Abstract

Background: Asthma is a highly prevalent disease affecting millions of death worldwide. It is characterized by sniffing, sneezing, coughing, bronchoconstriction, hyper-responsiveness, excessive mucus, and bronchial inflammation. *Onosoma bracteatum* commonly called as Gojihva, is recommended to cure asthma and respiratory disorders. It is constituted into various herbal formulations to treat respiratory and cough diseases. Flavonoids belong to a vast group of plant secondary metabolites with variable phenolic structures. These are reported to have anti-allergic, anti-inflammatory, anti-oxidative, anti-asthmatic activities and modify immune responses. Benzopyrone class of compounds occurs in nature as flavones, isoflavones, neoflavones, coumarins etc. Depending upon the type of lactone ring, benzopyrones are available as α-benzopyrone, β-benzopyrone and γ-benzopyrone of which α-benzopyrone (coumarines) and γ-benzopyrone (flavones, isoflavones, neoflavones) occur widely in nature.

Aim: The aim of present study was to carry out phytochemical and pharmacological evaluation of *Onosoma bracteatum* and synthesized flavones for prevention of respiratory and asthmatic disorders.

Methods: The plant drug sample was duly authentified, dried and extracted using pet ether and alcohol (70% in water) as solvents. The phytochemical testing for presence of flavonoid, phenolic, alkaloid and saponin contents were done. The acute toxicity study of prepared extracts were carried out, as per OECD 423 guidelines and the animals were observed for adverse symptoms and mortality, if any. Male Hartley Guinea pigs (n=4) were selected for further *in-vivo* studies. Citric acid (7.5%) was given to the animals to induce coughing. In chronic model for asthma, the animals were actively sensitized by intraperitoneal injection of 20 µg ovalbumin and 100 mg Al(OH)₃ dissolved in normal saline (0.2 ml) twice in a gap of seven days. Two weeks later, the animals were placed in Perspex chamber equipped with an ultrasonic nebulizer and challenged with 0.5% ovalbumin inhalation in order to verify the occurrence of sensitization. The animals showing airway hyper-responsiveness, to the inhaled antigen, were referred to as sensitized animals. Plant extracts in doses 100 and 200 mg/kg, *p.o.* and the standard drug Aminophylline (50 mg/kg, *i.p.*) were given. Ten compounds of benzopyrone class were synthesized and their docking studies were done to evaluate their binding on histamine (H₁) receptor. All the synthesized compounds at the dose 30 mg/kg, *p.o.* were tested for their anti-tussive effect using citric acid (7.5%) model in guinea pigs. On the basis of the results
of docking study and acute study of citric acid model, the most promising compounds were evaluated at 30 mg/kg, p.o. for their pharmacological effect on OVA induced asthma in Guinea pigs.

A successive solvent extraction of plant drug using solvents of graded polarities like pet. ether, chloroform, ethyl acetate, butanol were done. The fractions were prepared and TLC study was carried out. On the basis of TLC study, the ethyl acetate fraction was chromatographed in column to isolate phytoconstituents. The isolated phytoconstituents were further characterized using analytical tools like UV, IR, NMR etc. An in-vitro antioxidant assays of prepared fractions was also carried out. In-vitro antimicrobial assays of synthesized flavones were done.

**Results:** The yield of prepared pet. ether and hydroalcoholic extracts aerial parts of *O. bracteatum* were found to be 8.17% and 21.43% w/w respectively. The preliminary phytochemical screening of lyophilized hydroalcoholic extract mainly showed the presence of flavonoids, alkaloids, glycosides, and saponins. In present study, citric acid control animals showed significant decrease in latency time and increase in cough frequency as compared to normal untreated animals which depicted significant cough response in the form of sniffing and sneezing. However, on treatment with hydroalcoholic extract of *O. bracteatum* at doses 100 and 200 mg/kg produced reversible response as induced by the citric acid and evidenced as significant decrease in cough frequency and increase in latency time. The effect of hydroalcoholic extract may be due to the correction of activation of C fibres and A fibres. The pet. ether extract of *O. bracteatum* extract showed significant decrease in cough frequency and increase in latency time, and exhibited that the pet ether extract was less effective than hydroalcoholic extract. In ovalbumin model, administration of ovalbumin caused significant increase in cough frequency, decrease in latency time, increased cellular infiltration markers, TNF-alpha & interleukins level in BALF, oxidative stress markers like LPO & GSH in BALF & lung tissue. However, the hydroalcoholic extract of *O. bracteatum* at 100 and 200 mg/kg provided significant protection from asthmatic responses as evidenced by decreased cough frequency and increased latency time.

