IN VITRO ANTI DIABETIC ACTIVITY OF MORINDA TINCTORIA FRUITS EXTRACTS

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ABSTRACT

Objective: To evaluate the glucose uptake of (antidiabetic activity) crude pet-ether, benzene, chloroform, ethyl acetate, ethanol, and methanol fruits extracts of Morinda tinctoria.

Materials and Methods: Morinda tinctoria fruits extracts were subjected to inhibitory effect of glucose utilization using specific standard in vitro procedure.

Results: The results in six different fruits extracts revealed that, the methanol extract at a concentration of 50 g plant extract/l was found to be more potent than other extracts with the lowest mean glucose concentration of 211±1.43 mg/dl at the end of 27 hrs.

Conclusions: The present findings suggest that, the methanolic extract showed a significant inhibitory effect on glucose diffusion in vitro thus validating the traditional claim of the plant.

Keywords: Antidiabetic activity, Glucose diffusion method, Morinda tinctoria

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. According to WHO, it is estimated that 3% of the world’s population have diabetes and the prevalence is expected to double by the year 2025 to 6.3%. Management of diabetes without any side effect is still a challenge to the medical community. The use of the drugs is restricted by their pharmacokinetic properties, secondary failure rates and accompanying side effects. Thus searching for a new class of compounds is essential to overcome diabetic problems. There is continuous search for alternative drugs. Morinda tinctoria Roxb. (family: Rubiaceae) grows wildly and distributed throughout Southeast Asia, commercially known as Nunas and locally known as “Togaru”, is a small tree with immense medicinal properties. It is indigenous to tropical countries and is considered as an important folklore medicine. In the traditional system of medicine, leaves and roots of M. tinctoria are used as astringent, Deobstrent, Emmengogue and to relieve pain in the gout [4]. There is a greater demand for fruit extract of morinda species in treatment for different kinds of illness such as arthritis, cancer, gastric ulcer and other heart disease [5]. Anti Convulvant, analgesic, antiinflammatory, anti oxidant activity and cytotoxic effect of Morinda tinctoria leaves has been reported [4,6,8]. Not much work has been carried out on in vitro antidiabetic activity of the fruits of M. tinctoria and identifies the phytoconstituents responsible for the biological activities of different solvent extracts of M. tinctoria fruits.

Materials and Methods

Plant material
The fresh fruits of Morinda tinctoria were collected locally and authenticated by the Department of Botany, American College, Madurai.

Preparation of extracts
The shade dried powdered form of fruits of Morinda tinctoria was taken and subjected to successive extraction using petroleum ether, benzene, Ethyl acetate, Chloroform, Ethanol and methanol by continuous percolation process in soxhlet apparatus. Each extract was concentrated by distilling off the solvent and evaporated to dryness. The extracts were dissolved in 1% carboxy methyl cellulose (CMC) and used for the present study.

Effects of Various Extracts on In vitro Inhibitory Glucose Diffusion

A simple model system was used to evaluate the effects of Morinda tinctoria fruits extract on glucose movement in vitro. The model was adapted from a method described by Edwards et al. [9] which involved the use of a sealed dialysis tube into which 15ml of a solution of glucose and sodium chloride (0.15M) was introduced and the appearance of glucose in the external solution was measured. The model used in the present experiment consisted of a dialysis tube (6cmX15mm) into which 1ml of 50g/litre plant extract in 1% CMC and 1ml of 0.15M sodium chloride containing 0.22M D-glucose was added. The dialysis tube was sealed at each end placed in a 50ml centrifuge tube containing 45ml of 0.15M sodium chloride. The tubes were placed on an orbital shaker and kept at room temperature. The movement of glucose into the external solution was monitored at set time intervals.

Statistical Analysis

Data are expressed as mean ± S.E.M. Statistical comparisons between groups were done by one way analysis of variance (ANOVA) followed by Tukey Kramer multiple comparison tests to analyze the differences. p<0.001 were considered as significant.

