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# Antiarthritic studies on Nyctanthes arbor tristis and Maharasnadi ghan

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# ABSTRACT

Nyctanthes Arbor tristis and Maharasnadi ghan extracts were studied for arthritis using gouty arthritis and Freund's adjuvant Induced Polyarthritis. The plant was evaluated for acute toxicity studies at two different dose levels and it did not show any toxic or deleterious effects indicating low toxicity of the extracts even at high doses. In gouty arthritis a significant reduction in legged gait was observed as compared to control group whereas in poly arthritis model complete control was noticed in injected as well as uninjected paw. Significant reduction reduction in the elevated levels of serum lysosomal enzymes (SGPT, SGOT, and ALP) and lipid peroxidation was noted as compared to control group. The extracts enlighten the probable role in both acute and chronic arthritis models suggesting further histopatholgical studies are necessary to establish its anti-arthritic potential. The extracts of the plant in the formulation might be containing significant amount of flavonoids which might had played a good evaluator parameter in reduction of the inflammation and thereby reducing legged gait. In vision of further studies the extracts needs to be studied in details by focusing on histopathogical examinations.

Keywords: Maharasnadi Quathar, *Parijat*, Alkaline Phosphatase, Aspartate Aminotransferase, Alanine Aminotransferase.

#### **INTRODUCTION**

Ayurveda is a traditional Indian medicinal system being praticised for thousands of years. Considerable research on Pharmacognosy, Phytochemistry, and Pharmacology and Clinical therapeutics has been carried out on ayurvedic medicinal plants. Numerous drugs have entered the international pharmacopoeia through the study of ethno pharmacology and traditional medicine [1]<sup>-</sup> Ayurveda strongly recommends the use of the plant as a whole. Ayurveda has fundamental aspects for drug formulation. The herbs are selected according to the disease; other herbs are used to prevent the side effects arising from chief herb. Typical formulations for fever include bitter herbs, like *Parpatadi quath*, *Kirat-tikatadi quath*. The polyherbal have been used for centuries and some like *Triphala*, *Trikatu* are integral part of formulations marketed for digestive disorders [2].

Thus, it can be concluded that polyherbal formulations should not be dismissed only on the basis that they do not withstand modern research. Ayurveda and herbal medicine has roots in medicinal herbs and they have been practiced for centuries. Although colchicine which is very effective in treatment of gout is associated with major side effects like profuse diarrhoea, gastric hemorrhage etc. which limits its use in patients. The greatest disadvantage in the presently available potent synthetic anti- inflammatory drugs lies in their toxicity and reappearance of symptoms after discontinuation. Therefore, the search for safe anti-inflammatory agents is an unending process [3, 4].

According to Ayurveda Ghana is prepared from Quath (Kwath). Maharasnadi ghan is prepared form Maharasnadi quath (MRQ). Both MRG and MRQ have similar pharmacological properties. Maharasnadi quath: Rasna is the main component of this preparation. Because of its analgesic, antiphlogistic and anti-pyretic properties this drug has long been used for the treatment of rheumatism and arthritis [5, 6].

The Night-flowering Jasmine (*Nyctanthes arbor tristis*) is native to the Bengal region of India, where it is known as *Shephali* or *Parijat*. The tree is sometimes called the tree of sorrow because the flowers lose their brightness during daytime; the taxonomic name *arbortristis* also means "sad tree". The flowers can be used as a source of yellow dye for clothing. The flower is the official flower of the state of West Bengal, India, as well as the Kanchanaburi Province, Thailand [7].

Since scientifically controlled investigations have been not carried out, the present study was conducted in experimental models of rats to assess the anti-arthritic potential of polyherbal formulation Maharasnadi ghan .It's activity is compared with single herbal drug *Nyctanthes arbor tristis* Linn.

# MATERIALS AND METHODS

# Polyherbal formulation collection and extraction:

Parijat (NAT) and Maharasnadi ghan (MRG) were procured as a gift sample from Herb Pharmaceuticals, India. The NAT and MRG were extracted with hydroalcoholic solvent (80% methanol) using Soxhlet apparatus for 48 hrs at 50- 60°C. The extracts were filtered and evaporated under reduced pressure to give dry powders. The powders were stored in airtight ambered colored glass containers and further used for studies.

# Chemicals and reagents:

Anti-inflammatory Drugs for study:Maharasnadi ghan and Parijat: Herb Pharmaceuticals, Ahmedabad.Etoricoxib: Khandelwal Laboratories Ltd, Mumbai.Proinflammatory Agents:Freund's Adjuvant: Diclo Lab., Detroit, MI, USA.Monosodium Urate Crystals: Research Lab Mumbai.Diagnostic Kits:Alkaline Phosphatase (ALP): Accurex Biomedical Pvt Ltd, Mumbai.Glutamate Oxaloacetate Transaminase (GOT): Accurex Biomedical Pvt Ltd, Mumbai.Glutamate Pyruvate Transaminase (GPT): Accurex Biomedical Pvt Ltd, Mumbai.Chemicals:Sodium Carboxy Methyl Cellulose: Sd Fine Chem Ltd, Mumbai.

