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Evaluation of Anti-inflammatory, Analgesic and Antipyretic Potential of the Stem Barks Aqueous Extract of *Albizia ferruginea* (Guill. & Perr.) Benth. (Mimosaceae) in Rats and Mice

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MGMK and TD designed the study and wrote the protocol. Authors MTBN and CM performed the statistical analysis. Authors AFL and PDDD wrote the first draft of the manuscript. Authors MGMK, GAAK and BAK managed the analyses of the study. Authors BMM and TD managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present research was carried out to investigate the anti-inflammatory, analgesic and antipyretic potential of aqueous extract of *Albizia ferruginea* stem bark.
Place and Duration of Study: Department of Animal Biology and Physiology (Animal Physiology Laboratory), Faculty of Sciences, University of Yaoundé I. between March 2012 and June 2016.
Methods: Qualitative and quantitative phytochemical analyzes were done.The anti-inflammatory effect of the plant extract (100 and 200 mg/kg) was investigated on carrageenan, histamine, serotonin or dextran-induced paw oedema. The analgesic activity was evaluated on acetic acid-induced writhing, formalin-induced nociception, hot plate and tail immersion tests in Swiss albino mice. The antipyretic activity of *A. ferruginea* extract was assessed on brewer's yeast induced pyrexia.
Results: Qualitative phytochemical analysis of the AEAF revealed the presence of alkaloids, flavonoïds, phenols, saponins, tannins, glycosides, tannins and steroids. For quantitative phytochemical analysis, total flavonols represent 0.12±0.04 mg EQT/g dried extract and the total phenol content was 58.69±0.65 mg ECA/g dried extract. The total flavonoids content was 0.18±0.01mg EQT/g dried extract).The total alkaloids presented a grade of 27.45±0.14 mg EBER/g dried extract. Carrageenan, dextran, histamine and serotonin-induced inflammation were significantly inhibited by *A. ferruginea's* extract (200 mg/kg), exhibiting 55.47%, 50.26%, 62.88% and 42.59% inhibition, respectively. Acetic acid-induced writhing was significantly reduced by the plant extract. The extract of *Albizia ferruginea* (200 mg/kg) significantly reduced the second phase of formalin test. The analgesic tests revealed that *A. ferruginea* had only peripheral analgesic effect. Additionally, the plant's extract prevented brewer's yeast-induced pyrexia in rats.
Conclusion: Taken together, these results suggest that *A. ferruginea's* aqueous extract has anti-inflammatory, anti-nociceptive and antipyretic properties and this strongly supports the ethnopharmacological uses.

Keywords: Albizia ferruginea; analgesic; anti-inflammatory; antipyretic; ethnopharmacological; phyto-chemical.

1. INTRODUCTION

Inflammation is a local response of living mammalian tissues to injury. It is the body defence reaction in order to eliminate or limit the spread of injurious agents. It is considered as a primary physiological defence mechanism and is associated with the protection of the body against burns, infections, toxic chemicals, allergens and other harmful stimuli [1,2]. The signs of inflammation are warmth, redness, pain, swelling and loss of function [1]. Pain is a pathophysiological response of living tissue to undesirable stimuli. The pharmacology of pain has become a complex field [3]. There are several tissue factors that are known to be involved in the inflammation reactions such as release of histamine, bradykinin and prostaglandins. Non-steroidal anti-inflammatory drugs are used worldwide for the treatment of inflammation and pain. However, the side effects of currently available anti-inflammatory drugs include gastric ulcer, renal damage, bronchospasm and cardiac abnormalities have limited their use [4]. Therefore, development of new and more substantial analgesic, anti-inflammatory and antipyretic drugs with minimum side effects is necessary.

Cameroon is a rich source of medicinal plants and natural products are believed to be an important source of new chemical substances with potential therapeutic applicability. Several plant species are traditionally used as analgesics, anti-inflammatory and antipyretics. *Albizia ferruginea* is a tree widely distributed in Cameroon and other African countries [5]. Traditionally, the stem barks of *Albizia ferruginea* are used to treat diarrhoea, rheumatism, abdominal and tooth pain, headache, inflammation, pain and reduced fever. In Central Africa, a juice made from the leaves of *Albizia ferruginea* is used in emollient to soothe rashes, swellings, boils and carbuncles, the leaves are used to treat malaria, and leaves decoctions are used to treat headaches and lotion or steam inhalation against fever and toothache. In Cameroon, stem bark, gum from the trunk bark and seeds are used to relieve abdominal pain, rheumatism, diarrhoea, bronchitis, dysentery and treat haemorrhoids [6-8].

Sarkiyayi et al. 2011 showed that this plant contained flavonoïds, tannins, anthraquinones, cardiac glycosides, saponins, triterpens and carbohydrates and DL_{50} is 5 g/kg.

However, to the best of our knowledge, no scientific work has been carried out to ascertain the analgesic, anti-inflammatory and antipyretic properties of extract from *Albizia ferruginea*. In order to address these issues, the present study has been initiated to assess the effect of aqueous extract of *Albizia ferruginea* stem bark on chemical stimuli-induced inflammation and pain, thermal stimuli-induced pain and brewer's yeast-induced pyrexia in rats and mice.

2. METHODS

2.1 Plant Materials

The stem barks of *Albizia ferruginea* were collected at Angallé village in the South Region of Cameroon in March 2012. The plant materials were identified by Dr Barthélémy TCHIENGUE of the National Herbarium of Cameroon. A voucher specimen of the plant was deposited at the National Herbarium of Cameroon under the number 49871.

Fresh stem barks were air-dried and reduced to a fine powder. The powder (500 g) was macerated with 2.5 L of distilled water for 24 hours. The mixture was filtered with Whatman N°3 filter paper, concentrated under reduced pressure and lyophilized at 50°C for 48 hours. A dark brown solid (8.39 g) representing the stem barks aqueous extract of *Albizia ferruginea* was obtained (yield of 16.8%).

