

## Anti-Arthritic Activity of Plant *Acalypha Indica* Extract

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### ABSTRACT

*Acalypha Indica* is a species of plant having catkin type of inflorescence. It occurs throughout tropical Africa and South Africa, in India and Sri Lanka, as well as in Yemen and Pakistan.<sup>1</sup> This plant is held in high esteem in traditional Tamil Siddha medicine as it is believed to rejuvenate the body. Pharmacological investigation has shown that the plant has potent anti-bacterial, anti-fungal, anti-inflammatory, anti-osteoporotic, anti-oxidant, neuro protective, wound healing, post-coital antifertility activities. The present review article attempt to summarize the anti-arthritic activity of the plant<sup>2</sup>.

Anti-arthritic activity evaluation of different plant extracts against *Mycobacterium* induced developed arthritis was carried out. The results showed moderate to marked inhibitory effect against different extracts. The extract M-P06A001 showed an inhibition of 17.85% paw swelling in injected paw and 33.53% paw swelling in un-injected paw. M-P06-A001 showed an inhibition of 41.96% paw swelling in the injected paw and 39.63% paw swelling in un-injected paw. Standard drug Ibuprofen administered at 100mg/kg body weight produced inhibition of 49.10% paw swelling in injected paw and 43.90% swelling in un-injected paw. Both plant showed inhibitory effect on elevated alkaline phosphatase<sup>3</sup>.

Rheumatoid arthritis is a systemic autoimmune disease characterized by articular inflammation that eventually leads to the destruction of joints<sup>4</sup>. Rheumatoid arthritis (RA) is an autoimmune disease that affects approximately 1% of the population. Prevalence of RA increases with age, approaching 5% in women over the age of 55. The incidence and prevalence of RA is 2-3 times greater in women than in men. Effective treatment of RA has been impeded by a paucity of accurate diagnostic and prognostic tests, owing in part to the heterogeneity of the disease.

The present work is aimed to evaluate the anti-arthritic effect of plant *Acalypha Indica* extract by *Mycobacterium* induced arthritis in rats. The extracts showed inhibition in paw swelling in injected as well as un-injected paw which was compared with the standard drug ibuprofen. Both

the plant extract showed inhibitory effect on elevated alkaline phosphatase level.<sup>5</sup>

**Keywords:** Euphorbiaceae, Acalyphine, Pharmacological, and Anti-arthritic Activity.

## INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease of unknown etiology. The disease is characterized by articular inflammation and by the formation of an inflammatory and invasive tissue, rheumatoid pannus that eventually leads to the destruction of joints. Analgesia (painkillers) and anti-inflammatory drugs<sup>6</sup>, including steroids are used to suppress the symptoms, while disease-modifying antirheumatic drugs (DMARDs), newer therapies such as anti-tumour necrosis factor (TNF)- $\alpha$  therapy (etanercept, infliximab and adalimumab), anti-CD20 therapy (rituximab) and abatacept are often required to inhibit or halt the underlying immune process. However, all of these agents are associated with numerous side effects. In recent days, researchers are directed towards traditional system of medicine for the discovery of drugs that are long acting anti-inflammatory with minimum side effects. The present work is aimed to evaluate the anti-arthritic effect of plant *Acalypha Indica* extract by Mycobacterium induced arthritis in rats<sup>7</sup>.

## MATERIALS AND METHODS

### Plant extract

Plant *acalypha Indica* extracts(IIM P06 A001 and A002)  
IIM-P006-A001-250mg/kg of *Acalypha Indica* in ethanolic extract  
IIM-P006-A002-250mg/kg of *Acalypha Indica* in water extract

### Experimental animals

Balb/c mice(8-2 weeks old, weighing between 18-22mg)

Wister rats (12-16 weeks old, weighing between 130- 150 mg)

All the studies were conducted after obtaining prior approval from the institutional ethical committee in accordance with the National Institute Of Health“ Guide for care and use of laboratory animals”.(NIH publication no.86-93,1985). IIM /CA/ 1045/08 (Registration number that was approved for animals for this project.)

