SUMMARY
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The entire work has been presented in three parts with a general introduction. The general introduction contains brief account of historical background of the evolution of medicinal plants-based therapeutics citing examples of different ancient civilizations. It also contains brief description of history of Ayurveda, research on medicinal plants in India, present status and future prospects of medicinal plant research. In addition general data on *Vitex negundo* and *Vitex altissima* and plan of work have also been described.

Plants belonging to *Vitex* genus have been recommended for the treatment of inflammatory disorders in Ayurveda. Experimental studies have shown presence of significant anti-inflammatory activity in different parts of *Vitex negundo*. But details are not available on the mechanism of this anti-inflammatory effect. *Vitex altissima* is a closely related species and no studies have been reported on it. Hence, its leaves were screened for anti-inflammatory activity. After confirming the presence of anti-inflammatory activity, detailed investigations were undertaken to elucidate the mechanism of anti-inflammatory activity. Besides, anti-ulcer effect and ulcerogenic potential of both plants was assessed. General pharmacological screening of leaf of *Vitex altissima* was also undertaken to define its activity profile.

Part I

Since lot of intra and inter species variations have been observed in this genus pharmacognostical characterisations of the plant material selected for the study was undertaken. The main pharmacognostical features were as follows:

*Vitex altissima* :

**Macroscopic features** :

Leaves are 3-5 foliate, arise from the end of a common petiole. They are comparatively large with winged petioles, terminal leaflets are larger than lateral ones. Both glandular and non-glandular hairs are present, mainly on lower surface.

**Microscopic features** :

The following cytoarchitecture can be observed from microscopic observation of T.S. of leaflet at midrib:

a) Upper epidermis has a thick cuticle with 2-3 layered hypodermis.

b) Lower epidermal cells are comparatively large with an outgrowth of both glandular and non-glandular trichomes.
c) Stomata are present on the lower surface and are highly variable.

d) Vascular bundle of the midrib is horse-shoe shaped with 5-8 small groups of additional bundles inside and is protected by a sclerenchymatous bundle sheath.

The presence of multicellular, uniseriate, non-glandular as well as glandular hairs are the chief diagnostic features of the leaflets.

**Vitex negundo:**

**Macroscopic features:**

Leaves are similar to Vitex altissima except that they are smaller in size and possess characteristic aroma.

**Microscopic features:**

Transverse section (T.S.) of midrib region shows the following features under microscope.

a) Upper epidermis has a thin cuticle and a single layered hypodermis present beneath it.

b) Vascular bundles are horse-shoe shaped without a fibrous bundles sheath. Only 2-3 additional bundles are present inside, mainly at both ends.

c) The lower epidermis is completely clothed by hairs which are multicellular, uniseriate, non-glandular as well as glandular. Due to this covering of hairs stomats are not easily detectable. This is the main diagnostic feature.

**Acute toxicity studies:** LD$_{50}$ values with 95% confidential limits were found to be 5800 (4677-7192) mgkg$^{-1}$ (ip) for ME of VAL in mice. In rats LD$_{50}$ is more than 4000 mgkg$^{-1}$ since only 2/6 rats died at this dose. In ME of VNDL treated group no mortality could be observed up to the dose of 6000 mgkg$^{-1}$ in mice and up to 4000 mgkg$^{-1}$ in rats.

**Anti-Inflammatory study:**

**Section A**

A detailed account of various factors involved in the induction, maintenance and modulation of inflammatory process has been provided as an introduction to this section.

As a part of elucidation of mechanism of anti-inflammatory activity, the extracts of both the plants were evaluated in different models of experimental inflammation selected for predictability of effect on particular phase of inflammation. For this purpose both pharmacological and biochemical experimental techniques were employed.
Methanol extract (ME) of both *Vitex altissima* (VAL) and *Vitex negundo* (VNDL) leaves produced significant suppression of carrageenin-induced hind paw oedema in a dose dependent manner confirming anti-inflammatory activity in *Vitex negundo* and revealing presence of significant anti-inflammatory activity in *Vitex altissima*. ED$_{50}$ value was found to be 138.00$\pm$20.41 mgkg$^{-1}$ (ip) for ME of VAL and 407.40$\pm$71.45 mgkg$^{-1}$ (ip) for ME of VNDL. Time course studies showed that the extracts may be modulating the activity of the inflammatory mediators in second phase of carrageenin oedema formation. Both the extracts suppressed histamine and bradykinin-induced rat paw oedema. ME of VNDL suppressed prostaglandin E$_2$-induced oedema also but the suppression with ME of VAL was not statistically significant. ME of VNDL seems to modulate the activity of the above three mediators of inflammation. ME of VAL may be modulating the activity of the histamine and bradykinin with weak effect on PGE$_2$ activity. It is also possible that the extract may be modulating the release of these mediators. Neither of the extracts could suppress 5-hydroxytryptamine-induced paw oedema ruling out antiserotonin activity as one of the mechanisms of anti-inflammatory activity.

