9. SUMMARY AND CONCLUSION:

Andrographis paniculata Nees (AP) is a traditionally used medicinal plant in Korea, Thailand, China, Japan, South Africa, India, Pakistan and Sri Lanka for the treatment of fever, cold, inflammation, diarrhea and other infectious diseases. Since 175 BC, AP along with other plants in polyherbal preparations was recommended in Charak Samhita for treatment of jaundice. Indian pharmacopoeia mentioned that AP is a predominant constituent of at least 26 Ayurvedic formulations. Andrographolide one of the major active component of the plant AP has been extensively used in traditional herbal medicine in China, Southeast Asia, and the Arabian Gulf for the treatment of several diseases, including inflammatory diseases. Traditional use of AP as anti-inflammatory herb has been studied by various scientists and proved the mechanism. Various reports suggest that inflammatory responses are a critically a part of pathophysiology of several diseases including septic shock, cancer, atherosclerosis, rheumatoid arthritis and diabetes. So the manipulation of inflammatory responses may allow for prevention of serious diseases or symptoms.

The extracts of AP and its isolated compounds are also reported to have various pharmacological activities, including hepatoprotective, antidiabetic inhibition of replication of the HIV virus, antimalarial and principally anti-inflammatory properties. Andrographolide, one of the active constituent of AP has been reported to have anti arthritic effect. Production of pro-inflammatory mediators, such as COX-2, iNOS and cytokines has been reduced by AN. In clinical trials, Andrographis paniculata extract (30% AN) showed effectiveness for symptom relief and reduce serological parameters in patients with Rheumatoid Arthritis.

Several polyherbal formulations consisting AP as a major ingredient as anti-inflammatory and anti-arthritic are available in local Indian markets. In treatment of arthritis it is common practice that, along with the disease–modifying antirhumatic drugs (DMARDs) and nonsteroidal anti-inflammatory drugs (NSAIDs), herbal formulations are either taken with or without knowledge of health care provider by the patients for better therapeutic effects. This may lead to either beneficial or toxic effects. An increasing consumption of medicinal herbs, which are often administered in combination with conventional therapeutic drugs, it is likely that constituents in herbal preparations may be substrates, inhibitors, or inducers of cytochrome P450 enzymes (CYPs) and have an impact on the pharmacokinetics and pharmacodynamics of any co-administered drugs metabolized by this system. Many studies have been reported for interaction
between *Andrographis paniculata Nees* extract and AN with various synthetic drugs. Though CYP1A2 is the common CYP involved in the metabolism of AN, APE, ETO, NAB and NP in rats, unfortunately not a single attempt has been done to investigate interaction of APE and it’s one of the major constituent AN with ETO, NAB and NP after oral administration in rats. Previously various bioanalytical methods are reported in literature for the determination of AN alone and in combination with other drugs. Seldom bioanalytical methods were previously reported in literature for the determination of ETO, NAB and NP in rats and humans. However, there was no analytical method is available for simultaneous estimation of AN and ETO/NAB/NP. Prompted by the above findings, this study developed new validated HPLC methods for simultaneous determination of AN with ETO/NAB/NP in rat plasma and application of the developed methods for pharmacokinetic study in rats. In the present study we investigated the possible herb-drug interactions of APE and AN with ETO, NAB and NP through comparing their pharmacokinetic profiles and pharmacodynamic after oral administration in rats.

For ETO, a HPLC method with mobile phase consisting of the mixture of solvent A (Acetonitrile) and solvent B (Water) in the ratio of 55:45 (v/v) for 35 minutes at a flow rate of 0.5 mL/min and detection using UV detector at a wavelength of 225 nm for simultaneous determination of AN, ETO and IS was developed and validated as per USFDA guidelines. The developed HPLC method was applied for pharmacokinetic study in rats. After oral co-administration of APE (200 mg/Kg), AN (60 mg/kg) with ETO (10mg/kg) in rats respectively, drug concentrations in plasma were determined using HPLC method. The main pharmacokinetic parameters of Cmax, tmax, t1/2, MRT, Vd, CL, and AUC were calculated by non-compartment model. To evaluate further possible herb-drug interaction anti-arthritic study has been performed in the groups with co-administration of APE and AN with ETO respectively. Change in paw volume, mechanical nociceptive threshold, mechanical hyperalgesia, histopathology and hematological parameters were evaluated to study antiarthritic activity.