### Equipments used

Volume differential meter model 7101' screw gauge, plethysmometer

### Chemicals used

Ibuprofen (standard drug), mycobacterium tuberculosis, liquid paraffin, alkaline phosphate kit, gum acacia.

### Anti-arthritic activity evaluation (prophylactic assay)<sup>8</sup>

Chronic arthritis was induced in rats by injection of 0.05ml of 5%w/v suspension of heat killed mycobacterium tuberculosis, homogenized in liquid paraffin in left hind paw. The administration of the test compound was started one day before the injection of mycobacterium and continued till day 13. The volume of paw was measured on alternate days and the 1% inhibition was determined on day 13.

### Biochemical Assays<sup>9</sup>

### Alkaline phosphates (ALKP)

Elevation of alkaline phosphatase in plasma is found in hepatitis alkaline phosphatase activity has been reported in severe anemia, scurvy, kwashiorkor and cretinism.

#### Procedure

The reagent kit intended to use for in-vitro quantitative determination of Alkaline phosphatase in plasma according to the recommendation of the German Society for clinical Chemistry. The sample and the reconstituted reagent should be brought to room temperature prior to use. 30 µl of the sample was added to 1ml of reconstituted reagent, mix and read immediately.

### SGOT

Organs rich in SGOT are liver, heart, skeletal muscles. When these organs are damaged serum SGOT levels rises in proportion to severity of damage.

#### Procedure

The reagent kit intended to use for in vitro quantitative determination of SGOT activity in serum according to the recommendations of International Federation of Clinical Chemistry. The sample and the reconstituted reagent should be brought to room temperature prior to use. 100µl of sample was added to 1ml of reconstituted reagent, mix and read immediately.

### SGPT

Elevation of SGPT (ALT) activity is found in liver and kidney diseases like infectious or toxic hepatitis, infectious mononucleosis and cirrhosis. SGPT levels may be decreased in patients undergoing long term hemodialysis without supplemental vitamin therapy.

#### Procedure

The reagent kit intended to use for in vitro quantitative determination of SGOT activity in serum according to the recommendations of International Federation of Clinical Chemistry. The sample and the reconstituted reagent should be brought to room temperature prior to use. 100µl of sample was added to 1ml of reconstituted reagent, mix and read immediately.

### Anti-arthritic activity and evaluation<sup>10</sup>

In the experiment, one group of animals were kept as control and given only vehicle while another group or received a standard drug for comparison of activity and authenticity of the experiment. Paw volume was measured at different time intervals using a volume differential meter model 7101. Mean increase in the paw volume and standard error of the mean for each group was calculated and the results were expressed as percent inhibition of oedema as compared to control drugs. Drugs were freshly prepared as fine homogenized suspension in 20% gum acacia (w/v) for administration.

### Paw oedema measurement<sup>11</sup>

Paw oedema was measured by using volume differential meter in rats and by screw gauze in mice. Readings were taken both in injected and un-injected paws. Paw volume was measured in millilitres using plethysmometre. The initial paw volume was measured plethysmographically (in ml). Plant extract and ibuprofen were administered once daily for the duration of the experiment starting a day before the injection. Oes calculated on 13 day. The percentage increase in the paw volume over the initial reading was calculated. The paw is marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. This increase in paw volume in group 2, 3, 4 and 5 were compared with group 1.

The percentage inhibition of edema volume was calculated by using the formula,

$$\text{Percentage inhibition} = [1 - V_t/V_c] \times 100$$

$V_t$  = increase in paw volume in treated groups

$V_c$  = increase in paw volume in control groups

## RESULTS

See table 1, 2 & fig. 1-7.

## DISCUSSION

Anti-arthritic activity evaluation of different plant extracts against Mycobacterium induced developed arthritis was carried out. *Anti-arthritic activity evaluation was performed using prophylactic assay as well as biochemical assay. Biochemical assay such as ALKP, SGPT and SGOT was performed.* Anti-arthritic activity evaluation of plant extracts against Mycobacterium induced arthritis in injected and un-injected paws of rats was determined. Initial and final paw volume, oedema and percentage inhibition was determined<sup>12</sup>.

