IOSR Journal of Pharmacy
e-ISSN: 2250-3013, **p**-ISSN: 2319-4219, www.iosrphr.org
Vol. 2, Issue 6, Nov-Dec. 2012 PP. 14-19

Evaluation of hypoglycemic effect of compound(s) from petroleum ether fraction of ethanol extract of *Mangifera indica* red leaves

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***Abstract:* – The petroleum ether fraction of ethanol extract of Mangifera indica red leaves decreased fasting blood glucose (FBG) level of approx. 90% in alloxan induced diabetes rats (AIDRs). However, the specific compound(s) has not been clarified. The present study has been designed to separate hypoglycemic compound(s) using preparative thin layer chromatography (PTLC) from petroleum ether fraction of ethanol extract of Mangifera indica red leaves. Development of TLC demonstrated the presence of distinct three layers having the R_f values 0.86, 0.56 and 0.28 on solvent system, chloroform: n-hexane (80:20). Compounds with R_f values 0.86 and 0.56 reduced fasting blood glucose level by 92 and 96%, respectively in AIDRs. However, the compound with R_f value 0.28 did not have any significant antihyperglycemic effect. It was found from the experiment that the compound with R_f value 0.56 had predominant hypoglycemic effect than the other. Therefore, it can be a potential candidate for screening the lead for newer antidiabetic drugs that can offer a natural key to manage diabetes for the future.**

***Keywords:* –** *Alloxan, hypoglycemic, PTLC, TLC.*

I. INTRODUCTION

Diabetes is a global problem and number of those affected is increasing day by day. It is affecting nearly 10% of the population worldwide. [1] Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is associated with reduced life expectancy, significant morbidity due to specific diabetes related microvascular complications (retinopathy, nephropathy and neuropathy), increased risk of macrovascular complications (ischemic heart disease, stroke and peripheral vascular disease) and diminished quality of life. [2] The major mode of controlling diabetes can be achieved by diet, exercise, and insulin replacement therapy and/or by different oral hypoglycemic drugs. However, treatment with sulfonylureas and biguanides is associated with side effects and fail to alter the course of diabetic complications significantly. [3] In modern medical system, managing diabetes without side effects is still a challenge. The search for new pharmacologically active agents obtained by screening natural sources such as medicinal plants or their extracts has led to the discovery of many clinically useful drugs that play a major role in the treatment of human diseases. [4]

Now a days, scientists and researchers are very much interested on research of natural plant products all over the world and a large amount of substantiation have shown the immense potential of medicinal plants used traditionally. [5] In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries. [6] However, it should be kept in mind that phytotherapy is not an alternative but a complementary and supportive treatment to the conventional diabetes therapy. One reason may be due an inappropriate dosage regimens and inadequate supply of the exact compounds exists in the herbs. Therefore, a proper scientific evaluation of the plant by pharmacological tests followed by chemical investigations is necessary for the invention of active chemicals or leads for the development of new potential antidiabetic drugs. One of the most prominent antidiabetic drug metformin, already leading in the diabetologist's pharmacy is also generated from a plant originated from French Lilac (*Galega officinalis*) that is using successfully in diabetes treatment for the last five decades.

In the light of the literature, experimental animal study by the stem-bark aqueous extract of *Mangifera indica* Linn. suggested the management of adult-onset type 2 diabetes mellitus in some rural African communities. [7] We have previously reported the antidiabetic effects of various fractions (Petroleum ether, ethyl

acetate and chloroform) of the ethanol extract of *Mangifera indica* red leaves in AIDRs. [8] In the present work, we have attempted to find out hypoglycemic compound(s) from the petroleum ether fraction of *Mangifera indica* red leaves. PTLC plates were used for the collection of compound(s) and alloxan-induced diabetic rats were used for the evaluation of antidiabetic effects.

II. MATERIALS AND METHODS

2.1 Drugs and Chemicals

The standard antidiabetic agent, metformin hydrochloride was the generous gift samples from Square Pharmaceuticals Ltd., Pabna Bangladesh. Alloxan was purchased from Sisco Research Laboratories Ltd., Mumbai, India.

2.2 Plant materials

The fresh leaves of *Mangifera indica* (Local name- Aam) were collected from medicinal plant garden at Naogaon, Bangladesh. These were dried completely under the mild sun and ground with an electric grinder into coarse powder and used for cold extraction.

