

***In-Vitro* Antidiabetic Propensities, Phytochemical Analysis, and Mechanism of Action of Commercial Antidiabetic polyherbal formulation “Mehon” †**

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Abstract: Present investigation assessed the hypoglycemic potential of hydro-alcoholic (HAE) and aqueous (AQE) extracts of Mehon, a commercial antidiabetic polyherbal formulation using various *in vitro* techniques. HAE and AQE were analyzed for α -amylase and α -glucosidase inhibition, glucose adsorption, diffusion, and transport across yeast cells membrane. HAE showed higher α -glucosidase (IC₅₀:156.95 μ g/ml) inhibition. Glucose adsorption increased with increment in glucose concentration (5mM/L-100mM/L). Rate of glucose uptake into yeast cells was linear. Both extracts exhibited time dependent glucose diffusion. Calculated GDRI was 27.44 %(HAE) and 17.43 %(AQE) at 30 min which reduced over time, thereby confirming the antihyperglycemic propensities of Mehon.

Keywords: Antidiabetic polyherbal formulations; Mechanism; Glucose uptake; Enzyme inhibition.

1. Introduction

Diabetes mellitus is a disorder of multiple etiologies characterized by high blood glucose with abnormal carbohydrate, protein, and lipid metabolism [1]. Absence or/and insensitivity of insulin leads to accumulation of glucose in the blood, further causes various secondary macro-vascular and micro-vascular complications [2]. Prevalence of diabetes mellitus is predominantly increasing due to sedentary lifestyles and consequential upsurge in obesity. It has been estimated that about 171 million people worldwide suffer from diabetes mellitus [3]. Oral hypoglycemic agents, insulin and combinatorial approach are presently available pharmacotherapies for management of diabetes mellitus. Drawbacks of present therapies include toxic side effects and prolonged use leading to diminution [4]. With the associated side effects and limitations of present therapies, a continuous research on natural sources are being attempted to develop new formulations for effective management of diabetes and its related complications [3,4]. Plants bioactive compounds have shown digestive enzymes inhibition abilities with their capability to bind to enzyme protein. Additionally, dietary fibers and its gelatinous polysaccharides play major role in reduction of postprandial plasma glucose levels in diabetes mellitus [10,11]. Various previous reports has showed that appropriate glycaemic control reduced the prevalence of retinopathy, nephropathy and neuropathy etc. [7,8]

Hence, herbal sources are considered as an alternative for effective management of diabetes mellitus and its associated complications. Various commercial antidiabetic polyherbal formulations, with claimed antidiabetic effects, are available in Indian market. However, only a few have received equitable scientific and medical scrutiny in terms of their mode of actions, in attaining glucose

homeostasis [5,6]. Thus, systematic scientific studies to explore the mechanism of action for commercial antidiabetic polyherbal formulation are needed. Further advances in understanding the activities of chief carbohydrate metabolizing enzymes such as α -amylase, α -glucosidase and the role of dietary fibers have led to the development of new pharmacologic agents, therefore leading to an innovative area and target of researches for attaining glucose homeostasis [9].

Since there are various polyherbal antidiabetic formulations existing in the Indian market, they are rarely studied for their *in-vitro* mode of actions and hence limited data on the same are available. Therefore, the present study is aimed to explore the systematic *in-vitro* mechanism of actions of the commercial Antidiabetic Polyherbal formulation, Mehon for its antidiabetic potential.

2. Material and methods

2.1. Chemicals and reagents

Glucose oxidase peroxidase reagent was procured from Agappe Diagnostic Ltd, India. Dialysis bags (12,000 MW cut-off) were used from Himedia laboratories, India. Antidiabetic polyherbal formulation, Mehon was obtained from Local market, India. All the other chemicals used in the study were of analytical grade.

2.2. Preparation of extract

Mehon (5 g) was extracted with 150ml of 70% methanol and distilled water by cold maceration for 24 h. The hydro-alcoholic (HAE) and aqueous (AQE) extracts were filtered using Whatman filter paper #1. The filtrate was concentrated with rotary evaporator and lyophilizer respectively, further stored at 4°C for analysis [12].

