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In –Vitro Study of *Adhatoda Vasica Nees* on Glucose Uptake by Isolated Rat Hemi- Diaphragm

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Abstract

Aims: The present study is to evaluate the *in vitro* Anti-diabetic activity of the various extracts of *Adhatoda vasica Nees* on glucose uptake by isolated rat hemi-diaphragm. *Methods:* The *Adhatoda vasica Nees* was extracted by using n-hexane, chloroform, ethyl acetate, methanol, and aqueous. The different extracts were then used to study on *in vitro* glucose uptake by isolated rat hemi-diaphragm. *Results:* The ethyl acetate and methanol extracts of the plant extracts showed significant glucose uptake when compared with control. The result strongly suggest that Adhatoda vasica nees will be alternative choice for treatment of diabetes mellitus. *Conclusion:* In the in vitro uptake assay in rat hemi diaphragm, Ethyl acetate and Methanol extract and the isolated compound showed significant of glucose uptake (41.3653±0.657188, and 27.26±0.88) respectively. The results suggested that the extracts and compound enhanced the uptake of glucose by isolated rat hemi diaphragm which was more effective than insulin.

Keywords: *Adhatoda Vasica Nees;* Glucose Uptake by Isolated Rat Hemi-Diaphragm; Diabetes Mellitus, Nilgiris.

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Introduction

Diabetes mellitus (DM) is a chronic disease caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to the secreted insulin. Such a deficiency results in increased blood glucose level, which in turn can damage many of the body's systems, including blood vessels and nerves [1].

The isolated diaphragm of the rat is a suitable tool for the experimental study of the glucose uptake and glycogen synthesis by muscle tissue, demonstrated that these processes are stimulated by in vitro addition of insulin. Many investigators have used the method to study the hormonal control of carbohydrate metabolism. The uptake of glucose by muscle such as diaphragm occur in two stages: (i) penetration of the muscle cell by glucose, and (ii) metabolism of glucose with in the cell: and that it is the first of theses which is normally rate-limiting. It is the penetration of the muscle cell by glucose which is the rat-limiting step in the uptake of glucose by isolated diaphragm and that insulin or factors which inhibit oxidative phosphorylation augment glucose uptake by increasing penetration [2].

Many traditional plants have been reported in India for diabetes, but only a small number of these have received scientific and medical evaluation to assess their efficacy. On the basis of ethno medical/ tribal information *Adhatoda vasica nees* has been used to treat and prevent diabetes. *Adhatoda vasica Nees* possess a diverse number of pharmacological activities including antioxidant and free radical scavenging activity [3–5], anticholinesterase action

[6-7] and anti-inflammatory property.

However, the studies on anti-diabetic effects of *Adhatoda vasica* extracts were not focused on the enzyme inhibitory activity. Thus, the present study is designed to evaluate the *in-vitro* antidiabetic activity of *Adhatoda vasica* extracts and to understand how the extract acts against in vitro glucose uptake by isolated rat hemi diaphragm.

Materials and Methods

Plant Material

The entire plant of *Adhatoda vasica* was collected from the forests of Doddabetta in Nilgiris. The plant species was identified and authenticated by Botanist, Government Botanical garden, Ooty. The voucher specimen was deposited in the herbarium of the Department of Pharmacognosy, JSS College of Pharmacy, Ooty. The whole plant was used in the study.

Preparation of Crude Extract

The medicinal plant part (*Adhatoda vasica nees*) was sun dried and ground to a coarse powder and stored in an air tight container. This coarse powder was subjected to successive extraction with n-hexane (68^0C), chloroform (61^0C), ethyl acetate (77^0C), and methanol (64^0C) by continuous soxhlation and aqueous extracts by maceration process. After collection of extracts, it is kept at temperature 37^0C until solvent is completely evaporated. Then finally dried in desiccator.

Glucose Uptake by Isolated Rat Hemi-Diaphragm

Isolation of rat hemi-diaphragm: - Albino rats of sex weighing between 160-180gm were selected. The animals were maintained on a standard pellet diet and fasted overnight. The animals were sacrificed by decapitation and diaphragms were dissected out quickly with minimal trauma and divided into two halves. The hemi diaphragm were then rinsed in cold tyrode solution(without glucose) to remove any blood clots and were placed in small culture tubes containing 2ml tyrode solution with 2% glucose and incubated for 30 min at 37^0c in an atm of 100%0_2 with shaking [8].

