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Comparative antidiabetic activity of aqueous, alcoholic and hydro alcoholic leaf extract of *mangifera indica* using various *in vitro* models

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ABSTRACT

The aim of this study was to investigate the invitro antidiabetic activity and mechanism of action of aqueous, alcoholic and hydro alcoholic leaf extract extract prepared from *Magnifera indica*. The *in vitro* antidiabetic 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 aqueous dried leaf 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 aqueous leaf extract holds antidiabetic activity. This work indicates that aqueous leaf extracts of Magnifera indica have an important long term antidiabetic effect that can be well established to treat diabetes

Keywords: α-glucosidse, α-amylase, Magnifera indica and Anti diabetic activity.

INTRODUCTION

Diabetes derives its source from a Greek word 'diabaino' which means to go through, and "mellitus" means sweet or sugar. Hence, the passing of sugar with urine may be the crude meaning of the word diabetes mellitus.[1-3] The characteristic of Diabetes Mellitus is the lack of ability to control blood glucose, which finally passes through urine in excessive cases of diabetes mellitus. Diabetes Mellitus is a group of metabolic disorders characterized by abnormal high blood glucose (hyperglycemia) resulting from defects either in insulin secretion or insulin action or both.[4-7] For diabetes mellitus a treatment without any adverse effect is still remains as a biggest question for medical society. According to the worlds ethanobotanicals around 800 plants are used for prevention of diabetes mellitus. It has been clinically proven that only 450 among those plants possess antidiabetic properties and 109 of them has complete mode of action.[7-10]

There is a long history of traditional medicinal plants used for the treatment of diabetes in India and china. The antidiabetic activity of the medicinal plants is due to the presence of flavonoids, terpenoids, phenolic compounds, coumarin and other chemical constituents which leads to the reduction in blood glucose level. The intestinal digestive enzymes like alpha-glucosidase and alpha-amylase are played a vital role in the carbohydrate metabolism.[11-15] Alpha-glucosidase inhibitors are the saccharides that act as competitive inhibitors of enzymes needed for the carbohydrates regulation specifically alpha-glucosidase enzymes in the striated border of the small lintestines. The membrane bound intestinal alpha glucosidases hydrolyze oligosaccharides, trisaccharides and disaccharides to glucose and other monosaccharides in the small intestine.[15-17]

One antidiabetic therapeutic approach to reduce the postprandial glucose level in blood by the inhibition of alpha-glucosidase and alpha-amylase enzymes.[17-22] These can be an important strategy for the management of blood glucose level in the body. The aim of the present study was to investigate the phytochemical bioactive compounds of the various extract of Magnifera Indica leaves and its In-vitro antidiabetic activity. The result suggests that the presence of bioactive compounds could

be responsible for the versatile medicinal properties of this plant including diabetes and the extract exhibit the dose-dependent action by increasing the inhibitory effect on alpha-glucosidase enzyme and alpha-amylase enzyme.

Plant profile

The Mangifera indica (mango) is one of the choicest fruit crops of tropical and subtropical 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. Mangifera indica is the leading fruit crop in India. We used Leaf Part of Plant for this study.(Fig.1)

Fig 1: Leaf of Magnifera Indica

Chemical Composition

Mango contains a variety of phytochemicals and nutrients [23]. Mango peel and pulp contain other compounds, such as pigment carotenoids and polyphenols, and omega-3 and -6 polyunsaturated fatty acids [24]. 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 [25]. 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 .[26]

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, 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 40C for the further analysis. The percentage yield was calculated by using following formula Table.No1.

$$\textbf{Percentage Yield (\%w/w)} = \frac{\textbf{weight off extract obtained (gm)}}{\textbf{Weight of plant material used}} \times 100$$

Table 1: Percentage yield of different extracts of Mangifera indica leaves

Extract	Amount of plant material used (g)	Amount of extract obtained(g)	Percentage yield (%w/w)
EEMI	100	22.32	22.32
HAEMI	100	28.75	28.75
AEMI	100	31.85	31.85

EEMI:Ethanolic extract of Mangifera indica leaves,HAEMI:Hydroalcoholic extract of Mangifera indica leaves, AEMI:Aqueous extract of Mangifera indica leaves

