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In-Vitro Evaluation for Glucose Diffusion and Kinetics of Amylolysis of Rubiadin

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ABSTRACT

The standardized pure Rubiadin was studied for its effects on the assay of diffusion glucose and kinetics of amylolysis using in-vitro models. The results verified the possible antidiabetic potential of the standardized Rubiadin.

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INTRODUCTION

Rubiadin is an anthraquinone that has been isolated from the roots of *Rubia cordifolia* Linn (Family- Rubiaceae).**[1]** *Rubia cordifolia* is an important medicinal plant that is used for the treatment of wide ailments in the Ayurvedic system of medicine**.[2,3]** Rubiadin, isolated from the roots of *Rubia cordifolia* was found to have potent antioxidant properties**.[4]** In addition, rubiadin also have been found to inhibit lipid peroxidation **[5].** The plant *Rubia cordifolia* has been reported for anti-inflammatory**[6],** immunomodulatory**[7],** anticonvulsant and anxiolytic[8], and anti-tumor activities**.[9]**

A detailed study by Roa et al. indicates that rubiadin has a potent hepatoprotective action against carbon tetrachloride-induced hepatic damage in rats**.[10]** While the recent study by Shen et al., showed that a close correlation existed between chemical fingerprints with analgesic and anti-inflammatory activities, and alizarin, 6-hydroxyrubiadin, purpurin, and rubidium might be the active constituents of *Rubia cordifolia* L. extract**.[11]**

However, the mechanisms that underlying their analgesic effect remain unclear. In the present study, we, therefore, attempt to evaluate the central and peripheral mechanisms underlying the analgesic effects of Rubiadin in mice using the acetic acid-induced writhing and hot plate tests.

MATERIALS AND METHODS

Plant material

The Rubiadin [1,3-dihydroxy-2-methylanthracene-9,10-dione] purchased (Product code: R004, Lot. no.: T19D079; CAS No: 117-02-2) from Natural Remedies Pvt. Ltd., Bangalore. The purity of Rubiadin was determined by the manufacturer by HPLC area normalization and was certified above 94.80%.

Chemicals

A glucose oxidase peroxidase (GOP) 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.

Evaluation of the antidiabetic activity of Rubiadin using various in vitro methods

1. Effect of Rubiadin on in-vitro glucose diffusion

It was performed according to accordingly to the method described by Ahmed et al [12]. In 25 mL of glucose solution (20 mmol/ L) and the samples, LM (1%) were dialyzed using in dialysis bags in 200 ml of distilled water at 37 °C in a water bath attached to a shaker. The glucose content in the dialysate was measured at the duration of 30, 60, 120, and 180 min using the GOP kit. A control test was carried without any sample for comparison.

2. Effect of Rubiadin on in-vitro amylolysis kinetics [13]

In this test, 40 g of potato starch was added to 900 mL of 0.05 mol/L phosphate buffer (pH 6.5). The solution is stirred at 65°C for 30 min duration. Then the final volume is made up of 1000 ml gives 4% (w/v) starch solution. To 25 ml of the prepared starch solution, α-amylase (0.4%) and Rubiadin (1%) were dialyzed in dialysis bags against 200 ml of distilled water at 37 °C (pH 7.0) in a water bath attached to a shaker. The glucose content in the dialysate was also measured at the duration of 30, 60, 120, and 180 min. A control test was carried without any sample for comparison.

Glucose dialysis retardation index (GDRI) calculated by below formula-

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

Statistical analysis- All the determinations were carried out in triplicates and data was analyzed by ANOVA followed by student's T-test. Values were considered at $P<0.05$.

RESULTS AND DISCUSSION

RESULTS

Effect of Rubiadin on glucose diffusion

The effect of Rubiadin on retarding glucose diffusion across the dialysis membrane is shown in figures 1 and 2. The glucose diffusion rate increases with time from 30 to 180 min. In the present study, the glucose movement across the dialysis membrane was monitored once in 30 min until

180 min and it was found that Rubiadin demonstrate significant inhibition of movement of glucose into the external solution across the dialysis membrane in comparison to control.

