Chapter – 4

STUDIES ON TOXICITY AND ANTI-INFLAMMATORY ACTIVITY OF THE LEAVES OF *BISCHOFIA JAVANICA* AND *FRAXINUS FLORIBUNDA*

4.1 Introduction:

4.1.1 Toxicity Studies:

Toxicity studies are performed to find the safety of the drug before its screening for pharmacological actions. Acute, sub-acute, chronic and special toxicity studies are performed in at least two species usually a rodent and a non rodent to assure the safety of the drug before application of clinical trial. In the studies, first acute toxicity tests on mice are performed. The compound is administered only once orally or parenterally at various dose level to groups of five to ten mice of equal number of both the sexes keeping them on fasting overnight. Before the actual LD_{50} determination, pilot study is made on a small group of mice, mainly to select the dose range for the subsequent study. The compound is administered intravenously to pairs of mice in ascending and widely spaced doses. The injected mice should be observed continuously for two hours and then occasionally for further four hours and finally for overnight and mortality recorded. By observing the behavior of the injected animals carefully, valuable indications of the action of the drug may be obtained, which may be a guidance for further testing. Convulsions during injection can usually be felt as tremor in the tail, or as paddling of the feet.

A simple method which uses minimal number of animals known as the up and down or staircase method may also be applied. Two mice are injected with a particular dose and observed for 24 hrs for any mortality. The subsequent doses are then increased by a factor 1.5 if the dose was tolerated and decreased by a factor 0.7 if it was lethal. Once the approximate LD_{50} range between the maximum non-lethal and minimum lethal dose is found, a final reliable LD_{50} assay is planned using at least three or four dose levels within this range with larger number of animals in each group. It is performed by administering a single dose of a drug starting from minimal dose to each group consists of 10 animals preferably mice.[1] The animals are observed for lethality for 24 hrs, by which Median Lethal Dose (LD_{50}) of a drug can be determined. LD_{50} can also be determined by many other methods like Litchfield and Wilcoxon method, Miller unitary method and Trevan's

method. [2-4] It is useful for the determination of therapeutic index. Sub-acute study is performed by administering the drug daily for 2-3 weeks until toxic signs are observed. Here hematological and biochemical monitoring is carried out and blood level of the compound is checked to ensure its absorption. In chronic studies the drug is given daily for six months and the parameters like body weight, food intake, renal function, hepatic function, hematological and biochemical parameters are studied every 2 weeks. Special tests are performed to study the teratogenic and carcinogenic effect of the drug.

4.1.2 Anti-inflammatory Studies:

One of the common method of screening anti-inflammatory activity is inhibiting the edema which is induced by the injection of phlogistic agents (irritants). Carrageenan is a marine derived polysaccharide which is injected (0.1ml of 0.2% soln) on the hind limb of rats and the swelling or edema is measured by plethysmographically. Other commonly used phlogistic agents are given below [5]

0.05ml undiluted fresh egg white
0.1ml of 1% ova albumin solution
0.1ml of 1% formalin
0.1ml of 1-3% dextran solution
0.1ml of 0.1% trypsin solution
0.1ml of 0.1% collagenase solution etc.

The subcutaneous implantation of cotton pellets into experimental animals was originally introduced to asses the effects of drugs on the formation of granulation tissue. The initial phase of the response occurs within the first 3 hr and is described as transudative phase, which is characterized by the accumulation of low protein exudates. The second phase of the response involves increase in vascular permeability and the subsequent appearance of exudates. The third phase of the response is described as the proliferative phase and is characterized by the appearance of collagen in the granuloma on day 4 after pellet implantation. This model has also been used to determine the contribution of several inflammatory mediators to the cellular response seen in implanted sponges. [6]

Rheumatoid arthritis is a chronic inflammatory disorder of unknown etiology characterized by joint pain and swelling with multiple extra-articular manifestations. The adjuvant diseases, the manifestation of which is synovitis, similar to that of rheumatoid arthritis can be produced in rats injecting mineral oil containing cell protein from mycobacterium. In addition similar conditions in joint tissue can be produced in rabbits with streptococcal infections and in mice with mycobacterial infections.[7] The biochemical characteristics in Rheumatoid arthritis are due to chronic inflammatory parameters such as perturbations in serum proteins, low plasma level of histidine, increased fluid volume, color change of serum from pale yellow to white or dark yellow viscosity falls and poor clotting.

4.1.3 Evaluation of Mode of Action of Anti-Inflammatory Drugs:

There are different mechanisms by which drugs produce the anti-inflammatory activity. The most common mechanism is arachidonic acid pathway, where the target enzymes are COX and LOX. In experimental models both *in-vivo* and *in-vitro* assays are performed to study the mode of action. *In-vitro* COX enzyme assay,COX-2 protein extraction and analysis play the key role in screening of new anti-inflammatory drug. [8-9] In *in-vivo* assays inhibition of arachidonic acid induced inflammation in paw edema model in rats using standard drugs the probable mechanism for COX and LOX inhibition (dual inhibition) can be established for a test drug.

4.1.4 Anti-nociceptive Studies:

The mediators involved in production of inflammation have got close relationship to those induce pain and pyrexia. Hence the anti-inflammatory agents can have analgesic and anti-pyretic activities. In animal model pain can be induced by physical, thermal or chemical methods in mice or rats. On basis of their mechanism of action we can classify them as centrally acting and peripherally acting analgesic agents. Several methods are available for testing central analgesic activity. [10]

1. Heffner's tail clip method in mice
2. Tail flick or radiant heat method in mice
3. Tail immersion method in mice
4. Formalin test in rats

Tail immersion method has been developed selectively for morphine like compounds. The procedure is based on the observation that morphine like drugs are capable of prolonging the reaction time of the typical tail-withdrawal reflex in mice induced by immersing the end of the tail in water at 55°C.

The peripheral analgesic agents possess the anti-inflammatory activity and are some cases the antipyretic activity. The mode of action has been elucidated as an inhibition of COX in the PGs pathway. Pain is induced by injection of irritants in to the peritoneal cavity of mice. The animals react with a stretching behavior characterized by a wave of contraction of the abdominal musculature followed by extension of the hind limbs. Intra-peritoneal injection of 0.25 ml of 0.02% aqueous solution of phenylquinone or 0.6% acetic acid (1ml/100g b.w) in mice produces writhing with in 3-10 minutes. [11]

4.1.5 Antipyretic Studies:

Pyrexia is associated with the release of inflammatory mediators like PGs, TNF α and Interleukins in the hypothalamus region of brain. The drugs which are inhibiting the release of these mediators can reduce the elevated body temperature. To evaluate anti-pyretic activity, fever is induced in rabbits or rats by injection of lipo-polysaccharides or Brewer's yeast. [12]

4.2 Acute Toxicity Studies of the Leaves of *B.javanica*:

4.2.1 Animals:

Swiss albino mice (20-35g) of either sex were used in the study. They were housed in large propylene cages and kept at 22±2°C in 12 h dark-light cycle. The animals were fed with pellet food and water *ad libitum*. All animals were acclimatized to the laboratory environment for at least one week before the experimental session. The experimental

procedure performed on animals was approved by the Institute Animal Ethics Committee (IAEC) *No: HPI/07/60/IAEC/0006.*

4.2.2 Materials:

Methanol extract of leaves of *B.javanica* (BJ) was prepared (described in **Chapter 3)** and suspended in 2% CMC as test drug. All other chemicals were procured from local firm and were of analytical grade.

4.2.3 Method:

Swiss albino mice were divided in to ten groups of ten in each. The different doses of the test drug at 100, 200, 400, 800, 1600, 2400, 3200, 4000, 4800 and 5600mg/kg b.w p.o were administered to the groups I to X and the physiological signs and lethality were observed for 24hrs of drug administration. The percent mortality values are converted to probit values by reading the corresponding probit units from the probit table. LD_{50} study was done by Miller and Tainter method. [1], [13]

4.2.4 Results of the Acute Toxicity Studies of the Leaves of *B.javanica* :

The results of the acute toxicity studies of BJ are presented in the **Table 4.1 and 4.2.** On application of probit values, the LD_{50} for the methanol extract of *B.javanica* has been calculated as 3200mg/kgb.w. The physiological signs observed during this studies were decreased motor activity, depression and skeletal muscle relaxation >800mg/kgb.w. Approximately $1/10^{th}$ of the LD_{50} can be chosen as starting dose for pharmacological studies. [1] Hence the doses of 200,400 and 600mg/kg were selected for pharmacological screening. The LD_{50} of the isolated compound Fredelin-3α-acetate was considered from the earlier studies reported. [14]

4.3 Anti-inflammatory Studies for the Leaves of *Bischofia javanica*:

4.3.1 Animals:

Wistar albino rats (150-250g) of either sex were used in the studies of ant-inflammatory activity of the test drug.

4.3.2 Instruments and Chemicals:

Plethysmometer (Model-520-R, IITC Life sciences, USA) was available in the laboratory of HPI, Aspirin, phenidone, carrageenan, Freund's adjuvant and arachidonic acid were purchased from Sigma Chemicals. All other chemicals were of analytical grade and procured locally.

4.3.3 Methods:

4.3.3.1 Carrageenan Induced Paw Edema in Rats:

Acute inflammation was produced by injecting 1% solution of carrageenan in to plantar surface of rat on right hind paw at the dose of 0.1ml per 100g body weight. [15] Wistar albino rats were divided in to seven groups of six each. A 2% solution of CMC at a dose of 0.1ml/100g/p.o was administered to the group 1 as control. The methanol extract of *B.javanica* was administered to the animals of group 2,3and 4 at the dose range of 200,400 and 600mg/kg/p.o respectively and the isolated compound Fredelin-3α-acetate was administered at the doses of 10 and 20 mg/kg p.o to the groups 5 and 6 respectively. The standard drug aspirin was administered to the group 7 at the dose of 100mg/kg/p.o. After 30 minutes of these administration, carrageenan solution was injected to the animals of all the groups, the paw edema was measured at the intervals of 1, 2, 3 and 4h using Plethysmometer.

