6. SUMMARY

The genus *Barleria* of family Acanthaceae is a medicinally important plant of India and finds mention in ancient scriptures for various ailments like skin diseases, gout, rheumatism, pneumonia, inflammation and others. The genus is known to contain iridoids, anthraquinones, flavonoids, sterols and fatty acids. There is a continuing interest in iridoids as many of them have shown a host of biological and pharmacological activities. As *Barleria* is enriched in iridoids and reported to possess anti-inflammatory activity according to traditional claims, so the present work was planned to carry out the detailed studies on this plant covering phytochemical, chemical, analytical and biological aspects. Five species/varieties of *Barleria* were selected for the present work. In their natural habitat, *B. prionitis* is abundantly growing in plains; *B. cristata* var. *dichotoma*, *B. cristata* (Pink flower variety) and *B. cristata* (Blue flower variety) are found in plains as well as hills and *B. lupulina* in hilly areas.

The plant material was collected from the plants grown in Medicinal Plants Garden of University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh and the identity of all species/varieties was duly confirmed by National Institute of Science Communication and Information Resources (NISCAIR), New Delhi.

The present study had four parts: Chemical/qualitative, phytochemical, analytical and biological evaluation.

**Chemical/Qualitative studies:** The preliminary phytochemical screening was done using well known methods which showed the presence of flavonoids, sterols, triterpenoids, anthraquinones, iridoids and carbohydrates. The chemical profiling was done on different plant parts and extracts of the five selected species/varieties of *Barleria*. A large number of solvent systems were tried and many of them gave good resolution but only for specific extract/plant. Finally such solvent system was developed which gave optimally good resolution for all plant parts/extracts. This was a significant success in the present study as it made the comparative evaluation quick and easy. The TLC fingerprint profile exhibited few similarities and dissimilarities in the chemical profile of various species and varieties of
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Barleria as some components present in one specie were absent in other. B. prionitis, B. cristata var. dichotoma and B. lupulina showed similarities while B. cristata (Pink flower variety) and B. cristata (Blue flower variety) had different chemical profile. Further both pink and blue flower variety of B. cristata were found to be completely devoid of iridoid markers. This observation was also of advantage in correlating chemical profiling and biological results.

Phytochemical studies: The phytochemical studies were done on the whole plant material of B. prionitis as it had shown intense spots of the marker compounds and the plant is abundantly growing in and surrounding areas of Chandigarh. The three major compounds were isolated resorting to different chromatographic techniques viz., column chromatography, medium pressure liquid chromatography and preparative thin layer chromatography. The identity of the isolates was duly confirmed by the spectroscopic techniques as acetyl barlerin, barlerin and shanzhiside methyl ester.

Analytical studies: The quantitative analysis of three major shanzhiside iridoids, viz., acetyl barlerin, barlerin and shanzhiside methyl ester in different extracts and plant parts of Barleria was done by three well known analytical techniques - HPTLC, HPLC and UPLC. The methods were developed for all the three selected techniques and validated as per ICH guidelines. The content of the three selected iridoids was estimated using these techniques and further a comparison of the three techniques was made w.r.t. each marker content. The calculated per cent content of acetyl barlerin ranged between 0.16 to 3.82 % by HPTLC, 0.004 to 7.30 % by HPLC and 0.05 to 2.65 % by UPLC; barlerin content varied between 0.02 to 0.97 % by HPTLC, 0.04 to 6.05 % by HPLC and 0.02 to 2.53 % by UPLC and shanzhiside methyl ester was in range of 0.11 to 3.92 % by HPTLC, 0.17 to 4.27 % by HPLC and 0.10 to 2.78 % by UPLC. A critical analysis of the results obtained by three techniques showed that HPTLC gives more reproducible results if simultaneous analysis of acetyl barlerin, barlerin and shanzhiside methyl ester is desired. HPLC and UPLC techniques were found to have limitation for simultaneous determination of acetyl barlerin, barlerin and shanzhiside methyl ester.
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ester. However, the latter two techniques being highly sensitive gives more accurate results if individual quantification of the iridoids is desired.

