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IN VITRO ANTI-DIABETIC AND ANTI-INFLAMMATORY ACTIVITY OF *HELIOTROPIUM INDICUM* LEAF EXTRACT IN LPS-STIMULATED RAW 264.7 CELLS

Ekramul Hasan[1], Rajbongshi Lata[1], Abdur Rouf [1], Howlader Saurav[2*]

[1] Department of Pharmacy, Daffodil International University, Dhanmondi, Dhaka

[2*] South East University, Banani, Dhaka, Bangladesh.

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Abstract:

The aim of the present study was to evaluate the anti-diabetic, anti-inflammatory, and antioxidant potential of Heliotropium indicum leaf extracts. Anti-diabetic and anti-inflammatory activities were evaluated via protein tyrosine phosphatase (PTP1B) and rat lens aldose reductase inhibitory assays and cell-based lipopolysaccharide (LPS)-induced nitric oxide (NO) inhibitory assays in RAW 264.7 murine macrophages. In addition, scavenging assays using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical, and peroxynitrite ($ONOO^-$) were used to evaluate antioxidant potential of Heliotropium indicum. The leaf extracts of Heliotropium indicum showed significant antioxidant effects in DPPH, ABTS, and $ONOO^-$ scavenging assays. Likewise, methanol (MeOH) extracts of H. indicum inhibited LPS-induced NO production in a dose dependent manner that was further clarified by suppression of iNOS and COX-2 protein production. The MeOH extracts of H. indicum exhibited potent inhibitory activities against PTP1B and RLAR with the corresponding IC_{50} values of 277.31±0.22 and 1561.32±3.48 µg/mL, respectively. Our study establishes that Heliotropium indicum extract might be useful as a potent anti-diabetic, antioxidant, and anti-inflammatory agent.

***Keywords**: H. indicum; Protein tyrosine phosphatase 1B (PTP1B), Rat Lens Aldose Reductase (RLAR), Anti-diabetic, Anti-inflammation, Antioxidant.*

Corresponding author:

Howlader Saurav,

SouthEast University, Banani,

Dhaka, Bangladesh.

E-mail: sauravpharm@gmail.com

QR code

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1. INTRODUCTION:

Diabetes mellitus (DM) is one of the oldest diseases known to mankind and is a leading health concern throughout the world. Type 2 DM accounts for 90% of all diabetes cases, which in the near future is expected to rise to 552 million cases worldwide, stimulating the need for investigation into the mechanism of DM [1]. It has been proposed that diabetic complications like retinopathy, cataracts, neuropathy, atherosclerosis, nephropathy, embryopathy, and delayed healing of wounds are initiated or activated by a common mechanism of free radical generation, which results in the generation of a variety of reactive oxygen species, reactive nitrogen species, and heightened oxidative stress [2].

Aldose reductase (AR) is the first enzyme of the polyol pathway that reduces glucose to sorbitol in the presence of adenine dinucleotide phosphate (NADPH) as a cofactor. Sorbitol dehydrogenases oxidize sorbitol to fructose in the polyol pathway, leading to a loss of functional integrity of the lens, subsequent cataract formation, and other severe diabetic complications [3]. PTP1B is considered another important therapeutic target for the treatment of type 2 DM via an increase in glucose uptake from vessels into cells, leading to a decrease in postprandial hyperglycemia [4]. Thus, inhibiting AR and PTP1B activity has great therapeutic potential for the prevention of DM and its complications. Inflammation has been implicated as an important etiological factor in the development of both insulin resistance and type 2 DM, which has been predominantly drawn from studies demonstrating associations between elevated levels of circulating acute phase inflammatory markers and indices of insulin resistance and the development of type 2 DM[5]. Likewise, inflammation and stress are both responsible for the pathogenesis of DM, suggesting the potential importance of antioxidants and anti-inflammatory alternatives [6].