The results showed moderate to marked inhibitory effect against different extracts. The extract M-P06A001 showed an inhibition of 17.85% paw swelling in injected paw and 33.53% paw swelling in un-injected paw. M-P06-A001 showed an inhibition of 41.96% paw swelling in the injected paw and 39.63% paw swelling in un-injected paw. Standard drug Ibuprofen administered at 100mg/kg body weight produced inhibition of 49.10% paw swelling in injected paw and 43.90% swelling in un-injected paw. Both plant showed inhibitory effect on elevated alkaline phosphatase<sup>13</sup>.

## CONCLUSION

Rheumatoid arthritis is a systemic autoimmune disease characterized by articular inflammation that eventually leads to the destruction of joints<sup>14</sup>. The plant extract obtained from *Acalypha indica* used commonly in traditional Indian system of medicine produced an excellent *in vitro* anti-arthritic activity when tested using standard methods. However, treatment with plant extracts although may be have some unpredictability in the effectiveness; being non-toxic, side effect less alternative, purified plant extracts and their isolated phytoconstituents can be very useful against rheumatoid arthritis. Further to corroborate the *in vitro* anti-arthritic activity, studies are to be carried out *in vivo* to confirm their activity and also the active principles has to be identified and isolated to explore the possible mechanism by which this plant acts in protecting from autoimmune disease, rheumatoid arthritis.

The present work is aimed to evaluate the anti-arthritic effect of plant *Acalypha Indica* extract by Mycobacterium induced arthritis in rats<sup>15</sup>. The extracts showed inhibition in paw swelling in injected as well as un-injected paw which was compared with the standard drug ibuprofen. Both the plant extract showed inhibitory effect on elevated alkaline phosphatase level<sup>16</sup>.

