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Original Article

IN VITRO AND *IN VIVO* SCREENING OF ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC AND AQUEOUS EXTRACTS OF *ANOGEISSUS LATIFOLIA* LEAVES

SABA SHAIKH[1*], BADRUDDEEN[2], MOHAMMAD IRFAN KHAN[2], AJAZ AHMED[3]

[1]Department of Pharmacology, Anjuman-I-Islam Kalsekar Technical Campus, New Panvel, Navi Mumbai 410206, Maharashtra, India, [2]Herbal Bioactive Research Laboratory, Faculty of Pharmacy, Integral University, Lucknow, Uttar Pradesh, India, [3]Department of Pharmacognosy, Anjuman-I-Islam Kalsekar Technical Campus, New Panvel, Navi Mumbai 410206, Maharashtra, India
Email: shaikhsaba66@gmail.com

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ABSTRACT

Objective: To evaluate and compare anti-oxidant and anti-inflammatory activity of methanolic and aqueous extract of *Anogeissus latifolia* leaves

Methods: The *in vitro* antioxidant activity was investigated using nitric oxide radical inhibition activity assay, hydroxyl radical scavenging activity assay, DPPH free radical scavenging assay, and reducing power assay. The *in vitro* anti-inflammatory activity was investigated using erythrocyte membrane stabilization, inhibition of protein denaturation, and proteinase inhibitory activity the *in vivo* anti-inflammatory activity was investigated using carrageenan-induced rat paw edema. The biochemical parameters were evaluated in the blood, which included the determination of serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase values and in the liver, which includes the estimation of lipid peroxidation, reduced glutathione, and superoxide dismutase.

Results: The methanolic extract caused a significant dose depended on the reduction of inflammation when compared with the aqueous extract of *Anogeissus latifolia.* The anti-inflammatory activity of all groups was found to be comparable to the standard indomethacin group. The maximum percent inhibition in paw edema was found in methanolic extract of *Anogeisuss latifolia* at a dose of 500 mg/kg was 53.33%, with significant anti-inflammatory activity $p<0.001$.

Conclusion: The leaf extract of *Anogeissus latifolia* possesses anti-oxidant and anti-inflammatory activity. The therapeutic effect of *Anogeisuss latifolia* extracts will encourage its use in the treatment of inflammation.

Keywords: *Anogeissus Latifolia*, *In vitro* antioxidant activity, DPPH, *In vitro* anti-inflammatory activity, Carrageenan rat paw edema

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INTRODUCTION

Inflammatory diseases have become one of the leading causes of health issues throughout the world, having a considerable influence on healthcare costs. Inflammation or phlogosis is the pathological response of living tissue to injuries that leads to the local accumulation of plastic fluid and blood cells. Although it is a defense mechanism, the complex event and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases [1]. It is a complex phenomenon comprising biochemical as well as immunological factors.

Several civilizations across the globe have used herbs in different forms. Before the advent of modern medicine, herbs were used in the form of powders, pastes, tablets, galenicals like tinctures, infusion, decoction, oils saturated with herbs, oils, poultice, etc. Herbs have been used in India in Ayurveda, Siddha, and Unani systems of medicine, collectively known as the indigenous system of medicine. The modernized method of formulating herbal medicines has yielded a new system of herbal medicine, popularly known as a phytomedicine.

Many developed countries have realized the usefulness of herbs as a source of starting material (lead compounds) in the process of drug discovery. The chances of getting a new drug from herbs are higher than from a synthetic source because of the availability of literature on plant activity. Many biomarkers have been isolated and identified.

The indigenous system of medicine and modern medicine have fundamental differences. Modern medicines make use of single ingredients, whereas the indigenous system of medicine believes that different constituents of herbs have a synergistic effect. It has been shown that the mode of action differs when the effect of the isolation biomarkers is compared with the total extracts [2].

The literature review observed that many plants containing isoflavonoids, flavonoids, phytosterol, sesquiterpenoids, cineol, monoterpenoid, stigmasterol, β-sitosterol and tannins are responsible for reducing inflammation and pain [3]. The main purpose of this study was to investigate the mechanism of action anti-inflammatory activity of *Anogeissus latifolia.* The current study is a comparative study of investigation of the Anti-inflammatory activity of aqueous and methanolic extracts of leaves of *Anogeissus latifolia.*

MATERIALS AND METHODS

Procurement, identification of plant material

The leaves of *Anogeissus latifolia* (DC.), were collected from the National Research Institute of Basic Ayurvedic Sciences, Pune, India. The plant material was identified and authenticated by the Department of Botany, Guru Nanak Khalsa College Matunga, Mumbai 400019. The voucher specimen (rpd/130912) has been preserved for future reference.

Animal

Adult female Spargue Dawley rats (150-180 g) were procured from Glenmark Research Centre, Plot no. a-607, TTC Industrial Area M. I.D. C, Mahape, Navi Mumbai. The animal house was maintained on a 12 h light/dark cycle at 22±2 °C, relative humidity 60-70%, and the animals were provided with a standard laboratory diet and water ad libetum. The study protocol was approved by Institutional Animal Ethics Committee, Mumbai (approval no-IAEC/PR/2012/02) prior to the commencement of experimental work.

