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# Evaluating the influence of *Aloe barbadensis* extracts on edema induced changes in C-reactive protein and interleukin-6 in albino rats through *in vivo* and *in silico* approaches

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**The current study investigated the *in-vivo* and *in-silico* anti-inflammatory effect of *Aloe barbadensis* in edema induced rat and its blood biomarkers. 60 albino rats (160-200 g) were divided into 4 groups. The 1st group (control) comprised of 6 rats that were treated with saline. The 2nd group (standard) comprised of 6 rats that were treated with diclofenac. The 3rd and 4th experimental groups consisted of 48 rats, treated with *A. barbadensis* gel ethanolic and aqueous extracts respectively at doses of 50, 100, 200 and 400 mg/kg. According to paw sizes, groups III and IV showed 51% and 46% inhibition respectively at the 5th hour, as compared to group II with 61% inhibition. Correlation was negative between biomarkers in group III, while, positive in group IV. Blood samples were collected; C-reactive protein and interleukin-6 were measured using commercially available ELISA kits. Similarly, biomarkers showed significant effect in dose-dependent manner. In molecular docking, for CRP both ligands aloe emodin and emodin showed –7.5 kcal/mol binding energy as compared to diclofenac with –7.0 kcal/mol. For IL-1beta, both ligands showed –4.7 kcal/mol binding energy as compared to diclofenac –4.4 kcal/mol. Hence, we concluded that *A. barbadensis* extracts can be used as an effective drug for managing inflammation.**

**Keywords**: *A. barbadensis*, albino rats, carrageenan, inflammation, biomarkers, computational analysis

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**Abbreviations**: CRP, C-reactive protein; IL-6, Interleukin-6; NSAIDs, Non-Steroidal Anti-Inflammatory Drugs; TNF-α, Tumor necrosis factor alpha

## INTRODUCTION

Inflammation is an immunological reaction of vascular tissues to detrimental stimuli like foreign organisms and allergens (Riddle *et al.*, 2022) It involves an intricate array that modulates enzyme activation, mediator discharge, tissue damage and repair. Carrageenan is a phlogistic intermediary causing edema in pathophysiological reactions (Rahmawati *et al.,* 2022) Macrophage releases IL-6, in addition to resident cells into circulation at the site of infection. IL-6 stimulates the production of CRP in the liver. IL-1β and TNF-α also triggers IL-6. Increasing CRP levels causes a discharge of TNF-α, IL-1β and IL-6 leading to an inflammation cascade (Mahnashi *et al.,* 2021) Many NSAIDs or SAIDs encompass an edge to manage diseases but often pose serious health issues, so there should be remedies with natural origins with fewer adverse effects (Grzybowski *et al.,* 2018)

The traditional medical system is an ancient healthcare system dating back to 5000 years and includes many indigenous practices including Ayurveda, Siddha, and Unani (Maurya *et al.,* 2022) Research on remedial plants has been intended to authenticate its traditional exploitations in the management of different kinds of ailments through the inflection of molecular and biochemical pathways (Zhang *et al.,* 2019) According to the World Health Organization, 80% of the world's inhabitants use phytotherapeutic drugs to meet their basic health and 11% of essential therapeutic drugs are of plant origin (Annad *et al.,* 2019) Asphodelaceae family includes the largest genus "Alloeh," and 548 accepted species possessing potential biological activities. Among them, *Aloe barbadensis* possesses a legendary medicinal reputation. It is a multifunctional xerophytic medicinal plant having 75 bioactive compounds (Leitgeb *et al.,* 2018) The gel is transparent mucilaginous jelly present in parenchyma cells. It has the ability to penetrate the deepest body tissues and ensures good health. *A. barbadensis* has effective anti-inflammatory activity through the inflection of molecular and biochemical pathways (Alven *et al.,* 2021)

*A. barbadensis* is cultivated worldwide and broadly dispersed in arid regions. It grows in an extensive variety of habitats. PH ranges from 7.0 to 8.5 and the required temperature ranges from 4°C to 21°C. Fully drained grimy topsoil is considered to be ideal for the nurture of the Aloe plant. The Egyptians called it the "Plant of immortality" due to its ability to colonize and ameliorate severe conditions in completely denuded or less vegetated landscapes (Salehi *et al.,* 2018) *A. barbadensis* has abundant therapeutic applications like anti-inflammatory, anti-diabetic, antioxidant, antiseptic, anticancer, immunomodulatory, antipyretic and analgesic activity (Majumder *et al.,* 2019) These properties have been credited to a range (100) of secondary metabolites called phytochemicals present in them. The outer green epidermis consists of mostly glycosides, pre-anthraquinones and anthraquinones. Acemannan and phenolic compounds are the main component of the outer pulp. Salicylic acid, proteins, vitamins, minerals, and enzymes are present in the inner leaf pulp (Wu *et al.,* 2022)

*A. barbadensis* reduces the leukocyte adhesion and pro-inflammatory cytokines like IL-β, interleukin 17, TNF-α and interleukins by inhibiting cyclooxygenase pathway and lessens prostaglandin E2 production *via* arachidonic acid (Gupta *et al*., 2020) Increasing CRP levels causes the discharge of TNF-α, IL-1β and IL-6. Whilst, promoting edema, CRP has some anti-inflammatory effects by stimulating the discharge of anti-edemic mediators such as IL-1ra and IL-10 (Del Giudice *et al.,* 2018) Bioinformatics is a promising field based on the application of computational techniques to resolve biological problems. It is an effective approach that provides favors such as ligand-receptor binding affinity, time organization and drug-drug interactions. Hence, it aids researchers to design the latest drugs for disease management. Anti-edematous effect of *A. barbadensis* and the molecular docking of some of its constituents by inflammatory proteins identified its potential inhibitors (Dali *et al.,* 2019) The current study is designed to investigate the effect of *A. barbadensis* in the induced inflammatory rat model and its blood biomarkers with molecular docking assay.