Effect of Rubiadin on kinetic amylolysis

The effects of Rubiadin on the amlolysis kinetics are shown in figures 3 & 4. The GDRI for Rubiadin is calculated to be 58.52 % at a duration of 60 min that gradually reduced to 18.74 % at 120 min.

DISCUSSION

The glucose diffusion retardation can be due to the inhibiting α-amylase by Rubiadin thereby limiting the release of glucose from the starch. Ou et al. mention possible facts that are responsible for α-amylase inhibition like fiber concentration, presence of fiber inhibitors, starch encapsulation, and fiber enzymes, which may reduce the accessibility of starch to the enzyme and direct adsorption of enzyme on fibers ultimately resulting in a decrease in amylase activity [14]. An *in-vitro* index such as GDRI is used to predict the effectiveness of fiber for the delay in glucose absorption in the gastrointestinal tract. A high GDRI indicates a high retardation index of glucose by the Rubiadin [12-14].

Glucose diffusion rate in amylolysis kinetic experiments increases with the time duration such as from 30 to 180 minutes. Rubiadin shows significant inhibition of movement of glucose across dialysis membrane into the external solution as compared with control.

In conclusion, the results of this study suggest possible hypoglycemic activity of Rubiadin that could be mediated by a decreased rate of glucose diffusion rate.

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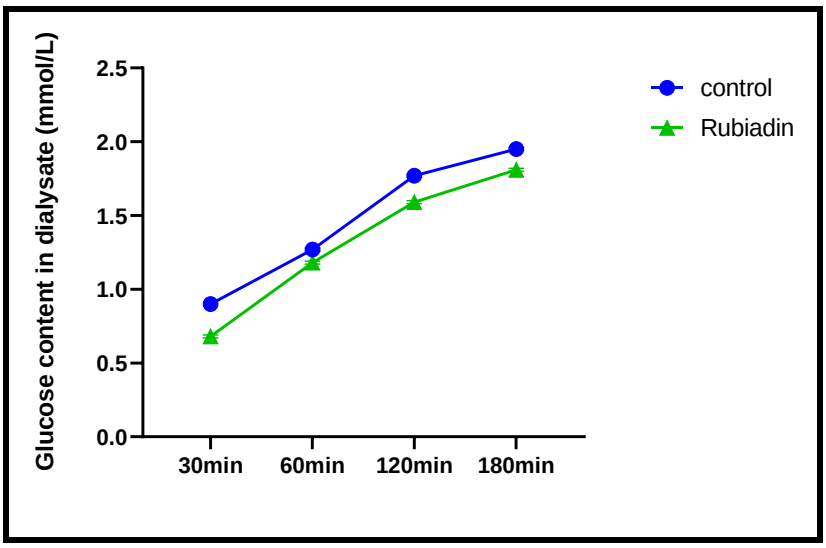

Figure No. 1: Effect of Leea macrophylla extracts on glucose diffusion.

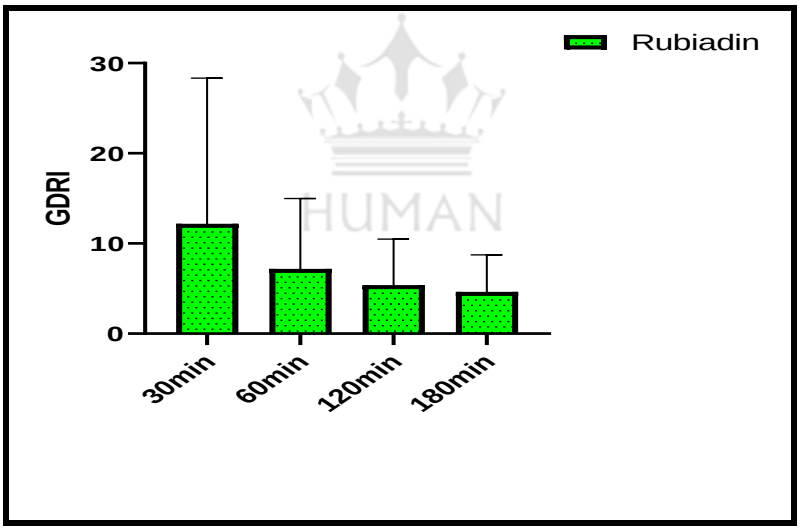

Figure No. 2: Effect of Leea macrophylla extracts on glucose GDRI.