Drugs: Carrageenan and diclofenac obtained from Sigma-Aldrich, Inc (Saint Louis, MO, USA); Acetic acid purchased from Pure Chem. Ltd., India. Morphine solution (Morphini. HCl. STEROP. Jayson pharmaceuticals Ltd., Dhaka, Bangladesh); Cyproheptadine (Periatine®, Laboratory TEOPHARMA S.r.l, Italy); Prométhazine (Phénergan, Pharmaceutical Laboratory FRILAB SA, Geneva); Cortancyl (Cortancyl®, Laboratory SANOFI-AVENTIS-WINTROP Industry, France); Brewer's yeast (Loba chem, Mumbai) and Aspirin were used in this study.

Animals: Wistar rats (200-250 g) and Swiss albino mice (25-30 g) of both sex were obtained from the Animal House unit of the Faculty of Science, of University of Yaounde I, Cameroon. They were maintained under standard environmental conditions (temperature 24±2°C) with dark and light circle 13/11h. They were fed with standard commercial diet and water was provided *ad libitum.*

2.2 Phytochemical Analysis

2.2.1 Qualitative phytochemical analysis

Qualitative phytochemical investigations of *Albizia ferruginea* aqueous extract were performed for alkaloids, flavonoids, saponins, phenols, Steroids, glycosides and tannins using standard methods previously described [9,10].

2.2.2 Quantitative phytochemical analysis

The quantitative phytochemical analysis of the stem barks of *Albizia ferruginea* was determined by the standard methods.

2.3 Determination of Total Phenolic Content

The total phenolic content of the plant extract was determined by the Folin-Ciocalteu method [11]. Briefly, 3 mg of diluted *A. ferruginea* extract (AEAF, 1 mg/mL), and 800 µL of freshly prepared Folin-Ciocalteu reagent were mixed with 2 mL of 7.5% sodium carbonate. The mixture was diluted into 7 mL of deionized water. The mixtures were kept in the dark at room temperature for 2 hours. The absorbance was measured at 765 nm. Total phenolic content was calculated using following equation y=0.0041x, R^2=0.97. Caffeic acid was used as standard and the results were expressed in mg equivalent of caffeic acid per g of dry extract (mg ECA/g dry extract).

2.4 Determination of Total Flavonoid Content

Total flavonoids content was determined using aluminum chloride (AlCl3). Quercetin (0.5 mg/mL, into ethanol) was used as standard [12]. Aqueous extract of *A. ferruginea* (0.1 mL of a solution freshly prepared at 1mg/mL) was added to 0.3 mL of distilled water, followed by a solution of $NaNO_2$ (5%; 0.03 mL). Five min later, a solution of aluminium trichloride (0.03 mL, 10%) was added to the medium. The mixture was kept at 25°C for 5 min. Five min later, the reaction mixture was treated with 0.2 mL of NaOH (1 mM). Finally, the reaction mixture was completed to 1 mL with water and the absorbance was read

at 510 nm. Total flavonoids content was calculated using the following equation y=4.5355X, R^2 =0.9956. The amount of flavonoids in extract was expressed in mg equivalent of quercetin per g of dry extract (mg EQT/g dry extract).

2.5 Determination of Total Flavonols

Total flavonols in the AEAF were estimated using a known method with modifications described by [13]. To 2.0 mL of sample (standard), 2.0 mL of 2% aluminium trichloride ethanol and 3.0 mL (50 g/L) sodium acetate solutions were added. The absorption at 440 nm was read after 2.5 h of incubation at 20°C. Extract samples were evaluated at a final concentration of 0.1 mg/mL. Total flavonols content was calculated as quercetin (mg/g) using the following equation y = 0.002x, R^2 = 0.9847, based on the calibration curve, where y was the absorbance and the concentration was expressed in mg extract equivalent of quercetin per g of dry extract (mg EQT/g dry extract).

2.6 Quantification of Alkaloid Content

Quantification of alkaloid content of AEAF was done as previously described [14]. One millilitre of the extract (1 mg/mL) was added to distilled water, 0.1 mL of $FeCl_3$ (2 mM $FeCl_3$ in 0,1 mL HCl , 2M) and 0.1 mL of 1,10-phenanthroline were added to the mixture. After incubation at 70°C for 30 min, the absorbance was measured at 500 nm. The alkaloid content was quantified from the Berberine (Ber) standard graph.

2.7 Anti-inflammatory Tests

2.7.1 Carrageenan-induced paw oedema in rats

Pedal inflammation in rats was induced as described by Winter et al. [15]. Briefly, animals were randomly divided into 4 groups of 5 rats each. They were subsequently treated with distilled water (10 mL/kg), *A. ferruginea's* aqueous extract (100 and 200 mg/kg, *p.o.*) or diclofenac (3.85 mg/kg). One hour after administration of test products, an injection of 0.1 mL of 1% carrageenan suspension (in NaCl 0.9%) was administered into the subplantar aponeuvrosis of each rat. Paw volume was determined measured using an electronic plethysmometer (Model Ugo Basil, N°37140) prior to carrageenan injection, and at 30 min, 1, 2, 3, 4, 5 and 6 hours after induction of inflammation. Oedema inhibition was calculated according to the following formula [16]:

$$\text{Percentage of inhibition (\%)} = \frac{(\overline{Vt}\text{-}\overline{Vo})c - (\overline{Vt}\text{-}\overline{Vo})e}{(\overline{Vt}\text{-}\overline{Vo})c} \times 100$$

Where $\overline{Vt}$ is the paw volume (mL) at a time t; Vo is the paw volume (mL) before carrageenan injection; $(\overline{Vt}\text{-}\overline{Vo})c$ is the difference of paw volume before and at a time "t" after carrageenan injection in control animals; $(\overline{Vt}\text{-}\overline{Vo})e$ is the difference of paw volume before and at a time "t" after carrageenan injection in treated rats.

