Research Article

Anti-Inflammatory and Analgesic Potential of Hot Water Extracts of Mulberry Leaves

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Abstract | Various diseases have been traditionally treated by utilizing different parts of mulberry plants. Mulberry leaves possess different physiological and biological potentials. Inflammation is the most common factor involved in various chronic diseases. This research study was conducted to evaluate anti-inflammatory and analgesic potentials of hot water extracts of mulberry leaves (mulberry tea) of two species i.e., *Morus alba* and *Morus nigra*. Mice were fed orally with mulberry leaves extract (200mg/kg, 100mg/kg and 50mg/kg) mixed in distilled water. Mice paw edema was induced by carrageenan injection while formalin was injected to mice paw for induction of pain. These extracts significantly decreased edema in mice paw. The level of TLC and RBCs was increased to normal. Platelets level was also increased in mice treated with mulberry leaves extracts. Furthermore, these extracts significantly reduced the inflammatory pain and licking response triggered by injection of formalin in mice paw. These findings showed that the hot water extracts of leaves of these two plant species possess analgesic and anti-inflammatory potentials.

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1. Introduction

Inflammation is a complex and dynamic response that can be defined by heat (hyperemia), redness (erythema), swelling (exudation), pain and loss of function (Placha and Jampilek, 2021; Pei *et al.*, 2023). It is of two basic types: Acute that starts rapidly, becomes severe in a short time and chronic inflammation that is slow and cause many chronic diseases (Ginwala *et al.*, 2019). Inflammatory processes are initiated by cell injury, infection (microbial) and trauma or toxins (Deshpande and Zou, 2020). Oxidative stress can also lead to inflammation (Hu *et al.*, 2020).

Inflammation is a common feature in most of the modern human diseases (Kotas and Medzhitov, 2015). Due to the enormous shift in the disease spectrum over the previous century, the World Health Organization (WHO) catalogued inflammatory disorders as the highest risk to human wellbeing (Kotas and Medzhitov, 2015; Logie and Vanden, 2020). Furthermore, most of the age-related diseases share an inflammatory pathogenesis (Franceschi and Campisi, 2014).

Inflammatory processes cause immune cells to release different molecules that sensitize nociceptor sensory neurons and induce pain and swelling (Pinho-Ribeiro *et al*., 2017). Leukocytes, particularly neutrophils are responsible for the initiation and maintenance of inflammation and the leukocytes that have infiltrated tissues continuously produce reactive chemicals that harm the structural and cellular components of tissues (Goldring *et al*., 2011; Franceschi and Campisi, 2014). Damaged cells and activated immune cells produce cytokines that alter the normal tissue function and phenotypes of nearby cells (Franceschi and Campisi, 2014). Tumor necrosis factor (TNF) and interleukins (IL-1, IL-6, and IL-12) produced by helper T cells and macrophages function as pro-inflammatory cytokines and blocking of either TNF or IL-1 or both leads to reduction in inflammation (Boleto *et al*., 2020; Rezagholizadeh *et al*., 2022; Shafeghat *et al*., 2022).

Currently non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to cure inflammation, but they also have a greater risk of heart attack, renal disease, and stomach ulcers and bleeding (Fanelli et al., 2013). Plant products are gaining popularity as natural remedies for the cure of different ailments including inflammation (Salehi *et al*., 2019, 2020; Alam *et al*., 2020; Poulsen *et al*., 2020). Plants are a rich source of medically important phytochemicals such as polyphenols, flavonols etc. (Selamoglu, 2017; Salehi *et al*., 2019).

Due to the higher therapeutic potential, low risk of side effects, availability, and connection to cultural practices, the herbal remedies are favored (Shah *et al*., 2018; Erarslan and Kültür, 2019; Süntar, 2020; Okaiyeto and Oguntibeju, 2021).

