

Anti-inflammatory Effects of the Stem Barks from *Albizia Ferruginea* (Mimosaceae) on Chronic Inflammation Induced in Rats

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Abstract

Albizia ferruginea is used in popular medicine for the treatment of inflammatory illnesses. The chronic anti-inflammatory effects of aqueous extract of *A. ferruginea* was assessed on the cotton pellet-induced granuloma, formalin and complete Freund's adjuvant-induced inflammation. The aqueous extract of *A. ferruginea* was administered orally at the doses of 100 and 200 mg/kg. It's showed that *A. ferruginea* extract (100 or 200 mg/kg) significantly ($p < 0.001$) inhibited the granuloma formation induced by cotton pellets by 41.70 % and 46.10 %, respectively. *A. ferruginea* extract, at the dose of 200 mg/kg inhibited ($p < 0.01$) the paw œdema induced by formalin (25.79 %) and Complete Freund's adjuvant (CFA) in rats (47.40 %). *A. ferruginea* extract (100 or 200 mg/kg) normalized the level of white blood cells, red blood cells, hemoglobin, and platelets of rats treated with CFA. Glutathione and superoxide dismutase levels were significantly increase (92.59% and 60.51%, respectively), catalase activity was significantly increased (42.03%) in the tissue of CFA rats treated with *A. ferruginea* (200 mg/kg). Malondialdehyde level was reduced by 72.88% ($p < 0.01$) in spleen of rats treated with *A. ferruginea* aqueous extract. The nitrite level was significantly decreased by 50.51% in liver of CFA rats treated with *A. ferruginea* (200 mg/kg). These results show that aqueous extract of *A. ferruginea* may possess anti-inflammatory effects on chronic inflammation, which could be partially attributed to its antioxidant properties.

Keywords: *Albizia ferruginea*, Anti-inflammatory, Cotton pellet, Complete Freund's adjuvant, Formalin.

Introduction

Inflammation is the defensive reaction of the living organisms as response to noxious stimulation. The Chronic inflammation is a response to prolonged stimulation in the tissue.^[1] The chronic inflammation is characterized by infiltration of leukocytes into the site of inflammation. This situation eventually leads to tissue deterioration, fibrosis and granuloma formation. The chronic inflammatory responses influences pathological evolution or increase of chronic illnesses. It is depicted or characterized by infiltration of these inflammatory cells, excessive production of cytokines, dysregulation of cellular signaling and loss of barrier function.^[2] The mechanism of chronic inflammation is attributed, in partly to release of reactive oxygen species from activated neutrophils and macrophages. This overproduction causes lipid peroxidation of membranes, which leads to tissue injury through damaging of macromolecules.^[3] In addition, ROS cause or extends inflammation by stimulating the release of cytokines such as

interleukin-1, tumor necrosis factor α and interferon α , which stimulates recruitment of additional neutrophils and macrophages.^[4] Thus, free radicals are important mediators that provoke and sustain inflammatory processes and consequently, their neutralization by antioxidants and radical scavengers can reduce inflammation.^[5] Among chronic illnesses, rheumatoid arthritis which is a greatly cause of disability, and is also considered the most common inflammatory disease.^[6] Attenuation of the chronic inflammatory response is a best strategy to fight against several human diseases. Although steroidal and non-steroidal anti-inflammatory drugs are currently used to treat chronic inflammation disorders, due to the fact that they cause serious side effects.^[7] Most of pharmaceutical drugs originate from plants and the contribution of plants in drug discovery process is remarkable. Therefore, there is an urgent need to find safer anti-inflammatory compounds in these plants. Traditional doctors uses many natural plant and unnatural as treatment options for a wide variety, including chronic inflammation.^[8] Natural products are

believed to be an important source of new chemical substance with potential therapeutic applicability.^[9] Cameroon is a rich source of medicinal plants and a number of these natural resources are used against diseases in the traditional medicine. *A. ferruginea* is a medicinal plants widely distributed in Cameroon and in other African countries.^[10]

Albizia ferruginea stem barks are used in Cameroon folk medicine to treat chronic inflammation without evidence of their anti-inflammatory effects. Traditionally, in Center region, Littoral and South regions of Cameroon, the stem barks of *A. ferruginea* are used to treat diarrhoea, rheumatism, abdominal and tooth pain, headache, bronchitis, dysentery and treat haemorrhoids and to draw out inflammation pain cause by fever.^[11] In Central Africa, a juice made from the leaves of *A. ferruginea* is used as emollient to soothe rashes, swellings, boils and carbuncles, the leaves are used to treat malaria.^[12] Leaves decoction is used as lotion or steam inhalation against fever, headaches and toothache.^[13]

Phytochemical work show on that plant the presence of flavonoids, tannins, anthraquinones, cardiac glycosides, saponins, triterpens and carbohydrates and this molecules have most effects known anti-inflammatory effects. Flavonoids and flavonols exhibit inhibition of arachidonic acid peroxidation, which chemical mediators are implicated in chronic inflammation.^[14] But no systematic research have been done on the search of anti-inflammatory effects. Therefore, the present study was designed to evaluate the anti-inflammatory effect of *A. ferruginea* on cotton pellet, formalin and Complete Freund's Adjuvant (CFA)-induced chronic inflammation in rats.

Materials and Methods

Plant material

The stem barks of *A. ferruginea* were harvested from 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, where a voucher specimen of the plant was deposited under the number 49871.

Preparation of plant extract

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 (84 g) representing the stem barks aqueous extract of *A. ferruginea* was obtained (yield of 16.8%).

Qualitative and quantitative phytochemical analysis

The qualitative phytochemical investigations of the stem bark aqueous extract of *Albizia ferruginea* were performed for alkaloids, flavonoids, saponins, phenols, steroids, glycosides and tannins using standard methods previously described.^[15,16] The quantity of flavonols, total phenols, total flavonoids and total alkaloids content of the stem barks of *A. ferruginea* was determined by the standard methods.^[17, 18, 19] Quantification of the ascorbic acid of the extract of *A. ferruginea* was made using protocol described by Ghate *et al.*^[20] Briefly, 1mg of the extract of *A. ferruginea* was dissolved in 1mL of distilled water. Then 1mL of 2,4-Dinitro-Phenylhydrazin reagent was added in the mixture. After 15 min of incubation at 95°C, 5mL of sulfuric acid (85%) were dripped to the mixture submerged in a cold water (5°C). After 30 minutes, the absorbance

was read at 520 nm with a spectrometer (URIT-810, London). The different tubes have been triplicated. The ascorbic acid content is estimated in mg of vitamin C/g of dry extract by using of vitamin C as standard.

Animals

Wistar albino 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 the University of Yaounde I, Cameroon. They were maintained under standard environmental conditions with dark and light circle 12/12h. They were fed with standard commercial diet and water was provided *ad libitum*. The experimental protocol was in conformity with guidelines of the Cameroon National Ethical Committee on the use of laboratory animals for scientific research (CEEC Council 86/609).