Heliotropium indicum Linn. commonly known as 'Indian heliotrope' belongs to family Boraginaceaeis, very common in India and Bangladesh as well as some parts of Africa, but also found in other countries. The whole plant is claimed to possess medicinal properties. The leaves are used for the treatment of ophthalmic disorders, erysipelas and pharyngodynia [7,8]. The roots are used as astringent, expectorant and febrifuge. The aqueous extract of leaves was proved to be active against Schwart's leukaemia [9]. Different extracts of *H. indicum* have been studied for possible biological activities in various animal models and reported to possess significant antimicrobial, antifertility, antitumor, antituberculosis, anti-inflammatory, histogastroprotective, anti-cataract, analgesic and wound healing activities. Literature suggests the methanolic extract of root of *Heliotropium indicum* was reported to have significant antihyperglycemic activity in Streptozotocin and alloxan induced diabetic rats [10]. However, there have been no studies on *H. indicum* responsible for anti-diabetic as well as anti-inflammatory activities on cellular model study. In this study, we aimed to determine the diabetes, diabetic complications, antioxidant and anti-inflammatory activity of the *H. indicum*. Our data suggest that *H. indicum* may represent a source to prevention or treatment associated with diabetes and oxidative damage-associated diseases.

2. MATERIALS AND METHODS:

2.1. Chemicals and Reagents

Lipopolysaccharide (LPS) from sulforaphane, protein tyrosine phosphatase (PTP1B), rat lens aldose reductase (RLAR), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino- bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), peroxynitrite ($ONOO^-$), Griess reagent, 3-(4,5-dimethylthiazol-2-yl)-2,5-di-phenyl tetrazolium bromide (MTT), ethylenediaminetetraacetic acid (EDTA), 2-amino-5,6-dihydro-6-methyl-4H-1,3-thiazine hydrochloride (AMT), p-nitrophenyl phosphate (pNPP), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), fetal bovine serum (FBS), and antibiotics were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Dulbecco's Modified Eagle's Medium (DMEM) was purchased from HyClone (Logan, UT, USA). Dihydrorhodamine 123 (DHR 123) was purchased from Molecular Probes (Eugene, OR, USA). Various primary antibodies (iNOS, COX-2, and β-actin) were obtained from Cell Signaling Technology, Inc. (Beverly, MA, USA). Polyvinylidene fluoride (PVDF) membrane (Immobilon-P) was obtained from Millipore Co. (Billerica, MA, USA). All other chemicals and solvents were purchased from trusted sources.

2.2. Plant Material

The plant sample of *H. indicum* leaves were collected in May, 2017 from local area of Bangladesh. Plants were then washed properly to remove dirty materials and air-dried for several days. These were then ground with a hammer grinder for better grinding. The dried leaves were ground into a coarse powder. Then, the dried powder was preserved in an airtight container against the re-absorption of moisture, oxidation, excessive heat or humidity, growth of moulds and bacteria and infestation by insects and

rodents.

2.3. Preparation of *H. indicum* extracts

H. indicum leaf extracts were refluxed with MeOH for 3 h (5 L×2). The total filtrate was then suspended to dryness in vacuo at 40°C in order to render the MeOH extracts (262.92 g, respectively).

2.4. DPPH radical scavenging activity

The DPPH radical scavenging activity was evaluated using the method of Blois [11], with slight modification. The *H. indicum* extracts and DPPH were dissolved in MeOH. One hundred sixty microliters of extracts at various concentrations (100 µM final concentration) were added to 40 µL DPPH (1.5×10^{-4} M). After mixing gently and standing at room temperature for 30 min, the optical density of the reactant was measured at 520 nm using a VERSAmax microplate spectrophotometer (Molecular Devices). The antioxidant activity of both samples is expressed in terms of IC_{50} values (µg/mL, required to inhibit DPPH radical formation by 50%), which was calculated from the log-dose inhibition curve. L-Ascorbic acid was used as the positive control.