The four prepared fractions obtained from successive plant drug extraction showed significant anti-oxidant activity on DPPH and H2O2 scavenging assays. Treatment with ten flavones in separate group of animals showed significant prevention of coughing response against citric acid in Guinea pigs as evidenced by decreased cough frequency and increased
cough latency. The effect produced by 5c, 5f and 5j were found to be most significant. Hence three compounds were subjected for anti-asthmatic evaluation over ovalbumin induced asthma model in guinea pigs. The acute toxicity study of these compounds was tested at 1000 and 3000 mg/kg doses as per OECD guidelines and observed for any adverse or toxic symptoms. In chronic antigen induced asthma model, the compounds 5c, 5f and 5j produced significant amelioration of asthmatic symptoms as evidenced by decreased cough frequency and increased cough latency. These compounds also decreased the cellular infiltration markers, pro-inflammatory cytokines: TNF-alpha & interleukins in BALF and restored antioxidant level in BALF and lung tissue.

The plant extract and the synthesized flavones 5f at optimized doses showed significant relaxation on histamine induced contraction in tracheal chain preparation. Interestingly, the relaxation was found to increase with increment in dose, whilst, control (at different dose) were found to show contraction due to induction of histamine. This revealed that 5(f) considerably inhibited the contractile effect of histamine thus produces bronchodilation. Therefore, compound 5(f) was found active in all the experiments, supporting each other.

The antimicrobial activity was determined in terms of minimum inhibitory concentration (MIC) of the synthesized compounds 5(a-j). The activity was determined against the gram-positive Bacillus subtilis, Stapylococcus aureus, the gram-negative Escherichia coli, Salmonella typhi and the fungi Candida albicans. The obtained data revealed that compounds inhibited the growth of the selected microorganisms in vitro showing MIC values between 3.1 to 100 µg/ml. Interestingly, compound having halogen substitution were found to show better inhibition against C. albicans in comparison to other compounds. However, very few of the synthesized compounds were found inactive against specific strains.

Most employed and popular software was used in the present study in order to visualize and optimize the binding affinity of synthesized new molecules on specific drug targets. The significant result of anti-asthmatic and antimicrobial activity prompted is to investigate the docking of molecules on well-known targets. The active site residues of human histamine H1 receptor according to co-crystal structure with inhibitors; Trp 428(A), Phe 432(A), Asp 107(A), Tyr 108(A), Ser 111(A), Tyr 431(A), Trp 158(A), Tyr 458(A), Asn 198(A), Thr 112(A), Thr 194(A), Phe 424(A), Phe 435(A) were taken to define binding pocket within target protein, whereas, active site residues of glucosamine-6-phosphate synthase according
to co-crystal structure with inhibitors (Cys 300, Gly 301, Thr 302, Ser 303, Ser 347, Gln 348, Ser 349, Thr 352, Val 399, Ser 401, Ala 602 and Lys 603) were taken to define binding pocket within target protein.

Protein ligand analysis also showed strong interactions with target protein and had four hydrogen bond interaction with different residues (Tyr 108, Ser 111, Tyr 431 and Tyr 458) in H1 receptor site, whereas, six hydrogen bond interaction with different residues (Ser 303, Ser 347, Thr 352, Thr 355 and Glu 488) in active site of antimicrobial and antifungal activity.

**Conclusion:** On the basis of the findings from present investigation it may be proposed following conclusions:

*O. bracteatum* was able to provide pharmacological effects on acute and chronic stages of cough and asthma in Guinea pigs which may be mainly due to presence of semi-polar and polar constituents like flavonoid, phenolics, alkaloid and glycosides. The constituents are able to reduce hyper-responsiveness of bronchial cells, cellular infiltration, pro-inflammatory mediators and oxidative stress. This may provide rationale pharmacological basis for its age old use to treat respiratory and cough disorders.

The synthesized flavones: 5c, 5f and 5j showed significant docking to histamine (H1) receptor and produced significant prevention of acute and chronic states of hypersensitivity and bronchoconstriction in asthma. The iminoflavones 5(f) having methyl substitution at para position was found to be the most active one during pharmacological and *in-silico* studies.