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Full Length Original Research Paper

An *in Vitro* investigation into the Mechanism of Anti-Diabetic activity of selected Medicinal Plants

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Abstract: Aim & Objective: *In vitro* analysis of the anti-diabetic effect of aqueous extracts from the medicinal plants *Hemidesmus indicus, Ficus benghalensis, Pterocarpus marsupium* roxb.Materials & Methods: An aqueous extract of each of the plants were prepared by soxhletion at 60° C. 1mL of the extract was then placed in a bio-membrane along with a glucose solution (0.22 mM in 0.15 M NaCl) and the bio-membrane was immersed in a beaker containing 40mL of 0.15 M NaCl + 10mL of distilled water. The control contained 1mL of 0.15 M NaCl containing 22 mM glucose and 1mL of distilled water. Half-hourly observations of the concentration of glucose in mg/dL in the beaker were done. Results:A significant hindrance to the flow of glucose across the bio-membrane was seen. *Pterocarpus marsupium*roxb and *Ficus benghalensis* showed a relative movement of 16.05% ($p<0.01$) and 78% ($p<0.01$) when compared to the aqueous control after 180 minutes. *Hemidesmus indicus* showed a relative movement of 84.5% ($p<0.01$) at 150 minutes and 134.2% ($p<0.01$) after 180 minutes when compared with the aqueous control. Conclusion: The medicinal plants were found to show potent inhibition of glucose diffusion across the membrane. These results indicate that these plants could quite possibly show hypoglycaemic activity due to this inhibitory action. However, further studies at a molecular level are essential to confirm this mechanism.

Keywords: *Pterocarpus marsupium* roxb, *Ficus benghalensis*,*Hemidesmus indicus,* Phytotherapy, Anti-diabetic plants, Mechanism of action, Hypoglycaemic plants, Hyperglycaemia, Ethnomedicine.

Introduction

Siddha system of medicine is a special, scientific and respected school of medicine practised in South India.[21]Its popularity is due to the fact that it provides better, cost-effective cures with fewer side effects than allopathic medicines for 4,448 diseases.[6,18]Diabetes mellitus is a common metabolic disorder characterised by hyperglycaemia induced by decreased cellular glucose uptake and metabolism.[3]Regulation of plasma glucose concentrations is extremely essential in order to decrease the incidence and severity of long term diabetic effects. [14]While external insulin is necessary for control of type 1 diabetes mellitus, the use of drug therapy in type 2 diabetes is initiated only after dietary and lifestyle modifications.[20]Dietary changes are most commonly considered for prevention of hyperglycaemia in diabetes.[20] Usage of high concentrations of plant derived dietary fibre and complex polysaccharides is recommended.[11]More than 400 plants world-wide have been documented as beneficial in the treatment of diabetes.[11] The majority of traditional anti-diabetic plants await proper scientific and medical evaluation for their ability to improve blood glucose control.[10,19]*Hemidesmus indicus,* commonly called *nannari*in Tamil, has been prescribed since a long time for diabetes. Its roots have been proven to show hypoglycaemic activity *in vivo* and anti-diabetic principles and bioactive molecules have been isolated from it.[13,17]*Ficus benghalensis* is commonly known as the

Indian Banyan and *Pterocarpus marsupium* roxbis commonly known as the Indian Kino tree. All the three plants are prescribed as anti-diabetic drugs in the *Siddha* system of medicine. Also, all of the plants show anti-microbial activity, anti-helminthic activity, anti-inflammatory activity and anti-oxidant activity besides their anti-diabetic activity.[9,1, 2,7,15]Despite extensive studies having been done on the anti-diabetic principles present on these plants, no study has been carried out to explain the mechanism of action of these plants.This study was essentially an effort to check whether the inhibition of glucose diffusion was a possible mechanism of the anti-diabetic action of these plants.

Materials and Methods

Chemicals and reagents: The dialysis membrane was purchased from HiMedia Laboratories, Mumbai, India. All other chemicals, reagents, kits and solvents used in this study were of analytical grade and procured locally.