Pharmacological tests

Cotton pellet-induced granuloma in rat

Cotton pellets-induced granuloma in rats was done according to the method of Sapna and Manish.^[21] Adult Wistar rats weighting 200-250 g were randomly divided into four groups of five in each. Rats were anaesthetized by valium (0.5 mL) and ketamine (0.25 mL), two sterilized cotton pellets weighing 10±1 mg were implanted subcutaneously and bilaterally in axillary region of each rat under sterile conditions. The *A. ferruginea* extract (100 or 200 mg/kg), diclofenac (3.85 mg/kg) was used as reference drug or distilled water (10 mL/kg) were administered orally 30 min before implantation of the cotton pellet and once daily for seven consecutive days. At the end of administration, animals were sacrificed under anesthesia (0.5 mL of valium and 0.25 mL ketamine). Group 1 received distilled water at the dose of 10 mL/kg (Control), group 2 and 3 received aqueous extract at the doses of 100 and 200 mg/kg respectively. Group 4 received diclofenac at the dose of 3.85 mg/kg (reference). All treatments were given by gastric gavage for seven consecutive days from the day of cotton implantation. On the eighth day, rats were anesthetized and the cotton pellets surrounded with the granuloma tissue were carefully removed surgically and made free from connective tissues (humid weight). The wet cotton pellets surrounded with the granuloma tissue were weighted and then dried in an oven at 40±0.5°C for 24h, after that the dried weigh were measured (dry weight). The anti-proliferative effects of aqueous extract of *A. ferruginea* were determined by comparison to the control group. Percentage inhibition was calculated as follows:

$$Pi (\%) = ((1-Gt)/(Gc)) \times 100$$

Pi = Percentage inhibition; GC= Average weight of pellets in control group and Gt= Average eight of pellets in treated group.

Formalin-induced paw oedema

This experiment was undertaken using the methodology previously described by Ighodaro *et al.*^[22] Inflammation was induced by subcutaneous injection of 0.1 mL of formalin (2% in 0.9% NaCl) in the right hind paw of the rats on the first and on the third day of the experiment. Adult Wistar rats weighting 200-250 mg/kg were used. Animals were then divided into four groups of five in each. *A. ferruginea* aqueous extract (100 or 200 mg/kg), diclofenac (3.85 mg/kg) or distilled water (10 mL/kg) were administered to rats 30 min before injection of formalin. The animals were treated once daily with the corresponding substances for 10 days. Group 1 (Control) received distilled water at 10 mL/kg. Group 2 and 3

received aqueous extract at the doses of 100 and 200 mg/kg, respectively. Group 4 received diclofenac at the dose of 3.85 mg/kg (reference). Inflammation was induced by injecting formalin (2%, 0.1 mL) into right hind limb of each rat under the subplantar aponeurosis on day zero and third day to induce progressive swelling of paw. The paw volume was determined with the plethysmometer (model 37140 UgoBasile, Italy) before and once daily for ten consecutive days after formalin injection. The ability of the drugs to reduce paw inflammation was expressed as a percentage of inhibition of paw oedema, calculated as follow:

$$Pi (\%) = \frac{(Vt-Vo)_{control} - (Vt-Vo)_{treated}}{(Vt-Vo)_{control}} \times 100$$

Where Vt = Average volume (mL) for each group after treatment; Vo = Average volume (mL) obtained for each group before treatment.

Complete Freund's adjuvant (CFA)-induced arthritis

Adjuvant-induced arthritis was done following the protocol described by Fotio *et al.*,^[23] with some modifications. Adult Wistar rats weighting 200-250 mg/kg were used. Animals were then divided into five groups of five in each. Animals of group 1 (normal control) were free of complete Freund's adjuvant, and were injected with 0.1 mL of 0.9% NaCl in the left hind paw. Group 2 (control) received distilled water (10 mL/kg). Group 3 and 4 received aqueous extract at the doses of 100 and 200 mg/kg, respectively. Group 4 received diclofenac (3.85 mg/kg, reference drug). The animal of other groups received complete Freund's adjuvant (0.1 mL/rat), injected in their left hind paw. Injection of normal saline or complete Freund's adjuvant were repeated once daily during four days. On the fifth day, arthritic rats were treated twice a day with *A. ferruginea* (100 or 200 mg/kg), diclofenac (3.85 mg/kg) or distilled water when normal control group received distilled water. The paw volumes of both sides of hind paws of all animals were recorded with plethysmometer (model 37140 UgoBasile, Italy) before, on day 1 before CFA injection and 1, 2, 3 and 4 h after CFA injection to see the degree of swelling. The volume of paw was measured every 2 days from day 9 to day 21. The treatment, excepted group 1, was initiated on day 9, when the joints were supposed to be well inflamed. Percentages of inhibition of oedema were obtained for each group as described above. The animals' body weight were recorded before and at the end of the study, on the twenty first day. Rats were killed by decapitation under

Table 1: Phytochemical of *Albizia ferruginea* the stem bark aqueous extract

<i>Albizia ferruginea</i>	Polyphenols	Flavonols	Flavonoïds	Ascorbic acid	Alkaloids
	(mg CA/g of dry extract)	(mg QT/g of dry extract)	(mg QT/g of dry extract)	(mg VC/g of dry extract)	(mg BER/g of dry extract)
Quantity	58.69±0.65	0.12±0.04	0.18±0.01	0.43±0.03	27.45±0.14

The results are expressed as mean ± SEM (n = 3). CA= caffeic acid, QT= quercetin, BER= berberine, VC= vitamine C.

Effects of the aqueous extract of *Albizia ferruginea* on cotton pellet-induced granuloma

The weight of the granuloma tissue of the control group was 152.16 ± 1.48 mg (figure 1). Treatment with aqueous extract of *A. ferruginea* inhibited in a dose dependent manner the proliferation of granulomatous tissues. At the doses of 100 and 200 mg/kg, the granuloma weight decreased to 88.72 ± 0.81 mg and 82.02±0.74 mg, respectively. Implantation of cotton pellet also causes

anesthesia (0.5 mL of valium and 0.25 mL ketamine) at the end of the study, and blood samples was collected from all groups for hematological analysis and separation of serum for nitrite serum determination. Liver spleen and kidney sample were collected and then homogenized (20%) in Tris-Hcl buffer (50 mM, pH 7.4) at 4°C to measure reduce glutathione (GSH), nitrites, catalase (CAT), proteins, superoxide dismutase (SOD), and malondialdéhéde (MDA) within 12 to 24 h.

Biochemical analysis

The total protein content was determined by the method of Buiret as described by Gornal *et al.*^[24] The glutathione (GSH) content was estimated by the method of Ellman.^[25] The superoxyde dismutase (SOD) activity in the tissues was determined by the method described of Misra and Fridovish^[26] and the catalase content was estimated by the method of Sinha^[27] The malondialdehyde (MDA) content in tissue was determined by the method of Wilbur *et al.*^[28] The nitrites levels were estimated by the method of Slack.^[29]

Ethics

The experimental protocol was in conformity with guidelines of the Cameroon National Ethical Committee on the use of laboratory animals for scientific research (CEEC Council 86/609).