## MATERIALS AND METHODS

### Sample collection and preparation of *A. barbadensis* gel extract

Fresh leaves of *A. barbadensis* were collected as a sample from the University of the Punjab, Lahore and were identified by the staff members of the botany department. After the identification, the leaves were washed with water. Lower 1 inch of leaf base and spines around the leaf were removed using a clean knife. Aloe gel was collected, dried, weighed and mixed with distilled water and 70% ethanol (semi-polar solvent) Bottles were placed at room temperature for 15 days. Extracts were filtered by Whatman no.1 paper and filtrate was permitted to peter out in rotary and glass Petri plates for 1 week as shown in Fig. 1A. When dried, the extract was scratched, measured and stored in labelled Eppendorf for further use (Abubakar *et al.,* 2020).

### Experimental rats

Albino rats of either sex weighing (160–200 g) were purchased from the University of Lahore and kept in polypropylene cages in the animal house of (UOL) Before experimental work, rats were kept in fasting condition. After that, they were given distilled water and balanced feed (Alyas *et al.,* 2020)

### Drugs used in the experiment.

*A. barbadensis* gel ethanolic and aqueous extracts were prepared at concentrations of 50, 100, 200 and 400 mg/kg. Diclofenac, normal saline was given at 10 ml/kg and 100 mg/kg respectively. Carrageenan for inducing inflammation was given at 100 mg/kg.

### Procedure

#### Carrageenan-induced paw edema.

1% carrageenan of 100 mg/kg was injected into all rat groups in the left hind paw in the sub-planter region for inducing edema. Paw sizes were taken with a Vernier caliper before and after the injection of carrageenan (Ou *et al.,* 2019)

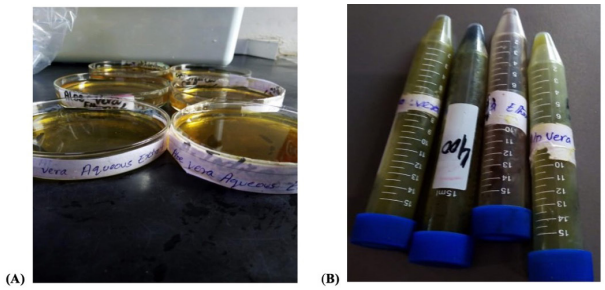

**Figure 1. (A) Filtrate of plant extract in glass Petri plates to dry at room temperature. (B) Prepared *A. barbadensis* gel extracts at 4 different concentrations of 50, 100, 200 and 400.**

### Grouping and treatment schedule

In group I (control) rats were injected with normal saline 10 ml/ kg in the sub-planter region of the hind paw. Paw size was measured with a Vernier calliper. At first, paw size increased due to edema but after 4or 5 hours it begins to decrease and normalized after 18 hours.

In group II (standard) rats were injected with diclofenac 100 mg/kg in sub-planter region of the hind paw. Paw size was measured with a Vernier calliper. At first paw, size was increased due to edema but after injecting diclofenac, it has begun to decrease and got normalized after 3 hours.

In group III and IV *A. barbadensis* gel extracts at doses of 50, 100,200 and 400 mg/kg, were injected Fig. 2A. Paw size was measured with a Vernier calliper. The Formula for calculating the anti-inflammatory activity is given below (Choudhury *et al.,* 2016)

$$\% \text{ inhibition} = \frac{\text{control mean} - \text{treated mean}}{\text{control mean}} \times 100$$

### Collection of blood samples and assessment of inflammatory biomarkers

At the end of the experiment blood samples were collected *via* cardiac puncture in EDTA tubes exclusive of anticoagulant, Fig. 2B, left for 10 minutes. Tubes were centrifuged at 4000 r/min for 10 minutes and obtained serum was put at –20ºC for further treatment (Sakr *et al.,* 2019) Different values of edema biomarkers like C-reactive protein and interleukin-6 were examined using commercially available ELISA kits (DiaMetra, Italy) Different concentrations of inflammatory biomarkers were evaluated at different concentrations of dose. Protocols of the above-mentioned parameters are attached in annexure-I and annexure-II.

### Anti-inflammatory activity model

Inflammation in the albino rats' paw was caused by the carrageenan and reduced by the diclofenac and ethanolic leaf extracts of *C. paradisi* (50, 100, 200 and 400 mg/kg)

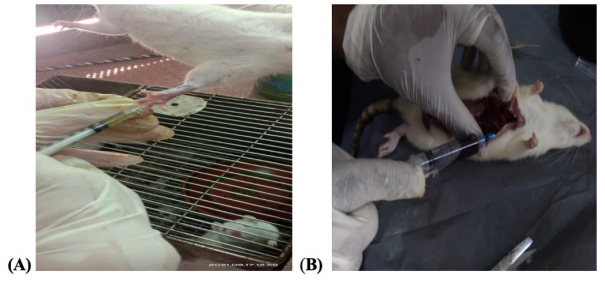

**Figure 2. (A) Rats were injected with *A. barbadensis* gel extract in the sub-planter region of the left hind paw. (B) Blood samples collection by cardiac puncture for blood biomarker.**

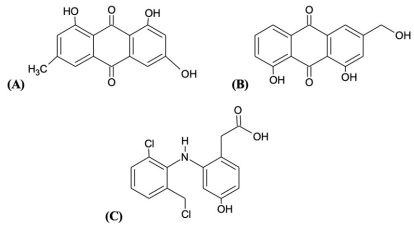


**Figure 3. 3D structure of ligand molecules.**
(**A**) Emodin and (**B**) Aloe-emodin are chemical compounds of aloe gel, selected as ligand molecules to target inflammatory proteins. (**C**) Diclofenac is a standard drug to target edema proteins.

