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Evaluation of antidiabetic effect of hydro alcoholic flower extract of mangifera indica using various in vitro models

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ABSTRACT

The aim of this study was to investigate the anti-diabetic activity and mechanism of action of alcoholic flower extract prepared from *Magnifera indica*. The *in vitro* anti-diabetic study was done by evaluating the inhibitory effect of magnifera on the activities of alpha-amylase, Alpha amylase inhibition assay, Alpha glucosidase inhibition assay, yeast cell glucose uptake and inhibition of glucose diffusion assay methods. The significant results were observed in the alcoholic flower extracts. The α-glucosidse & α-amylase inhibition was in a dose dependent manner and glucose transport differs with the sample and glucose concentration. From the results of the study, it is inferred that, Magnifera indica flower possesses anti-diabetic activity. This work indicates that Flowers of Magnifera indica have an important long term anti-diabetic effect that can be well established to treat diabetes

Keywords: α-glucosidse, α-amylase and Magnifera indica.

INTRODUCTION

The prevalence of diabetes worldwide raised from 2.8% in 2000 to 4.4% in 2030. It is estimated that the overall number of diabetic people will jump to over 366 million cases in 2030[1-2]. Diabetes comes with several complications that threatened people's lives like chest pain, heart attack, stroke, cardiovascular problems (coronary artery disease), atherosclerosis, and neuropathy.[3] Postprandial hyperglycemia (PPHG) is a condition in which blood glucose level remains high after consuming meal, and it is an important factor to be considered in the management of diabetes mellitus and diabetes related secondary complications such as diabetic retinopathy, diabetic neuropathy, cardiovascular diseases, etc.[4]. Glycosidic linkages of α-D-(1,4) in carbohydrates are cleaved by α-amylase to produce oligosaccharides, which are further cleaved to monosaccharide glucose by α-glucosidase[5]. Therefore, inhibitors of these enzymes can delay the increase in blood glucose level in people who consume carbohydrate-rich food, and keep the PPHG under control[6].

One of the therapeutic approaches for Diabetic management is to reduce the postprandial hyperglycemia by delaying the digestion and absorption of carbohydrates such as alpha-glucosidase and alpha-amylase enzyme in the digestive tract. Inhibitors of alpha- glucosidase and alpha-amylase enzyme delays the digestion of carbohydrate and prolong the carbohydrate digestion rate, which causes a reduction in the absorption rate of glucose and consequently decrease the postprandial hyperglycemia[7-12]. Therefore, the current study was undertaken to evaluate the alcoholic extracts from the flowers of Magnifera Indiaca for α-amylase and α-glucosidase inhibiting activities in vitro and glucose lowering activity in vivo using Metformin as reference.[12-15] The *Mangifera indica* (mango) is one of the choicest fruit crops of tropical andsubtropical regions of the world, especially in Asia. Its popularity and importance can easily be realized by the fact that it is often referred as 'King of fruits in the tropical world.

Part used

Fig 1: Flower of Magnifera Indica

Chemical Composition

Mango contains a variety of phytochemicals and nutrients [16]. Mango peel and pulp contain other compounds, such as pigment carotenoids and polyphenols, and omega-3 and -6 polyunsaturated fatty acids [17]. Mango peel pigments have biological effects, including carotenoids, such as the provitamin A compound, beta-carotene, lutein and alphacarotene, polyphenols such as quercetin, kaempferol, gallic acid, caffeic acid, catechins, tannins and the unique mango xanthonoid, mangiferin which are under preliminary research for their potential to counteract various disease processes [18-21]. Phytochemical and nutrient content appears to vary across mango cultivars. Up to 25 different carotenoids have been isolated from mango pulp, the densest of which was beta- carotene, which accounts for the yellow-orange pigmentation of most mango cultivars [22]