Statistics

The values are expressed as Mean ± SEM. The data were analyzed by one way ANOVA followed by Dunnett's test using Graph pad prism (5.03) software. P values less than 0.05 were considered statistically significant.

Results

Phytochemical analysis

Phytochemical analysis of the aqueous extract of *A. ferruginea* stem bark showed that it contain polyphenols, flavonols, flavonoids, vitamin C and alkaloids. Total flavonols represented 0.12±0.04 mg equivalent of quercetin per gram of dried extract. Total phenol content was 58.69±0.65 mg of caffeic acid per gram of dried extract. Total flavonoids content was 0.18±0.01 mg equivalent of quercetin per gram of dried extract. Total alkaloids was 27.45±0.14 mg equivalent of berberine per gram of dried extract and vitamin C represented 0.43±0.03 mg equivalent of vitamin C per gram of dry extract (Table 1).

transudation of fluid from neighboring tissues estimated at 494.37± 4.40 mg. The effects of aqueous extract (100 and 200 mg/kg) on the transudative phase were significantly different compare to control. These inhibitory effect were 41.70 % and 46.10 %, respectively, compared to control. Diclofenac (3.85 mg/kg) also significantly inhibited ($p < 0.001$) proliferation of granulomatous tissues by 58.72 % and transudation by 52.59 %.

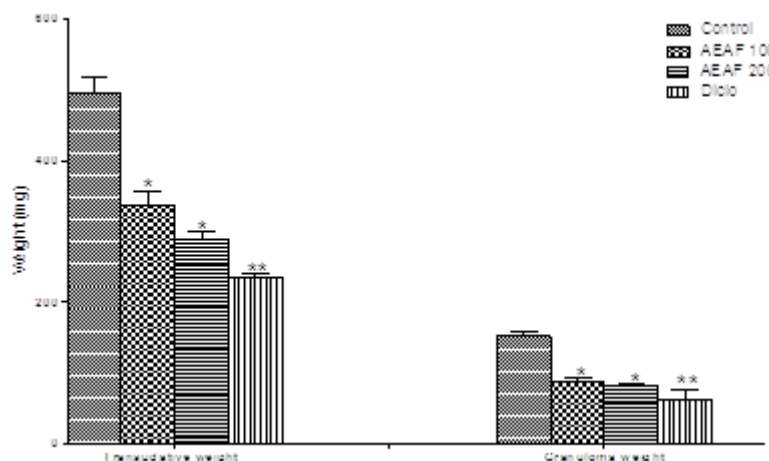


Fig.1: Effects of *Albizia ferruginea* aqueous extract on wet and dry weight of cotton pellets

Data are expressed as mean \pm SEM, n = 5; * p < 0.01, ***p < 0.001, compared to control group (distilled water). Aq ext 100 or 200= aqueous extract of *A. ferruginea* at the dose of 100 or 200 mg/kg.

Effects of the aqueous extract of *Albizia ferruginea* on formalin-induced paw oedema

Subplantar injection of formalin induced oedema, which increased progressively and reached a maximum value of 5.01 ± 0.06 mL, 5 days after induction. Administration of *A. ferruginea* (100 mg/kg) failed to reduce significantly oedema induced by formalin. At the

dose of 200 mg/kg the aqueous extract significantly reduced oedema from day four till the end of the study. The higher inhibition percentage (25.79 %) was recorded on the seventh day. Diclofenac inhibited ($p < 0.001$) oedema induced by formalin compared to control. The maximal inhibition (34.26 %) was recorded on the fifth day after induction of inflammation (figure 2).

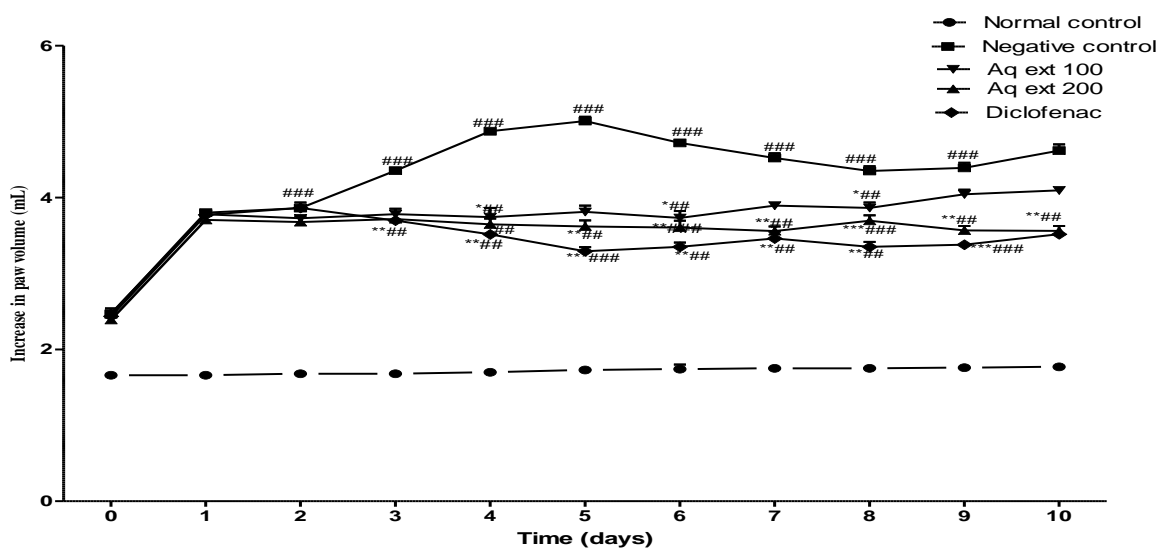


Fig. 2: Effects extracts of *Albizia ferruginea* on formalin-induced paw oedema

Data are expressed as mean \pm SEM, n = 5; # p < 0.05, ### p < 0.01, #### p < 0.001, compared to normal control group, * p < 0.05, ** p < 0.01, compared to arthritic control group. Aq ext 100 or 200= aqueous extract of *A. ferruginea* at the dose of 100 or 200 mg/kg.

Table 2: Effect of *Albizia ferruginea* extract on injected right hindpaw

Treatment	Doses (mg/kg)	(oedema ΔV in mL)						
		(% Inhibition of paw oedema)						
		J9	J11	J13	J15	J17	J19	J21
Normal control		0.06	0.07	0.08	0.09	0.10	0.12	0.14
Arthritic Control	10 mL/kg	3.06 ± 0.03 ##	3.07 ± 0.03 ##	3.10 ± 0.04 ##	3.2 ± 0.04 ###	3.19 ± 0.11 ##	3.07 ± 0.04 ##	3.02 ± 0.10 ##
<i>Albizia ferruginea</i> (aqueous)	100	2.78 ± 0.03 ## (15.16)	2.79 ± 0.03 ##* (32.82)	2.78 ± 0.01 ## (20.88)	3.00 ± 0.02 ##* (35.81)	2.98 ± 0.02 ##* (36.64)	2.88 ± 0.01 ## (25.46)	2.76 ± 0.01 ## (27.29)

	200	2.60±0.01#* (33.71)	2.67±0.04##** (44.44)	2.66±0.02#* (33.13)	2.90 ± 0.03###** (44.02)	2.85±0.04## ** (47.40)	2.75±0.1#* (37.56)	2.65±0.05#* (38.27)
Diclofenac	3.85	2.44±0.01## ** (50.58)	2.57±0.02##* * (54.36)	2.46±0.01###* * (53.81)	2.60 ± 0.03###** (69.98)	2.60±0.03## *** (69.08)	2.50±0.02##* * (61.77)	2.42±0.02## ** (61.60)

Each value represents mean ± SEM, n = 5; #p <0.05,##p <0.001, compared to normal control group,*p <0.05, **p<0.01,compared to arthritic control group.

