SUMMARY

Brief account of evolution of therapeutic practice based on medicinal plant preparations, brief historical background of Ayurveda and importance of continued research on medicinal plants for evolving drugs for some of the diseases for which pharmacotherapy is not available have been provided in the introduction.

Strobilanthes heyneanus is one of the extensively used plants in Kerala and is equated with the Sahachara of classics. Review of literature revealed that inspite of extensive use of medicinal preparations prepared from this plant no data on its pharmacological activity profile and phytochemical composition are available. This prompted the author to initiate preliminary screening on leaf, stem and root extracts. Based on the results of this screening stem extracts were selected for further studies.

Extracts were prepared from pharmacognostically identified plant material with serial extraction in a soxhlet apparatus with petroleumether, - 60-80 °C, chloroform, methanol and distilled water. Aqueous and methanol extracts (ME) were subjected to detailed pharmacological investigations.

As a first step pharmacognostic features of the material selected for the study were defined since many Strobilanthes species resemble each other quite closely. This was followed by preliminary phytochemical screening to obtain details about the phytochemical composition of the plant material. The extracts were screened in a series of pharmacological tests to define their activity profile. In the next phase, since the extracts showed significant anti-inflammatory activity and inflammatory disorders being the important therapeutic indication for the plant, detailed investigations were undertaken employing both biochemical and pharmacological techniques to elucidate the probable mechanism of anti-inflammatory activity noted with them. Propensity to cause damage to the gastric mucosa is one of the main limiting factors for the prolonged use of majority of the nonsteroidal anti-inflammatory agents (NSAIDs). Hence, it has become a normal convention with majority of the investigators to assess the ulcerogenic potential of any new anti-inflammatory agent. The extracts were evaluated for both anti-ulcer and ulcerogenic properties in pylorus ligated rats. The entire study was undertaken in four phases within the above frame work.

Part I

Preliminary phytochemical screening and pharmacognostical study on Strobilanthes heyneanus stem

Pharmacognostical study revealed that the presence of cystolith in the cortex and pith, a distinct endodermis, thick walled medullary ray cells, sclers in the pith and scattered acicular crystals of calcium oxalate to be the chief diagnostic features of Strobilanthes heyneanus stem.

Preliminary phytochemical screening

Phytochemical screening of aqueous (AQE) and methanol extracts (ME) revealed presence of sugars, alkaloids and phenolic compounds in both the extracts. Presence of terpenoid in AQE
and flavonoid in ME was also noted. ME after fractionation through column chromatography and preliminary TLC gave a single spot on reacting with Dragendorff's reagent.

Part II

General pharmacological screening and acute toxicity studies on aqueous (AQE) and methanol extracts (ME).

From acute toxicity studies the LD50 with 95% confidential limits were found be:

AQE: 1000.00 (667.67 - 1500.00) mg kg\(^{-1}\) (ip) in mice. 920.00 (634.48 - 1334.00) mg kg\(^{-1}\) (ip) in rats.

ME: No mortality could be observed up to 4000 mg kg\(^{-1}\) (ip) in both rats and mice.

The effect of AQE and ME was studied on gross behaviour, spontaneous motor activity (SMA), forced motor activity (FMA) and evaluated for antipsychotic, antidepressant, antiparkinsonian, anticonvulsant, analgesic, anti-inflammatory, hepatoprotective, adaptogenic, immunomodulatory, antivenom, antimicrobial, diuretic and antifertility. They were screened for hypoglycaemic, hypolipidaemic activity by noting their effect on serum glucose, total cholesterol, HDL-cholesterol and triglyceride levels. In addition, the effect of extracts on rectal temperature in rats, blood pressure in anaesthetized dogs, isolated frog heart, guinea pig ileum and tracheal spiral, rat uterus and rabbit jejunum was also studied.

AQE and ME did not affect gross behaviour, SMA and FMA. ME prolonged duration of pentobarbitone (PBN) sleep in mice and decreased rectal temperature in rats. The activity profile of CNS suggests that the extracts are devoid of CNS depressant effect. Though, ME prolonged PBN sleep, it does not seem to represent CNS depression since it did not affect other parameters which are supposed to have predictive value for sedative activity. The PBN sleep prolongation may be due to augmentation of hypothermia. The extracts suppressed acetic acid induced writhing without affecting the pain threshold for radiant heat stimuli indicating presence of peripheral analgesic activity. Both extracts produced significant anti-inflammatory effect in carrageenin hind paw oedema test. Significant antipyretic activity against peptone induced pyrexia in rats was also noted. The extracts produced significant suppression of antibody formation against SRBC in mice. Unlike the significant diuretic activity reported with other Strobilanthes species, significant fluid retention effect was observed in hydrated rats with AQE. It also produced hypoglycaemia in rats. The extracts did not produce any significant activity in other test models studied.

