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Evaluation of anti-asthmatic and antioxidant potential of *Boerhavia procumbens* in toluene diisocyanate (TDI) treated rats

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ABSTRACT

Aim of the study: Asthma is an ailment of airways characterized by activation of the T helper (Th) 2 lymphocytes and subsequent movement of inflammatory cells. *Boerhavia procumbens* of family Nyctaginaceae is locally used for the treatment of asthma, cough, hemorrhoids, dropsy, cardiac, eyes and kidney problems. We have evaluated its methanol extract (BPM) as a therapeutic candidate for asthma against toluene diisocyanate (TDI) allergic model in rat.

The BPM extract was obtained from the whole plant of *B. procumbens* in methanol. Sprague-Dawley male 36 rats (200–250 g) were categorized into 6 groups having six rats in each category. The animals were provoked (10%) and sensitized (5%) by TDI. Animals of groups I–III were vehicle control (ethyl acetate), diseased control (TDI) and reference control (TDI+dexamethasone {2.5 mg/kg bw}), respectively. Animals of group IV (TDI+200 mg/kg bw) and group V (TDI+400 mg/kg bw) were administered with BPM whereas group VI was administered with 400 mg/kg bw alone of BPM. Protective effects of BPM were determined by counting the number of leucocytes and estimation of interleukines in blood, bronchoalveolar lavage (BAL) and in *in vitro* culture of spleen cells. Estimation of antioxidant enzymes, lipid peroxides and H_2O_2 and histopathology of lungs were carried out for antioxidant potential of plant extract used.

Results: Methanol extract of *B. procumbens* suppressed the asthmatic symptoms and inhibited the infiltration of eosinophils and lymphocytes in lungs of TDI provoked rats. Administration of BPM to TDI provoked rats, dose dependently, inhibited the release of interleukins (IL)-2 in serum and IL-4, IL-6 interferon gamma (IFN-γ) in bronchoalveolar lavage (BAL) and in *in vitro* culture of spleen cells, and ameliorated the oxidative stress in lung tissues. Quantitative scoring of the lung histopathology exhibited protective effects of BPM and the inflammation, mucus, thickening of peribronchial smooth muscle layer and subepithelial deposition of collagen induced with TDI were ameliorated.

The BPM has the anti-inflammatory properties that may be used to treat the asthma and inflammatory related ailments.

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1. Introduction

Asthma is a respiratory disease caused by chronic inflammation that is mediated by the influx and activation of various inflammatory cells. Its symptoms usually include the reversible airflow obstruction. Two stages, early and late, have been recognized on the basis of mediators and the associated cells. During early phase mast cell degranulation occurs while the late phase is characterized by the obstruction of airways (Elliot et al., 2009). During the development of asthma pro-inflammatory cytokines such as IL-4, IL-5 and IL-13 secreted by $CD4^+$ Th2 cells play a critical role in the initial phase (Holgate, 2008). IL-4 is reported to be involved in the recruitment of eosinophils via the upregulation of endothelial vascular cell adhesion molecule-1 (VCAM-1). Further, $CD4^+$ Th1 cells augment the situation by release of IFN-γ that inhibits the differentiation of Th2 cells (Dai et al., 2009). Ultimately the accumulation of macrophages, neutrophils, eosinophils, lymphocytes, and mast cells in airway takes place leading to airway hyperresponsiveness.

Release of reactive oxygen species (ROS) during the development of asthmatic response further deteriorated the situation while causing lung injuries in diseased animals. Hyperactivity of airways induced with ROS elicits the histamine release from mast cells and mucous secretion from epithelial cells (Lee et al., 2011). During acute asthma exacerbation these changes are associated with cell shedding, enhanced release of arachidonic acid and chemo-attractants (Calabrese et al., 2000) and increased release of hydrogen peroxide

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and nitrous oxide during exhale (Wewel et al., 2006; Nagaraja et al., 2012).

Asthmatic response is triggered through various agents including viral infections, air pollutants and allergens. Toluene diisocyanate (TDI) is used in various industries and is considered as low molecular weight occupational sensitizer. TDI exposure in both acute and chronic is toxic to human. Malfunctioning and severe injuries are being induced with acute exposure to TDI in the respiratory, gastrointestinal and central nervous systems. It also induces severe irritation of the skin and eyes. However, its chronic exposure may provoke inflammatory response of airways that may develop into asthma-like symptoms (Pisati et al., 1993; Padoan et al., 2003).

To combat the oxidative stress various non-enzymatic antioxidants, vitamin C, glutathione, vitamin E and albumin exist in the lung lining fluid. In mild form of asthmatic response concentration of the antioxidants is enhanced due to epithelial permeability. Aside from non-enzymatic antioxidants the crucial enzymatic antioxidants are also involved to ameliorate the oxidative stress. Among the enzymatic antioxidant catalase, superoxide dismutase, peroxidase and glutathione peroxidase are the key biomolecules to combat and neutralize the deleterious effects of ROS. However, during exacerbation episodes of asthma antioxidant system of the lungs is suppressed. Plant species having antioxidant constituents can be added to the system to scavenge the free radicals. Diverse reports have indicated the beneficial effects of fruits, vegetables and medicinal plants in stress related ailments in experimental animals (Sahreen et al., 2014).

