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In-Vitro Evaluation of glucose diffusion and amylolysis kinetics of Niranthin

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Abstract: *The standardized Niranthin was studied for its effects on glucose diffusion and amylolysis kinetics using invitro models. The results verified the antidiabetic potential of the standardized Niranthin..*

Key Words: *Niranthin; glucose diffusion; amylolysis kinetics.*

1. INTRODUCTION:

Niranthin, a lignan isolated from the aerial parts of the plant Phyllanthus amarus, exhibits a wide spectrum of pharmacological activities [1]. Niranthin also exhibits anti-inflammatory and anti-allodynic properties (1) and has been shown to possess anti-viral activity against human hepatitis B virus in vitro (2). The lignan-rich fraction containing niranthin from aerial parts of Phyllanthus amarus exhibits cytotoxic effects in the K-562 cell line (3). Chowdhury et al have shown niranthin capability as a potent anti-leishmanial agent (4). Recently Conrado et al have reaffirmed Antileishmanial and Antitrypanosomal Activity activity of niranthin and other lignans from Niranthin (5). Recently study by Chopade et al indicates that the Niranthin may have potential clinical applications in the management of anxiety (6). The present study was undertaken to verify the antidiabetic potential of Niranthin using various in vitro techniques and also as an attempt to predict its mechanism of action.

2. MATERIALS:

Plant marker: The Niranthin [6-[(2R,3R)-3-[(3,4-dimethoxyphenyl)methyl]-4-methoxy-2-(methoxymethyl)butyl]-4-methoxy-1,3-benzodioxole] purchased (Product code : N006, Lot. no. : T18C277) from Natural Remedies Pvt. Ltd., Bangalore. Purity of Niranthin was determined by the manufacturer by HPLC area normalization and was certified above 95.0%.

Chemicals: Glucose oxidase peroxidase kit was procured from Pathozyme Diagnostics, Kagal, India. Dialysis bags (12 000 MW cutoff; Himedia laboratories, India) were used. All the chemicals used in the study were of extra pure analytical grade.

3. METHOD:

3.1. Evaluation of antidiabetic activity of Niranthin using various in vitro methods

1. Effect of Niranthin on in-vitro glucose diffusion

It was performed according to the method stated by Ahmed et al (7). A total of 25 mL of glucose solution (20 mmol/ L) and the samples of plant extracts (1%) were dialyzed in dialysis bags against 200 mL of distilled water at 37 °C in a shaker water bath. The glucose content in the dialysate was determined at 30, 60, 120 and 180 min using glucose oxidase peroxidase diagnostic kit. A control test was carried out without sample.

3.2. Effect of Niranthin on in-vitro amylolysis kinetics (8)

A total of 40 g of potato starch was added to about 900 mL of 0.05 mol/L phosphate buffer (pH 6.5). The solution after stirring at 65 °C for 30 min was made up to a final volume of 1 000 mL to give a 4% (w/v) starch solution. And 25 mL of the above starch solution, α-amylase (0.4%), and the plant extracts (1%) were dialyzed in a dialysis bags against 200 mL of distilled water at 37 °C (pH 7.0) in a shaker water bath. The glucose content in the dialysate was determined at 30, 60, 120 and 180 min. A control test was carried out without sample.

Glucose dialysis retardation index (GDRI) was calculated using the following formula-

$$\textbf{GDRI} = 100 - \left[\frac{\text{Glucose content with addition of sample (mg/dl)}}{\text{[Glucose content of the control (mg/dl)]}} \times 100\right]$$

Statistical analysis- All the determinations were carried out in triplicates and data were analyzed by ANOVA followed by students T test. Values were considered at $P<0.05$.

4. DISCUSSION:

The retardation of glucose diffusion may also be due to the inhibition of α-amylase by t Niranthin thereby limiting the release of glucose from the starch. Ou et al. have mentioned several possible factors that may be responsible for α-amylase inhibition such as fiber concentration, the presence of inhibitors on fibers, encapsulation of starch and 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 (9). GDRI is a useful in vitro index to predict the effect of a fiber on the delay in glucose absorption in the gastrointestinal tract. A higher GDRI indicates a higher retardation index of glucose by the sample (7,8). The GDRI was found to be 40.31% at 30 min.

Amylolysis kinetic experimental model the rate of glucose diffusion was found to increase with the time from 30 to 180 minutes and both the extracts demonstrated significant inhibitory effects on movement of glucose into external solution across dialysis membrane as compared to control.