2.7.2 Dextran-induced paw oedema in rats

Rats were randomly divided into five groups of five animals each, treated with distilled water (10 mL/kg); *A. ferruginea's* extract (100 and 200 mg/kg); cyproheptadine (1 mg/kg) or diclofenac (3.85 mg/kg). All treatments were given by oral route. Thirty minutes later, a solution of dextran (0.1 mL, 1% w/v in normal saline) was injected into the subplantar surface of the right hind paw of each rat [17]. Paw volume was measured before and at 30 min, 1 and 2 hours after dextran injection. The percentages of inhibition of paw oedema were assed as previously described [18].

2.7.3 Histamine and serotonin-induced paw oedema

The rats treated with distilled water, the plant extract (100 and 200 mg/kg), cyproheptadine (1 mg/kg), cotancyl (1 mg/kg) or diclofenac (3.85 mg/kg) were injected subcutaneously with freshly prepared solution of histamine (0.1 mL, 1%) or serotonin (0.1 mL, 1mg/mL) in normal saline. Cyproheptadine and cortancyl were used as reference drugs for histamine and serotonin-induced paw oedema respectively. The paw volume was measured before induction of inflammation and at one hour after histamine injection or 30 min after serotonin injection [18]. The percentage of inhibition of paw oedema was calculated as previously reported.

2.8 Analgesic Activity

2.8.1 Acetic acid-induced writhing response in mice

Mice were ramdomly divided into 7 groups of five animals each. All group were injected subcutaneously with acetic acid (1%, 0.5 mL/kg).

The animals were treated with distilled water, AEAF (100 and 200 mg/kg, *p.o.*), aspirin (100 mg/kg, *p.o.*) morphine (5 mg/kg, *i.p*) or a combination of *A. ferruginea's* extract (200 mg/kg) or morphine with naloxone (0.4 mg/kg, *i.p*) one hour before administration of acetic acid. The writhing response was observed during 30 minutes following acetic acid injection. The analgesic effect of the extract was calculated using the following formula [16]:

$$PAA\ (\%) = \frac{\overline{Nc} - \overline{Nt}}{\overline{Nt}} \times 100$$

Where PAA is the percentage analgesic activity, Nt the average writhing count of control group and Nc the average writhing count of treated group.

This behavior was observed after 5 min of administration of acetic acid and counted for total 20 minutes before and after the administration of the drug. Writhing movement was accepted as contraction of the abdominal muscles accompanied by stretching with a jerk and the hind limb.

2.8.2 Formalin-induced nociception in mice

Mice organised into groups of 5 animals each were treated as described above and injected with formalin (0.05 mL of 1%) into the subplantar surface of their right hind paw. The time spending by each mouse licking its paw was recorded from 0 to 5 minutes (neurogenic phase) and from 15 to 30 minutes (inflammatory phase), after the formalin injection. The analgesic effect of the extract was calculated according to the following equation [16]:

$$PAA\ (\%) = \frac{\overline{Tc} - \overline{Tt}}{\overline{Tc}} \times 100$$

PAA = Percentage analgesic activity
$\overline{Tc}$ = Average time of control group
$\overline{Tt}$ = Average time of treated group

2.9 Hot Plate Test

Albino mice were divided into six groups of five animals each. The animals were placed into the perspex cylinder on a heated surface (55 ±0.5°C) of the hot plate and the time taken for either sitting on its licking of the forepaw or jumping response, or blowing its fore paw was taken as the reaction time or latency time. The animals were treated with distilled water (10 mL/kg, *p.o.*), the plant extract (100 and 200 mg/kg, *p.o.*), morphine (5 mg/kg, *i.p*). Two groups of mice received respectively AEAF (200 mg/kg) combined with naloxone (0.4 mg/kg, *i.p*) or morphine combined with naloxone. The cut-off period was taken at 15 seconds. The latency time was recorded prior administration of test compounds and at 30 min 1, 2, 3, 4, 5 and 6 hours after the treatment of animals. The analgesic activity of test compounds was determined as according to the following equation [16]:

$$PAA\ (\%) = \frac{\overline{Tf} - \overline{Ti}}{\overline{Ti}} \times 100$$

PAA = Percentage analgesic activity
$\overline{Ti}$ = Mean initial time
$\overline{Tf}$= Mean final time

2.10 Tail Immersion Test

Tail immersion was conducted as described by Deraniyagala et al. [18]. Rats of either sex were divided into six groups of five animals each. The animal was kept in vertical position to hang the tail, with up to 2.5 cm portion of the tail immersed into a water bath containing water maintained at temperature 55 ± 0.5°C. Within a few minutes, the rats reacted by withdrawing the tail. The reaction time was recorded with stopwatch. Group 1 received distilled water (10 mg/kg, orally), group 2 and 3 received aqueous extract of *A. ferruginea* (100 and 200 mg/kg, *p.o.*) and group 4 received morphine (5 mg/kg, *i.p.*). Groups 5 and 6 received respectively morphine (5 mg/kg, *i.p*) combined with naloxone (0.4 mg/kg, *i.p*) or aqueous extract of *A. ferruginea* (200 mg/kg, *p.o.*) combined with naloxone (0.4 mg/kg, *i.p*). The time spent to withdraw the tail from hot water was taken as the reaction time (Ta). The reading was taken as 30 min, 1, 2, 3, 4, 5 and 6 hours after administration of test products. The percentage analgesic activity of this test was calculated as previously mentioned.

