Research Article

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Evaluation of influence of *Butea monosperma* floral extract on inflammatory biomarkers

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Abstract: *Butea monosperma* is a deciduous tree, widely distributed throughout India, Burma, and Ceylon. The present study was intended to investigate the anti-inflammatory effects of *Butea monosperma* on the induced inflammatory model by evaluating pro-inflammatory biomarkers and their computational analysis. The anti-inflammatory activity may be attributed to the phyto-constituents for inhibitory effects on the two pro-inflammatory mediators (IL-8 and TNF-α). For this purpose, rats (n = 48) were equally divided in each group, i.e., 8 each in the negative and positive control and 32 in the experimental group with 8 rats for each dose, i.e., 50, 100, 200, and 400 mg/kg. TNF-α and IL-8 were tested by serum enzyme-linked immunosorbent assay (ELISA). The ELISA results showed 400 mg/kg dose as the potent anti-inflammatory. The binding sites of target proteins (TNF-α and IL-8) were docked with the active compounds (butrin and butein) of *Butea monosperma*. The butrin (target: TNF-α) and butein (target: IL-8) showed −8.4 and −6.0 kcal/mol binding energies, respectively, compared to the (diclofenac) standard drug with −6.8 kcal/mol binding energy. Hence, we concluded that *Butea monosperma* can be subjected to as a useful anti-inflammatory drug.

Keywords: *Butea monosperma*, induced inflammation, biomarkers, *in silico*

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1 Introduction

The flame tree, *Butea monosperma*, is a member of the Fabaceae family's Caesalpinioideae subfamily (formerly Leguminosae). In India, the plant is frequently referred to as a Palash tree. Along with the South Asian peninsula, it grows all over India. It is a deciduous tree of medium size. It increases in height by 10–15 m. Due to increased branching, it resembles a tiny shrub when it is between 1 and 2 m tall. During the spring blooming season, its unscented, reddish-colored flowers are produced along with trifoliate leaves. Flowers are mostly used to treat digestive issues, stomachaches, and other conditions related to the stomach. It also treats other disorders like leprosy, sanguinary, skin issues, and thirst [1]. The majority of rangelands and grasslands naturally include *Butea monosperma*, a gum-yielding tree that grows best in arid and semi-arid climates. It is a crucial multipurpose tree for the rural population since it offers shade, fodder, fiber, firewood, gum, and medication. It is the most common species in Bundelkhand and is primarily found in open woodlands, degraded/pasture areas, forests, and farmers' holdings [2]. Numerous active components found in medicinal plants have the potential to be used in the manufacture of therapeutic drugs. Therefore, for drug development, it is required to identify and isolate phytochemical groups and/or single chemical entities from them because these substances frequently operate as single agent or as a group of phytocompounds (purified extracts) to provide the desired therapeutic effect. The biological actions of plant bioactive chemicals cover a broad range, including antibacterial, antioxidant, and anti-inflammatory effects [3]. *Butea monosperma* exhibits promising anti-inflammatory and analgesic properties attributed to its diverse array of bioactive compounds, including flavonoids, steroids, glucosides, and aromatic compounds. *Butea monosperma* extracts exhibit several

in vitro and *in vivo* activities including wound healing, anti-hyperglycemic, anti-inflammatory, hepatoprotective, and anti-tumor activities [4]. By blocking the activation of NF-kB, the butrin, isobutrin, and butein isolated from *Butea monosperma* flowers significantly decreased the inflammatory gene expression and production of TNF-α, IL-6, and IL-8 in macrophages caused by phorbol 12-myristate 13-acetate and calcium ionophore A23187 [5]. The purpose of the present research was to explore the anti-inflammatory and analgesic properties of *Butea monosperma*, commonly known as the Palash tree. The research was driven by the recognition of the plant's historical use in traditional Asian remedies, and the increasing global interest in natural treatments as an alternative to synthetic drugs. The study also acknowledged the need for a detailed scientific investigation into the chemical components and bioactivities of medicinal plants, particularly their potential anti-inflammatory effects. Through an *in vivo* study, it was hypothesized that the plant extracts can effectively modulate inflammatory mediators and demonstrated pain-relieving effects. Molecular docking simulations further elucidated the interaction between bioactive compounds from *Butea monosperma* and key proteins involved in inflammation, providing insights for potential drug development in the treatment of inflammatory disorders.