Effect of Albizia ferruginea aqueous extract on inflammation induced by Complete Freund’s adjuvant in rat hindpaw

Table 2 shows the results of aqueous extract of *Albizia ferruginea* on the swelling of injected paw which is the primary response to the injection of Complete Freund’s adjuvant (CFA). Injection of CFA caused a paw oedema that increased progressively to reach a maximal value of 3.20±0.04 mL, on the 15th day after induction. The oedema of the animals treated with *A. ferruginea* aqueous extract (200 mg/kg) and diclofenac (3.85 mg/kg) group began to subside gradually (p<0.001) when compared with arthritic control. This primary response to CFA was inhibited by the *A. ferruginea* aqueous extract in a dose-dependent manner. The effect of *A. ferruginea* aqueous extract (100 or 200 mg/kg) was significantly different from arthritic control. The maximal inhibition was 47.40 % registered on the 17th day following CFA injection. Administration of diclofenac (3.85 mg/kg) significantly reduced

the oedema induced by CFA from the 9th day till the end of the study. The maximal effect of diclofenac was 69.98 % observed on the 15th day after induction of inflammation.

Effect of Albizia ferruginea extract on non-injected left hind paw

The latent response to the CFA injection was registered on the left hind paw of arthritic rats (Table 3). This response which occurred few days after induction was characterized by joint swelling and nodule formation in the contralateral left hind and was significantly different to normal control from the 9th day. Administration of *A. ferruginea* extract (100 or 200 mg/kg) protects in a dose dependent manner against contralateral joint swelling compared to arthritic rats. These effects were significant (p<0.05) from day 11 to 17 with a maximal inhibition of 36.16% on the 17 day. Diclofenac (3.85 mg/kg) significantly reduces the secondary response to CFA injection.

Table 3: Effect of Albizia ferruginea extract on non-injected left hind paw

Treatment	Doses (mg/kg)	(oedema ΔV in mL) (% Inhibition of paw oedema)						
		J9	J11	J13	J15	J17	J19	J21
Normal control	10 mL/kg	0.07±0.04	0.07±0.01	0.08±0.11	0.09 ± 0.05	0.10±0.01	0.12±0.01	0.14±0.04
Arthritic Control	10 mL/kg	0.66±0.03##	0.72±0.05##	0.69±0.03##	0.79 ± 0.10##	0.86±0.11##	0.77±0.10##	0.76±0.10##
<i>Albizia ferruginea</i> (aqueous)	100	0.39±0.12 (08.94)	0.45±0.02 (09.40)	0.41±0.02 (09.70)	0.52 ± 0.03 (13.06)	0.58±0.02 (19.27)	0.50±0.02 (12.69)	0.48±0.02 (11.84)
	200	0.36±0.02 (12.54)	0.36±0.03 (21.10)	0.37±0.01 (14.95)	0.40 ± 0.05## (27.78)	0.43±0.01## (36.16)	0.46±0.02 (17.61)	0.44±0.02 (17.39)
Diclofenac	3.85	0.27±0.04##** (55.44)	0.20±0.02##** (57.87)	0.16±0.02###* (58.68)	0.12 ± 0.03#** (62.04)	0.18±0.04###* (66.49)	0.20±0.03##* (59.84)	0.19±0.02##* (55.22)

Each value represents mean ± SEM, n = 5; #p <0.05,##p <0.001, compared to normal control group,**p<0.01,compared to arthritic control group.

Table 4: Effect of Albizia ferruginea extract on body weight of arthritis rats

Treatment	Body weight ± ESM (g)		% increase in body weight
	Initial	Final	
Normal control	205.70±0.17	234.61±1.17	14.04
Arthritic control	205.64±1.37	218.78±2.473##	6.37
AEAF 100 mg/kg	205.78±0.40	228.04±2.39*	10.84
AEAF 200 mg/kg	205.32±0.37	231.17±0.50*	12.40
Diclofenac (3.85 mg/kg)	205.44±0.91	225.16±1.19#*	8.67

Each value represents mean ± SEM, n = 5; #p <0.05,##p <0.001, compared to normal control group,*p <0.05, compared to arthritic control group.

Table 5: Effect of *Albizia ferruginea* extract on hematological profiles of arthritic rats

Treatment	Hematological parameters				
	WBC (10 ³ /μL)	Lym (10 ³ /μL)	RBC (10 ⁶ /μL)	Hb (g/dL)	PLAT (10 ³ /μL)
Normal control	6.60±0.62	1.45±0.21	8.77±0.16	12.35± 0.79	4.79±0.75
Arthritic control	8.88±0.04	1.97±0.02	6.39±0.17 [#]	10.96±0.51 [#]	3.46±0.47 ^{##}
AEAF 100 mg/kg	6.80±0.34 [*]	1.73 ± 1.81 [*]	8.38±0.35 [*]	11.48±0.65	4.52±0.55
AEAF 200 mg/kg	6.72±0.26	1.75 ±1.66	8.69±0.25	11.55±0.49	4.66±0.96
Diclofenac 3.85 mg/kg	6.72±0.33	1.47 ±0.29	7.62±0.44	11.14±0.74	4.26±0.50

Each value represents mean ± SEM, n = 5; [#]p <0.05, ^{##}p <0.001, compared to normal control group, ^{*}p <0.05, ^{**}p <0.01, compared to control group (arthritic rats); WBC=White blood cell; Lym= Lymphocytes; RBC= Red blood cell; Hb=Hemoglobin; .PLAT=Platelets.

Effect of *Albizia ferruginea* extract on body weight of arthritic rats

An increase in body weight was noted in all groups of animals. *A. ferruginea* extract (100 or 200 mg/kg) has improved the growth of treated rats compared to controls groups (Table 4).

Effect of *Albizia ferruginea* extract on hematological profiles of arthritic rats

The table 5 illustrates hematological profiles of experimental rats. Increase in number of white blood cell and decrease in number of red blood cell were found in arthritic control compare to normal control. *A. ferruginea* extract (100 mg/kg and 200 mg/kg) tend to normalize the level of these parameters. Result also indicates that Diclofenac at 3.85 mg/kg fell to stabilize the level of red blood cell.