RESULTS

Effect on Glucose Diffusion:

With the distinctive traditional medical opinions and natural medicines mainly originated in herbs, traditional medicine offers good clinical opportunities and shows a bright future in the therapy of diabetes mellitus and its complications. The effect of Morinda tinctoria fruits as anti-diabetic agents has been studied. All extracts showed varying effect on glucose utilization. These extracts caused a significant decrease in glucose concentration during the experiment. The effects of Morinda tinctoria fruits extract on glucose diffusion inhibition were summarized in Table 1. At the end of 27 hrs, glucose movement of control (without plant extract) in the external solution had reached a plateau with a mean glucose concentration above 400mg/dl (423±1.72). It was evident from the table that the ethanol and methanol extracts were found to be potent inhibitors of glucose...
diffusion (p<0.001) compared to control. The methanol extract was found to be more potent than other extracts showing the lowest mean glucose concentration of 211±1.43 mg/dl at the end of 27 hrs (Table.1)

**DISCUSSION**

Diabetes mellitus is a debilitating and often life threatening disorder with increasing incidence throughout the world. There is a steady rise in the rate of incidence of Diabetes mellitus and estimated that 1 in 5 may be diabetic by 2025[10]. Antihyperglycemic activities of most effective plants were in part explained by the ability of the phytoconstituents to increase glucose transport and metabolism in muscle and/ or to stimulate insulin secretion [11]. In the present study, research has been carried out to evaluate the potential of various extracts to additionally retard the diffusion and movement of glucose in the intestinal tract [12,13 &14]. A decoction of *Morinda tinctoria* leaves is used worldwide for the treatment of various ailments including antidiabetic. The numerous polyphenolic compounds, triterpenoids and other chemical compounds present in the plant may account for the observed antidiabetic effects of the leaf extracts. A Decoction of *Morinda tinctoria* leaves was screened for hypoglycaemic activity on alloxan-induced diabetic rats. In both acute and sub-acute tests, the water extract, at an oral dose of 250 mg/kg, showed statistically significant hypoglycaemic activity [8]. The treatment with *Morinda tinctoria* aqueous leaf extract (0.01-0.625 mg/ml) showed significant inhibition on LDL glycation in a dose-dependent manner. Tannins, flavonoids, pentacyclic triterpenoids, quercetin, and other chemical compounds present in the plant are speculated to account for the observed hypoglycaemic and hypotensive effects of the leaf extract [8].

**Table 1: Effect of Morinda tinctoria fruits extracts (50g/litre) on the movement of glucose out of dialysis tube over 27hr incubation period.**

<table>
<thead>
<tr>
<th>Extract</th>
<th>1h</th>
<th>3h</th>
<th>5h</th>
<th>24h</th>
<th>27h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (in the absence of extract)</td>
<td>123.1±1.22</td>
<td>190.1±3.14</td>
<td>247.13±1.86</td>
<td>329.25±1.76</td>
<td>423±1.76</td>
</tr>
<tr>
<td>Pet ether extract (50g/l)</td>
<td>103.4±1.21**</td>
<td>173±1.71**</td>
<td>238.13±1.18**</td>
<td>254.32±1.73**</td>
<td>301.26±1.86**</td>
</tr>
<tr>
<td>Benzene extract (50g/l)</td>
<td>82.14±1.91***</td>
<td>139±0.43**</td>
<td>177.57±1.43**</td>
<td>228.12±2.84**</td>
<td>268±1.42**</td>
</tr>
<tr>
<td>Chloroform extract (50g/l)</td>
<td>85.15±0.27**</td>
<td>118±1.64**</td>
<td>167.24±1.62**</td>
<td>214±1.52**</td>
<td>251±1.42**</td>
</tr>
<tr>
<td>Ethylacetate extract (50g/l)</td>
<td>77.14±1.23**</td>
<td>113±0.98**</td>
<td>147.48±1.52**</td>
<td>209±2.23**</td>
<td>236±1.76**</td>
</tr>
<tr>
<td>Ethanol extract (50g/l)</td>
<td>74.14±1.66**</td>
<td>105±1.88**</td>
<td>141.61±1.36**</td>
<td>197±1.59**</td>
<td>223±1.72**</td>
</tr>
<tr>
<td>Methanol extract (50g/l)</td>
<td>75.11±0.25**</td>
<td>104±1.69**</td>
<td>139.78±1.42**</td>
<td>196±1.52**</td>
<td>211±1.43**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of triplicate; Data were analysed using one way ANOVA followed by Tukey-Kramer multiple comparison test; ***P<0.001 compared to control.

**CONCLUSION**

The present study demonstrates the ability of various extracts of *Morinda tinctoria* to inhibit glucose diffusion using an in vitro model of glucose absorption. In particular, ether and methanol extracts represent potential inhibitory of glucose diffusion supplements that may be useful for allowing flexibility in meal planning in type 2 diabetes. Further studies are required to elucidate whether in vitro effects represent therapeutic potential by limiting postprandial glucose absorptions and for improving glycemic control in type 2 diabetic subjects.

**Conflict of interest statement**

We declare that we have no conflict of interest.

**Acknowledgement**

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