A total of 36 albino rats were equally divided into three groups (control, standard, and experimental) In the control group, rats were treated with normal saline. In the standard group, rats were treated with diclofenac (100 mg/kg), and in the experimental group, rats were treated with the *C. paradisi* leaf aqueous extract. First, the size of the hind paw of rats was measured, followed by injecting the carrageenan doses (50, 100, 200, and 400 mg/kg) into the hind paw at the sub-planter region. The carrageenan doses start producing inflammation in the rat's hind paws. After 3 hours of carrageenan injection, the doses of *C. paradisi* leaf extract and diclofenac (12.5, 25, 50, and 100 mg/kg) were injected into the hind paw, and the paw size was measured at the time interval of 1, 2, and 3 h.

## Anti-pyretic Activity Model

Pyrexia is induced in antipyretic activity in albino rats by normal saline and yeast and then treated with leaf ethanolic extracts of *C. paradisi* (50, 100, 200 and 400 mg/kg) for reducing fever. The rats in this model were also divided according to the anti-inflammatory activity model. In the control group, rats were treated with normal saline (2 ml/kg) below the nape of the neck, and in the standard group, rats were treated with normal saline + brewer's yeast. In the experimental group, rats were treated with doses of ethanolic leaf extracts of *C. paradisi*. After injecting yeast, fever developed after 21 hours, and the highest temperature, 101.81 Fahrenheit was measured.

## *In silico* anti-inflammatory activity of *A. barbadensis*

To begin with *in silico* analysis, different computational tools including chemskech, chimera, pymol, pyrx, depth residue and discovery studio were used in support of the anti-inflammatory effect of *A. barbadensis*.

### Selection and preparation of ligands

Aloe emodin and emodin are chemical compounds of the anthraquinone family present in the Aloe gel, which possesses an anti-inflammatory role. Therefore, analysis was done targeting these intermediates using as it may control the undue inflammation and consequently prevents acute inflammatory infections. Three ligand molecules emodin, aloe emodin and diclofenac were selected for molecular docking studies. ChemSketch was assessed for the 3D structure of ligand molecules as shown in Fig. 3A, B, C.

### Protein preparation and prediction of the active site

CRP and IL-1β were selected for *in silico* studies as target proteins. The 3D structures of CRP and IL-1β were occupied from Swiss prot, subsequent to open in Chimera. Out of four, one chain of IL-1β was selected. All unnecessary ions and metals were removed. The geometry of all hetero groups was assured and the structure of the protein was saved as a PDB file. The active sites of CRP and IL-1β were found in depth residue.

### Protein-ligand docking

To identify the potential anti-inflammatory drug or ligand molecule for a particular protein, PyRx was used for virtual screening. In this procedure, both target proteins CRP and IL-1b and ligand molecules were opted from pdb files; fasten to the binding pocket of the receptor protein. Following to docking run, results were estimated on the strength of bond interaction, binding site energy and ligand-molecule interface. Discovery studio was used to fashion complexes of proteins-ligand molecules and 3D structure of complex for high-quality observation respectively. The Chimera tool was operated to generate the 3D structure of the complex for high-quality observation.

Emodin, aloe-emodin and diclofenac have molecular formulas $C_{15}H_{10}O_5$, $C_{15}H_{10}O_5$ and $C_{14}H_{11}Cl_2NO_2$ with PubChem ID 3220, 10207 and 3033 respectively.

## Statistical analysis

The data were subjected to homogeneity of variance to check normality and preceded to one-way ANOVA

**Table 1. % age inhibition of paw size in group III (*A. barbadensis* gel ethanolic extracts) in dose-dependent manner exhibiting significant $p<0.05$ decrease at 5th hour (mean±S.D $30.83^{d}$±0.60) with 51% inhibition $p$-value<0.0001. While no such effect was demonstrated in group I (control) (mean ±S.D. $62.33^{a}$±1.67).**

| Treatment | Reaction Time with mean ±S.D. (% inhibition) per hour | | | | |
|---|---|---|---|---|---|
| | 1-hour | 2-hour | 3-hour | 4-hour | 5-hour |
| Group I | $45.17^{a}$±1.66 | $47.83^{a}$±2.29 | $55.67^{a}$±2.58 | $64.17^{a}$±1.66 | $62.33^{a}$±1.67 |
| Group II | $33.17^{c}$±1.19 (48%) | $34.33^{d}$±0.80 (46%) | $33.50^{c}$±2.03 (52%) | $29.50^{d\pm}$1.06 (54%) | $25.00^{e}$±0.73 (61%) |
| Dose 50 mg/kg | $39.50^{b}$±0.76 (17%) | $40.17^{b}$±1.08 (27%) | $40.33^{b}$ ±1.20 (29%) | $37.00^{b}$±0.58 (21%) | $36.00^{b}$±0.52 (23%) |
| Dose 100 mg/kg | $37.50^{b}$±0.76 (18%) | $38.00^{bc}$±0.58 (21%) | $39.00^{b}$±1.00 (30%) | $35.50^{bc}$±0.56 (44%) | $34.50^{bc}$±0.43 (45%) |
| Dose 200 mg/kg | $34.00^{c}$±0.58 (47%) | $38.83^{bc}$±0.60 (39%) | $41.00^{b}$ ±1.29 (36%) | $36.17^{b}$±1.62 (43%) | $32.67^{cd}$±0.33 (49%) |
| Dose 400 mg/kg | $33.17^{c}$±0.60 (46%) | $35.50^{cd}$±0.76 (43%) | $38.50^{b}$ ±1.02 (38%) | $32.50^{cd}$±0.43 (49%) | $30.83^{d}$±0.60 (51%) |
| $p$-value | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |

Superscripts on different means within the column differ significantly at $p\leq0.05$.

**Table 2. % age inhibition of paw size in group IV (*A. barbadensis* gel aqueous extracts) in dose-dependent manner demonstrating significant $p \leq 0.05$ decrease at 5th hour (mean ±S.D. $34.50^{e}$±0.56) with 46% inhibition *p*-value<0.0001.While, no such surge was shown in group I (control) (mean ±S.D. $62.33^{a}$±1.67).**

| Treatment | Reaction Time with mean±S.D (% inhibition) per hour | | | | |
|---|---|---|---|---|---|
| | 1-hour | 2-hour | 3-hour | 4-hour | 5-hour |
| Group I | $45.17^{ab}$±1.66 | $47.83^{a}$±2.29 | $55.67^{a}$±2.58 | $64.17^{a}$±1.66 | $62.33^{a}$±1.67 |
| Group II | $33.17^{d}$±1.19 (48%) | $34.33^{c}$±0.80 (46%) | $33.50^{c}$±2.03 (52%) | $29.50^{e}$±1.06 (54%) | $25.00^{f}$±0.73 (61%) |
| Dose 50 mg/kg | $48.00^{a}$±0.73 (12%) | $50.33^{a}$±0.80 (19%) | $51.17^{a}$±1.54 (20%) | $45.83^{b}$±1.11 (27%) | $45.50^{b}$±0.43 (29%) |
| Dose 100 mg/kg | $45.00^{ab}$±0.58 (18%) | $47.67^{a}$±0.67 (14.5%) | $46.17^{b}$±1.35 (28%) | $43.17^{bc}$±0.83 (33%) | $41.17^{c}$±0.60 (36%) |
| Dose 200 mg/kg | $42.33^{bc}$±0.88 (34%) | $44.17^{b}$±0.91 (31%) | $45.17^{b}$±1.01 (29%) | $40.50^{c}$±0.76 (36%) | $38.17^{d}$±0.60 (41%) |
| Dose 400 mg/kg | $39.50^{c}$±0.76 (38%) | $41.67^{b}$±0.76 (35%) | $41.67^{b}$±1.05 (35%) | $35.33^{d}$±1.02 (45%) | $34.50^{e}$±0.56 (46%) |

Superscripts on different means within the column differ significantly at $p<0.05$.

using PROC GLM in SAS software (version 9.1) Duncan's multiple range test, Pearson correlation method and Dunnett's *t*-test were used considering $p \leq 0.05$.

## RESULTS

In this study, the anti-edematous effect of *A. barbadensis* gel ethanolic and aqueous extracts at different concentrations (50, 100, 200 and 400 mg/kg) was observed against rat models of acute edema. The results showed a significant difference between the groups of rats treated with the extracts and the control group.

### Effect of *A. barbadensis* on paw sizes in rat

All groups showed a significant increase in paw edema volume injected with 1% carrageenan. Group I showed the most pronounced increase in paw volume ($45.17^{a}$±1.66, $47.83^{a}$±2.29, $55.67^{a}$±2.58, $64.17^{a}$±1.66 and $62.33^{a}$±1.67) after 1 to 5 hours of injection. While, group III and IV treated with *A. barbadensis* gel extracts at different concentrations (50, 100, 200 and 400 mg/kg) reduced paw edema at 1 to 5 hours as compared to group I. showed an improvement in the edema volume as compared to group I. The improvement was more pronounced at the 5th hour in a dose-dependent manner with PI 23, 45, 49 and 51% closer to diclofenac treated group 61% as shown in Table 1. Group IV treated with *A. barbadensis* gel aqueous extracts showed ($p<0.05$) % inhibition activity 29, 36, 41 and 46 % closer to group III. *A. barbadensis* gel ethanolic extract (group III) showed the most pronounced effect as compared to *A. barbadensis* gel aqueous extracts (group IV) as shown in Table 2.

### Effect of *A. barbadensis* on immunological and biochemical parameter

Carragenan is an intermediary of edema cascade. C-reactive protein is produced in the liver by IL-6 and is involved in edema and innate immune reactions. In response to inflammation, IL-6 is triggered by macrophages and neutrophils into the bloodstream and increases CRP and IL-6 levels in the blood. Hence, both CRP and IL-6 are broadly recognized as edema biomarkers. To additionally expose the means of the action of *A. barbadensis* gel extracts, the immunological and biochemical parameters in inflammation and pro-inflammatory cytokines like CRP and IL-6 were analyzed by ELISA method. *A. barbadensis* gel extracts significantly ($p \leq 0.05$) reduced the elevated levels of CRP and IL-6 in blood. CRP biomarker values significantly differ in group III ($4.75^{d\pm}0.02$) and IV ($6.94^{d\pm}0.04$) as compared to group I (control) with elevated levels of CRP ($9.48^{a\pm}0.22$) IL-6 values also differ significantly ($p \leq 0.05$) at different doses in extract-treated groups III ($13.48^{d}\pm0.02$) and IV ($14.23^{c}\pm0.03$) as compared to group I control ($17.21^{a}\pm0.10$) as shown in Table 3.

