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Research Article

DIPEPTIDYL PEPTIDASE-IV INHIBITORY ACTIVITIES OF MEDICINAL PLANTS: *TERMINALIA ARJUNA, COMMIPHORA MUKUL, GYMNEMA SYLVESTRE, MORINDA CITRIFOLIA, EMBLICA OFFICINALIS*

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ABSTRACT

Objective: The present study was designed to screen the dipeptidyl peptidase-IV (DPP-IV) inhibitory ability of hydroalcoholic extracts of *Terminalia arjuna*, *Commiphora mukul*, *Gymnema sylvestre, Morinda citrifolia*, and *Emblica officinalis* and compare their inhibitory activity with the synthetic DPP-IV inhibitors (Sitagliptin and Vildagliptin). The aim of the study was to identifying indigenous sources of DPP-IV inhibitors for the management of type II diabetes mellitus as alternatives to their synthetic counterparts.

Methods: The hydroalcoholic extract of *T. arjuna, C. mukul, G. sylvestre, M. citrifolia, E. officinalis* and synthetic DPP-IV inhibitors (Sitagliptin and Vildagliptin) were tested *in vitro* for DPP-IV inhibitory activity.

Results: The DPP-IV inhibitory activity of synthetic drugs Vildagliptin was found to be 90.42±7.84% and Sitagliptin 84.67±8.21%. The DPP-IV Inhibitory activity of *T. arjuna* was found to be 83.39±7.58%, *C. mukul*: 92.97± 8.45%, *G. sylvestre*: 16.98±1.69%, *M. citrifolia*: 24.64±2.24%, and *E. officinalis*: 85.95±7.16%. *C. mukul* extract showed superior inhibitory activity than reference standard drugs (Sitagliptin and Vildagliptin).

Conclusion: *C. mukul, T. arjuna,* and *E. officinalis* extracts possess significant DPP-IV Inhibitory activity while *G. sylvestre* and *M. citrifolia* failed to markedly inhibit DPP-IV enzyme.

Keywords: Type II diabetes mellitus, Dipeptidyl peptidase-IV inhibitors, Plant extracts, *In vitro* assay.

INTRODUCTION

The World over, one of the major public health challenges of the 21st century is undisputedly type II diabetes. Recently, considerable interest has been generated by a novel class of antihyperglycemic agents that act at distinct levels of the incretin pathway. The glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) are incretin hormones, released from the intestine in response to the ingestion of nutrients [1]. which increase post-prandial insulin release from pancreatic beta-cells in a glucose-dependent manner [2]. The continuous infusion of this peptide decreases plasma glucose and improves beta-cells function. However, both peptides have very short half-lives because of their rapid degradation by dipeptidyl peptidase-IV (DPP-IV). DPP-IV, enzyme belongs to the family of serine proteases; cleaves the alanine and proline from the N-terminal ends of GLP-I and GIP making them biologically inactive [3]. Administration of DPP-IV inhibitors blocks the enzyme and thereby prolongs the half-life GLP-I and GIP. Therefore, inhibiting DPP-IV prolongs the action of GLP-1 and GIP is one of the newest pharmaceutical targets for type II diabetes treatment [4].

DPP-IV inhibitors seem to represent an efficient and well-tolerated resourceful new class of oral normoglycemic agents being widely used clinically [5]. Several synthetic DPP-IV inhibitors are available in the market such as Sitagliptin, Vildagliptin, Saxagliptin, and Alogliptin; although the drugs are efficacious studies have shown that on the prolonged usage of these medications cause unacceptable adverse effects such as pancreatitis, angioedema, infective disorders, pancreatic cancer, and thyroid cancer [6-9]. Moreover, these synthetic drugs are expensive as they have to be used on a regular basis for the management of chronic disease like diabetes, and patients belonging to weaker sections of the society may be non-complaint in therapy on a long-term basis. In this scenario, it would be a boon if we could have DPP-IV inhibitors from natural sources that may be a lack or minimal undesirable effects and are less expensive. With this point of view, the study was designed. The medicinal plant extracts (*Terminalia arjuna* [Arjuna], *Commiphora mukul* [Gugglu], *Gymnema sylvestre* [Gurmar], *Morinda citrifolia* [Noni], and *Emblica officinalis* [Amla]) were screened for assessing their *in vitro* DPP-IV inhibitory activity. The inhibitory activities of these medicinal plants extract were also compared to the synthetic DPP-IV Inhibitors: Vildagliptin and Sitagliptin. Although the antidiabetic activity of these medicinal plants has been reported. however, its ability to inhibit the DPP-IV activity had not been studied before. This is the first report of the DPP-IV inhibitory activity of these medicinal plants extract. Therefore, these plants have the potential to be developed as a natural alternative to synthetic DPP-IV inhibitors. In terms of utilization by the pharmaceutical companies, research outcomes of this study may play a key role in the development of natural indigenous DPP-IV inhibitors.

