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In-vitro anti-inflammatory activity of *Indigofera heterantha* roots

Abstract

The current study was carried out to investigate the anti-inflammatory activity of the *I. heterantha* roots using membrane stabilizing assay by HRBC. The n-hexane fraction showed minimum stabilization 25.44% at 100µg/ml and maximum stabilization 55.61% at 400µg/ml while the EtoAc fraction showed minimum stabilization 48% at 100 µg/ml and maximum stabilization 71.88% at 400 µg/ml as compared to standard drug 64.70% at 100 µg/ml and 92.29% at 400µg/ml. These results revealed that this plant is very important from medicinal point of view and it needs further phytochemical exploitation to isolate phytochemical constituents having anti-inflammatory activity.

Keywords: *Indigofera heterantha*, anti-inflammatory, HRBC

Volume 6 Issue 4 - 2018

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Received: January 28, 2017 | **Published:** August 06, 2018

Introduction

The *Fabaceae* family (*Leguminosae*) consists of approximately 650 genera and 18,000 species, it is one of the largest *Angiosperm* families.[1,2] The genus *Indigofera* comprises around 700 species that are distributed geographically in tropical regions.[3] *I. heterantha* locally known as ghoreja or kainthaye (in Pashtho), Jangli methi (in Urdu) and Himalayan Indigo (in English) extensively spread in Northern regions of Pakistan and possessing high medicinal importance in the indigenous system of medicine. It is a shrub of 30 to 60cm tall and the leaves are imparipinnately compound, while the fruits are long cylindrical 1.5cm with 10-12 seeds.[4] *I. heterantha* is used as herbal medicine as well as folk medicine to treat gastrointestinal disorder and abdominal pain.[4] Various constituents have been isolated from the *I. heterantha* exhibited inhibitory activity against the enzyme lipoxygenase.[5] *I. heterantha* roots showed antioxidant, free radical scavenging activity and antidiabetic activity using Glucose uptake in yeast cells assay.[6] The crude fractions of the aerial parts of *I. heterantha* showed brine shrimp (Artemia salina Leach) cytotoxicity activity.[7] The metabolites and crude extracts of the genus *Indigofera* were found to exhibit various bioactivities including, antimicrobial, insecticidal, phytotoxic, antiulcergenic, hepatotoxic, teratogenic and cytotoxicity.[8] Other compounds like saponins, quinines, tannins, garlic acid, caffeic acid, myricetin, quercetin myricetin and galangin were also reported.[9] The chemical constituents like kaempferitrin,[10] louisfieserone, indigotin,[11] (S) indispicine,[12] benzofuran, dibenzofuran,[13] arabinofuranoside,[14] 12-oleanen-3, 11- dione, afromosin, genistein, isoliquiritigenin,[15] rutin[16] endecaphyllin A_1 and hiptagin,[17] have been isolated and reported from various species of genus *Indigofera*. To provide scientific evidence to the ethnobotanical uses of *I. heterantha*. The aim of current study was to investigate the importance of *I. heterantha* roots as an important medicinal plant for its anti-inflammatory potential.

Materials and methods

Plant material

I. heterantha roots were collected during the month of September, 2015 from District Swat, K.P.K, Pakistan. The identification of plant was done at the Department of Botany, Hazara University, Mansehra, Pakistan. The plant material was washed and dried in shade for fifteen days then chopped and powdered using a grinder.

Extraction and fractionation

The powdered plant materials (5Kg) was extracted by maceration in methanol for 10 days with (10L) solvent at room temperature, and the extract was concentrated in vacuum to yield 900g of residue. The crude extract of was further suspended in water and partitioned successively with organic solvents with increasing order of polarity for obtaining n-hexane, chloroform, ethyl acetate, methanol and aqueous fractions respectively. From each fraction the corresponding solvent was evaporated by rotary evaporator keeping specific temperature for each solvent. By this method, gummy residue of each fraction was obtained.