2.5. Trolox equivalent antioxidant capacity (TEAC)

This assay is based on the ability of different substances to scavenge ABTS radical cation ($ABTS^{\cdot+}$) as compared to the Trolox positive control [12]. To oxidize colorless ABTS to blue-green $ABTS^{\cdot+}$, a 7.0 mM ABTS stock solution was mixed with 2.45 mM potassium per-sulfate (1:1, v/v) and left at room temperature in the dark for 12 to 16 h until the reaction was complete and the absorbance was stable. The blue/green $ABTS^{\cdot+}$ solution was diluted in ethanol (EtOH) to an absorbance of 0.70±0.02 at 734 nm for measurement. The photometric assay was conducted with 180 µL of the $ABTS^{\cdot+}$ solution and 20 µL of sample dissolved in EtOH solution (100 µM final concentration) that was stirred for 30 s. The optical density was measured at 734 nm after 2 min using a VERSAmax microplate spectrophotometer (Molecular Devices). The antioxidant activity was calculated by determining the decrease in absorbance at different concentrations using the following equation:

$E=[A_c-A_t/A_c]\times100$

Where A_t and A_c are the absorbance with and without extracts, respectively. Trolox was used as the positive controls. The TEAC results are expressed as IC_{50} values (µg/mL).

2.6. Assay for $ONOO^-$ scavenging activity

The $ONOO^-$ scavenging activity was assessed by a modified Kooy's method, which involves the monitoring of highly fluorescent rhodamine 123, which is rapidly produced from non-fluorescent dihydrorhodamine (DHR) 123 in the presence of $ONOO^-$ [13]. In brief, the rhodamine buffer (pH 7.4) consisted of 50 mM sodium phosphate dibasic, 50 mM sodium phosphate monobasic, 90 mM sodium chloride, 5.0 mM potassium chloride, and 100 M diethylenetriamine pentaacetate. The final DHR 123 concentration was 5.0 µM. The assay buffer was prepared prior to use and placed on ice. The extracts were dissolved in 10% DMSO (100 µM final concentration). The background and final fluorescent intensities were measured 5 min after treatment with and without the addition of authentic $ONOO^-$ (10 µM), dissolved in 0.3 N sodium hydroxide (NaOH). The fluorescence intensity of the oxidized DHR 123 was evaluated using a fluorescence microplate reader (FL×800, Bio-Tek Instruments Inc., Winooski, VT, USA) at excitation and emission wavelengths of 480 and 530 nm, respectively. The values of $ONOO^-$ scavenging activity were calculated as the final fluorescence intensity minus the background fluorescence via the detection of DHR 123 oxidation. L-penicillamine was used as the positive control.

2.7. Assay for PTP1B inhibitory activity

Inhibitory activity of the *H. indicum* extracts against PTP1B was evaluated using *p*NPP [14]. To each well in a 96-well plate, 2.0 mM *p*NPP and PTP1B were added in a buffer containing 50 mM citrate (pH 6.0), 0.1 M NaCl, 1.0 mM EDTA, and 1.0 mM DTT with or without sample. The plate was pre-incubated at 37°C for 10 min, and then 50 µL *p*NPP buffer was added. Following incubation at 37°C for 30 min, the reaction was terminated with the addition of 10 M NaOH. The amount of *p*-nitrophenyl produced after enzymatic de-phosphorylation was estimated by measuring the absorbance at 405 nm using a VERSAmax microplate spectrophotometer (Molecular Devices). The non-enzymatic hydrolysis of 2.0 mM *p*NPP was corrected by measuring the increase in absorbance at 405 nm obtained in the absence of PTP1B enzyme. The percent inhibition was calculated as:

Inhibition (%)=$[A_c-A_s/A_c]\times100$

Where A_c was the absorbance of the control, and A_s was the absorbance of the sample. Ursolic acid was used as the positive control.

