

ACUTE AND SUBACUTE MODELS OF INFLAMMATION OF *NYCTANTHES ARBOR TRISTIS* AND MAHARASNADI GHAN

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ABSTRACT

Aqueous Methanolic extracts of *Nyctanthes Arbor tristis* and *Maharasnadi ghan* were studied for a inflammation parameter using carrageenin induced hind paw edema and cotton pellet granuloma. The natural plant was screened for acute toxicity and it did not show any toxic or deleterious effects indicating low toxicity of the extract even at high doses at two different dose levels. In carrageenin induced hind paw edema a significant reduction in paw volume was observed as compared to control group whereas in cotton pellet granuloma model marked inhibition in granuloma formation, 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 exhibited profound anti-inflammatory activity in both acute and subacute animal models warranting further investigations to establish its anti-inflammatory potential. The activity was thought due to flavonoids which might be present in the formulation which could play a significant role in preventing the release of histamine, leukotrens and prostaglandins. In future the extract needs to be studied for cellular line models of inflammation.

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

1. INTRODUCTION

In the early 19th century, scientific methods became more advanced and preferred, and the practice of botanical healing was dismissed as quackery. In the 1960s, with concerns over the iatrogenic effects of conventional medicine and desire for more self-reliance, interest in “natural health” and the use of herbal products increased. Recognition of the rising use of herbal medicines and other non-traditional remedies led to the establishment of the office of Alternative Medicine by the National Institute of Health USA in 1992.

Worldwide, herbal medicine received a boost when the WHO encouraged developing countries to use traditional plant medicine to fulfill needs unmet by modern systems¹.

Today it is estimated that more than 500 million people around the world are suffering from different forms of arthritis. As inflammation, itself a very complex process, involves many different cells and various mediators, permits a variety of strategies in developing anti-inflammatory drugs. Due to the unavailability of the most satisfying and

perfect anti-inflammatory drug, the intense search for discovering newer NSAIDs is continues with the hope of discovering ideal remedies for inflammatory disorders².

Nyctanthes arbor tristis belonging to Oleaceae has each and every part's wide utility in India. The leaves are antibacterial, anti-inflammatory and anthelmintic. The flowers are bitter astringent, ophthalmic, stomachic and carminative. It is an expectorant, bitter and tonic, febrifuge, and mild purgative. It is used in bilious and obstinate remittent fever, sciatica, and rheumatism. It is also very useful in constipation in children³. Maharasnadi ghan contains active constituents as that of Maharasnadi Quathar (MRQ). MRQ is a polyherbal formulation consists of 26 different plants. These are used in traditional medicine for a variety of purposes such as reduction of pain and inflammation, improve appetite and digestion, and act as immunostimulants and laxatives⁴.

Since scientifically controlled investigations have been not carried out so far, the present study was conducted in experimental models of rats to assess the anti-inflammatory potential of polyherbal formulation Maharasnadi ghan (MRG). Its activity is compared with single herbal drug *Nyctanthes arbor tristis* Linn. (NAT).

2. MATERIAL AND METHODS

2.1 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.

2.2 Chemicals and reagents

Anti-inflammatory Drugs for study:

Maharasnadi ghan and Parijat: (Herb Pharmaceuticals, Ahmedabad)

Diclofenac Sodium: Themis Pharmaceuticals, Mumbai.

Proinflammatory agents:

Carrageenan Sigma Chemical Co, St Louis, MO, USA

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.

2.3 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°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 ((Animal house reg. no. 25/ 1999/ CPCSEA) 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.

2.4 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 420.

2.5 Anti-inflammatory activity evaluation

2.5.1 Acute model of inflammation Carrageenan Induced Hind Paw Edema in Rats⁵

Albino Wistar rats weighing in the range of 100- 150gm were divided into six groups of 6 each. The rats were injected subcutaneously 0.1 ml of 1% (w/v) of injection) + 0.1 ml of 1% (w/v) of carrageenan subcutaneously. carrageenin 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.

Group-1: Served as control, which received 1% Sodium CMC solution (1ml/kg orally) + 0.1 ml of 1% (w/v) of carrageenan subcutaneously.

Group-2: Received MRG at 250mg/kg orally (one hour prior to carrageenin

Group-3: Received MRG at 750mg/kg orally (one hour prior to carrageenin injection) + 0.1 ml of 1%(w/v) of carrageenan subcutaneously.

Group-4: Received NAT at 250mg/kg orally (one hour prior to carrageenin injection) + 0.1 ml of 1% (w/v) of carrageenan subcutaneously.

Group-5: Received NAT at 750mg/kg orally (one hour prior to carrageenin injection) + 0.1 ml of 1% (w/v) of carrageenan subcutaneously.