Effect of the extracts on vascular permeability was assessed by noting their effect on Evans blue extravasation into carrageenin-injected paw and on fluid exudation in carrageenin-induced pleurisy and PVC cup-implanted rats. Both extracts suppressed dye extravasation into rat paw and fluid exudation into PVC cup-implant. ME of VAL produced significant inhibition of exudation of fluid in rat pleurisy test. ME of VNDL did not produce significant suppression. The results indicate presence of significant vascular permeability inhibitory effect. When considered as a whole.

Both extracts significantly inhibited leukocyte migration into PVC cup implants in rats. Formaldehyde-induced arthritis is supposed to represent proliferative phase of inflammation. Both extracts produced significant suppression of formaldehyde-induced paw oedema, granulation tissue formation in implanted cotton pellets and carrageenin granuloma in rats suggesting presence of significant inhibitory effect in extracts on proliferative phase of inflammation. The extracts inhibited fluid exudation in carrageenin granuloma test, further corroborating inhibitory effect on fluid exudation.

Neither of the extracts could suppress primary oedema in Freund's adjuvant-induced arthritis in rats. Secondary oedema was suppressed by ME of VNDL. The reason for not observing suppression of primary oedema in this model in spite of noting significant anti-inflammatory activity in acute inflammatory models is not known. Since both extracts produced significant suppression of SRBC-induced immunological oedema in mice, it could be indicative of their efficacy in only selective types of inflammations of immunological origin. The different responses obtained in the two models may also be indicative of species difference.
ME of VAL suppressed oedema formation when injected locally together with carrageenin into the rat paw. The suppression observed with ME of VNDL was not significant. This may be indicative of weak local anti-inflammatory activity in ME of VNDL since it did not potentiate, like counter-irritants, oedema formation. It can be inferred that counter-irritation does not play any role in the anti-inflammatory activity observed with the extract.

ME of VAL caused adrenal weight gain in Granuloma Pouch bearing (GP) rats. ME of VNDL increased adrenal weight in both GP and cotton pellet-implanted (CP) rats. Both extracts caused decrease in adrenal ascorbic acid content and signs of weak to moderate adrenal stimulation were observed on histological examination. Both the extracts produced significant anti-inflammatory activity in adrenalectomised rats though the magnitude of activity was less in comparison to intact rats, especially with ME of VAL. The results indicate that though the extract may stimulate adrenal-hypophyseal axis it does not seems to play major role in the anti-inflammatory activity.

Both extracts suppressed nystatin-induced paw oedema in rats and hyposaline-induced lysis of human red blood cells (HRBC) in vitro. Further, the extracts also effectively antagonised carbachol and egg albumin-induced mast cell degranulation. This suggests that enhancement of membrane stability may be one of the mechanisms of anti-inflammatory activity observed with the extracts.

ME of both VAL and VNDL produced significant inhibition of castor oil-induced diarrhoea in rats indicating a modulatory effect on prostaglandin (PG) formation. The extracts did not antagonise H2O2-induced rat red blood cell lysis suggesting that they are devoid of superoxide scavenging effect. However, since even the standard drugs were ineffective in this model, it would be prudent to evaluate the extracts in other models to arrive at an unequivocal conclusion.