METHODS

Test drugs

Hydroalcoholic dried extracts of the *T. arjuna, C. mukul, G. sylvestre, M. citrifolia*, and *E. officinalis* were procured from Sanat Pharmaceutical, New Delhi. These are standardized extract that are available commercially.

Chemicals used

DPP-IV activity assay kit was purchased from Sigma Chemical Co., St Louis, USA. Vildagliptin and Sitagliptin were purchased from authenticated sources.

Quality analysis of the medicinal plant extracts

The *T. arjuna* bark., *C. mukuloleo*-gum-resin, *G. sylvestre* leaves, *M. citrifolia* fruit, *and E. officinalis* fresh fruit were used for extraction. The pH of 1% w/v aqueous solution, loss on drying value at 105°C by infrared balance and total ash content was done for bio-standardization of each plant extracts.

DPP-IV assay reaction

DPP-IV assay was performed using DPP-IV assay kit procured from Sigma-Aldrich. In this assay, DPP-IV activity was determined by the cleavage rate of 7-amino-4-methylcoumarin (AMC) from the synthetic substrate H-glycyl-prolyl-AMC. One unit of DPP-IV is the amount of enzyme that hydrolyzes the DPP-IV substrate to yield 1.0 U mole of AMC per minute at 37°C. The standard curve of free AMC was generated using 0-50 mM AMC (Sigma). DPP-IV activity was expressed as the amount of cleaved AMC per minute per ml (nmol/min/ml). The 1% (w/v) extract of *T. arjuna, C. mukul*, *G. sylvestre, M. citrifolia*, and *E. officinalis* plants in distilled water was used for the assay. While Sitagliptin and Vildagliptin were used as a reference drugs and control was prepared without inhibitors/plant extracts. Experiments were done in triplicates. A decrease in DPP-IV activity is measured for inhibition [10]. The percent inhibition was calculated using following formula:

$$\% \text{ Inhibition} = \frac{\text{Control} - \text{Inhibitor}}{\text{Control}} \times 100$$

Statistical analysis

All values are represented as mean±standard deviation. The percentage DPP-IV inhibitory activity was calculated using the above-mentioned formula.

RESULTS

Quality analysis of the medicinal plant extracts

The pH of 1% w/v aqueous solution of *T. arjuna, C. mukul, G. sylvestre, M. citrifolia,* and *E. officinalis* extracts were found to be 4.52, 7.80, 5.98 4.50, and 2.92, respectively. Loss on drying value at 105°C by infrared balance for *T. arjuna, C. mukul, G. sylvestre, M. citrifolia,* and *E. officinalis* extracts were: 3.2% w/w, 2.6% w/w, 5.6% w/w, 5.6% w/w, and 5.50% w/w, respectively. Total ash content for *T. arjuna* extract was 4.95% w/w, *G. sylvestre* extract; 9.30% w/w, *M. citrifolia;* 8.86% w/w, and *E. officinalis* extracts; 7.36% w/w.

DPP-IV inhibitory activity the medicinal plant extracts

The 1% hydroalcoholic extract of *T. arjuna, C. mukul*, *G. sylvestre, M. citrifolia,* and *E. officinalis* was tested for DPP-IV inhibitory activity and compared with the synthetic DPP-IV inhibitors (Sitagliptin and Vildagliptin) by *in vitro* assay. DPP-IV inhibitory activity of synthetic drugs Vildagliptin and Sitagliptin were found to be 90.42±7.84% and 84.67±8.21%, respectively. The hydroalcoholic extracts of DPP-IV inhibitory activity *T. arjuna* showed 83.39±7.58%, *C. mukul* 92.97±8.45%, *G. sylvestre* 16.98±1.69%, *M. citrifolia* 24.64±2.24%, and *E. officinalis* 85.95±7.16%. Among these plant extracts, *T. arjuna, C. mukul,* and *E. officinalis* showed comparable DPP-IV inhibitory effects compared to DPP-IV inhibitory activity of reference standards Vildagliptin and Sitagliptin. *C. mukul* showed superior inhibitory activity with percent inhibition 92.97±8.45% than reference standard drugs (Sitagliptin and Vildagliptin), reflecting the potential benefits of developing Indigenous DPP-IV inhibitors (Fig. 1).