2 Materials and methods

2.1 Sample collection

The plant material used in this research was the mature flowers of *Butea monosperma* directly collected in March 2021 as a sample from various locations in the Bahawalpur district of Pakistan. The samples were identified by the Botany Department of The University of Lahore (UOL), Pakistan. The flowers were cleaned and air-dried in stainless steel tray covered with aluminum foil at 37°C in an incubator for about 1 week and then chopped into smaller pieces.

2.2 *Butea monosperma* flower extract

The chopped flowers of *Butea monosperma* were soaked in a sterile glass bottle. The flowers were immersed in acetone, well shaken for 4–5 min, and then placed at room temperature for 15 days. After this time, the mixture was filtered and poured into Petri dishes by placing them at room temperature for the next 7 days until dry. The dried paste was saved in an Eppendorf tube by scratching it with a blade from a Petri dish.

2.3 Experimental rats

For appraising anti-inflammatory activity, albino rats (female or male) of weight 200 g were purchased from the Institute of Molecular Biology and Biotechnology's (UOL) animal home for the experiment. The experimental permission was taken from the ethical committee of the University of Lahore. Rats were kept in polypropylene cages at the University of Lahore animal house. Rats were acclimatized with our Department's animal house for about a week and were supplied with water and balanced feed. Rats were off-feed for about 24 h and off-water 12 h before the start of the experiment.

2.4 Drugs/chemicals with doses

Butea monosperma acetonic extracts @ 50, 100, 200, and 400 mg/kg, Diclofenac @ 100 mg/kg, Carrageenan (inflammation inducer) [6] @ 400 mg/kg, and normal saline @ 10 ml/kg were used.

2.5 Evaluation of anti-inflammatory effect

For the evaluation of anti-inflammatory effects of *Butea monosperma*, 48 albino rats were divided into 3 groups, i.e., 8 each in group I (negative control) and group II (positive control/standard-diclofenac), and 32 in group III (8 for each dose of *Butea monosperma* acetonic extract).

2.6 Procedure

2.6.1 Carrageenan-induced rat paw edema

Carrageenan was subcutaneously administered in each rat's paw in all the groups to cause edema. Group I or negative control was treated with simple normal saline @ 10 ml/kg. Group II or Positive control was treated with diclofenac sodium @ 100 mg/kg, subcutaneous injection. Whereas the rats in group III were treated with different concentrations of acetonic flower extracts of *Butea monosperma* @ 50, 100, 200, and 400 mg/kg by oral administration. The paw volume was measured, using a plethysmometer, before the injection and then after 1–4 h post-injection. The rats were placed in different cages during the activity.

2.6.2 Blood samples

Blood samples were collected by cardiac puncture in Eppendorf tubes and were centrifuged at 2,683*g* for 10 min to obtain serum. The serum was stored at −20°C.

2.6.3 Assessment of anti-inflammatory biomarkers

Pro-inflammatory biomarkers IL-8 and TNF-α protein were analyzed by using commerciallyavailable ELISA kits and sandwich ELISA was performed. Concentrations of inflammatory biomarkers were obtained for the negative control, standard, and various doses of *Butea monosperma* flower acetone extract. TNF-α estimation was performed using Biospes kit (Chongqing Biospes Co., Ltd, Catalog No. BEK1212). Briefly, the experiment involved setting up a pre-coated plate with standard, test sample, and control wells, each measured in duplicate. Standard wells received 0.1 ml aliquots of standard solutions, while the control well received a diluent buffer. Test sample wells received 0.1 ml of properly diluted samples and underwent incubation at 37°C for 90 min. After discarding the plate content, Biotin-conjugated anti-Human TNFα antibody was added, followed by incubation at 37°C for 60 min. The plate underwent washing, and ABC working solution was added and incubated. After additional washes, TMB substrate was added and incubated at 37°C in the dark. To stop the reaction, Stop solution was added, resulting in a color change. Optical density at 450 nm was read, and relative O.D.450 was calculated. A standard curve was plotted, and Human TNFα concentrations in samples were interpolated from the curve.