5. RESULT:

5.1. Effect of Niranthin on in vitro glucose diffusion:

The effect of Niranthin on retarding glucose diffusion across the dialysis membrane is shown in figures 1 and 2. The rate of glucose diffusion was found to increase with time from 30 to 180 min. In the present study, the movement of glucose across the dialysis membrane was monitored once in 30 min till 180 min and it was found that, Niranthin demonstrated significant inhibitory effects on movement of glucose into external solution across dialysis membrane compared to control.

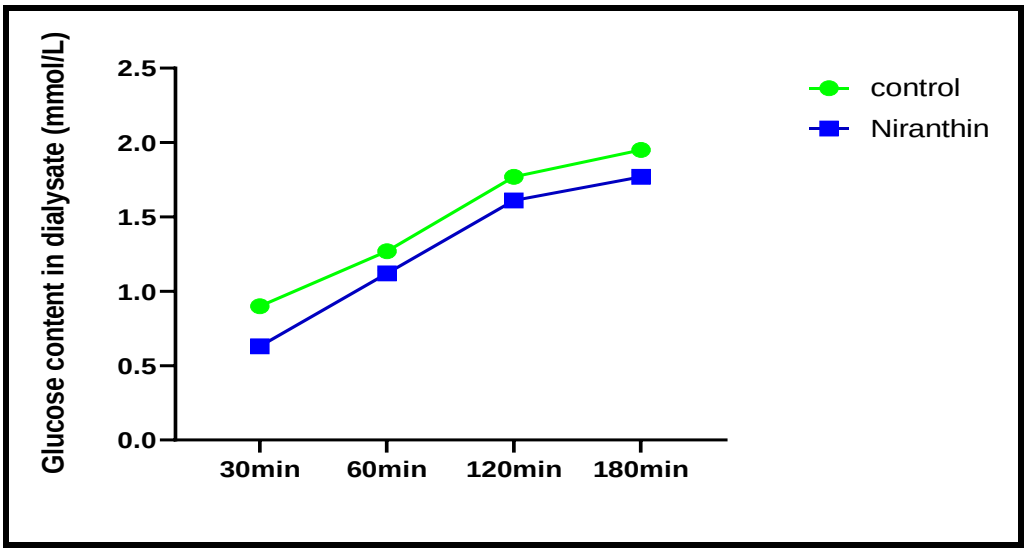

Figure 1: *Effect of Niranthin on glucose diffusion.*

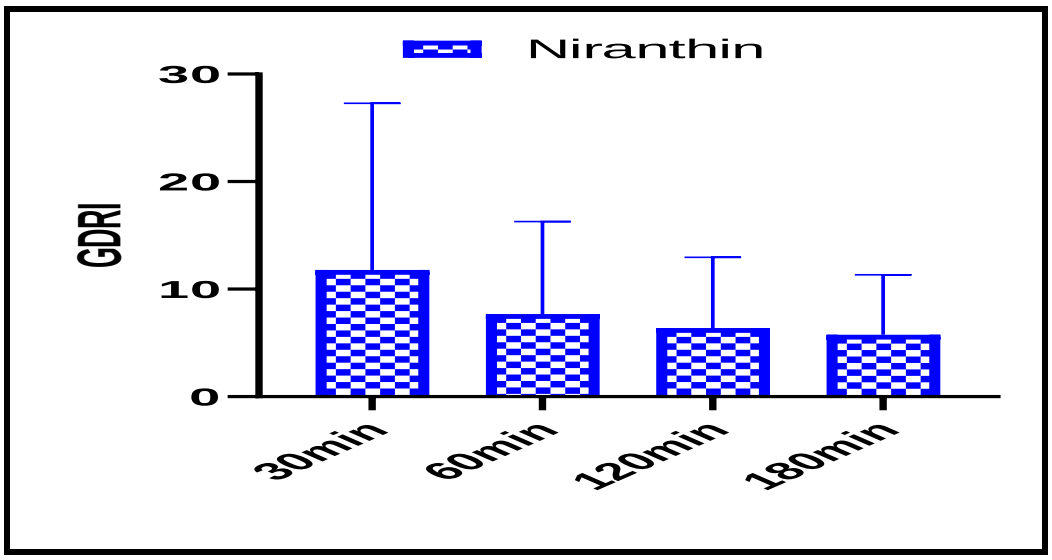

Figure 2: *Effect of Niranthin on glucose GDRI.*

5.2. Effect of Niranthin on in vitro amylolysis kinetics:

The effects of Niranthin on the amylolysis kinetics are shown in the figures 3 & 4. The GDRI was found to be 70.82 % at 60 min which gradually got reduced to 37.41 % at 120 min.