MATERIALS AND METHODS

Extraction of the plant material

100 g of finely powdered flower powder was extracted with 1 L of petroleum ether in a soxhlet apparatus for 48 h, obtained marc was further extracted with 1 L of ethyl acetate in soxhlet apparatus for 48 h, and obtained marc was again extracted with ethanol in a soxhlet apparatus for 48 h. After extraction the extracts were separately concentrated by distillation and dried at room temperature until get viscous solid mass. The obtained crude extracts were weighed and stored at 4^0C for the further analysis. The percentage yield was calculated by using following formula Table 1.

$$\text{Percentage Yield (\%w/w)} = \frac{\text{Weight of extract obtained (gm)}}{\text{Weight of Plant material used}} \times 100$$

Table 1: Percentage yield of different extracts of *Mangifera indica* flowers

Extract	Amount of Plantmaterial used (g)	Amount of extract obtained (g)	Percentage yield (% w/w)
PEEMI	100	3.42	3.42
EAEMI	95	11.55	12.16
EEMI	80	20.18	25.23

PEEMI: Petroleum ether extracts of *Mangifera indica* flowers
EAEMI: Ethyl acetate extracts of *Mangifera indica* flowers
EEMI: Ethanolic extract of *Mangifera indica* flowers

Qualitative phytochemical analysis

Preparation of test sample

A small quantity of the extract was dissolved in 5ml of distilled water and filtered. The filtrate was tested to detect the presence of various phytochemical constituents Table 2. in the sample.[23]

Test for carbohydrates

Molisch's test

Few drops of Molisch's reagent was added to 2-3ml of filtrate, followed by addition of concentrated sulphuric acid along the sides of the test tube. Formation of violet colour ring at the junction of two liquids indicates the presence of carbohydrates.

Fehling's test

1ml of Fehling's-A (copper sulphate in distilled water) was added to 1ml of Fehling's-B (potassium tartarate and sodium hydroxide in distilled water) solution, boiled for one minute. To this added1ml of filtrate and heated gently. Formation of brick red precipitate indicates the presence of reducing sugars.

Benedict's test

Few ml of filtrate was mixed with equal volume of Benedict's reagent (alkaline solution containing cupric citrate complex)

and heated in boiling water bath for 5min. Formation of the reddish brown precipitate infers the presence of reducing sugars.

Test for alkaloids

Small amount of extract mixed with few ml of dilute hydrochloric acid. Shaken well and filtered. Following tests were performed with the obtained filtrate.

Dragendorff's test

A few drops of Dragendorff's reagent (potassium bismuth iodide solution) was added to 2-3ml of filtrate. Orange red precipitate indicates the presence of alkaloids.

Mayer's test

A few drops of Mayer's reagent (potassium mercuric iodide solution) was added to 2-3ml of filtrate.Cream (dull white) precipitate was formed.

Wagner's test

A few drops of Wagner's reagent (solution of iodine in potassium iodide) were added to 2-3ml of filtrate. Reddish brown precipitate was obtained.

Hager's test

A few drops of Hager's reagent (Picric acid) were added to 2-3 ml of filtrate. Yellow precipitate was obtained

Test for triterpenoid

Libermann-Burchard test

A small quantity of extract was treated with few drops of acetic anhydride, followed by a few drops of concentrated sulphuric acid. A brown ring was formed at the junction of two layers and the upper layer turns green color, infers the presence of phytosterols and formation of deep red color indicates the presence of triterpenoids.

Salkowski test

A small quantity of the extract was treated with chloroform and few drops of concentrated sulphuric acid and allowed to stand for few minutes. Yellow colour at the lower layer indicates the presence of triterpenoids.

Test for glycosides

Legal's test

1ml of pyridine and 1 ml of sodium nitroprusside was added to 1 ml of extract. Pink to red color indicates the presence of glycosides.

Keller-Killiani test

Glacial acetic acid was added to 2 ml extract, followed by the addition of trace quantity of ferric chloride and 2 to 3 drops of concentrated sulphuric acid. Reddish brown colour appears at the junction of two liquid indicates the presence of cardiac glycosides.

Baljet test

2 ml of extract was added to sodium picrate solution. Yellow to orange colour formation indicates the presence of glycosides.