# **Experimental animals:**

Albino mice of Swiss strain (20-25kg) were purchased from Bharat Serum and Vaccines, Thane. The animals were housed in polypropylene cages and maintained under standard conditions (12 hours light/12 hours dark cycle;  $25 + 3 \circ C$ ; humidity 35-60 %). They were fed with Amrut brand pelleted standard diet manufactured by Nav Maharashtra Chakan oils, Ltd., Maharashtra and drinking water *ad libitum*. The animals had free access to water all the time and were allowed to adapt to the animal house conditions by keeping them for a period of 8-10 days prior to using them for the experiments. The study was conducted after seeking clearance from the Institutional animal ethical committee.

### Acute Toxicity Studies:

Albino Wistar rats, weighing in the range of 100-150gm were divided into various groups of 3 each of both sex. MRG and NAT were weighed and dissolved in appropriate amount of distilled water, using a mortar and pestle. The different groups of rats were administered the following doses of test formulation viz, 2000mg/kg, 5000mg/kg per oral. The rats were critically observed for clinical signs, gross behavioral changes and mortality, if any, following the administration of the test formulation at different time intervals like 30min, 1hr, 2hr, 4hr, 24hr and then 48 hr up to 72 hrs period as per OECD guidelines.

# Anti-arthritic activity evaluation:

# Acute model of gouty arthritis:

### a. Preparation of monosodium urate crystals [4]:

0.01M Sodium Hydroxide solution was prepared by dissolving 0.4g of sodium hydroxide pellets in 400ml of distilled water in a glass beaker and 1.68g (0.01M) Uric Acid is added. The resulting opaque solution is allowed to remain overnight at room temperature. On the next morning Sodium Urate Crystals were harvested by decanting supernatant solution and washed three times with cold phosphate buffered saline (PBS). The resulting crystals were dried and resuspended in PBS and sterilised in an autoclave at 120 °C, for half an hour. Suspensions for injection were kept in rubber stoppered multidose vials.

#### b. Injection of monosodium urate crystals:

Albino Wistar rats weighing in the range of 100- 150gm were divided into six groups of 6 each. MRG and NAT were evaluated at two dose levels; Etoricoxib was used as standard antiinflammatory drug for comparison. MRG, NAT and Diclofenac Sodium were administered orally to the rats one hour before monosodium urate crystal injection. The rats were anesthetized and monosodium urate crystals were injected into the synovial space of the right knee in a volume of 50µl sterile phosphate buffered saline equivalent to 0.5-2.5 mg/joint. Accordingly size of joint was evaluated using micrometer.

Group-1: Served as control, which received 1% Sodium CMC solution at 1ml/kg orally + 50µl Urate crystals in PBS intraarticularly.

Group-2: Received MRG at 250mg/kg orally (one hour prior to urate crystals injection) + 50µl Urate crystals in PBS intraarticularly.

Group-3: Received MRG at 750mg/kg orally (one hour prior to urate crystals injection) + 50µl Urate crystals in PBS intraarticularly.

Group-4: Received NAT at 250mg/kg orally (one hour prior to urate crystals injection) + 50µl Urate crystals in PBS intraarticularly.

Group-5: Received NAT at 750mg/kg orally (one hour prior to urate crystals injection) + 50µl Urate crystals in PBS intraarticularly.

*Group-6: Received Etoricoxib at 10mg/kg orally (one hour prior to urate crystals injection)* +  $50\mu l$  Urate *crystals in PBS intraarticularly.* 

Further, histopathological studies of the joints were carried out.

### Chronic model of inflammation

### Freund's Adjuvant Induced Polyarthritis in Rats [8]:

Albino Wistar rats weighing in the range of 100- 150gm were divided into six groups of 6 each. The rats were injected subcutaneously 0.1ml of complete Freund's adjuvant into the plantar region of hind-paw. MRG and NAT were evaluated at two dose levels; Diclofenac Sodium was used as standard anti-inflammatory drug for comparison. The control animals were treated with vehicle instead of drugs. MRG, NAT and Diclofenac Sodium were administered orally for 14 days from the day of Freund's adjuvant injection.

*Group-1: Served as control, which received 1% Sodium CMC solution at 1ml/kg orally + 0.1 ml of complete Freund's adjuvant subcutaneously.* 

*Group-2:* Received MRG at 250mg/kg orally (one hour prior to carrageenin injection) + 0.1 ml of complete Freund's adjuvant subcutaneously.