Moraceae family of flowering plants is ecologically important having 38 genera and 1500 species (Christenhusz and Byng, 2016; de Sousa *et al*., 2016). Genus Morus of this family includes 24 species and widely distributed across Asia, Europe, America, and several areas of Africa (Ionica *et al*., 2017). Plants of this genus can be grown under cultivation and contain some medically important species, particularly red mulberry (*M. rubra*), white mulberry (*M. alba*) and black mulberry (*M. nigra*) (Sarkhel *et al*., 2020; Jan *et al*., 2021).

In Asia, various mulberry plant parts, including the fruits, leaves and root bark have often been used as traditional medicines to cure a variety of human health impairments (Yuan and Zhao, 2017). Mulberry leaves are palatable, low cost and valuable materials that are used for raring silkworm (*Bombyx mori*) and as feed for dairy animals (Rohela *et al*., 2020; Jaiswal *et al*., 2021). Different mulberry leaves-derived products such as ice-cream and tea are now commercially available (Thaipitakwong *et al*., 2018). These leaves are also used for reducing the risks associated with cardiovascular system, nervous system and obesity (Ignat *et al*., 2021; Weng *et al*., 2021). Mulberry leaves extracts have anti-inflammatory, anti-hyperglycemic and antimicrobial activity in addition to lowering the blood lipids in mild hyperlipidemic patients (Sharma *et al*., 2020; Hao *et al*., 2022; Zhang *et al*., 2022). Mulberry leaf hot water extracts and methanolic extracts prevent oxidative damage and reduce the level of inflammatory cytokines (Hameed *et al*., 2020; Boro *et al*., 2021).

M. alba and *M. nigra* are originated in China and Iran respectively but frequently farmed widely in subtropical and tropical areas of the world (Aljane et al., 2016; Luo *et al*., 2019). Different mulberry species especially *Morus nigra, M. alba, M. laevigata and M. macroura* are present in Pakistan and their leaves are extensively used for silkworm rearing (Mutebi, 2022). The aim of the current study was to assess the nociceptive and anti-inflammatory properties of a hot water extract of *M. nigra* and *M. alba* leaves against carrageenan induced inflammation and formalin induced pain by using Swiss albino mice as model animal.

2. Materials and Methods

2.1 Plant material

The leaves of the two mulberry species *Morus nigra* and *M. alba* were obtained from Government College University Lahore Botanical Garden (31° 33' 24.102" N and 74° 19' 40.8432" E). Leaves were rinsed with tap water and shade dried for 3 days at 30°C. Dried leaves of both plant species were ground to fine powder and kept in a hermetic seal separately until further use (Katsube *et al*., 2009).

2.2 Preparation of extract

Mulberry powder (10g) was added to 100ml of distilled water and solution was heated on hot plate

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at 40°C for 20 minutes. Further extraction was done by mixing the solution in Flask Shaker for 24 hours at room temperature. The Whatmann's filter paper (9cm Diameter and 11µm Particle retention) was used to filter the extract and filtrate containing soluble constituents was obtained (Arabshahi-Delouee and Urooj, 2007). Extracts were mixed with distilled water to prepare the required dose of 200mg/kg, 100mg/kg and 50mg/kg.

2.3 Model animal

Mus musculus (Swiss albino mice) with an average weight of 25-30gm were chosen as model animal. Mice were bought from Veterinary Research Institute Lahore, Pakistan. They were kept in standard-sized boxes (28" x 18" x 18") with regulated climatic conditions, such as 26 to 30 °C temperature range, humidity range of 40 to 50%, and a 12-hour cycle of light and dark alternatively. All the animals were given a regular diet. Saw dust was utilised as bedding which was changed on daily basis. The experiment was performed at Animal House of Government College University Lahore after ethical approval from Institutional Bioethics Committee of GC University, Lahore (No. GCU-IIB-450) (Bayliak *et al*., 2021).