Group-6: Received Diclofenac Na at 30mg/kg orally (one hour prior to carrageenin injection) + 0.1 ml of 1% (w/v) of carrageenan subcutaneously.

The paw edema volumes were measured using plethysmometer at various time intervals like 1, 2, 3, 6 and 24 hr after carrageenin injection. The hind paw edema inhibition at different doses of MRG, NAT and Diclofenac Sodium was calculated by comparing with vehicle treated control rats.

Following formula was used:

% inhibition of paw edema =

$$\frac{(Vt-Vo)_{\text{control}} - (Vt-Vo)_{\text{treated}}}{(Vt-Vo)_{\text{control}}} \times 100$$

Where,

Vt is the rat paw volume at time 't'.

Vo is the initial rat paw volume (before carrageenin injection)

$(Vt-Vo)_{\text{control}}$ is edema produced in control group and

$(Vt-Vo)_{\text{treated}}$ is edema produced in treatment groups.

The result was compared with control group.

2.5.2 Subacute model of inflammation Cotton Pellet Granuloma Formation in Rats⁶:

Albino Wistar rats weighing in the range of 120- 200gm were divided into six groups of 6 each. Four sterile cotton pellets (10mg) were implanted subcutaneously in the ventral region 2 on either side, in each rat under light ether anesthesia. MRG and NAT was 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.

Group-1: Served as control, which received 1% Sodium CMC solution at 1ml/kg daily for 8 days following subcutaneous implantation of cotton pellet.

Group-2: Received MRG daily at 250mg/kg orally for 8 days following subcutaneous (S.C) implantation of cotton pellet.

Group-3: Received MRG daily at 750mg/kg orally for 8 days following subcutaneous (S.C) implantation of cotton pellet.

Group-4: Received NAT daily at 250mg/kg orally for 8 days following subcutaneous (S.C) implantation of cotton pellet.

Group-5: Received NAT daily at 750mg/kg orally for 8 days following subcutaneous (S.C) implantation of cotton pellet.

Group-6: Received Diclofenac Na daily at 10mg/kg orally for 8 days following subcutaneous (S.C) implantation of cotton pellet.

On 9th day, the animals were sacrificed, cardiac puncture was carried out, the cotton pellets along with granuloma tissue removed and weighed immediately for wet weight. Then pellets were dried in an incubator at 60°C until a constant weight was obtained. The granuloma tissue formation and exudate formation was calculated.

Following formula was used:

Measure of granuloma tissue formation = constant dry weight – initial weight of pellet

Measure of exudates formation = wet weight - constant dry weight of pellet

The blood collected was allowed to coagulate at room temperature and serum separated by centrifugation at 2500rpm for 15min. The serum separated was analyzed for lysosomal enzymes such as Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT) and Alkaline Phosphatase (ALP). The liver collected was homogenized and assayed for lipid peroxidation.

Statistical analysis:

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

3. RESULTS

3.1 Acute Toxicity Studies

MRG and NAT did not show any toxic or deleterious effects upto 5000mg/ kg oral dose. As the rats were administered the maximal possible dose, the LD₅₀ value of the MRG and NAT could not be determined. (Table -1 and 2)

3.2 Anti inflammatory Activity

3.2.1 Acute Model

Effect on carrageenin induced hind paw edema in rat:

In acute anti-inflammatory model i.e. carrageenin induced paw edema in rats MRG at dose of 750mg/kg and 250mg/kg and NAT at dose of 750mg/kg caused significant inhibition of paw edema viz : 52%, 28 % and 45% respectively as compared to control at 6th hr after

carrageenin injection. (Figure no.1 , Table No.3)

3.2.2 Subacute Model

Cotton pellet granuloma

In cotton pellet granuloma the MRG and NAT was found to be effective at exudatory and granulatory phases of inflammation. MRG at 750mg/kg and 250mg/kg was found to inhibit granuloma wet weight by 27 and 14% respectively, while NAT at 750mg/kg inhibition was found to be 22%. MRG at 750mg/kg was found to inhibit granuloma formation by 26%, while at 250mg/kg inhibition was found to be 14%. NAT at 750mg/kg dry weight inhibition was found to be 18%. (Figure no.2)

3.2.3 Determination of Various Biochemical Parameters in Subacute model

3.2.3.1 Aspartate Aminotransferase (SGOT)

The MRG at 750 mg/kg dose significantly reduced the level of lysosomal enzyme SGOT by 24 %, while NAT at 750 mg/kg reduced the respective parameter by 23% (Figure no.3)