2.3 Preparation and fractionation of crude extracts

The coarse powder was submerged in ethanol (96%) and allowed to stand for several days (7-10) with occasional shaking and stirring. When the solvent become concentrated, the liquid alcohol content was filtered through cotton and then through filter paper (Whatman filter paper no. 1). Then the solvents were allowed to evaporate using rotary evaporator at 40 – 45^{o}c and the highly concentrated crude extracts were obtained. They were then fractionated using petroleum ether. The dried fractionated extracts were then preserved at 4^{o}c for the experimental use. [9]

2.4 Preparation of TLC and PTLC plates

One dimensional thin layer chromatographic (TLC) technique was used for the initial screening of compounds from chloroform and petroleum ether fraction. TLC and PTLC plates were made on glass plates (20 cm X 5 cm for TLC and 20 cm X 20 cm for PTLC) with silica gel (Kiesel gel 60 PF 254). These plates were thoroughly washed and dried in an oven. These dried plates were then swabbed with acetone to remove any fatty residue. To make the slurry silica gel 60 PF-254 and appropriate volume of distilled water (2ml/ gm of silica for TLC and 3 gm silica/ plate for PTLC) were mixed in a conical flask and the flask was gently shaken. Slurry was then evenly distributed over the plates using TLC spreader. After air-drying the coated plates were subjected to activation by heating in an oven at 110^{o}c for 70 minutes.

2.5 Development of TLC plates

Using trial and error assumption different solvents in different ratios were tried. TLC tank with airtight lid was used for the development of chromatoplates. Filter paper was introduced into the tank and allowed to soak in the solvent. Then the tank was kept airtight for few minutes to saturate the internal atmosphere with the solvent vapor. A small amount of dried extracts (chloroform and petroleum ether fractions) were dissolved in a suitable solvent to get a solution. A vertical line was drawn in the middle position on it and spotted on the plate with capillary tube just 1 cm above the lower edge of the plate. Then it was dried and drawn a straight-line 2 cm below from the upper edge of the plate. Spotted plate was then placed in the TLC tank contain solvents with selected proportion and allowed it for developing. Running the solvent(s) to the marked upper layer it was taken out and air-dried. The compounds were then detected from the developed plates. The following techniques were used to detect the compounds in TLC plates; Visual detection, UV- light, Iodine chamber. R_f value of the compounds were calculated by the following equation:

$$R_f \text{ Value} = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent system}}$$

2.6 Experimental design on Rat model

Total 30 rats were used in this study and divided into six groups. Rats were collected from ICCDR, Bangladesh. Each group contains 5 rats. After induction of chemical diabetes (group II to group IV), group II was selected for diabetic control, which did not receive either metformin, or plant extracts. Group III was for metformin control in which metformin was administered intraperitoneally at a dose of 150 mg/kg body weights. Group IV, V and VI received plant extracts with R_f values 0.28, 0.56 and 0.86 layer containing compound(s), respectively. Group I was normal control group, also did not receive either metformin or plant extracts. The plant extracts were administered intraperitoneally at a same dose of metformin standard. The blood samples

were analyzed for blood glucose content at different time points as indicated in figures. Blood glucose level was estimated in all the experiments by using glucometer (Bioland-423, Germany).

2.7 Statistical Analysis:

Data were expressed as mean ± standard error of mean (SEM). Statistical comparisons were performed by one-way analysis of variance (ANOVA), or students paired or unpaired *t*-test where appropriate. Results were considered to be significant when *p* values were less than 0.05 ($p<0.05$). Statistical calculations and the graphs were prepared using Graph Pad Prism version 5.00 for Windows (Graph Pad Software, San Diego, CA, USA).

III. RESULTS

3.1 Antihyperglycemic effects of petroleum ether fraction on AIDRs

Petroleum ether fraction of ethanol extract reduced fasting blood glucose level to 70.21%, 58.26%, and 13.64% in 2, 6, and 16 hrs, respectively. Maximum reduction of blood glucose level by 86.36% was observed in 16 hr time points during the experimental period.