Plant material and extract powder: The plant material was obtained from forests in and around Chitteri Hills, Keeraipatty Village, Harur, Dharmapuri District, Tamil Nadu. It was identified and authenticated by the local forest officer. Bark of *Pterocarpus marsupium* roxb and *Ficus benghalensis* were taken while root of *Hemidesmus indicus* was taken. Aqueous extracts of the plant material were prepared using soxhletion. 10 g of plant material was soxhleted with 250mL of water at 60°C for 6 hours. This was then filtered through a Whatman No. 2 filter paper and used directly.

Glucose diffusion inhibitory study: To test the diffusion of glucose across the bio-membrane, 3cm strips of the dialysis membrane (12000MW, HiMedia Laboratories, Mumbai, India) were cut and filled with 1mL of 0.15 M NaCl containing 22mM glucose and 1mL of plant extract. They were then tied at both ends using a nylon thread and placed in a 100mL beaker containing 40mL of 0.15 M NaCl and 10mL of distilled water to equalise the strength of internal and external media. These beakers were then placed on an orbital shaker(The I L E Company, Chennai, Tamil Nadu, India) and kept at room temperature(37°C).[19, 16]The control contained 1mL of 0.15M NaCl containing 22 mM glucose and 1mL of distilled water. It was devoid of plant extract. Samples were taken from each beaker and glucose concentration in them was tested every half an hour. Three replications of this procedure were done for 3 hours.

Statistical Analysis

Statistical analysis was carried out using Microsoft Excel 2010. Error values of 5% were assumed for calculating the concentration of glucose and 1% for the relative movement of glucose.

Results and Discussions

The results of the glucose diffusion inhibitory study are given in Table 1, 2 & 3. As inferred from the tables, the plants retard the diffusion of glucose across a dialysis membrane. *Pterocarpus marsupium* roxb showed maximum inhibition to the diffusion of glucose. The relative movement with respect to control was only 16.05% after 180 minutes. *Ficus benghalensis* also showed continued retardation of movement of glucose. *Hemidesmus indicus* showed effective inhibition of glucose diffusion till 150 minutes, but after this, there was a sudden increase in the movement of

glucose. The results indicate that glucose diffusion inhibition across a membrane is a possible mechanism of hypoglycaemic activity of the anti-diabetic principles of these plants. This is especially true for *Pterocarpus marsupium* roxb and *Ficus benghalensis* as they show continued inhibition to the diffusion of glucose across a membrane. *Hemidesmus indicus* does not showed continued inhibition to the diffusion of glucose. Its anti-diabetic principle may act by some other mechanism like a-amylase inhibition. It is also possible that the anti-diabetic principle of *Hemidesmus indicus* is not stable for long. Since this was a simple study, it used a normal dialysis membrane to test the mechanism. In the body, there are various transporters which work in synchronization with other molecules to transport glucose. Glucose was prepared in NaCl as glucose molecules need a carrier molecule to diffuse across cells. In the body, this is typically achieved by sodium ions.[12]Further studies using GLUT family of transporters in liposomes are planned.

Table 1: This table shows the effect of aqueous plant extract (40g/L) of *Pterocarpus marsupium* roxbon diffusion of glucose out of a bio-membrane over 180 minutes.

Concentration of Glucose (mg/dL)			
TIME (Minutes)	CONTROL Mean ± SEM*	*Pterocarpus marsupium*roxb Mean ± SEM*	Relative Movement %**
30	0.550 ± 0.034	0.34 ± 0.045	61.80%
60	1.167 ± 0.110	0.74 ± 0.045	63.40%
90	2.134 ± 0.200	1.20 ± 0.120	56.23%
120	3.250 ± 0.025	2.54 ± 0.067	78.15%
150	4.200 ± 0.267	3.01 ± 0.089	71.40%
180	5.120 ± 0.123	4.21 ± 0.290	16.05%

Values are mean (SEM) for groups of 3 observations.*p < 0.05**p<0.01

Table 2: This table shows the effect of aqueous plant extract (40g/L)of *Ficus benghalensis* on diffusion of glucose out of a bio-membrane over 180 minutes.