When the extracts were co-administered in sub-effective dose with sub-effective dose of phenylbutazone, synergism in their anti-inflammatory activity was observed indicating that they may have similar mechanism of action. Pre-treatment of rats with ascorbic acid did not modify the anti oedema effect of the extracts. Similar effect was observed with adrenaline indicating non-involvement of endogenous adrenaline in the anti-inflammatory activity of the extract. Reserpine pretreatment potentiated the anti-inflammatory effect of ME of VNDL and antagonised the anti-inflammatory activity of ME of VAL.
Section B:

Both extracts decreased serum protein content in CP rats. ME of VAL at both the dose levels and ME of VNDL at lower dose level increased liver protein content significantly in CP rats. This indicates presence of microsomal enzyme induction effect. Studies on transaminase activities in serum and liver of both GP and CP rats and in granulation tissue in GP rats failed to reveal any uniform correlation between changes in their activity and anti-inflammatory activity. Both extracts significantly inhibited acid phosphatase (ACpase) activity in serum from CP rats.

Elevation of acute phase proteins in serum is one of the changes observed during inflammation. This elevation is reported to be inhibited by disease modifying anti-rheumatic drugs but is not influenced by NSAIDs. The effect of extracts was studied on serum orosomucoid level and ceruloplasmin activity, two representative acute phase proteins. Statistically non-significant decrease was observed in serum orosomucoid level in CP rats after the administration of both the extracts. In GP rats, ME of VAL produced significant decrease. The extracts did not influence ceruloplasmin activity. The effect of extracts on serum orosomucoid level indicates that ME of VAL may possess disease-modifying effect. But ME of VAL did not produce anti-inflammatory activity in Freund's adjuvant-induced arthritis in rats. Hence it is desirable to subject the extract to further evaluation.

Both the extracts decreased hydroxyproline content in granulation tissue when the data were presented as absolute values. However the decrease noted was statistically significant only in ME of VNDL-treated group when the data were expressed as relative values (i.e. ug/g of granulation tissue). The results indicate that ME of VNDL may have modulatory effect on hydroxyproline biosynthesis. The decrease noted in absolute values in ME of VAL treated group may be indicative of decrease in other granulation tissue components without affecting hydroxyproline biosynthesis. ME of VAL decreased hexosamine content in granulation tissue when the data were expressed as absolute values. None of the drugs administered could affect relative hexosamine content. This indicates that the extracts donot modulate biosynthesis of hexosamine. Decrease in RNA and DNA content in granulation tissue was observed in both the extracts-administered groups. This indicates that the extract may decrease the cellular content of the granulation tissue.

Evaluation of ME of VAL and VNDL in a battery of tests representing different phases and aspects of inflammation revealed that both possess significant anti-inflammatory activity. They have modulatory effect on fluid exudation, cell emigration proliferation of inflammatory cells and connective tissue formation. Enhancement of membrane stability, inhibition of PG formation and modulation of certain types of cell mediated immunity also contribute to the anti-inflammatory activity of the extract. The extracts appear to share similar mechanism of anti-inflammatory activity, although some quantitative and qualitative differences were observed.
Part II

Evaluation of ME of VAL and VNDL for antiulcer effect or ulcerogenic potential.

Non-steroidal anti-inflammatory drugs (NSAIDs) have the propensity to cause gastric and intestinal ulcerations. This is one of the major limiting factors for their prolonged use. Hence, any new drug found to possess anti-inflammatory activity is normally assessed for ulcerogenic potential.

Anti-ulcer or ulcerogenic potential in the test extracts was evaluated by employing two experimental protocols.

1) Extracts were administered to rats in the dose producing anti-inflammatory activity for 7 days followed by pyloric ligation for 18 h.

2) Extracts were administered in two doses, concomitantly with phnarylbutazone prior to and after pyloric ligation and sacrificed 8 h later.

Effect of extracts on the following parameters was assessed in both the sets: Gastric secretion, gastric acidity, ulcer index, total proteins, total carbohydrate and total hexosamine content in gastric juice.

ME of VNDL produced significant decrease in ulcer index. It did not affect gastric secretion, acidity and hexosamine content significantly. However, it produced significant inhibition of peptic activity. Increase observed in total protein and total carbohydrate content was not statistically significant. ME of VAL decreased gastric secretion, acidity and peptic activity. However, the decrease in ulcer index was not statistically significant. It also caused decrease in total hexosamine content and increase in total protein and total carbohydrate content. Analysis of the results show that the extracts do not share the ulcerogenic propensity of the NSAIDs. On the contrary, moderate antiulcer effect in ME and weak antiulcer effect in ME of VAL was observed.