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 leaves

PHYTOCONSTITUENTS	EEMI	HAEMI	AEMI
Alkaloids	Present	Present	Present
Flavonoids	Present	Present	Present
Tannins	Present	Present	Present
Steroids	Absent	Absent	Absent
Triterpenoids	Absent	Absent	Absent

Carbohydrates	Monosaccharide	Absent	Absent	Present
	Polisaccharide	Absent	Absent	Present
Saponins		Present	Present	Present
Glycosides		Present	Present	Present
Proteins and Aminoacids		Absent	Absent	Present

Alpha amylase inhibitory assay by dnsa color assay

The α-amylase inhibitory activity was assessed using method described by Elsnoussi et al.[27] . 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. [28]

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

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

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

S.no	Concentration (µg/ml)	Percentage of inhibition EEMI(%)	Percentage of inhibition HAEMI(%)	Percentage of inhibition AEMI(%)	Percentage of inhibition of Acarbose(%)
1	100	60.38	68.2	58.22	75.82
2	200	70.22	73.45	62.17	77.49
3	300	72.15	75.25	64.33	79.06
4	400	76.33	79.07	70.11	85.49
5	500	77.85	80.49	72.53	88.56
6	IC_{50} (µg/ml)	85.28	78.39	90.19	63.28

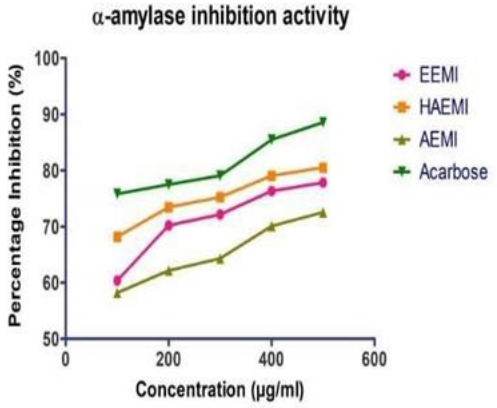

Fig 2: Effect of different extracts of Mangifera indica leaves and acarbose on α-amylase inhibition activity

Inhibition of α-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 No 4.and Figure.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 leaves and Acarbose

S.no	Concentration (µg/ml)	Percentage of inhibition EEMI(%)	Percentage of inhibition HAEMI(%)	Percentage of inhibition AEMI(%)	Percentage of inhibition of Acarbose(%)
1	100	17.91	19.85	16.22	88.47
2	200	33.75	38.33	29.87	90.66
3	300	47.67	49.21	31.56	92.21
4	400	63.86	69.55	44.28	93.18
5	500	76.36	78.27	60.11	96.27
6	IC_{50} (µg/ml)	318.21	304.05	456.24	59.55

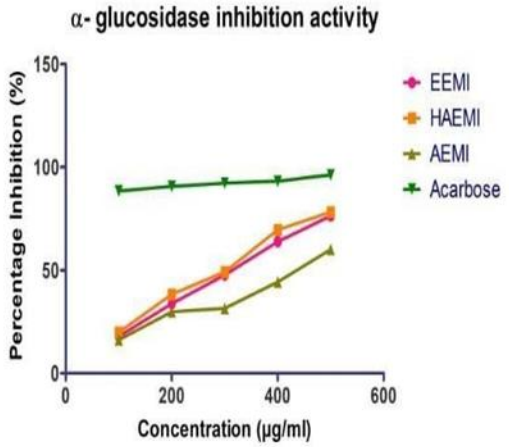

Fig 3: Percentage inhibition of Alpha glucosidase enzyme on different extracts of Mangifera indica leaves and Acarbose

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, 5 min) 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 No 5.and Figure.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. [29]

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

Sample	Glucose in external solution(mM)				
	30mins	60mins	90mins	120mins	180mins
Absence of drug (control)	55.00±2.22	64.59±1.18	66.28±1.11	115.64±1.08	129.89±1.77
Metformin	18.91±0.31	27.49±0.66	38.41±1.88	51.69±1.12	65.19±1.19
EEMI	19.26±0.56	29.66±1.02	44.70±0.67	62.18±0.51	77.61±2.35
HAEMI	18.36±0.56	27.46±2.18	42.08±0.71	60.19±0.18	69.61±1.54
AEMI	27.28±0.16	39.63±1.11	48.00±0.35	72.23±0.12	99.68±2.21