DISCUSSION

DPP-IV inhibitors represent a unique approach in the treatment of type II diabetes. Inhibition of DPP-IV prolongs and also enhances the activity of endogenous GLP-1 and GIP, which serves as an important prandial stimulator of insulin secretion and blood glucose regulator. In spite of their beneficial therapeutic effects, they have limitations: High cost and reported adverse effects such as pancreatitis, angioedema, infective disorders, pancreatic cancer, and thyroid cancer. In this scenario, it would be beneficial if we could have DPP-IV inhibitors from natural sources that lack or minimal unacceptable adverse effects. Moreover, natural DPP-IV inhibitors would be less expensive than their synthetic counterparts. Cost is an important aspect to be considered for chronic disease like diabetes were the medicines have to be taken on a long-term basis.

In this light, the natural DPP-IV-based therapy may be an alternative that needs to be exploited considering the rich biodiversity, flora, and fauna that India is blessed with.

Various *in vitro* and molecular docking studies have reported that DPP-IV inhibitory activity of medicinal extracts as well as isolated compounds. Bharti *et al.* (2012) demonstrated that seed extract of *castanospermum australe* showed potent DPP-IV activity with IC 50 value 13.96 ug/ml and the molecular docking analysis with Gold (2005) software showed that among the three alkaloids (7-Deoxy-6-epi-castanospermine, castanospermine, and australine) from the seeds of *C. australe*, 7-Deoxy-6-epi-castanospermine is a potent DPP-IV inhibitor similar to berberine [11]. Increased in DPP-IV levels may contribute to elevated glucose levels in diabetic rats, which in turn results in increased DPP-IV activity. It is interesting that daily used spices: Cinnamon, clove, and tea play an important role in management diabetes by highly effective DPP-IV inhibition [12]. The crude extracts of tree turmeric (*Berberis aristata)* were tested using Diprotein as the standard inhibitors. In our study, we were using marketed synthetic DPP-IV inhibitors Sitagliptin and Vildagliptin as the standard inhibitors for comparison [13]. Al-masri *et al.* (2009) reported that berberine (an alkaloid) isolated from plants such as *B. aristata*, *Berberis aquifolium,* and *Hydrastis canadensis* showed effective inhibition against the DPP-IV enzyme [14]. Yogisha *et al.* (2010) demonstrated that the methanolic extract of *Mangifer aindica* leaves inhibited DPP-IV mediated degradation of GLP-1 *in vitro* [10]. The previous study deals with some antidiabetic plants, methanolic extracts of *Ocimum sanctum* (Tulsi) leaves, and *Momordica charantia* (Karela/bitter melon) fruit contain DPP-IV inhibition activity with cytoprotective potential [15]. Bisht *et al.* (2014) also demonstrated that *Desmodium gangeticum* alpha-glucosidase and DPP-IV inhibitory effects to contribute the understanding of their mechanism of action type II Diabetes mellitus [16]. *In vivo* study proposed by Kilari *et al.* (2014) reported that aqueous peel extract of *Punica granatum* given orally to diabetic rats exhibited an antidiabetic effect by lowering of blood glucose levels through the inhibitory activity on DPP-IV enzyme [17].

Several studies have been reported that antidiabetic activity of these medicinal plants extract, but this is the first report indicated that hydroalcoholic extracts of *T. arjuna, C. mukul,* and *E. officinalis* plants extract showed significant DPP-IV Inhibitory activities. The inhibitory activities of these medicinal plants *C. mukul, T. arjuna,* and *E. officinalis* were comparable to the synthetic DPP-IV inhibitors Sitagliptin and Vildagliptin. Thus, on the basis of observed results, it can be concluded that nature can be a source of indigenously developed DPP-IV inhibitors. They may serve as a natural alternative to synthetic DPP-IV inhibitors that are already marketed.

C. Mukul, family Burseraceae, has been used to treat various conditions such as inflammation, hyperlipidemia, diabetes, and diabetic cardiomyopathy [18].Shirazi *et al.* (2013) demonstrated the antidiabetic and hypolipidemic activity of *C. mukul* extract [19]. Bellamkonda *et al.* (2011) demonstrated the protective role of *C.* mukul against