2.3. Estimation of total phenol content (TPC)

Mehon extracts 1ml was pre-incubated with 1.5 ml of Folin ciocalteu (FC) reagent for 15min followed by addition of 2ml Na₂CO₃ (7.5%), incubated in dark for 30 min. Absorbance was read at 765nm and Gallic acid was used as standard [6].

2.4. Estimation of total flavonoid content (TFC)

TFC was measured by adding 0.5ml of tested extracts (1mg/ml), 0.5ml of NaNO₃ (5%), incubated for 5 min followed by addition of AlCl₃ (10%) and Absorbance was read at 430nm Quercetin was used as standard [6].

2.5. α -amylase inhibition assay

DNSA method was used to determine the α -amylase inhibition activity with slight modification [6]. The reaction mixture composed of 250 μ l of extracts (200-1000 μ g/ml) with 250 μ l of porcine pancreatic α -amylase enzyme (1unit) was pre-incubated for 20min at 37°C. Reaction was initiated by addition of 250 μ l of 1% potato soluble starch followed by incubated for 10min at 37°C. Reaction was stopped with the addition of 0.5 μ l of DNS reagent, incubated in boiling water bath for 10min, the tubes were cooled and absorbance taken at 540nm, Acarbose was considered as positive control. The percentage of inhibition was calculated with following equation.

$$\% \text{ Inhibition: } \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

2.6. α -glucosidase inhibition activity

α -glucosidase inhibitory activity was assessed [6] with slight modifications. The reaction mixture containing 50 μ l of tested extracts (200-1000 μ g/ml) and 240 μ l of yeast α -glucosidase enzyme (1Unit/ml) was pre-incubated for 20mins at 37°C. The reaction was initiated with addition of 40 μ l PNPG (5mM) incubated for 10mins followed by addition of 750 μ l Na₂CO₃ (0.2M). Absorbance was

recorded at 405nm and acarbose was considered as positive control. The percentage of inhibition was calculated.

$$\% \text{ Inhibition: } \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

2.7. Effect of tested extracts on glucose adsorption capacity

HAE and AQE (250 mg) were separately added to 25 mL of glucose solution of increasing concentrations (5, 10, 20, 50 and 100 mM). The reaction mixture was agitated and incubated in a shaker incubator at 37°C for 6 h, centrifuged at 4,000 × g for 20 min. Glucose content in the supernatant solution was determined by Glucose oxidase- peroxidase method. Absorbance was read at 520nm and Acarbose was taken as positive control. Glucose adsorption capacity was determined according to the following formula [12].

$$\text{Glucose Bound} = \frac{(\text{Glucose}_1 - \text{Glucose}_6) \times \text{Volume of solution}}{\text{Weight of the extracts}}$$

Glucose₁: Concentration of glucose original solution.

Glucose₆: Concentration of glucose after 6 hrs.

2.8. Effect of tested extracts on in-vitro glucose diffusion

Twenty mM glucose solution (25 ml) and 0.25 g of Mehon extracts and Acarbose were dialyzed against 200ml of distilled water at 37°C [12]. Further, glucose concentration in the dialysate was determined at time intervals i.e., 30, 60, 120 and 180 min using glucose oxidase peroxidase kit. Control test without addition of the extract was also performed. Glucose dialysis retardation index (GDRI) was calculated according to the following formula.

$$\text{GDRI (\%)} = \frac{(100 - \text{Glucose content with the addition of extract})}{\text{Glucose content of the control}} \times 100$$

2.8. Effect of tested extracts on in-vitro amylolysis kinetics

Twenty-five millilitres of 4% starch solution with 0.4% of α-amylase and 1% of Mehon extracts were dialysed against 200 ml of distilled water at 37°C (pH-7). Concentration of glucose in the dialysate was determined at various time intervals 30, 60, 120 and 180 min and a control test without addition of the extract was also performed [12].

2.9. Effect of tested extracts on glucose uptake by yeast cells

Commercial baker's yeast (EasyGrow Baker's) was washed in distilled water with repeated centrifugation (3,000 × g; 5 min) till a clear supernatant was obtained; further 10% (v/v) suspension was prepared with the same. Different concentrations of both the extracts (1–5 mg) were added to 1 mL of glucose solution (5–25 mM), mixture was incubated for 10 min at 37 °C. Reaction was initiated by adding 100µL of yeast suspension, vortexed and incubated at 37 °C. After 60 min, tubes were centrifuged (2500 × g, 5 min) and glucose was estimated in the supernatant. Percent increase in glucose uptake by yeast cells was calculated using the following formula [12].