**Biological studies:** In this part of the study, the toxicological and biological evaluation was carried out. Acute toxicity was performed as per the guidelines issued by OECD (2008) for acute toxicity studies. The median lethal dose of methanol extract of *B. prionitis* after *per oral* administration in rats was found to be greater than 2000 mg/kg. Therefore, the three dose levels (100, 200 and 400 mg/kg) for various extracts were selected to carry out various biological activities. The two most commonly and abundantly available species of *Barleria* viz., *B. prionitis* and *B. cristata* var. dichotoma were taken up to generate the biological profile. The biological studies were not done on *B. lupulina* (as its chemical profile was similar to *B. prionitis*), *B. cristata* var. pink flower and *B. cristata* var. blue flower (as they were devoid of the iridoid markers). The pharmacological investigations done in the present study were: anti-inflammatory, analgesic, antiulcer and antiarthritic activity. The anti-inflammatory studies were carried out using different inflammogens in acute (carrageenan, histamine and dextran), sub-acute (cotton pellet) and topical (croton oil) models of inflammation. Also, the estimation of NO was done in sub-acute model of inflammation in the active extracts of *B. prionitis* and the isolated shanzhiside markers. Further, anti-inflammatory evaluation involved using different standard drugs viz., ibuprofen, celecoxib, dexamethasone and nimesulide. The selection of standard drugs was done keeping in mind different mechanisms of actions exhibited by these drugs to know the probable mechanism of anti-inflammatory action of *Barleria* iridoids. The anti-inflammatory evaluation using different inflammogens and different standard drugs was done with a view -

(i) to confirm the anti-inflammatory potential of *Barleria*

(ii) to confirm the anti-inflammatory potential of pure compounds i.e. acetyl barlerin, barlerin and shanzhiside methyl ester isolated from *Barleria*

(iii) to have a view of the inflammatory disorders against which *Barleria* pure compounds are expected to be effective

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(iv) to assess the mechanism of anti-inflammatory action of Barleria and its pure compounds

To carry out the planned anti-inflammatory study, different extracts of B. prionitis and B. cristata were prepared and evaluated at the above mentioned doses. The isolated pure compounds were also subjected to various inflammation models. A 1 mg/kg was selected as the defined dose of pure compounds for all the activities as it showed maximum activity profile in preliminary anti-inflammatory screening. A significant protection was observed in all the tested models of inflammation at different time intervals and dose levels. Of all the extracts of the two species, the best activity was demonstrated by methanol extract of B. prionitis in different models except for topical model where chloroform extract showed the maximum effect at respective dose levels. Although both the species showed good activity profile but the overall therapeutic efficacy of B. prionitis was found to be more than B. cristata. The analgesic activity was also carried out with the same extracts of the two species of Barleria and on the isolated pure compounds using central and peripheral pain models. The active extracts and pure compounds were further investigated for antiulcer effect using pyloric ligation induced gastric ulcer model. The extracts as well as pure iridoids also showed mild to moderate analgesic activity with good gastric protection.

The antiarthritic potential of the active extracts and pure compounds was evaluated in adjuvant induced arthritis model at a selected single dose of 200 mg/kg and 1 mg/kg, respectively. From the results, it was observed that iridoid enriched methanol and butanol extracts and pure iridoids not only directs towards the control of arthritis progression and/or the inflammation associated with it, but also prevents bone destruction of the arthritic joints of adjuvant induced arthritis rats. The serum levels of TNF-α and IL-1β were also significantly decreased by the extracts/pure compounds indicating their potential role in lowering various cytokines which are released during arthritis.

Further, the biological activity has been correlated to the generated TLC fingerprint profiles. The extracts showing intense spots of three iridoid markers showed more
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activity as compared to extracts having no or less intense spots. This was further proved when these isolated markers were tested individually. The mechanism of action of anti-inflammation, analgesic, antiulcer and antiarthritic activity has also been proposed based on the above findings.

Hence, the present study justifies the traditional claims of the plant mentioned in Ayurveda. It clearly indicates that *Barleria* is a potent anti-inflammatory plant with good gastric protection. It does not cause gastric disorders, so the problem of change in pH or ulcer formation is not there. Further, the analgesic studies shows that plant possess mild to moderate analgesic activity and has promising potential to cure arthritis. This clearly indicates that *Barleria* and its pure compounds can be safely used in chronic disorders like arthritis, psoriasis etc where pain is often associated with the inflammatory condition of the disease. In addition to the significant activities shown by the plant extracts, the three isolated pure iridoids *viz.*, acetyl barlerin, barlerin and shanzhiside methyl ester have also exhibited significant and promising anti-inflammatory, antiulcer, antiarthritic and mild to moderate analgesic activity. The biological potential shown by pure compounds as good anti-inflammatory and antiarthritic compounds with added advantage of mild to moderate analgesic activity and no gastric side effects makes these iridoids as potential lead molecules for the development of safer drugs especially for chronic inflammatory diseases.

Thus from a critical analysis of the investigations done in the present study, it is clear that all species/varieties of *Barleria* do not contain iridoids and hence it is the selected species/varieties only which holds potential to be used in formulations by herbal industry. The selection of species, plant part and iridoid enriched extracts for the isolation of different iridoids has also been clearly demonstrated. The significant results further indicate that *Barleria* holds promise to be taken up in drug discovery programmes especially to treat chronic inflammatory disorders.