Chemicals

Indomethacin (Sigma), Carrageenan (Sigma), Erba SGOT and SGPT kit, and all other chemicals were of analytical grade.

Extraction of plant

Freshly collected leaves of *Anogeissus latifolia* (DC.) were dried under shade. Dried leaves were ground to a coarse powder. The

powder was extracted using two different laboratory-grade solvents (distilled water and methanol).

Preparation of aqueous extract-Powdered leaves of *Anogeissus latifolia* (DC.) were extracted with distilled water by maceration for 24 h. The solvent was filtered and the marc was re-extracted another two times. The combined filtrate was concentrated using rotary a evaporator and kept in desiccators to obtain the dried extract.

Preparation of methanolic extract-*Anogeissus latifolia* (DC.) powdered leaves were extracted with methanol using the soxhlet apparatus. The solvent from the extract was evaporated using a rotary evaporator and kept in desiccators to obtain the dried extract.

***In vitro* antioxidant activity**

Evaluation of *in vitro* antioxidant activity was carried out using the following assays

- **Nitric oxide radical inhibition activity assay**

3.0 ml of 10 mmol sodium nitroprusside in phosphate buffer was added to 1 ml of different concentrations (25-1000µg/ml) of extract and reference. The resulting solution was then incubated at 25 °C for 60 min. A similar procedure was repeated with distilled water instead of extract, which served as a control. 5 ml of Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dihydrochloride, 2% H_3PO_4) was added to 5 ml of the incubated sample. The absorbance of chromophores formed during diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylene-diamine dihydrochloride was measured at 546 nm using UV-spectrophotometer. Ascorbic acid was used as reference material. All tests were performed in triplicate [4].

- **Hydroxyl radical scavenging activity assay**

The assay was performing by adding 0.1 ml EDTA, 0.01 ml $Fecl_3$, 0.1 ml H_2O_2, 0.36 ml of deoxyribose, 1 ml of different concentrations (25-1000µg/ml) of extract and reference. 0.33 ml of phosphate buffer (50 mmol, pH 7.4) and 0.1 ml of ascorbic acid in the sequence. The mixture was then incubated at 37 °C for 1 hour.1.0 ml of the incubated mixture was mixed with 1 mmol of 10% trichloroacetic acid and 1.0 ml of 0.5% thiobarbituric acid to develop the pink chromogen, which was measured at 532 nm using UV-spectrophotometer, against control preparation containing deoxyribose and buffer instead of extract. Ascorbic acid was used as reference material. All tests were performed in triplicate [5].

- **DPPH free radical scavenging assay**

2.0 ml of 0.1 mmol solutions of DPPH prepared in methanol was added into 1.0 ml of different concentrations of (25-1000 µg/ml) of extract and reference material. After 30 min, absorbance was measured at 517 nm using UV spectrophotometer. A similar procedure was repeated with distilled water instead of extract, which served as control. Ascorbic acid was used as reference material. All tests were performed in triplicate. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity [6].

- **Reducing power assay**

2.5 ml of phosphate buffer and 2.5 ml of potassium ferricyanide (1%) were mixed with 1.0 ml of different concentrations of (25-1000 µg/ml) of extract and reference material. The mixture was incubated at 50^0C for 20 min. 2.5 ml of trichloroacetic acid (10%) was added to the mixture and centrifuged at 3000rpm for 10 min. 2.5 ml of upper layer of the mixture was mixed with 2.5 ml of distilled water and 0.5 ml of FeCl3 solution (0.1%). The absorbance was measured at 700 nm using a UV spectrophotometer. Ascorbic acid was used as reference material. All tests were performed in triplicate [7].

***In vitro* anti-inflammatory activity**

- **Erythrocyte membrane stabilization**

The blood was collected from rats by orbital plexus route in a vial containing heparin (anti-coagulant). 20 µl of fresh blood was added to a vial containing 1 ml of PBS (phosphate buffer solution pH=7.4). Test drugs or standards in PBS were added to these vials so as to achieve final concentrations of 100-1000µg/ml and PBS (15 µl) was added to control vials and mixed properly. Prior to the addition of the drugs solution to vials, the drug solutions were subjected to centrifugation at 3000× g for 10 min to give a clear solution. The vials containing the reaction mixture were incubated at 37 °C FOR 15 min; the mixture was then heated at 54 °C for 25 min. The mixture was centrifuged to give a clear supernatant. The absorbance of the supernatant was measured at 540 nm using UV visible spectrophotometer [8].

- **Inhibition of protein denaturation**

The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and0.05 ml of drug or standard (100-1000µg/ml). pH was adjusted to 6.3 using a small amount of 1 N HCL. The samples were incubated at 37 °C for 20 min and then heated at 57 °C for 3 min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm. For control tests, 0.05 ml distilled water was used instead of drugs, while product control tests lacked bovine serum albumin. The control represents 100% protein denaturation. The results were compared with standard-treated samples [9].