4.3.3.2 Cotton Pellet Induced Granuloma in Rats:

Two autoclaved cotton pellets weighing 10±1mg were implanted in to both sides of the groin region of each rat [16]. The animals were divided into seven groups of six each. The control group (group1) was received 2% CMC solution at the dose of 0.1ml/100g/p.o.The test groups (group 2, 3 and 4) were treated with methanol extract of *B.javanica* at the doses of 200, 400 and 600mg/kg/p.o respectively. The isolated compound Friedelin-3α-acetate was administered at the doses of 10,20mg/kg to the groups 5 and 6.The standard group (group7) received aspirin at the dose of 100mg/kg p.o daily for seven days. After seven days animals were sacrificed by cervical dislocation and the cotton pellets along with the granuloma tissues were collected and dried in a oven at

60°C, weighed and resulted weights were compared with the control. The percentage inhibition of granuloma by the test drug was determined.

4.3.3.3 Freund's Adjuvant Induced Arthritis in Rats:

Male albino rats were divided in to seven groups six in each. On day one 0.1ml of Freund's adjuvant was injected in to the right plantar pad of each rat. The control group (group1) received 0.1ml/100g/p.o of 2% CMC solution consecutively for 21 days. The test groups i.e. 2, 3 and 4 were treated with the methanol extract of *B.javanica* at the dose of 200, 400, 600mg/kg/p.o respectively. The isolated compounds Friedelin-3α-acetate was administered at the doses of 10, 20 mg/kg for 21 days to the group 5 and 6 respectively. The standard group received aspirin at the dose 100mg/kg/p.o for 21 days. [17] The paw edema of each group was measured using Plethysmometer on day 1 before and on day 22 after the drug administration. The percentage inhibition of arthritis (Paw edema) was calculated.

4.3.3.4 Arachidonic acid Induced Paw Edema in Rats:

Paw edema was induced by single injection of 0.1ml of 0.5% arachidonic acid in 0.2 M carbonate buffer (pH8.4) in to right hind paw of rats. [18] The methanol extract of *B.javanica* was administered at the dose range of 200,400, 600 mg/kg, p.o to the animals of group 2,3 and 4 respectively and the isolated compound Friedelin-3α-acetate was injected to group 5 and 6 at the doses of 10, 20 mg/kg p.o respectively. The standard drug phenidone at the dose of 200mg/kg p.o was administered to standard group (group7) and 2% CMC 0.1ml/100g p.o to the control group (group1) at 2hrs before the arachidonic acid injection to different groups of animals. The paw volume was measured 1hr after arachidonic acid injection using plethysmometer. The paw volumes among the different group of animals were compared.

4.3.4 Results of the Anti-inflammatory Studies of the Leaves of *B.javanicai*:

The results of the carrageenan induced paw edema studies are presented in **Table 4.3** and **Figure 4.1**. The percentage inhibition of paw edema in the animals was determined using the following equation

$$\% \text{ Inhibition of Paw Edema} = \frac{Vc-Vt}{Vc} \times 100$$

Where, Vc -- Paw edema of control animals

Vt --- Paw edema of drug treated animals

The percent inhibition of paw edema at different doses of the pure compound along with the control and the standard is presented graphically in **Figure4.1**. From the figure it is evident that the test drugs inhibited the paw edema in dose and time dependent manner. BJ at the dose of 600mg/kg b.w inhibited the paw edema by 41.83% at 4 hr of drug administration. Friedelin-3α-acetate the isolated compound of *B.javanica* at the dose of 20mg/kg b.w inhibited the paw edema by 53.08% more potentially than standard drug aspirin (45.83%) at 4 hr.

The result of the studies of cotton pellet induced granuloma in rats is produced in **Table 4.4**. The percentage inhibition of granuloma in rats has been determined and from the study it is evident that the isolated compound Friedelin-3α-acetate at 20mg/kg b.w showed maximum (55%) compared to the standard drug aspirin (60%) followed by the methanol extract at 600mg (50.27%).The activity index is shown in the **Figure 4.2.**

Table 4.5 depicted that BJ and isolated compound of *B.javanica* have significant anti-inflammatory effect in Freund's adjuvant induced arthritis. BJ at the dose of 600mg/kg b.w inhibited arthritis by 50.24% and the isolated compound at the dose of 20mg/kg b.w inhibited by higher rate (65.21%) than the standard drug aspirin (61.2%) at the dose of 100mg/kg b.w. It also revealed from the graphical representation presented in the **Figure 4.3**. The mode of action of anti-inflammatory activity was studied by arachidonic acid

induced paw edema in rats, the results are shown in **Table 4.6** and **Figure 4.4.** The crude drug BJ inhibited the archidonic acid induced paw edema in rats significant manner (75.27%) at the dose of 600mg/kg b.w compared to the isolated compound Friedelin-3α-acetate 20mg/kg b.w (59.27%).The standard dual-inhibitor drug Phenidone has the maximum inhibition of arachidonic acid induced paw edema (77.81%) in this study.

4.4 Anti-nociceptive Studies of the Leaves of *Bischofia javanica*:

4.4.1 Animals:

Swiss albino mice (20-40g) of either sex were used in the studies of anti-nociceptive activity of the test drug.

4.4.2 Drugs and Chemicals:

Aspirin was purchased from Sigma Chemicals. Pentazocine was purchased from Ranbaxy Laboratories, New Delhi, India. All other chemicals were of analytical grade and procured locally.

4.4.3 Methods:

4.4.3.1 Tail-immersion Test:

Swiss albino mice of either sex (20-35g) were used in the study. Animals were divided in to seven groups of six each. Group1 received 0.1ml of 2%CMC solution as control. The test drug methanol extract of *B.javanica* was administered at the dose of 200,400 and 600mg/kg p.o to the groups 2, 3, 4 respectively and the isolated compounds Friedelin-3α-acetate at the doses of 10,20mg/kg was administered to the group 5 and 6 respectively. The standard drug Pentazocine was administered to the group 7 at the dose of 5mg/kg i.p .The animals were held in a suitable restrainer with tail extending out. The tail up to 5 cm was then dipped into a pot of water maintained at 55±0.1°C. [19] The time taken for the mouse to withdraw the tail in seconds was considered as the reaction time. The reading was recorded after 30, 60 &120 min of administration of drugs and control.

4.4.3.2 Writhing Test:

Animals were divided in to seven groups of six each. The control group (group1) received 0.1 ml of 2% CMC solution p.o. The test groups (group 2, 3, 4) were treated with 200, 400 & 600 mg /kg/p.o. of methanol extract of *B.javanica* and the isolated compound Friedelin-3α-acetate (group 5 and 6) at the doses of 10,20mg/kg. The standard group (group 7) received aspirin at the dose of 100mg/ kg/p.o. After 30 minutes of drug administration 0.7% acetic acid was given to each mouse at the dose of 0.1 ml/10g bodyweight i.p [20]. Number of writhing (stretching episode of belly and touching on the floor when moving) was counted for 15 minutes. The percentage inhibition of writhing offered by the drug samples to the animals was calculated and compared with the control.

4.4.4 Results of the Anti-nociceptive Studies of the Leaves of *B.javanica*:

The results of anti-nociceptive studies revealed that the test drugs have significant anti-nociceptive activities in both Tail-immersion test and Writhing test. In Tail-immersion test, BJ at the dose of 600mg/kg b.w showed tail-flick for 12 and 13 seconds at 60 and 120 minutes which was higher than the isolated compound Friedelin-3α-acetate at the dose of 20mg/kg b.w (9 and 12 sec) as shown in **Table 4.7**. Tail-fick response is also depicted graphically in the **Figure 4.5**. Writhing test results are presented in **Table 4.8** and **Figure 4.6**. In this study the inhibition of writhing by the test drug BJ at 600mg/kg is 50.26% compared to the standard drug aspirin 57.50% at a dose of 100mg/kg. The isolated compound Friedelin-3α-acetate protected writhing by 45.25% at a dose of 20mg/kg.

4.5 Anti-pyretic Studies of the Leaves of *B.javanica*:

4.5.1 Animals:

Wistar albino rats (150-250g) of either sex were used in the studies of ant-inflammatory activity of the test drug.

4.5.2 Instruments and Chemicals:

Digital tele thermometer (Shreeji Pharmaceutical Instruments, Mumbai). Paracetamol and Brewer's yeast were purchased from Sigma Chemicals. All other chemicals were of analytical grade and procured locally.

4.5.3 Method:

4.5.3.1 Brewer's Yeast Induced Pyrexia in Rats:

Albino rats of either sex (150-250g) were chosen for the study. Initial rectal temperature was recorded using digital tele-thermometer. Brewer's yeast in the aqueous suspension (20%) was administered (10ml/kg) subcutaneously in the neck region of all the rats. The raise in body temperature by 1°C or more were included in the study. [21] Animals were divided in to seven groups of six in each. After recording rectal temperature at 18hr, the test drug methanol extract of *B.javanica* at the doses of 200,400 and 600mg/kgp.o and the isolated compound Friedelin-3α-acetate at the doses of 10,20mg/kg p.o was administered to the groups 2,3,4,5 and 6 respectively. The standard drug paracetamol 100mg/kg p.o was administered to the standard group (group 7). The control group (group 1) was administered 0.1ml of 2% CMC suspension in water. The rectal temperature was measured at 1hr interval up to 3hr after administration of drugs.

4.5.4 Results of Anti-pyretic Studies of the Leaves of *B.javanica*

Anti-pyretic studies of the leaves of *B.javanica* is represented in the **Table 4.9**. The isolated compound of *B.javanica* at the dose of 20mg/kgb.w and the standard drug paracetamol at the dose of 100mg/kg b.w decreased the elevated rectal temperature of rats after Brewer's yeast administration by 1.1°C after 1 hr of (19hr after yeast administration) of drug administration whereas the crude extract BJ decreased the elevated rectal temperature by 0.9°C. After 2hr of drug administration all the drugs have decreased the rectal temperature within the range of 0.2 to 0.4°C.

4.6 Acute Toxicity Studies of the Leaves of *Fraxinus floribunda:*

4.6.1 Animals:

Swiss albino mice (20-35g) of either sex were used in the study. They were housed in large propylene cages and kept at 22±2°C in 12 h dark-light cycle. The animals were fed with pellet food and water *ad libitum*. All animals were acclimatized to the laboratory environment for at least one week before the experimental session.