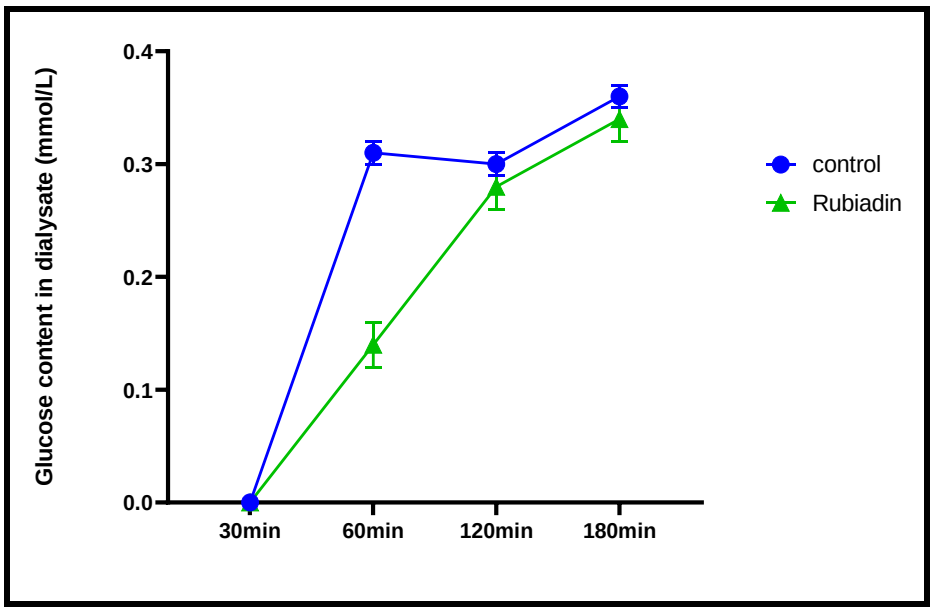

Figure No. 3: Effect of selected samples on starch digestibility.

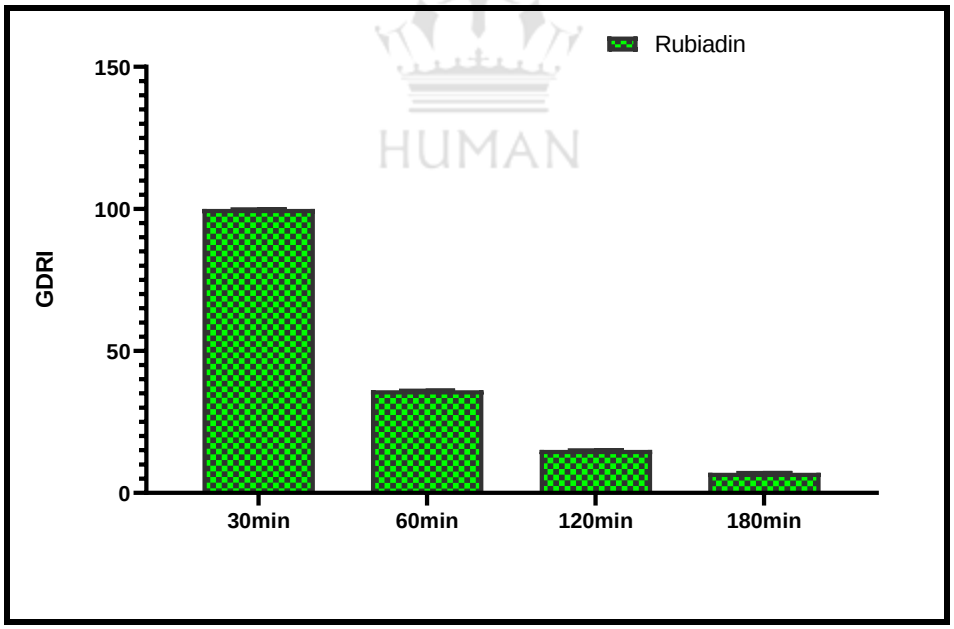

Figure No. 4: Effect of selected samples on starch GDRI.