3.2.3.2 Alanine Aminotransferase (SGPT)

The MRG at 750 mg/kg dose significantly reduced the level of lysosomal enzyme SGPT by 32 %, while NAT at 750 mg/kg reduced the respective parameter by 24% (Figure no.5.3)

3.2.3.3 Alkaline Phosphatase (ALP)

The MRG at 750 mg/kg and 250mg/kg dose significantly reduced the level of lysosomal enzyme ALP by 42 and 24 % respectively, while NAT at 750 mg/kg reduced the respective parameter by 36% (Figure no.3)

3.2.3.4 Estimation of Lipid peroxides Formed in vivo

The MRG at 750 mg/kg and 250mg/kg dose significantly reduced the level of lipid peroxide by 61 and 40 % respectively, while NAT at 750 mg/kg and 250 mg/kg reduced the respective parameter by 52 and 30% respectively. (Figure no.4)

4. DISCUSSION AND CONCLUSION

We have tried to evaluate the anti-inflammatory activity of MRG and NAT 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 LD₅₀ value of both drugs could not be determined.

In acute anti-inflammatory model carrageenin induced paw edema in albino rats the formation of paw edema depends upon the release of kinins and polymorpho leukocytes with their proinflammatory mediators including prostaglandins. The development of edema in paw of rats is a biphasic event. The initial phase during the 1st hour is attributed to release of histamine and serotonin, while the 2nd phase occurs after 3-4 hrs, which is said to be essentially mediated by prostaglandins⁷. The MRG at doses of 750 and 250mg/kg exhibited significant inhibition of paw edema in a dose dependent manner while NAT shows

significant inhibition of paw edema at a dose of 750mg/kg but did not show significant activity at 250mg/kg. The activity was pronounced at the 6th hour of inflammatory response, which corresponds to the phase of prostaglandins and thereby may exhibit inhibitory activity on cyclooxygenase enzyme.

In order to assess the efficacy of MRG and NAT against exudative and proliferative phase of inflammation in which tissue degeneration and fibrosis occur, the widely used cotton pellet granuloma test was employed. During the repair process of inflammation, there is proliferation of macrophages, neutrophils, fibroblasts and multiplication of small blood vessels, which are the basic sources of forming a highly vascularised reddish mass, termed granulomatous tissue^{8,9}. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma whereas the dry weight corresponds to the amount of granulomatous tissue formed. The MRG at doses of 250 mg/kg, 750mg/kg and NAT at 750 mg/kg caused significant inhibition of exudation and granuloma tissue formation, when compared with control. Activity obtained for NAT at the dose of 250 mg/kg was not significant in this model.

In conclusion, the data obtained in the present investigation suggests MRG and NAT are potential anti-inflammatory agents. 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.

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Acute Toxicity Studies

1. Maharasnadi ghan (MRG)

Table 1: Toxicological observations for Maharasnadi ghan

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

++

Moderate

+++ Marked

2. *Nyctanthes arbor tristis* (NAT)**Table 2:** Toxicological observations for *Nyctanthes arbor tristis*