Decrease in tmax of AN in APE and pure AN on co-administration with ETO was observed. No change in tmax of ETO on co-administration with pure AN was observed whereas tmax of ETO on co-administration with APE was increased to 6 h. The Cmax of AN and ETO were decreased significantly after co-administration of AN and APE with ETO than AN, APE and ETO individually administered groups. Co-administration of pure AN with ETO significantly (P < 0.05) decreased the AUClast, AUC∞, AUMClast, AUMC∞, t1/2, MRTlast and MRT∞ of AN and
ETO when compared to AN and ETO alone groups. There was significant increase in CL of ETO and AN, significant decrease in Vd of AN while increase in Vd of ETO was observed in co-administered group when compared to respective alone groups. In anti-arthritic study, from increase in change in paw volume and decrease in mechanical nociceptive threshold and mechanical hyperalgesia, it was observed that ETO treated group showed better activity than APE+ETO treated group. The results of histopathological and hematological study supports the findings and revealed the dose dependent effects of AN and APE.

For NAB, a HPLC method with mobile phase of 0.01N acetic acid (Solvent A) and acetonitrile (Solvent B) with gradient as follows: 0 to 9 min 70% A and 30% B, from 10 to 35 min 30% A and 70% B at a flow rate of 0.5 mL/min and detection using UV detector at a wavelength of 225 nm was developed and validated as per USFDA guidelines. Pharmacokinetic study in rats was carried out using the developed HPLC method. After oral co-administration of APE (200 mg/Kg), AN (60 mg/kg) with NAB (7.5 mg/kg) in rats respectively, drug concentrations in plasma were determined using HPLC method. The main pharmacokinetic parameters of Cmax, tmax, t1/2, MRT, Vd, CL, and AUC were calculated by non-compartment model. To evaluate further possible herb-drug interaction anti-arthritic study has been performed in the groups with co-administration of APE and AN with NAB respectively. Change in paw volume, mechanical nociceptive threshold, mechanical hyperalgesia, histopathology and hematological parameters were evaluated to study antiarthritic activity.

As per previously published studies on pharmacokinetics of NAB, its active metabolite-6-MNA was detected intact in plasma instead of the parent compound NAB. 6-MNA was absorbed into the circulatory system and reached its peak concentration approximately 1.5 h after administered individually. T_max of group co-administered AN and NAB has been changed to 2 hr but T_max of group co-administered APE and NAB was the same. The C_max of 6-MNA was decreased significantly after co-administration of AN and APE with NAB compared to group administered with NAB alone. Co-administration of AN with NAB significantly (P < 0.05) decreased the C_max, AUC0-t, AUC0-∞, T1/2, MRT0-t, MRT0-∞, and Vd of 6-MNA whereas increased T_max and CL of 6-MNA in comparison with group treated with NAB alone. Co-administration of APE with NAB significantly (P < 0.05) decreased the C_max, AUC0-t, and AUC0-∞ of 6-MNA whereas increased the T1/2, MRT0-t, MRT0-∞, Vd and CL of 6-MNA when compared to group
treated with NAB alone. From anti-arthritic study, it was observed that NAB treated group showed better activity than all other groups. Increase in change in paw volume, decrease in mechanical nociceptive threshold and mechanical hyperalgesia, absence of infiltration of inflammatory cells and fibrous tissue in histopathology and increased levels of Hb, and RBC with concomitant decrease in ESR, WBC and platelet count in hematological study of NAB (7.5 mg/kg) support the findings. Further, even though the dose of AN is equal in both groups treated with standardized APE+NAB and AN+NAB, there was better activity observed with APE+NAB treated group.

For NP, a HPLC method with mobile phase consisting of the mixture of solvent A (Acetonitrile), solvent B (MeOH) and solvent C (0.01 M Acetic acid) in the ratio of 20:30:50 (v/v/v) for 25 minutes at a flow rate of 0.5 mL/min and detection using UV detector at a wavelength of 225 nm was developed and validated as per USFDA guidelines. After oral co-administration of APE (200 mg/Kg), AN (60 mg/kg) with NP (7.5 mg/kg) in rats respectively, drug concentrations in plasma were determined using HPLC method. The main pharmacokinetic parameters of Cmax, tmax, t1/2, MRT, Vd, CL, and AUC were calculated by non-compartment model. To evaluate further possible herb-drug interaction anti-arthritic study has been performed in the groups with co-administration of APE and AN with NP respectively. Change in paw volume, mechanical nociceptive threshold, mechanical hyperalgesia, histopathology and hematological parameters were evaluated to study antiarthritic activity.

