6. CONCLUSION

In the current study two medicinal plants namely *Artemisia amygdalina* Decne and *Gentiana kurroo* Royle belonging to two different families were worked out for determining their anti-inflammatory potential. The study was carried out using whole plant material extracts from both plants. The whole plant material was successively extracted with petroleum ether, ethyl acetate, methanol and water in the order of increasing polarity of the solvents respectively. The study on inflammation involved two approaches: (1) *In vivo* studies involving – (i) carrageenin induced paw edema and adjuvant induced arthritis for the study of acute and chronic inflammation and, (ii) haemagglutination titre and delayed type hypersensitivity methods for demonstrating the potential against humoral and cell mediated immunity respectively. (2) *In vitro* studies using (i) mice peritoneal macrophages treated with LPS for investigation of pro-inflammatory mediators like NO, TNF-α, IL-6 and NFκB and (ii) splenocytes treated with LPS and Con-A for evaluating the B-cell and T-cell proliferation respectively.

From the present *in vivo* and *in vitro* studies of *G. kurroo* and *A. amygdalina* following conclusion can be arrived at:

- All these extracts possessed the potential to decrease the acute inflammatory response with methanolic extract from both plants having the maximum potential. This was evident from the decrease in edema produced in carrageenin model by these extracts.

- The primary and secondary humoral immune response was suppressed in the extract treated animals with the effect being higher in the latter. Thus all these extracts had the inhibitory effect on the humoral immunity. Methanolic extract from both plants again being most potent among them.
The response in cellular immunity after 24h and 48h was less in treated as compared to the untreated animals with the effect being higher in the latter. So, both the plants had negative effect on the cellular immune response as well with maximum effect shown by their methanolic extract.

The edema formation was less with increase in the methanolic doses in carrageenin induced inflammation. Also the suppression in humoral and cellular immune responses was high with higher doses of methanolic extracts. Thus the methanolic extracts from both the plants showed dose dependent effect against the acute inflammation and immune response.

The serum TNF-α level in Balb/C mice treated with methanolic extracts from both plants was less as compared to untreated control mice. Since the methanolic extracts suppress the immune response, so they might inhibit the production of TNF-α from immune cells in Balb/C mice.

The methanolic extracts from both the plants demonstrated a decrease in the arthritic symptoms in mycobacterium induced adjuvant arthritis. These extracts at higher doses showed higher anti-arthritic potential thus suggesting their role in alleviating the arthritic symptoms.

In case of in vitro study, the levels of NO, TNF-α and IL-6 was less in the supernatants collected from macrophage culture treated with different concentrations of methanolic extracts as compared with control group. Thus, production of these pro-inflammatory mediators is inhibited in presence of methanolic extracts.

The production of NFkB in the cultured macrophages was less in presence of the methanolic extracts.

The B-cell and T-cell proliferation got inhibited in presence of the methanolic extracts. So, these extracts had an anti-proliferative effect on the B and T-cells.
The above observations were recorded in both *A. amygdalina* and *G. kurroo* methanolic extracts. But the effects shown by *G. kurroo* methanolic extract were higher as compared to the *A. amygdalina* methanolic extract. LC-MS of methanolic extracts of both plants was carried to identify the principle active compounds in them. LC-MS analysis of *Artemisia amygdalina* showed the presence of compounds from terpenes and flavones in it, while the LC-MS analysis of *Gentiana kurroo* showed presence of glycosides and flavonoids.

Our research results demonstrate that methanolic extracts of *A. amygdalina* and *G. kurroo* possess anti-inflammatory activity. They suppressed humoral and cell mediated immune response by inhibiting the B and T-cell proliferation. They also inhibited the production of NO and attenuated the induction of pro-inflammatory cytokines (TNF-α, and IL-6) by LPS. More importantly, the methanolic extracts of these two plants exert anti-inflammatory effects *in vitro*, which results from the inhibition of NF-κB activation in macrophages, thereby, inhibiting the production of iNOS and pro-inflammatory cytokines. Thus, present findings showing the inhibition of inflammatory response both *in vivo* and *in vitro* increase our understanding of the novel pharmacologic aspects of these plants and suggest their potential as a novel therapeutic candidate for managing inflammatory disorders. Further, detailed work is to be carried out for the isolation of bioactive compounds from these extracts.