CHAPTER: 7 SUMMARY AND CONCLUSION

Rheumatoid arthritis is the most common form of chronic and systemic inflammatory disease of unknown etiology marked by synovial hyperplasia with local invasion of bone and cartilage leading to joint destruction. Henceforth, this opens for the big challenge for researchers to carry out the research based on treatment and pathophysiology of the disease. This study was designed to find out and prove the claims put forth by the traditional and folk medicine of applicability of *C. zedoaria* root that have been used in crippling arthritis and frozen joints in siddha and ayurveda system of medicine.

The aim of the present study was to investigate the phytochemical presence in petroleum ether, chloroform, methanol, ethanol and aqueous extracts of *C. zedoaria* roots and evaluation of potential anti-arthritic and acute anti-inflammatory effect of *C. zedoaria* extracts and its formulations obtained from potent extracts of *C. zedoaria* (SHF-A, SHF-B, SHF-C and SHF-D) in FCA-induced arthritis in rats and carrageenan and histamine induced-acute inflammation in rat paw edema. In addition the isolation, purification and characterization of active constituents of the highly potent extract was carried out by TLC, column chromatography, preparative TLC, HPTLC, HPLC, UV, IR, $^1$HNMR, and LCMS.

Preliminary phytochemical data revealed that petroleum ether, chloroform and ethanol extracts of *C. zedoaria* showed the presence of steroids, terpenoids, glycosides, alkaloids, tannins and other phenolic compounds (curcuminoid).

Acute toxicity studies showed that non-toxic nature of each extract of *C. zedoaria*. There was no lethal or toxic reaction observed at 2000 mg/kg. Hence, 1/10th (200mg/kg.) and 1/5th (400 mg/kg.) of the lethal dose taken as effective dose (therapeutic dose) were selected for further study.
Intra-articular injection of FCA-induced arthritic model was suitable for long testing anti-arthritic activity of *C. zedoaria* root extracts. The petroleum ether, chloroform and ethanol extracts at 200 and 400 mg/kg showed anti-arthritic activity and acute anti-inflammatory activities. In the present study, the FCA-induced chronic inflammation in joints of control group is manifested as a progressive increase in paw edema. It is noteworthy that the inhibitory effect of petroleum ether, chloroform, and ethanol extracts of *C. zedoaria* roots in rat paw edema were observed from third day to last day of study and recovery in health status such as body weight, ESR, Hb, RBC and WBC. Moreover, reduction was seen in the nitric oxide level and vascular permeability. In addition, protective effects were observed in rat joints. The result of behavior studies confirms its ability to overcome stress, anxiety, and abnormality in mobility. However, methanol and aqueous 200 and 400 mg/kg treated groups have been failed in these aspects. On the basis of biochemical data it was observed that no toxic effect was found in any of the *C. zedoaria* extract. The organ to body weight ratio showed that improvement in the change in organ weight in petroleum ether, chloroform and ethanol groups.

Petroleum ether, chloroform and ethanol extracts at both doses of *C. zedoaria* showed significant reduction in the paw edema from 2nd hours to 6th hours of the study. However methanol and aqueous extracts showed no significant reduction in paw edema in rats. Hence Petroleum ether, chloroform and ethanol extracts showed potent acute anti-inflammatory and anti-arthritic effects.

Amongst these three extracts, the petroleum ether extract showed the highest effect on acute anti-inflammatory activity. So, the further phytochemical study of petroleum ether extract of *C. zedoaria* was carried out.
On the basis of preliminary phytochemical data, petroleum ether extract showed the presence of steroids, terpenoids, and phenolic compounds (curcuminoids: curcumin, bismethoxycurcuminoid and dismethoxycurcuminoid). Literature survey revealed that these active constituents steroid, terpenoids and curcuminoid responsible for anti-arthritic and acute anti-inflammatory activities.

In the present study the crude curcuminoid was identified and separated from the petroleum ether extract of *C. zedoaria* by the comparison with herbal standard curcuminoid using preparative thin layer chromatography technique. The separation of curcumin from crude curcuminoid which was obtained from petroleum ether extract of *C. zedoaria* by using column chromatography and identified by TLC, HPLC and HPTLC techniques. Separated fractions were collected and recrystallized. Fractions were subjected to spectroscopic analysis like UV, IR, LCMS, and $^{1}$HNMR. All spectral data’s indicates that the separated compound was curcumin.

The single herb formulation from potent extracts obtained from *C. zedoaria* root was developed. Pharmacological evaluation of anti-arthritic and acute anti-inflammatory activity of *C. zedoaria* extracts showed that petroleum ether, chloroform, and ethanol root extracts have potent anti-arthritic and anti-inflammatory of *C. Zedoaria*. Using these three potent extracts by mixing with additives in a standard ratio, four formulations prepared in a suspension form and named as SHF-A, SHF-B SHF-C and SHF-D. All formulations were subjected to acute toxicity study. Acute toxicity studies of formulations showed no sign of toxicity at the dose of 2000 mg/kg.p.o. Hence 1/10th of the lethal dose was selected to each formulation for anti-arthritic and acute anti-inflammatory activities.

Evaluation of anti-arthritic and acute inflammatory activities of formulations SHF-A, SHF-B SHF-C and SHF-D were carried on female Wistar rats by using FCA induced arthritis and carrageenan and histamine induced acute inflammation.
Formulations SHF-A, SHF-C and SHF-D at 200 mg/kg showed anti-arthritic activity and acute anti-inflammatory activity. In the present study, SHF-A, SHF-C and SHF-D at 200 mg/kg showed significant reduction in the FCA-induced paw edema in rats from third day to last day of study compared with control group paw edema. Recoveries were observed in health status such as body weight, ESR, Hb, RBC and WBC. Moreover, reduction in nitric oxide level and vascular permeability was observed. In addition, protective effects were observed in rats joint and behavior studies which confirm its ability to overcome stress, anxiety and abnormality in mobility. However, SHF-B treated groups showed non-significant results in these aspects. Biochemical and organ to body weight ratio studies indicates that no toxic effects were observed in any formulations but SHF-A showed highly potent anti-arthritic activity.

Formulations SHF-A, SHF-C and SHF-D at 200 mg/kg dose of C. zedoaria showed significant reduction in the paw edema from 2nd hour to 6th hour of the study. However SHF-B showed non-significant effect in the reduction of paw edema in rats. SHF-A, SHF-C and SHF-D showed acute anti-inflammatory activity.

In conclusions, it can be stated that the petroleum ether, chloroform and ethanol extracts of C. zedoaria roots and its formulations SHF-A, SHF-C and SHF-D have beneficial effects in long lasting reduction in rat paw edema, recovery in hematological changes, inhibitory effects on nitric oxide synthesis and vascular permeability. It also showed a protective effect on arthritic rat joints without any toxic effect. The mechanism may be mediated via the inhibition of prostaglandin synthesis as well as central inhibitory mechanism, this, justifying the claim made by Siddha and Ayurveda classics.