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Research Article

ANTI INFLAMMATORY AND ANTI NOCICEPTIVE EVALUATION OF ROOTS EXTRACTS OF *BAUHINIA PURPUREA* LINN

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ABSTRACT

The present study was carried out to evaluate the anti nociceptive activity of the roots of the *Bauhinia purpurea* Linn plant. The rats weighing around 150-200g were selected, the ethanolic extracts of dried roots of *Bauhinia purpurea* Linn were administered at following dose of 100, 200, 400 mg/kg body weight were used. The study was conducted as per the "Tail flick Method" and "Acetic acid induced writhing method". *Bauhinia purpurea* Linn roots have shown significant Anti nociceptive activity at 200 and 400 mg/kg in both the models, whereas 100mg/kg dose did not produce significant results when compared with control. (P<0.001) .The results of anti inflammatory activity of ethanolic extract produced significant results at doses of 200 & 400mg/kg (P<0.001) in "carrageenan induced paw edema model" and" cotton pellet Granuloma pouch method". The findings indicated the Anti inflammatory & Anti nociceptive evaluation of root bark extract of *Bauhinia purpurea* Linn.

Key words: Analgesic activity, Anti nociceptive, Anti inflammatory activity.

INTRODUCTION

Bauhinia purpurea Linn. Is a flowering plant belonging to the family (Caesalpiniaceae /Fabaceae) native to South China, Malaysia and India [1]. The plant is popular in India,. The bark was reported as anti mycobacterial, antimalarial, antifungal, cytotoxic, and antiinflammatory activities [2] . The leaves were reported to possess antinociceptive, anti inflammatory and antipyretic properties [3] , while the stem was found to have anti-diabetic and adrenergic properties [4] . Bauhiniastatins, isolated from leaves and bark was reported to inhibit human cancer cell lines. [5]

Bauhinia purpurea Linn (Caesalpiniaceae/Fabaceae) is a medicinal plant traditionally used to treat various ailments, In order to establish pharmacological properties of the roots of *Bauhinia purpurea*; studies were performed on anti inflammatory and antinociceptive activity in ethanolic extract.

Traditionally this plant is used in the treatment of dropsy, pain, rheumatism, convulsions, delirium, septicemia, etc [6]. The bark of the plant is used as an astringent in the treatment of diarrhoea. Extensive literature survey indicated the presence of phytoconstituents like triterpenoids, steroids, and saponins from the whole plant .The aerial parts of the plant are reported to contain flavanones glycosides,foliar flavonoids, 6-butyl-3-hydroxy flavanone, phenyl fatty ester, lutine and β-sitosterol [07-10,]. They are reported to exhibit various pharmacological activities such as CNS activity, cardio tonic activity,

lipid-lowering activity, anti-oxidant activity, hepatoprotective activity, hypoglycemic activity, etc.[11] Eleven new secondary metabolites, together with two known flavanones and five known bibenzyls, were isolated from the root extract of *Bauhinia purpurea* [12] .Preliminary phytochemical studies have revealed that the genus *Bauhinia* is mainly constituted of steroidal glycosides, terpenoids, lactones and flavonoids [13].

The roots of *Bauhinia purpurea* linn were collected from the rural areas of Mangalore, Karnataka, and were authenticated by Dr Noeline J Pinto. Professor in botany, St Agnes College, Mangalore. The roots were air dried and powdered. The powdered roots were extracted using ethanol as solvents in a Soxhlet apparatus until complete extraction. The extract was collected and vacuum dried followed by desiccation to get the constant weight. Extracts were subjected to qualitative analysis for the various phytoconstituents such as alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids and flavonoids.

The experimental protocols were approved by Institutional Animal Ethics Committee (KSHEMA/AEC/03/2011dated 20-06-11) for the use of albino wister rats (200-250 g). swiss albino mice (17-25g) The animals were maintained in an animal house recognized by the Committee for the Purpose of Control and Supervision on Experiments on Animals.