*Group-3:* Received MRG at 750mg/kg orally (one hour prior to carrageenin injection) + 0.1 ml of complete Freund's adjuvant subcutaneously.

*Group-4:* Received NAT at 250mg/kg orally (one hour prior to carrageenin injection) + 0.1 ml of complete Freund's adjuvant subcutaneously.

*Group-5:* Received NAT at 750mg/kg orally (one hour prior to carrageenin injection) + 0.1 ml of complete Freund's adjuvant subcutaneously.

*Group-6: Received Diclofenac Na at 10mg/kg orally (one hour prior to carrageenin injection) + 0.1 ml of complete Freund's adjuvant subcutaneously.* 

The changes in the paw volume were measured up to 21 days following Freund's adjuvant injection. On the 21st day, the animals were sacrificed, cardiac puncture was carried out. The liver and serum were collected for further biochemical estimations.

Statistical analysis:

The data was analyzed using One-way ANOVA followed by Dunnett's test with SPSS packages (version 6.0).

# RESULTS

#### Acute Toxicity Studies:

MRG and NAT did not show any toxic or deleterious effects upto 5000 mg/kg oral dose. As the rats were administered the maximal possible dose, the LD<sub>50</sub> value of the MRG and NAT could not be determined. (Table -1 and 2)

Acute Toxicity Studies:

1. Maharasnadi ghan (MRG):

Characters observed	Dose mg/kg	
	2000	5000
Stimulant effects		
Hyperactivity	-	-
Irritability	-	-
Tremor	-	-
Convulsions	-	-
Piloerection	-	-
Depressant effects		
Sedation	-	-
Loss of righting reflex	-	-
Analgesia	-	-
Straub's tail	-	-
Loss of muscle co-ordination	-	-
Loss of pinna reflex	-	-
Autonomic effects		
Labored respiration	-	-
Blenching	-	-
Reddening	-	-
Abnormal secretions	-	-
Mortality	-	-
Absent + Mild ++ M	oderate +	++ Marked

Table 1: Toxicological observations for Maharasnadi ghan

# 2. Nyctanthes arbor tristis (NAT):

Table 2: Toxicological observations for Nyctanthes arbor tristis

	Dose mg/kg	
Characters observed	2000	5000
Stimulant effects		
Hyperactivity	-	-
Irritability	-	-
Tremor	-	-
Convulsions	-	-
Piloerection	-	-
Depressant effects		
Sedation	-	-
Loss of righting reflex	-	-
Analgesia	-	-
Straub's tail	-	-
Loss of muscle co-ordination	-	-
Loss of pinna reflex	-	-
Autonomic effects		
Labored respiration	-	-
Blenching	-	-
Reddening	-	-
Abnormal secretions	-	-
Mortality	-	-
sent + Mild ++	Moderate	++-

Anti arthritic Activity: Acute Model: Urate induced synovitis: Complete 3 legged gaits was observed in rats of control group after injection of monosodium urate crystals in the knee joints due to irritation produced by these crystals. In MRG and NAT of dose 750mg/kg each treated groups complete 3 legged gaits was observed up to 1 hour after monosodium urate crystal injection. However after 5 hours the rats showed occasional 3 legged gaits and after 6 hours only limping were observed.

Significant control over edema produced in monosodium urate crystal injected rat knee was observed at 750mg/kg of MRG and NAT oral dose of hydroalcoholic extract (Figure no.1).









Histopathological estimations showed presence of few inflammatory cells mainly neutrophils in the synovial membrane. However the results were inconclusive due to absence of prominent inflammatory symptoms in control as well as treated groups after 24 hours of monosodium urate crystal injection.

### Chronic model:

#### Freund's adjuvant induced poly arthritis:

MRG and NAT at dose 750mg/kg showed significant control on paw edema volume in both injected and uninjected paw. (Figure no. 2 and 3).

