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

Introduction
The Fabaceae family (Leguminosae) consists of approximately 650 genera and 18,000 species, it is one of the largest Angiosperm families. The genus Indigofera comprises around 700 species that are distributed geographically in tropical regions. 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. I. heterantha is used as herbal medicine as well as folk medicine to treat gastrointestinal disorder and abdominal pain. Various constituents have been isolated from the I. heterantha exhibited inhibitory activity against the enzyme lipoxygenase. I. heterantha roots showed antioxidant, free radical scavenging activity and antidiabetic activity using Glucose uptake in yeast cells assay. The crude fractions of the aerial parts of I. heterantha showed brine shrimp (Artemia salina Leach) cytotoxicity activity. The metabolites and crude extracts of the genus Indigofera were found to exhibit various bioactivities including, antimicrobial, insecticidal, phytotoxic, antiluercigenic, hepatotoxic, teratogenic and cytotoxicity. Other compounds like saponins, quinines, tannins, garlic acid, caffeic acid, myricetin, quercetin myricetin and galangin were also reported. The chemical constituents like kaempferitin, lousifeserone, indigotin, indigocyanin (S) indiscipine, benzoferuran, dibenzoferuran, arabinofuranoside, 12-oleanen-3, 11- dione, afroosmon, genistin, isooliquiritigenin, rutin, endcaphyllin A and hiptagin, have been isolated and reported from various species of genus Indigofera. To provide scientific evidence to the ethnomedical 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 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.

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\text{Percent (\%)} = \left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}\right) \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

Conflict of interest

Authors have none to declare.

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References