No significant effect could be observed on blood pressure in anaesthetized dog and isolated frog heart. The extracts did not produce any effect per se on guinea pig ileum and tracheal spiral and also did not modify responses to various spasmogens studied. ME without affecting spontaneous rhythmicity produced moderate antagonism towards isoprenaline induced relaxation in rabbit jejunum. It also produced significant antagonism of both oxytocin and PGF2a induced contractions in rat uterus. AQE produced potentiation of PGF2a induced contraction in rat uterus.
Presence of significant peripheral analgesic and anti-inflammatory activity provides pharmacological basis to the use of medicinal preparations of Strobilanthes heyneanus in inflammatory disorders. Presence of antipyretic activity justifies their therapeutic use for reducing fever. However, the study could not provide pharmacological basis to the therapeutic applications of medicinal preparations of the plant as adaptogen and antimicrobial agents.

The results of the present study indicate that stem extracts possess analgesic, antipyretic, anti-inflammatory and immunosuppressant activities. AQE possess hypoglycaemic activity in rats and ME antispasmodic effect on rat uterus. AQE was found to be more toxic in comparison to ME.

PART III

Studies on the anti-inflammatory activity of the stem extracts

since the medicinal preparations of the plant are extensively used for treating inflammatory disorders and anti-inflammatory activity was observed during preliminary screening, it was thought worthwhile to elucidate the mechanism of action of the anti-inflammatory activity of the extracts. This was done by employing both pharmacological and biochemical techniques.

The extracts were evaluated in different models of experimental inflammation selected as representative of different phases of inflammation. Besides, the effect of extracts was also studied on certain biochemical parameters supposed to be altered during inflammation.

Both AQE and ME significantly suppressed carrageenin induced rat paw oedema in a dose dependent manner. The ED50 was calculated to be $112.20 \pm 130$ mgkg$^{-1}$ for AQE and $281.18 \pm 12.53$ mgkg$^{-1}$ for ME. The extracts were more effective in suppressing the later phase of carrageenin oedema formation than early phase. The extracts did not suppress 5-hydroxytryptamine induced rat paw oedema. AQE at higher dose level decreased histamine induced rat paw oedema at 4h after phlogistic injection. However, both the extracts significantly suppressed bradykinin and PGE2 induced oedema. The results indicate that the extract may be producing oedema suppression through modulation of release or activity of the mediators in the second phase of oedema formation.

Effect of extracts on vascular permeability was studied employing different models. AQE and ME did not decrease Evans blue extravasation into carrageenin injected rat paw significantly. However, they significantly inhibited fluid exudation in carrageenin induced pleurisy and PVC cup implant in rats. The extracts also inhibited leukocyte emigration into PVC cup implant.

Effect of extracts on proliferative phase of inflammation was studied by noting their effect on formaldehyde induced oedema, cotton pellet granuloma and carrageenin induced granuloma pouch in rats. The extracts produced significant suppression of formaldehyde oedema and granulation tissue formation in both cotton pellet implanted and carrageenin pouch bearing rats. In carrageenin granuloma pouch rats the extracts suppressed fluid accumulation also. Granulation tissue formation involves cell emigration, fibroblast proliferation and new blood vessel formation. The results suggest that the extracts possess significant inhibitory effect on both exudative and proliferative phases of inflammation.
Fruend's adjuvant induced arthritis in rats is supposed to be produced by cell mediated hypersensitivity. Because of this immunological involvement it is presumed to have predictive value for screening drugs for anti-rheumatic effect. AQE, like dexamethasone suppressed both primary and secondary oedema. Whereas ME like phenylbutazone suppressed only secondary oedema, both extracts suppressed SRBC induced oedema in presensitised mice, this indicates that the extracts possess inhibitory effect on cell mediated immunity.

AQE when injected locally into the rat paw together with carrageenin produced oedema suppression. This and the fact that it is effective even on oral administration rules out counter irritation as the possible mechanism of anti-inflammatory activity. ME did not produce significant suppression on local injection. Since it did not potentiate oedema formation, counter irritation as the main mechanism of activity can be ruled out.

Significant weight gain in adrenal weight, non-significant decrease in adrenal ascorbic acid were noted in rats receiving the extracts. AQE produced anti-inflammatory activity in adrenalectomised rats but it was less in comparison to the activity obtained in intact rats. ME did not produce significant suppression in adrenalectomised rats. Histological examination of the adrenals obtained from extract treated rats showed signs of weak to moderate stimulation. The results suggest that at least part of the anti-inflammatory activity may be mediated through stimulation of adrenal pituitary axis. Both extracts suppressed nystatin induced rat paw oedema. Only AQE antagonised hyposaline induced human red blood cell (HRBC) lysis. However, both extracts antagonised carbachol and egg albumin induced mast cell degranulation.