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

2.10. Statistical analysis

All the experimental works were carried out in triplicates and the obtained data were analysed by ANOVA. Graphs were plotted using Graph Pad Prism 8 software.

3. Results

3.1. Phytochemical analysis

In the present study, both the extracts i.e., HAE and AQE of plant based formulation were analysed for their phytochemical composition (Table 1). The data revealed HAE exhibited higher TPC (95.44±0.22 GAE/mg) and TFC (86±0.15 QE/mg) when compared to AQE.

Table 1. TPC and TFC values are represented as Mean ± SE, IC₅₀ values of HAE and AQE against α-amylase and α-glucosidase inhibition

Mehon Extracts	TPC GAE/mg	TFC QE/mg	α-amylase inhibition IC ₅₀ µg/ml	α-glucosidase inhibition IC ₅₀ µg/ml
HAE	95.44±0.22	86±0.15	581.5	156.95
AQE	47.87±0.29	38.82±0.15	872.88	800.63

3.2. Bioactivity assays

α-Amylase inhibitory activity data from the present study showed the variable inhibitory effect of HAE and AQE extracts, IC₅₀ values of extracts are summarized in Table 1. HAE exhibited significant comparable α-amylase inhibition (IC₅₀ 581.5 µg/ml) compared to standard drug Acarbose (IC₅₀ 523.12 µg/ml).

α-Glucosidase activity was assessed by the release of p-nitrophenol from PNPG *in vitro*. IC₅₀ (µg/mL) values of active extracts are presented in Table 1. Among the extracts Mehon HAE (IC₅₀ 156.95 µg/ml) showed potent inhibitors of α-glucosidase compared to acarbose (476.64 µg/mL).

3.3. Effect of Tested Extracts on Glucose Adsorption Capacity

HAE and AQE extracts of Mehon were assessed for their glucose adsorption capacity. A directly proportional relationship between glucose concentration and increase in bound glucose concentration was established (Fig 1). Greater adsorption capacity was observed with HAE (74.56±1.26) as compared to AQE (73.86±0.816). Acarbose (83.75±0.95 mM) was considered as positive control .

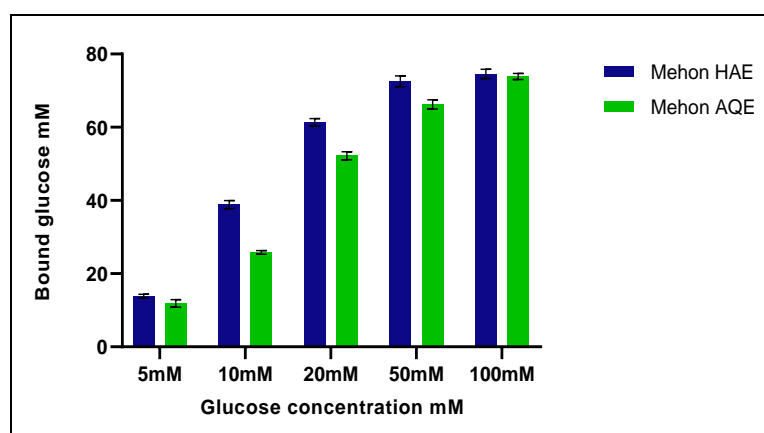


Figure 1. Glucose binding capacity of HAE and AQE at different glucose concentrations. Values are mean ± SE of triplicate determinations. Significant values (P<0.0001).

3.4. Effect of Tested Extracts on In-Vitro Glucose Diffusion

Both HAE and AQE extracts exhibited significant inhibitory effects on movement of glucose into external solution when compared to control. The rate of glucose diffusion across the dialysis membrane, was found to be directly proportional with time. The GRDI reduced over time for both the extracts with highest values observed at 180 min, (Table 2).