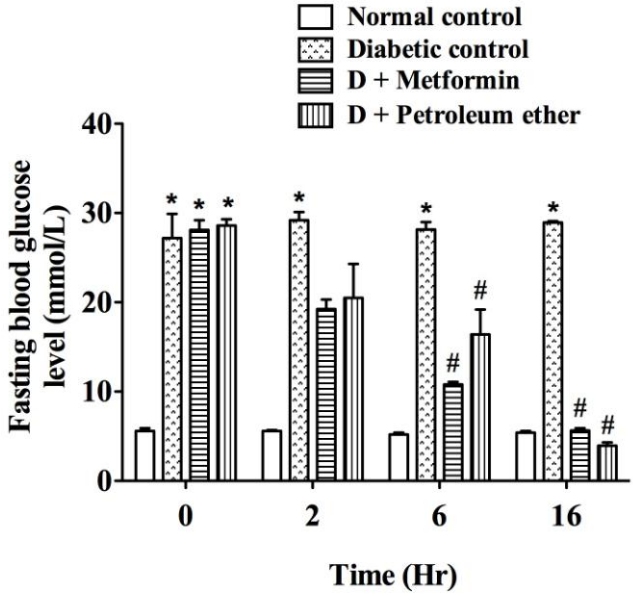

Fig. 1 Effect of petroleum ether fraction of ethanol extract on the FBG level in diabetic rats compared to normal rats. ***** indicates significant changes (increase) of blood glucose level compared with normal control group. **#** indicates significant changes (decrease) of FBG level in diabetic rats after treatment compared with zero hr treatment group. The results are expressed as means ± SEM.

3.2 Isolation of compounds from the petroleum ether fraction of Mangifera indica

TLC plates were used to detect different compounds from the petroleum ether fraction of *Mangifera indica* ethanol extract using chloroform and n-hexane in the ratio 80:20. Three layers were detected with R_f values 0.86, 0.56 and 0.28 as shown in fig. 2 (A, B). Compounds with R_f values 0.86 and 0.56 were visually detected on developed TLC plate but that of R_f 0.28 only responded under the UV light and Iodine chamber. In iodine chamber it absorbed iodine and became brown. The three separated layers were scraped (compounds with silica gel) from PTLC plate and collected in closed bottle (R_f 0.28; prevent oxidation) and open beakers (R_f 0.86 and 0.56), respectively. Methanol and ethyl acetate solvent at a ratio of 3:7 were used to separate compounds from silica gel. Then these solvents were evaporated to get solid mass. Approximately 200 PTLC plates were used to get sufficient amount of yields. Total yield was 400 mg approximately. These solid masses were dissolved in dimethyl sulfoxide (DMSO) solution and applied to rat model for the evaluation of antihyperglycemic effects.

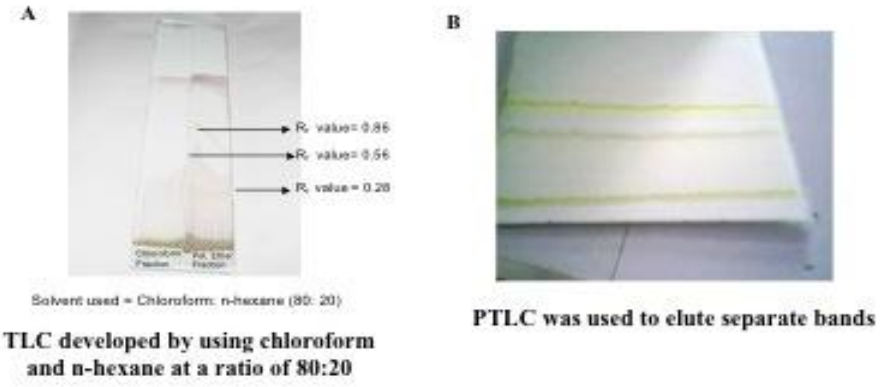

Fig. 2 Petroleum ether fraction shows three distinct layers on the TLC plate using the solvent system chloroform: n-hexane (80:20) with R_f values 0.86, 0.56, and 0.28 (A). Compounds with R_f values 0.86 and 0.56 were visually detected on developed TLC plate but that of R_f 0.28 only responded under the UV light and Iodine chamber (B).

3.3 Antidiabetic effects of the separated crude extracts on rat model

The parameters of fasting blood glucose (FBG) level was measured to search for the antidiabetic compound (s) from petroleum ether fraction of the ethanolic extracts of *Mangifera indica* on normal and alloxan induced diabetic rats using metformin as standard antidiabetic agent. The effects of metformin and different compounds separated form petroleum ether fraction of *Mangifera indica* on FBG level in normal and alloxan induced diabetic rats are shown in the fig. 3. The significant decrease of FBG observed from 4 hr after treatment and sustained the reduction until 16 hr. Maximum reduction of FBG level was 95.5% and 92% for the layer R_f 0.56 and R_f 0.86, respectively at 16 hr of the experimental period. However, the compound for the layer Rf 0.28 was ineffective all over the treatment period.