- **Proteinase inhibitory activity**

The reaction mixture (2.0 ml) contained 0.06 mg trypsin, 1.0 ml 20 mmol tris HCL buffer (pH 7.4) and 1 ml aqueous drugs or standard suspension (100-1000µg/ml of final volume). The mixture was incubated at 37 °C for 5 min. Then 1.0 ml of 0.8% (w/v) casein was added. The reaction was then incubated for an additional 20 min. 2.0 ml of 70% perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged. The absorbance of the supernatant was recorded at 210 nm against buffer as control [10].

***In vivo* anti-inflammatory activity by carrageenan-induced rat paw edema method**

Female Sprague dawley rats weighing between 170-180g are fasted 16 h before treatment with water *ad libitum*. The animals were divided into 6 groups of 6 animals each. Paw edema was induced by injecting 0.1 ml of 1% carrageenan in sterile saline subcutaneously into the sub-plantar region of the rat right hind paw of animals which were pretreated with normal saline 2 ml/kg (Control), indomethacin 10 mg/kg (Standard), MALL 250 mg/kg, MALL 500 mg/kg, AALL 250 mg/kg and AALL 500 mg/kg, 30 min before the carrageenan injection. The paw volumes are measured plethysmographically immediately after injection of Carrageenan at 0, 0.5, 1, 2, 3, 4, 5 and 6 h. Inhibition of Inflammation was calculated as the increase in volume (ml) of the paw after treatment. The percentage inhibition of edema was calculated by the following equation.

$$\% \text{ Inhibition of edema } = 100\left(1 - \frac{Vt}{Vc}\right)$$

Where Vc is the edema volume in the control group and Vt is the edema volume in the tested group. The difference between the initial and subsequent reading gives the actual edema volume [11].

Biochemical investigations

1. Biochemical parameter on blood

After recording the paw edema the blood samples were collected by the retro-orbital route. Serum was separated from each sample and used for the biochemical analysis. The collected serum was used for the estimation of SGOT and SGPT enzymes using commercially available standard enzymatic kits.

a. Serum glutamate oxaloacetate transaminase (SGOT)

Pipette into a clean, dry Test tube, 0.8 ml of an enzyme reagent (L1) and 0.2 ml of sample then incubate at the 25 ℃/30 ℃ temperature for 1 minute. After incubation, add 0.2 ml of starter reagent (L2). Mix well and read the initial absorbance A_0 and repeat the absorbance reading after every 1, 2, and 3 min. calculate the mean absorbance change per minute (ΔA/min). Wavelength/filter: 340 nm, Lightpath: 1 cm.

b. Serum glutamate pyruvate transaminase (SGPT)

Pipette into a clean, dry Test tube, 0.8 ml of an enzyme reagent (L1) and 0.2 ml of sample, then incubate at the 25 ℃/30 ℃ temperature for 1 minute. After incubation, add 0.2 ml of starter reagent (L2). Mix well and read the initial absorbance A_0 and repeat the absorbance reading after every 1, 2, and 3 min. calculate the mean absorbance change per minute (ΔA/min). Wavelength/filter: 340 nm, Lightpath: 1 cm

2. Biochemical parameters on liver

After blood collection, the animals were sacrificed. Immediately after sacrificing the animals, livers were separated, wash with pH 7.4 buffer, blotted with dry filter paper and liver weight was recorded. A part of the liver was minced and then homogenized in pH 7.4 buffer to prepare 10%w/v tissue homogenate. The homogenate was centrifuged at 3000rpm for 15 min at 4 °C and supernatant was used for estimation of Lipid peroxidation (LPO) and Reduced glutathione (GSH). Another part of the liver was minced and then homogenized in pH 7.0 buffer to prepare 10%w/v tissue homogenate. The homogenate was centrifuged at 3000rpm for 15 min at 4 °C and supernatant was used for estimation of Superoxide dismutase (SOD). The following procedures were used to estimate LPO, GSH and SOD.

A) Lipid peroxidation

Reagents-

1) TCA-TBA-HCL Reagent: 15g of TCA (trichloroacetic acid), 0.375g (thiobarbituric acid) were dissolved in 100 ml 0.25N HCL. This solution was slightly heated to assist in the dissolution of TBA.

2) Phosphate buffer pH 7.4.

3) Test samples 10%w/v liver homogenate in phosphate buffer pH 7.4.

Procedure

Add 1 ml of tissue homogenate and 2 ml of TCA-TBA-HCL Reagent. Vortex tube for a few seconds. Heat for 15 min in a boiling water bath. Cool to room temperature, then centrifuged at 1000g for 10 min. Pipette out supernatant in cuvette. Read O. D at 535 nm against blank. For blank, add 1 ml of distilled water and 2 ml of TCA-TBA-HCL Reagent [12].

B) Reduced glutathione content (GSH)

GSH content of test samples was calculated by the extrapolation method using the standard graph. The result was expressed in µmol/g. To prepare of a standard curve. Prepare different concentration of GSH as 5, 10, 15, 25 and 30 µg/ml. Take five test tube, add 0.2 ml of different concentrations of GSH. Add 1.8 ml of distilled water and then add 3 ml of precipitating solution. Stand for 5 min, filter, 2 ml filtrate added to 2 ml of Phosphate solution (in cuvette) and 1 ml of DTNB. Read O. D at 412 nm. For the test sample, add 0.2 ml of liver homogenate, add 1.8 ml of distilled water and then add 3 ml of precipitating solution. Stand for 5 min, filter, 2 ml filtrate added to 2 ml of Phosphate solution (in cuvette) and 1 ml of DTNB. Read O. D at 412 nm. For blank, add 3 ml of precipitating solution. Stand for 5 min, filter, 2 ml filtrate added to 2 ml of Phosphate solution (in cuvette) and 1 ml of DTNB. Read O. D at 412 nm [13].