2.8. Assay for RLAR inhibitory activity

Rat lens homogenate was prepared according to a slightly modified method from Hayman and Kinoshita [15]. The lenses were removed from the eyes of Sprague-Dawley rats weighing 250 to 280 g. The lenses were homogenized in sodium phosphate

buffer (pH 6.2), which was prepared from sodium phosphate dibasic ($Na_2HPO_4.H_2O$, 0.66 g) and sodium phosphate monobasic ($Na_2HPO_4.H_2O$, 1.27 g) in 100 mL double distilled H_2O. The supernatant was obtained by centrifugation of the homogenate at 10,000 rpm at 4°C for 20 min and was frozen until use. A crude AR homogenate with a specific activity of 6.5 U/mg was used in the enzyme inhibition evaluations. The reaction solution consisted of 620 µL 100 mM sodium phosphate buffer (pH 6.2), 90 µL AR homogenate, 90 µL 1.6 mM NADPH, and 91 µL sample. The substrate included 90 µL DL-glyceraldehyde (50 mM). The AR activity was determined by measuring the decrease in NADPH absorption at 340 nm over a 4 min period on an Ultrospec2100 pro UV/Visible spectrophotometer with SWIFT II Applications software (Amersham Biosciences). Quercetin, a well-known AR inhibitor, was used as a reference. The percent inhibition was calculated as:

Inhibition (%)= (1- ΔA sample/min-ΔA blank/min/ΔA control/min-ΔA blank/min) × 100

Where ΔA sample/min represents the reduction in absorbance over 4 min for the test sample and substrate, and ΔA control/min represents the same, but with 100% DMSO instead of the test sample. The 50% inhibition concentration is expressed as the mean ± SEM.

2.9. Cell Culture

RAW 264.7 murine macrophages were obtained from the American Type Culture Collection (Rockville, MD, USA). RAW 264.7 cells were cultured in DMEM supplemented with 10% FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37°C with humidified air containing 5% CO_2.

2.10. Cell Viability Assay

Cell viability was assessed using the MTT assay. Briefly, RAW 264.7 cells were seeded into 96-well plates at a density of 1.0×10^4 cells per well and incubated at 37°C for 24 h. The cells were then treated with various sample concentrations (400 to 1,600 µg/mL). After incubation for an additional 24 h at 37°C, 100 µL MTT (0.5 mg/mL in PBS) was added to each well and the incubation continued for another 2 h. The resulting color was assayed at 540 nm using a microplate spectrophotometer (Molecular Devices).

2.11. Assay for inhibition of cellular NO production

The nitrite concentration in the medium was measured using Griess reagent as an indicator of NO production. Briefly, RAW 264.7 cells (1.0×10^5 cells/well in a 24-well plate with 500 µL culture medium) were pretreated with samples (400 to 1,600 µg/mL) for 2 h and incubated for 18 h with LPS (1.0 µg/mL). After incubation, the nitrite concentration of the supernatants (100 µL/well) was measured by adding 100 µL Griess reagent. To quantify the nitrite concentration, standard nitrite solutions were prepared. The absorbance values of mixtures were determined using a micro-plate spectrophotometer (Molecular Devices) at 540 nm. The iNOS inhibitor AMT was used as a positive control.

2.12. Analysis for inhibition of iNOS and COX-2 protein production

Western blotting was used to measure iNOS and COX-2 protein levels. First, RAW 264.7 cells were cultured in 100 mm culture dishes in the presence or absence of LPS (1.0 µg/mL) with or without test samples (400 to 1,600 µg/mL) for 18 h. Afterwards, the cells were washed twice with ice-cold PBS and lysed with cell lysis buffer on ice for 30 min. Cell extracts were obtained by centrifugation at 14,000 *g* at 4°C for 20 min. Cytosolic proteins were electrophoretically separated on SDS-PAGE and transferred to PVDF membranes. Membranes were immediately blocked with 5% (w/v) non-fat dry milk in Tris-buffered saline containing 0.1% Tween-20 (pH 7.4) (TBST) buffer at room temperature for 1 h. The membranes were then washed three times (10 min each) in TBST buffer and incubated with primary antibody, diluted 1:1000 in 5% (w/v) non-fat dry milk in TBST buffer at 4°C overnight. After three washings in TBST buffer (10 min each), the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody diluted 1:2000 in 5% (w/v) non-fat dry milk in TBST buffer at room temperature for 1 h. After three washings in TBST buffer (10 min each), antibody labeling was visualized with SuperSignal West Pico Chemi-luminescent Substrate (Pierce, Rockford, IL, USA) according to the manufacturer's instructions and was exposed to X-ray film (GE Healthcare Ltd., Amersham, UK). Pre-stained blue protein markers were used for molecular weight determination. Bands were visualized using a Luminescent image analyzer (Fujifilm Life Science, Tokyo, Japan) and quantified by densitometry analysis using PDQuest software (Version 7.0, BIO-RAD, Hercules, CA, USA).