Both AQE and ME inhibited castor oil induced diarrhoea in rats indicating that they may modulate PG formation. Inability of the extracts to inhibit $H_2O_2$ induced rat red blood cells lysis indicates lack of superoxide scavenging effect in them. However, since even the standard drugs were ineffective in this model it would be necessary to reevaluate the extracts in other models to arrive at an unequivocal conclusion.

Sub-effective dose of AQE potentiated the anti-inflammatory effect of sub-effective dose of phenylbutazone indicating similarity of action. Ascorbic acid and adrenaline did not enhance the anti-inflammatory activity of the extracts. However, reserpine pretreatment significantly potentiated the anti-inflammatory activity of the extracts.

Biochemical studies on the anti-inflammatory activity of strobilanthes heyneanus stem extracts.

AQE decreased serum protein content. Liver protein content increased in both the extracts administered rats indicating liver microsomal enzyme induction property in them. Studies on transaminase revealed that decrease or increase in their activity did not uniformly correlate with the anti-inflammatory activity. Significant decrease in elevated serum acid phosphatase (ACPase) activity was observed in extracts administered cotton pellet implanted (CP) rats. However, effect on ACPase activity in liver and granulation tissue were not consistent. Significant decrease in hydroxyproline and hexosamine content in the granulation tissue was observed in extract treated groups when the values were expressed as absolute values. When the values were expressed as relative values ($\mu g/g$ tissue) reduction in hydroxyproline content was observed in AQE treated group and reduction in hexosamine content was observed in ME treated groups. This indicates
that AQE may be modulating the biosynthesis of hydroxyproline and ME biosynthesis of hexosamine. Reduction in RNA and DNA content of the granulation tissue in extracts treated group indicates decrease in cellular content. Unlike the standard anti-inflammatory agents, the disease modifying anti-rheumatic drugs are reported to decrease the elevated level of acute phase reactants in different models of chronic inflammation. Orosomucoid is one of the acute phase reactants, its serum level significantly elevated in CP and GP rats. The extracts antagonised this elevation. However, the decrease was statistically significant only in GP rats. Reference standard drugs did not affect serum orosomucoid levels. This indicates that the extracts may possess disease modifying effect. This need to be explored further. Ceruloplasmin, another acute phase reactant was also found to be elevated in CP and GP rats. But the extracts did not decrease ceruloplasmin activity.

Evaluation of AQE and ME in a battery of tests revealed that they possess significant anti-inflammatory activity. They seems to influence all the three phases of inflammatory response. They suppress fluid exudation, cell migration, proliferation of cells and connective tissue formation. Enhancement of membrane stability, inhibition of prostaglandin biosynthesis, stimulation of adrenal-pituitary axis and suppression of both antibody and cell mediated immunity are the probable mechanisms through which anti-inflammatory activity is produced.

Part IV

Studies on the effect of extracts on gastric ulcer in rats

Gastric mucosal damaging property is one of the major limiting factors for the prolonged use of nonsteroidal anti-inflammatory agents (NSAIDs). Hence it has almost become a convention to evaluate ulcerogenic property of any substance showing anti-inflammatory activity.

The extracts were evaluated by employing two experimental protocol.

(1). Extracts were administered for seven days followed by pyloric ligation for 18 h.
(2). Extracts were administered in two doses along with phenylbutazone prior to and after pyloric ligation, which lasted for 8 h.

In both the sets, effect of treatment of extracts on gastric secretion, gastric acidity and ulcer index, total protein (TP), total carbohydrate (TC), TC:TP ratio and total hexosamine content of the gastric juice was observed.

In the first set, AQE showed statistically non-significant decrease in ulcer index. Ulcer index in ME treated group did not differ from control group. This shows that the extract donot possess antiulcer effect and since no increase in ulcer index was observed it can be concluded that the extracts are devoid of ulcerogenic property. AQE (at 100 mgkg⁻¹) produced significant decrease in gastric secretion, gastric acidity and peptic activity. Inspite of this activity profile the antiulcer effect with AQE was not significant. The reason for this might be decrease noted in hexosamine content, which represents mucosal secretion.

In second set, PBZ per se produced significant ulceration, as evident from significant increase in ulcer index. ME did not increase ulcer index. Increase in ulcer index noted with AQE
was not statistically significant. Concomitant administration of AQE with PBZ lead to decrease in ulcer index. ME did not modify ulcer index when administered with PBZ. This clearly indicates that the extracts do not share the ulcerogenic propensity of PBZ. Effect of extracts per se and in combination with PBZ on other parameters was complex in this test.

The results of the study indicate that the extracts act in a complex manner on the gastric mucosa. And they do not produce significant damage to the gastric mucosa.