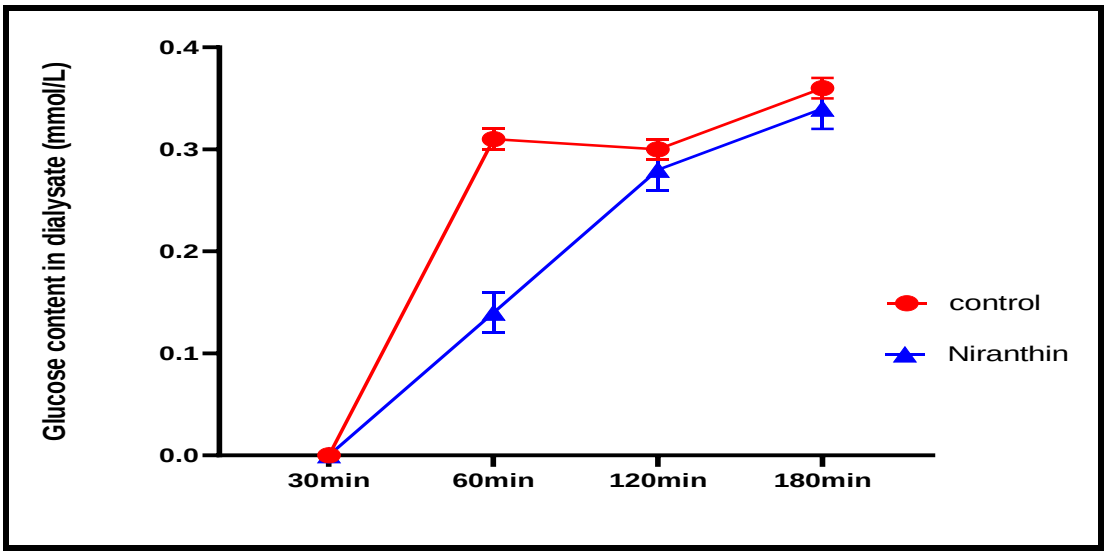

Figure 3: *Effect of Niranthin on starch digestibility.*

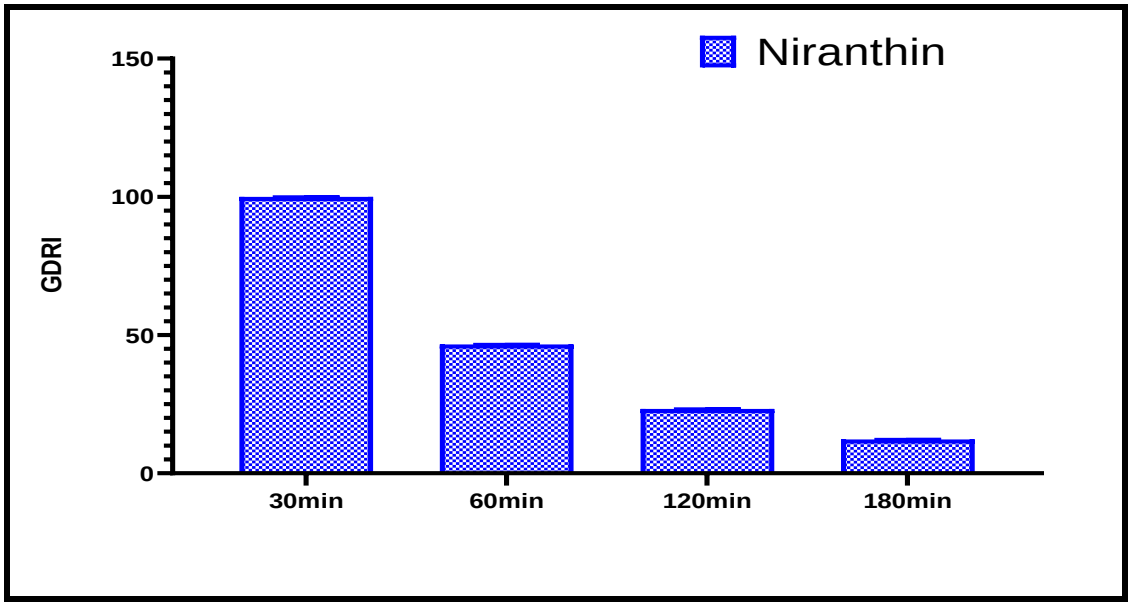

Figure 4: *Effect of Niranthin on starch GDRI.*

6. CONCLUSION:

To conclude, the results of the present study suggest hypoglycemic effect of Niranthin might be mediated by decreasing glucose diffusion rate.

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