Test for steroids and sterols

Liebermann- Burchard reaction

2 ml of extract was mixed with chloroform. To that mixture added 1-2 ml of acetic anhydride and 2 drops of concentrated sulphuric acid along the sides of the test tube. The solution becomes; red, then blue and finally bluish green colour.

Salkowski reaction

2 ml of extract was mixed with 2 ml of chloroform and 2 ml concentrated sulphuric acid. Shaken well Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

Test for phenols

Ferric chloride test

1 ml of the alcoholic solution of the extract was added to 2 ml of distilled water followed by few drops of 10% ferric chloride. Formation of blue or green colour indicates the presence of phenols.

Lead acetate test

Diluted 1 ml of alcoholic solution of extract with 5 ml distilled water and to this added few drops of 1% aqueous solution of lead acetate. Formation of yellow colour precipitate indicates the presence of phenols.

Test for Tannins

Lead acetate test

A few drop of lead acetate was added to 5 ml of extract. Formation of yellow or red color precipitate indicates the presence of tannins.

Test for Saponins

Foam Test

1ml of test sample was diluted with 20 ml of distilled water and shaken it in a graduated cylinder for 3minutes. Foam of 1 cm after 10 min indicates the presence of saponins.

Froth test

5 ml of test sample was added to sodium bicarbonate solution. After vigorous shaking the mixture, kept it for 3 min. A honey comb like froth formation indicates the presence of saponins.

Test for flavonoids

Alkaline reagent test

A few drop of sodium hydroxide solution was added to the extract. Formation of an intense yellow colour, which turns to colourless on addition of few drops of dilute hydrochloric acid, indicates the presence of flavonoids.

Table 2: Preliminary phytochemical evaluation of different extracts of *Mangifera indica* Flowers

Phytoconstituents		PEEMI	EAEMI	EEMI
Alkaloids		Absent	Present	Present
Flavonoids		Absent	Present	Present
Tannins		Absent	Absent	Present
Steroids		Absent	Absent	Absent
Triterpenoids		Present	Present	Present
Carbohydrates	Monosaccharide	Absent	Absent	Absent
	Polysaccharide	Absent	Absent	Absent
Saponins		Absent	Present	Present
Glycosides		Present	Present	Present
Proteins and amino acids		Absent	Absent	Absent

ALPHA AMYLASE INHIBITORY ASSAY BY DNSA COLOR ASSAY

The α-amylase inhibitory activity was assessed using method described by Elsnoussi et al.[25] Briefly, 500 ml of each of the varying extracts dilutions (0.1–0.5 mg/ml) was mixed with 500 ml of 0.02 M sodium phosphate buffer (pH 6.9) containing 0.5 mg/mL of α-amylase solution. The mixture was pre-incubated in test tubes at 25 0C for 10 min. Thereafter, 500 mL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each test tube at timed intervals. The reaction mixtures were incubated at 250C for 10 min and stopped with 1.0 mL of DNSA reagent. The tubes were incubated in a boiling water bath for 5 min and left to cool at 250C. Then 15 mL of distilled water was used to dilute the reaction mixtures, and the absorbance was measured at 504 nm using a spectrophotometer. Similar procedure was repeated for acarbose which serves as the positive control by preparing it in distilled water at same concentrations (0.1–0.5 mg/ml) as extracts. The result of the triplicate determinations of α-amylase inhibitory activity was expressed as % inhibition. The concentration of the extract causing 50% inhibition (IC50) of α-amylase activity was calculated from its standard calibration curve. [26]

$$\text{Percentage inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The results were shown in Table No 3.and Figure.2.