#### Fig no.3: Effect on changes in the volume of uninjected paw Freund's adjuvant induced Poly arthritis



Determination of Various Biochemical Parameters in Chronic model: Aspartate Aminotransferase (SGOT)

The MRG at 750 mg/kg dose significantly reduced the level of lysosomal enzyme SGOT by 28 %, while NAT at 750 mg/kg reduced the respective parameter by 23% (Figure no.4)

# Alanine Aminotransferase (SGPT)

The MRG at 750 mg/kg and 250mg/kg dose significantly reduced the level of lysosomal enzyme SGPT by 22and 15 %, while NAT at 750 mg/kg reduced the respective parameter by 22% (Figure no.4)

# Alkaline Phosphatase (ALP)

The MRG at 750 mg/kg dose significantly reduced the level of lysosomal enzyme ALP by 17 % respectively, while NAT at 750 mg/kg reduced the respective parameter by 12% (Figure no.4)

# Estimation of Lipidperoxides formed in vivo

The MRG at 750 mg/kg and 250mg/kg dose significantly reduced the level of lipid peroxide by 42 and 27 % respectively, while NAT at 750 mg/kg and 250 mg/kg dose reduced the respective parameter by 37 and 21% respectively. (Figure no. 5)



Fig. no.4: Effect of MRG and NAT on biochemical parameters in Freund's adjuvant induced arthritis

Fig. no.5: Effect of MRG and NAT on lipid peroxidation in Freund's adjuvant Induced arthritis



#### **DISCUSSION AND CONCLUSION**

We have tried to evaluate the anti-arthritic of Maharasnadi ghan and Nyctanthes arbor tristis by using various *in vivo* animal models. We have also tried to compare the activities of these two different drugs one a polyherbal and another single herb, to evaluate the synergistic effect, if any, of polyherbal formulation.

Acute toxicity testing is necessary to evaluate the toxic effects after administration of a single large dose of the drug. Acute toxicity of both the drugs was evaluated. MRG and NAT did not show any toxic or deleterious effects upto 5000mg/ kg oral dose indicating low toxicity of the drugs at high doses. As the rats were administered upto maximal possible dose, the  $LD_{50}$  value of both drugs could not be determined.

Urate induced synovitis model is used to evaluate efficacy of test drugs in treatment of inflammation produced due to deposition of urate crystals in the joints. Gouty arthritis is associated with the deposition of urate crystals in the synovial cavity. Monosodium urate crystals produced a time dependent inflammation of knee joint when injected intra-articularly [9]. MRG and NAT at dose 750mg/kg showed inhibition of edema produced in rat knee joint at 6<sup>th</sup> hour as compared to control group. Histopathological evaluations showed that the inflammatory symptoms had subsided by 24 hours after mono sodium urate crystal injection in all the groups. Although the results were inconclusive, MRG and NAT has shown potential to control the pain and irritation produced due to monosodium urate crystal injection.

Freund's adjuvant induced arthritis model is based on a syndrome which is more akin to rheumatoid arthritis than any other test. Freund's adjuvant is a mixture of dead *Mycobacteria* with liquid paraffin. The method involves both infectious and immunological factors responsible for development of arthritis [10]. Hence it provides an important means which can detect the efficacy of test drug in controlling both the infectious as well as immunological factors. In the control (untreated) group, paw swelling was constantly increased upto 14<sup>th</sup> day. On the 14<sup>th</sup> day paw volume was maximum. This was due to the immunological response to the dead *Mycobacteria* present in the Freund's adjuvant. MRG and NAT showed inhibition of paw swelling at the dose of 750mg/kg. The inhibition was observed even after 13<sup>th</sup> day showing the efficacy of the MRG and NAT in control of the immunological factors. The findings indicate the potential of MRG and NAT in controlling the inflammatory as well as immunological factors involved in rheumatoid arthritis.

Lipid peroxidation process initiated by Reactive Oxygen Species (ROS) is said to participate in the process of inflammation. The ROS produced by poly morpho leukocytes that infiltrate the site of injury can injure cellular biomolecules such as nucleic acids, protein, carbohydrates and lipids causing cellular and tissue damage, which in turn augments the inflammation [11]. In subacute and chronic form of inflammation MRG and NAT at 750 and 250mg/kg dose was found to be effective in lowering the levels of lipid peroxides, this effect of both drugs could probably be due to its anti oxidant activity.

Lipid peroxidation results in damage and loss of functional integrity of the cell membrane resulting in leakage of lysosomal enzymes SGOT, SGPT and ALP in the serum, which are simple but effective tools in assessing arthritic activity [12]. In Freund's adjuvant induced arthritis model, the lysosomal enzyme levels of SGOT, SGPT and ALP were increased significantly. The MRG and NAT at a dose of 750mg/kg was found to be effective in lowering the elevated levels of serum lysosomal enzymes (SGOT, SGPT and ALP) which may be a consequence of stabilization of plasma membrane. Thus the MRG and NAT was not as effective as diclofenac but was effective enough in significantly reducing the various inflammatory parameters when compared with control.

Thus in conclusion, data obtained in the present investigation suggests MRG and NAT are potential antiarthritic agent. Hence it is essential to investigate the exact underlying molecular mechanism(s) of action of both the drugs and also long term toxicity studies in different animal species. After completing the preclinical studies the herbal products need to be standardized on human patients to establish its therapeutic efficacy and safety.

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