2.4 Evaluation of anti-inflammatory potential

Carrageenan solution (1%) was freshly prepared before the experiment. Animals were starved for 2 hours and divided into eight groups with five animals each. Treatment doses were given orally to animals of each group with help of oral gavage. Positive and negative control groups were treated orally with indomethacin (5mg/kg) and water respectively. Out of six treatment groups, three were treated with 200mg/kg, 100mg/kg and 50mg/kg of *M. alba* leaves hot water extract mixed in dH_2O and remaining three groups with hot water extracts of *M. nigra* leaves. After 1 hour of treatment dose 50µl of 1% carrageenan in 0.9% saline solution was injected to left hind tibio-tarsal joint of all animals. The diameter of ankle joints was utilized to assess the level of inflammation (Winter *et al*., 1962). Paw circumference was assessed by cotton thread at 0, 1, 2, 3, 4, 5 and 6 hours post carrageenan injection to assess the inflammation or paw edema (Akindele and Adeyemi, 2007; Gupta *et al*., 2015).

2.5 Effect on leukocytes and complete blood count

After 6 hours of carrageenan injection the blood was drawn directly by a cardiac puncture and stored in EDTA. K_3 vacuum tubes at 4°C for 2 hours. Complete blood count was performed in auto blood analyzer.

2.6 Histopathology of Paw

Animals were sacrificed at 6 hours post treatment and to assess histopathological changes their paws were taken precisely. Paw samples were decalcified after being washed with 10% Formalin. Furthermore, tissues were fixed in paraffin and cut into thin pieces. Hematoxylin-Eosin (HE) stain was used to determine the level of inflammation in these thin sections.

2.7 Analgesic activity

A day before experiment, each animal was habituated for an hour in the observation chamber. Experimental design was similar as mentioned in anti-inflammatory assay except positive and negative control groups that were treated orally with Diclofenac sodium (10mg/kg) and water respectively. Formalin induced method of nociception is used to access analgesic activity (Hunskaar and Hole, 1987). After 30 minutes of oral dose, 20 µl (10ml/kg) of 1% formalin prepared in normal saline was injected subcutaneously into the planter tissue of dorsal hind paw of the mice with a 26-gauge needle. Individual animal was then put back into the chamber and licking response time was measured with stop watch for 30 min.

Intensive licking activity was divided in two distinct periods and both periods were scored separately. The first period for 0-5 minutes and the second period for almost 15-30 minutes after the injection of formalin. Acute pain in first period is non-inflammatory and due to the direct activation of nociceptors by formalin, while pain in second period is inflammatory and appears due to the combination of inflammatory reactions in tissue (Warren, 1990).

2.8 Statistical analyses

Results were observed as decrease in licking time and increase in paw edema. Results of both anti-inflammatory and analgesic activity were computed by using one way ANOVA followed by Tukey's test to assess the differences between treatment groups and negative control. $P < 0.05$ was considered as significant value.

3. Results and Discussion

3.1 Formalin induced nociception

Formalin injection in paw tissues resulted in severe pain and paw licking activity immediately after the administration. Licking response was severe during first 5 minutes and then decreased afterwards. There was a latency period of almost 10 minutes when animals did not show nociception responses. After the latency period, animals again showed licking response from 15-30 minutes post formalin injection. Mulberry leaves extracts of *M. alba* and *M. nigra* (200mg/kg, 100mg/kg and 50mg/kg) caused non-significant ($P>0.05$) decrease in licking and biting activity of mice in the first phase of acute pain (0-5 minutes) as compared to negative control (Figure 1A). In second phase of nociceptive activity (15-30 minutes) there was significant ($P<0.05$) reduction of licking response in treated groups as compared to negative control. However, Diclofenac Sodium showed highest anti-nociception activity in second phases (Figure 1B).