NP was absorbed into the circulatory system and reached its peak concentration approximately 2 h after administered individually. T\text{max} of groups co-administered AN+NP and APE+NP has been changed to 1.5 hr and 1 h respectively. The C\text{max} of NP was decreased significantly with the groups administered with AN + NP and APE + NP than the group administered NP individually. Co-administration of AN with NP significantly (P < 0.05) decreased the C\text{max}, T\text{max}, AUC\text{0-4}, T\text{1/2} MRT\text{0-4}, CL and increased the AUC\text{0-}\infty, MRT\text{0-}\infty and Vd of NP when compared to NP alone group. Co-administration of APE with NP significantly (P < 0.001) decreased the C\text{max}, T\text{max}, AUC\text{0-4}, AUC\text{0-}\infty and increased the MRT\text{0-4}, MRT\text{0-}\infty, CL and Vd of NP when compared to NP alone group. In anti-arthritic study from increase in change in paw volume, decrease in mechanical nociceptive threshold and mechanical hyperalgesia, it was observed that NP treated group showed better activity than NP+AN and NP+APE treated groups.
Summary and conclusion

The minimum infiltration of inflammatory cells and fibrous tissue in histopathology and increased levels of Hb, and RBC with concomitant decrease in ESR, WBC and platelet count in the hematological study of NP+AN (10+60 mg/kg) supports the findings.

In conclusion, the results obtained from this study suggested that ETO, NAB and NP with APE and pure AN existed pharmacokinetic herb-drug interactions in rat which is correlated with anti-arthritic study. Significant decrease in Cmax, AUC, t1/2 and increase in Vd, CL of ETO was observed after co-administration with pure AN and APE. Co-administration of ETO with APE and pure AN decreased systemic exposure level of each compound in vivo. In anti arthritic studies, even though the dose of AN is equal in both groups treated with APE and AN there was better activity observed with APE treated group.

Significant decrease in Cmax, AUC0-t and AUC0-∞ of 6-MNA was observed after co-administration with pure AN and APE. Increase in elimination of 6-MNA was observed with reduced T1/2 when co-administered with AN. Increase in CL and decrease in Vd of AN+NAB treated group indicated the increase in elimination. Decrease in MRT support the findings. Whereas T1/2 of 6-MNA increases on co-administration with APE suggesting decrease in elimination. Co-administration of NAB with APE and pure AN decreased systemic exposure level of NAB in vivo. The decrease in Cmax and AUC of 6-MNA is more with AN+NAB treated group compared to APE+NAB treated group. Increase in MRT, Cl and Vd of 6-MNA in APE+NAB treated group reveals that other components of APE other than AN might be interfering in metabolism of NAB. In anti arthritic studies, even though the dose of AN is equal in both groups treated with standardized APE+NAB and AN+NAB there was better activity observed with APE+NAB treated group.

Significant decrease in Cmax, tmax, AUC0-t, of NP was observed after co-administration with pure AN and APE. Co-administration of NP with APE and pure AN decreased systemic exposure level of NP in vivo. Fast elimination of NP was observed with decreased T1/2 when co-administered with AN. But decrease in CL and MRT0-t and increase in Vd and MRT0-∞ indicate changes in elimination pattern of NP on co-administration with AN. Whereas T1/2 of NP remained same when co-administered with APE and increase in CL, Vd, MRT0-t and MRT0-∞ has been observed. Co-administration of NP with AN and APE decreased the systemic exposure level of NP in vivo with decreasing Cmax and AUC0-t but this decrease is significantly more with AN+NP treated group compared to APE+NP treated group. In anti arthritic studies, even though
the dose of AN was equal in both groups treated with standardized APE and AN there was better activity observed with AN+NAB treated group.

The study observed that co-administration of AN and APE changes pharmacokinetics and pharmacodynamics of ETO, NAB and NP. Further studies should be done to understand the effect of other herbal ingredients of APE on ETO/NAB/NP as well as to predict the herb-drug interaction in humans. Physicians and patients using A. paniculata should have the knowledge about its possible herb–drug interaction with ETO, NAB and NP. The study provided valuable information for rational use of herbal remedies in the treatment of arthritis.