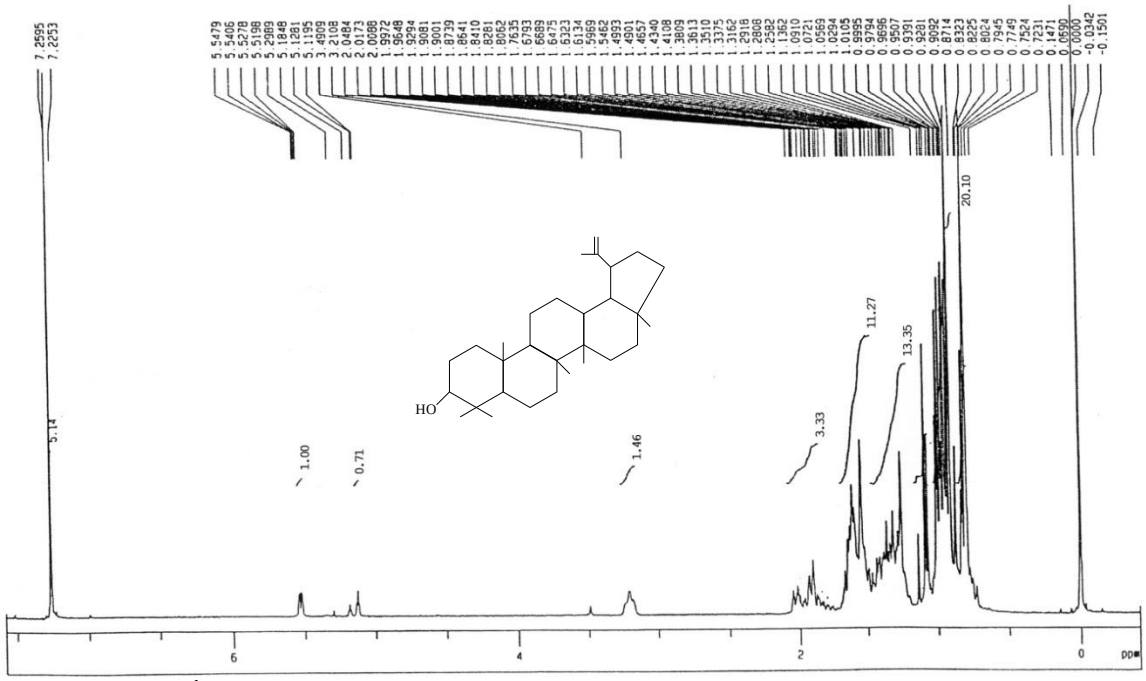


Figure- 6 ^{1}H NMR (400 MHz in $CDCl_3$) spectrum of isolated compound

Conclusion

Antihyperglycemic activities of two extracts and isolated compound from the bark of *M. elengi*were investigated on adrenaline induced hyperglycemic rats. The effective doses were observed 2 g/kg body weight for each extract and 2mg/kg body weight for isolated compound. The aqueous extract showed the optimum antihyperglycemic effect at 1 hr

(19.94% reduction, $p<0.01$) and 2 hr (21.61% reduction, $p<0.01$), and 70% ethanol extract decreased blood glucose level at 1 hr (30.11% reduction, $p<0.01$) and 2 hr (35.25% reduction, $p<0.01$) compared with control group. From these results, 70%ethanol extract was found to be more potent than aqueous extract in antihyperglycemic activity. On the other hand, the effective reduction percent of blood glucose levels of isolated compound showed 19.44% ($p<0.01$) at 2 hr.

In acute toxicity test, the maximum dose for each extract was found to be 8 g/kg body weight. From these result, LD_{50} was more than 8 g/kg body weight and it was inferred that both extracts were free from acute toxic or harmful effects.

In brief, according to this scientific investigations, the bark of *M. elengi* may be used as medicine for the treatment of diseases concerning with hyperglycemia.

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