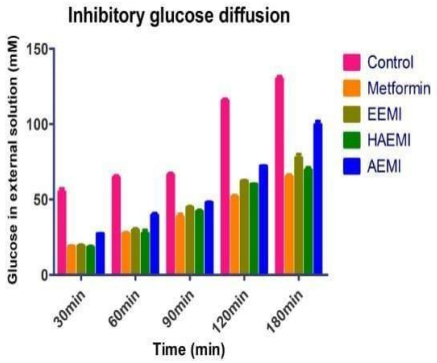

Fig 4: In vitro inhibitory glucose diffusion of different extracts of Mangifera indica leaves and Metformin

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 Figure.5-7[28]

Table 6: Percentage inhibition of Glucose uptake in yeast cells with different concentration of EEMI

S.no	Concentration (µg/ml)	Percentage of inhibition EEMI(%)		
		5mM glucose	10mM glucose	25mM glucose
1	1	15.17±0.85	18.15±0.28	17.61±1.33
2	2	33.34±1.01	34.48±1.81	35.34±0.45
3	3	44.05±0.41	48.26±1.07	39.15±1.18
4	4	58.54±0.58	53.71±1.31	45.22±0.97
5	5	67.58±1.07	61.35±1.02	56.21±0.48

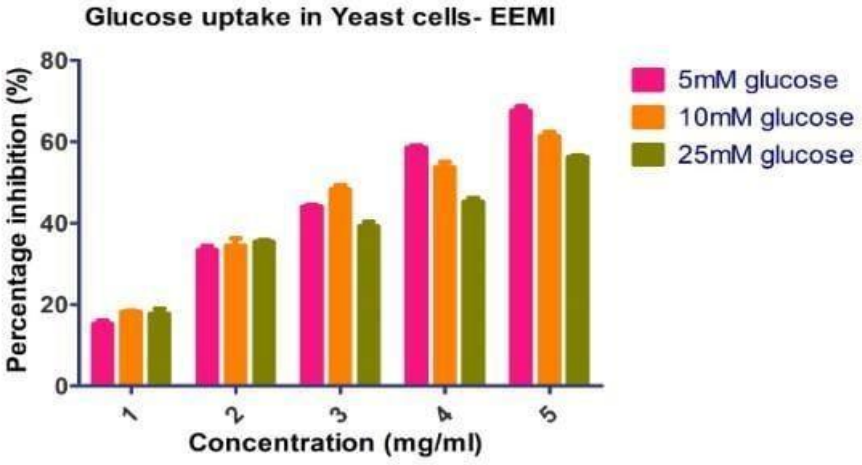

Fig 5: Percentage inhibition of Glucose uptake in yeast cells with different concentration of EEMI

Table 7: Percentage inhibition of Glucose uptake in yeast cells with different concentration of HAEMI

S.no	Concentration (µg/ml)	Percentage of inhibition HAEMI(%)		
		5mM glucose	10mM glucose	25mM glucose
1	1	14.18±0.15	15.17±1.81	16.11±0.83

2	2	28.24±0.91	29.45±1.85	39.22±0.51
3	3	39.15±0.18	42.61±0.37	47.15±1.22
4	4	49.54±1.08	52.18±1.38	52.12±0.71
5	5	60.11±0.27	64.32±0.27	59.12±0.41

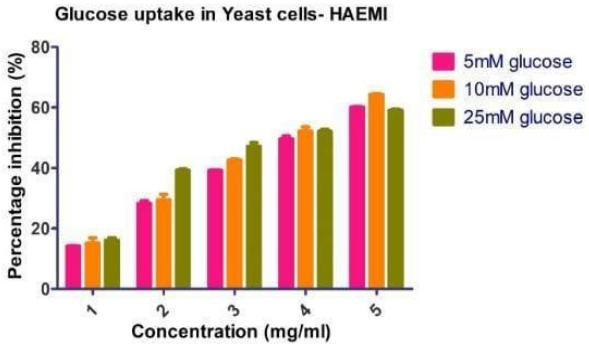

Fig 6: Percentage inhibition of Glucose uptake in yeast cells with different concentration of HAEMI

Table 8: Percentage inhibition of Glucose uptake in yeast cells with different concentration of AEMI

S.no	Concentration (µg/ml)	Percentage of inhibition AEMI(%)		
		5mM glucose	10mM glucose	25mM glucose
1	1	11.28±1.05	10.11±0.11	13.22±1.03
2	2	19.49±1.11	20.55±0.95	25.25±1.11
3	3	28.11±1.18	27.18±1.33	34.18±0.22
4	4	39.33±1.38	35.22±1.48	49.17±0.91
5	5	50.00±1.27	51.09±1.27	60.15±0.18