4.6.2 Materials:

Methanol extract of leaves of *F.floribunda* (FF) was prepared (described in **Chapter 3)** and suspended in 2% CMC as test drug. All other chemicals were procured from local firm and were of analytical grade.

4.6.3 Method:

Swiss albino mice were divided in to ten groups of ten in each. The different doses of the test drug at 100, 200, 400, 600, 800, 1600, 2400, 3200, 4000 and 4800mg/kg b.w p.o were administered to the groups I to X. The physiological signs and lethality were observed for 24hrs of drug administration. The percent mortality values are converted to probit values by reading the corresponding probit units from the probit table. LD_{50} study was done by Miller and Tainter method. [1], [13]

4.6.4 Results of the Acute Toxicity Studies of the Leaves of *F.floribunda*

The results of the acute toxicity studies of FF are presented in the **Table 4.10 and 4.11.** On application of probit values, the LD_{50} for the methanol extract of *F.floribunda* has been evaluated as 2,400mg/kg b.w. The physiological signs observed during acute toxicity studies were stimulation followed by depression, increased secretion and hypnosis at the dose of >600mg/kgb.w. Approximately $1/10^{th}$ of the LD_{50} can be chosen as starting dose for pharmacological studies. [1] Hence the doses of 100, 200 and 400mg/kg b.w were selected for pharmacological screening. The LD_{50} of the isolated compound β-amyrin was considered from the earlier studies reported. [22-23]

4.7 Anti-inflammatory Studies of the Leaves of *F.floribunda:*

4.7.1 Animals:

Wistar albino rats (150-250g) of either sex were used in the studies of ant-inflammatory activity of the test drug.

4.7.2 Instruments and Chemicals:

Plethysmometer (Model-520-R, IITC Life sciences, USA) was available in the laboratory of HPI. Aspirin, phenidone, carrageenan, Freund's adjuvant and arachidonic acid were purchased from Sigma Chemicals. All other chemicals were of analytical grade and procured locally.

4.7.3 Methods:

4.7.3.1 Carrageenan Induced Paw Edema in Rats:

Acute inflammation was produced by injecting 1% solution of carrageenan in to plantar surface of rat on right hind paw at the dose of 0.1ml per 100g body weight. [15] Wistar albino rats were divided in to seven groups of six each. A 2% solution of CMC at a dose of 0.1ml/100g/p.o was administered to the group 1 as control. The methanol extract of *F.floribunda* was administered to the animals of group 2,3and 4 at the dose range of 100,200 and 400mg/kgb.w p.o respectively and the isolated compound β-amyrin was administered at the doses of 10 and 20 mg/kg p.o to the groups 5 and 6 respectively. The standard drug aspirin was administered to the group 7 at the dose of 100mg/kg/p.o. After 30 minutes of these administrations, carrageenan solution was injected to the animals of all the groups, the paw edema was measured at the intervals of 1, 2, 3 and 4h using Plethysmometer.

4.7.3.2 Cotton Pellet Induced Granuloma in Rats:

Two autoclaved cotton pellets weighing 10±1mg were implanted in to both sides of the groin region of each rat. [16] The animals were divided into seven groups of six each. The control group (group 1) received 2% CMC solution at the dose of 0.1ml/100g/p.o.The test groups (group 2, 3 and 4) were treated with methanol extract of *F.floribunda* at the doses of 100, 200 and 400mg/kg/p.o respectively. The isolated

compound β-amyrin was administered at the doses of 10, 20 mg/kg to the groups 5 and 6. The standard group (group 7) received aspirin at the dose of 100mg/kg p.o daily for seven days. After seven days animals were sacrificed by cervical dislocation and the cotton pellets along with the granuloma tissues were collected and dried in an oven at 60°C, weighed and resulted weights were compared with the control. The percentage inhibition of granuloma by the test drug was determined.

4.7.3.3 Freund's Adjuvant Induced Arthritis in Rats:

Male albino rats were divided in to seven groups six in each. On day one 0.1ml of Freund's adjuvant was injected in to the right plantar pad of each rat. The control group (group1) received 0.1ml/100g/p.o of 2% CMC solution consecutively for 21 days. The test groups i.e. 2, 3 and 4 were treated with the methanol extract of *F.floribunda* at the dose of 100, 200, 400mg/kg/p.o respectively. The isolated compounds β-amyrin was administered at the doses of 10, 20 mg/kg for 21 days to the group 5 and 6 respectively. The standard group received aspirin at the dose 100mg/kg/p.o for 21 days. [17] The paw edema of each group was measured using Plethysmometer on day 1 before and on day 22 after the drug administration. The percentage inhibition of arthritis (Paw edema) was calculated.

4.7.3.4 Arachidonic acid Induced Paw Edema in Rats:

Paw edema was induced by single injection of 0.1ml of 0.5% arachidonic acid in 0.2 M carbonate buffer (pH8.4) in to right hind paw of rats. [18] The methanol extract of *F.floribunda* was administered at the dose range of 100,200, 400 mg/kg, p.o to the animals of group 2,3 and 4 respectively and the isolated compound β-amyrin was administered to group 5 and 6 at the doses of 10, 20 mg/kg p.o respectively. The standard drug phenidone at the dose of 200mg/kg p.o was administered to standard group (group7) and 2% CMC 0.1ml/100g p.o to the control group (group1) at 2hrs before the arachidonic acid injection to different groups of animals. The paw volume was measured 1hr after arachidonic acid injection using Plethysmometer. The paw volumes among the different group of animals were compared.

4.7.4 Results of the Anti-inflammatory Studies of the Leaves of *F.floribunda:*

The results of the carrageenan induced paw edema studies are presented in **Table 4.12.** The percentage inhibition of paw edema in the animals were determined using the following equation.

$$\% \text{ Inhibition of Paw Edema} = \frac{Vc-Vt}{Vc} \times 100$$

Where, Vc -- Paw edema of control animals

Vt -- Paw edema of drug treated animals

The percent inhibition of paw edema at different doses of the pure compound along with the control and the standard is presented graphically in **Figure 4.7.** From the figure it is evident that the test drugs inhibited the paw edema in dose and time dependent manner. FF at the dose of 200 and 400mg/kg b.w inhibited the paw edema by 51.40% and 55.14% at 4 hr of drug administration. β-amyrin the isolated pure compound of *F.floribunda* at the dose of 20mg/kg b.w inhibited the paw edema by (69.02%) more potentially than standard drug aspirin at the dose of 100mg/kg b.w p.o (64.48%) at 4 hr.

The results of the studies of cotton pellet induced granuloma in rats is produced in **Table 4.13.** The percentage inhibition of granuloma in rats has been determined and from the study it is evident that the isolated compound β-amyrin at 20mg/kgb.w showed maximum (49.33%) compared to the standard drug aspirin (41.88%) followed by the methanol extract at 400mg (35.72%). The activity index is shown in the **Figure 4.8.**

Table 4.14 depicted that FF and the isolated compound of *F.floribunda* have significant anti-inflammatory effect in Freund's adjuvant induced arthritis. FF at the dose of 400mg/kg b.w inhibited arthritis by 13.91% and the isolated compound β-amyrin at the dose of 20mg/kg b.w inhibited by higher rate (51.23%) than the standard drug aspirin (45.36%) at the dose of 100mg/kg b.w. It also revealed from the graphical representation presented in the **Figure 4.9.** The mode of action of anti-inflammatory activity was studied by arachidonic acid induced paw edema in rats, the results are shown in **Table**

4.15 and **Figure 4.10**. The crude drug FF at the dose of 400mg/kg b.w and the isolated compound at the dose of 20mg/kg b.w inhibited the archidonic acid induced paw edema in rats by 30.86 and 34.97%.

4.8 Anti-nociceptive Studies of the Leaves of *Fraxinus floribunda*:

4.8.1 Animals:

Swiss albino mice (20-40g) of either sex were used in the studies on anti-nociceptive activity of the test drug.

4.8.2 Drugs and Chemicals:

Aspirin was purchased from Sigma Chemicals. Pentazocine was purchased from Ranbaxy Laboratories, New Delhi, India. All other chemicals were of analytical grade and procured locally.

4.8.3 Methods:

4.8.3.1 Tail-immersion Test:

Swiss albino mice of either sex (20-35g) were used in the study. Animals were divided in to seven groups of six each. Group1 received 0.1ml of 2%CMC solution as control. The test drug methanol extract of *F.floribunda* was administered at the dose of 100,200 and 400mg/kg p.o to the groups 2, 3, 4 respectively and the isolated compounds β-amyrin at the doses of 10,20mg/kg was administered to the group 5 and 6 respectively. The standard drug pentazocine was administered to the group 7 at the dose of 5mg/kg i.p. The animals were held in a suitable restrainer with tail extending out. The tail up to 5 cm was then dipped into a pot of water maintained at 55±0.1°C. [19] The time taken for the mouse to withdraw the tail in seconds was considered as the reaction time. The reading was recorded after 30, 60 &120 min of administration of drugs and control.

4.8.3.2 Writhing Test:

Animals were divided in to seven groups of six each. The control group (group1) received 0.1 ml of 2% CMC solution p.o. The test groups (group 2, 3 and 4) were treated with 100, 200 & 400 mg /kg/p.o. of methanol extract of *F.floribunda* and the isolated

compound β-amyrin at the doses of 10,20mg/kg. The standard group (group 7) received aspirin at the dose of 100mg/ kg/p.o. After 30 minutes of drug administration 0.7% acetic acid was given to each mouse at the dose of 0.1 ml/10g bodyweight i.p. [20] Number of writhing (stretching episode of belly and touching on the floor when moving) was counted for 15 minutes. The percentage inhibition of writhing offered by the drug samples to the animals was calculated and compared with the control.

