Modes of inhibition of human protein-tyrosine phosphatase 1b and aldose reductase by *moringaoleifera* lam leaves extract

Abstract

Insulin resistance is the main feature of Type 2 diabetes, due to attenuated or diminished signaling from insulin receptors, resulting in hyperglycemia. Inhibition of protein tyrosine phosphatase 1B (PTP1B) has been suggested as an attractive target to improve insulin sensitivity in different cell types. In addition, hyperglycemia activates the intracellular polyol pathway causing accumulation of sorbitol. This results in cellular water and electrolyte imbalance and oxidative injury. Aldose reductase inhibitors block the rate-limiting enzyme of the polyol pathway, decreasing the accumulation of sorbitol. *Moringaoleifera* is a plant widely used in traditional Indian and African medicine, eg. for its antidiabetic effect. However, studies about the inhibitory effect of *M. oleifera* on diabetic complications mediated via diverse enzymes as PTP1B and aldose reductase have not been carried out yet. To potentially identify targets responsible for this antidiabetic effect, we conducted kinetic studies to investigate the inhibitory capacity of *M. oleifera* extract (MOE) over PTP1B. MOE possess the highest inhibitory activity against PTP1B (IC_{50} value of 346.8 μ g/mL). We also found that the extract inhibited the recombinant human aldose reductase (rhAR) activity with an IC_{50} of 3.55 μ g/mL. Subsequent kinetic analysis revealed that MOE behaved as an uncompetitive inhibitor (K_i of 19.65 μ g/mL) against PTP1B, since it markedly modified K_m and also modified V_{max} to a lesser extent. As for the rhAR, the extract behaved as mixed inhibitor ($K_i=18.6 \mu$ g/mL, $\alpha=1.3$). Our results help to understand the inhibition mechanism of PTP1B and rhAR by phenolic compounds and flavonoids present in MOE, which is essential for the development of improved natural inhibitors.

Keywords: moringaoleifera, PTP1B, rhAR, enzymatic inhibitors, diabetic complications

Volume 1 Issue 1 - 2017

Marie Jazmin Sarabia-Sanchez,² Maria Alejandra Sanchez-Munoz,¹ Jesus Ricardo Perez-Velazquez,¹ Monica Andrea Valdez-Solana,¹ Claudia Avitia-Dominguez,² Alfredo Tellez-Valencia,² Erick Sierra-Campos¹

¹Facultad de Ciencias Químicas, Universidad Juárez del Estado de Durango, Mexico

²Facultad de Medicina y Nutrición, Universidad Juárez del Estado de Durango, Mexico

Correspondence: Facultad de Ciencias Químicas, Universidad Juárez del Estado de Durango, Av Artículo 123 S/N Fracc. Filadelfia, Gómez Palacio, Durango, CP 35010, Mexico, Tel and Fax (+52) 8717158810, Email ericksier@gmail.com

Received: March 01, 2017 | **Published:** April 25, 2017

Introduction

The protein tyrosine phosphatase (PTP) member of super family enzymes functions in a coordinated manner with protein tyrosine kinases. It plays a critical role in the regulation of a broad spectrum of cellular processes such as growth, proliferation and differentiation, metabolism, immune response, cell-cell adhesion, and cell matrix contacts. Among these, Protein tyrosine phosphatase 1B (PTP1B) is a major non-transmembrane phosphotyrosine phosphatase widely expressed in human skeletal muscle, liver, adipose tissue and brain.¹

In regard to type2 diabetes (T2DM), PTP1B acts by dephosphorylating specific phosphotyrosine residues on the insulin receptor (IR) and insulin receptor substrate proteins 1 (IRS-1) and 2 (IRS-2). Phosphorylated IRS activates several signaling cascades that mediate the biological effects of insulin including glucose uptake and glycogen synthesis. Thus, a number of genetic and biochemical studies have demonstrated that PTP1B is a major negative regulator of insulin receptor signaling.² For instance, PTP1B deficiency enhances insulin signaling and sensitivity in skeletal muscle and liver.³ PTP1B-null mice displayed enhanced tyrosine phosphorylation of IR and IRS-1 in muscle and liver as a consequence of increased systemic insulin sensitivity. Moreover, these PTP1B-null mice were less prompt to develop T2DM and obesity when fed with a high fat diet. In addition, liver-specific deletion of PTP1B attenuates metabolic syndrome and diet-induced endoplasmic reticulum stress in mice.⁴ Based on these findings, inhibition of PTP1B is expected to improve insulin action, and PTP1B inhibitors have received considerable attention as novel therapies to T2DM and obesity.