Concentration of Glucose (mg/dL)			
TIME (Minutes)	CONTROL Mean ± SEM*	*Ficus benghalensis* Mean ± SEM*	Relative Movement %**
30	0.550 ± 0.034	0.116 ± 0.023	28.0%
60	1.167 ± 0.110	0.467± 0.078	46.7%
90	2.134 ± 0.200	1.043 ± 0.078	80.0%
120	3.250 ± 0.025	2.183 ± 0.058	81.0%
150	4.200 ± 0.267	2.450 ± 0.034	69.0%
180	5.120 ± 0.123	4.000 ± 0.045	78.0%

Values are mean (SEM) for groups of 3 observations.*p < 0.05**p<0.01

Table 3: This table shows the effect of aqueous plant extract (40g/L) of *Hemidesmus indicus* on diffusion of glucose out of a bio-membrane over 180 minutes.

Concentration of Glucose (mg/dL)			
TIME (Minutes)	CONTROL Mean ± SEM*	*Hemidesmus indicus* Mean ± SEM*	Relative Movement %**
30	0.550 ± 0.034	0.417 ± 0.023	75.80%
60	1.167 ± 0.110	1.000 ± 0.067	85.70%
90	2.134 ± 0.200	1.250 ± 0.058	58.60%
120	3.250 ± 0.025	2.700 ± 0.030	83.07%
150	4.200 ± 0.267	3.550 ± 0.034	84.50%
180	5.120 ± 0.123	6.870 ± 0.577	134.20%

Values are mean (SEM) for groups of 3 observations.*p < 0.05**p<0.01

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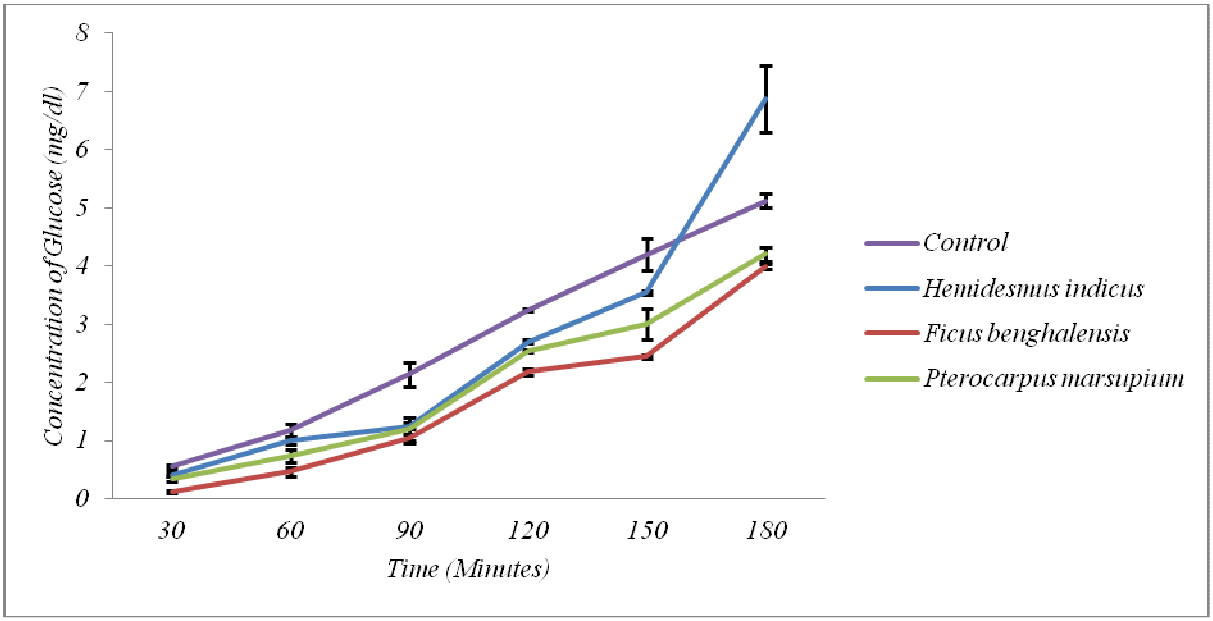

Fig 1: Effect of aqueous extracts of *Hemidesmus indicus, Pterocarpus marsupium* roxb& *Ficus benghalensis* on the diffusion of glucose out of a bio-membrane as compared to aqueous control. Values are Mean ± SEM for groups of 3 observations with their standard errors indicated by vertical bars.