Procedure

Group 1: 2ml of tyrode solution with 2%glucose and regular insulin 0.62 ml of 0.4 U/ml solutions.

Group 2: 2ml of tyrode solution with 2%glucose and 1.38ml of n-hexane extract of *Adhatoda vasica ness.*

Group 3: 2ml of tyrode solution with 2%glucose and 1.38ml of n-hexane extract of *Adhatoda vasica ness* and 0.62ml of regular insulin.

Group 4: 2ml of tyrode solution with 2%glucose and 1.38ml of chloroform extract of *Adhatoda vasica ness.*

Group 5: 2ml of tyrode solution with 2%glucose and 1.38ml of chloroform extract of *Adhatoda vasica ness* and 0.62ml of regular insulin.

Group 6: 2ml of tyrode solution with 2%glucose and 1.38ml of Ethyl acetate extract of *Adhatoda vasica ness*

Group 7: 2ml of tyrode solution with 2%glucose and 1.38ml of Ethyl acetate extract of *Adhatoda vasica ness* and 0.62ml of regular insulin.

Group 8: 2ml of tyrode solution with 2%glucose and 1.38ml of methanol extract of *Adhatoda vasica ness*

Group 9: 2ml of tyrode solution with 2%glucose and 1.38ml of methanol extract of *Adhatoda vasica ness* and 0.62ml of regular insulin.

Group 10: 2ml of tyrode solution with 2% glucose and 1.38ml of aqueous extract of *Adhatoda vasica ness.*

Group 11: 2ml of tyrode solution with 2% glucose and 1.38ml of aqueous extract of *Adhatoda vasica ness* and 0.62ml of regular insulin.

Results

Glucose uptake by isolated rat hemi-diaphragm of different extracts of n-hexane, chloroform, ethyl acetate, methanol and aqueous extracts of *Adhatoda vasica ness* are given in table1. Ethyl acetate and methanol showed significant glucose uptake when compared with control. The results showed that ethyl acetate and methanol had better efficacy than insulin alone

Discussion

The treatment goal of diabetes patients is to maintain glycemic level in control, in both the fasting and post-prandial states. Many natural resources have been investigated for the suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine [11].

Table 1: Effect of extracts and fractions of*Adhatoda vasica Nees*on glucose uptake in rat hemi diaphragm

S. No	Group	GLUCOSE UPTAKE (mg/g/30 min)
1	Insulin	53.51±0.95
2	n-hexane	6.69±0.66
3	n-hexane+insulin	4.36±0.24
4	Chloroform	0.87±0.07
5	Chloroform+insulin	0.56±0.88
6	Ethyl acetate	41.36±0.65
7	Ethyl acetate+insulin	37.35±0.56
8	Methanol	27.26±0.88
9	Methanol+insulin	26.94±0.65
10	Aqueous	10.45±0.31
11	Aqueous+insulin	9.59±0.4276

Value are expressed as Mean ± SEM, (n=5)

In this study we have investigated the anti-diabetic potential of the *Adhatoda vasica*, which is used in traditional ayurvedic medicine for the treatment of several diseases [12]. This valuable herb was not previously investigated for its *in vitro* anti-diabetic activity.

In the in vitro uptake assay in rat hemi diaphragm, Ethyl acetate and Methanol extract and the isolated compound showed significant of glucose uptake (41.3653±0.657188, and 27.26±0.88) respectively. The results suggested that the extracts and compound enhanced the uptake of glucose by isolated rat hemi diaphragm which was more effective than insulin [13].

Among the five extracts and the compound, Ethyl acetate and Methanol extracts possessed significant enzyme inhibition, hypoglycemic activity and significant glucose uptake in skeletal muscle (rat hemi diaphragm). This would suggest the presence of hypoglycemic and insulin sensitizer components in ethyl acetate and Methanol extract [14].

Conclusion

In the in vitro uptake assay in rat hemi diaphragm, Ethyl acetate and Methanol extract and the isolated compound showed significant of glucose uptake (41.36±0.65 and 27.26±0.88) respectively. The results suggested that the extracts and compound enhanced the uptake of glucose by isolated rat hemi diaphragm which was more effective than insulin.

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