MATERIAL AND METHODS

Chemicals

Carrageenan solution (1%w/v), Sodium carboxy methyl cellulose (vehicle for antiinflammatory activity),

Diclofenac sodium (standard). & Pentazocin (standard).

Plant collection

The *Bauhinia purpurea* linn root was collected from a local supplier at paneer Deralalakte Mangalore and authenticated at Botany Department, St Agnes college Mangalore After collection roots were washed thrice with water, dried under shade for a period of one month, powdered, passed through 60# sieve and stored in air tight container.

Preparation of the alcoholic extract.

Roots were collected from local supplier. Shade dried coarsely powdered *Bauhinia purpurea* Linn of was extracted with ethanol in Soxhlet apparatus. The marc will be dried and shaken with warm distilled water and boiled. Each of the extractives obtained will subject evaporate to dryness under vacuum. The extract is stored in refrigerator for further use. The shade dried powdered roots (5 kg) were extracted in Soxhlet apparatus with ethanol (95%). The solvent from the total extract was distilled off and the concentrate was evaporated on a water bath to a syrupy consistency and then evaporated to dryness (350 gm).

Preliminary phytochemical screening

The preliminary photochemical studies were performed for testing the different chemical groups present in ethanolic extract. The chemical group tests were performed [14]

Animals

Healthy male Wistar rats (200-250g) were housed in CPCSEA approved animal house in Groups of five in polypropylene cages. They were maintained at 25 ± 2° C, relative humidity of 45 to 55% and under standard environmental conditions (12hrs light 12 hrs dark cycle). The animals had free access to food and water *ad libitum*. All the procedures were performed in accordance with the Institutional Animal Ethical Committee constituted as per the directions of the CPCSEA.

Pharmacological investigation of the roots of *Bauhinia purpurea* linn

1. Anti nociceptive activity (Analgesic activity) [15]

(A). Tail-flick method

Procedure: Acute nociception was assessed using a tail flick apparatus (Analgesiometer). The Each animal was placed in a restrainer, before treatment the basal reaction time was measured by keeping distal one-third portion of the animal tail. The extracts were orally administered immediately after this step

and reaction time was measured at every 30 minutes intervals after the drug administration for 2h.10seconds cut off time was used in order to prevent the tissue damage[16].In the present study, adult male/female Sprague- Dawley rats (100-150 g, 5-6weeks old)were selected. They were divided into 5 groups of 6 animals each. The first group served as control and received 0.6% Na CMC. The second group was treated with Pentazocine (at a dose of 10 mg/kg body weight, i.p.). The third, fourth and fifth groups received 100, 200 and 400 mg/kg body weight of ethanolic extract of the roots of the plant *Bauhinia purpurea Linn.* as a suspension in 0.6% Na CMC. Reaction time was recorded after every 30 minutes for 2 h and the average values of reaction time after each time interval are calculated and compared with the pretest value by analysis of significance.

(B). Acetic acid induced writhing method [17]

Procedure: Pain was produced by injection of acetic acid into peritoneal cavityof mice. The animals react with characteristic stretching behavior, which is called writhing. In this method adult male/female albino mice (20-25g, 3-4 weeks old) were selected for the study. The animals were divided into 5 groups of 6 animals each. First group of animals received acetic acid 0.6 % v/v i.p. and served as control. Second group served as positive control and received Diclofenac sodium (10 mg/kg body weight, i.m.) The third, fourth and fifth groups of animals received 100, 200 and400mg/kg body weight of ethanolic extract of the roots of plant *Bauhinia purpurea* Linn as a suspension in 0.6% Na CMC. 30 minutes prior to the administration of acetic acid injection. The writhing effect was indicated by the stretching of abdomen with simultaneous stretching of at least one hind limb. This was observed for 30minutes

II. Anti-inflammatory screening method

***(A).* Carrageenan induced rat paw oedema (acute- inflammatory model)**

The method of Winter *et* al[18] was used to study anti- inflammatory activity using a plethysmograph apparatus, Plethysmometer (Paw Volume) IITC Life Science Inc to measure the paw volume. Adult male/female Sprague-Dawley rats (100-150 g, 5-6 weeks old) were used for the study. The rats were divided into 5 groups of 6 animals each.