Table 3: Effect of different extracts of *Mangifera indica* flowers and acarbose on α-amylase inhibition activity

S.no	Concentration (µg/ml)	Percentage inhibition PEEMI(%)	Percentage inhibition EAEMI (%)	Percentage inhibition EEMI (%)	Percentage inhibition of Acarbose (%)
1	100	18.15	59.25	55.37	77.88
2	200	30.22	63.58	59.18	79.38
3	300	36.28	71.29	63.37	81.55
4	400	47.99	80.33	70.44	88.37
5	500	52.85	83.47	73.49	88.56
	IC_{50} (µg/ml)	454.22	88.27	80.18	65.29

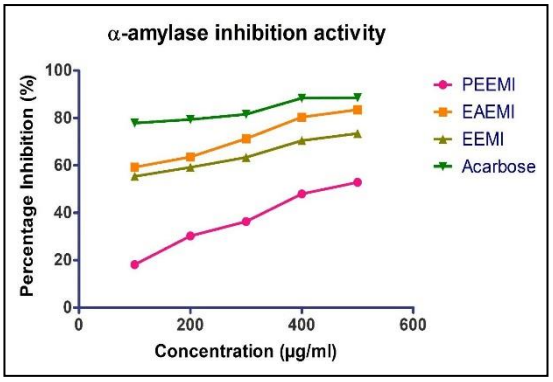

Fig 2: Effect of different extracts of *Mangifera indica* flowers and acarbose on α-amylase inhibition activity

INHIBITION OF A-GLUCOSIDASES ENZYME

The inhibitory activity was determined by incubating 1 ml of maltose solution (2% w/v maltose) with 0.2 M tris buffer (pH 8) and various concentrations of sample (0.1-0.5 mg/ml). The reaction mixture was incubated at 37°C for 10 min. The reaction was initiated by adding 1 ml of α-glucosidase enzyme (1 U/ml) to it and incubation at 35°C for 40 min. Then the reaction was terminated by the addition of 2 ml of 6 N HCl. The intensity of the color was measured at 540 nm using spectrophotometer. The results were expressed as % inhibition using the formula and the inhibitory concentration (IC50) value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions and the results were shown in Table 4. and Fig 3. [27]

$$\text{Percentage inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

Table 4: Percentage inhibition of Alpha glucosidase enzyme on different extracts of *Mangifera indica* flowers and Acarbose

S.no	Concentration (µg/ml)	Percentageinhibition PEEMI (%)	Percentageinhibition EAEMI	Percentageinhibition EEMI	Percentageinhibition Acarbose
1	100	5.28	21.85	24.22	85.71
2	200	11.45	39.47	30.65	88.34
3	300	26.35	45.59	38.48	91.18
4	400	30.64	59.58	44.09	92.29
5	500	41.78	68.38	55.29	94.07
IC_{50} (µg/ml)		598.21	361.25	465.38	61.29

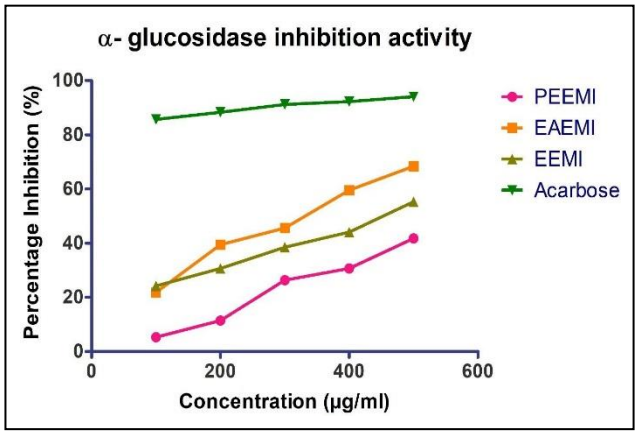

Fig 3: Percentage inhibition of Alpha glucosidase enzyme on different extracts of *Mangifera indica* flowers and Acarbose

GLUCOSE UPTAKE IN YEAST CELLS

The commercial baker's yeast in distilled water was subjected to repeated centrifugation (3,000×g, 5 min) until clear supernatant fluids were obtained and a 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of plant extracts (1- 5 mg/ml) were added to 1ml of glucose solution (5, 10 and 25 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 µl of yeast suspension followed by vortex and further incubation at 370C for 60 min. After 60 min, the tubes were centrifuged (2,500×g, 5min) and amount of glucose was estimated in the supernatant. Metformin was used as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula and the results were shown in Table 5 and Fig 4.