On the other hand, aldose reductase (AR) is NADPH-dependent also-keto oxidoreductase, which is the first enzyme in the polyol pathway. This enzyme catalyzes the reduction of glucose to sorbitol via the oxidation of NADPH.⁵ During hyperglycemia, the increased glucose flux enters to polyol pathway leading to an overflow of sorbitol and its metabolites (i.e. fructose), followed by accumulation within the cells due to their poor permeation across membranes and inefficient metabolism making this pathway one of the leading causes of diabetic complications.⁶ Aldose reductase inhibitors (ARIs) have been studied *in vivo* to evaluate their effectiveness in the prevention of diabetes-related complications in experimental animals and clinical trials. Although several synthetic ARIs exhibit potent inhibitory effects, either their use is limited, or they have been withdrawn from clinical trials because of their relatively low efficacy, poor pharmacokinetics and potential side effects. Furthermore, ARIs may be able to prevent or delay the onset of cardiovascular complications related to secondary diabetic complications.⁷

It is well established that natural products are excellent sources of chemicals that may be therapeutically attractive agents for the prevention and/or treatment of diabetic complications. Previous studies on *Moringaoleifera* have revealed that it possess potent antioxidant, antimicrobial and antipyretic properties which have been used in traditional Indian and African medicine for centuries.⁸ The plant is credited to contain important amounts of phenolic compounds and flavonoids like quercetin, rutin, kaempferol, astragal in and luteolin that possess diverse pharmacological and therapeutic actions.⁹ Among these, much attention has been paid to the influence of *M. oleifera* on insulin action, which may provide benefits for

diabetic patients. Moreover, there is increasing interest in the use of *M. oleifera* to treat some pathological conditions linked to oxidative disorders that include diabetes,¹⁰ and cancer.¹¹

Some studies showed that MOE decreased streptozotocin-induced hyperglycaemia since it possesses potent hypoglycemic effects through the normalization of elevated hepatic pyruvate carboxylase (PC) enzyme and regeneration of damaged hepatocytes and pancreatic β cells. In addition, it has been reported to contain compounds with strong inhibitory capacity against carbohydrate and lipid digestive enzymes.¹² More recently, it was reported the inhibition of angiotensin I-converting enzyme and arginase activities in a dose-dependent pattern with IC_{50} values of 303.03 and 159.59 $\mu\text{g/mL}$, respectively.¹³ Although some studies have demonstrated the potential inhibitory activity of phenolic compounds and flavonoids present in MOE, only few studies discussed about the inhibition mode of these compounds, none examined its inhibitory activities against AR, and PTP1B. Therefore, the aim of this study was to investigate its inhibitory activity of *M.oleifera* over PTP1B and AR *in vitro*.

Materials and methods

Reagents

DL-glyceraldehyde, the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), p-nitro phenyl phosphate (pNPP) and recombinant human aldose reductase (rhAR) were purchased from Sigma-AldrichQuimica, S.L (Mexico).

Expression and Purification of recombinant PTP1B

Cloning PTPN1 gene into over expression plasmid PET28a using *E. coli* BLR strain produced recombinant human PTP1B; enzyme purification was made by affinity chromatography as detailed elsewhere.¹⁴

Preparation of MOE

Hydro alcoholic extract of dried *M. oleifera* leaves was prepared as described in our previous report.⁹ Briefly, the extract was prepared using 23g of dry-ground sample and 260mL of 80% methanolic aqueous solution by successive maceration. The mixtures were shaken in a magnetic grid at room temperature for 21h, and then filtered through a Whatman filter paper number 1. The final extract was concentrated on a rotary evaporator, placed in a deep freezer for 24h and freeze-dried to obtain a powdered extract.

