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Research Paper

Dipeptidyl Peptidase IV inhibitory activity of *Mangifera indica*

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ABSTRACT

The insulinotropic hormone, glucagon-like peptide 1 (GLP-1), which has been proposed as a new treatment for type 2 diabetes, is metabolized extremely by Dipeptidyl peptidase IV (DPP-IV). Inhibitors of DPP IV enhance the level of GLP-1, which have improved glucose tolerance and increased insulin secretion. To understand the therapeutic *activity* of *Mangifera indica* for the treatment of non-insulin dependent diabetes mellitus (NIDDM). The methanolic extract of *Mangifera indica* leaves were tested *in-vitro* for DPPIV inhibitory activity. The extract showed potent activity with an IC_{50} value of 182.7µg/ml. Diprotin-A was used as reference standard. The results confirm the inhibitory effect of *M. indica* on DPPIV, and the potential to be a novel, efficient and tolerable approach for the diabetes.

Keywords: GLP-1; DPPIV; NIDDM; Diprotin-A; In-vitro.

INTRODUCTION

A novel approach for treatment of type- 2 diabetes is based on the gut hormone glucagon-like peptide-1 (GLP-1), which is ant-diabetic due to its combined action to stimulate insulin secretion, increase beta-cell mass, inhibit glucagon secretion, reduce the rate of gastric emptying and induces satiety. The peptide is rapidly inactivated by the enzyme dipeptidyl peptidase-IV (DPP-4), resulting in a half-life of active GLP-1 of only approximately 1-2 minutes.

Inhibition of DPP-IV increases the levels of endogenous active GLP-1 and prolongs its half-life. The studies on animals showed genetic deletion of DPP-IV, which have improved glucose tolerance and increased insulin secretion in response to oral glucose. Recent studies in subjects with type 2 diabetes have shown that prolonged DPP-IV inhibition for up to 1 year is anti-diabetogenic because fasting and postprandial glucose as well as HbA1c levels are reduced. Hence, DPP-IV inhibition has the potential to be a novel, efficient and tolerable approach to treat type 2 diabetes (Mentlein, 1999 and Bo Ahrén, 2005).

Mangifera indica L. (Family-*Anacardiaceae*) is one of the most popular of all tropical fruits. Most parts of the tree [Fruit, seeds, pulp, stem bark, roots and leaves] have medicinal properties (Sathyavathi, et al., 1987). It is native to tropical Asia and

has been cultivated in the Indian subcontinent for over 4000 years and is now found naturalized in most tropical countries. *Mangifera indica* contains vitamins A and C, β-carotene, xanthophylls, humulene, elemene, indicine, terpinine, tannins, flavonoids, linalool, nerol, gallic acid, ethyl gallate, methyl gallate & mangiferin (Aderibigbe, et al., 1999 and Wagner, 1996).

The leaves of *Mangifera indica* were assessed for antidiabetic properties using normoglycaemic, glucose-induced hyperglycemia and streptozotocin (STZ) induced diabetic mice (Umezawa, et al., 1984). This experiment designed to evaluate the anti-diabetic property of *Mangifera indica* leaves on DPPIV inhibitory activity. The aims of this study were to clarify methanolic extract *Mangifera indica* leaves inhibits DPPIV to determine inhibitory activities and to compare these activites in Diprotin, a reference standard. The present study explains one of the targets for diabetes and it helps in improved glucose tolerance and insulin secretion.

MATERIALS AND METHODS

***Chemicals used*:** DPPIV from porcine kidney, Gly-pro-p-nitroanilide, Diprotein-A (Ile-pro-ile), Tris-HCl Buffer. All chemicals were purchased from M/s Sigma, St Louis, USA.

Sample preparation: Fresh leaves of *Mangifera indica* L. (Anacardiaceae) were collected from Bangalore in November 2007 and the sample was authenticated [Reference no.MI/PRO/04/07]. The powdered plant materials of 600 g were extracted with methanol using Soxhlet apparatus. The resulting extracts were evaporated in vacuum and finally lyophilized into solid mass devoid of solvent (Yield = 20 %) and stored in desiccators for future use.

Dipeptidyl peptidase IV assays (in-vitro): The assay was performed as per Kojima et al (7). In brief, the assay was performed in 96 micro well plates. A pre-incubation volume 250µl contained 100mM Tris HCl buffer pH 8.4, 7.5µl of DPP IV enzyme (0.2U/ml) and various concentration of test material/reference inhibitor. This mixture was incubated at 37°C for 30mins, followed by addition of 10µl of 1.4 mM Gly-pro-p-nitroanilide (substrate). The reaction mixture was incubated for 30 mins at 37°C and absorbance was measured at 410nm. Diprotein-A (Ile-Pro-Ile) was used as reference inhibitor (7).