4.8.4 Results of the Anti-nociceptive Studies of the Leaves of *F.floribunda*:

The results of the anti-nociceptive studies revealed that the test drugs have significant anti-nociceptive activities in both Tail-immersion test and Writhing test. In Tail-immersion test, FF at the dose of 400mg/kg b.w showed tail-flick for 12 and 13 seconds of tail immersion at 60 and 120 minutes after drug administration which was higher than the isolated compound β-amyrin at the dose of 20mg/kg b.w (9.8 and 12 sec) shown in **Table 4.16**. Tail-fick response is also depicted graphically in the **Figure 4.11**. Writhing test results are presented in **Table 4.17** and **Figure 4.12.** In this study the inhibition of writhing by the test drug FF at the dose of 400mg/kg b.w was 48.27% compared to the standard drug aspirin 62.68% at a dose of 100mg/kg. The isolated compound β-amyrin protected writhing by 58.35% at a dose of 20mg/kg.

4.9 Anti-pyretic Studies of the Leaves of *F.floribunda*

4.9.1 Animals:

Wistar albino rats (150-250g) of either sex were used in the studies of ant-inflammatory activity of the test drug.

4.9.2 Instruments and Chemicals:

Digital tele thermometer (Shreeji Pharmaceutical Instruments, Mumbai). Paracetamol and Brewer's yeast were purchased from Sigma Chemicals. All other chemicals were of analytical grade and procured locally.

4.9.3 Method:

4.9.3.1 Brewer's Yeast Induced Pyrexia in Rats:

Albino rats of either sex (150-250g) were chosen for the study. Initial rectal temperature was recorded using digital tele thermometer. Brewer's yeast in 20% aqueous suspension was administered (10ml/kg) subcutaneously in the neck region of all the rats. The raise in body temperature by 1°C or more were included in the study. [21] Animals were divided in to seven groups of six in each. After recording rectal temperature at 18hr, the test drug methanol extract of *F.floribunda* at the doses of 100, 200 and 400mg/kg p.o and the isolated compound β-amyrin at the doses of 10,20mg/kg p.o was administered to the groups 2,3,4,5 and 6 respectively. The standard drug paracetamol 100mg/kg p.o was administered to the standard group (group 7). The control group (group 1) was administered 0.1ml of 2% CMC solution in water. The rectal temperature was measured at 1hr interval up to 3hr after administration of drugs.

4.9.4 Results of Anti-pyretic Studies of the Leaves of *F.floribunda:*

Anti-pyretic studies of the leaves of *F.floribunda* is represented in the **Table 4.18**.The isolated compound β-amyrin at the dose of 20mg/kgb.w and the standard drug paracetamol at the dose of 100mg/kg b.w decreased the elevated rectal temperature of rats after Brewer's yeast administration by 1.2 and 1.3°C after 1 hr of (19hr after yeast administration) of drug administration whereas the crude extract FF decreased the elevated rectal temperature by 0.7°C. After 2hr of drug administration all the drugs have decreased the rectal temperature with in the range of 0.1 to 0.3°C.

Table 4.1 Acute toxicity study of methanol extract of the leaves *B. javanica* in mice

Group No	Treatment (mg/kg,p.o)	Log dose	Mice died (No)	% Mortality	Corrected	Probit
1	BJ-100	2.000	0	0	2.5*	3.04
2	BJ-200	2.3010	0	0	2.5*	3.04
3	BJ-400	2.6020	0	0	2.5*	3.04
4	BJ-800	2.9030	1	10	10	3.72
5	BJ-1600	3.2041	2	20	20	4.16
6	BJ-2400	3.3802	3	30	30	4.48
7	BJ-3200	3.5051	5	50	50	5.00
8	BJ-4000	3.6020	8	80	80	5.84
9	BJ-4800	3.6812	9	90	90	6.25
10	BJ-5600	3.7481	10	100	97.5*	6.96

BJ-Methanol extract of *Bischofia javanica*

*Corrected for 0% dead = 100(0.25/n) and 100%dead = 100 X (n-0.25/n)

n – Number of animals, (n=10)

Table 4.2 Observed physiological signs and lethality during acute toxicity study of the methanol extract of *B.javanica* in mice

Treatment (mg/kg,p.o)	Observations
BJ 100-400	No specific alterations in the physiological signs
BJ 800-2400	Decreased motor activity Decreased response to Stimuli
BJ 3200-5600	Decreased motor activity Depression Skeletal muscle relaxation,(Studied by using Rota-rod apparatus)

BJ- Methanol extract of *Bischofia javanica*

Table 4.3 Effect of methanol extract of the leaves of *Bischofia javanica* on carrageenan induced paw edema in rats

Group No	Treatment (mg/kg, p.o)	Paw volume in ml Mean± SEM (% Inhibition of paw edema)			
		1hr	2hr	3hr	4hr
1	Control	1.19±0.02	1.27±0.02	1.32±0.02	1.36±0.02
2	BJ-200	1.05±0.06*(11.76)	0.99±0.05*(22.04)	0.95±0.04*(27.65)	0.89±0.04* (33.94)
3	BJ -400	0.99±0.05*(16.38)	0.97±0.07*(23.10)	0.84±0.05*(35.75)	0.81±0.05*(40.80)
4	BJ -600	1.02±0.12*(13.78)	0.93±0.03*(26.53)	0.81±0.16*(38.13)	0.79±0.10* (41.83)
5	Friedelin-3α-acetate-10	0.98±0.04*(17.64)	0.92±0.04*(26.90)	0.82±0.03*(37.75)	0.68±0.02*(49.38)
6	Friedelin-3α-acetate -20	0.95±0.01*(20.16)	0.87±0.03*(31.33)	0.78±0.05*(40.41)	0.63±0.04*(53.08)
7	Aspirin-100	0.96±0.040*(19.32)	0.90±0.019*(36.67)	0.77±0.083*(41.66)	0.73±0.260*(45.83)

Each value represents Mean± SEM, (n=6). Statistical significance test with control was done *P<0.001, BJ-Methanol extract of *Bischofia javanica*

Table 4.4 Effect of methanol extract of the leaves of *Bischofia javanica* on cotton pellet induced granuloma in rats

Group No	Treatment (mg/kg, p.o)	Weight of dry cotton pellet granuloma (mg)	Inhibition of granuloma formation (%)
1	Control (2% CMC)	36.0±2.4	-------
2	BJ-200	29.1±1.4*	19.16
3	BJ-400	22.2±1.2***	38.33
4	BJ-600	17.9±1.0***	50.27
5	Friedelin-3α-acetate-10	19.3±1.2***	46.38
6	Friedelin-3α-acetate -20	16.2±0.8***	55.00
7	Aspirin- 100	14.1±0.6***	60.83

Each value represents Mean ± SEM (n=6). Statistical significance test with control was done *P<0.05, P***<0.001, BJ- Methanol extract of *Bischofia javanica*

Table 4.5 Effect of methanol extract of the leaves of *Bischofia javanica* on Freund's adjuvant induced arthritis in rats

Group No	Treatment (mg/kg, p.o)	Paw volume in ml	% Inhibition of arthritis
1	Control(2% CMC)	2.07±0.05	----------
2	BJ-200	1.81±0.04**	12.56
3	BJ-400	1.45±0.06***	29.95
4	BJ-600	1.03±0.04***	50.24
5	Friedelin-3α-acetate-10	1.21±0.04***	41.54
6	Friedelin-3α-acetate -20	0.72 ±0.05***	65.21
7	Aspirin-100	0.80±0.05***	61.20

Each value represents Mean ± SEM (n=6). Statistical significance test with control was done **P<0.01, ***P<0.001, BJ- Methanol extract of *B.javanica*

Table 4.6 Effect of methanol extract of leaves of *B.javanica* on arachidonic acid induced inflammation in rats

Group No	Treatment mg/kg (p.o)	Paw volume in ml at 1 hr after arachidonic acid injection	% Reduction in Paw volume
1	Control (2% CMC)	2.75±0.03	----------
2	BJ-200	1.51±0.02***	45.09
3	BJ-400	1.29±0.05***	53.09
4	BJ-600	0.68±0.03***	75.27
5	Friedelin-3α-acetate-10	1.32±0.03***	52.00
6	Friedelin-3α-acetate -20	1.12±0.05***	59.27
7	Phenidone-200	0.61±0.03***	77.81

Each value represents Mean ± SEM (n=6). Statistical significance test with control was done, ***P<0.001, BJ- Methanol extract of *B.javanica*

Table 4.7 Effect of methanol extract of leaves of *Bischofia javanica* on thermally induced nociception in mice

Group No	Treatment (mg/kg, p.o)	Tail flick at 30 minutes (sec)	Tail flick at 60 minutes (sec)	Tail flick at 120 minutes (sec)
1	Control (2% CMC)	2.98±0.46	2.78±0.33	2.75±0.33
2	BJ- 200	4.05±0.34	3.78±0.46	4.73±0.14***
3	BJ -400	5.38±0.34**	6.45±0.83**	8.08±0.41***
4	BJ- 600	7.54±0.36***	12.28±1.26***	13.18±0.50***
5	Friedelin-3α-acetate-10	5.18±0.24**	6.83±0.54**	9.72±0.63***
6	Friedelin-3α-acetate -20	7.18±0.35***	9.32±0.87***	12.11±0.76***
7	Pentazocine 5 i.p	11.12±2.28***	15.43±0.81***	15.46±0.89***

Each value represents Mean± SEM, n=6. Statistical significant test with control was done *P<0.05, **P<0.01, ***P<0.001 BJ-Methanol extract of *B.javanica*

Table 4.8 Effect of methanol extract of leaves of *Bischofia javanica* on acetic acid induced writhing in mice

Group No	Treatment (mg/kg, p.o)	Number of writhing for 15 minutes	Inhibition of writhing (%)
1	Control(2% CMC)	35.53±3.09	--------
2	BJ 200	25.17±1.41*	29.15
3	BJ 400	20.00±1.93**	43.70
4	BJ 600	17.67±1.85***	50.26
5	Friedelin-3α-acetate-10	28.13±1.15*	20.82
6	Friedelin-3α-acetate -20	19.45±1.46**	45.25
7	Aspirin 100	15.17±1.36***	57.30

Each value represents Mean± SEM, n=6. Statistical significant test with control was done *P<0.05,**P<0.01,***P<0.001, BJ-Methanol extract of *B.javanica*

Table 4.9 Anti-pyretic activity of methanol extract of *Bischofia javanica* on Brewer's yeast induced pyrexia in rats