Effect of *Albizia ferruginea* aqueous extract on biochemical parameters

Effects on tissue level of protein and malondialdehyde

Result shows a significant increase of total protein in liver, kidneys and spleen of arthritic rats. An increasing level of 68.81 % in liver,

58.72 % in kidney and 52.52 % in spleen was recorded at the end of the experiment compared to arthritic control. Treatment with *A. ferruginea* aqueous extract at the doses of 100 or 200 mg/kg caused a reduction of the total protein in these tissues. The effect of aqueous extract was significant (p <0.01) in the kidney compared to arthritic rats. Treatment with diclofenac (3.85 mg/kg) normalized the level of total protein in all tissues (figure 3). The level of malondialdehyde was significantly increased in liver, kidney and spleen of animal treated with the Complete Freund's Adjuvant (CFA) compared to normal control (figure 4). Administration of *A. ferruginea* aqueous extract led to a significant decrease of the MDA level in these tissues. The effect of aqueous extract was dose-dependent. *A. ferruginea* aqueous extract (200 mg/kg) reduced the MDA level by 40.63%, 46.50% (p<0.05) and 72.88% (p<0.01), respectively in the liver, kidney and spleen compared to arthritic rats. Diclofenac (3.85 mg/kg) caused a decrease of MDA level by 56.18%, 51.07% (p<0.01) and 33.88% (p<0.05) in the liver, kidney and spleen, respectively, compared to arthritic control.

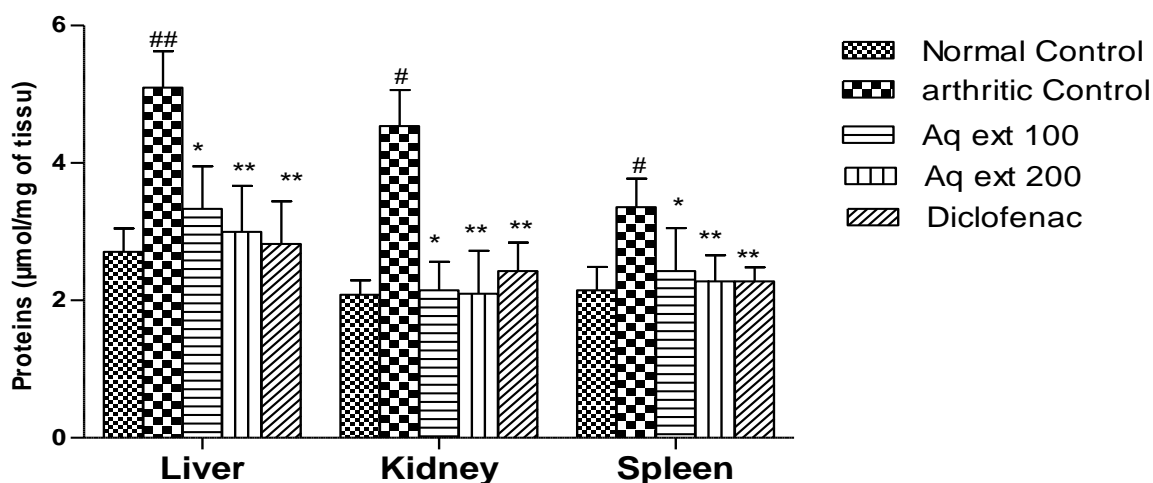


Fig.3: Effects of aqueous extract of *Albizia ferruginea* on tissue level of protein

Each value represents mean ± SEM, n = 5; [#]p <0.05, ^{##}p <0.001, compared to normal control group, ^{*}p <0.05, ^{**}p <0.01, compared to arthritic control group. Aq ext 100 = aqueous extract of *A. ferruginea* dose 100; Aq ext 200 = aqueous extract of *A. ferruginea* dose 200

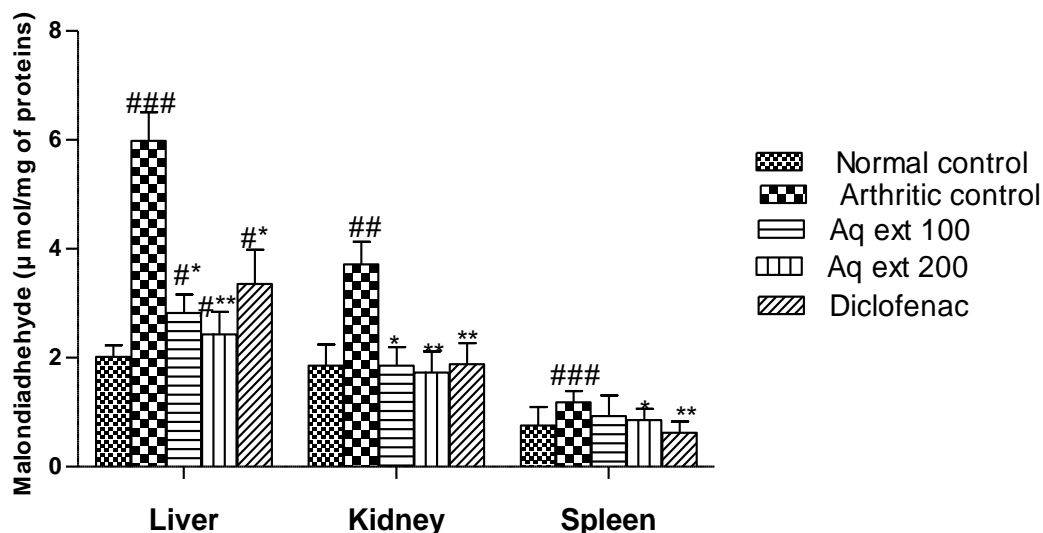


Fig 4: Effects of aqueous extract of *Albizia ferruginea* on tissue level of malondialdehyde

Each value represents mean \pm SEM, n = 5; #p <0.05, ###p <0.001, compared to normal control group, *p <0.05, **p <0.01, compared to arthritic control group. Aqext 100= aqueous extract of *A. ferruginea* dose 100; Aqext200= aqueous extract of *A. ferruginea* dose 200.

Effects on superoxyde dismutase and catalase activity

Results indicated a significant decrease of superoxide dismutase (SOD) activity in rat tissue following treatment with complete Freund adjuvant compared to normal control (figure 6). Administration of *A. ferruginea* (100 and 200 mg/kg) causes a dose dependent increase in the SOD activity in rat tissue. In liver, kidney and spleen, the SOD activity increased by 42.72%, 60.51% and 30.86%, respectively, compared to arthritic control. Diclofenac (3.85 mg/kg) referent substance has increase the SOD activity by

43.36%, 63.31% and 33.15% compared with arthritic control animals.

Catalase activity decreased significantly in tissues of animals treated with CFA compared to normal control. In the liver, catalase activity of normal control was 0.80 ± 0.05 mmolmin⁻¹/mg of protein compared to 0.31 ± 0.05 mmolmin⁻¹/mg of protein registered in arthritic group. When treated with *A. ferruginea* aqueous extract at the doses of 100 or 200 mg/kg, catalase activity increased (p<0.01) in dose dependent manner. This effect was significantly different compared to arthritic group and the maximal effect was 40.94%, 42.03% and 35.29%, respectively in liver, kidney and spleen. Treatment with diclofenac (3.85 mg/kg) caused an elevation of the catalase activity by 44.11%, 41.17% and 33.63%, respectively in liver, kidney and spleen, compared to arthritic control (figure 5).