2.7 *In silico* anti-inflammatory activity

2.7.1 Selection of ligands

For *in silico* investigation of monosperma anti-inflammatory activity, (Chimera) https://www.cgl.ucsf.edu/chimera/cgi-bin/secure/chimera-get.py, (PyRx) and https://sourceforge.net/projects/pyrx/, (ApoHoloproteinsearch) http://apoholo.cz/ were used and visualized with (discovery studio) https://discover.3ds.com/discovery-studio-visualizer-download. TNF-α and IL-8 proteins 3D structures were retrieved from a protein data bank (PDB).

2.7.2 Preparation of ligands

The ligand molecules were selected from the bioactive constituents of *Butea monosperma* that have anti-inflammatory potential. These ligands were discovered in the literature of phytochemical databases [7]. The docking analysis of anti-inflammatory efficacy used two ligands of *Butea monosperma*: butrin and butein and the standard drug diclofenac. The 3D structures of the ligand molecules were assessed from PubChem (PubChem CID of butein is 5281222 and butrin is 164630).

2.8 Protein preparation

TNF-α and IL-8 PDB files were opened in chimera and selected chains were incorporated for further characterization. The extra ions, water, and metals were eliminated before dispensing the protein. Furthermore, the hydrogen atoms were added if needed in any structure and the geometry of all the hetero groups was assured. The protein structures were saved in PDB files.

2.9 Prediction of the active site

TNF-α and IL-8 active sites were found in the apo Holo protein search by submitted the protein id file (1ilq,5uui) after few sec online tool generate binding sites file which are further downloaded.

2.9.1 Protein-ligand docking

PyRx was utilized for virtual screening to find a potential anti-inflammatory drug or ligand molecule for a certain protein. Both target protein TNF-α (PDB ID = 5UUI) and IL-8 (PDB ID = 1ILQ) binding pockets were coupled with the ligands molecules by using their respective PDB IDs. The active sites of the target protein (TNF-α and IL-8) were discovered using the depth residue prediction data source. The strength of binding contact was estimated as a consequence of the docking run, binding site energy, and the ligand-molecule interface. Following that, the discovery studio tool was used to create protein complexes containing ligand compounds. The Chimera tool was used to create the 3D structure complex for high-quality observation.

2.10 Statistical analysis

Results were statistically analyzed by applying Graph-Pad-Prism v.6.0. For paw size variation in “mm” at various

doses of acetone flower extract and at various time points, two-way ANOVA was applied. For multiple comparisons among groups at various time points, Tukey's multiple comparison tests was promulgated. Whereas, for comparing pro-inflammatory cytokines, one-way ANOVA was applied and Dunnett's multiple comparison test was considered for group comparison. Significant values were given asterisk (*) sign and non-significant values were denoted with "ns." Tukey's multiple comparison tests were applied with a significance level (α) set at 0.05. The tests were two-tailed, and degrees of freedom were adjusted accordingly. Tukey's multiple comparison test was chosen due to its ability to effectively control the group-wise error rate in situations involving multiple group comparisons. Tukey's multiple comparison test was chosen due to its ability to effectively control the group-wise error rate in situations involving multiple group comparisons. The selected α level of 0.05 was deemed appropriate for maintaining a balance between Type I and Type II errors in our study.

3 Results

3.1 Anti-inflammatory activity

3.1.1 Carrageenan-induced paw edema

The various concentrations of acetonic flower extracts of *Butea monosperma* showed non-significant anti-inflammatory effects (IL-8 and TNF-α) on rats when compared with standard (diclofenac) [1] (Figure 1).