PTP1B activity assay

The enzyme reactions were carried out using standard conditions essentially as described by Goldstein and Bittner-Kowalczyk.¹⁵ The assay conditions were as follow. Appropriately diluted inhibitors (4 different concentrations: diluted 1, 3, 9, and 27 fold) were added to the reaction mixtures containing different concentrations of the substrate, p-nitrophenyl phosphate (pNPP) (usual range: 1 to 20mM, final assay concentration). The assay buffer 50mMHepes pH 7.0, contained 150mM sodium chloride, 1mMdithiothreitol (DTT), and 1mM EDTA (final volume of 500 μL). The reaction was started by the addition of the enzyme and carried out at 37°C for 5min. The enzyme activity was determined by measuring the changes in absorbance at 405nm with appropriate corrections for absorbance of the MOE and pNPP. The data were analyzed using nonlinear regression hyperbolic fit to classical Michaelis-Menten enzyme kinetic models. Inhibition is expressed as K_i values in $\mu\text{g/mL}$.

Kinetics of rhAR inhibition by MOE

Reaction mixtures consisted of 0.1M potassium phosphate (pH 6.0), 0.2mM NADPH, 1 $\mu\text{g/mL}$ of rhAR with varied concentrations of DL-glyceraldehyde (DL-GAL; 40-400 μM) in a total volume of 600 μL . Enzyme activity was assayed spectrophotometrically by measuring the decrease absorbance at 340nm after NADPH addition using Hatch 5000 spectrophotometer. Line weaver-Burk plot analysis was determined calculated from the data according to Michaelis-Menten kinetics in order to understand the probable mode of inhibition using Sigma plot 12.3 software. Samples were prepared in replicates and analyzed two twice.

Inhibition studies

PTP1B activity was measured by addition of 10 μL of 100mmol/LpNPP (as substrate) into a buffer solution (pH 7.0) containing 50mMHepes, 100mMNaCl, 1mM EDTA, 1mM DTT, DMSO 10% and 50 $\mu\text{g/mL}$ recombinant PTP1B, along with or without different concentrations of MOE diluted in reaction medium. After incubation for 5min at 37°C, the reactions were stopped with 0.1M NaHCO_3 . The amount of p-nitrophenol (pNP) was measured by UV absorbance at 405nm. The percentage of inhibition was calculated considering the activity in the absence of MOE as 100%. The IC_{50} value was determined by non-linear regression analysis plot of percent inhibition versus log inhibitor concentration.

Results

Typically, high-throughput screening assays seek a total inhibitor concentration that reduces the activity by 50 % (IC_{50}). So initially, we determined the IC_{50} for the MOE. The PTP1B inhibition studies demonstrated that MOE had inhibitory activity. In all experiments, control samples were prepared without any plant extract and were compared with test samples containing various concentrations of the plant extract. The concentration of substrate used was 25mM, and PTB6 small compound inhibitor was used as the positive control (data not shown). As shown in Figure 1, results of inhibitory assays on PTP1B activity showed that MOE had moderate activity, with an IC_{50} value of $346.8 \pm 2.5 \mu\text{g/mL}$. While, MOE showed the highest inhibitory activity against rhAR ($IC_{50} = 3.55 \mu\text{g/mL}$, Figure 1B).

Figure 1 Inhibition of human PTP1B and rhAR by MOE. A) Inhibition of PTP1B activity by MOE at various concentrations (100-500 $\mu\text{g/mL}$). The PTP1B inhibition was analyzed by measuring p-NP released from p-NPP at 405nm after 5min of incubation at 37°C. B) Inhibition of rhAR activity by MOE at various concentrations (0.5-50 $\mu\text{g/mL}$). The rhAR inhibition was analyzed by measuring NADP^+ released from NADPH at 340nm after 2 min of incubation at 25°C. $IC_{50} = 3.55 \mu\text{g/mL}$. Results are expressed as mean of percent inhibition $\pm$ SEM against log 10 concentration of inhibitor.