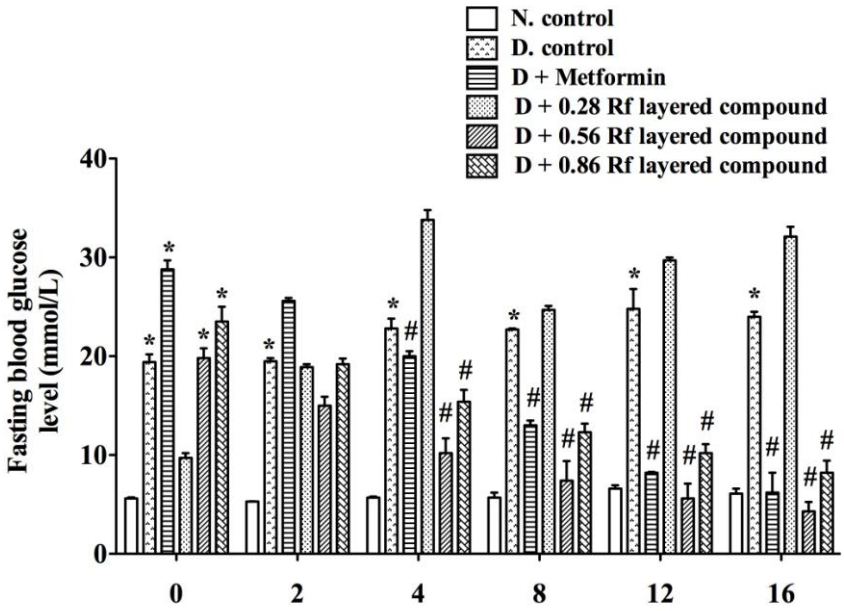

Fig. 3 Effect of different separated compounds on the FBG level on diabetic rats compared to normal rats. ***** indicates significant changes (increase) of blood glucose level compared with normal control group. **#** indicates significant changes (decrease) of FBG level in diabetic rats after treatment compared with zero hour treatment group. The results are expressed as means ± SEM.

IV. DISCUSSION

Numerous oral hypoglycemic drugs exist alongside insulin; still there is no promising therapy to cure diabetes. [10] In recent years, many traditional medicinal plant**s** were tested for their antidiabetic potential in the experimental animals. [11] In the light of the literature on *Mangifera indica* we made an attempt for the first time

to isolate compounds from petroleum ether fraction of *Mangifera indica* ethanolic extract and to study the effect of specific compound(s) of partitionates in alloxan induced hyperglycemic rats.

We have isolated three layers of compounds with R_f values 0.86, 0.56 and 0.28 (Fig. 2). Among them compound (R_f 0.56) reduced FBG of 95.5% and compound (R_f value 0.86) reduced FBG of 92% in alloxan induced diabetic rats (AIDR). The significant antidiabetic activity of compounds (R_f value 0.56) and (R_f value 0.86) of petroleum ether fraction of ethanol extract of *Mangifera indica* has been shown in Fig. 3.

In this study, the antihyperglycemic action of the *Mangifera indica* was better (especially R_f 0.56 fraction) than that of the standard drug, metformin. The possible mechanism by which *Mangifera indica* brings about its antidiabetic action may be by potentiating the insulin effect of plasma by stimulating insulin release from the remnant pancreatic β-cells or its release from the bound form. [12] Beside this, it might involve in extra-pancreatic action in these alloxan-diabetic rats, which might include the stimulation of peripheral glucose utilization or enhancing glycolytic and glycogenic processes with concomitant decrease in glycogenolysis and gluconeogenesis. [13]

Lastly, the antihyperglycemic activity of *Mangifera indica* may be due to the presence of hypoglycemic saponins, tannins, triterpines, alkaloids, flavonoids etc. [7] On the basis of the current investigation it was noted that the compounds (R_f value.56) and (R_f value.86) of petroleum ether fraction of ethanol extract of *Mangifera indica* acted in a similar fashion to metformin (standard drug) and it can be suggested that these results provide pharmacological evidence for its folklore claim as an anti-diabetic agent.

V. CONCLUSOINS

From the current study, it is concluded that the compounds (R_f value 0.56) and (R_f value 0.86) of petroleum ether fraction of ethanol extract of *Mangifera indica* has potent antidiabetic effects in a similar extent as that of metformin. Therefore, we believe that the study of specific hypoglycemic compound(s) from petroleum ether fraction of *Mangifera indica* ethanol extract offer a natural key to unlock a diabetologist's pharmacy for the future. However, further study is necessary for structure elucidation of the respective antidiabetic compounds.

ACKNOWLEDGEMENTS

We thank Pharmacy Department of Rajshahi University for providing necessary facilities to carry out this research work. The present work was supported by the National Science and Information and Communication Technology (NSICT), Dhaka, Bangladesh for financial assistance to the second author and the authors would like to extend their gratitude to the Director, Animal Research Centre (ARC), ICDDR, B for providing necessary facilities.

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