In the second set of experiments, phenylbutazone (PBZ) per se significantly increased ulcer index in comparison to normal 8 h pylorus ligated rats. Neither of the extracts per se significantly influenced degree and incidence of ulceration. ME of VAL when administered together with PBZ inhibited the latter's ulcerogenic effect as observed from the significant decrease in ulcer index and incidence of ulceration. ME combined with PBZ did not modify ulcer index and incidence of ulceration. PBZ treatment alone did not influence gastric secretion, acidity and peptic activity. It increased total protein content and decreased total...
carbohydrate to total protein ratio (TC:TP). It did not affect total carbohydrate and total hexosamine content significantly. The extracts per se increased gastric secretion and this increase was not observed when they were administered together with PBZ. The extracts were effective in restoring the decreased TC:TP ratio. The extracts when administered alone produced significant decrease in peptic activity and total hexosamine content. However, this decrease was not seen when they were co-administered with PBZ. The complex nature of the effect of extracts on gastric constituents makes it difficult to draw a general inference on the nature of their effect on the ulcerogenic effect of PBZ. Since the extracts did not increase ulcer index it is clear that they do not have ulcerogenic effect. Further more, ME of VAL was effective in significantly decreasing PBZ induced ulceration indicating the possibility of combining the extracts with PBZ for treating inflammatory disorders.

Part III

General pharmacological screening of Vitex altissima:

General pharmacological screening of Vitex altissima was undertaken with a view to define the pharmacological activity profile of Vitex altissima. Methanol extract (ME) of its leaf was screened for different pharmacological activities. The effect of ME of VAL was studied on gross behaviour, spontaneous motor activity (SMA), forced motor activity (FMA) and evaluated for anti-psychotic, antidepressant, antiparkinsonian, anticonvulsant, analgesic, antipyretic, hepatoprotective, adaptogenic, immunomodulation, antivenom, antimicrobial, diuretic and antifertility effects. It was also screened for hypoglycaemic and hypolipidaemic activities. Besides, effect 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.

The extract did not produce any discernible effect on gross behaviour. SMA was moderately depressed. FMA was not affected but shortening of pentobarbitone sleep was observed at higher dose level. It did not modify d-amphetamine stereotypy, the primary screening test for anti-psychotic activity. However, it suppressed exploratory behaviour of mice. It did not affect rectal temperature in rats and failed to protect rats against electroconvulsions and mice against chemooconvulsions. It is also devoid of anti-parkinsonian and anti-depressant effects.

The extracts suppressed acetic acid writhing in mice but did not elevate pain threshold in radiant heat test indicating that it has only peripheral analgesic activity. It also produced antipyretic activity.

It did not antagonies CCl4-induced increase in PBN sleep in mice and did not affect liver volume, weight and transaminase activity significantly indicating lack of hepatoprotective effect.
The extract enhanced swimming endurance in mice but did not affect antibody formation against SRBC. It also did not protect mice against cobra envenomation. No effect could be observed on urine formation and excretion of sodium, potassium and chloride in hydrated rats.

It did not affect blood glucose level in normoglycaemic rats but produced significant decrease in both total cholesterol and triglyceride levels in serum. The extract exhibited weak anti-fertility effect in rats.

The extract did not produce any significant effect per se on blood pressure in anaesthetized dogs. It also did not affect normal bracketing responses significantly except showing moderate attenuation of response to noradrenaline.

The extract did not produce any effect on isolated frog heart and also did not alter the responses of the heart to adrenaline and acetylcholine. It had no effect on isolated guinea pig ileum, tracheal spiral and rabbit jejunum. However, it produced significant antagonism of oxytocin and PGF2α induced contraction in rat uterus. This indicates that it may be useful as a uterine depressant.

Analysis of the results reveal that the pharmacological activity profile of ME of VAL is narrow. Since it did not produce any marked effect on CNS and CVS chances of occurrence of untoward effects involving these systems seem to be limited.

Preliminary phytochemical analysis of ME of Vitex altissima and Vitex negundo revealed the presence of flavonoid, tannins and sugar fraction in them. (Table 69)