Group No	Drug(mg/kg,p.o)	Rectal Temperature in °C after yeast administration				
		0hr	18hr	19hr	20hr	21hr
1	Control(2%CMC)	37.4±0.1	38.5±0.4	38.6±0.2	38.8±0.1	38.9±0.3
2	BJ-200	37.5±0.2	38.6±0.1	38.2±0.1**	38.1±0.1***	37.9±0.3***
3	BJ-400	37.2±0.1	38.3±0.2	38.1±0.3***	37.7±0.2***	37.5±0.2***
4	BJ-600	37.3±0.2	38.5±0.4	37.6±0.4***	37.5±0.3***	37.1±0.5***
5	Friedelin-3α-acetate-10	37.6±0.1	38.7±0.3	38.1±0.5***	37.7±0.4***	37.5±0.2***
6	Friedelin-3α-acetate-20	37.7±0.1	38.9±0.3	37.8±0.2***	37.4±0.4***	37.3±0.1***
7	Paracetamol-100	37.4±0.2	38.7±0.3	37.6±0.2***	37.4±0.1***	37.3±0.3***

Each value represents Mean ± SEM, n=6. Statistical significant test was done *P<0.05, **P<0.01,***P<0.001, BJ-Methanol extract of *B.javanica*

Table 4.10 Acute toxicity study of methanol extract of the leaves of *F.floribunda* in mice

Group No	Drug (mg/kg p.o)	Log Dose	Mice Died(no)	%Mortality	Corrected	Probit
1	FF-100	2.000	0	0	2.5	3.04
2	FF-200	2.3010	0	0	2.5	3.04
3	FF-400	2.6020	0	0	2.5	3.04
4	FF-600	2.7781	1	10	10	3.72
5	FF-800	2.9030	2	20	20	4.16
6	FF-1600	3.2041	4	40	40	4.75
7	FF-2400	3.3802	5	50	50	5.00
8	FF-3200	3.5051	7	70	70	5.52
9	FF-4000	3.6020	9	90	90	6.28
10	FF-4800	3.6812	10	100	97.5	6.96

FF-Methanol extract of *Fraxinus floribunda*

*Corrected for 0% dead = 100(0.25/n) and 100%dead = 100 X (n-0.25/n)

n – Number of animals, (n=10)

Table 4.11 Observed physiological signs and lethality during acute toxicity study of the methanol extract of *F.floribunda* in mice

Group No	Drug (mg/kg, p.o)	Observations
1	FF-100-400	No specific alterations in the physiological signs
2	FF-600-2400	Increased secretions Increased motor activity followed by depression
3	FF- 3200-4800	Decreased motor activity Ataxia Hypnosis

FF-Methanol extract of *Fraxinus floribunda*

Table 4.12 Effect of methanol extract of leaves of *Fraxinus floribunda* on carrageenan induced paw edema in rats

Group No	Treatment (mg/kg,p.o)	Paw volume in ml(% Inhibition of Paw Edema)			
		1h	2h	3h	4h
1	Control(2%CMC)	1.52±0.04	1.84±0.06	2.11±0.01	2.14±0.07
2	FF-100	1.41±0.04*** (7.2)	1.34±0.01*** (27.17)	1.26±0.12*** (40.28)	1.24±0.02*** (42.05)
3	FF-200	1.32±0.01*** (13.15)	1.25±0.02*** (32.06)	1.10±0.03*** (42.86)	1.08±0.03*** (51.40)
4	FF-400	1.24±0.05*** (18.42)	1.14±0.03*** (38.04)	1.08±0.01*** (48.81)	0.96±0.01*** (55.14)
5	β-amyrin-10	1.12±0.04*** (26.31)	1.02±0.02*** (44.56)	0.93±0.04*** (55.92)	0.72±0.03*** (66.35)
6	β-amyrin-20	1.02±0.07*** (32.89)	0.94±0.03*** (48.91)	0.79±0.01*** (62.55)	0.65±0.02*** (69.62)
7	Aspirin-100	1.02±0.01*** (32.89)	0.86±0.02*** (53.26)	0.79±0.02*** (62.35)	0.76±0.05*** (64.48)

Data represent Mean ± SEM,(n=6). Statistical significance was done with control was done ***P<0.001. FF-Methanol extract of leaves of *Fraxinus floribunda*

Table 4.13 Effect of methanol extract of leaves of *Fraxinus floribunda* on cotton pellet induced granuloma in rats

Group No	Treatment (mg/kg p.o)	Weight of dry cotton pellet granuloma (mg)	% Inhibition of granuloma
1	Control (2%CMC)	37.03±1.92	-------
2	FF-100	31.90±0.64*	13.86
3	FF-200	29.01±0.78**	21.65
4	FF-400	23.80±0.77**	35.72
5	β-amyrin-10	24.65± 0.59**	33.43
6	β-amyrin-20	18.76±0.86***	49.33
7	Aspirin-100	21.52±0.82***	41.88

Data represent Mean ± SEM of 6 animals. Statistical significance with control was done *P<0.05, **P<0.01 and ***P<0.001. FF-Methanol extract of leaves of *Fraxinus floribunda*

Table 4.14 Effect of methanol extract of leaves of *Fraxinus floribunda* on Freund's adjuvant induced arthritis in rats

Group No	Treatment (mg/kg, p.o)	Paw volume in ml	% Inhibition of arthritis
1	Control(2%CMC)	1.94±0.07	----------
2	FF-100	1.75±0.02*	9.79
3	FF-200	1.71±0.02**	11.85
4	FF-400	1.67±0.01**	13.91
5	β-amyrin-10	1.18±0.02***	39.17
6	β-amyrin-20	0.94±0.02***	51.23
7	Aspirin-100	1.06±0.03***	45.36

Data represent Mean±SEM of 6 animals. *P<0.05, **P<0.01 and ***P<0.001 compared to control (student's t-test). FF-Methanol Extract of leaves of *Fraxinus floribunda*

Table 4.15 Effect of methanol extract of the leaves of *F.floribunda* on arachidonic acid induced inflammation in rats

Group No	Treatment (mg/kg, p.o)	Paw volume in (ml) at 1 hr after arachidonic acid injection	% Reduction in Paw volume
1	Control(2%CMC)	2.43±0.04	----------
2	FF-100	2.21±0.0.35^{NS}	9.05
3	FF-200	1.94±0.04*	20.16
4	FF-400	1.68±0.02*	30.86
5	β-amyrin-10	1.72±0.02*	29.21
6	β-amyrin-20	1.58±0.01*	34.97
7	Phenidone-200mg/kg i.p	0.65±0.02***	73.25

Data represent Mean ±SEM of 6 animals. NS-Non Significant, *P<0.05, and ***P<0.001 compared to control (student's t-test). FF- Methanol extract of leaves of *Fraxinus floribunda*

Table 4.16 Effect of methanol extract of leaves of *Fraxinus floribunda* on thermally induced nociception in mice

Group No	Treatment(mg/kg, p.o)	Tail flick at 30 minutes (sec)	Tail flick at 60 minutes (sec)	Tail flick at 120 minutes (sec)
1	Control(2%CMC)	2.78±0.45	2.59±0.34	2.43±0.314
2	FF 100	3.15 ± 0.34^{NS}	$4.18\pm0.46^{**}$	$4.78\pm0.14^{***}$
3	FF 200	$5.38\pm0.35^{**}$	$6.45\pm0.83^{**}$	$8.08\pm0.41^{***}$
4	FF 400	$8.54\pm0.35^{***}$	$12.28\pm1.26^{***}$	$13.18\pm0.49^{***}$
5	β-amyrin-10	$4.34\pm0.43^{**}$	$5.69\pm0.64^{**}$	$9.43\pm.47^{***}$
6	β-amyrin-20	$7.83\pm0.43^{***}$	$9.80\pm0.82^{***}$	$12.41\pm1.16^{***}$
7	Pentazocine 5 i.p	$12.12\pm2.26^{***}$	$14.43\pm0.81^{***}$	$15.46\pm0.89^{***}$

Data represent Mean ± SEM of 6 animals. ^{NS}Non Significant, $^{**}P<0.01$ and $^{***}P<0.001$ compared to control (student's t-test). FF-Methanol extract of leaves of *Fraxinus floribunda.*

Table 4.17 Effect of methanol extract of leaves of *Fraxinus floribunda* on acetic acid induced writhing in mice

Group No	Treatment(mg/kg,p.o)	Number of writhing for 20 minutes	% Inhibition of Writhing
1	Control(2%CMC)	32.51±3.14	----------
2	FF 100	25.17±1.43*	22.57
3	FF 200	22.03±1.94**	32.23
4	FF 400	16.62±1.84***	48.87
5	β-amyrin-10	18.14±1.36***	44.20
6	β-amyrin-20	13.54±1.62***	58.35
7	Aspirin 100	12.13±1.47***	62.68

Data represent Mean ± SEM of 6 animals.*P<0.05,**P<0.01 and ***P<0.001 compared to control. FF-Methanol extract of leaves of *Fraxinus floribunda*

Table 4.18 Anti-pyretic activity of methanol extract leaves of *Fraxinus floribunda* on Brewers yeast induced pyrexia in rats

Group. No	Drug (mg/kg,p.o)	Rectal Temperature in °C after yeast administration				
		0hr	18hr	19hr	20hr	21hr
1	Control(2%CMC)	37.3±0.2	38.2±0.4	38.4±0.2	38.6±0.3	38.7±0.4
2	FF-100	37.2 ± 0.3	38.3±0.3	38.2±0.3*	38.1±0.1**	37.9±0.1***
3	FF-200	37.2±0.4	38.7±0.5	38.0±0.4**	37.9±0.2***	37.8±0.2***
4	FF-400	36.9±0.2	38.3±0.2	37.6±0.1***	37.5±0.3***	37.4±0.1***
5	β-amyrin-10	37.4±0.1	38.7±0.4	37.9±0.2**	37.7±0.6***	37.7±0.2***
6	β-amyrin-20	37.5±0.2	38.9±0.1	37.7±0.1***	37.5±0.5***	37.4±0.1***
7	Paracetamol-100	37.6±0.3	38.8±0.4	37.5±0.4***	37.3±0.3***	37.2±0.2***

