Summary and Conclusion
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*Operculina turpethum* (Linn.) (Sanskrit: Trivirt) is commonly used in the treatment of rheumatism and inflammatory conditions by the practitioners of Indian system of medicine. In the present study extract of stem bark *Operculina turpethum* has been evaluated for its anti-inflammatory, anti-arthritis, antioxidant and anti-ulcer activities.

The various pharmacognostical findings with hydroalcoholic (HAOP) and methanolic (MOP) extracts of stem bark of *Operculina turpethum* presented in the thesis are summarized as follows.

**Pharmacognostical and phytochemical studies**

Pharmacognostical studies plays a key factor in establishing the authenticity of the plant material. The botanical identity of the aerial parts and root was established by examining its anatomical features. The anatomical examination of aerial parts and root exhibited important microscopical features like, presence of uniseriate, unbranched, thick walled epidermal trichomes with a rosette of pedestal epidermal cells. The anatomical examination also showed occurrence of spindle shaped, elongated calcium corborate cystoliths in the lithocysts of the epidermal cells of all aerial organs. Distribution of rod shaped calcium oxalate crystals in the ground cells of the petiole, stem and root was another distinguished observation made in the study.
The plant material was standardized as per the specifications of ayurvedic macroepia of India by subjecting the plant material for determination of physiochemical constants like ash value, extractive value and loss on drying.

Extracts of stem bark (HAOP) and (MOP) were prepared by hot percolation method using soxhlet apparatus and screened for the presence of phytochemicals by subjecting the extracts to various chemical tests and High Performance Thin Layer Chromatography (HPTLC). The chemical examination of the extracts revealed the presence of phytoconstituents like reducing and non-reducing sugars, steroids, triterpenes, flavonoids, tannins etc.

HPTLC fingerprinting gave well-resolved peaks with HAOP and MOP and can be used as a laboratory guide to validate the authenticity of the plant materials for future study.

**Acute and chronic toxicity studies**

In acute toxicity study, no mortality was observed up to a dose level of 2000 mg/kg body weight for both the extracts HAOP and MOP. As per the ranking system used by the European Economic Community (EEC) for acute oral toxicity, the LD50 dose of 2000 mg/kg and above is categorized as unclassified (EC Directive 83/467/EEC, 1983), hence further studies were not conducted with higher doses. The administration of HAOP and MOP at dose of 1000 mg/kg for 90 days in chronic toxicity studies did not produce any significant
changes in the food intake, water intake, body weight of animals and did not alter the normal haematological and biochemical parameters. Histopathological examination of the internal organs like liver, kidney, spleen, heart, testis and ovary of treated and control groups showed normal architecture suggesting no detrimental changes and morphological disturbances were caused by the daily oral administration of HAOP and MOP for 90 days.

**Anti-nociceptive, Anti-inflammatory and Antipyretic studies**

HAOP and MOP at dose of 100 mg/kg/p.o b.wt significantly (p<0.001) reduced the number of abdominal constrictions and stretching of hind limbs induced by the injection of acetic acid and increased the reaction time of animals towards the thermal source in hot plate and tail flick test in a dose-dependent manner, which establishes the fact that the extracts exhibit anti-nociceptive activity by central as well as peripheral mechanism(s). The activity of MOP was predominant in both chemical and thermal method than HAOP.

Both extracts exhibited significant (p<0.001) anti-inflammatory activity in both carrageenan induced paw edema and cotton pellet granuloma methods in a dose-related manner. In carrageenan method the extracts HAOP (100 mg/kg), MOP (100 mg/kg) and Diclofenac sodium (5 mg/kg) exerted maximal anti-inflammatory effects at 3 hr after carrageenan administration.
In cotton pellet granuloma method treatment with HAOP and MOP at both doses and diclofenac inhibited both wet and dry weight of granuloma, they also reduced the lipid peroxide levels in liver and exudates and reverted the elevated levels of lysosomal enzymes, acid and alkaline phosphatase in serum and liver. The extracts exert their anti-inflammatory action probably by suppressing the infiltration of neutrophils into the granuloma by inhibiting either the formation of chemotactic mediators or by suppressing the ability of inflammatory cells to respond to a chemotactic stimulus mediated through oxygen free radicals.

In antipyretic studies HAOP (100 mg/kg), MOP (100 mg/kg) and Paracetamol (100 mg/kg) showed a significant \( p<0.05 \) reduction in pyrexia induced by TAB vaccine.

The anti-nociceptive, anti-inflammatory and antipyretic activity exhibited by HAOP and MOP may be due to its inhibition of cyclooxygenase pathway interfering with prostaglandin biosynthesis.