C) Superoxide dismutase (SOD)

A 10%w/v tissue homogenate was prepared in 0.1M phosphate buffer (pH 7.0). The reaction was initiated by the addition of 0.5 ml of hydroxylamine hydrochloride to the reaction mixture containing 2.0 ml of nitroblue tetrazolium (NBT) and 0.1 ml of liver homogenate. Change in absorbance was measured spectro-photometrically at 560 nm. The enzyme activity was expressed as a unit of SOD/min/gm liver wt [14].

Statistical analysis

Results were expressed as mean±Standard Error Mean (SEM). Differences were considered significant at ***P<0.001, or **P<0.01 or*P<0.05 when compared test groups v/s control group. Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by Dunnett's t-test. * P<0.05 was considered statistically significant, and all graphs were made by using Graph Pad Prism 6 software.

RESULTS

***In vitro* antioxidant activity**

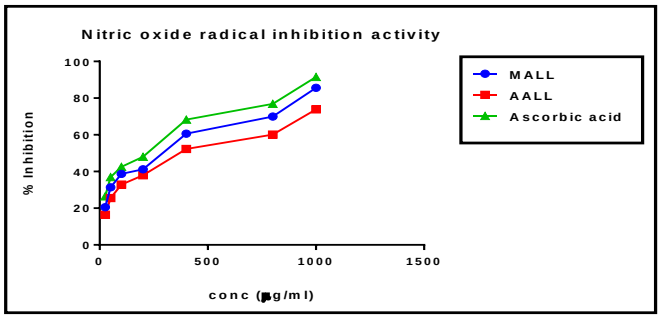

Fig. 1: Result of nitric oxide radical scavenging activity of MALL (Methanolic Extract of *Anogeissus latifolia* leaves), AALL (Methanolic extract of *Anogeissus latifolia* leaves) and ascorbic acid, values are mean±SEM (n=3)

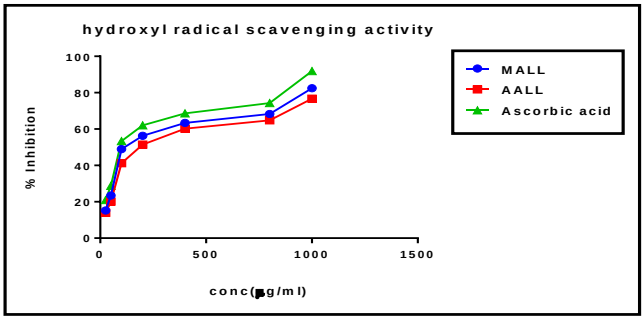

Fig. 2: Result of hydroxyl radical scavenging activity of MALL, AALL, and ascorbic acid, values are mean±SEM (n=3)

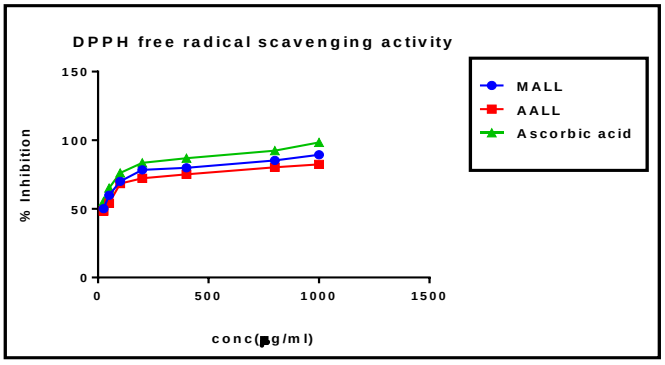

Fig. 3: Result of DPPH scavenging activity of MALL, AALL, and ascorbic acid, value are mean±SEM (n=3)

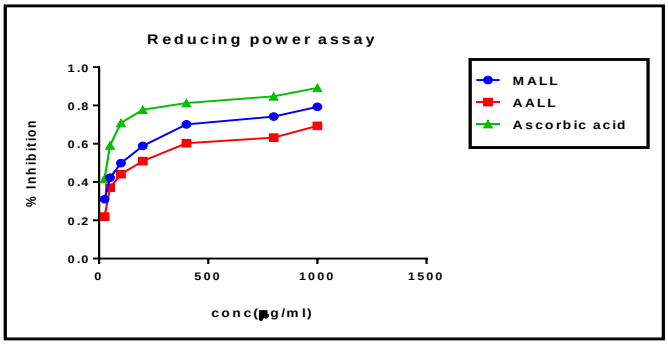

Fig. 4: Result of reducing power assay of MALL, AALL, and ascorbic acid, values are mean±SEM (n=3)