2.11 Antipyretic Effect

The Antipyretic activity of *A. ferruginea* was evaluated on Brewer's yeast-induced pyrexia in rats according to the methods described by Metowogo et *al*. with some modifications [19]. Briefly; after an overnight fasting, rats were divided into 4 groups of 5 animals each. Pyrexia was induced by subcutaneous administration of

0.2 mL/100 g of 20% aqueous suspension of brewer's yeast at the level of the neck, before treating the animals. Only rat with body temperature greater than 36.5 °C were taken into the test. Animals of the control group were treated with distilled water. Rats of groups 2 and 3 were treated with the plant extract at 100 and 200 mg/kg (*p.o.)*, respectively; while those of group 4 were treated with aspirin (100 mg/kg, *p.o*). Twenty four hours after induction of pyrexia, rectal temperatures recorded by insertion of a digital thermometer (ADDTO1-A-Glaxo Smith Kline) with automatic alarm, accuracy ± 0.2°C. The temperature of animals was taken at 30 min, 1, 2, 3, 4, 5 and 6 hour post-dosing. The percentage reduction of pyrexia was calculated according to the following formula:

$$PRP\ (\%) = \frac{\overline{Tb} - \overline{Ta}}{\overline{Ta}} \times 100$$

Where $\overline{Tb}$ was the temperature after induction of pyrexia, Tb was the temperature after 1, 2, 3, 4, 5 and 6 hours and $\overline{Ta}$ was the normal body temperature.

2.12 Statistical Analysis

Values were presented as the mean ± SEM and data were analyzed statistically by one way analysis of variance (ANOVA) followed by Dunnett's test. P value less than 0.05 was considered significant.

3. RESULTS

3.1 Phytochemical Analysis

3.1.1 Qualitative phytochemical analysis

The phytochemical analysis of the AEAF revealed the presence of alkaloids, flavonoids, phenols, saponins, tannins, glycosides, tannins and steroids (Table 1).

3.1.2 Quantitative phytochemical analysis

Total flavonols represent 0.12±0.04mg EQT/g dried extract and the total phenol content was 58.69±0.65 mg ECA/g dried extract. The total flavonoids content was 0.18±0.01mg EQT/g dried extract).The total alkaloids presented a grade of 27.45±0.14 mg EBER/g dried extract (Table 2).

3.2 Anti-inflammatory Effects of *Albizia ferruginea* Extract

3.2.1 Effects of *A. ferruginea* aqueous extract on carrageenan-induced paw oedema

The results of carrageenan-induced rat paw oedema are presented in Table 3. Animals of the control group presented a slow oedema evolution, reaching peak inflammatory response (3.27 ± 0.14 mL) three hour after injection of carrageenan. Administration of *A. ferruginea* (100 and 200 mg/kg) reduced paw oedema to 2,36±0,06 mL and 2,18±0,05 mL, 1 and 2 hours after induction of inflammation, representing (48.12 and 55.47%) inhibition respectively. The maximum inhibitory effect (60.74%) of diclofenac was recorded at two hours (p<0.01).

3.2.2 Effects of stem barks of *A. ferruginea* on dextran-induced paw oedema

Table 4 shows that subplantar injection of 0.1 mL of dextran solution induced an increase in paw volume in rats. *A. ferruginea* significantly inhibited oedema formation during the two first hours of experiment, with a maximum inhibition of 50.26% time and dosage of the plant extract. The effect of *A. ferruginea* extract (continue the sentence, comparing the effect of the plant with that of both drugs Cyproheptadine or diclofenac significantly reduced inflammation with a maximum inhibition at the second hour after injection of dextran.

3.2.3 Effects of *Albizia ferruginea* on histamine-induced and serotonin-induced paw oedema

The injection of histamine induced an increase in the volume of the rat paw from 1.65 ± 0.01 mL to 2.30 ± 0.01 mL (Table 4a). The *A. ferruginea* extract (100 and 200 mg/kg) and Promethazine (1 mg/kg) significantly (p<0.01) reduced histamine-induced inflammation, by 56.13%, 62.88% and 72.08%, respectively. Table (4b) shows the effects of the plant extract on paw oedema induced by serotonin. The inflammatory oedema induced by serotonin was significantly inhibited by *A. ferruginea* with 42.59% inhibition at 200 mg/kg (p<0.05). The effect of Cortancyl (1 mg/kg) was highest (66.66%), compare to the effect of plant extract.

3.3 Analgesic Effects of *A. ferruginea* Aqueous Extract

3.3.1 Acetic acid induced writhing response in mice

A. ferruginea aqueous extract at the doses of 100 and 200 mg/kg body weight and Aspirin (100 mg/kg) decreased in a significant manner (p<0.001) the number of writhes induced by acetic acid (Fig. 1). Exhibiting 43.86%, 69.61% and 78.87% inhibition, respectively. Morphine induced an inhibition of 88.93% compared to control group. The treatment of the mice with naloxone didn't modify the analgesic effect of plant extract.

3.3.2 Effects of aqueous extract of *A. ferruginea* on formalin test

Oral administration of AEAF at the doses of 100 and 200 mg/kg showed no significant effects on the first phase of formalin-induced pain. The plant extract (200 mg/kg) significantly (p<0.05) inhibited the second phase of formalin test (47.26%). Morphine significantly reduced the licking time of paw during the 1st and 2nd phases, exhibiting 68.16% and 77.81%, respectively. Diclofenac also reduced by 51.07% and 68.16% the first and the second phases of formalin-induced pain, respectively (Table 5).

3.3.3 Effects of *A. ferruginea* on hot plate induced pain in mice

The results of the hot plat test revealed that the effect of the plant extract was dose dependent and the maximum effect (33.46%) was observed after 2 hours as shown in Table 6. The maximum protective effect (70.03%) of morphine (p<0,001) was noticed at 2 hours after its administration. Naloxone did not modify the effect of the plant extract. Naloxone administration reduced the effect of morphine during all time of test.