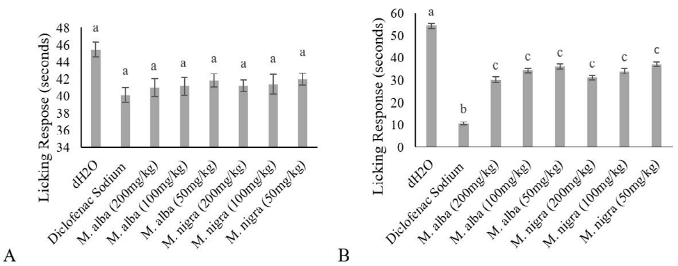

Figure 1: A: Analgesic activity (0-5 min post formalin injection); B: Analgesic activity (15-30 min post formalin injection).

Note: Similar superscripts show non-significant differences, while different superscripts show significant differences.

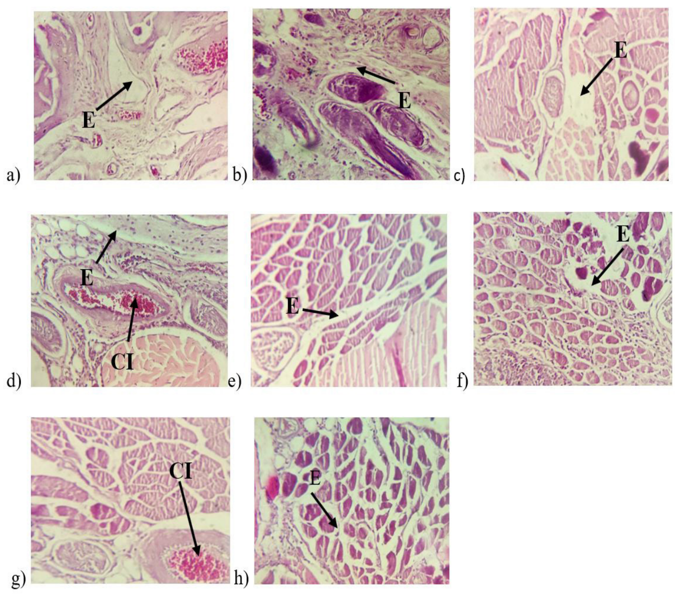

Figure 2: (a) Sub-planter tissue of mice in negative control group, (b) positive control group, (c) *M. alba* 2%, (d) *M. alba* 1%, (e) *M. alba* 0.5%, (f) *M. nigra* 2%, (g) *M. alba* 1% and (h) *M. nigra* 0.5%. "E" represents edema and "CI" represents cell infiltration.

3.2 Anti-inflammatory activity

The injection of carrageenan into planter tissues of tibio-tarsal joints of mice paw resulted in immediate swelling of paw. The paw circumference increased gradually with time and was the highest in all groups at 4th hour of post carrageenan injection (Table 2). Mice of negative control showed highest increase in paw width (3.14mm ± 0.148) while positive control (Indomethacin) showed lowest increase (2.2mm ± 0.119) at 4th hour post treatment. Just after the carrageenan injection (0 hour), there were non-significant differences of paw width among all groups ($F_{7,32}$= 0.409; $P>0.05$). However, there were statistically significant differences ($P<0.01$) in paw width of all treatment groups against negative control at 1st ($F_{7,32}$= 0.409), 2nd ($F_{7,32}$= 2.464), 3rd ($F_{7,32}$= 4.071), 4th ($F_{7,32}$= 3.597), 5th ($F_{7,32}$= 5.087) and 6th ($F_{7,32}$= 0.409) hours, post carrageenan injection. However, there were non-significant differences ($P>0.05$) among Indomethacin and mulberry extracts treated groups.

3.3 Histological results

The histological sections of paw edema tissues after the carrageenan injection were observed and the results of histological changes including different inflammatory markers are shown in Figure 2. High tissue damage was observed in negative control as compared to treatment groups. The level of swelling and collection of fluid was also high in negative control. Moreover, there was accumulation of infiltrating cells, especially polymorphonuclear leukocytes (PMNs) in intercellular spaces. While the treatment groups showed less damage and swelling. The number of infiltrating cells was reduced as well as the intercellular spaces. Infiltrating cells were only confined to spaces near to vascular areas. Indomethacin, a standard drug had shown similar results to treatment groups.