Statistical Analysis: All data are expressed as the mean ± SEM. The Statistical data were evaluated by using Graph pad Prism4 software. The % inhibition was calculated using the formula, control – test/control x 100. The IC_{50} value was determined by non-linear regression curve fit using Graph pad Prism4.

RESULTS

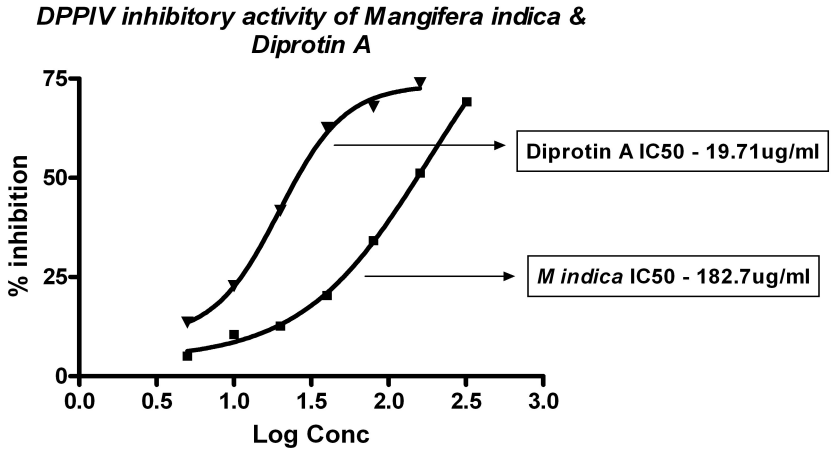
The methanolic extract of *Mangifera indica* leaves were tested in Dipeptidyl peptidase IV inhibitory assay (in-vitro). A rapid and simple micro dilution technique on 96-well micro plate based on enzyme inhibition mechanism was optimized and validated for screening of antidiabteic activity.

The highest concentration tested for the *Mangifera indica* in DPPIV assay was 320µg/ml. The 50% inhibition was exhibited at 160µg/ml. *Mangifera indica* leaves methanolic extract inhibited porcine kidney DPP-IV with an IC_{50} of 182.7µg/ml. The data pertaining to the antidaibetic potential of the *Mangifera indica* leaves are presented in Table 1 & Fig 1 respectively. The 95% confidence interval indicates the IC_{50} value was found to be within the range of tested concentration.

Table-1: DPPIV inhibitory activity of *Mangifera indica* & Diprotin A.

S. No.	Tested material	Concentration µg/ml	% inhibition ± S.E.M	IC50 (µg/ml) (95% C.I)[a]
1	*Mangifera indica* (n=3)	0	1.12 ± 1.10	182.7 (82.35-284.61)
		5	9.47 ± 1.20	
		10	14.22 ± 1.50	
		20	27.44 ± 0.45	
		40	39.55 ± 0.80	
		80	48.12 ± 1.10	
		160	49.22 ± 1.64	
		320	68.22 ± 1.14	
2	Diprotin-A (n=3) (Ile-Pro-Ile) positive control	0	1.21 ± 1.20	19.71 (9.7-32.79)
		2.5	13.75 ± 2.34	
		5	22.71± 1.34	
		10	41.72 ± 1.67	
		20	62.57 ± 1.74	
		30	67.88 ± 1.80	
		40	73.83 ± 0.94	

Figure-1



DISCUSSION

GLP-1 is a substrate for the enzyme Dipeptidyl peptidase IV (DPP-IV), a serine protease which degrades GLP-1 into its inactive form. Exogenous GLP-1 administration has been shown to be useful in the treatment of type 2 diabetes. However, the short half-life makes GLP-1 unattractive for chronic therapy of type 2 diabetes. DPP-IV inhibition is an approach to prolong the circulating half-life of GLP-1, thus making DPP-IV inhibitors a promising target for the treatment of type 2 diabetes.

Dipeptidyl peptidase-IV (DPP-IV) is involved in the inactivation of glucagon-like peptide-1 (GLP-1), a potent insulinotropic peptide. Thus, DPP-IV inhibition can be an effective approach to treat type 2 diabetes mellitus by potentiating insulin secretion (Umezawa, et al., 1984 & Aderibigbe, et al., 2001).This study describes the biological effects of a natural plant extracts *Mangifera indica in-vitro*. In addition, the *M. indica* methanolic extract inhibited DPP-IV mediated degradation of GLP-1 *in- vitro*. *M.*

indica methanolic extract exhibited competitive type of enzyme inhibition. The anti-diabetic property of *Mangifera indica* leaves makes this study unique (Wagner, 1996). The result explains inhibitory activities on DPP IV and may have therapeutic potential on type 2 diabetes.

The present study underlines that *Mangifera indica* inhibits the DPPIV and enhances the GLP-1 for type 2 diabetes. This study demonstrates that *Mangifera indica* methanolic leaves extract could be a good lead for further development as a new anti-diabetic agent.

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