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

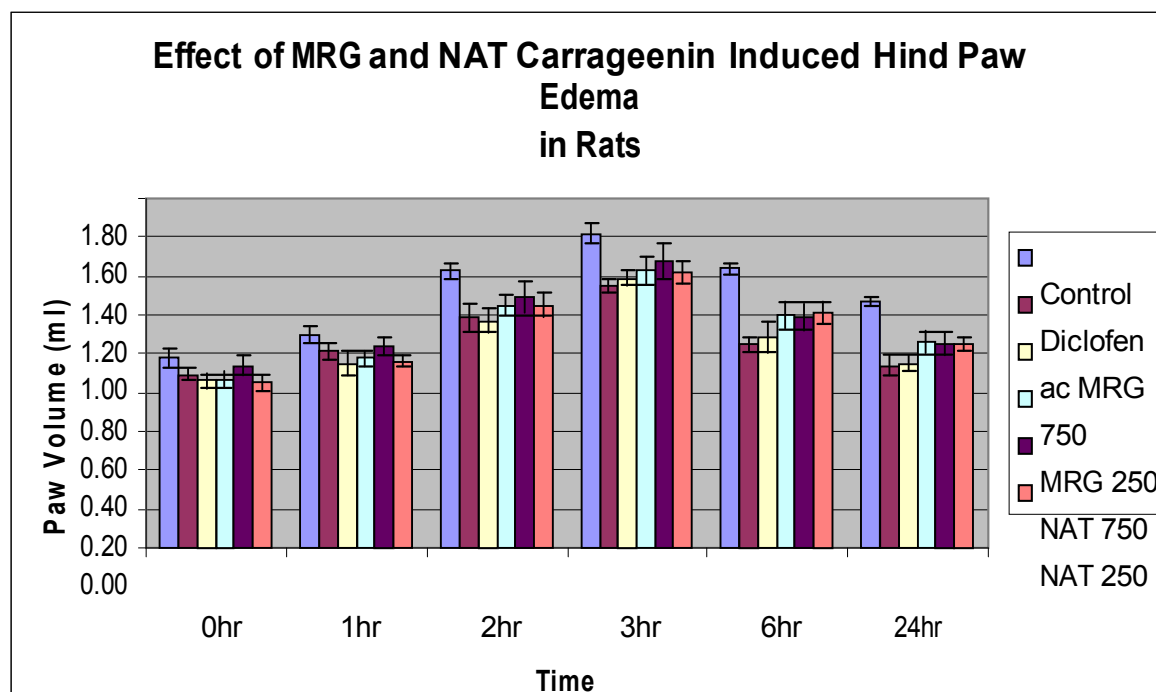
+ Mild

++ Moderate

+++ Marked

Table 3: Effect of MRG and NAT on carrageenin induced hind paw edema in rat

Sr. No.	Group/Treatment Dose (mg/kg)	Paw edema volume (ml) Time interval (in hrs)					
		0	1	2	3	6	24
1.	Control	0.98 ±0.05	1.1 ±0.04	1.43 ±0.04	1.62 ±0.05	1.44 ±0.03	1.27 ±0.02
2.	Diclofenac Sodium (30mg/kg)	0.89 ±0.03	1.01 ±0.04 (0)	1.19 ±0.07* (33)	1.35 ±0.04** (28)	1.05 ±0.03** (65)	0.94 ±0.05** (82.00)
3.	MRG (750mg/kg)	0.86 ±0.04	0.95 ±0.06 (25)	1.17 ±0.06* (31)	1.39 ±0.04* (17)	1.08 ±0.08** (52)	0.95 ±0.04** (68)
4.	MRG (250mg/kg)	0.87 ±0.04*	0.98 ±0.04 (8)	1.25 ±0.05 (15)	1.43 ±0.07 (12)	1.2 ±0.07* (28)	1.01 ±0.06** (51)
5.	NAT (750mg/kg)	0.94 ±0.05	1.04 ±0.05 (16)	1.29 ±0.08 (22)	1.48 ±0.09 (15)	1.19 ±0.07* (45)	1.05 ±0.06** (62)
6.	NAT (250mg/kg)	0.85 ±0.04	0.96 ±0.03 (8)	1.25 ±0.06 (11)	1.42 ±0.06 (10)	1.21 ±0.06 (21)	1.05 ±0.04* (31)