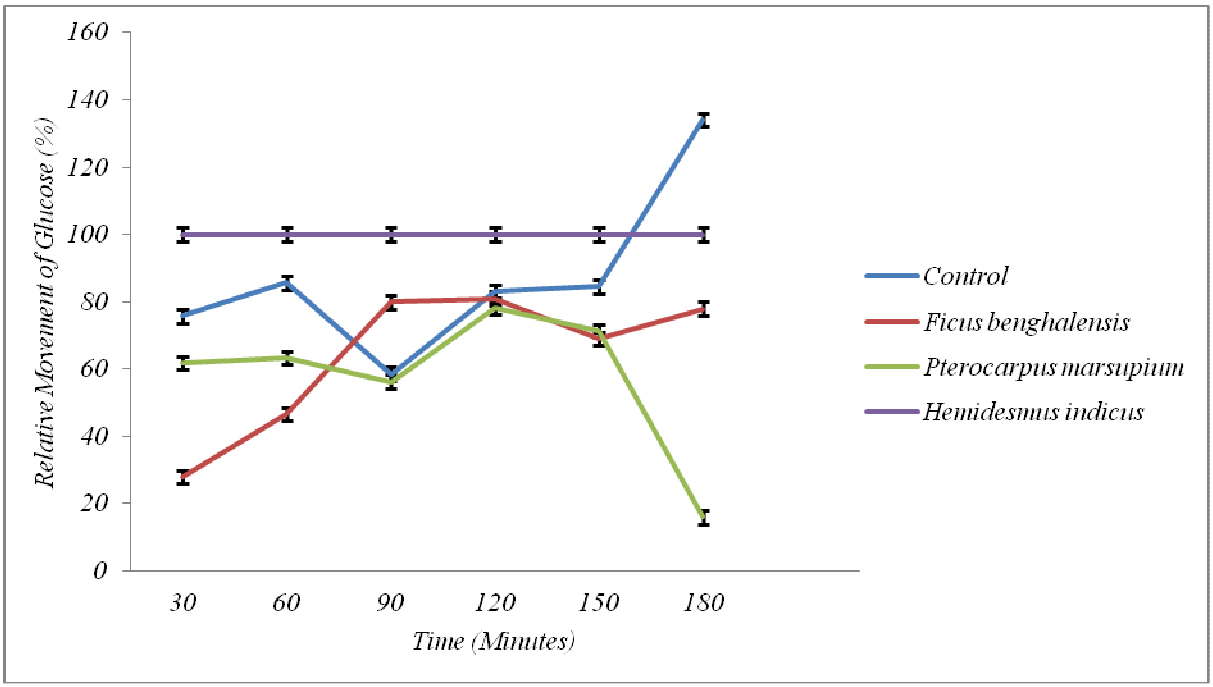

Fig 2: Relative movement of glucose across bio-membrane with respect to control under the influence of aqueous extracts of *Hemidesmus indicus, Pterocarpus marsupium* roxb& *Ficus benghalensis*. Values are percentages with their standard errors indicated by vertical bars.

Figure 1 shows the anti-diabetic activity of the plants in terms of concentration of glucose. It is clear that the amount of glucose diffusing out of extract containing membrane is always less than the amount of glucose diffusing out of control membrane except for *Hemidesmus indicus* after 180 minutes.

Figure 2 shows the anti-diabetic activity of the plants in terms of relative movement of glucose out of the membrane. Here, the amount of glucose escaped out of the control membrane is

taken as 100%. The other concentrations as then expressed as percentages of the control value. Again, they are always less than the control values except for *Hemidesmus indicus* after 180 minutes.

Conclusion

The results of this study show a strong possibility that the selected medicinal plants show hypoglycaemic activity by inhibiting the diffusion of glucose, thereby reducing its uptake in the intestine. Further studies with liposomes or cell lines are required to confirm these findings. However, the glucose diffusion inhibitory effects are clear and cannot be discounted. They may exhibit hypoglycaemic activity by inhibiting glucose diffusion in conjugation with inhibition of gluconeogenic enzymes like α-amylase and β-glucosidase. Due to their natural origin, drugs developed from these plants are likely to show less side effects and find easier acceptance by people when compared to allopathic drugs.

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