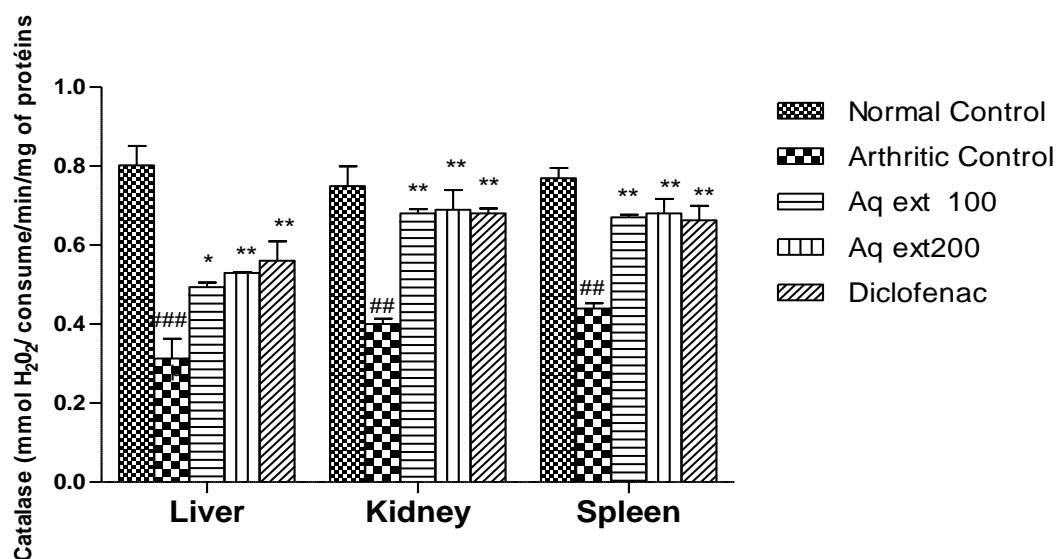


Fig.5: Effects of aqueous extract of *Albizia ferruginea* on catalase activity

Each value represents mean \pm SEM, n = 5; #p <0.05, ###p <0.001, compared to normal control group, *p <0.05, **p <0.01, compared to arthritic control group. Aq ext 100= aqueous extract of *A. ferruginea* dose 100; Aq ext 200= aqueous extract of *A. ferruginea* dose 200.

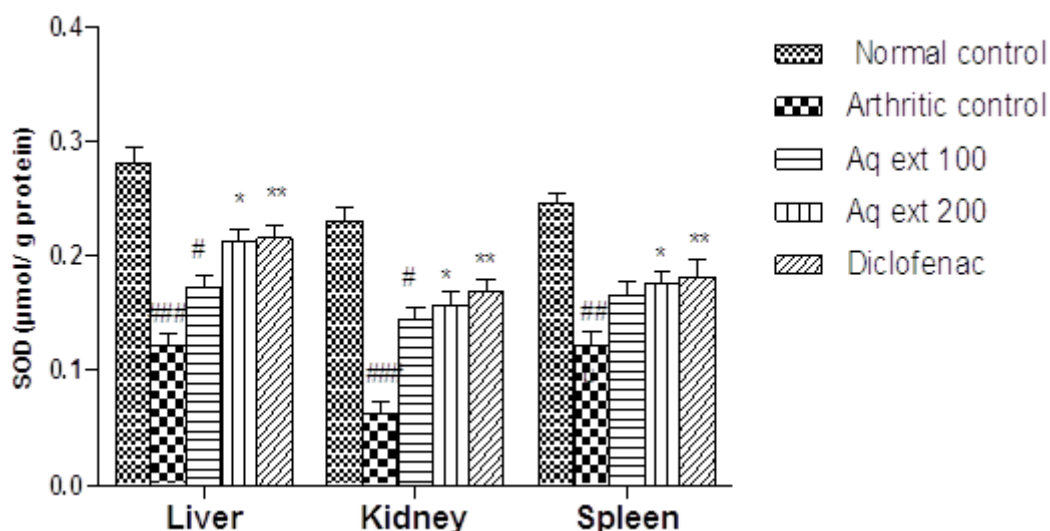


Fig.6: Effects of aqueous extract of *Albizia ferruginea* on superoxide dismutase activity

Each value represents mean \pm SEM, n = 5; #p <0.05, ##p <0.001, compared to normal control group, *p <0.05, **p <0.01, compared to arthritic control group. Aqext 100= aqueous extract of *A. ferruginea* dose 100; Aq ext 200 = aqueous extract of *A. ferruginea* dose 200.

Effects on tissue level of glutathione

Treatment of rats with Complete Freund's Adjuvant induced after 21 days, a significant reduction of the level of glutathione in liver, kidney and spleen by 65.92%, 50.16% and 23.25%, respectively, compare to normal control (figure 7). Administration of *A. ferruginea* (100 or 200 mg/kg) and diclofenac (3.85 mg/kg) result in an increase in the level glutathione. The aqueous extract acts in

dose dependent manner. The effect of the most effective dose of *A. ferruginea* extract (200 mg/kg) was comparable to that of diclofenac. The level of glutathione of arthritic rats compared to those treated with *A. ferruginea* extract (200 mg/kg) raised from 0.26 ± 0.01 to 0.52 ± 0.01 mmol/g of protein in the liver, from 0.31 ± 0.01 to 0.55 ± 0.01 mmol/g of protein in the kidney and from 0.76 ± 0.01 to 0.91 ± 0.02 mmol/g of protein in the spleen.

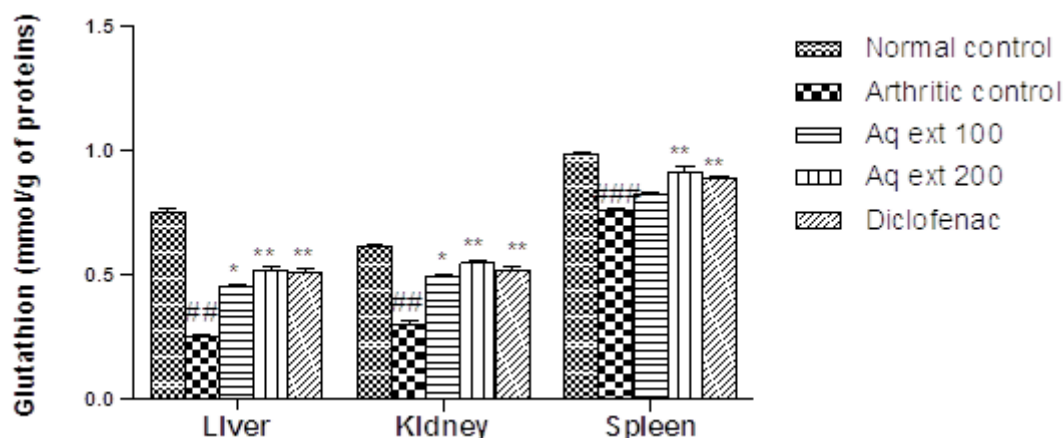


Fig.7: Effects of aqueous extract of *Albizia ferruginea* on tissue level of glutathione

Each value represents mean \pm SEM, n = 5; #p <0.05, ##p <0.001, compared to normal control group, *p <0.05, **p <0.01, compared to arthritic control group. Aq ext 100 = aqueous extract of *A. ferruginea* dose 100; Aq ext 200 = aqueous extract of *A. ferruginea* dose 200.