Once the IC_{50} assays had revealed that MOE have a moderate and high inhibitory activity against both enzymes respectively, and that the IC_{50} value depends on enzyme and substrate concentrations, and type of inhibitor along with other experimental conditions. It was necessary to determine the K_i value and the inhibition mechanism performed by MOE. Thus, to assess the mechanism of MOE inhibition on PTP1B and rhAR activities, the potential effects of MOE on the Michaelis constant (K_m) and/or the maximal velocity of the reaction (V_{max}) were determined by increasing concentrations of substrates (pNPP and DL-GAL) and 50 or 200 $\mu\text{g/mL}$ of MOE as inhibitor.

Figure 1 Inhibition of human PTP1B and rhAR by MOE.

As shown in Figure 2A, the rate of pNPP decomposition by human PTP1B displayed a Michaelis-Menten behavior. An estimation of V_{max} and K_m can be made from these data. In the absence of MOE, the V_{max} was calculated to be 129.9 ± 0.05 mmol/min/mg using nonlinear regression analysis with the function provided by Sigma plot program

v12.3. In addition, nonlinear curve fitting of the data to the Michaelis-Menten equation yielded a K_m of 2.2 ± 0.3 mM, a K_{cat} of 12.9 ± 0.5 s⁻¹ and a K_{cat}/K_m of 5.8 s⁻¹ mM⁻¹. Our results are similar to that reported by Sarmiento.¹⁶

Figure 2 Kinetic analysis of PTP1B inhibition by MOE.

To elucidate the inhibition mode, the inhibition kinetics of MOE was analyzed by the Line weaver-Burk method in order to establish whether the double-reciprocal plots are parallel or convergent. In the presence of MOE, steeper lines obtained in a double-reciprocal plot were parallel (Figure 2B). Both the V_{max} (129.9 ± 0.5 mmol/min/mg for control; 58.3 ± 1.6 mmol/min/mg for $200 \mu\text{g/mL}$ of MOE) and k_m (2.25 mM for control; 0.8 ± 0.15 mM for $200 \mu\text{g/mL}$ of MOE) decreased in the presence of MOE. The results indicate that the inhibition of PTP1B by MOE was uncompetitive, so MOE is more likely to bind to the substrate-enzyme complex.

Figure 2 Kinetic analysis of PTP1B inhibition by MOE (A) PTP1B was assayed with various concentrations of pNPP (1-15 mM) in the absence (black dots) or presence (white dots) of MOE (0.2 mg/mL). The kinetics assays were performed after incubating the mixture at 37°C for 5 min. (B) Line weaver-Burk plot of the effect of MOE on PTP1B. Line weaver-Burk double reciprocal plot of $1/v$ versus $1/S$ at the presence (white dots) or absence (black dots) of MOE.

To assess the aldose reductase inhibition activities of MOE, rhAR was used. This enzyme is available in a pure form and has been widely used in medical research as a model for screening potential inhibitors. In the present study, we have evaluated the potential inhibitory activity of the MOE against rhAR. MOE showed a high percentage inhibition (with IC_{50} value of $0.55 \mu\text{g/mL}$) on rhAR activity (Figure

3). Hyperbolic initial velocity as a function of substrate concentration (DL-GAL) was plotted as a linear function of reciprocal of initial velocity against the reciprocal of DL-GAL concentration (Line weaver-Burk plot) at the presence or absence of MOE. The double-reciprocal plots showed a family of lines intersecting to the left on the y-axis (Figure 3B), which is consistent with mixed inhibition. The kinetics parameters of rhAR were V_{max} of 498.6 nmol/min/mg, K_m of 0.223 mM, K_{iof} $18.6 \mu\text{g/mL}$, and α factor of 1.3 .

Figure 3 Kinetic analysis of rhAR inhibition by MOE. (A) rhAR was treated with various concentrations of DL-GAL ($40\text{--}400 \mu\text{M}$) in the absence (black dots) or presence (white dots) of MOE ($5 \mu\text{g/mL}$). The kinetics assays were performed after incubating the mixture at 25°C for 1 min. (B) Line weaver-Burk plot of the effect of MOE on rhAR. Line weaver-Burk double reciprocal plot of $1/v$ versus $1/S$ at the presence (white dots) or absence (black dots) of MOE.