**Table 3. Effect of *A. barbadensis* gel extracts on biomarkers CRP (ng/ml) and IL-6 (pg/ml) in group III and IV representing significant p-value<0.0001 reduction in edema as compared to group I (control).**

| Treatment | CRP(ng/ml) | | IL-6(pg/ml) | |
|---|---|---|---|---|
| | Group III | Group IV | Group III | Group IV |
| Group I | $9.48^{a\pm}0.22$ | $9.48^{a\pm}0.22$ | $17.21^{a}\pm0.10$ | $17.21^{a}\pm0.10$ |
| Group II | $2.48^{e\pm}0.08$ | $2.48^{e\pm}0.08$ | $10.42^{e}\pm0.04$ | $10.42^{f}\pm0.04$ |
| Dose 50 mg/kg | $5.28^{b\pm}0.01$ | $7.76^{b\pm}0.08$ | $14.08^{b}\pm0.05$ | $14.92^{b}\pm0.06$ |
| Dose 100 mg/kg | $5.17^{bc\pm}0.01$ | $7.55^{bc\pm}0.03$ | $13.80^{c}\pm0.03$ | $14.60^{c}\pm0.02$ |
| Dose 200 mg/kg | $4.93^{cd\pm}0.04$ | $7.39^{c\pm}0.03$ | $13.58^{d}\pm0.02$ | $14.43^{d}\pm0.03$ |
| Dose 400 mg/kg | $4.75^{d\pm}0.02$ | $6.94^{d\pm}0.04$ | $13.48^{d}\pm0.02$ | $14.23^{e}\pm0.03$ |
| *p*-value | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |

Superscripts on different means within the column differ significantly at $p<0.05$.

**Table 4. Comparisons of CRP biomarker means through Dunnett's *t*–test**

| Group III (Ethanolic extracts) | | | | |
|---|---|---|---|---|
| Treatment comparison | Difference between means | Simultaneous 95% confidence limits | | Significance |
| Dose 50 *vs* group I | –4.1967 | –4.5680 | –3.8253 | *** |
| Dose 100 *vs* group I | –4.3050 | –4.6763 | –3.9337 | *** |
| Dose 200 *vs* group I | –4.5483 | –4.9197 | –4.1770 | *** |
| Dose 400 *vs* group I | –4.7300 | –5.1013 | –4.3587 | *** |
| Dose 50 *vs* group II | 2.79833 | 2.64663 | 2.95004 | *** |
| Dose 100 *vs* group II | 2.69000 | 2.53829 | 2.84171 | *** |
| Dose 200 group II | 2.44667 | 2.29496 | 2.59837 | *** |
| Dose 400 *vs* group II | 2.26500 | 2.11329 | 2.41671 | *** |
| Group IV (Aqueous extracts) | | | | |
| Dose 50 *vs* group I | –1.7183 | –2.1149 | –1.3218 | *** |
| Dose 100 *vs* group I | –1.9233 | –2.3199 | –1.5268 | *** |
| Dose 200 *vs* group I | –2.0900 | –2.4866 | –1.6934 | *** |
| Dose 400 *vs* group I | –2.5367 | –2.9332 | –2.10401 | *** |
| Dose 50 *vs* group II | 5.27667 | 5.06355 | 5.48979 | *** |
| Dose 100 *vs* group II | 5.07167 | 4.85855 | 5.28479 | *** |
| Dose 200 group II | 4.90500 | 4.69188 | 5.11812 | *** |
| Dose 400 *vs* group II | 4.45833 | 4.24521 | 4.67145 | *** |

In a comparison of the experimental group IV with the control and standard group, our results showed a significant difference where, ***$p<0.001$.

The most significant ($p\leq0.05$) effect of both extracts was observed at a dose of 400 mg/kg closer to group II (standard) treated with diclofenac drug ($2.48^{e\pm}0.08$) in a dose-dependent manner, where $p$-value<0.0001.

### Comparison study of biomarker CRP

By comparing CRP biomarker in group III and IV doses *vs* group I and II, there is a significant $p<0.001$ mean difference between treated groups at different doses. Group III was treated with a dose of 400 mg/kg *vs* control and standard group has a mean difference of –4.7300 and 2.26500 respectively. Group IV was treated with a dose of 400mg/kg *vs* control and standard group has a mean difference of -2.5367 and 4.45833 respectively as shown in Table 4.

### Comparison study of biomarker IL-6

By comparing IL-6 biomarker at different doses, group III treated with a dose of 400 mg/kg *vs* control and standard has a mean difference of –3.73167 and 3.05667, respectively. Group IV treated with a dose of 400 mg/kg *vs* control and standard group has a mean difference of –3.19264 and 3.80333, respectively as shown in Table 5.

### Correlation study

The study of correlations is fundamental to the comprehension of biological methods. Correlation analysis is abundantly used to interpret, quantify, and visualize the association between measured values.