However, the effect of extracts on decreasing paw size in response to carrageenan was highly significant ($P < 0.0001$) at various time points (Figure 2). 50, 100, and 200 mg/kg doses (0 h), 50 and 200 mg/kg doses (1, 3, and 4 h), 400 mg/kg (2 h) provided high-significant paw size reduction when compared with standard (diclofenac) drug (Figure 2). These results in relation to the negative control samples, which were treated with normal saline. Actually, negative control is typically used to establish a baseline and assess the impact of any treatment, it is also valid to focus on the positive control (diclofenac in this case) and compare the experimental groups to it. The negative control was included to establish a baseline level of inflammation induced by Carrageenan, and our primary interest was in comparing the efficacy of the acetonic flower extract of *Butea monosperma* with the well-known anti-inflammatory drug diclofenac (positive control). The negative control was not intended for direct comparison, as it only represents the baseline inflammation without any treatment.

3.1.2 *In Silico* analysis

3.1.2.1 Chemistry

Butrin is a flavanone glycoside that is butin substituted by two beta-D-glucopyranosyl residues at positions 7 and 3′, respectively (Figure 3a). It has a role as an anti-inflammatory agent and a plant metabolite. It has a molecular formula of $C_{27}H_{32}O_{15}$ and molecular weight of 596.5 g/mol. Butein is a flavonoid obtained from the seed of *Cyclopia subalternate* having molecular formula $C_{15}H_{12}O_5$ and molecular weight of 272.25 g/mol (Figure 3b). Diclofenac is used as a standard anti-

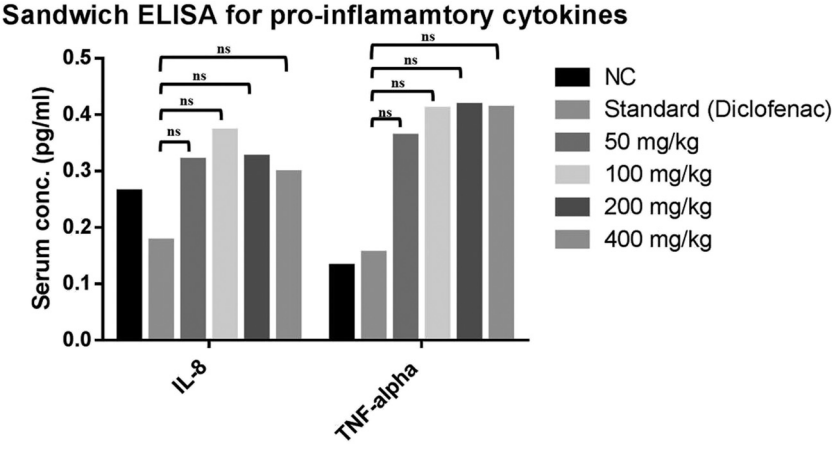

Figure 1: Sandwich ELISA for pro-inflammatory cytokines.

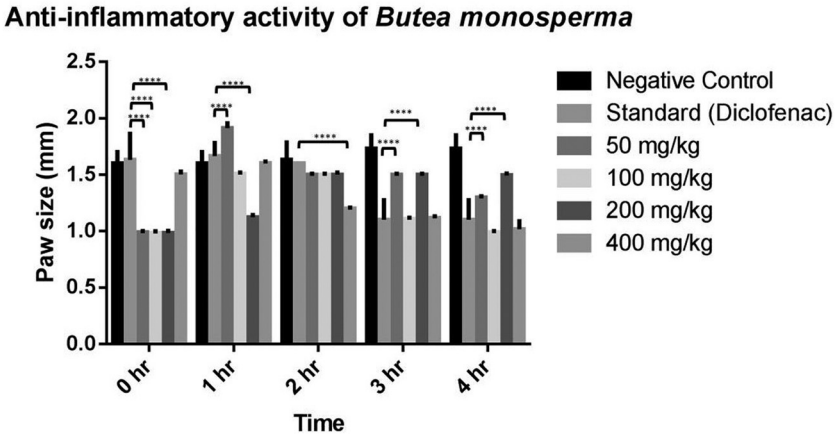

Figure 2: Shows the anti-inflammatory activity of *Butea monosperma*.

inflammatory chemical with molecular formula $C_{14}H_{11}Cl_2NO_2$ and molecular weight of 296.1 g/mol (Figure 3c).