Data represent mean ± SEM of 6 animals.*P<0.05,**P<0.01 and ***P<0.001 compared to control. FF- Methanol Extract of leaves of *Fraxinus floribunda*

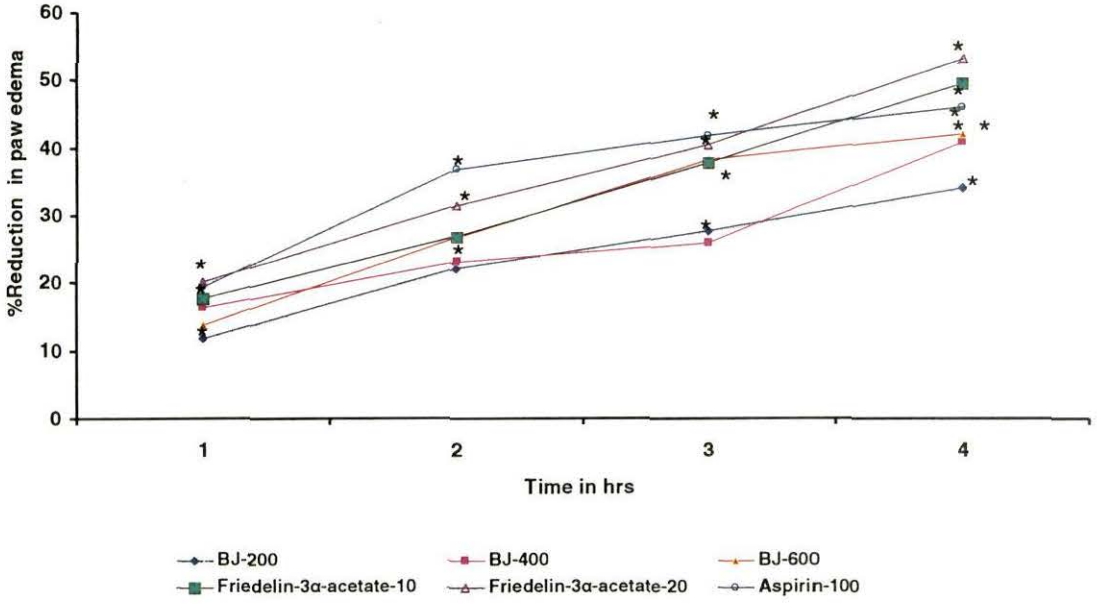

Figure 4.1 Anti-inflammatory activity of leaves of *Bischofia javanica* on carrageenan induced paw edema in rats

Data represents Mean±SEM, n=6. Statistical significance was done with the control,*P<0.001

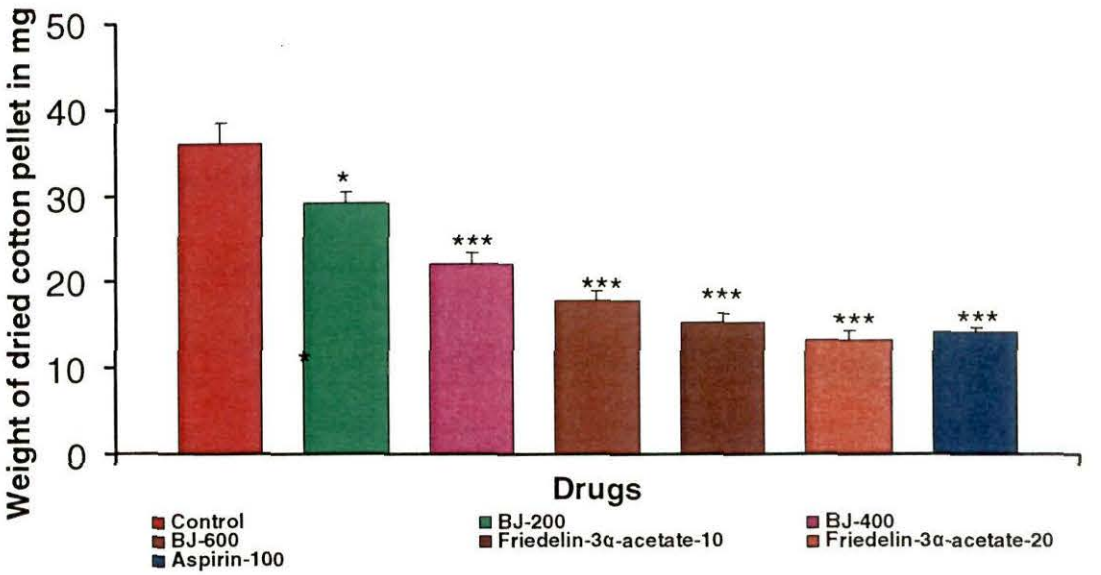

Figure 4.2 Anti-inflammatory activity of leaves of *Bischofia javanica* on cotton pellet induced granuloma in rats

Data represents Mean±SEM, n=6, Statistical significance was done with the control,*P<0.05, ***P<0.001

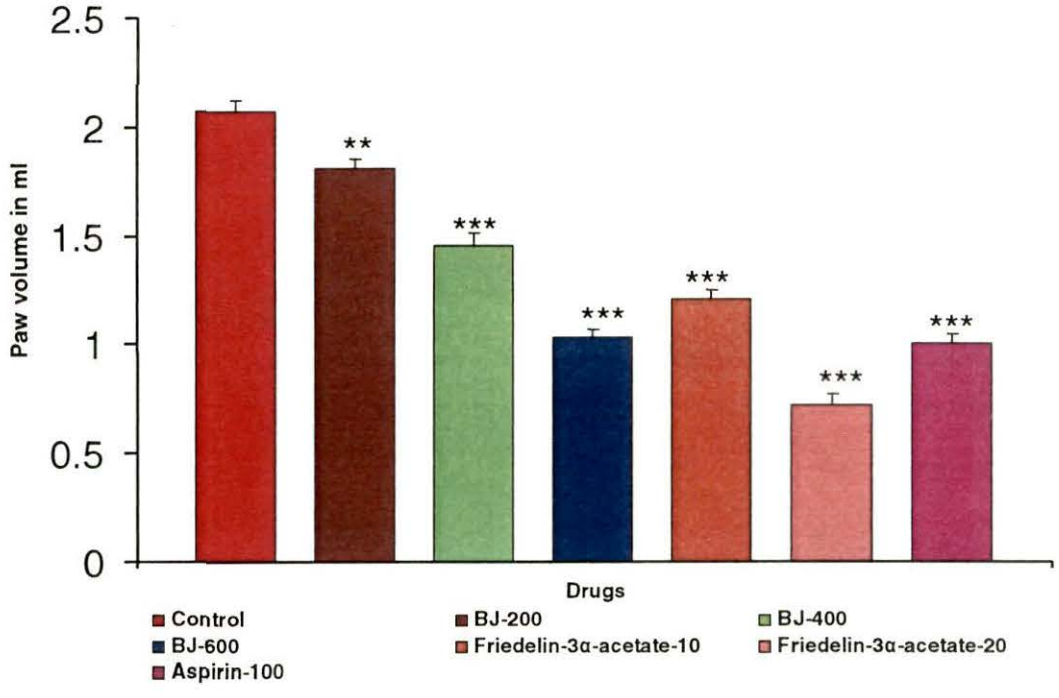

Figure 4.3 Anti-inflammatory activity of leaves of *Bischofia javanica* on Freund's adjuvant induced arthritis in rats

Data represents Mean±SEM, n=6,Statistical significance was done with the control,**P<0.01,***P<0.001

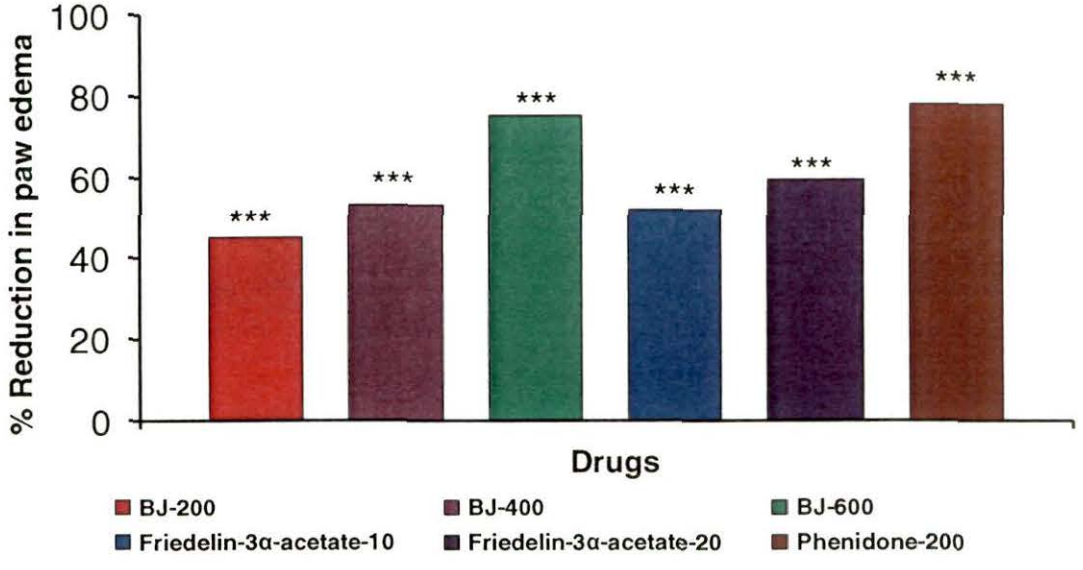

Figure 4.4 Anti-inflammatory activity of leaves of *Bischofia javanica* on arachidonic acid induced paw edema in rats