According to Pearson's correlation, there was a strong positive correlation (0.19) between biomarker CRP and IL-6 ($p<0.05$) in the control group. While, it was negative (–0.18) in the standard group and all doses of 50, 100, 200 and 400 mg/kg as highly strong negative correlation (–0.71), weak negative (–0.07), strong negative correlations (–0.27) and (–0.29) correspondingly. In group IV there was a strong negative correlation at higher doses of 200 and 400mg/k (–0.69) and (–0.06) respectively.

A positive correlation was observed between the IL-6 marker and paw size where the correlation was significant at ($p<0.05$) A strongly positive association was evident with increasing dose rate at 100, 200 and 400 mg/kg i.e. 0.18, 0.58 and 0.89 in group III and 0.2, 0.21 and 0.74 in group IV correspondingly.

Based on the results, CRP marker and paw sizes depicted a negative correlation of –0.37 in both the control group and the standard group. At the 4th hour, there was a positive correlation at a dose of 50 and 100 mg/kg as (0.22) and (0.4) However, the correlation was weakly negative at a dose of 200 and strongly negative at 400 mg/kg i.e. –0.06 and –0.51 correspondingly. At the end of the 4th hour, there was a negative correlation between the biomarker IL-6 and paw sizes ($p<0.05$) in the experimental group IV at doses 50, 100 and 200 as (–0.9), (–0.36) and (–0.05), respectively as shown in Table 6.

### *In-silico* analysis

*In-silico* analysis examined the potential of *A. barbadensis* to target cytokine IL-1b and CRP. The molecular binding energy of ligands against CRP scored –7.5Kcal/mol for aloe emodin and emodin and –7.0 Kcal/mol for diclofenac. IL-1b has –4.7Kcal/mol energy for aloe emodin and emodin and –4.4 Kcal/mol for diclofenac. While aloe emodin exhibited the highest score among the three as shown in Fig. 4.

#### Protein structure analysis and binding pocket with ligands

IL-1b has one chain, and CRP has five chains. All ligand molecules and proteins were adjusted in the grid box.

**Table 5. Comparisons of IL-6 biomarker means through Dunnett's *t*-test**

| Group III (Ethanolic extracts) | | | | |
|---|---|---|---|---|
| Treatment comparison | Difference between means | Simultaneous 95% confidence limits | | Significance |
| Dose 50 *vs* group I | −3.13667 | −3.33813 | −2.93521 | *** |
| Dose 100 *vs* group I | −3.41000 | −3.61146 | −3.20854 | *** |
| Dose 200 *vs* group I | −3.63167 | −3.83313 | −3.43021 | *** |
| Dose 400 *vs* group I | −3.73167 | −3.93313 | −3.53021 | *** |
| Dose 50 *vs* group II | 3.65167 | 3.51733 | 3.78601 | *** |
| Dose 100 *vs* group II | 3.37833 | 3.24399 | 3.51267 | *** |
| Dose 200 group II | 3.15667 | 3.02233 | 3.29101 | *** |
| Dose 400 *vs* group II | 3.05667 | 2.92233 | 3.19101 | *** |
| Group IV (Aqueous extracts) | | | | |
| Dose 50 *vs* group I | −2.28833 | −2.49598 | −2.08069 | *** |
| Dose 100 *vs* group I | −2.61500 | −2.82264 | −2.40736 | *** |
| Dose 200 *vs* group I | −2.78000 | −2.98764 | −2.57236 | *** |
| Dose 400 *vs* group I | −3.19264 | −2.98500 | −2.77736 | *** |
| Dose 50 *vs* group II | 4.50000 | 4.35519 | 4.64481 | *** |
| Dose 100 *vs* group II | 4.17333 | 4.02852 | 4.31814 | *** |
| Dose 200 group II | 4.00833 | 3.86352 | 4.15314 | *** |
| Dose 400 *vs* group II | 3.80333 | 3.65852 | 3.94814 | *** |

**Table 6. Correlation between Paw sizes, CRP and IL-6**

| Treatment | CRP and IL-6 | | IL-6 and paw sizes | | CRP and Paw sizes | |
|---|---|---|---|---|---|---|
| | Group III | Group IV | Group III | Group IV | Group III | Group IV |
| Control | 0.19 | 0.19 | 0.34 | 0.33 | −0.37 | 0.04 |
| Standard | −0.18 | −0.18 | 0.06 | 0.06 | −0.37 | −0.37 |
| Dose 50 mg/kg | −0.71 | 0.09 | −0.33 | 0.12 | 0.22 | −0.9 |
| Dose 100 mg/kg | −0.07 | 0.45 | 0.18 | 0.2 | 0.4 | −0.36 |
| Dose 200 mg/kg | −0.27 | −0.69 | 0.58 | 0.21 | −0.06 | −0.05 |
| Dose 400 mg/kg | −0.29 | −0.06 | 0.89 | 0.74 | −0.51 | 0.04 |

[1]Correlation was significant at $p \leq 0.05$.