$$\text{Increase in glucose} = \frac{\text{Absorbance of sample} - \text{Absorbance of control uptake (\%)}}{\text{Absorbance of sample} \times 100}$$

Absorbance of the control reaction (containing all reagents except the test sample) and Absorbance of sample is the absorbance of the test sample. All the experiments were carried out in triplicates. [28]

Table 5: *In vitro* inhibitory glucose diffusion of different extracts of *Mangifera indica* flowers and Metformin

Sample	Glucose in external solution (mM)				
	30mins	60mins	90mins	120mins	180mins
Absence of drug (Control)	54.11±1.18	65.54±0.29	75.28±3.25	125.65±1.16	147.89±2.77
Metformin	18.01±0.42	25.91±1.08	36.47±0.88	53.78±0.62	66.33±0.99
PEEMI	49.35±1.26	52.22±1.35	60.71±0.37	95.38±0.56	112.18±2.32
EAEMI	21.35±1.62	29.45±1.33	39.48±1.07	57.26±1.38	72.21±0.66
EEMI	28.25±1.36	40.14±0.99	47.55±1.27	65.13±2.13	87.78±1.90

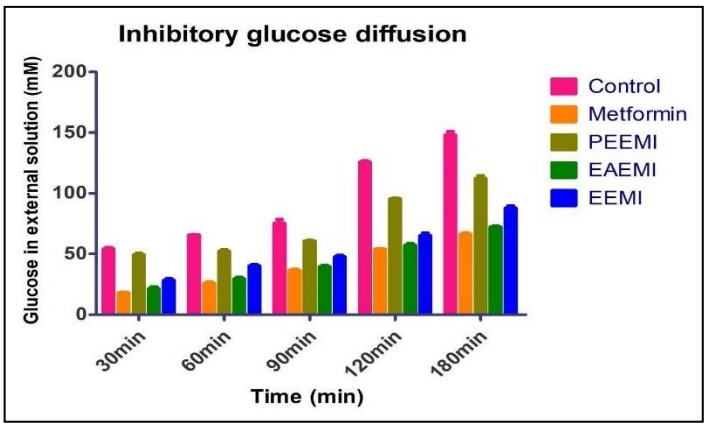

Fig 4: *In vitro* inhibitory glucose diffusion of different extracts of *Mangifera indica* flowers and Metformin

Inhibition of Glucose Diffusion Assay

This model was adapted from a method described by Edwards et al., which involved the use of a sealed dialysis tube into which 15 ml of a solution of glucose and NaCl (0.15 M) was introduced and the appearance of glucose in the external solution was measured. The model used in the present experiments consisted of a dialysis tube (6 cm ±15 mm) into which 2 ml of 0.15 M NaCl containing 0.22 mM D-glucose was added. The dialysis tube was sealed at each end and placed in a 50 ml centrifuge tube containing 45 ml of 0.15 M NaCl. The tubes were placed on an orbital shaker and kept at room temperature (20 ±2°C). The movement of glucose into the external solution was monitored at set time intervals. The effects of 50 mg/ml different plant extracts on glucose diffusion were compared to control tests conducted in the absence of plant extract. At the end of the experimental period, the concentrations of glucose within the dialysis tubing were measured. All tests were carried out in triplicate and the results were shown in Table No 6-8.and Fig 5-7.[28]

Table 6: Percentage inhibition of Glucose uptake in yeast cells with differentconcentration of PEEMI

S. No	Concentration (mg/ml)	Percentage inhibition of PEEMI (%)		
		5mM glucose	10mM glucose	25mM glucose
1	1	3.18±1.15	5.13±0.19	6.61±0.13
2	2	9.21±0.92	10.48±2.17	11.41±1.66
3	3	15.78±0.18	18.27±2.14	19.27±0.78
4	4	29.34±1.08	33.41±0.81	35.65±1.66
5	5	35.38±0.87	40.15±0.82	44.15±0.55