Effects on nitrites level

A significant increase of serum nitrites level, compared to normal control was noted after treatment of rats with complete Freund adjuvant. This effect was inhibited in a significant manner by administration of *A. ferruginea* aqueous extract (100 and 200 mg/kg). Inhibitory effects were 36.79% and 39.15%, respectively, compared to arthritic control. Diclofenac also reduce significantly nitrite production induced by CFA (figure 8a). Induction of

arthritis by CFA also lead to significant nitrites production in liver, kidney and spleen of animals compared to normal control. Administrated at the doses of 100 and 200 mg/kg, *A. ferruginea* aqueous extract significantly decreased nitrites production in liver, kidney and spleen by 50.51%, 28.48% and 34.33%, respectively, compared to arthritic control. Diclofenac (3.85 mg/kg) exhibited 53.35%, 30.81% and 38.24% of inhibition respectively in the liver, kidney and spleen, compared to arthritic control (figure 8b).

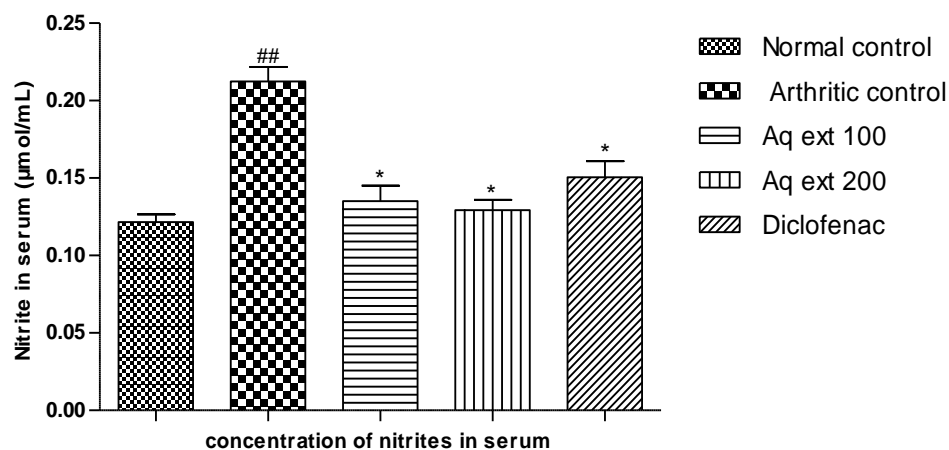


Fig.8a: Effects of aqueous extract of *Albizia ferruginea* on serum level of nitrites

Each value represents mean \pm SEM, n = 5; ^{##}p <0.01, compared to normal control group, ^{*}p <0.05, compared to arthritic control group. Aq ext 100= aqueous extract of *Albizia ferruginea* dose 100; Aq ext 200= aqueous extract of *Albizia ferruginea* dose 200.

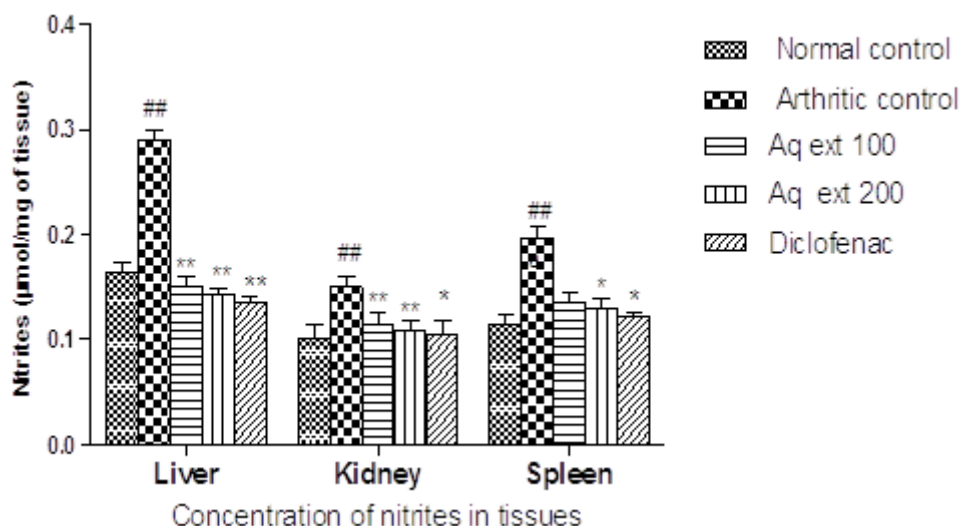


Fig.8b: Effects of aqueous extract of *Albizia ferruginea* on tissue levels of nitrites

Each value represents mean \pm SEM, n = 5; [#]p <0.05, ^{##}p <0.001, compared to normal control group, ^{*}p <0.05, ^{**}p <0.01, compared to arthritic control group. Aq ext 100= aqueous extract of *A. ferruginea* dose 100; Aq ext 200= aqueous extract of *A. ferruginea* dose 200.

Discussion

Chronic inflammation model, induced by subcutaneous implantation of foreign bodies, is used commonly in experimentations. It is the most suitable method for the evaluation of drug efficiency against proliferative phase of inflammation.^[3] Subcutaneous implantation of the cotton pellet causes granulomatous tissue formation. This granulomatous tissue is due firstly to the accumulation of macrophages, neutrophils and lymphocytes around the foreign particles, which is followed by proliferation of fibroblast cells.^[30] The cotton pellet stimulates the immune system to produce interleukins and antibodies that consequently, stimulate proliferation of lymphocytes and accumulation of cells around the pellets to form granulomatous tissue.^[31] Thus, inhibition of granulomatous tissue formation indicates an anti-proliferative effect of a drug. The result of this study shows that *Albizia ferruginea* extract (100 or 200 mg/kg) inhibited accumulation of cells and substances around the foreign body. This may be due to its anti-proliferative properties.