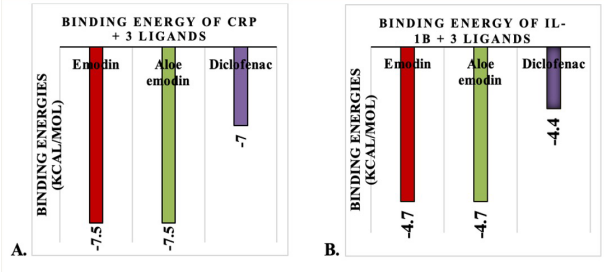


**Figure 4. Graphical representation of binding energy of (A) CRP and (B) IL-6 with 3 ligand molecules: Emodin; red, Aloe-emodin; green and diclofenac: purple.**

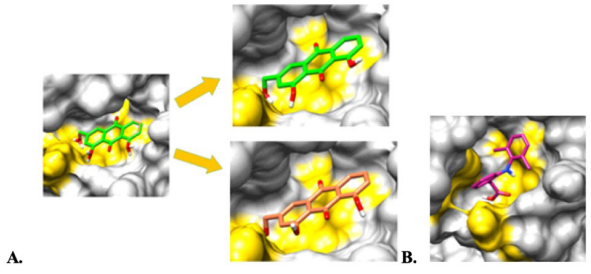


**Figure 5. C-reactive protein and ligand molecules (A) emodin and aloe emodin (B) diclofenac.**

Docking analysis with hydrogen bonds and binding energies

**CRP and IL-1beta with ligand molecules interaction.** CRP is represented by silver color, the binding pocket: golden color, ligand molecules aloe emodin: green, emodin: reddish brown and diclofenac purple as shown in Fig. 5. Diclofenac with CRP has 2 hydrogen bonds at TRP-204 (2.86 Å) and ARG-205 (2.14 Å) It has 2 hydrophobic bonds at PHE-197 (4.71 Å) and ASP-175 (3.56 Å) Emodin with CRP has 1 hydrogen bond at ASN-203(1.90 Å) and 2 hydrophobic interactions at TRP-204(4.04 Å) and ASP-175(5.20 Å) Aloe-emodin with CRP has 1 hydrogen bond at ARG (2.90 Å) and 2 hydrophobic interactions at ASP-175(3.77Å) and TRP-205 (5.24 Å) as shown in Fig. 6.

IL-1β is represented by grey color, the binding pocket: golden and ligand molecules aloe emodin: green, emodin: red and diclofenac purple as shown in Fig. 6. Di-

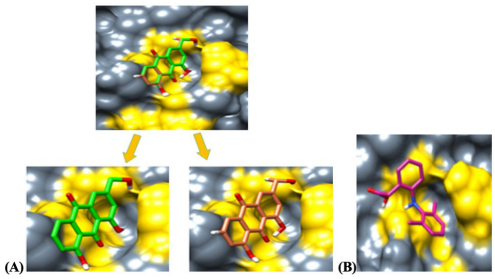


**Figure 6. Interlukin-1β and ligand molecules (A) emodin and aloe emodin (B) diclofenac**

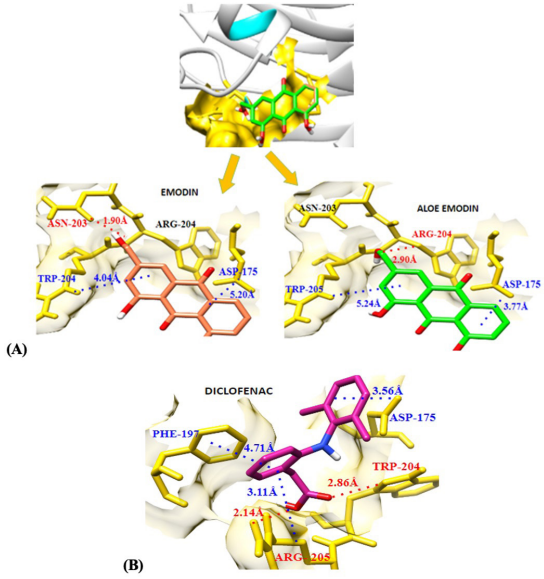


**Figure 7. Binding affinities of CRP with ligand molecules (A) emodin and aloe emodin (B) diclofenac.**
The dotted red lines represent the hydrogen bonds. While dotted blue lines represent hydrophobic interactions.

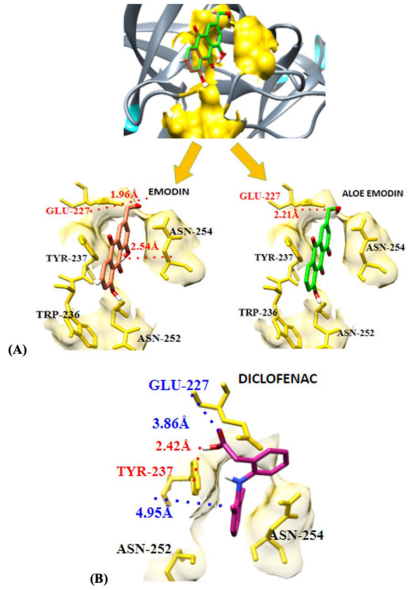


**Figure 8. Binding affinities of IL-1β with ligand molecules (A) emodin and aloe emodin (B) diclofenac.**
The dotted red lines represent the hydrogen bonds. While, dotted blue lines represents hydrophobic interactions.

clofenac with IL-1β has 1 hydrogen bond at TYR-237 (2.42 Å) and 2 hydrophobic bonds at GLU-227 (3.86 Å) and TYR-237 (4.95 Å) Emodin with IL-1β has 2 hydrogen bonds at GLU-227 (1.96 Å) and ASN-254 (2.54 Å) Aloe-emodin with IL-1β has 1 hydrogen bond at GLU-227 (2.21 Å) shown in Fig. 7.