**Fig. 1:** Effect of MRG and NAT on carrageenin induced hind paw edema in rats

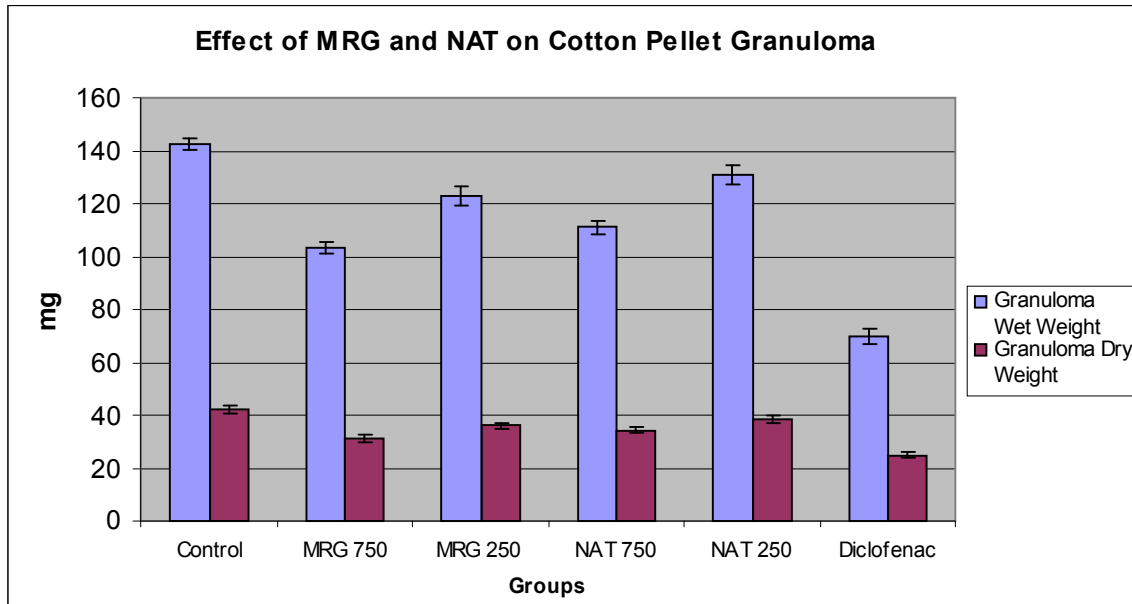


Fig. 2: Effect of MRG and NAT on Cotton Pellet granuloma in albino rats

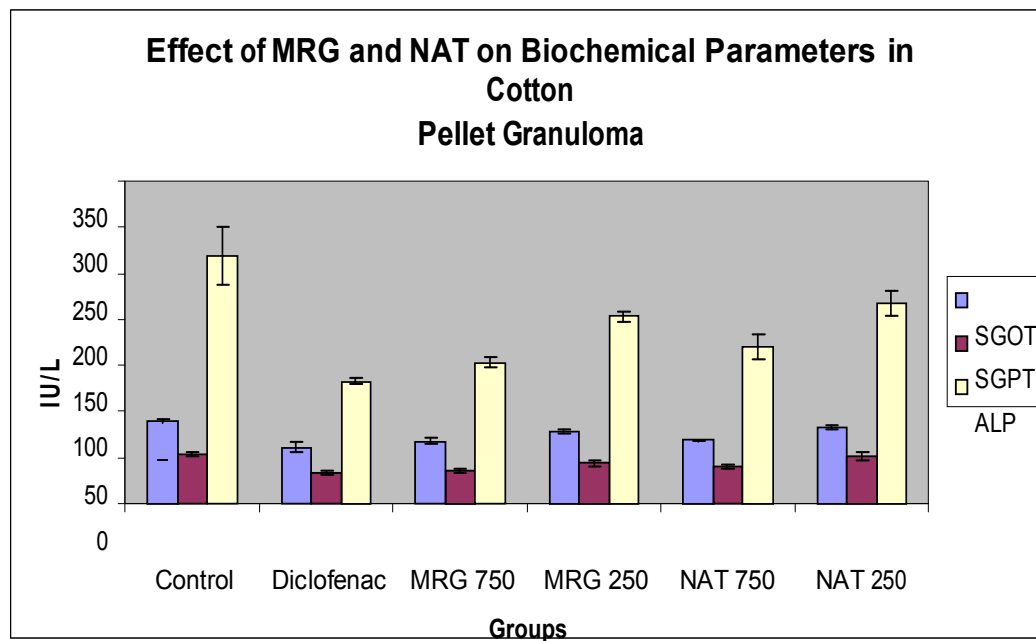


Fig. 3: Effect on serum enzyme levels in cotton pellet induced granuloma model

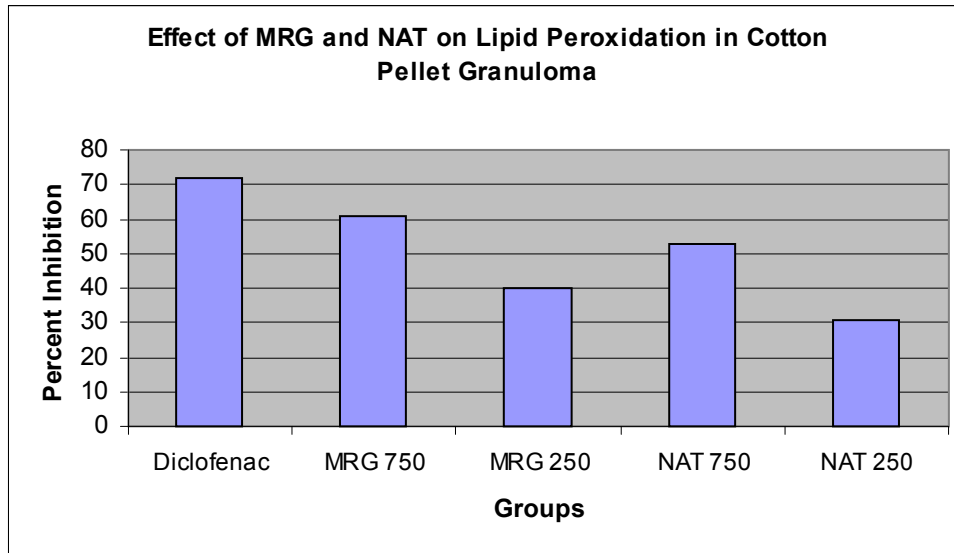


Fig 4: Effect on lipid peroxidation in cotton pellet induced granuloma model