Table 2. Effect of Mehon extracts on glucose diffusion and GDRI

Sample	Glucose content in dialysate (mM)			
	30 min	60 min	120 min	180min
Control	0.929±0.081	1.31±0.043	1.503±0.04	1.744±0.13
AQE	0.767±0.05(17.44)	1.21±0.053 (7.66)	1.398±0.07 (7)	1.502±0.014(13.87)
HAE	0.674±0.05 (27.48)	1.062±0.05(19.08)	1.283±0.043 (14.63)	1.488±0.04(14.67)

Values in parenthesis indicate GDRI. Represented as Mean ± SD (n = 3)

3.5. Effect of tested extracts on in-vitro amylolysis kinetics

Effect of tested extracts on starch digestibility and glucose dialysis retardation index were presented in Table 3. The rate of glucose diffusion were analysed at every 30 min interval, diffusion rate proliferated with increase in time, maximum GDRI was observed at 180min for both the extracts. The present investigation showed GRDI value reduced steadily as time increases.

Table 3. Effect of Mehon extracts on extracts on starch digestibility and GDRI

	Glucose content in dialysate (mM)			
	30 min	60 min	120 min	180min
Control	0.0	0.28±0.005	0.354±0.003	0.446±0.009
AQE	0.0 (100)	0.134±0.03 (52.5%)	0.228±0.027 (34.19%)	0.38±0.07 (14.25%)
HAE	0.0 (100)	0.152±0.06 (45.7%)	0.24±0.047 (30.86%)	0.364±0.049(18.59%)

Values in parenthesis indicate GDRI. Represented as Mean ± SD (n = 3)

3.6. Effect of Tested Extracts on Glucose Uptake by Yeast Cells

Glucose transport across yeast cell membrane system is depicted (Figure. 2a and 2b). A linear uptake of glucose was observed for both the extracts, where HAE exhibited higher uptake activity

(Figure 2a) when compared to AQE. Percent increase in glucose uptake by the yeast cells was found to be inversely proportional to glucose concentration.

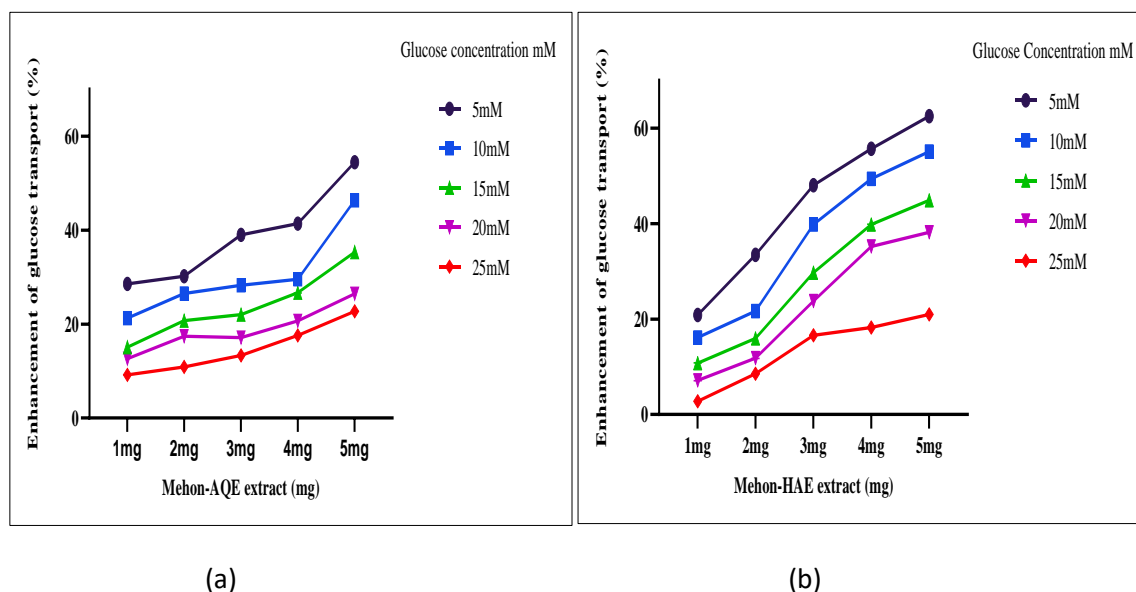


Figure 2. Effect of Mehon extract on the uptake of glucose by yeast cells. Values are mean of triplicate determinations. (a)AQE- Aqueous extract; (b)HAQ- Hydroalcoholic extract