Group I Animals serve as control and received vehicle orally.

Group II Animals were administered with standard drug Diclofenac at a dose of 10mg/kg body weight, i.p

Group III, IV and V Animals received 100, 200, 400-mg/kg-body weight ethanolic root extract of the plant *Bauhinia purpurea Linn.* A mark was made on both the hind paws just below the tibio-tarsal junction so that every time the paw could be dipped in the mercury column of the plethysmograph upto the mark to ensure constant paw volume. After 30 min of above treatment an oedema was induced in the left hind paw by injection of 0.1 ml of 1% of carrageenan solution in the plantar tissue of the paw ofall the animals. The right paw served as a reference to non- inflammed paw for comparison. The initial paw volume was measured plethysmographically with in 30sec. of the injection. The relative increase in paw volume was measured in control, standard and treated group at 30, 60, 90,120 & 180min after carrageenan injection. The percentage increase in the paw volume over the initial reading was calculated. This increase in the paw volume in the animal's treated with standard drug and the different doses of the ethanolic extract of the roots of *Bauhinia purpurea* Linn*.* Were compared with increase in paw volume of untreated control.

(B). Cotton pellet granuloma method (Sub-acute inflammatory model)

The method of Goldstein[19] was used with few modifications. Sterilized cotton pellet of 20 mg were implanted beneath the abdominal skin in axilla and groin region of the rat through a single incision along the midline. The duration of implantation varied from 1-14 days. Adult male Wistar rats (200g, 6-7 weeks old) were selected for the study. They were divided into 5 groups of 6 animals in each. The first group received (0.6% w/v sodium CMC) and served as a control. Second group was

treated with Diclofenac sodium at a dose of 10 mg/kg body weight, i.p . Third, fourth and fifth groups received ethanolic extract of the roots of *Bauhinia purpurea* Linn at a dose of 100, 200, 400mg/kg body weight for seven days. On the eighth day the animals were sacrificed with ether and the implanted pellet along with granulation tissue were removed, freed from extraneous tissues and dried in an oven at 60^0C for 24hours. The dried pellets were weighed and the gain in weight in each group was calculated. The difference in granulation tissue weights of the treated group and the control group were noted.

RESULTS

I. Phytochemical investigation

The preliminary phyto chemical screening of the ethanolic extract of the roots of *Bauhinia purpurea* linn revealed the presence of Triterpenoids, Flavonoids, and Steroids. **(Table-1)**

II. Acute toxicity studies

No death was observed even at the maximum administered dose of 4000mg/kg body weight. However there was a dose dependent increase in the magnitude of certain autonomic responses..

Selection of dose

The LD_{50} of the ethanol extract *Bauhinia purpurea* linn was more than 4000 mg/kg body weight in all the cases. Hence a dose of 100, 200 and 400 mg/kg body weight were chosen for the study.

III. ANTI NOCICEPTIVE ACTIVITY (ANALGESIC ACTIVITY)

Tail-flick Method

Treatment of rats with the ethanolic extract of the roots of *Bauhinia purpurea* linn at a dose of 100,200,400mg/kg body weight significantly increased (P<0.001) the tail flick latency compared to control. Tail flick latency was maximum (120min) at a dose of 400mg/kg body weight **(Table-2) (Figure-1, 2, 3, 4.)**

Acetic Acid Induced Writhing Method.