According to our results, there are remarkable differences on the inhibitory potency of MOE against PTP1B and rhAR. These differences can be attributed to the inhibition mechanism produced and as a result of different degrees of bulk tolerance for these enzymes. However, it is essential to perform comprehensive examinations of flavonoids presents in MOE that function as inhibitors against PTP1B and rhAR.

Figure 3 Kinetic analysis of rhAR inhibition by MOE.

Discussion

Protein Tyrosine Phosphatases (PTPs) are essential in the metabolic system to regulate physiological functions of cells. However, a number of diseases like diabetes, obesity and cancer are reported to be induced by over-activity of PTPs.¹⁷ Hence, regulation of PTP1B activity through specific inhibitors is an excellent target to the development of new treatments for diabetes.

Natural products gained attention for long-term treatment of metabolic diseases due their multifunctional components and multipronged mode of action. To date, the reports on the inhibitory activities of medicinal plants extracts against PTP1B are still limited. However, several PTP1B inhibitors have been isolated from medicinal plants to develop an alternative drug with higher potency and lesser adverse effects.¹⁸ *M. oleifera* has been used since ancient times in traditional medicine to treat diabetes, and it was found to have insulin sensitizing properties.¹⁹ Along with the antidiabetic effect, its leaves have been reported to possess antioxidant activity,²⁰ and anti-dyslipidemic properties.²¹ Hence, their traditionally reported antidiabetic activity could be due to PTP1B inhibition.

To our knowledge, there is no report on the inhibitory potential role of MOE on PTP1B activity. In our study, methanolic extract of *M.oleifera* was tested to evaluate its PTP1B inhibitory potential. The extract remarkably inhibited the activity of PTP1B with an IC_{50} value in the range of 322–378 μ g/mL (Figure 1). This activity is lower than the reported values for a variety of vegetables²² and seaweed.²³

Previous photochemical studies on *Moringaoleifera* revealed the presence of polyphenols, flavonoids and other compounds.²⁵ Our previous results of photochemical analysis of *M. oleifera* also showed the presence of flavonoids and phenolic compounds.⁹ Here we provide evidence that MOE containing metabolites inhibited PTP1B activity, while no effects were observed by isolated compounds (caffeic acid, catechins and siringic acid), except by rutin and Gallic acid which presented moderate inhibition effects (data not shown). Further investigation is necessary to confirm which of the components from MOE were responsible to inhibit the PTP1B. However, it is highly probable that a synergic effect from the combination of its bioactive components does contribute to the strong inhibitory effect.

There are studies that suggested that individual components asursolic acid, tormentic acid, palmitic acid, and flavonoids isolated from the roots of the *Broussonetiapapyrifera* inhibited PTP1B,²⁶ but the mechanism involved in the inhibition remained to be defined.

Nevertheless, it was reported that glisoflavone isolated from the roots of *Glycyrrhizauralensis* showed the strongest inhibitory activity of PTP1B, with an IC_{50} of 27.9 μ mol/L, in a mixed-type manner.²⁷ Furthermore, MeOH extract of *Selaginellatariscina* was found to inhibit PTP1B at 30 μ g/mL, and its bioassay-guided fractionation of the extract produced amentoflavone, a non-competitively inhibitor with K_i value of 5.2 μ M.²⁸ In a similar manner, MOE showed a strong inhibitory activity against PTP1B with $i_{0.5}$ of 40 μ g/mL in a mixed-uncompetitive type manner.

Our data are also in agreement with other studies in which several natural products were shown to inhibit PTP1B, including phenylated flavonoids from the root bark of *Erythrinamilbraedii*,²⁹ isoprenylated flavonoids from *Ficustikoua*,³⁰ and tea catechins.³¹ Therefore, significant results of inhibitory capacity in MOE can be attributed to the presence of diverse compounds. No obstant, further studies are needed to understand how *M. oleifera* leaf extract mediate the inhibitory effects on PTP1B and determine whether phenolic compounds, flavonoids and triterpenoids are the major active components responsible for MOE inhibitory potential as well as to identify other potential compounds involved.