***In vitro* anti-inflammatory activity**

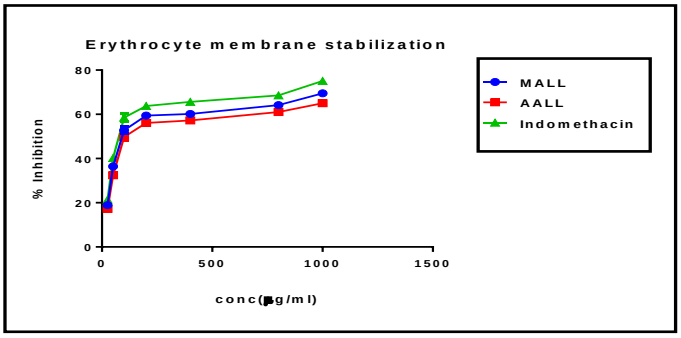

Fig. 5: Result of Effect of Indomethacin, MALL, and AALL on heat-induced haemolysis of rat erythrocytes, values are mean±SEM (n=3)

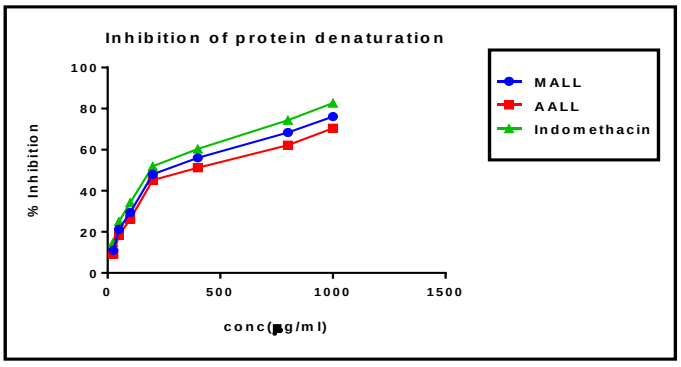

Fig. 6: Result of Effect of indomethacin, MALL, and AALL on protein denaturation, Values are mean±SEM (n=3)

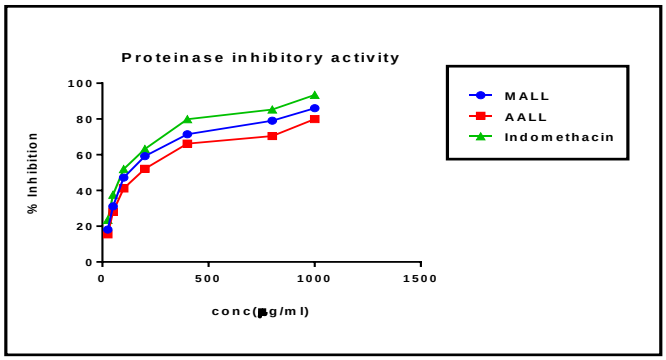

Fig. 7: Result of the effect of indomethacin, MALL, and AALL on proteinase inhibition, values are mean±SEM (n=3)

Table 1: *In vivo* anti-inflammatory activity by carrageenan-induced rat paw edema method, all values are mean±SEM, (One-way ANOVA followed by dunnett's t-test: n=6 in each group). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ when compared with control

Edema volume (ml)						
Time (H)	**Control (Vehicle, p. o.)**	**Indomethacin (10 mg/kg)**	**MALL (250 mg/kg)**	**AALL (250 mg/kg)**	**MALL (500 mg/kg)**	**AALL (500 mg/kg)**
0	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.27±0.04	0.24±0.03 (11%)	0.26±0.01 (3.70%)	0.26±0.02 (3.70%)	0.25±0.05 (7.40%)	0.25±0.04 (7.40%)
1	0.34±0.02	0.25±0.04 (26.47%)	0.28±0.05 (17.64%)	0.29±0.01 (14.70%)	0.26±0.03* (23.52%)	0.27±0.02 (20.58%)
2	0.55±0.05	0.34±0.01 (38.18%)	0.38±0.03 (30.90%)	0.40±0.01 (27.27%)	0.36±0.02** (34.54%)	0.37±0.01 (32.72%)
3	0.75±0.02	0.31±0.02 (58.04%)	0.38±0.01* (49.33%)	0.40±0.02 (46.66%)	0.35±0.03*** (53.33%)	0.37±0.01 (50.66%)
4	0.62±0.07	0.29±0.04 (52.70%)	0.36±0.03* (41.93%)	0.37±0.01 (40.32%)	0.32±0.05 (48.83%)	0.33±0.02 (46.77%)
5	0.51±0.02	0.26±0.05 (48.00%)	0.33±0.02 (35.29%)	0.35±0.01 (31.37%)	0.29±0.04 (43.13%)	0.31±0.03 (39.21%)
6	0.45±0.03	0.29±0.04 (35.55%)	0.38±0.02 (15.55%)	0.41±0.01 (8.88%)	0.34±0.03 (24.44%)	0.36±0.02 (20.00%)