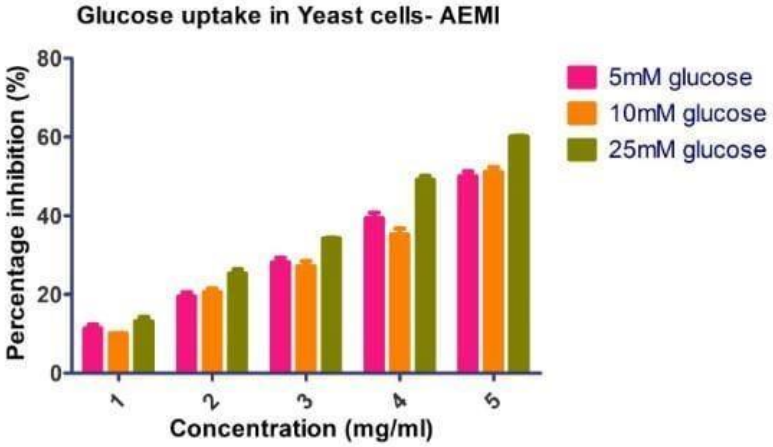

Fig 7: Percentage inhibition of Glucose uptake in yeast cells with different concentration of AEMI

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

S.no	Concentration (µg/ml)	Percentage inhibition of Metformin (%)		
		5mM glucose	10mM glucose	25mM glucose
1	1	5.85±1.18	16.81±0.64	34.22±0.14
2	2	20.18±1.12	35.27±0.48	45.84±0.55
3	3	29.19±1.33	55.11±0.39	54.48±0.81
4	4	38.03±0.91	61.08±0.77	64.85±0.29
5	5	58.33±1.05	69.36±0.94	70.46±0.65

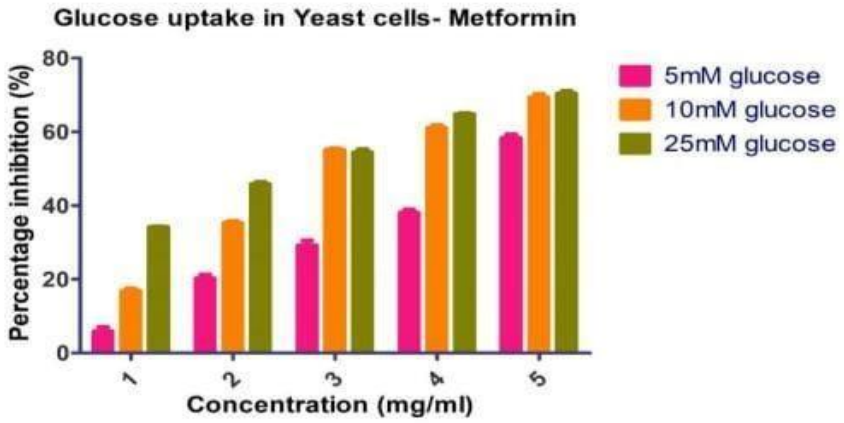

Fig 8: Percentage inhibition of Glucose uptake in yeast cells with different concentration of Metformin

CONCLUSION

An extensive research in ethno pharmacology has taken place throughout the world. The leaves of *Mangifera indica* was traditionally claimed for a large number of pharmacological actions and medicinal uses. In present investigation, the COMPARATIVE INVITRO ANTI-DIABETIC study revealed that the among the various extracts such as aqueous, alcoholic, and hydro alcoholic extracts of dried leaves of *Mangifera indica* the aqueous extract is found to have more ANTIDIABETIC action when compared to the other extracts. The antidiabetic activity may be attributed due to the phytoconstituents present in it. The present study offered a scientific proof to the traditional use of *Mangifera indica*. Further phytochemical studies are needed to isolate the active compounds responsible for these pharmacological activities. The comparative study showed that the hydro alcoholic extract of *Mangifera indica* leaves has more ANTIDIABETIC activity when compared to aqueous and alcoholic extracts. The present study is attempted to develop a novel plant based antidiabetic drug. Extensive research will be needed to determine the individual component responsible for the antidiabetic activity and molecular mechanism responsible for the same.

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