4. Discussion

The present study was focused towards investigating the potential effects of selected commercial antidiabetic polyherbal formulation, Mehon for total phenolic and total flavonoid contents and ability to inhibit key carbohydrate hydrolysing enzymes, namely, α -amylase and α -glucosidase. Furthermore, the ability of the extracts to adsorb, entrap, transport glucose and amylolysis kinetics were also evaluated by *in-vitro* methods.

Quantitative phytochemical analysis showed HAE exhibited higher TPC and TFC. TPC was measured with FC reagent i.e., initially not specific for phenol as it is grounded on color formation therefore 90 min incubation, assures color change and measures the existence of phenolic compounds. Total flavonoid content was determined using aluminium chloride method. Aluminium chloride will form stable complex with carbonyl group at C4 and hydroxyls at C3 (flavonols) and C5 in flavonols and flavones [13]. Our results are in accordance with previous studies on *Caesalpinia bonduc* (L.) Roxb [14].

α -Amylase and α -glucosidase are key carbohydrate hydrolysing enzymes responsible for breaking α ,1-4 bonds in disaccharides and polysaccharides, liberating glucose [15, 16]. Hyperglycemia, the hallmark of DM can be maintained by inhibition of these chief enzymes where it was observed that both the extracts of Mehon showed satisfactory inhibitory activities. α -glucosidase is the vital enzyme involved in digestion of polysaccharides or disaccharides to monosaccharide's, increasing the blood glucose level. Hence delay in digestion of polysaccharides can be considered as one of the primary mechanisms to control hyperglycemia, which can be achieved by inhibition of α -glucosidase [23, 24]. Several previous scientific reports have shed light on the inhibition of these key enzymes [17,14].

The results of the study revealed higher adsorption capacity of the Mehon extracts (HAE and AQE). Both insoluble and soluble constituents such as fibers and bioactive compounds might have attributed to the adsorption capacity; earlier reports have confirmed constituents from different

sources to adsorb glucose. The results also showed that the AQE and HAE extracts of Mehon can bind glucose at lower glucose concentrations (5 mM), thereby decreasing the volume of accessible glucose for transport across the intestinal lumen, therefore reducing the postprandial glucose level [18].

Glucose dialysis retardation index (GDRI) is a suitable *in-vitro* criterion to evaluate glucose absorption in the gastrointestinal tract [19]. A higher GDRI indicates a higher retardation index of glucose by the sample. α -amylase inhibition can be considered as an important step in glucose diffusion retardation by the extracts under study, thus gradually controlling the release of glucose from the starch [19,20]. Previous reports have mentioned several possible factors that may be responsible for GDRI i.e., inhibition of α -amylase enzyme, fiber concentration, the presence of inhibitors on fibers, encapsulation of starch/enzyme by the fibers present in the sample, thereby reducing accessibility of starch to the enzyme, and direct adsorption of the enzyme on fibers, leading to decreased amylase activity [20, 18].

The mechanism of glucose transport across the yeast cell membrane has been receiving attention as an important method for *in vitro* screening of hypoglycemic effect of various compounds/medicinal plants [18,21]. It was observed that both the extracts of Mehon promoted glucose transport across the membrane of yeast cells. The rate of glucose uptake into the yeast cells was linear in all the five glucose concentrations considered in the study. Our results are in accordance with previous reports [21,22].

5. Conclusion

The observed results in the present study validate the antidiabetic activities of HAE and AQE of Mehon by several *in-vitro* methods viz. Total phenolic, total flavonoid contents, α -amylase and α -glucosidase inhibitory activities, glucose adsorption, glucose diffusion and glucose uptake at cellular levels by *in-vitro* yeast cells model. Thus, our study primarily emphasizes on various mechanisms for the hypoglycemic activity by which an antidiabetic polyherbal formulation, Mehon might be managing blood glucose levels, thus authenticating claims for the same.

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Conflicts of Interest: The authors declare no conflict of interest.

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