The AR is implicated in the development of diabetic complications by catalyzing the reduction of glucose to sorbitol.³² It participates in diabetes associated mitochondrial disorder and damage via the activation of p53.³³ Moreover, polymorphic markers of the human AR gene demonstrate a strong association with susceptibility to develop diabetic complications. Although a number of ARIs have been tested or are currently undergoing to clinical trials, the clinical efficacy is uncertain and there are concerns with associated adverse effects such as hepatic damage.⁷ Hence, it is necessary to found new natural compounds.

A number of common plant/natural products used in Indian gastronomy have been evaluated for their AR inhibitory potential. Among the 22 dietary sources tested, 10 showed considerable inhibitory potential against both rat lens and rhAR with an approximate IC_{50} of 0.2 mg/mL.³⁴ Although the *M. oleifera* is an Indian native tree and is part of its population's diet, it was not considered in this study. Previous studies have shown that the active components of the aqueous extract from *Emblia officinalis* fruit penetrate the lens and substantially delay the progression of cataracts through aldose reductase 2 inhibition.³⁵

Kinetics studies have suggested that is flavones isolated from *Caesalpinia pulcherrima* can interact and inhibit the AR in a non-

competitive manner, suggesting its potential to combine with both, the free enzyme and the enzyme-substrate complex.³⁶ As reported by Tae Hyeon Kim et al.,³⁷ through bioassay-guided fraction of an EtOH extract of the kernel from purple corn (*Zea mays* L.) showed that hirsutrin have rhAR inhibitory activity (IC₅₀, 4.78 μM) and kinetic analyses using Line weaver-Burk plots hirsutrin showed a competitive inhibition against rhAR. However, it was reported that compounds that selectively bind to the enzyme-nucleotide complex are more effective than those bind to free enzyme.³⁸

In our study, MOE showed a significant inhibition of rhAR with an IC₅₀ value of 3.55 μg/mL in a mixed manner, which suggests that compounds present in this extract may be bind both the enzyme-nucleotide complex and free enzyme. In addition, these results suggest that MOE could serve as source of leading compounds for potential analogues with improved inhibitory activity. Therefore, the presence of biologically important photochemical in this extract may be useful for pharmaceutical therapies. Further studies towards fractioning, isolation and purification of active principles from *M. oleifera* extract are in progress in our laboratory.

Conclusion

The data obtained in the present study confirmed that themethanolic extract of *M. oleifera* possess inhibitory potential against both PTP1B and rhAR activities. Our findings suggest that MOE have potential to be used in the treatment of diseases associated with over activity of PTP1B. These results also indicate that MOE is an effective inhibitor of rhAR. Moreover, the information provided by this analysis suggest that the antidiabetic effects of *M. oleifera* extract are attributable not only to the insulin receptor but may also block the participation of AR in diabetes-associated mitochondrial disorder and damage via the activation of p53, confirming the use of *M. oleifera* as a treatment of diabetes in traditional medicine.

Acknowledgements

The authors acknowledge the financial support for the development of this research to the Consejo Nacional de Ciencia y Tecnologia (CONACyT) grant 128499 to ATV, to Programa para el Desarrollo Profesional Docente (PRODEP) grant DSA/103.5/15/7028 to CAD, and grant DSA/103.5/15/11103 to CAD and ATV. The authors are also grateful to the Mexican Moringa producers (Akuanandi) for the samples supplied for this study. The authors are very thankful to Jorge A. Sosa-Gutierrez for helpful discussions and critical reading of this paper. Marie Jazmin Sarabia Sanchez thanks CONACyT for the financial support for her Ph. D. studies, Grant 271541. Maria Alejandra Sanchez Munoz thanks CONACyT for financial support for her Masters studies, Grant 601630. All authors contributed equally to this work.

Conflict of interest

The authors declare that there is not conflict of interests regarding the publication of this paper.