Table 1. Qualitative phytochemical of *Albizia ferruginea* stem bark

No	Constituents	Results
1	Alkaloids	+
2	Flavonoids	+
3	Saponins	+
4	Phenols	+
5	Steroids	+
6	Glycosides	+
7	Tannins	+

Key: (+) presence

Table 2. Quantitative phytochemical of *Albizia ferruginea* stem bark

Albizia ferruginea	Polyphenols	Flavonols	Flavonoids	Alkaloids
Presence	+	+	+	+
Quantification	58.69±0.65 (mg/ECA/g DE)	123.5±4.27 (mg EQT/g DE)	180.79±14.45 (mg EQT/ g DE)	27.45±0.14 (mg EBER/ g DE)

The results are expressed as mean ± SEM (n = 3). Mg equivalent of caffeic acid per g of dry extract (mg ECA/g dry extract), mg equivalent of quercetin per g of dry extract (mg EQT/g dry extract) and mg equivalent of berberine per g of dry extract (mg EBER/g dry extract)

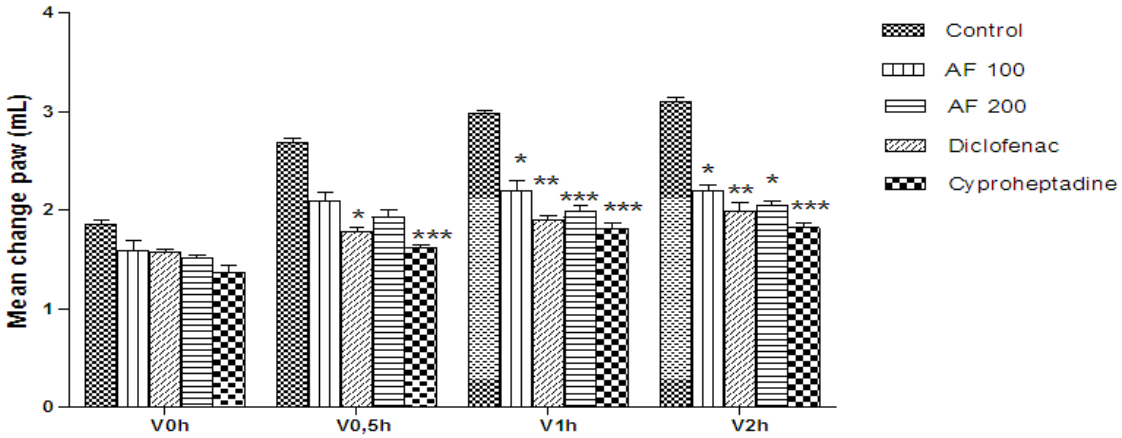

Fig.1. Effects of oral treatment of *Albizia ferruginea* on dextran-induced paw oedema

*Results expressed as mean ± SEM (n = 5); *p<0.05, **p<0.01, ***p<0.001 when compared to distilled water group. AEAF: Aqueous extract of Albizia ferruginea*

Table 3. Effects of oral treatment of *Albizia ferruginea* on carrageenan-induced rat paw oedema in rats

Treatments	Change in paw volume (h)							
	0 h	1/2 h	1 h	2 h	3 h	4 h	5 h	6 h
Distilled water (10 mL/kg)	1.81±0.01	2.31 ± 0.02	2.62 ± 0.01	2.83 ± 0.04	3.27± 0.14	3.19 ± 0.09	3.07 ± 0.11	3.15±0.16
AEAF (100 mg/kg)	1.84 ±0.01	2.19 ± 0.04 (30.00)	2.26 ±0.03* (47.76)	2.36±0.1* (48.12)	2.64 ± 0.05* (45.13)	2.66 ± 0.03* (40.11)	2.76 ± 0.04 (26.47)	2.80±0.05 (26.87)
AEAF (200 mg/kg)	1.82±0.04	2.15 ± 0.04 (34.40)	2.18±0.05** (55.47)	2.32±0.04* (50.69)	2.68 ± 0.03* (40.60)	2.56±0.06* (45.93)	2.68± 0.08 (31.57)	2.64±0.08 (37.55)
Diclofenac (3.85 mg/kg)	1.79±0.04	2.08±0.08* (41.60)	2.21±0.01* (47.51)	2.19±0.01** (60.74)	2.46±0.02** (53.63)	2.44±0.08** (52.32)	2.47±0.05* (45.61)	2.47±0.05* (48.09)

*Each value represents the average volume of the paw ± SEM (n = 5); the values in parentheses represent percentage of inhibition. *p<0.05, **p<0.01 when compared to distilled water group. AEAF: Aqueous extract of Albizia ferruginea*

Table 4. Effects of oral treatment of *Albizia ferruginea* on the oedema induced by histamine and serotonin

Treatments	Change in paw volume (h)	
	0 h	1 h
Distilled water (10 mL/kg)	1.65±0.01	2.30 ± 0.01
AEAF (100 mg/kg)	1.80±0.06	2.09 ± 0.07* (56.13)
AEAF (200 mg/kg)	1.69±0.11	1.94±0.12** (62.88)
Prométhazine (1 mg/kg)	1.63±0.07	1.81±0.07*** (72.08)
Diclofenac (3.85 mg/kg)	1.65±0.01	2.10±0.03 (30.67)

(a)

Treatments	Change in paw volume (h)	
	0 h	1/2 h
Distilled water (10 mL/kg)	2.35±0.03	3.00±0.11
AEAF (100 mg/kg)	1.70±0.01	2.13±0.04 (33.02)
AEAF (200 mg/kg)	1.72±0.01	2.10±0.03* (42.59)
Cortancyl (1 mg/kg)	2.28±0.10	2.50±0.08** (66.66)
Diclofenac (3.85 mg/kg)	1.68±0.02	1.95±0.03* (57.09)

(b)

*Each value represents the mean ± SEM (n = 5); values in parentheses represent the percentage of inhibition. *p<0.05, **p<0.01, ***p<0.001 when compared to distilled water group. AEAF: Aqueous extract of Albizia ferruginea.*