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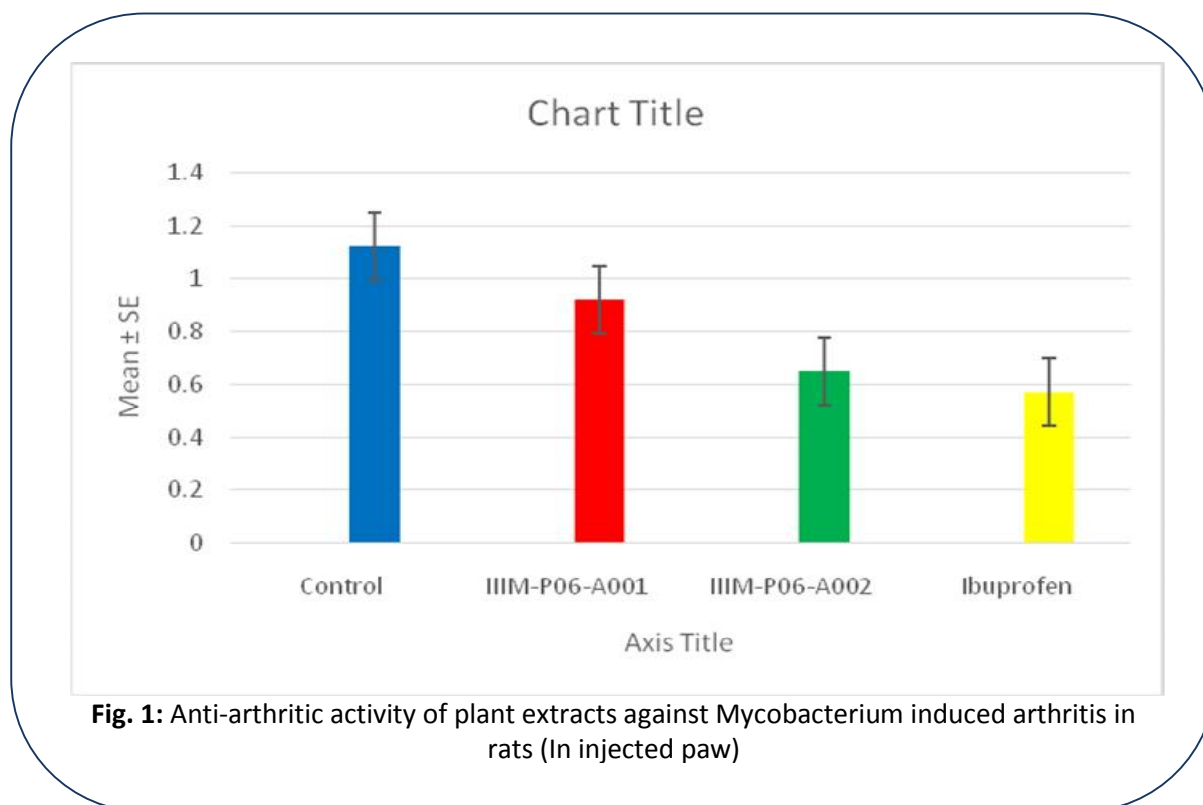
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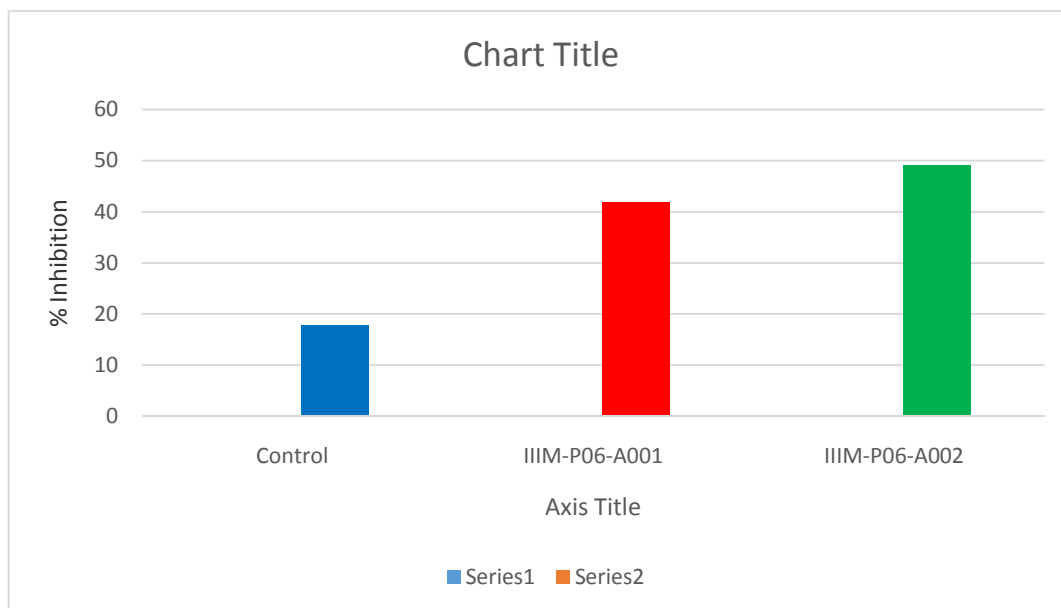
**Table 1.** Anti-arthritic activity evaluation of plant extracts against Mycobacterium induced arthritis in rats (in injected paw)

Treatment	Dose mg/kg	Initial paw vol (ml) mean $\pm$ SE DAY 0	Final paw vol (ml) mean $\pm$ SE DAY 13	Oedema mean $\pm$ SE DAY 13	% Inhibition
Control	-	1.10 $\pm$ 0.05	2.22 $\pm$ 0.07	1.12 $\pm$ 0.02	-
IIIM-P06-A001	250	1.0 $\pm$ 0.06	1.98 $\pm$ 0.04	0.92 $\pm$ 0.08	17.85
IIIM-P06-A001	250	1.10 $\pm$ 0.05	1.75 $\pm$ 0.05	0.65 $\pm$ 0.05	41.96
Ibuprofen	100	1.04 $\pm$ 0.02	1.61 $\pm$ 0.03	0.57 $\pm$ 0.04	49.10

**Table 2.** Anti-arthritic activity evaluation of plant extracts against Mycobacterium induced arthritis in rats (un-injected paw)