## DISCUSSION

The present study was evaluated to assess the medicinal capacity of *A. barbadensis* against induced inflammation in rat paw. Plant-derived remedies exhibit high medicinal properties when extracted *via* organic solvents. Previous studies reported carrageenan for its biphasic outcomes triggered by the production of endogenous mediators in addition to the discharge of serotonin and histamine in the earliest hour compelling vasodilatory cores, following period is mediated by the discharge of prostaglandins, free radicals, interferons, interleukins, tumor necrosis factor, lysozyme and bradykinin in the progression of inflammation from two to six hours post carrageenan injection. Major cells of the immune system are concerned in these processes including mast cells, basophils, and neutrophils. This time is insightful to evaluate the anti-edematous effect of natural and clinically efficient drugs. These natural antioxidants have the ability to detoxify and neutralize free radicals to protect against oxidative stress-induced cellular injuries (Jyothi *et al.,* 2022; Rosales *et al.,* 2020)

In our study, inflammation was significantly ($p<0.05$) reduced in groups III and IV in dose-dependent manner. A higher dose of 400 mg/kg showed 51% and 46% inhibition of paw edema effective as imitating the action of the drug diclofenac with 61% inhibition. High concentration of anthraquinones, like aloe-emodin, carboxylic acid and polysaccharides in aloe-gel may trigger an immune response, while lower dose do not persuade such an effect. Results of another study were concurrent with our findings (Gupta *et al.,* 2020; Pradhan *et al.,* 2021)

Previous studies have shown that edema may be clarified by reducing cyclooxygenase and PGE2 levels from arachidonic acid. Presence of phytoconstituents, phenolic compounds, flavonoids like quercetin and salicylic acid, choline, vitamins, folic acid, B1, B2, B6, C, β-carotene and α-tocopherol in aqueous extract of *A. barbadensis* gel may be accountable for anti-inflammatory activity in subacute edema model. The results of another study were concurrent with our findings (Gul *et al.*, 2023; Naureen *et al.*, 2023, Naveed *et al.*, 2022b; Jhundoo *et al.,* 2020) CRP is an acute phase protein and IL-6 is a reliable biomarker (cell signaling agent) which amplifies at the inflammation site. The previous studies reported CRP as a key role in inflammatory courses and host retorts to infections together with the complement pathway, nitric oxide (NO) release, apoptosis, phagocytosis, and the assembly of cytokines, predominantly tumor necrosis factor-α and interleukin-6. Group I demonstrated a noteworthy elevation in serum levels of CRP and IL-6 post-carrageenan injection. In a similar trend, others reported remarkable elevation in serum levels of IL-6 and CRP in rats subsequent to 4 hours post carrageenan injection (Acharya *et al.,* 2019)

A higher dose of extract (400 mg/kg) has exposed an enhanced inhibitory effect in IL-6 than the lower dose (50mg/kg) in groups III and IV. For CRP, it demonstrated inhibition of increased serum cytokines and proteins in dose-dependent manner. The use of *A. barbadensis* in some inflammatory cascades confirmed the occur-

rence of active biochemical compounds and minerals and gallic acid related to these edema inhibition activities (Asci *et al.*, 2017) The correlation was positive between biomarkers CRP and IL-6 in group IV in our findings. Similarly, other reports are in concurrence with our data set showing that classic and trans-pathways together take part in the inflammatory bout, although, both pathways are diverse. Through classic signaling in the liver, IL-6 up-regulates the production of CRP, with lipolysis. IL-6 correlates well with the severity of other inflammatory processes interceding the edema reaction (Vreugdenhil *et al.,* 2018)

Molecular docking is a new strategy to analyze binding energy or interaction. Results evidenced that phytoconstituents emodin and aloe-emodin showed interaction with active sites of protein concerned with inflammation and have utmost inhibitory potential against CRP and IL-1beta and could be practiced in the treatment of edema disorders. The binding interaction of the phytoconstituents of *A. barbadensis* and standard drug diclofenac against edema cytokines like IL-1beta and protein CRP exhibited maximum affinity –7.5 by aloe emodin, diclofenac with –7.0 Kcal/mol and emodin with –4.5 Kcal/mol binding score. Aloe-emodin demonstrated the utmost part for inhibiting edema, which are in accordance with our findings (Syed *et al.,* 2023; Sana *et al.,* 2023; Khushnuma *et al.,* 2023; Aziz *et al.,* 2023; Ahmad *et al.,* 2023; Naureen *et al.,* 2023; Kaloni *et al.,* 2019; Modak *et al.,* 2021; Saleem *et al.*, 2023; Naveed *et al.*, 2022a)

## CONCLUSIONS

Use of SAID and NSAIDS possibly helpful in disease management but their adverse impacts pretence health problems. Therefore, therapies with safe natural sources are mandatory. Our study concluded that potentially bioactive phytochemicals in *A. barbadensis* detoxify and counteract free radicals to reduce edema. Hence, our study supports the traditional use of *A. barbadensis* gel extract as an effective and safe drug against inflammation.

### Declarations

**Ethical Approval.** Ethical approval for this research work was granted by the Institute of Molecular Biology and Biotechnology of the University of Lahore. Animal maintenance and experimental protocols were carried out in accordance with guidelines approved by the Care Committee of Animal House at the University of Lahore.

**Conflict of Interest.** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. No conflict of interest. All the authors declare no conflict of interest.

**Author Contributions.** Conceptualization, B.R, S.I, S.A and B.Z.; methodology, T.A; software, B.R, S.I, S.A and B.Z.; validation, M.A., and T.A.; formal analysis, A.A.S; investigation, M.A.; resources, T.A.; data curation, A.S,.; writing – original draft preparation, A.S and N.U; writing – review and editing, M.A. and A.A.S; visualization, N.U. supervision, T.A.; project administration, M.A, A.F.A and A.A.S.; funding acquisition, T.A.

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