3.1.2.2 Prediction of active sites and interaction of binding pockets with ligands

Active sites of TNF-α contained five amino acids (TYR 86, TYR 87, VAL 91, LEU 94, and ILE 97) and the binding of diclofenac and butrin molecule (Figure 2a). Active sites of IL-8 contained four amino acids (GLY 31, SER 30, ARG 26, and ILE 40) and the binding of active sites revealed that all ligands were confined in the binding region of the target protein. All of the docked ligand structures were superimposed to evaluate their binding interactions in the binding pockets of all targeted proteins (TNF-α and IL-8). All the ligands have similar binding interactions and are shown by the results (Figure 4a and b).

3.1.2.3 Hydrogen bonding analysis, docking results of TNF-α and IL-8

Hydrogen and hydrophobic bonds are used to evaluate the bonding interactions of docking complexes. Diclofenac and butrin with TNF-α interactions were supported by 7 H-bonds at GLY 24, SER 65, SER 65, LEU 142, PHE 14, ALA 14, and GLN 14. One hydrophobic bond on PHE 14 with a bond distance of 4.793 Å (Table 1).

Ligands (Figure 5a), IL-8, and butein interactions are shown by eight H-bonds with GLN8, ARG 26, ARG 26, ARG 26, ARG 26, ILE 28, GLU 29, and GLU 38. Figure 5c shows binding interactions between the target protein IL-8 and one ligand.

3.1.2.4 Binding energies

The standard drug diclofenac and the plant bioactive compound (butrin) were docked of TNF-α using PyRx. The top

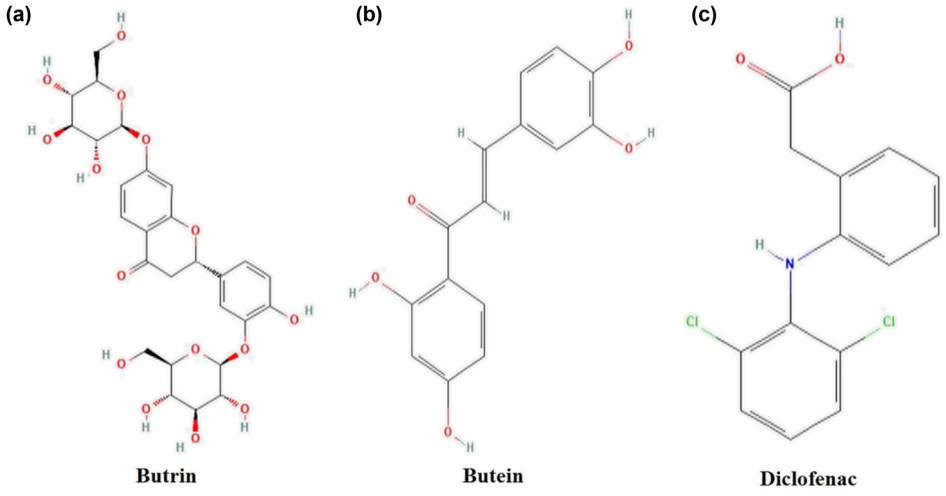

Figure 3: Structures of compounds (a) butrin, (b) butein, and (c) diclofenac used in the study.

docking energy was −8.4 kcal/mol by butrin while the standard drug diclofenac showed a binding score of −6.8 kcal/mol (Figure 5b). Bio-active compound (butein) was docked of IL-8 using PyRx and has binding energy of 6.0 kcal/mol (Figure 5d).

Table 1: Docking complex of pro-inflammatory cytokines

Ligands	Amino acids	Bond distance (Å)
TNF-α		
Butrin	Glycine 24	2.05
	Serine 65	1.92
	Serine 65	1.64
Diclofenac	Leucine 142	2.21
	Phenylalanine 14	2.18
	Alanine 14	1.95
	Glutamine 14	1.97
IL-8		
Butein	Glutamine8	2.49
	Arginine26	1.93
	Arginine26	1.40
	Arginine26	1.84
	Arginine26	2.03
	Isoleucine28	2.08
	Glutamic acid	2.42
	Glutamic acid	2.19

4 Discussion

Traditional medicines are in use in various regions of the world for ages. Medicinal plants are used to treat pain, fever, and many other infections [8–23]. The present study was conducted to estimate the effect of *Butea monosperma* in carrageenan-induced inflammation and investigation of anti-inflammatory biomarkers in albino rats, using the carrageenan-induced paw edema, which is the most widely used primary model for the screening of new anti-inflammatory agents. Carrageenan induced edema is a multi-mediated phenomenon that liberates diversity of mediator like histamine, 5-HT, kinins, and prostaglandins at various time intervals [24].