Treatment	Dose mg/kg	Initial paw vol (ml) mean $\pm$ SE DAY 0	Final paw vol (ml) mean $\pm$ SE DAY 13	Oedema mean $\pm$ SE DAY 13	% Inhibition
Control		1.10 $\pm$ 0.05	2.74 $\pm$ 0.01	1.64 $\pm$ 0.02	-
IIIM-P06-A001	250	1.06 $\pm$ 0.02	2.15 $\pm$ 0.13	0.09 $\pm$ 0.17	33.53
IIIM-P06-A001	250	1.08 $\pm$ 0.02	2.07 $\pm$ 0.11	0.99 $\pm$ 0.20	39.63
Ibuprofen	100	1.04 $\pm$ 0.04	1.96 $\pm$ 0.21	0.92 $\pm$ 0.16	43.90



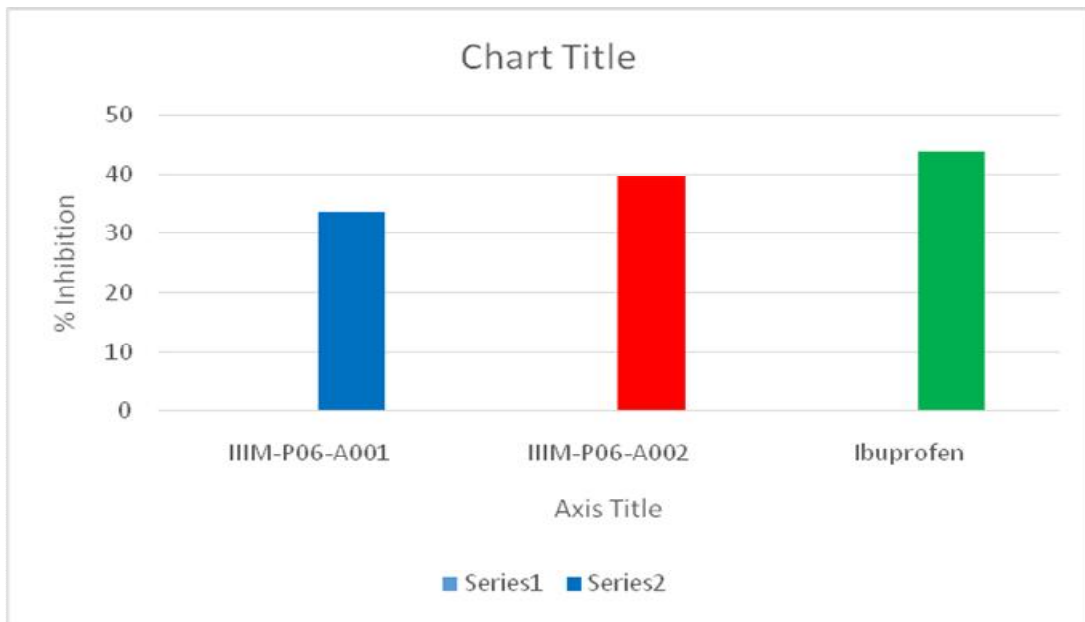


**Fig. 2:** Percentage inhibition of arthritis by plant extracts, paw volume taken after 13 days of *Mycobacterium tuberculosis* injection. (Injected paw)