Pretreatment of mice with the ethanolic extract of the roots of *Bauhinia purpurea* linn at doses of 100, 200, 400 mg/kg body weight produced significant reduction (P<0.01) in writhing induced by acetic acid compared to control. However it exhibited dose dependent analgesic activity. **(Table-3) (Figure- 5.)**

IV. ANTI-INFLAMMATORY ACTIVITY

Carrageenan induced paw oedema (*Acute inflammatory model*)

The rats treated with oral administration of ethanolic extract of the *Bauhinia purpurea* Linn roots , reduced acute paw oedema volume as compared to the control, There was a significant inhibition (P<0.01) in paw oedema volume at a dose of 100,200,400mg/kg body weight.**(Table-4)(Figure-06,07,08,09,10.)**

Cotton pellet Granuloma method (Sub-acute inflammatory model)

The rats treated with oral administration of the ethanolic extract of the roots of *Bauhinia purpurea* Linn *significantly* reduced (P<0.01) the granulation mass formation at a dose of 100,200,400mg/kg body weight. However it exhibited a dose dependent Anti- inflammatory activity. **(Table-5)(Figure-11)**

DISCUSSION

The preliminary phytochemical screening of the ethanolic extract of roots of plant *Bauhinia purpurea* linn revealed the presence of triterpenoids, Flavonoids,Steroids. The presence of the various phytochemical constituents in the plant namelyflavonoids, steroids, triterpenoids showed the plant to be a potential source of crude drug that can positively serve as source of modern drugs. flavonoids of medicinal plant origin were found to possessed significant pharmacological activities: antidiarrheal, analgesic and anti-inflammatory among others in the animal body systems [20].Toxicological studies carried out revealed that, The LD50 (median lethal dose) of the plant (4000)mg extract / kg body weight *i.p*) showed that the plant is relatively safe to the experimental model (mice) used. A New Approach to Practical Acute Toxicity Testing has confirmed [21] that, substance with LD50 value of about 5000mg/ kg was considered relatively safe,

the plant has a wide margin of safety. The analgesic activity may be attributed due to the presence of triterpenoids,Flavonoids, Steroids. The present study demonstrated that ethanolic extract of plant *Bauhiniapurpurea* linn roots has intrinsic analgesic activity which needs to be investigated with more information on the bioactive principles responsible for the action. The results indicate that the plant *Bauhinia purpurea* linn possesses significant analgesic activity. Furthermore, Carrageenan induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing antiinflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover the experimental model exhibits a high degree of reproducibility. Carrageenan induced edema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins whereas the second phase is related to the release of prostaglandin and slow reacting substance which peak at 3 hrs. The *Bauhinia purpurea* linn ethanolic extract of root was evaluated by carrageenan induced rat paw edema. They produced significant inhibition of rat paw edema induced by carrageenan. The inhibition was however, less than that of standard drug Diclofenac sodium. From these overall results, we can conclude that the 200 & 400 mg/k.g/ b.w ethanolic extract of roots of *Bauhinia purpurea* linn possesses significant anti-inflammatory and analgesic effect, (P≤0.001) which can be useful for the treatment of acute pain and local inflammation. Preliminary phytochemical investigation of this plant showed the presence, phytosterols, flavonoid, which might be in part responsible for analgesic and anti-inflammatory effects. It could be concluded and confirmed that the ethanolic extracts of plant of *B. Purpurea* has analgesic and anti-inflammatory effects comparable with standard drugs, which is effective against pain of humans. Further, in future it is necessary to identify and isolate the possible active phytoconstituents responsible for the analgesic and anti-inflammatory effects activity and study its pharmacological actions.

CONCLUSION

Wide range of NSAID's is used in the management of pain, and inflammation which has side effects such as gastrointestinal complications and newer antiinflammatory and analgesic agents has cardio toxic and hepatotoxic side effects. There is no Single drug which has proved to be safe. There is a need of safe and efficacious drug for the management of pain. Traditional medical practitioners are using the plant *Bauhinia purpurea* linn for treating pain which is very safe. There is no much published data available on anti inflammatory & analgesic activity on roots of *Bauhinia purpurea* linn. Hence present study was under taken. The present study was aimed to screen the anti inflammatory & analgesic activity of *Bauhinia purpurea* linn roots. Preliminary phytochemical screening of the plant was carried out on ethanolic extract of *Bauhinia purpurea* linn roots revealed the presence of triterpenoids, tannins, and steroids. The anti inflammatory activity and analgesic activity may be due to presence of these active ingredients. Toxicological studies carried out revealed that, The LD50 (median lethal dose) of the plant (4000.5 mg extract / kg body weight *i.p*) showed that the plant is relatively safe to the experimental model (mice) used. The anti inflammatory activity and analgesic activity carried out at 03 dosage levels (100, 200, 400 mg/kg/b.w) Plant extract has shown significant antiinflammatory activity by carrageenan induced paw edema and cotton Granuloma pouch method. at 200 & 400 mg/kg/b.w (P≤0.001).Plant also possesses significant analgesic activity by tail flick method and acetic acid induced writhing method at 200& 400 mg/kg.