References

- Bourdeau A, Dube N, Tremblay ML. Cytoplasmic protein tyrosine phosphatases, regulation and function: the roles of PTP1B and TC-PTP. *Curr Opin Cell Biol*. 2005;17(2):203–209.
- Klaman LD, Boss O, Peroni OD, et al. Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. *Mol Cell Biol*. 2000;20(15):5479–5489.
- Delibegovic M, Bence KK, Mody N, et al. Improved glucose homeostasis in mice with muscle-specific deletion of protein-tyrosine phosphatase 1B. *Mol Cell Biol*. 2007;27(21):7727–7734.
- Delibegovic M, Zimmer D, Kauffman C, et al. Liver-specific deletion of protein-tyrosine phosphatase 1B (PTP1B) improves metabolic syndrome and attenuates diet-induced endoplasmic reticulum stress. *Diabetes*. 2009;58(3):590–599.
- Suzen S, Buyukbingol E. Recent studies of aldose reductase enzyme inhibition for diabetic complications. *Curr Med Chem*. 2003;10(15):1329–1352.
- Ahmed N, Thornalley P. Advanced glycation endproducts: what is their relevance to diabetic complications. *Diabetes Obes Metab*. 2007;9(3):233–245.
- Tang WH, Martin KA, Hwa J. Aldose reductase, oxidative stress, and diabetic mellitus. *Front Pharmacol*. 2012;3:87.
- Popoola JO, Obembe OO. Local knowledge, use pattern and geographical distribution of *Moringa oleifera* Lam. (Moringaceae) in Nigeria. *J Ethnopharmacol*. 2013;150(2):682–691.
- Valdez-Solana MA, Mejia-Garcia VY, Tellez-Valencia A, et al. Nutritional content and elemental and phytochemical analyses of moringa oleifera grown in Mexico. *Journal of Chemistry*. 2015;2015:1–9.
- Al-Asmari AK, Al-Elaiwi AM, Athar MT, et al. A review of hepatoprotective plants used in Saudi traditional medicine. *Evid Based Complement Alternat Med*. 2014;2014:890842.
- Krishnamurthy PT, Vardarajulu A, Wadhvani A, et al. Identification and characterization of a potent anticancer fraction from the leaf extracts of *Moringa oleifera* L. *Indian J Exp Biol*. 2015;53(2):98–103.
- Abd El Latif A, El Bialy BES, Mahboub HD, et al. Moringa oleifera leaf extract ameliorates alloxan-induced diabetes in rats by regeneration of β cells and reduction of pyruvate carboxylase expression. *Biochem Cell Biol*. 2014;92(5):413–419.
- Obboh G, Ademiluyi AO, Ademosun AO, et al. Phenolic Extract from *Moringa oleifera* Leaves Inhibits Key Enzymes Linked to Erectile Dysfunction and Oxidative Stress in Rats' Penile Tissues. *Biochemistry research international*. 2015;2015:175950.
- Sarabia-Sanchez M. *Diseno, Sintesis y Evaluacion in silico e in vitro de derivados de bencimidazol como inhibidores de la proteina fosfatasa de tirosina 1B*. Mexico: Universidad Juarez del Estado de Durango; 2016.
- Goldstein BJ, Bittner-Kowalczyk A, White MF, et al. Tyrosine dephosphorylation and deactivation of insulin receptor substrate-1 by protein-tyrosine phosphatase 1B Possible facilitation by the formation of a ternary complex with the Grb2 adaptor protein. *J Biol Chem*. 2000;275(6):4283–4289.
- Sarmiento M, Zhao Y, Gordon SJ, et al. Molecular basis for substrate specificity of protein-tyrosine phosphatase 1B. *J Biol Chem*. 1998;273(41):26368–26374.
- Gupta SC, Phromnoi K, Aggarwal BB. Morin inhibits STAT3 tyrosine 705 phosphorylation in tumor cells through activation of protein tyrosine phosphatase SHP1. *Biochem Pharmacol*. 2013;85(7):898–912.
- Wang LJ, Jiang B, Wu N, et al. Small molecules as potent protein tyrosine phosphatase 1B (PTP1B) inhibitors documented in patents from 2009 to 2013. *Mini Rev Med Chem*. 2015;15(2):104–122.
- Dieye AM, Sarr A, Diop SN, et al. Medicinal plants and the treatment of diabetes in Senegal: survey with patients. *Fundam Clin Pharmacol*. 2008;22(2):211–216.
- Elangovan M, Dhanarajan MS, Rajalakshmi A, et al. Analysis of Phytochemicals, Antibacterial and Antioxidant activities of *Moringa oleifera* Lam. Leaf extract—an in vitro study. *International Journal of Drug Development and Research*. 2014;6(4):173–180.