In this study, phytochemical analysis was also performed to screen and study all the phytochemical constituents of *Butea monosperma* and selective potentially bioactive molecules for molecular docking analysis. Phyto-constituents characterized in the current study are known to be beneficial in medicinal sciences. The results of this study can be anticipated as decipher in the search of novel and economically valued drug molecules [25]. Hence, we concluded that acetonic *Butea monosperma* extract can be subjected as a useful drug in the treatment of inflammation. *Butea monosperma* (L.) is the reservoir for many potentially active chemical compounds which acts as drugs against various diseases and disorders. Different bioactive substances, such as alkaloids, flavonoids, amino acids, resin, saponin, etc., are present in Butea monosperma [26]. The acetonic flower extract of *Butea monosperma* showed a significant result in anti-inflammatory activity. The maximum inhibition activity was shown by a concentration of 400 mg/kg which was 60% in contrast to a standard which was 90% of the same concentration and the control is 0%. The plant showed 50, 50, and 10% at respective concentrations of 50, 100, and 200 mg/kg. The standard group showed inhibition activity of 40, 10, and 30% at respective concentrations of 50, 100, and 200 mg/kg while the control group could not give any inhibitory

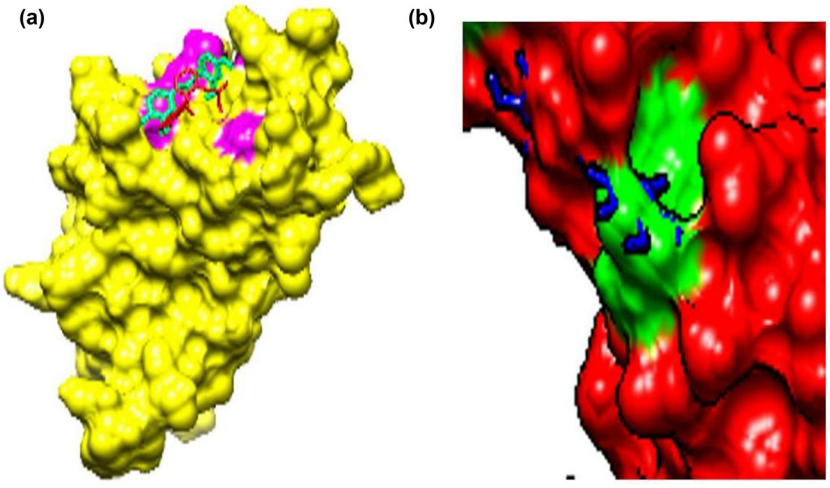

Figure 4: (a) TNF-α protein (yellow), binding pocket (hot pink), and two ligand molecules – diclofenac (crayon blue) and butrin (red). (b) IL-8 protein (red), binding pocket (green), and one ligand molecule (dark blue).