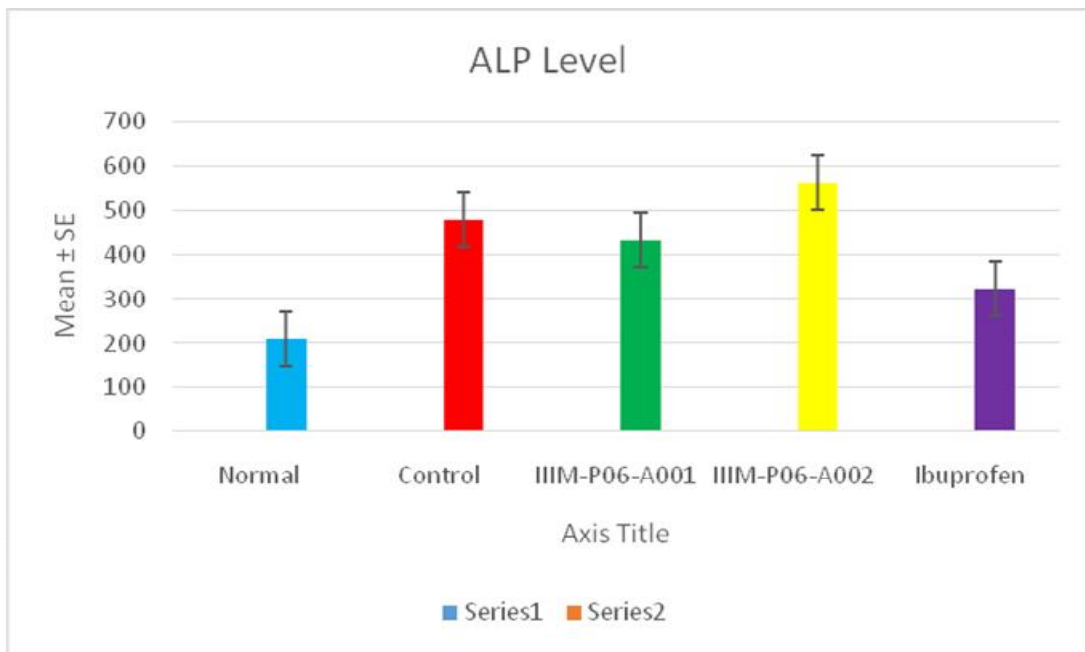


**Fig. 3:** Anti-arthritic activity evaluation of plant extracts against *Mycobacterium* induced arthritis in rats (un-injected paw)