Fig 5: Percentage inhibition of Glucose uptake in yeast cells with differentconcentration of PEEMI

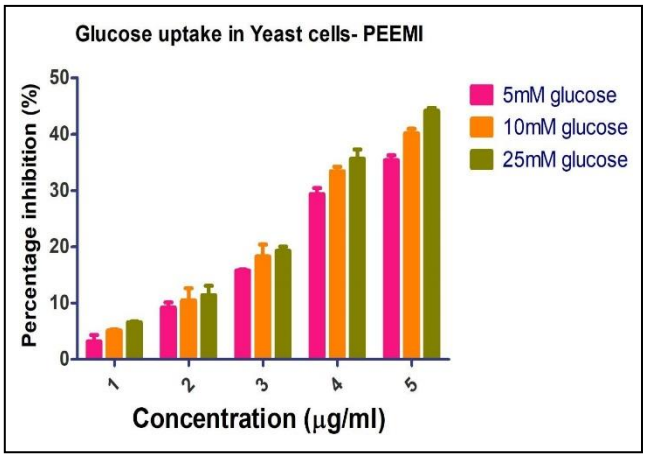

Table 7: Percentage inhibition of Glucose uptake in yeast cells with differentconcentration of EAEMI

S.no	Concentration (mg/ml)	Percentage inhibition of EAEMI (%)		
		5mM glucose	10mM glucose	25mM glucose
1	1	15.19±1.05	16.28±0.62	18.12±1.63
2	2	28.19±1.11	28.95±0.66	35.08±0.69
3	3	37.25±1.18	38.64±1.33	45.23±1.28
4	4	47.44±0.98	49.19±0.96	54.32±0.66
5	5	59.21±0.97	61.33±0.67	64.22±0.31

Fig 6: Percentage inhibition of Glucose uptake in yeast cells with differentconcentration of EAEMI

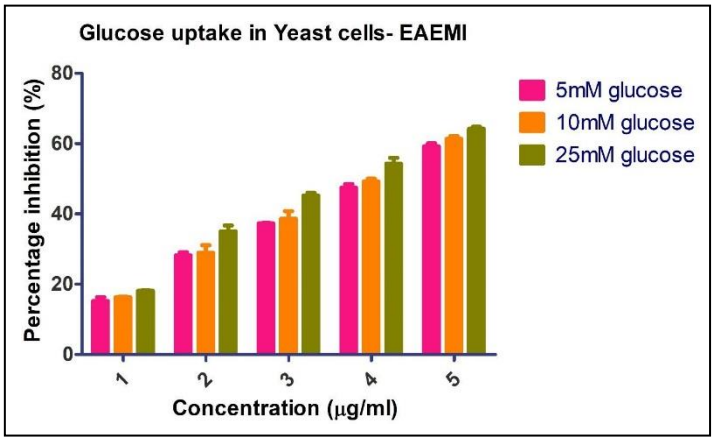

Table 8: Percentage inhibition of Glucose uptake in yeast cells with differentconcentration of EEMI

S.no	Concentration (mg/ml)	Percentage inhibition of EEMI (%)		
		5mM glucose	10mM glucose	25mMglucose
1	1	10.29±1.05	11.55±1.28	12.11±0.94
2	2	20.29±2.08	21.25±1.07	23.27±2.02
3	3	31.21±0.97	33.20±0.81	35.19±0.35
4	4	38.47±0.68	37.31±0.69	41.18±1.07
5	5	52.65±1.95	55.72±0.79	56.77±1.28

Fig 7: Percentage inhibition of Glucose uptake in yeast cells with differentconcentration of EEMI