Data represents Mean±SEM,n=6,Statistical significance was done with the control

***P<0.001

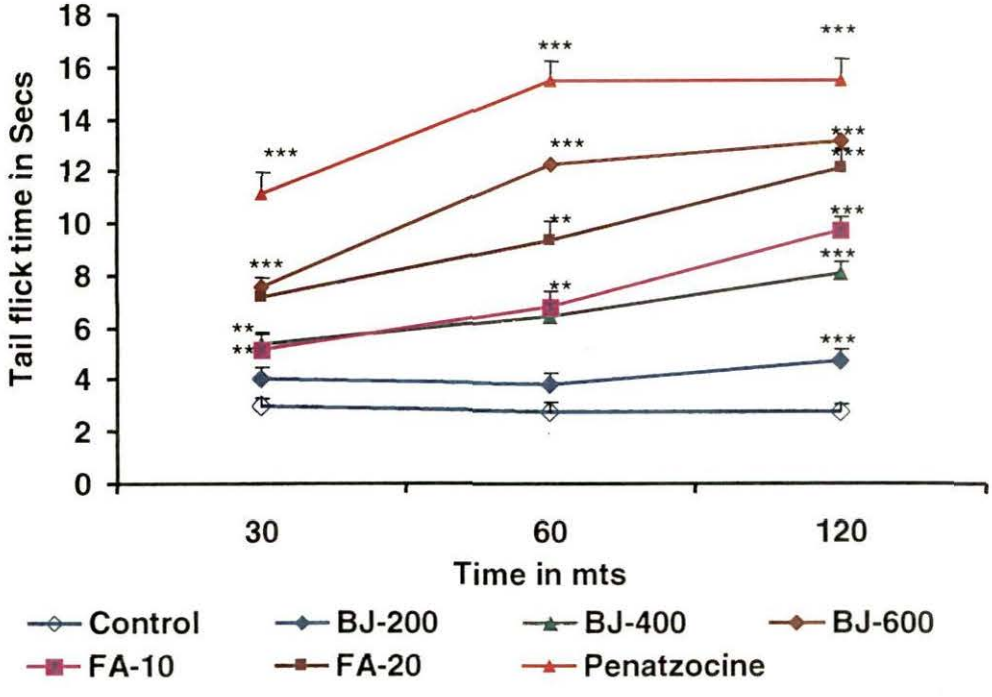

Figure 4.5 Analgesic activity of leaves of *Bischofia javanica* by tail immersion test on mice

Data represents Mean±SEM, n=6. Statistical significance was done with the control, **P<0.01, ***P<0.001

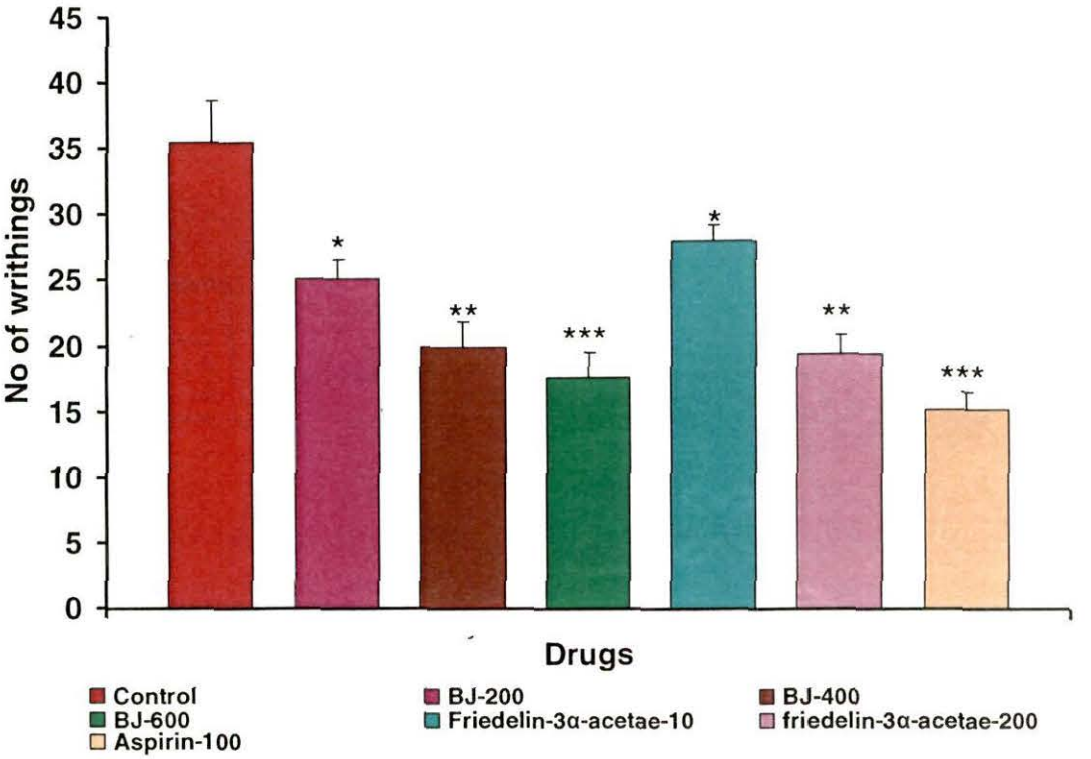

Figure 4.6 Analgesic activity of leaves of *Bischofia javanica* by acetic acid induced writhing in mice

Data represents Mean±SEM, n=6, Statistical significance was done with the control,*P<0.05, **P<0.01, ***P<0.001

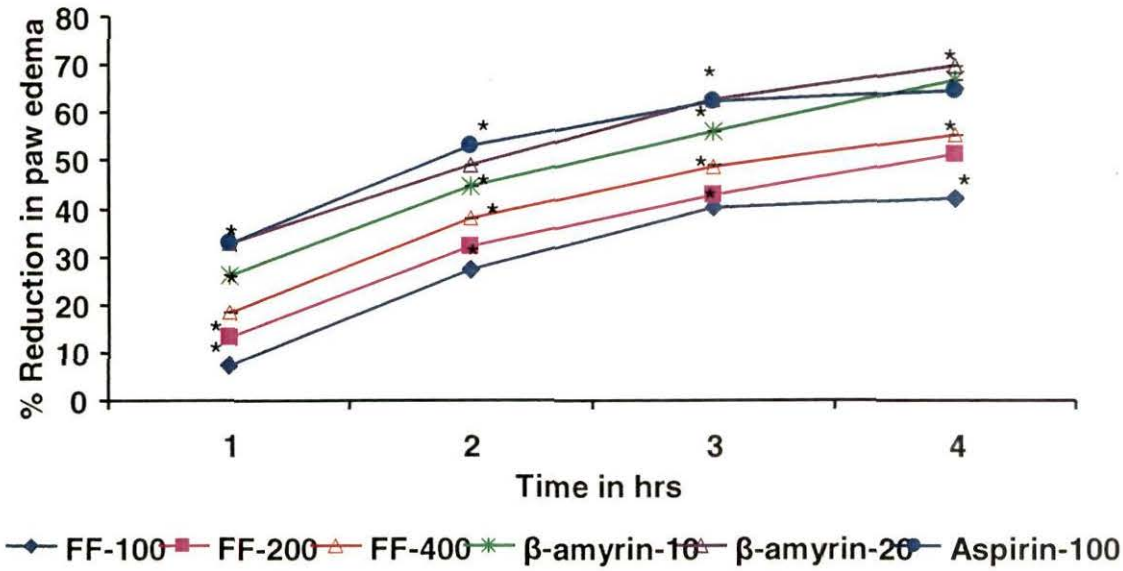

Figure 4.7 Anti-inflammatory activity leaves of *Fraxinus floribunda* on carrageenan induced paw edema in rats

Data represents Mean±SEM, n=6, Statistical significance was done with the control, *P<0.001

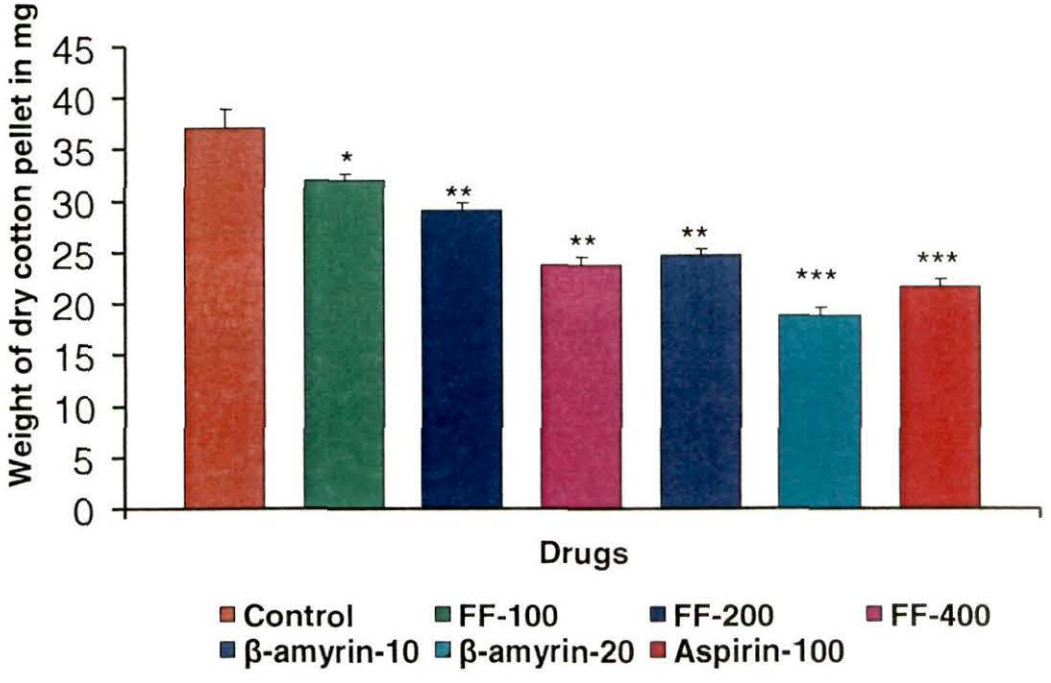

Figure 4.8 Anti-inflammatory activity of leaves of Fraxinus floribunda on cotton pellet induced granuloma in rats

Data represents Mean±SEM, n=6, Statistical significance was done with the control,*P<0.05, **P<0.01, ***P<0.001