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Table 1:

S. No	Tests	Inference
1	Alkaloids a) Dragendorff's test b) Hager's test c) Wagner's test d) Mayer's test	 -ve -ve -ve -ve
2	Carbohydrates a) Anthrone test b) Benedict's test c) Fehling's test d) Molisch's test	 -ve -ve -ve -ve
3	Flavanoids a) Shinoda's test	 +ve
4	Glycosides a) Molisch's test	 -ve
5	Triterpenoids a) Liebermann – Burchard test	+ve
6	Resins	-ve
7	Saponins	+ve
8	Steroids a)Liebermann -Burchard's test b)Salkowski reaction	 +ve +ve
9	Tannins	-ve

Table 2: Anti nociceptive (Analgesic) activity of ethanol extract of the roots of *Bauhinia purpurea Linn.* By Tail flick method

Group	Treatment	Dose mg/kg	Reaction time in seconds			
			30 min	60 min	90 min	120 min
I	Tween-80	5 ml	3.5 ±0.1	3.8 ±0.1	4.0 ±0.2	3.5 ±0.1
II	Standard drug Pentozocine	10	3.2 ±0.1***	6.0 ±0.3***	6.8 ±0.2 ***	7.3 ±0.2***
III	*B.purpura extract*	100	2.7 ±0.2	3.3±0.1	4.2±0.2	4.6 ±0.2*
IV	*B.purpura extract*	200	3.4 ±0.312	4.0 ±0.2	4.6 ±0.2	5.6±0.3***
V	*B.purpura extract*	400	2.6 ±0.283	3.5 ±0.1	5.0 ±0.202*	6.0 ±0.2***

Values are expressed as Mean ± SEM; n = 06 animals in each group; **p≤0.01 (significant)
***p≤0.001 (highly significant)

Table 3: Anti nociceptive (Analgesic) activity of ethanol extract of the roots of *Bauhinia purpurea Linn.* By Acetic acid induced writhing method

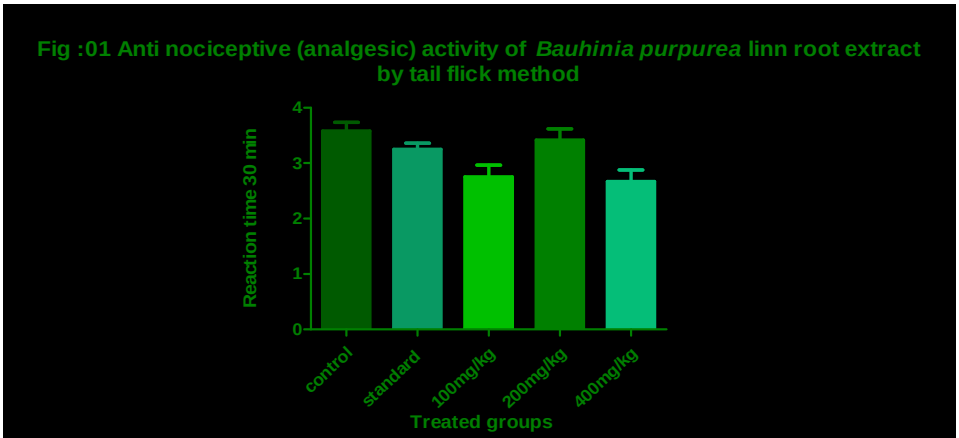
S. No	Treatment	Dose mg/kg	Number of writhings Mean ±SEM
1.	Tween-80	0.1 ml	62.83±1.92
2.	Standard drug Diclofenac sodium	10	4.33±0.72 ***
3.	B.Purpurea Extract	100	66.50±1.67
4.	B.Purpurea Extract	200	20.83±1.53***
5.	B.Purpurea Extract	400	20.17±1.42***