3.4 Hematology

Results of the hematological studies (Table 1) showed that the total number of leukocytes, platelets, RBCs, lymphocytes and neutrophils in blood of negative control group mice were significantly lower than mulberry treated and positive control groups ($F_{7,23}$= 13.026; $P<0.01$; $F_{7,23}$= 18.86; $P>0.05$; $F_{7,23}$= 17.92; $P<0.01$; $F_{7,23}$= 3.47; $P<0.05$; $F_{7,23}$= 9.19; $P<0.01$). However, there were non-significant differences in level of monocytes ($F_{7,23}$=1.49; $P>0.05$) and eosinophils ($F_{7,23}$= 1.25; $P>0.05$) in the mice of all experimental groups ($P>0.05$). The total number of leukocytes, lymphocytes and platelets did not differ significantly

Table 1: Effect of mulberry leaves extracts on different blood parameters of mice injected with carrageenan solution.

Treatments	RBCs ($1x10^6/\mu l$)	TLC ($1x10^3/\mu l$)	Lymphocytes ($1x10^3/\mu l$)	Neutrophils ($1x10^3/\mu l$)	Monocytes ($1x10^3/\mu l$)	Eosinophils ($1x10^3/\mu l$)	Platelet count ($1x10^8/\mu l$)
Negative Control	$6.4^a \pm 0.11$	$4.56^a \pm 0.2$	$3.48^a \pm 0.08$	$1.72^a \pm 0.04$	$0.056^a \pm 0.004$	$0.059^a \pm 0.004$	$961.53^a \pm 11.32$
Indomethacin	$8.8^c \pm 0.08$	$6.38^b \pm 0.16$	$4.92^b \pm 0.07$	$2.60^b \pm 0.1$	$0.092^a \pm 0.004$	$0.096^a \pm 0.003$	$1219^b \pm 12.97$
M. alba (200mg/kg)	$8.37^{bc} \pm 0.2$	$6.07^b \pm 0.1$	$4.01^{ab} \pm 0.12$	$2.24^a \pm 0.09$	$0.072^a \pm 0.01$	$0.076^a \pm 0.01$	$1181.33^b \pm 12.74$
M. alba (100mg/kg)	$7.92^b \pm 0.1$	$5.95^b \pm 0.08$	$3.88^{ab} \pm 0.11$	$2.12^a \pm 0.12$	$0.069^a \pm 0.005$	$0.071^a \pm 0.004$	$1154.22^b \pm 14.21$
M. alba (50mg/kg)	$7.81^b \pm 0.17$	$5.72^b \pm 0.09$	$3.76^{ab} \pm 0.13$	$2.06^a \pm 0.13$	$0.067^a \pm 0.007$	$0.069^a \pm 0.007$	$1143^b \pm 20.7$
M. nigra (200mg/kg)	$8.46^{bc} \pm 0.12$	$6.1^b \pm 0.11$	$4.07^{ab} \pm 0.13$	$2.15^a \pm 0.1$	$0.072^a \pm 0.007$	$0.075^a \pm 0.009$	$1189.67^b \pm 11.77$
M. nigra (100mg/kg)	$8.13^{bc} \pm 0.12$	$6.03^b \pm 0.1$	$3.97^{ab} \pm 0.11$	$2.04^a \pm 0.12$	$0.071^a \pm 0.01$	$0.072^a \pm 0.009$	$1161.67^b \pm 17.19$
M. nigra (50mg/kg)	$7.97^{bc} \pm 0.12$	$5.84^b \pm 0.09$	$3.83^{ab} \pm 0.13$	$2.01^a \pm 0.12$	$0.068^a \pm 0.008$	$0.069^a \pm 0.01$	$1153^b \pm 14.43$

Note: Data shows Mean ± SE; Similar superscripts on each value within a column show non-significant differences, while different superscripts show significant differences.