Transudate material is correlated to the wet weight of the cotton pellets.^[32] Our results show an inhibiting effect of the aqueous extract of *A. ferruginea* on the transudative phase of inflammation. This may suggest its antiphlogistic effects. This plant effect was closer to that of diclofenac, a non-steroidal anti-inflammatory drug (NSAID). It is demonstrated that NSAIDs reduce the size of granuloma and the quantity of transudation through inhibition of pro-inflammatory mediator's production (inflammatory cytokines and other lipid mediators of inflammation like leukotrienes and prostaglandins), inhibition of cells infiltration (leucocytes) and prevention of fibroblast proliferation and collagen fibres and mucopolysaccharides synthesis.^[33, 34]

The aqueous extract of *A. ferruginea* was further tested on chronic inflammation induced by formalin. It is well known that oedema induced by formalin in rats is one of the most suitable investigations to screen anti-arthritis and anti-inflammatory agents, because this inflammation model is similar to human arthritis. Arthritis induced by formalin is used for the evaluation of an eventual anti-

proliferative effects of natural substances.^[22] In this study, we demonstrated that *A. ferruginea* aqueous extract significantly inhibited formalin induced inflammation. This result in accordance with that of granuloma induced cotton pellet which demonstrated anti-proliferative and antiarthritic properties of this plant extract. Previous study proved that, chronic inflammation induced by formalin results from cell damage which induces production of endogenous mediators such as histamine, serotonin, prostaglandins, and bradykinin.^[35] Therefore, it may be suggest that *A. ferruginea* extract should be involved in the inhibition of the production and/or the activities of these inflammatory mediators. These result corroborate previous study on the effects of the stem bark of aqueous extract of *A. ferruginea* on inflammation induce in rat's paw by carrageenan, dextran, serotonin and histamine.

The most study chronic model of inflammation is the arthritis induced by a complete Freund's adjuvant. One of the reasons justifying the wide exploitation of this model is the strong correlation existing between the efficacy of therapeutic agents on this model and on the human's rheumatoid arthritis. Moreover, Freund's adjuvant-induced arthritis is a very rapid erosive disease.^[36] The first reaction after injection of Freund's adjuvant heat killed (*Mycobacterium tuberculosis* in paraffin oil) which is due to irritant effects of the adjuvant is the soft tissue thickening at the depot site. This initial response is followed by an immunologic events.^[37]

Dudhgaonkar *et al.*^[38] demonstrated that after CFA injection in a rat hind paw induces a primary response characterized by swelling and hyperalgesia of the injected paw. This reaction is followed by a delayed systemic response which occurs few days later on the controlateral paw and characterized by joint swelling and nodule formation in the tail.

In this present study, both initial and delayed responses were observed after injection of a complete Freund' adjuvant. Treatment of rats with *A. ferruginea* extracts significantly reduce oedema of the injected paw compared to arthritic rats. This extract also inhibits the delayed hypersensitive response which is a latent secondary systemic response occurring few days on the controlateral paw, by preventing significant increase in right paw oedema as compared to normal rats, thus indicating it anti-inflammatory effect.

Arthritis induced by CFA is characterized by a reduction of body weight and It is showed that the loss of body weight observed in this case is associated with higher production of pro-inflammatory cytokines like inter-leukin-1 and tumor necrosis factor- α .^[37,39] Result from our study confirmed this hypothesis and revealed a better improvement effect of *A. ferruginea* extract compared to that of diclofenac, on the body weight of rats within the experimental period. This observation suggest that antiarthritic effect of *A. ferruginea* aqueous extract could be due to it inhibitory effect on the release of pro-inflammatory cytokines.

Tissue damage is related to membrane lesion and impairment of the membrane permeability due to lipid peroxidation. Lipid peroxidation is assessed through determination of the malondialdehyde level (MDA). Our result showed that, administration of CFA resulted in a significant increase in the level of MDA in the tissues of arthritic rats. Treatment with aqueous

extract of *A. ferruginea* significantly decreased the MDA levels. This activity could be due to an anti-oxidative effect of this plant extract.

The earliest cells of the immunological system that contribute to the defense of the body against pathogens are neutrophils. However, the activity of these immune cells may lead to tissue damages in chronic inflammatory diseases. Interaction between neutrophils and other components like resident cells, local inflammatory mediators and extracellular matrix at the site of the inflammation induces liberation of degrading enzymes, reactive oxygen and nitrogen species as well as additional inflammatory mediators which increase inflammatory process especially through oxidative stress.^[2] Chronic inflammation induced either by CFA causes oxidative stress and damage to several organs including the liver,^[40] the kidney^[41] and the spleen.^[42] Endogenous enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase are involve in the protection against free radical induced tissue damages cause by the superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2).^[43] Glutathione peroxidase degrade the organic hydroperoxydes and hydrogen peroxide into harmless compounds by using reduced glutathione (GSH) as a substrate. The aqueous extract of *A. ferruginea* significantly increased the levels of SOD and the activities of CAT and GSH suggesting it anti-oxidative effect.

It has also been established that CFA could activates of the inducible NO-synthase for massive production of nitric oxide. Nitrite is a stable product of NO which is a mediator of inflammation involving in the synthesis of prostaglandins, cytokines and reactive oxygen species.^[44,45] The result of this study showed the inhibitory effect of *A. ferruginea* aqueous extract on the inducible NO-synthase activity, corroborating the anti-oxidative activity of this plant extract.

The phytochemical analysis of the aqueous extract of *A. ferruginea* stem backs shows that it contains compounds like polyphenols, flavonoids, flavonols, alkaloids and ascorbic acid. These compound have extensive pharmacological effects such as antioxidant, inhibition of platelet aggregation and anti-inflammatory.^[46] Previous study has shown that ascorbic acid is an antioxidant that reduces lipid peroxidation directly or indirectly through regeneration of vitamins E,^[47] or by binding free radicals species via a transfer of electron with a view to protect biological membranes from peroxide damage.^[48] It is demonstrated that, ascorbic acid could normalize the level of malondialdehyde, potentiate the activities of superoxide dismutase.^[49]

Flavonoids possess an antioxidant property which confers to this important class of compound a potential anti-inflammatory effects.^[50] Study showed that flavonoids inhibit production of lipid derivative inflammatory mediators such as prostaglandins, leukotrienes through inhibition of their substrate or their catalytic enzyme.^[50,51] Flavonoids inhibit neutrophil degranulation and reduce complement activation.^[48] It has been reported that polyphenols may inhibit lipid peroxidation and enzymes involve in the degradation of the arachidonic acid into inflammatory mediators.^[52] The effect of the *A. ferruginea* extract may be partly related to the presence of these compounds. Nevertheless, further study are require to understand the mechanism of the *A. ferruginea* aqueous extract on inflammatory diseases.

Conclusion

The results of the present study show that the aqueous extract of *A. ferruginea* possesses an anti-inflammatory effect. *A. ferruginea* aqueous extract inhibits transudative and proliferative phases of cotton pellet induced granuloma. This extract also reduces oedema induced by formalin and complete Freund's adjuvant. These anti-inflammatory effects of the aqueous bark extract of *A. ferruginea* on chronic inflammation may be due to its inhibitory effect on pro-inflammatory mediators and its antioxidant properties. These findings therefore supporting the use of the stem bark of this plant in the folk medicine for the treatment of anti-inflammatory diseases. *A. ferruginea* could be a potential source of new anti-inflammatory compounds. Thus, further studies are needed to assess the possible activities on joint destruction causes by arthritis and to evaluate the safety of this extract.

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Abbreviations

A.ferruginea = *Albizia ferruginea*; CA= caffeic acid, QT= Quercetin, BER= berberine, VC= vitamine C; GSH: Glutathione; SOD: Superoxyde dismutase, CAT: Catalase; MDA: Malondialdehyde.

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