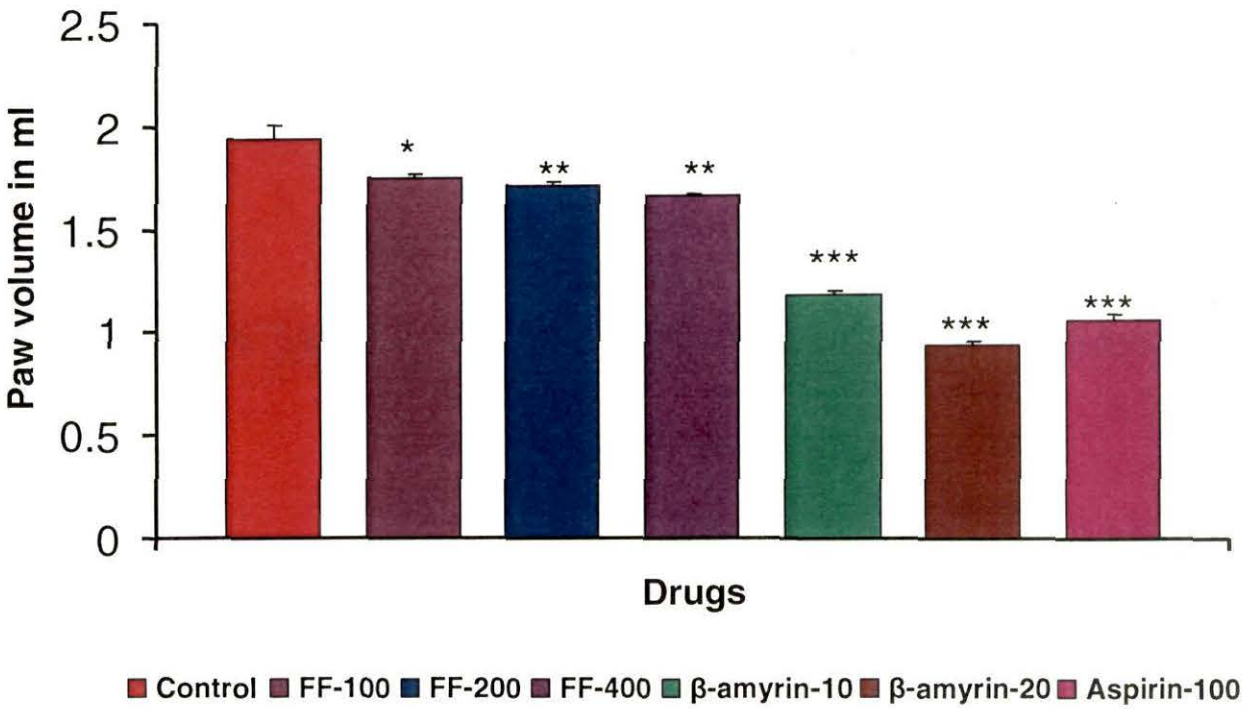

Figure 4.9 Anti-inflammatory activity of leaves of *Fraxinus floribunda* on Freund's adjuvant induced arthritis in rats

Data represents Mean±SEM, n=6, Statistical significance was done with the control,*P<0.05, **P<0.01, ***P<0.001

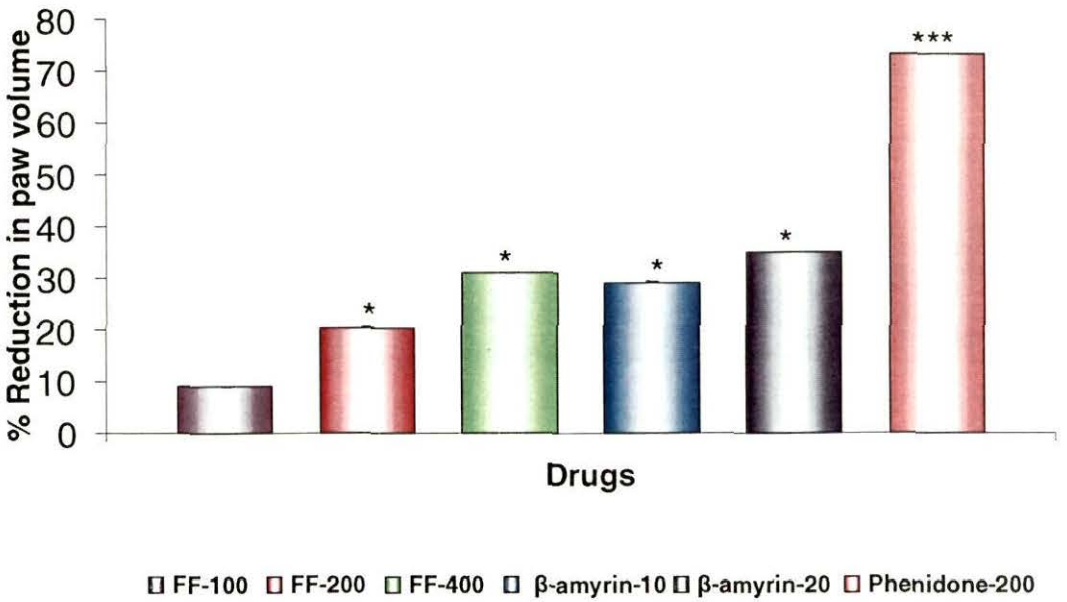

Figure 4.10 Anti-inflammatory activity of leaves of *Fraxinus floribunda* on arachidonic acid induced arthritis in rats

Data represents Mean±SEM, n=6. Statistical significance was done with the control,*P<0.05, ***P<0.001

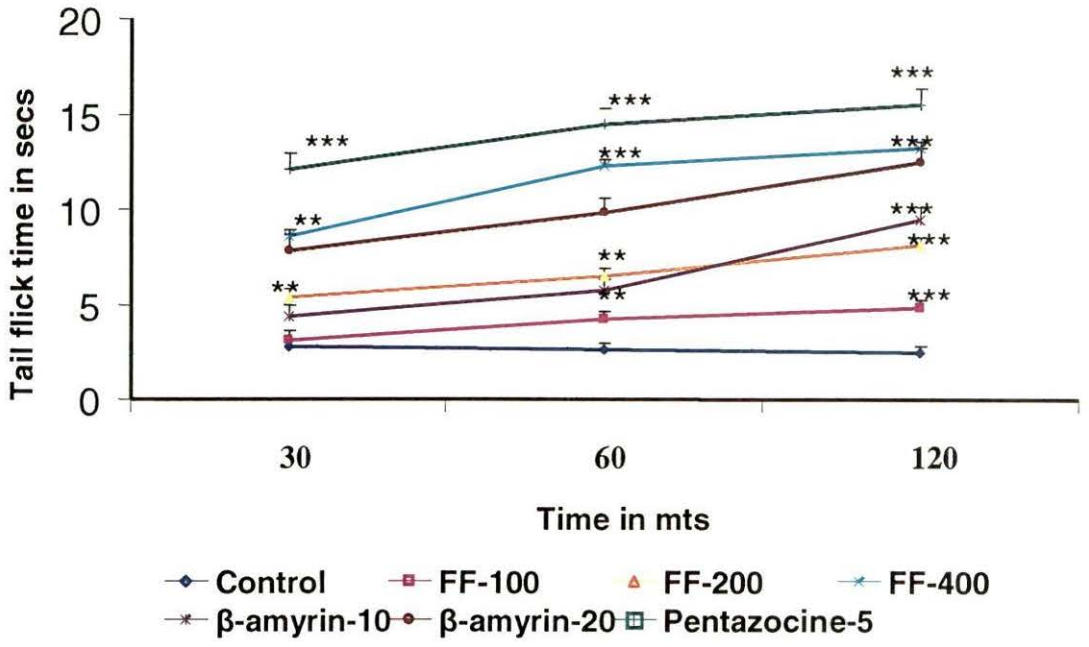

Figure 4.11 Analgesic activity of leaves of *Fraxinus floribunda* by tail immersion test in mice

Data represents Mean±SEM, n=6. Statistical significance was done with the control, **P<0.01, ***P<0.001

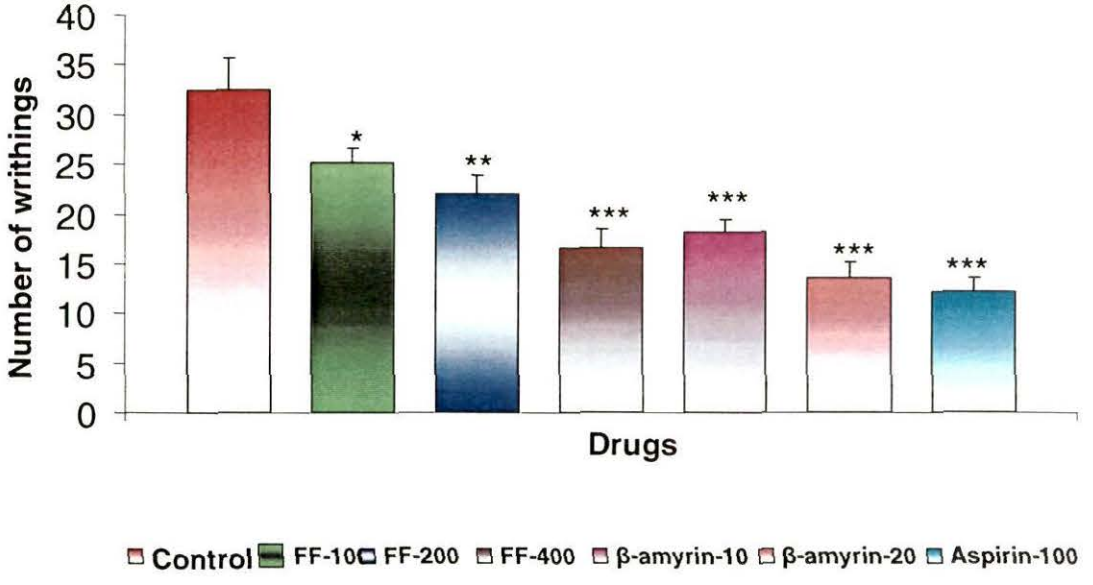

Figure 4.12 Analgesic activity of leaves of *Fraxinus floribunda* on acetic acid induced writhing in mice

Data represents Mean±SEM, n=6. Statistical significance was done with the control,*P<0.05, **P<0.01,***P<0.001

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