Table 2: Effect of mulberry leaves extracts on paw edema or increase in paw circumference of mice in control and treatment groups.

Groups	Increase in paw circumference						
	0 h	1 h	2 h	3 h	4 h	5 h	6h
Negative Control	$4.56^a \pm 0.186$	$2.32^a \pm 0.130$	$2.46^a \pm 0.128$	$2.89^a \pm 0.154$	$3.14^a \pm 0.148$	$2.53^a \pm 0.138$	$2.18^a \pm 0.112$
Indomethacin	$4.1^a \pm 0.159$	$1.47^b \pm 0.184$	$1.59^b \pm 0.175$	$1.86^b \pm 0.107$	$2.2^b \pm 0.119$	$1.42^b \pm 0.163$	$0.79^b \pm 0.074$
M. alba (200mg/kg)	$4.31^a \pm 0.152$	$1.59^{ab} \pm 0.172$	$1.9^{ab} \pm 0.178$	$2.05^b \pm 0.148$	$2.31^b \pm 0.144$	$1.55^b \pm 0.127$	$0.83^b \pm 0.081$
M. alba (100mg/kg)	$4.36^a \pm 0.177$	$1.6^{ab} \pm 0.162$	$1.72^{ab} \pm 0.165$	$2.14^b \pm 0.155$	$2.36^b \pm 0.148$	$1.57^b \pm 0.131$	$0.85^b \pm 0.087$
M. alba (50mg/kg)	$4.38^a \pm 0.197$	$1.63^{ab} \pm 0.176$	$1.8^{ab} \pm 0.172$	$2.28^{ab} \pm 0.157$	$2.35^b \pm 0.120$	$1.75^b \pm 0.113$	$0.87^b \pm 0.077$
M. nigra (200mg/kg)	$4.29^a \pm 0.195$	$1.51^b \pm 0.151$	$1.64^b \pm 0.149$	$1.96^b \pm 0.149$	$2.27^b \pm 0.171$	$1.53^b \pm 0.166$	$0.81^b \pm 0.091$
M. nigra (100mg/kg)	$4.34^a \pm 0.128$	$1.57^{ab} \pm 0.088$	$1.68^{ab} \pm 0.095$	$2.11^b \pm 0.097$	$2.34^b \pm 0.084$	$1.56^b \pm 0.110$	$0.82^b \pm 0.058$
M. nigra (50mg/kg)	$4.38^a \pm 0.213$	$1.6^{ab} \pm 0.157$	$1.82^{ab} \pm 0.174$	$2.02^b \pm 0.150$	$2.35^b \pm 0.167$	$1.6^b \pm 0.157$	$0.86^b \pm 0.068$

Note: Data shows Mean ± SE; Similar superscripts on each value within a column show non-significant differences, while different superscripts show significant differences.

(P>0.05) among mulberry treated groups and positive control group. While there were non-significant differences (P>0.05) in the level of neutrophils among mulberry treated groups and negative control. The number of RBCs was significantly lower in mice treated with lower concentrations of *M. alba* leaves extract (100mg/kg and 50mg/kg) as compared to mice treated with mulberry leaves extracts of *M. nigra* and positive control group.

The anti-inflammatory potentials of mulberry leaves were investigated by using carrageenan induced mice paw edema as it is considered the most suitable inflammatory model to evaluate various anti-inflammatory products (Pathak *et al.*, 2020; Nigussie *et al.*, 2021). Carrageenan induced inflammatory response is non-specific and biphasic, characterized on the basis of mediator release (Meacock and Kitchen, 1976; Hvattum and Ekeberg, 2003). Serotonin and histamine are released in the first phase and cause increase in vascular permeability, kinins in second phase while various pro-inflammatory mediators including prostaglandins, TNF-α and different cytokines in third phase (Sriramula, 2020; Zhang and Kurashima, 2021; Mo *et al.*, 2022). Anti-inflammatory effects of various extracts of mulberry leaves had already been reported (Souza *et al.*, 2018). However, these extracts were prepared in toxic solvents like methanol and ethanol that further require complete separations.