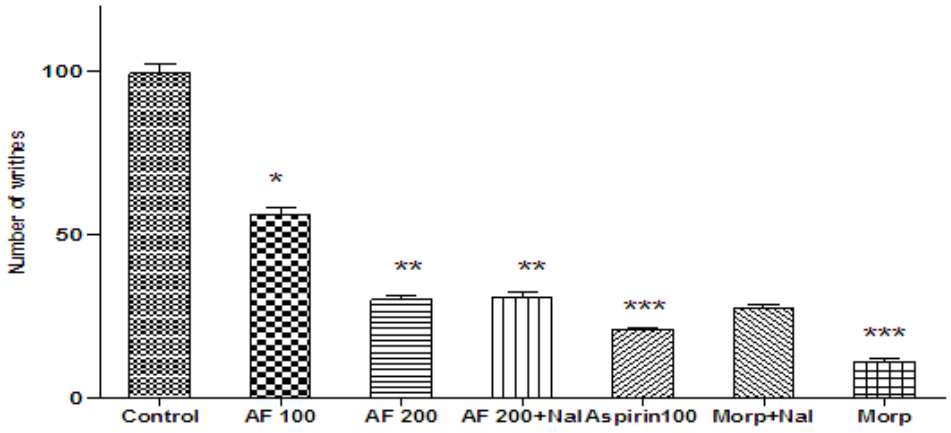

Fig. 2. Effects of oral treatment of *Albizia ferruginea* on acetic acid induced writhing test response in mice

*Each column represents the means writhing number±SEM (n=5); *p<0.05, **p<0.01, ***p<0.001 when compared to control group. AEAF: Aqueous extract of Albizia ferruginea, AEAF+Nal: Aqueous extract of Albizia ferruginea combined with naloxone, MorNal: Morphine combined with naloxone*

Table 5. Effects of oral treatment of *Albizia ferruginea* on the pain caused by formalin

Treatments	First phase	Second phase
Distilled water (10 mL/kg)	74.40±1.20	62.20±1.98
AEAF (100 mg/kg)	60.60±2.85 (18.54)	42.80 ± 3.16 (31.2)
AEAF (200 mg/kg)	56.46±1.36 (24.1)	32.80±0.86* (47.26)
Morphine (5 mg/kg)	23.60±1.80** (68.27)	13.80±1.52** (77.81)
AEAF+Nal (200 + 0.4 mg/kg)	57.8 0±0.37 (22.31)	36.54±6.33* (41.25)
MorNal (5 + 0.4 mg/kg)	56.60+1.02 (23.92)	44.80+1.24 (27.97)
Diclofenac (3.85 mg/kg)	36.40±1.07* (51.07)	19.80±1,24** (68.16)

*Each value represents the mean±SEM of abdominal contractions (n = 5); values in parentheses represent percentage of inhibition. *p<0.05, **p<0.01 when compared to distilled water group. AEAF: Aqueous extract of Albizia ferruginea, AEAF+Nal: Aqueous extract of Albizia ferruginea combined with naloxone, MorNal: Morphine combined with naloxone*

3.4 Tail Immersion Test

The results given in Table 7 showed the effects of the AEAF on pain caused by immersion of the rat's tail into hot water. The plant extract at 200 mg/kg present maximum effect (23.88 %) at 3hours. The maximum analgesic effect was noticed at 2 hours (79.5%) for morphine.

3.5 Antipyretics Effects of Aqueous Extract of *A. ferruginea*

The Brewer's yeast induced pyrexia model is a classical method for screening antipyretic action of plant extracts. Oral administration of AEAF produced a significant (p<0.05) antipyretic activity at 100 mg/kg (47.41%) 4 hours post-therapy. Aspirin (100 $mg.kg^{-1}$) significantly reduced hyperthermia, exhibiting a highest (p<0.01) antipyretic activity (66.66%) 2 hours after its administration (Table 8).

4. DISCUSSION

The aim of the present work was to evaluate the anti-inflammatory, analgesic and antipyretic effect of aqueous extract of *Albizia ferruginea.* The results showed that *A. ferruginea* extract significantly protected mice against chemical and thermal pain, inflammatory stimuli and brewer's yeast induced pyrexia.

Pain and inflammation are associated with pathophysiology of various diseases like arthritis, cancer and vascular diseases. Inflammation is body's response to disturbed homeostasis caused by infection, injury or trauma and results in systemic and local effects [20,21]. In living animal, inflammatory processes involve the release of several mediators including prostaglandins, histamine, cytokines, proteinases, as well as substances that regulate adhesion of molecules and the processes of cell

migration, activation and degranulation [22]. A number of natural products are used in traditional medicine to relief symptoms of pain and inflammation [23]. *Albizia ferruginea* is reported to contain chemical constituents such as flavonoid, alkaloids and tanins which may be responsible for analgesic, anti-inflammatory and antipyretic activity. Flavonoids were reported to have a role in analgesic activity primarily by targeting prostaglandins [24].

Carrageenan induced inflammation model is a predictive test for anti-inflammatory agents that inhibit the mediators of acute inflammation [25]. The development of oedema induced by carrageenan is a triphasic event; the early phase (0-2 h) of inflammation is due to release of histamine and serotonin. The second phase (2-3 h) is associated with the activation of kinin-like substances and the later phase (3 h-6 h) is characterized by release of prostaglandins, proteases and lysosome [26]. Carrageenan induced paw oedema has been used to evaluate the effect of non-steroidal anti-inflammatory agent which primarily inhibits cyclooxygenase involved in prostaglandin synthesis [27]. Based on these reports, the protective effect of *A. ferruginea* extract on carrageenan-induced inflammation may be attributed to the presence of flavonoids in the extract [28]. Flavonoids inhibit prostaglandin biosysnthesis, endoperoxidases, enzymes like protein kinases and phosphodiesterases that are involved in the inflammatory process [29]. The effect of the plant extract was comparable to that of diclofenac, a well-known inhibitor of inflammation. The aqueous extract of *A. ferruginea* significantly inhibited carrageenan-induced paw oedema in the first phase, suggesting an inhibitory effect on the release of histamine and/ or serotonin. The aqueous extract of *A. ferruginea* showed significant inhibition of the oedema in all the three phases To ascertain the effect of the aqueous extract of *A. ferruginea* on the activities of the mediator, it was tested on inflammation induced by histamine and serotonin, characterised by increased vascular permeability. It was observed that the aqueous extract of *A. ferruginea* was capable of inhibiting oedema induced by histamine and serotonin.