Values are expressed as Mean ± SEM; n = 06 animals in each group;
***p≤0.001 (highly significant)

Table 4: Effect of ethanol extract of the roots of *Bauhinia purpurea Linn.* On carrageenan induced inflammation in albino rats

Group	Dose Mg/kg	Increase in paw volume Mean SEM				
		30	60	90	120	180
Control		0.39±0.01	0.56±0.01	0.60±0.01	0.67±0.01	.0.74±0.01
Standard Diclofenac sodium	10	0.21±0.01	0.32±0.01 ***	0.34±0.01 ***	0.31±0.01 ***	0.30±0.01 ***
Bauhinia purpurea	100 mg	0.38±0.01	0.56±0.22	0.56±0.02	0.63±0.11	0.66±0.01
Bauhinia purpurea	200 mg	0.40±0.01	0.35±0.16 ***	0.32±0.01 ***	0.31±0.01 ***	0.31±0.01 ***
Bauhinia purpurea	400 mg	0.37±0.02	0.40±0.01 ***	0.51±0.01 **	0.58±0.01 ***	0.50±0.04 ***

Values are expressed as Mean ± SEM; n = 06 animals in each group; ***p≤0.001 (highly significant)



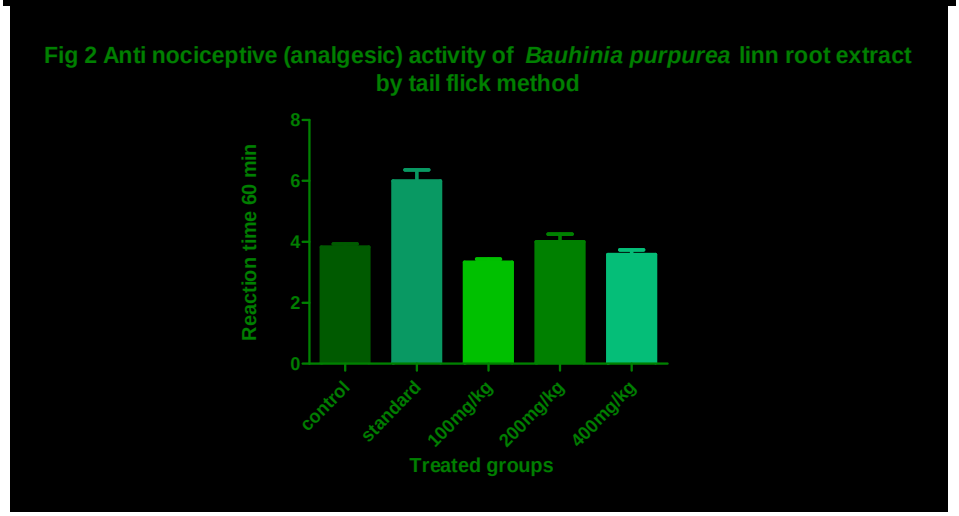


Fig 3 Anti nociceptive(analgesic) activity of *Bauhinia purpurea* linn root extract by tail flick method

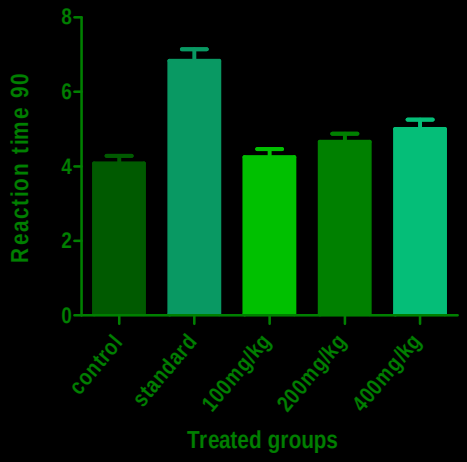


Fig 04 Anti nociceptive(analgesic) activity of *Bauhinia purpurea* linn root extract by tail flick method