Anti-inflammatory activity

5ml Blood was obtained from volunteer in heparinized tubes for preparation of red blood cells suspension, who have not used non-steroidal anti-inflammatory drugs for at least a couple of weeks before the experiment. The blood was transferred to the centrifuge tubes. The tubes were centrifuged at 3000rpm for 15min at room temperature. The blood was washed with equal volume of normal saline 0.9% NaCl solution pH 7.4 (w/v) for a few times, along with removing supernatant carefully until supernatant became clear. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline. Solution with final concentration of 4.5ml having 1ml Phosphate buffer saline, pH 7.4, 2ml hypo saline (0.25% w/v), 0.5ml human red blood cells suspension and 1ml sample solution with varying concentration (100µg, 200µg, 300µg, 400µg) in normal saline was prepared. The prepared solutions and suspensions were incubated for 30minutes at 37ºC. After the incubation period the prepared solutions and suspensions were centrifuged at 3000rpm for 20minutes. At the end the absorbance of supernatant was measured using UV 5100B spectrophotometer at 560nm. The percent stabilization of human red blood cells membranes was calculated using the following formula.

$$\text{Percent }(\%)\text{ HRBC membrane stabilization} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Results and discussion

Anti-inflammatory activity

The reticence of hypotonicity induced HRBC membrane lysis i.e., stabilization of HRBC membrane was affianced as a measure of the

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anti-inflammatory activity. The percentage of membrane stabilization for crude fractions and standard drug Indomethacin were done at 100µg/ml, 200µg/ml, 300µg/ml and 400µg/ml respectively as given in Figure 1. The *n*-hexane faction exhibits minimum stabilization 25.44% at 100µg/ml and maximum stabilization 55.61% at 400µg/ml. The Chloroform fraction showed minimum stabilization 45.87% at 100µg/ml and maximum stabilization 67.32 at 400µg/ml. Similarly, the EtoAc fraction showed minimum stabilization 48% at 100µg/ml and maximum stabilization 71.88% at 400µg/ml and the MeOH fraction showed minimum stabilization 42% at 100µg/ml and maximum stabilization 67% at 400µg/ml. while the aqueous fraction shows maximum stabilization 39% at 100µg/ml and maximum stabilization 60% at 400µg/ml. As compared to standard drug 64.70% at 100µg/ml and 92.29% at 400µg/ml. With the increase in concentration the membrane hemolysis is lowered and membrane protection/stabilization is amplified as shown in Figure 1. Thus, the anti-inflammatory activity of the crude fractions was found to be concentration dependent.

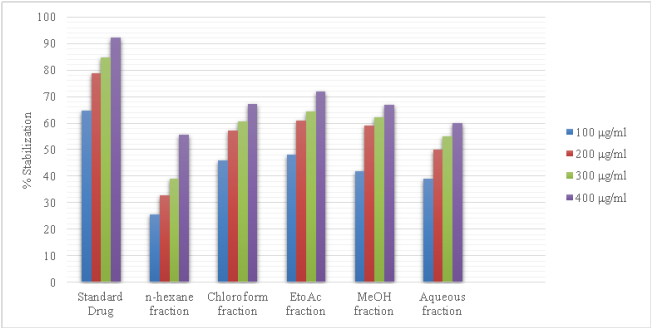

Figure 1 Anti-Inflammatory activity of I. heterantha roots.

Conclusion

In the current study the roots of *I. heterantha* were investigated to explore its medicinal importance. The results obtained exhibit that the EtoAc fraction of this plant showed highest activity among other crude fractions. The EtoAc fraction of this plant needs further phytochemical exploitation to isolate phytochemical constituents having anti-inflammatory activity.

Acknowledgements

Authors are grateful to Department of Biochemistry, Hazara University, Mansehra, Pakistan and Department of Chemistry, Abdul Wali Khan University, Mardan, Pakistan for providing research facilities.

Conflict of interest

Authors have none to declare.

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