**Anti-ulcerogenic activity**

The anti-ulcer studies were conducted to determine whether the extracts are ulcerogenic or ulcer protective in nature. HAOP and MOP were screened for anti-ulcer activity at a dose of 100 mg/kg b.wt using aspirin + pyloric ligation induced ulcer model. Both extracts significantly \( p<0.001 \) reduced the acid secretary parameters (i.e., total and free) acidity as well as the gastric volume and ulcer index in aspirin + pyloric ligated rats. They also
significantly increased the total carbohydrate:protein ratio indicating their mucosal protective activity.

Histopathological examination of stomach mucosa revealed that treatment with HAOP, MOP and ranitidine protects the mucosal epithelium from the damage caused by aspirin. In HAOP and MOP treated groups the mucosa was found to be almost normal with mild erosion in muscularis mucosa. Ranitidine treated section shows normal mucosa with no ulcer, edema in the submucosa.

At the same time HAOP alone (100 mg/kg) and MOP alone (100 mg/kg) treated groups did not produce any significant changes in the biochemical parameters and they preserved the normal architecture of the stomach mucosa by significantly reducing the gastric volume and ulcer index as compared to PL control animals. This further establishes the fact that the extracts thereby are ulcer protective in nature.

**Anti-arthritis and Antioxidant activity**

The anti-arthritis activity of HAOP (100 mg/kg/p.o) and MOP (100 mg/kg/p.o) has been evaluated in adjuvant-induced arthritis using Complete Freund's Adjuvant. HAOP and MOP exhibited significant anti-arthritic action in both developing and developed arthritis. These results are further substantiated by the evaluation of biochemical parameters.

Treatment with HAOP and MOP has ameliorated the decrease in body weight in arthritic rats. In arthritic rats there was a significant increase in
haematological parameters like ESR and WBC with significant decrease in RBC and Hb. Alterations in haematological parameters were normalized upon treatment with HAOP and MOP.

The acute phase proteins like serum ceruloplasmin and fibrinogen were elevated in arthritic animals, similarly there was an increase in serum creatinine, globulin, blood urea nitrogen and blood glucose levels with a decrease in albumin and uric acid levels in arthritic animals. All these changes in arthritic rats were reversed after treatment with HAOP and MOP.

HAOP and MOP treatment decreased the elevated levels of marker enzymes like ALT, AST, ALP and LDH in arthritic animals. Glycoproteins and lysosomal enzymes were significantly increased in arthritic animals. Treatment with HAOP and MOP reversed the elevated levels of these enzymes due to its membrane stabilizing ability. HAOP and MOP treatment also brought back the abnormalities in lipid metabolism to normal levels.

HAOP and MOP treatment significantly inhibited lipid peroxidation in kidney, spleen and plasma of arthritic rats and reverted the decreased levels of liver lipid peroxides. The treatment also protected the enzymic and non-enzymic antioxidant defense mechanism in arthritic rats. These effects may be attributed to the free radical scavenging property of the extracts.

HAOP and MOP exhibited significant (p<0.001) *in vitro* antioxidant activity by inhibiting the oxidation of linoleic acid in both FTC
and TBA methods with HAOP having greater activity in TBA method and MOP having greater activity in FTC method. Incidentally the activities of both HAOP and MOP were found to be better than the standards Vitamin E and C in both methods. The results of the *in vitro* antioxidant activity of the extracts showed correlation with the *in vivo* results.

Membrane bound ATPases and collagen levels were significantly decreased in arthritic animals. HAOP and MOP treatment restored the levels of above parameters to normal levels in arthritic rats. The increased collagen level indicates that HAOP and MOP treatment prevents bone destruction and increases collagen metabolism.

Radiological studies showed narrowing of the joint space and mild soft tissue swelling and erosions of the cartilage representing bony destruction in joints of arthritic animals. HAOP and MOP treated animals showed less destruction of joints and there was retardation or reversal of bony erosions and joint space narrowing which are good indices that HAOP and MOP administration is beneficial in the treatment of RA.

Histopathological studies of the proximal interphalangeal joints showed the joints of arthritic rats were edematous, degenerated, with partial erosion of cartilage, destruction of bone marrow and extensive infiltration of inflammatory exudates in the articular surface. HAOP and MOP treated animals showed normal bone marrow with less destruction of cartilage and scanty cellular infiltrate.
From the results it can be concluded that both extracts HAOP and MOP exhibit anti-arhritic activity to greater extent in both developing and developed arthritis. The activity of the extracts was more predominant in developing arthritis than developed arthritis. When compared between the two extracts MOP exhibited more significant anti-arhritic potential than HAOP.

The present study and its findings establish the rationale of using the aqueous extracts in various dispensing forms in traditional system of medicine for the treatment of inflammatory joint diseases and rheumatoid arthritis. However, further studies are required to identify the active principles(s) responsible for the therapeutic action and multi-central clinical trials are necessary to ensure the clinical efficacy and safety of the extracts before they can be prescribed as an anti-arhritic agent.