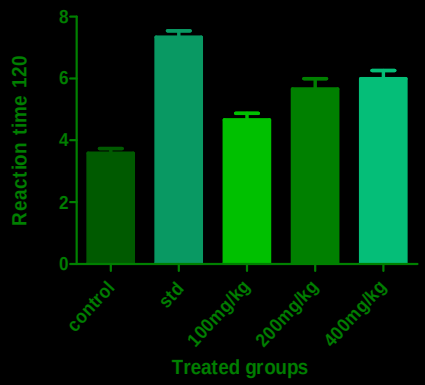


Fig 05 Anti nociceptive (analgesic) activity Bauhinia purpurea linn roots by acetic acid induced writhing method

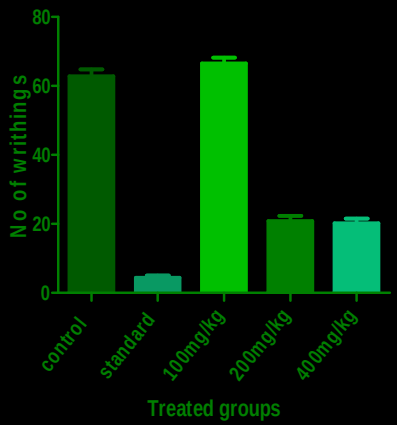


Fig:06 Antiinflammatory activity of *Bauhinia purpurea* linn root extract in carragenan induced paw edeme method

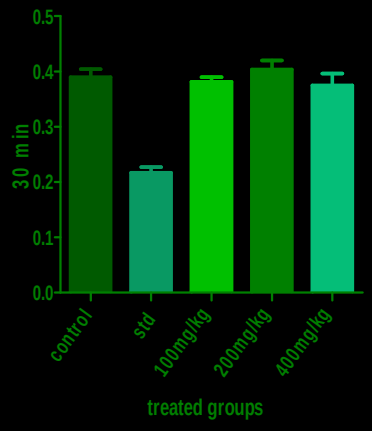


Fig:07Antiinflammatory activity of *Bauhinia purpurea* linn root extract in carragenan induced paw edeme method