2.13. Statistical analysis

Data are presented as the mean ± SEM of at least four independent experiments. Statistical comparisons between groups were performed using one-way ANOVA followed by Student's *t*-test. A *P* value less than or equal to 0.05 was considered statistically significant.

3. RESULTS:

3.1. Scavenging effect of DPPH, ABTS and $ONOO^-$ radical from *H. Indicum*

The radical-scavenging activities of MeOH extracts from H. indicum are shown in Table 1. *H. indicum* showed potent activity in DPPH scavenging activity with an IC_{50} value of 696.43±2.58 µg/mL as compared to the positive L-ascorbic acid control (IC_{50} = 2.31±0.03 µg/mL). The ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating and chain breaking antioxidants. The methanolic extract of *H. indicum* showed potent activity in ABTS scavenging activity with an IC_{50} value of 373.23±2.42 µg/mL as compared to the positive control Trolox (IC_{50}=3.58±0.09 µg/mL). The $ONOO^-$ scavenging activities are shown in Table 1. The formation of $ONOO^-$ detectable by the oxidation of DHR 123 to rhodamine 123 from decomposition of authentic $ONOO^-$ was scavenged by methanolic extracts of *H. indicum* to a variable degree. *H. indicum* showed potent activity in $ONOO^-$ scavenging assay with an IC_{50} value of 118.13±0.16 µg/mL as compared to the positive control L-Penicillamine (IC_{50}=1.31±0.17 µg/mL).

Table 1: Antioxidant activities of the methanolic extract from *H. indicum*

Parameters	IC_{50} (µg/mL)		
	DPPH	ABTS	$ONOO^-$
H. indicum	696.43±2.58	372.23±2.42	118.13±0.16
Trolox[b]		3.58±0.09	
L-Ascorbic acid[c]	2.31±0.03		
L-Penicillamine[d]			1.31±0.17

[a] The concentration that caused 50% inhibition (IC_{50}) is given as the mean ± SEM of triplicate experiments
[b] Used as positive control in ABTS scavenging assay
[c] Used as positive control in DPPH assay
[d] Used as positive control in $ONOO^-$ assay

3.2. PTP1B and RLAR inhibitory activity of the MeOH extract of *H. indicum*

To evaluate the anti-diabetic potential of *H. indicum*, the MeOH extract was tested in *in vitro* PTP1B and RLAR inhibition assays. The inhibitory activities of the MeOH extract against PTP1B and RLAR are shown in Table 2. The MeOH extract showed significant inhibitory activity against PTP1B with IC_{50} values of 277.31 ± 0.22 µg/mL compared to the positive controls of ursolic acid with an IC_{50} values of 5.29 ± 0.43 µg/mL. Furthermore, The MeOH extract showed significant inhibitory activity against RLAR with IC_{50} values of 1561.32 ± 3.48 µg/mL compared to the positive controls of quercetin with an IC_{50} value of 0.48 ± 0.02 µg/mL, respectively.

Table 2: PTP1B and RLAR inhibitory activities of the methanolic extract from *H. indicum*

Parameters	IC_{50} (µg/mL)	
	PTP1B	RLAR
H. indicum	277.31±0.22	1561.32±3.48
Ursolic acid[b]	5.29±0.43	
Quercetin[c]		0.48±0.02

[a] The concentration that caused 50% inhibition (IC_{50}) is given as the mean ± SEM of triplicate experiments
[b] Used as positive control in PTP1B inhibitory assay
[c] Used as positive control in RLAR inhibitory assay