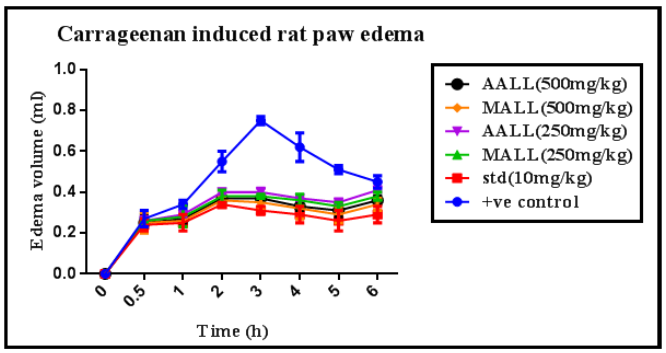

Fig. 8: Effect of MALL, AALL, and indomethacin on carrageenan-induced rat paw edema. Results are expressed as mean±SEM, n=6 in each group. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ when compared with control

Biochemical parameter on serum

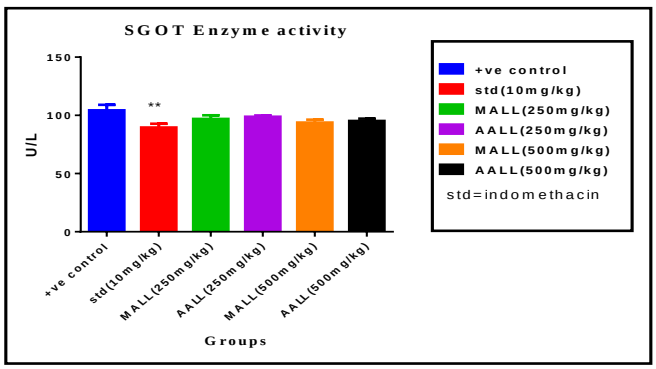

Fig. 9: Result of effect of MALL, AALL and indomethacin on serum glutamate oxaloacetate transaminase (SGOT) level, all values are mean±SEM. (One-way ANOVA followed by Dunnett's t-test: n=6 in each group). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ when compared with control

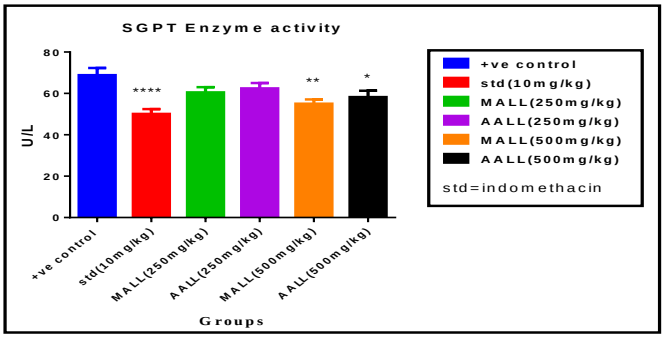

Fig. 10: Result of the effect of MALL, AALL and indomethacin on serum glutamate pyruvate transaminase (SGPT) level; all values are mean±SEM. (One-way ANOVA followed by dunnett's t-test: n=6 in each group). *p<0.05, **p<0.01, ***p<0.001 when compared with control

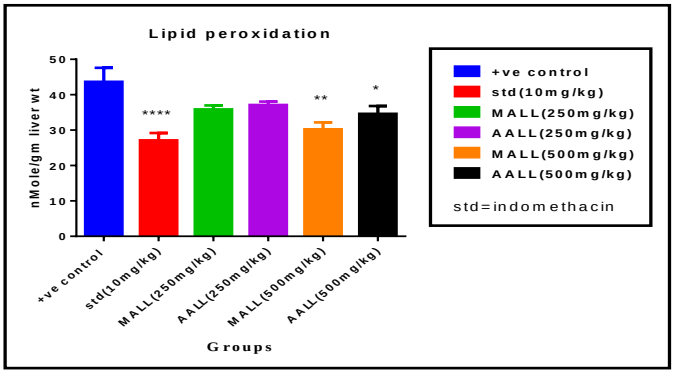

Fig. 11: Result of Biochemical Parameter on Liver Lipid peroxidation assay of MALL, AALL, and Indomethacin; all values are mean±SEM. (One-way ANOVA followed by dunnett's t-test: n=6 in each group). *p<0.05, **p<0.01, ***p<0.001when compared with control

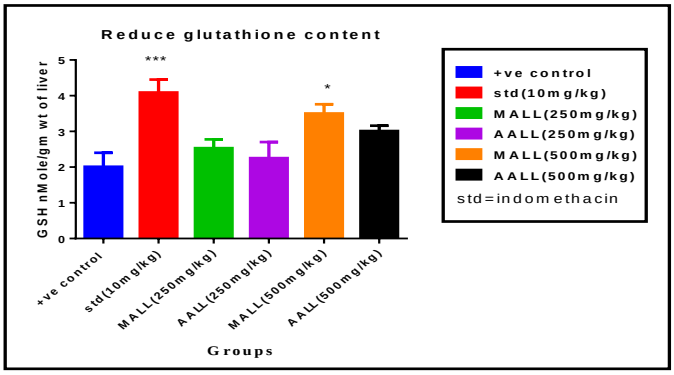

Fig. 12: Result of reduced glutathione (GSH) assay of MALL, AALL, and Indomethacin; all values are mean±SEM. (One-way ANOVA followed by dunnett's t-test: n=6 in each group). *p<0.05, **p<0.01, ***p<0.001when compared with control