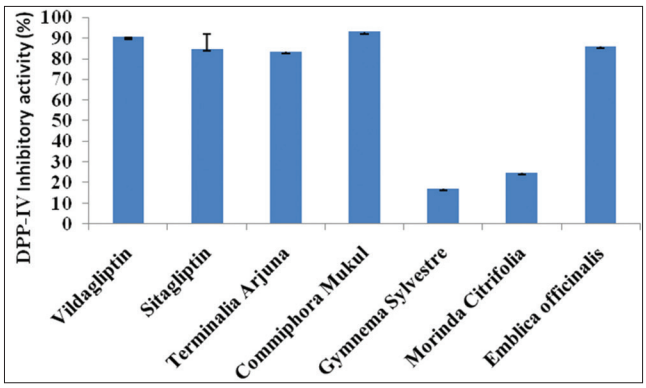

Fig. 1: Dipeptidyl peptidase-IV inhibitory activity of medicinal plants extract. *Terminalia arjuna, Commiphora mukul, Gymnema sylvestre, Morinda citrifolia,* and *Emblica officinalis* as compared to Vildagliptin and Sitagliptin. Each vertical bar represents the mean±standard error of mean (n=3)

streptozotocin (STZ)-induced diabetic oxidative stress; by modulating blood glucose, enzymatic activities (aspartate aminotransferase), (alanine aminotransferase) in liver and kidney, oxidative markers such as lipid peroxidation and protein oxidation in pancreas and heart. Hence, *C. mukul* plant could be used as an adjuvant therapy with standard antidiabetic drugs for the prevention and/or management of diabetes [20]. Although the antidiabetic activity of *C. mukul* has been reported, its ability to alter the DPP-IV pathway had not been studied so far.

E. officinalis belong to a family Euphorbiaceae commonly known as "Amla" effective in ameliorating the of diabetes mellitus induced changes. Various researchers concluded that *E. officinalis* supplement is effective in reducing the fasting and postprandial blood glucose levels and HbA1c levels in type II diabetic patients [21]. Nain *et al*. (2012) reported that hydromethanolic extract of *E*. *officinalis* showed antidiabetic activity in STZ induced diabetes at dose-dependent manner [22]. However, its ability to inhibit the DPP-IV activity had not been studied before. This is the first report of the DPP-IV Inhibitory activity of *E. officinalis.*

T. arjuna belongs to family Combretaceae, commonly known as Arjuna has been traditionally used for several medicinal purposes; cardiotonic, antidiabetic, antidysenteric, antipyretic, astringent. Morshed *et al*. (2011) and several results of the experimental study clearly demonstrate that the bark extract of *T. arjuna* possesses potent antidiabetic activity. In addition, to the hypoglycemic effect of *T. arjuna* beneficial effect was also observed on lipid profile [23]. Parveen *et al.* (2011) reported that *T. arjuna* possesses hypoglycemic, hypolipidemic, and antioxidant effects by reducing fasting blood glucose, glycated hemoglobin, alter lipid profile in high-fat diet diabetic rats. Studies have reported that *T. arjuna* is effective in reducing hyperglycemia, hyperlipidemia, and oxidative stress related to the pathogenesis of diabetes mellitus [24]. Thus, *T. arjuna* possesses therapeutic potential as a DPP-IV-based therapy for the treatment of type II diabetes mellitus. This is the first report of the DPP-IV inhibitory activity of *T. arjuna.* This could explain the multiple pathways with which *T. arjuna* could possibly interact to produce its beneficial effects. The result explains inhibitory activities on DPP-IV and may have therapeutic potential on type II diabetes.

The DPP-IV inhibitory activity of *T. arjuna, C. mukul,* and *E. officinalis e*xtracts was found to be comparable to the synthetic inhibitors: Sitagliptin and Vildagliptin. These results may represent novel findings which if further developed may address the safety issues with synthetic DPP-IV inhibitors in the management of Type II diabetes mellitus. The results of the study provide scientific support for the development of *C. mukul, T. arjuna,* and *E. officinalis* as natural DPP-IV inhibitors. This is the first report of the DPP-IV inhibitory activity of *C. mukul T. arjuna,* and *E. officinalis.* Most interestingly, the DPP-IV inhibitory activity of *C. mukul* was found to be superior that the synthetic DPP-IV inhibitors: Vildagliptin and Sitagliptin. If these beneficial effects can be established in diabetic patients, these findings may represent a novel therapy for diabetes mellitus.

CONCLUSION

The *C. mukul, T. arjuna,* and *E. officinalis* extracts possess significant DPP-IV inhibitory activity. *G. sylvestre* and *M. citrifolia* extracts lacked any significant DPP-IV inhibitory activity. The highlight of the study is that *C. mukul* extract showed superior inhibitory activity than reference standard drugs (Sitagliptin and Vildagliptin), reflecting the potential benefits of developing DPP-IV Inhibitors from Indigenous sources for the management of type II diabetes mellitus.

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