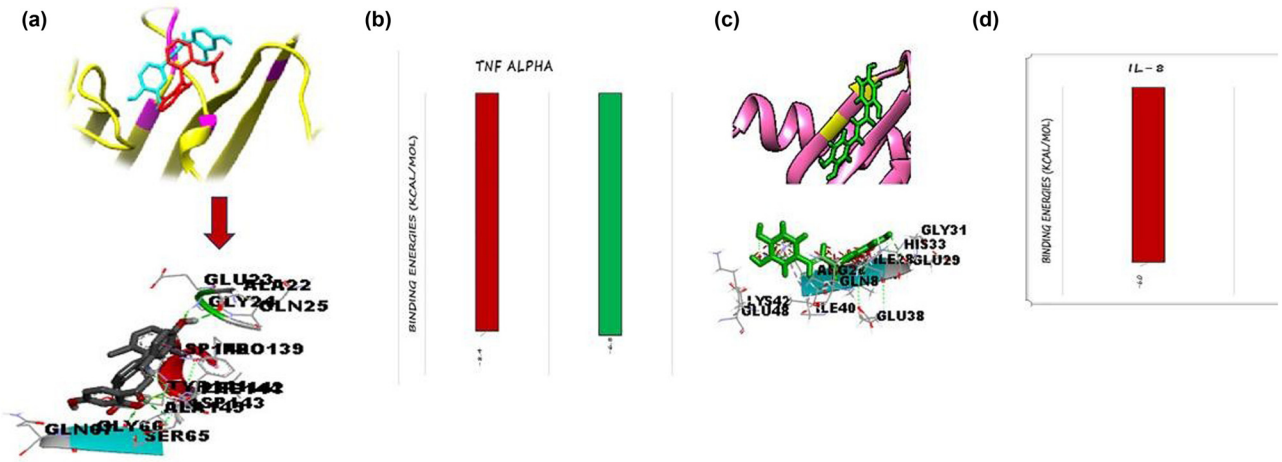

Figure 5: (a) Docking complex of TNF-α in which butrin and diclofenac showed seven hydrogen bonds and one hydrophobic bond. (b) Hydrogen bond is shown by green dotted lines and hydrophobic bond represented by pink dotted lines. Binding energies of TNF-α with two ligand molecules butrin (red) and diclofenac (green). (c) Docking complex of IL-8 shows eight hydrogen bonds represented by green dotted lines. (d) Binding energy of IL-8 with one ligand molecule butein (red).

activity after the carrageenan injection. All the results were significant ($P < 0.001$). Blood biomarkers showed highly significant activity at the dose of 400 mg/kg. The phytochemical analysis presented the occurrence of glycosides, tannins, flavonoid saponins, anthraquinones, and phenols. *Butea monosperma* has a variety and number of bioactive compounds having different therapeutic applications. In our study, butrin and butein found in the flower part of *Butea monosperma* were studied in *in silico* analysis with TNF-α and IL-8, it is believed that butrin and butein have a significant contribution to anti-inflammatory activity by inhibiting the TNF-α and IL-8. The more negative values represent more affinity to bind with protein. The butrin and butein showed −8.4 and −6.0 kcal/mol binding energies, respectively, as compared to the standard drug diclofenac with −6.8 kcal/mol binding energy to IL-8 and TNF-α. In our results, butrin showed the greatest binding affinity as compared to butein.

5 Conclusion

In conclusion, the acetonic flower extracts of *Butea monosperma* demonstrated non-significant anti-inflammatory effects in terms of IL-8 and TNF-α compared to the standard diclofenac. However, the extracts exhibited a high-significant reduction in paw size in response to carrageenan-induced paw edema, particularly at doses of 50, 100, and 200 mg/kg at various time points. Molecular docking studies revealed that the bioactive compound butrin from *Butea monosperma* exhibited superior binding energies with TNF-α compared to diclofenac, and butein displayed favorable interactions with IL-8. The identified amino acids involved in binding interactions and the hydrogen bonding patterns were elucidated. These findings suggest the potential anti-inflammatory properties of *Butea monosperma* extracts and its bioactive compounds, particularly butrin and butein, through modulation of pro-inflammatory cytokines. However, further research is warranted to validate these results *in vivo* and explore the underlying mechanisms of action. Additionally, the study provides insights into the molecular interactions of the compounds with key inflammatory markers, contributing to the understanding of their therapeutic potential. Nonetheless, the limitations of the study include the need for more extensive clinical investigations and the consideration of potential side effects or toxicity. Further research directions could involve exploring the broader pharmacological profile of *Butea monosperma* extracts, conducting clinical trials, and investigating potential synergistic effects with conventional anti-inflammatory drugs for enhanced therapeutic outcomes.

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Zahid: Visualization, Software. Muhammad Ahsan Naeem: Writing – review & editing. Abid Sarwar: Methodology, Investigation. Tariq Aziz: Methodology, Investigation. Metab Alharbi: Visualization, Software. Thamer H Albekairi: Formal analysis. Abdullah F Alasmari: Writing – review & editing. Tariq Aziz: Writing – review & editing, Supervision: Tariq Aziz.

Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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