Results of our study showed that the hot water extracts of mulberry leaves have suppressive effects on carrageenan induced edema. Chung et al. (2003) had also reported similar effects. In the present study the inhibition was mild in first four hours of post carrageenan injection, but more intense at 5th hour and onward, as suggested by Aouey et al. (2016). The decrease in edema during the first phase could be due to inhibition of histamine and serotonin as well as neutrophil migration (Channa *et al.*, 2006; Patil *et al.*, 2019). While the possible mechanism for the intense late inhibition in our study could be due to inhibition of cyclooxygenases (involved in prostaglandin synthesis), cytokines and other pro-inflammatory mediators (Channa *et al*., 2006; Babu *et al*., 2009). Anti-histaminic effect may be related to inhibition of degranulation of mast cells (Barbosa *et al*., 2009; Vanderlei *et al*., 2011). While Sadeghi (2013) reported that the reduction in pro-inflammatory mediators is due to decreased influx of inflammatory cells like neutrophils. Moreover, the effectiveness of *M. nigra* and *M. alba* leaves extracts against inflammation was similar to Indomethacin, a standard anti-inflammatory drug.

The TLC was decreased in mice injected with carrageenan solution. It may be due to migration of leukocytes towards the inflammatory sites, causing the decreased leukocyte count in peripheral blood (Eric and Dick, 1996; Patil and Suryavanshi, 2007). In our study the mice treated with mulberry leaves extracts and Indomethacin showed higher TLC. Vaisbuch et al. (2002) reported that anti-inflammatory drugs like Betamethasone lower the clearance of leukocytes from peripheral blood and increase their production and release from bone marrow. The mulberry leaves extracts in our study showed increased TLC level. Chen et al. (2013) reported that oxyresveratrol present in mulberry leaves inhibits leukocyte migration and suppress inflammation. On the contrary it had been reported that some anti-inflammatory substances *i.e.*, plant extracts cause the decrease in TLC (Rached *et al*., 2010; Al-Sadoon *et al*., 2012; John and Shobana, 2012).

RBCs level of untreated mice in our study was low as inflammation causes clearance of RBCs. According to Straat (2012) the possible mechanisms involved could be phagocytosis and increased adherence of RBCs to endothelial walls. In our study the mice treated with mulberry leaves extracts showed comparatively high levels of RBCs. The exact possible mechanisms responsible for this effect are unknown. While the platelet level of mice in our study increased significantly in mulberry leaves extract treated groups. However, Henriques (1987) reported the great increase in circulating platelets after 48 hours of carrageenan injection. Histopathological observations of mice paw tissue showed that the carrageenan induced various histological alterations such as swelling and influx of inflammatory cells (Ma *et al*., 2013). Paw tissues of treated mice exhibited reduced histological alterations. According to Sadeghi (2013) the reduction in histological alterations is due to the inhibition of vascular permeability and pro-inflammatory mediators production.

In present study the formalin induced licking response in mice was used to evaluate the analgesic effect of mulberry leaves extracts as previous studies have suggested that this model has the ability to differentiate between peripheral and central analgesic mechanism (Bouaziz *et al*., 2005). Formalin 1% solution was used for induction of pain because lower concentrations did not induce biphasic pain (Rosland *et al*., 1990; Hasriadi *et al*., 2022). Pain during the first phase is due to peripheral inflammatory response and central sensitization of pain stimuli; while histamine, serotonin, bradykinin and prostaglandins are involved in pain of second phase (Dou *et al*., 2021; Miranda *et al*., 2022). It had been reported that the flavonoids, 1-Deoxynojirimycin (DNJ) and phenols present in mulberry leaves are responsible for their functional properties (Thaipitakwong *et al*., 2018; Eruygur and Dural, 2019).