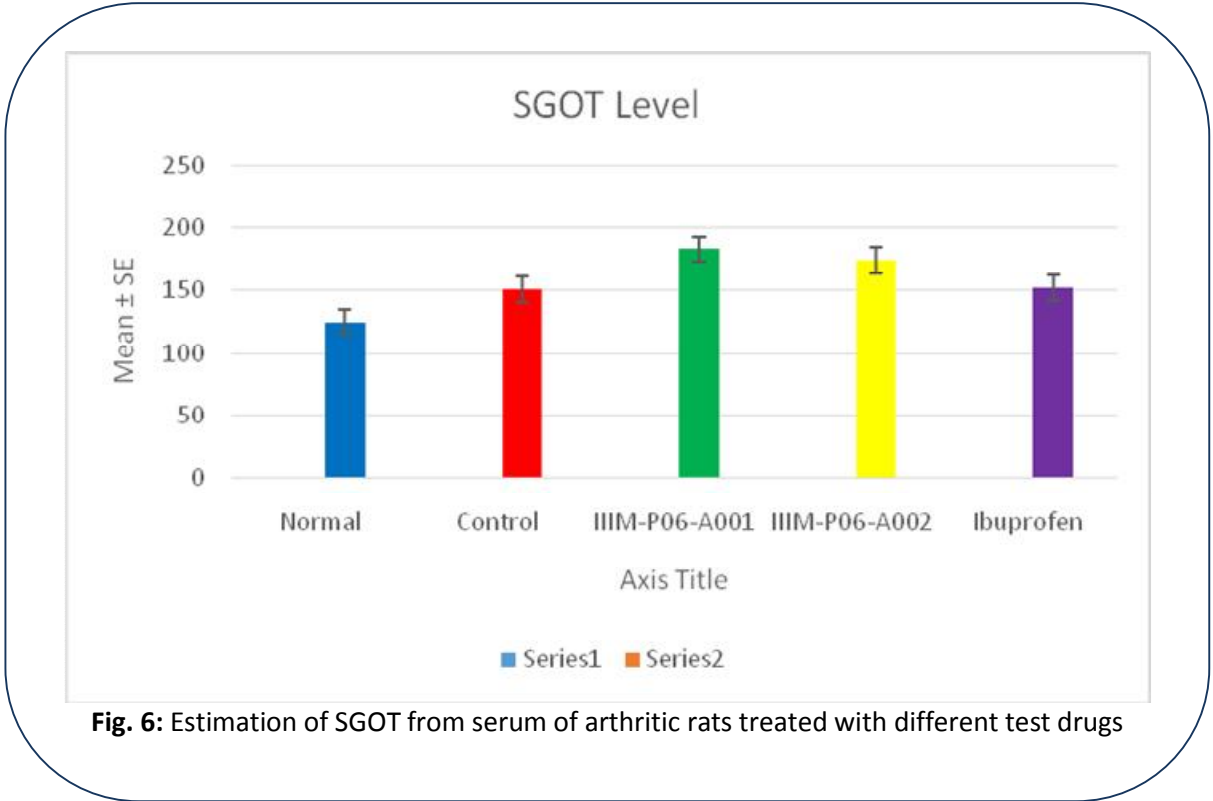


**Fig. 4:** Percentage inhibition of arthritis by plant extracts, paw volume taken after 13 days of Mycobacterium tuberculosis injection. (Un-injected paw)

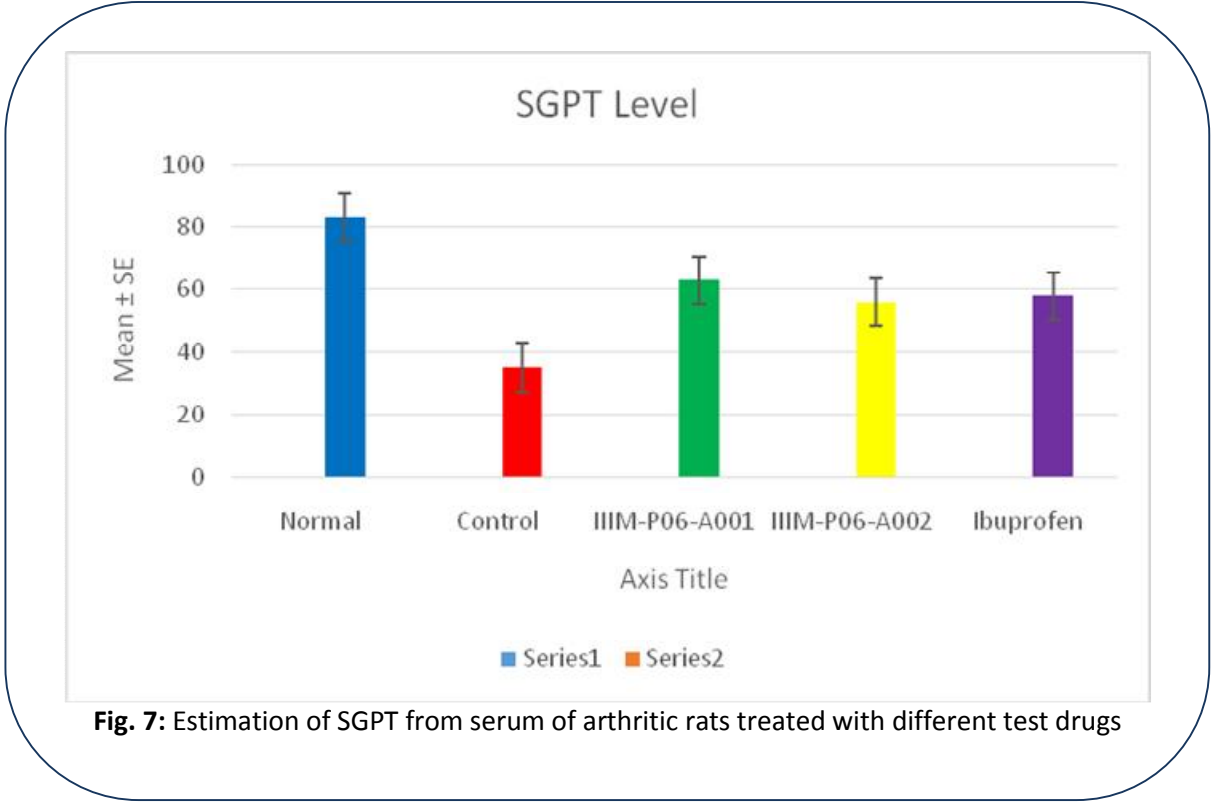


**Fig. 5:** Estimation of ALP from serum of arthritic rats treated with different test drugs.





**Fig. 6:** Estimation of SGOT from serum of arthritic rats treated with different test drugs



**Fig. 7:** Estimation of SGPT from serum of arthritic rats treated with different test drugs