Boerhavia procumbens Banks ex Roxb. of family Nyctaginaceae is used in the local system of medicine to treat various ailments. Katewa and Galav (2005) reported the decoction of roots as eye tonic and the use of root paste as poultice in swelling of body, joints and in scorpion sting. Whole plant of *B. procumbens* is used in dysmenorrhea (Qureshi and Bhatti, 2008). Traditional healers use the whole plant of *B. procumbens* in anemia, bronchitis, piles, asthma, night blindness, jaundice and in inflammation (Akhtar and Begum, 2009). Its roots are used to cure jaundice (Hussain et al., 2010; Shaheen et al., 2012). The paste of whole plant is externally applied as poultice to treat paralysis (Shaheen et al., 2012). Infusion of whole plant is used to treat dropsy, menstrual flow dysregulation and gonorrhea (Srivastava et al., 2012). Ahmad et al. (2012) reported that whole plant of *B. procumbens* is used as a decoction and infusion in kidney failure, hematuria, as blood purifier and in septic conditions. Hussain et al. (2012) reported the use of whole plant of *B. procumbans* in asthma, edema, eye diseases, heart disease, hemorrhoids jaundice and in various other disorders. The decoction of the root is taken as a remedy in cough, asthma, hernia, dropsy, chest-pain, piles, and swellings. It is also used for gonorrhoea, and internal inflammations (Hussain et al., 2012; Nisar et al., 2014). Kayani et al. (2014) reported that local healers use the whole plant, leaves and roots for the treatment of asthma and cough.

Its phytoconstituents have been determined in the methanol extract and its derived fractions (Bokhari et al., 2015). HPLC analysis of *B. procumbens* was found to be comprised of rutin, kaempferol, catechin, quercetin, caffeic acid and myricetin. Experimentally the plant has shown the antioxidant in various *in vitro* free radical scavenging assays and anti-inflammatory potential in carrageenan induced paw edema in rat (Bokhari et al., 2015). On account of folklore use of whole plant of *B. procumbens* by traditional healers in asthma we have evaluated its anti-asthmatic potential against the toluene diisocyanate (TDI) induced allergic asthma in rat. In this regard we have provoked the rats with TDI and assessed the anti-inflammatory effects of crude methanol extract of *B. procumbens* by assessing the infiltration of leucocytes, to determine the amount of various cytokines and through antioxidant effects in lung tissues.

2. Material and methods

2.1. Plant extract

In August–September 2010 the whole plant of *B. procumbens* was collected from the campus of Quaid-i-Azam University Islamabad and deposited (#059133) at the herbarium of Pakistan Museum of Natural History, Islamabad. The plant after shade drying was pulverized at 60-mesh size (5 kg) and extracted twice in 95% methanol at 25 °C for 48 h. The filtrate obtained was concentrated on rotary evaporator at 40 °C to get the extract (BPM) 3.5% of the dry powder.

2.2. Animal treatment

The experiment was conducted on 36 Sprague-Dawley male rats (200–250 g); divided equally into 6 different groups (06 animals) and were maintained at room temperature (25 ± 3 °C) with 12 h dark/light cycle. The animals had free access to standard laboratory feed and water given *ad libitum*. The experimental use of rats was approved by the Ethical Committee of Quaid-i-Azam University, Islamabad.

Animals of Group I were treated with intranasal ethyl acetate for two rounds of 7 days with middle 7 days of rest. After a week of rest these animals were provoked by administering 5 μl of 5% TDI for 7 days (vehicle control). Animals of Group II–V were treated for 7 consecutive days by dropping 5 μl of 10% TDI in to each nostril. Having a rest of 7 days these animals were again sensitized for 7 days. For provocation 5 μl of 10% TDI was administered after a week of 2nd sensitize course. Rats of Group-II were remained as such (diseased control); whereas 2.5 mg/kg body weight (bw) dexamethasone was administered to Group III animals (reference control). Animals of Group IV and Group V were treated with BPM (200 mg/kg bw and 400 mg/kg bw) respectively. In order to evaluate toxicity induced with BPM, rats of Group VI were administered with BPM (400 mg/kg bw; orally) alone. Animals of the respective groups were administered the drug and the plant sample on daily basis orally a week prior to the experiment and it remained continuous for the rest of the experimental period. The last treatment was administered 2 h prior to the provocation dose.

2.3. Cytokines determination in serum

After provocation of asthmatic response the blood was collected from the cervical vein of animals, anesthetized with intraperitoneal injection of sodium phenobarbital. For the estimation of cytokines, serum was prepared from the blood and stored at −80 °C.

2.4. Determination of cell count and cytokines in bronchoalveolar lavage (BAL)

To determine the cytokine concentration and the accumulation of leukocytes in the fluid of lungs, rats were sacrificed, bronchoalveolar lavage (BAL) was collected thrice via trachea with 1 ml of PBS. Cells were separated after centrifugation of the BAL fluid at $500 \times g$ (4 °C). The cell pellet was suspended in 1000 μl of PBS to determine the total cell and differential count. The leukocytes were stained with hematoxylin and eosin (Sigma-Aldrich, MO, USA). Total cell number and differential cell count was made using a Neubauer hemocytometer. The supernatant was used for estimation of cytokines.

2.5. 3.15. Spleen cell culture

From each animal spleen was excised under aseptic conditions and single cell suspension was prepared by pressing between two

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