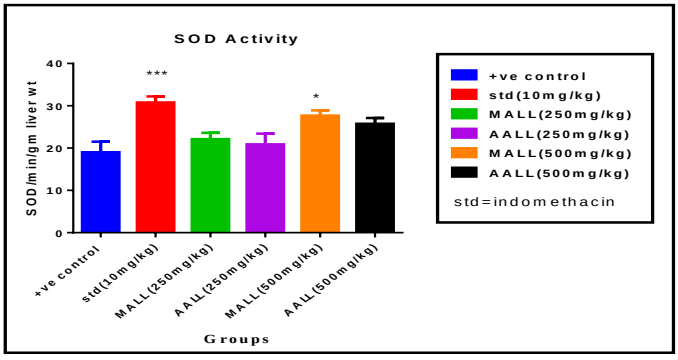

Fig. 13: Result of Superoxide Dismutase (SOD) assay of MALL, AALL, and Indomethacin, all values are mean±SEM. (One-way ANOVA followed by Dunnett's t-test: n=6 in each group). *p<0.05, **p<0.01, ***p<0.001when compared with control

DISCUSSION

Inflammation and the immune system are closely intertwined. Inflammation is a complex web of responses to tissue injury and infection, characterized by classic signs of rubor (redness), color (heat), tumor (swelling), dolor (pain), and funtiolaesa (loss of function). The immune system comprises the cells and soluble factors, such as antibodies and complement proteins, that mediate the inflammatory response; these cells and factors both eliminate the inciting inflammatory stimulus and initiate the process of immunologic memory [15]. Because of this associated complexity in inflammation, the present study was undertaken to investigate and compare the anti-inflammatory activity of aqueous and methanolic extract of *Anogeissus latifolia* DC. (Combretaceae) using the carrageenan-induced rat paw edema method. In the present study, in addition to *in vivo* studies, some *in vitro* anti-inflammatory and some *in vitro* antioxidant assays were carried out to get an insight into mechanisms involved in the pharmacological actions of herbs. In the present study, some *in vitro* anti-oxidant assays were carried out. *Anogeissus latifolia* DC was evaluated for nitric oxide scavenging activity. From fig. 1, *Anogeissus latifolia* DC showed moderately good nitric oxide scavenging activity between 25 and 1000 µg/ml. The percentage of inhibitions was increased with increasing concentration of the extracts. The hydroxyl radical is extremely reactive in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging biomolecules of living cells. Hydroxyl radical produced in the body are very reactive and one of the strongest oxidizing agents that reacts with almost all biomolecules found in living cells at a high rate constant, and is involved in many pathological processes, including DNA strand breakage and K^+loss from the cell membrane [16]. The results are shown in the fig. 2. Fig. 3 shows the DPPH scavenging activity of the methanolic and aqueous extract of *Anogeissus latifolia* DC compared with ascorbic acid, as standard. The reducing power of the extracts was measured by the direct electron donation in the reduction of $[Fe(CN)_6]^{3-}$ to $[Fe(CN)_6]^{4-}$. The product was visualized by the addition of free Fe^{3+}ions after the reduction reaction by forming an intense Prussian blue colour complex, $(Fe^{3+})_4[Fe^{2+}(CN)_6]^{3-}$, and quantified by absorbance measurement at 700 nm. A direct correlation between antioxidant activity and reducing properties are generally associated with the presence of reductones, which have been shown to exert anti-oxidant action by breaking the free radical chain by donating hydrogen atom [17]. The presence of reductants (i. e anti-oxidant) in the plant extract causes the reduction of Fe^{3+}/ferrocyanide complex to the ferrous form. Therefore, the Fe^{2+}can be monitored by measuring the formation of the pearl's Prussian blue at 700 nm. Fig. 4 shows the reductive capabilities of the plant extract compared to ascorbic acid. In the present study, some *in vitro* anti-inflammatory assays were carried out to get an insight into mechanisms involved in the pharmacological actions of herbs. The vitality of cells depends on the integrity of their membranes. Since the RBC membrane is similar to that of the lysosomal membrane [18], inhibition of RBC haemolysis provides good insights into the inflammatory process especially as both events are also a consequence of injury. Injury to the lysosomal membrane usually triggers the release of phospholipase A_2 that mediates the hydrolysis of phospholipids to produce inflammatory mediators [19]. It is therefore expected that compounds with membrane stabilizing properties should offer significant protection of cell membranes against injurious substances and thereby exhibit anti-inflammatory activity. This notion is consistent with the observations that the breakdown of biomembranes leads to the formation of free radicals, which in turn enhance cellular damage. Hence, protection against hypotonicity or heat-induced lysis of RBCs can be used as a biochemical index of anti-inflammatory activity. Results in fig. 5 demonstrate that both extracts were able to protect heat-induced rat erythrocyte haemolysis in a dose-dependent manner. Hence it may be inferred that stabilization of lysosomal membranes is one of the mechanisms by which test herb mediates its anti-inflammatory action.