3.3. Effect of *H. indicum* leaf extracts on cell viability and NO production in LPS-induced RAW 264.7 cells

The cytotoxicity was assessed via MTT assay. As shown in Fig. 1, the methanolic extract of *H. indicum* did not exhibit significant toxicity even at a concentration of 1,600 µg/mL. Thus, we further evaluated NO scavenging using this non-toxic concentration. Increased NO production is a typical phenomenon that occurs in LPS-stimulated macrophages and serves as an indicator of a typical inflammatory response. Nitrite, a stable metabolite of

NO, was used as an indicator of NO production in the medium. During the 18 h incubation with LPS (1.0 µg/mL), NO production in RAW 264.7 cells increased dramatically. Pretreatment with the MeOH extract resulted potent activity to inhibit NO production in a dose-dependent manner (Fig. 1). With respect to the cellular NO production assay, the MeOH extract resulted in a 47.23% inhibition compared with the positive AMT control (90% at 0.5 µg/ mL).

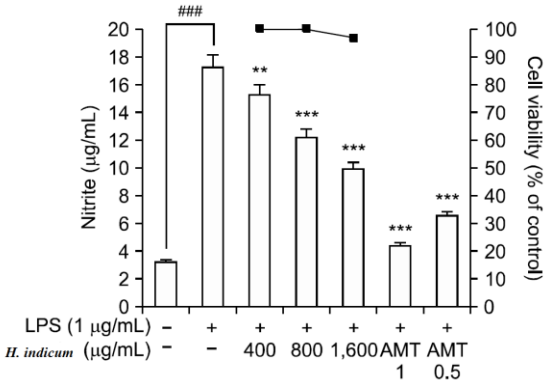

Fig. 1. Effect of *H. indicum* extracts on cell viability and LPS-induced NO production in RAW 264.7 cells. Cell viability was determined by MTT assay. Cells were seeded in 96-well plates at a density of 1.0×10^4 cells/well and incubated at 37°C for 24 h. The cells were then treated with various concentrations of sample (400 to 1,600 µg/mL). After an additional 24 h of incubation at 37°C, 100 µL MTT (0.5 mg/mL in PBS) was added to the wells and mixed. Cells were pre-treated with the indicated concentrations for 2 h, after which LPS (1.0 µg/mL) was added. After 18 h of incubation, the NO in the culture supernatants was measured using Griess reagent. Values are expressed as the mean ± SEM of triplicate experiments. ###P<0.001 indicates a significant difference from the unstimulated control group, *P <0.05, **P<0.01, and ***P<0.001 indicate significant differences from the LPS-stimulated control group.

3.3. Effect of *H. indicum* extract on iNOS and COX-2 production in RAW 264.7 cells

Western blot analysis was conducted to assess the activity of *H. indicum* extract on the production of iNOS and COX-2, which are well-characterized markers of NF-κB-responsive inflammation. As shown in Fig. 2, iNOS and COX-2 protein production in unstimulated RAW 264.7 cells was almost undetectable, but it was significantly stimulated upon treatment with 1.0 µg/mL of LPS. Pretreatment with the *H. indicum* MeOH extract at concentrations of 400, 800, and 1,600 µg/mL significantly down-regulated the production of iNOS and COX-2 protein in a dose dependent manner. *H. indicum* had a greater effect on the inhibition of COX-2 protein production than on iNOS production, which was further elucidated by densitometry analysis showing the potent inhibition of COX-2 expression in a dose-dependent manner.

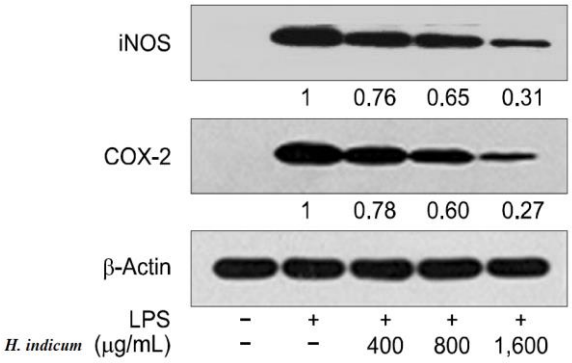

Fig. 2. Effect of *H. indicum* on the production of iNOS and COX-2 protein in RAW 264.7 cells. Cytosolic lysates from RAW 264.7 cells stimulated for 18 h were separated by SDSPAGE and the protein levels of iNOS, COX-2, and β-actin were detected by Western blot.