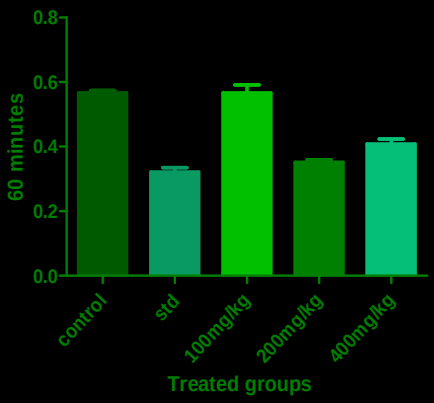
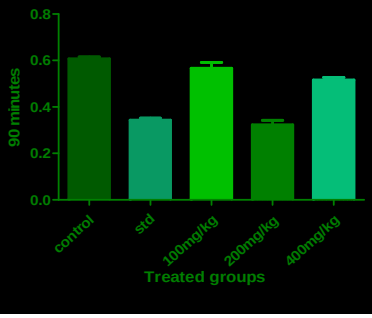
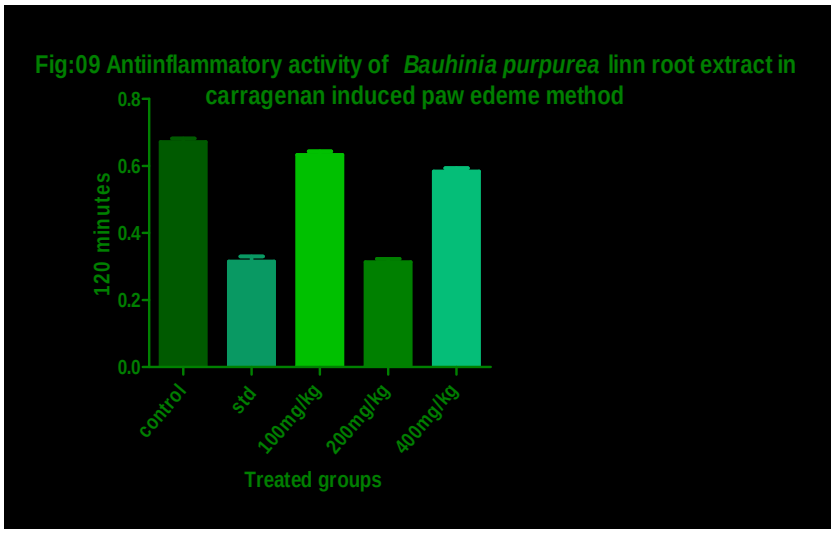
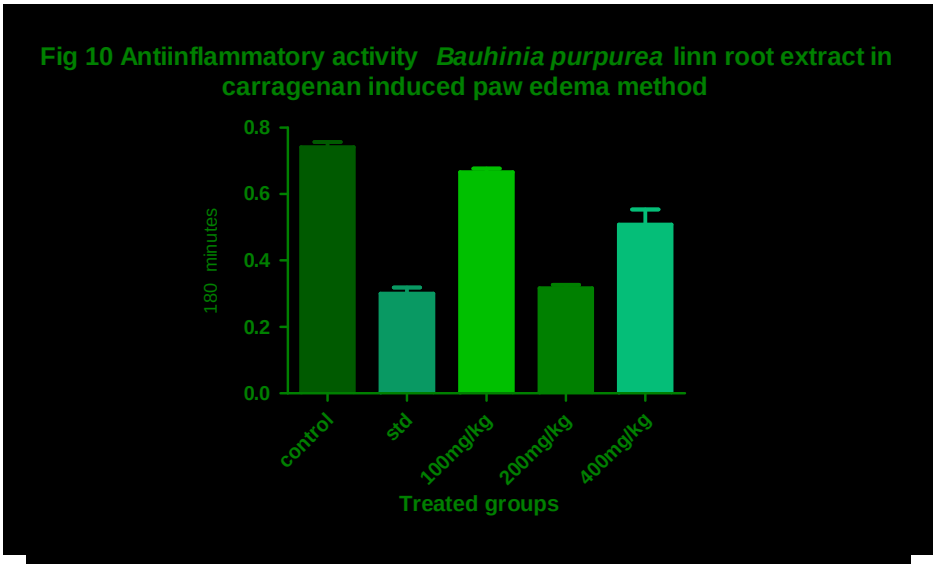
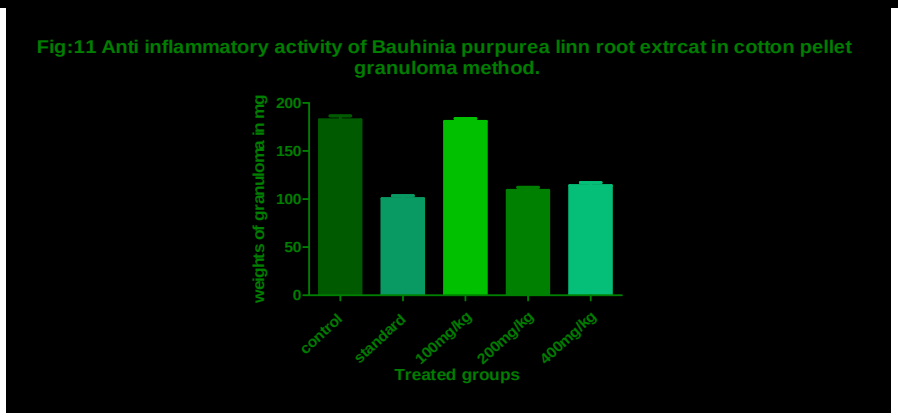


Fig 08.Anti-inflammatory activity of Bauhinia purpurea linn root extract in carragennan induced paw edema method









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