It has been observed that chronic inflammation is accompanied by protein denaturation [20]. Denaturation of proteins is one of the causes of rheumatoid arthritis and is well documented [21]. Production of autoantigens in certain rheumatic diseases may be due to *in vivo* denaturation of proteins. The mechanism of denaturation involves the loss of hydrogen bonding and disulphide bonding [22]. From the results of the present study (fig. 6) it can be stated that both the extracts are capable of controlling the production of autoantigens due to inhibition of *in vivo* denaturation of proteins in rheumatic diseases, which possibly contributes to its usefulness as an anti-inflammatory drug. Proteinases (also known as Endopeptidases) are of protease enzymes [23], which selectively hydrolyses a peptide bond in a polypeptide chain of a target molecule. Neutrophils are rich sources of proteinases, which carry in their lysosomal granules many neutral serine proteinases. Proteinases are involved in essential steps in the homeostasis of both cartilage and bone. Proteinases participate in the cartilage remodelling process by degrading the damaged matrix to permit repair. Failure to regulate the synthesis, activation and inhibition of proteinases favors the deleterious effects of MMPs (Matrix Metallo-Proteinases, a type of proteinase), which finally lead to joint destruction, which is the main reason for the disability observed in rheumatoid arthritis, osteoarthritis, and spondyloarthritis. *Anogeissus latifolia* DC showed significant anti-proteinase activity in a dose-dependent manner, which indicates the contribution of this action to its usefulness as an anti-inflammatory agent. The results are shown in fig. 7. The distinct phases of edema response obtained in our study subsequent to the injection of carrageenan were in confirmation with these reports as shown in table 1 fig. 8. There was a gradual increase in edema paw volume in the control group. The oral pre-treatment with methanolic and aqueous extract of *Anogeissus latifolia* DC showed inhibition of edema formation at the 1st phase and also in the 2nd phase of carrageenan evoked hind paw edema. The intensity of inhibitory activity of both, low (250 mg/kg) and high (500 mg/kg) doses, of both the extracts of *Anogeissus latifolia* DC on the first phase, was found to be almost similar but, the inhibition of the second phase of edema response was increased at a higher dose (500 mg/kg) as compare to low dose (250 mg/kg). *In vivo* anti-inflammatory activity was found in both the extract but the anti-inflammatory activity was found more significant in the methanolic extract of *Anogeissus latifolia* DC than the aqueous extract of *Anogeissus latifolia* DC. These results suggest that the methanolic and aqueous extract of *Anogeissus latifolia* DC leaves exhibits the anti-inflammatory property in the acute phase of inflammation but the anti-inflammatory activity is more significant in methanolic extract than aqueous extract and the mechanism of action may be associated with inhibition of the some of the inflammatory mediators like histamine, serotonin, bradykinins, and prostaglandins. Glutamic oxaloacetic transaminases (GOT) is a mitochondrial enzyme present in the highest amount in heart muscle, skeletal muscle, liver, brain, and kidney, whereas glutamic pyruvic transaminase (GPT) is a cytosolic enzyme primarily present in the liver [24]. Upon damage to the tissues, an appreciable amount of these enzymes escapes into the bloodstream and this is bringing out a sharp rise in the serum concentration of these enzymes. The raised activity of these enzymes was reduced in the animals pretreated with indomethacin and the extracts. The results are shown in the fig. 9 and fig. 10. The effect of methanolic and aqueous extracts of *Anogeissus latifolia* DC as compared to indomethacin on lipid peroxidation is brought out by the results summarized in fig. 11. Thus results suggest that the methanolic extract of decreases the lipid peroxidation level more than the aqueous extract *Anogeissus latifolia* DC. Glutathione (GSH) is one of the most abundant naturally occurring tripeptides, a non-enzymatic biological antioxidant presents in the liver. Its functions are concerned with the removal of free radicals such as H_2O_2 and superoxide radicals, maintenance of membrane protein, detoxification of foreign chemicals and biotransformation of drugs. In inflamed tissues, GSH content and SOD activity are decreased. Tissue damage related to oxidative stress can be reversed via the SOD enzyme and GSH. The action of these parameters limits the cytotoxic effects of toxic free radicals. The effect of both extracts as compared to indomethacin on GSH enzyme activity and SOD level are summarized in fig. 12 and 13, respectively. Methanolic extract shows promising results as compared to the aqueous extract of *Anogeissus Latifolia* leaves. The study hence proves that methanolic extract has more anti-inflammatory activity.

CONCLUSION

The leaf extracts of *Anogeissus latifolia* have both antioxidant and anti-inflammatory activity. *In vitro* anti-oxidant and *In vitro* anti-inflammatory activities were found in both methanolic and aqueous extracts of *Anogeissus latifolia* (DC.) and also *In vivo* anti-inflammatory activity was found in both the extract. When we compare both the extracts, it was observed that the anti-inflammatory activity was found more significant in the methanolic extract of *Anogeissus latifolia* (DC.).

ABBREVIATION

MALL (Methanolic Extract of *Anogeissus latifolia* Leaves), AALL (Aqueous Extract of *Anogeissus latifolia* Leaves).

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All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors have no conflicts of interest regarding this investigation.

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