21. Jain PG, Patil SD, Haswani NG, et al. Hypolipidemic activity of *Moringa oleifera* Lam., Moringaceae, on high fat diet induced hyperlipidemia in albino rats. *Revista Brasileira de Farmacognosia*. 2010;20(6):969–973.
22. Wang LJ, Jiang B, Wu N, et al. Natural and semisynthetic protein tyrosine phosphatase 1B (PTP1B) inhibitors as anti-diabetic agents. *RSC Advances*. 2015;5(60):48822–48834.
23. Sharifuddin Y, Chin YX, Lim PE, et al. Potential Bioactive Compounds from Seaweed for Diabetes Management. *Mar Drugs*. 2015;13(8):5447–5491.
24. Cortes A, Cascante M, Cardenas ML, et al. Relationships between inhibition constants, inhibitor concentrations for 50% inhibition and types of inhibition: new ways of analysing data. *Biochem J*. 2001;357(1):263–268.
25. Stohs SJ, Hartman MJ. Review of the Safety and Efficacy of *Moringa oleifera*. *Phytother Res*. 2015;29(6):796–804.
26. Chen RM, Hu LH, An TY, et al. Natural PTP1B inhibitors from *Broussonetia papyrifera*. *Bioorg Med Chem Lett*. 2002;12(23):3387–3390.
27. Li S, Li W, Wang Y, et al. Prenylflavonoids from *Glycyrrhiza uralensis* and their protein tyrosine phosphatase-1B inhibitory activities. *Bioorg Med Chem Lett*. 2010;20(18):5398–5401.
28. Na M, Kim KA, Oh H, et al. Protein tyrosine phosphatase 1B inhibitory activity of amentoflavone and its cellular effect on tyrosine phosphorylation of insulin receptors. *Biological and Pharmaceutical Bulletin*. 2007;30(2):379–381.
29. Jang J, Na M, Thuong PT, et al. Prenylated flavonoids with PTP1B inhibitory activity from the root bark of *Erythrina mildbraedii*. *Chem Pharm Bull*. 2008;56(1):85–88.
30. Wu LQ, Lei C, Gao LX, et al. Isoprenylated Flavonoids with PTP1B Inhibition from *Ficus tikoua*. *Nat Prod Commun*. 2015;10(12):2105–2107.
31. Ma J, Li Z, Xing S, et al. Tea contains potent inhibitors of tyrosine phosphatase PTP1B. *Biochem Biophys Res Commun*. 2011;407(1):98–102.
32. Borhani D, Harter TM, Petrash JM. The crystal structure of the aldose reductase. NADPH binary complex. *J Biol Chem*. 1992;267(34):24841–24847.
33. Tang WH, Stitham J, Jin Y, et al. Aldose Reductase-Mediated Phosphorylation of p53 Leads to Mitochondrial Dysfunction and Damage in Diabetic Platelets Clinical Perspective. *Circulation*. 2014;129(15):1598–1609.
34. Saraswat M, Muthenna P, Suryanarayana P, et al. Dietary sources of aldose reductase inhibitors: prospects for alleviating diabetic complications. *Asia Pac J Clin Nutr*. 2008;17(4):558–565.
35. Suryanarayana P, Kumar PA, Saraswat M, et al. Inhibition of aldose reductase by tannoid principles of *Embllica officinalis*: implications for the prevention of sugar cataract. *Mol Vis*. 2004;10:148–154.
36. Kumar MP, Sankeshi V, Naik RR, et al. The inhibitory effect of Isoflavones isolated from *Caesalpinia pulcherrima* on aldose reductase in STZ induced diabetic rats. *Chemico-biological interactions*. 2015;237:18–24.
37. Kim TH, Kim JK, Kang YH, et al. Aldose reductase inhibitory activity of compounds from *Zea mays* L. *Biomed Res Int*. 2013;2013:727143.
38. Bohren KM, Grimshaw CE. The sorbinil trap: a predicted dead-end complex confirms the mechanism of aldose reductase inhibition. *Biochemistry*. 2000;39(32):9967–9974.