DISCUSSION:

There have been several recent attempts devoted to the discovery of new and effective anti-diabetic agents for the treatment of DM, including sulfonylurea derivatives, biguanides, thiazolidinedione, and alpha-glucosidase inhibitors [16]. However, the use of these anti-diabetics is limited due to toxic side effects. Thus, we are trying to investigate the possible development of natural drug alternatives derived from natural sources.

H. indicum showed potent inhibitory activity against DPPH, ABTS, and $ONOO^-$, which can be attributed to the presence of potent compounds such as steroids, saponins, triterpenes, and alakloids [17]. Free radicals ultimately have been reported to induce inflammation that further contributes to the onset and progression of inflammation in distant organs [18]. Our results demonstrated that *H. indicum* possess dose-dependent anti-inflammatory activity. Further, the *H. indicum* extract inhibited the production of iNOS and COX-2 protein, which further supports it as a candidate for an anti-inflammatory agent. Enhanced production of iNOS and COX-2 proteins is associated

with the inflammatory response. In our study, we demonstrated the suppression of both iNOS and COX-2 protein production by *H. indicum* for the first time. This result demonstrates that *H. indicum* inhibited NO production through the suppression of both iNOS and COX-2 production in LPS-stimulated RAW 264.7 cells.

PTP1B, which is localized to the cytoplasmic face of the endoplasmic reticulum and acts as a key regulator of both the insulin and leptin signaling pathways, is ubiquitously produced in classical insulin-targeted tissues including the liver, muscle, and fat [19]. It is known that PTP1B overexpression in multiple tissues in obesity is regulated by inflammation [20]. Our study investigated potent PTP1B inhibitors present in the MeOH extracts of *H. indicum*. DM associated complications can also arise from increased flux of glucose through the polyol pathway. The increased polyol pathway flux leads to accumulation of sorbitol in the lens fiber, which causes an influx of water, the generation of osmotic stress, and subsequent cataract formation [21]. Therefore, inhibition of AR has emerged as an important therapeutic approach for preventing and reducing long term diabetic complications. In our investigation, the *H. indicum* extract showed reasonable RLAR inhibitory activity which highlights its potential anti-diabetic activity. Usually, the activity of extracts cannot be correlated with individual compounds due to the presence of a diverse range of compounds, with each of the compounds differing in the range of activity. Thus, although RLAR activity was reasonable in our study, the individual compounds present in the *H. indicum* may be more potent. Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of both types of DM. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to the damage of cellular organelles that can promote the development of complications of DM [22]. Our results show that potent anti-diabetic activity can be correlated with potent antioxidant properties. Our study reveals the potent activity of *H. indicum*, establishing them as an alternate source of anti-diabetic agents.

New therapeutic innovations that are dedicated to prevent DM and DM complications are of interest. Our study revealed the effectiveness of *H. indicum* extract and found potent anti-diabetic (PTP1B and RLAR), antioxidant (DPPH, ABTS, and $ONOO^-$), and anti-inflammatory (NO) activity. Furthermore, presence of natural constituents like triterpene, alkaloid etc. demonstrated antioxidant activity with the potential to prevent inflammation and DM. Thus, the multifactorial activity of *H. indicum* extracts shows promising anti-diabetic activity which can be used as a new therapeutic agent for the treatment of DM as well as its complications.

CONCLUSION:

From the above results it is concluded that *Heliotropium indicum* exhibited potential anti-diabetic, antioxidant, and anti-inflammatory activity. *H. indicum* possesses various phytochemical constituents, which may be responsible for the anti-diabetic, antioxidant as well as anti-inflammatory activity. Further work has to be carried out to investigate the active compounds from *Heliotropium indicum*.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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