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EVALUATION OF *IN-VITRO* GLUCOSE DIFFUSION AND AMYLOLYSIS KINETICS OF *WRIGHTIA TINCTORIA* EXTRACTS

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ABSTRACT The standardized aqueous and methanolic extracts of *Wrightia tinctoria* were studied for their effects on glucose diffusion and amylolysis kinetics using in-vitro models.
The results showed the anti-diabetic potential of the *Wrightia tinctoria*.

KEYWORDS : *Wrightia tinctoria;* glucose diffusion; amylolysis kinetics.

INTRODUCTION:

The plants belonging to the genus Wrightia are widely distributed throughout the world.[1-3] *Wrightia tinctoria* plant is being used in folk medicine for treatment of several diseases.[1-3] The review of the literature suggests that good number of preclinical studies have confirmed the medicinal use of various *Wrightia tinctoria* species that have been mentioned traditionally.[1-3]

The present study was undertaken to verify the antidiabetic potential of *Wrightia tinctoria* using various in vitro techniques and also as an attempt to predict its mechanism of action.

MATERIALS AND METHODS:

Plant Material- The leaves *of Wrightia tinctoria* were collected from Jalgaon state Maharashtra. The plant was identified and authenticated by T. Chakraborty Joint Director, Botanical survey of India, Western circle, Pune.

Extracts of *Wrightia tinctoria*- The leaves of *Wrightia tinctoria* was collected shade dried and then pulverized in grinder. The powered leaves utilized for extraction procedure was passed through 60-120 mesh to remove fine powder and course powder was used for extraction. The extraction was started with non-polar solvent like methyl alcohol. Extraction was carried out by continuous hot extraction method using Soxhlet apparatus till all constituent are removed. The completion of extraction was confirmed by taking sample out of siphon tube on TLC plate and placing it in iodine chamber. Absence of colored spot on plate indicated complete extraction. After completion of extraction methyl alcohol was distilled off and concentrated extract was air dried. The extract was stored in desiccators. The marc was refluxed for about 3 hours with distilled water to obtain aqueous extract.

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.

Evaluation of antidiabetic activity of *Wrightia tinctoria* extracts using various in vitro methods

1. Effect of *Wrightia tinctoria* extracts on in-vitro glucose diffusion

It was performed according to the method stated by Ahmed et al.[4]. 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.

2. Effect of *Wrightia tinctoria* extracts on in-vitro amylolysis kinetics[5]

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.

Statistical analysis-

All the determinations were carried out in triplicates and data is represented as Mean ± SEM (standard error of mean).

Results

Effect of *Wrightia tinctoria* extracts on in vitro glucose diffusion

The effect of the plant extracts on retarding glucose diffusion across the dialysis membrane is shown in Table 1. The rate of glucose diffusion was found to increase with time from 30 min 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, both the samples of *Wrightia tinctoria* extracts demonstrated significant inhibitory effects on movement of glucose into external solution across dialysis membrane compared to control.

Effect of *Wrightia tinctoria* extracts on in vitro amylolysis kinetics

The effects of *Wrightia tinctoria* on the amylolysis kinetics are shown in the Table 2. The GDRI was found to be 47.44% and 38.88 % in aqueous and methanolic extracts respectively at 60 min which gradually got reduced to 26.51% and 16.11 % respectively at 120 min.

Discussion

The retardation of glucose diffusion may also be due to the inhibition of α-amylase by the plant extracts 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.[5]

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. 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.

To conclude, the results of the present study suggest hypoglycemic effect of *Wrightia tinctoria* extracts are mediated by the by decreasing glucose diffusion rate.

Table 1- Effect of selected samples on glucose diffusion and GDRI.

Sample	Glucose content in dialysate (mmol/L)			
	30 min	60 min	120 min	180 min
Control	0.88 ± 0.01	1.27 ± 0.01	1.77 ± 0.01	1.99 ± 0.01
Aqueous extract	0.58 ± 0.02	1.12 ± 0.02	1.61 ± 0.02	1.85 ± 0.03
GDRI	27.24	18.17	13.14	12.33
Methanol extract	0.77 ± 0.01	1.19 ± 0.01	1.59 ± 0.01	1.91 ± 0.01
GDRI	32.14	16.10	11.22	8.56

Values are mean of three readings (n=3)

Table 2- Effect of selected samples on starch digestibility and GDRI.

Sample	Glucose content in dialysate (mmol/L)			
	30 min	60 min	120 min	180 min
Control	0.0	0.22 ± 0.01	0.31 ± 0.01	0.41 ± 0.01
Aqueous extract	0.0	0.13 ± 0.02	0.35 ± 0.02	0.41 ± 0.02
GDRI	100	47.44	26.51	12.24
Methanol extract	0.0	0.15 ± 0.02	0.38 ± 0.02	0.32 ± 0.02
GDRI	100	38.88	16.11	7.12

Values are mean of three readings (n=3)

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