The oedema lead by the dextran, a wellknwon experimental model where the oedema is one consequence of the simultaneous spread out of histamine and serotonin by mastocytes [30]. Those results showed that the essence of 100 mg/kg and 200 mg mg/kg dose of *Albizia ferruginea* have significally inhibit the oedema cause by the dextran during the first hours. The same as the cyproheptadine, an anti-histaminic and anti-sérotoninergious. Those results want to show that this essence contain some composite able to inhibit synthesis of histamine and serotonin the same as cyproheptadine. The same results have been obtained by the Perianayagam et al. [31] used in the study of the anti-inflammatory effects of essence of the *Trichodes maindicum* bark. This effects have been evaluated independenly on the oedema cause by histamine or serotonin.

Same as promethazine, the essence of *Albizia ferruginea* bark can probably decreases the capillary permeability by inhibition of catecholamines capture and competitive inhibition of H_1 histaminic receptors. Anti-inflammatory effects of this essence can be explained by the presence of steroids, triterpens and saponines [32,33]. Mastocytes cells participated in inflammation and allergy reactions by secreting inflammatory mediator like histamine and pro-inflammatory cytokines.

Pharmacological action of flavonoids present in *Albizia ferruginea*, bark show that it would be interesting in the cure of inflammation by under-regulate those mastocytes. Histamine secretion is inhibited by flavonoids. Actually, this could be probably due to the presence of tannin in *Albizia ferruginea* dark.

Serotonin has the capability to increase vascular permeability. Compare to cortancyl, a referent anti-serotonin, Different essence of plant in the dose of 100 and 200 mg/kg have caused a little inhibition of oedema caused by serotonin. Histamine, one of the important inflammation mediators, is a potent vasodilator substance and increases the vascular permeability [31]. This study showed that all doses of the aqueous extract of *A. ferruginea* significantly suppressed the oedema produced by histamine at 1 h, so it may be suggested that its anti-inflammatory activity is possibly backed by its antihistaminic effects as prométhazine. The mediators include serotonin, histamine, kinins, leucotrienes and prostaglandins, all of which also cause pain and fever. The abdominal contortions test induced by acetic acid-a visceral pain model-despite its high sensitivity and low specificity is described as a typical model to relieve inflammatory pain. The pain induction caused by liberating endogenous substances as well as some other pain mediators such as arachidonic acid via cyclooxygenase and prostaglandin biosynthesis

Table 6. Effects of aqueous extract of *Albizia ferruginea* on pain reaction time in hot plate test in rat

Treatments	Latency period (s)							
	0 h	1/2 h	1 h	2 h	3 h	4 h	5 h	6 h
Distilled water (10 mL/kg)	9.76±0.28	10.74±0.52	11.55±0.16	11.30±0.40	11.46±0.22	11.18±0.49	11.30±0.31	11.33±0.19
AEAF (100 mg/kg)	10.74±0.15	12.76±0.04 (18.81)	12.90±0.25 (20.11)	13.12±0.33 (22.16)	12.82±0.20 (19.37)	12.72±0.48 (18.44)	12.08±0.10 (12.48)	12.00±0.20 (11.73)
AEAF (200 mg/kg)	10.76±0.53	13.40±0.75 (24.54)	14.34±0.19 (33.27)	14.36±0.53 (33.46)	13,48±0,48 (25.28)	13,18±0.37 (22.49)	14.00±0.51 (30.11)	13.60±0.55 (26.39)
Morphine (5 mg/kg)	10.01±0.35	14.64±0.40*** (46.25)	15.76±0.29*** (57.44)	17.02±0.32*** (70.03)	16.84±0.41*** (68.23)	15.04±0.56*** (50.25)	13.06±0.27 (30.47)	12.64±0.50 (26.27)
AEAF+Nal (200 + 0.4 mg/kg)	10.43±0.75	12.08±0.65 (15.78)	13.26±0.21 (27.13)	13.46±0.21 (29.05)	12.54±0.29 (20.23)	13.96±0.36 (24.26)	12.78±0.54 (22.53)	12.88±0.53 (23.49)
MorNal (5 + 0.4 mg/kg)	12.37±0.62	13.30±0.23 (7.52)	13.80±0.38 (11.56)	14.20±0.31 (14.79)	14.12±0.17 (14.15)	13.92±0.34 (12.53)	13.94±0.34 (12.69)	13.89±0.19 (12.26)

*Each value represents the mean±SEM of abdominal contractions (n = 5); values in parentheses represent percent inhibition. ***p<0.001 when compared to distilled water group. AEAF: Aqueous extract of Albizia ferruginea, AEAF+Nal: Aqueous extract of Albizia ferruginea combined with naloxone, MorNal: Morphine combined with naloxone*

Table 7. Effects of aqueous extract of *Albizia ferruginea* on pain reaction time in tail immersion test in rat

Treatments	Latency period (s)							
	0 h	1/2 h	1 h	2 h	3 h	4 h	5 h	6 h
Distilled water (10 mL/kg)	8.73±0.31	8.72±0.16	8.11±0.36	8.16±0.33	8.11±0.42	8.35±0.43	7.68±0.18	7.97±0.29
AEAF (100 mg/kg)	9.07±0.52	9.22±0.47 (5.75)	9.39±0.21 (15.83)	9.52±0.32 (16.77)	9.07±0.45 (11.91)	9.03±0.30 (8.19)	8.44±0.21 (9.84)	8.69±0.11 (9.08)
AEAF (200 mg/kg)	8.35 ±0.44	9.47±0.24 (8.69)	9.07±0.24 (11.83)	10.09±0.22 (23.73)	10.04±0.20 (23.88)	9.15±0.37 (9.58)	8.6±0.11 (11.97)	8.65±0.19 (8.57)
Morphine (5 mg/kg)	8.37±0.57	12.23±0.47 (40.33)	12.76±0.16*** (57.34)	14.64±0.19*** (79.49)	12.23±0.42*** (50.90)	11.68±0.61 (39.97)	10.63±0.11 (38.41)	10.55±0.55 (32.27)
AEAF+Nal (200 + 0.4 mg/kg)	8.33±0.61	9.26±0.14 (6.21)	8.87±0.21 (9.44)	9.89±0.30 (21.26)	9.66±0.18) (19.17)	9.19±0.25 (10.11)	8.64±0.16 (12.52)	8.57±0.13 (7.57)
MorNal (5 + 0.4 mg/kg)	8.37±0.64	10.32±0.03 (18.37)	10.07±0.03 (24.16)	10.47±0.13 (28.47)	9.95±0.27 (22.72)	10.13±0.51 (21.44)	9.21±0.66 (19.97)	9.43±0.61 (18.28)