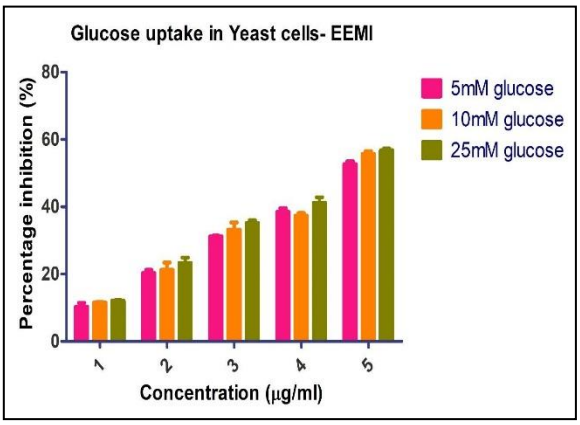

Table 9: Percentage inhibition of Glucose uptake in yeast cells with differentconcentration of Metformin

S.no	Concentration (mg/ml)	Percentage inhibition of Metformin (%)		
		5mM glucose	10mMglucose	25mMglucose
1	1	15.25±0.17	15.99±0.36	24.18±1.17
2	2	19.19±1.12	33.29±1.37	35.64±1.56
3	3	30.158±1.31	54.26±1.47	44.58±0.85

4	4	38.87±1.02	59.33±0.94	62.83±0.78
5	5	59.33±1.07	66.35±1.03	68.16±0.63

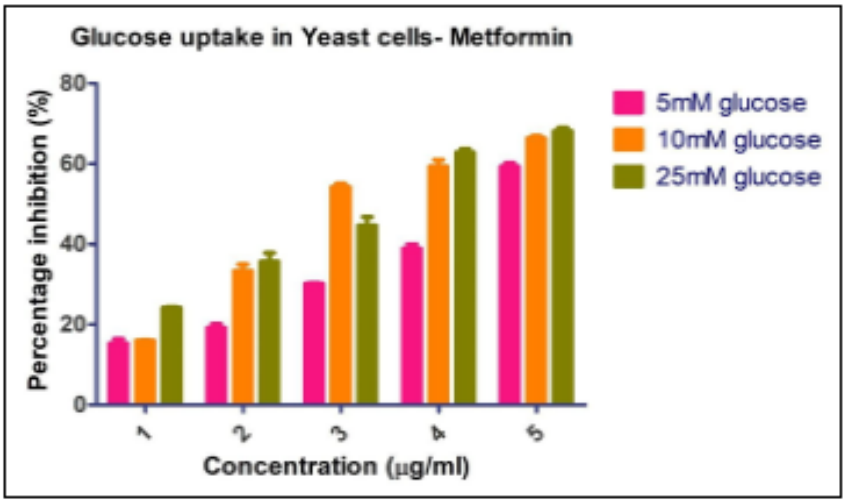

Fig 8: Percentage inhibition of Glucose uptake in yeast cells with differentconcentration of Metformin

CONCLUSION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia caused by defective insulin secretion, resistance to insulin action, or a combination of both. DM has reached epidemic proportions in the US and more recently worldwide. The morbidity and mortality associated with diabetes is anticipated to account for a substantial proportion of health care expenditures. Although there are several drug treatments currently available, the need for new herbal agents for treatment of diabetes are required. *Mangifera indica* Linn, locally known as mangotree has been claimed to possess antidiabetic properties by many investigators. The present study was undertaken to screen the antidiabetic activity of "*Mangifera indica"* flowers. In this study the plant material is extracted with petroleum ether, Ethyl acetate and ethanol. The extract is tested for phytochemical constituents and resulted the presence of alkaloids, flavanoids, Tannins, Triterpinoids, Saponins, Glycosides. The anti-diabetic activity of *Mangifera indica* flowers is evaluated by vitro methods such alpha amylase inhibition assay, Alpha glucosidase inhibition assay, Yeast cell glucose uptake and inhibition of glucose diffusion assay. The results demonstarted that the *Mangifera indica* flower extracts showed significant anti-diabetic activity. Hence, this review will be valuable for the scientific world to develop herbal drugsor clinical candidates in various biological areas. Based on the available study results, *Mangifera indica* flowers can be considered as one of the promising herbal drugs that can overcome future problems.

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