*Results are tabulated as mean±SEM (n=5); values in parentheses represent the percentage of reduction. ***p<0.001 when compared to distilled water group. AEAF: Aqueous extract of Albizia ferruginea, AEAF+Nal: Aqueous extract of Albizia ferruginea combined with naloxone, MorNal: Morphine combined with naloxone*

Table 8. Effect of aspirin and aqueous extract of Albizia ferruginea on Brewer's yeast-induced pyrexia in rats

Treatments	Rectal temperature in °C at different time period (h)							
	Ti	T0 h	T1 h	T2 h	T3 h	T4 h	T5 h	T6 h
Distilled water (10 mL/kg)	36.54±0.05	38.12±0.16	38.30±0.21	38.30±0.19	38.12 ± 0.21	38.38±0.17	38.04±0.15	38.24±0.24
AEAF (100 mg/kg)	36.56±0.08	37.48±0.18 (32.47)	37.64±0.25 (26.66)	37.62±0.18 (30.00)	37.54 ± 0.14 (18.19)	37.58±0.20* (47.41)	37.48±0.15 (22.23)	37.62±0.03 (20.66)
AEAF (200 mg/kg)	36.54±0.06	37.10±0.24 (37.23)	37.26±0.37 (30.00)	37.24±0.16 (33.33)	37.14± 0.17 (27.71)	37.28±0.18 (38.58)	37.18±0.15 (04.58)	37.30±0.16 (13.25)
Aspirin (100 mg/kg)	36.52±0.05	37.46±0.16 (22.95)	37.34±0.22* (46.66)	37.46±0.05** (66.66)	37.28±0.19** (65.81)	37.32±0.22** (65.05)	37.32±0.25* (51.64)	37.44±0.19* (42.88)

*Each value is the mean±SEM (n=5); values in parentheses represent the percentage of reduction, *p<0.05, **p<0.01 when compared to distilled water group. Ti: Initial temperature, To: Temperature after induction of fever 18 hours after yeast injection*

[32,33]. The local peritoneal receptor could be the cause of abdominal writhings [34]. These prostaglandin and lipoxygenase products cause inflammation and pain by increasing capillary permeability. The substance inhibiting the writhings will have analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition [35]. Regarding the results of in acetic acid-induced abdominal constriction assay, a prominent inhibition of writhing reflux was observed. These findings strongly recommend that AEAF has peripheral analgesic activity and their mechanisms of action may be mediated through inhibition of local peritoneal receptors which may be the involvement of cyclooxygenase inhibition potential. The profound analgesic activity of *A. ferruginea* may be due to the interference of their active principle with the release of pain mediators. The test did not indicate if the potential resulted from central and/or peripheral actions. To clarify that, formalin test was conducted. This test showed that the extract had only peripheral analgesic effects. The nociceptive effect of formalin is biphasic. This test showed that the extract had only peripheral analgesic effects (inhibition of inflammatory pain which is phase 2) rather than central analgesic effects (inhibition of non-inflammatory pain which is phase 1), an early neurogenic component followed by a later tissue mediated response [36]. *A. ferruginea* blocked the licking response in phase 2, indicating that the extract is also effective against formalin-induced nociception. Since the extract's effect was on inflammatory pain, it meant its possible site and mechanism of action was inhibition of inflammatory mediator's synthesis as well as receptor blockade. The anti-inflammatory activity of *A. ferruginea* may be due to their content of flavonoids and tannins which inhibits the cyclooxygenase activity.

Thermal nociception models such as hot plat and the tail immersion tests were used to evaluate central analgesic activity. In hotplate test any significant results noted, (a test suitable for identifying centrally and not peripherally acting analgesics), were not due to central acting effects of the extract since naloxone did not block the action of extract. The tail immersion test differentiates central opioid-like analgesic from peripheral analgesics. The AEAF showed no significant analgesic effect in both the hot plat and tail immersion tests, suggesting both no spinal and no supraspinal analgesic pathways. Any significant results was noted in hot plate were not due to central acting effects of the extract since naloxone did not block the actions of the extract. This meant, there was no opioid-like receptor mediation involved.

The antipyretic effects of EAEF was expected since from the anti-inflammatory and analgesic tests, the extract had consistently shown to act peripherally on inflammatory mediators especially prostaglandins. The blockade of second phase of formalin test was typical of substances, which antagonise cyclo-oxygenase. These enzymes which produces prostaglandins responsible for the genesis of fever. Aspirin, reference antipyretic drug used in this study is known to inhibit cyclooxygenase enzymes I and II which are implicated in the production of inflammation mediating agent prostaglandin (PGE2) from arachidonic acid [37,38]. The AEAF demonstrate effective antipyretic activity as evident in the inhibition of temperature elevation in the yeast model. The antipyretic action of the aqueous extract may possibly be through inhibition of prostaglandin production, leading to suppression of elevated plasma level especially since the aqueous extract had been shown to possess analgesic and anti-inflammatory activities.

5. CONCLUSION

From these investigations it may be concluded that AEAF stem barks is analgesic, anti-inflammatory and antipyretic effects, and it justifies the traditional use of this plant in the treatment of various types of pains and inflammation.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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