In our study the hot water extracts of *M. alba* and *M. nigra* leaves did not show significant analgesic effects during the first phase of acute pain. However, all the doses of hot water extracts of both mulberry species as well as Diclofenac Sodium showed significant analgesic response during second phase of pain. Parr and Bolwell (2000) reported the NSAIDs are also not effective to reduce pain in the first phase but significantly reduce the pain during second phase. Mohammadifar et al. (2016) reported that alcoholic extract of *M. alba* leaves showed analgesic effect in both first and second phase of pain. Analgesic potential of other mulberry leaves extracts like ethanol had also been reported (Chauhan *et al*., 2015). According to Denny (2013) the flavonoids and phenols of mulberry leaves could be responsible for analgesic potentials. The exact mechanisms are still needs to be identified. It has been reported that the possible mechanism involved could be dual inhibition of arachidonic acid pathway and inhibition of prostaglandin synthesis (Duarte *et al*., 1988; Ferdous *et al*., 2008). The analgesic activity of mulberry leaves extracts was not as strong as Diclofenac Sodium, a standard analgesic drug. It is concluded that the crude extract of leaves of *M. alba* and *M. nigra* possess anti-inflammatory and analgesic potentials. However, further mechanistic studies are required in this regard to find exact constituents and their mechanisms responsible for these potentials.

Conclusions and Recommendations

It is concluded that the crude extract of leaves of M. alba and M. nigra possess anti-inflammatory and analgesic potentials. However, further mechanistic studies are recommended in this regard to find exact constituents and their potential mechanisms responsible for these results.

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Novelty Statement

The Hot Water Extract of Mulberry Leaves (Mulberry Tea) has the potential to reduce inflammation.

Author's Contribution

Aamir Ali: Designed the study, executed the experimentation.
Hafiz Muhammad Tahir and Azizullah: Designed and supervised the study.
Shaukat Ali and Muhammad Summer: Analyzed the data and wrote the manuscript.
Ali Haidar Gormani and Saira Nawaz: Edited the manuscript.
Ayesha Muzamil: Analyzed the data and wrote the manuscript.

Conflict of interest
The authors have declared no conflict of interest.

References

Akindele, A.J., and Adeyemi, O.O., 2007. Antiinflammatory activity of the aqueous leaf extract of *Byrsocarpus coccineus*. *Fitoterapia*, 78(1): 25-28. https://doi.org/10.1016/j.fitote.2006.09.002

Alam, W., Khan, H., Shah, M.A., Cauli, O. and Saso, L., 2020. Kaempferol as a dietary anti-inflammatory agent: current therapeutic standing. Molecules, 2020; 25(18): 4073. https://doi.org/10.3390/molecules25184073

Aljane, F., Sdiri, N.J., Laamari, R., Ochatt, S., and Ferchichi, A., 2016. Morphological, phytochemical and antioxidant characteristics of white (*Morus alba* L.), red (*Morus rubra* L.) and black (*Morus nigra* L.) mulberry fruits grown in arid regions of Tunisia. *Journal of New Sciences*, 35(1): 1940-1947.

Al-Sadoon, M.K., Fahim, A., Salama, S.F., and Badr, G., 2012. The effects of LD50 of Walterinnesia aegyptia crude venom on blood parameters of male rats. *African Journal of Microbiology Research*, 6(3): 653-659.

Aouey, B., Samet, A.M., Fetoui, H., Simmonds, M.S., and Bouaziz, M., 2016. Anti-oxidant, anti-inflammatory, analgesic and antipyretic activities of grapevine leaf extract (*Vitis vinifera*) in mice and identification of its active constituents by LC–MS/MS analyses. *Biomedicine and Pharmacotherapy*, 84(2016): 1088-1098. https://doi.org/10.1016/j.biopha.2016.10.033

Arabshahi-Delouee, S., and Urooj, A., 2007. Antioxidant properties of various solvent extracts of mulberry (*Morus indica* L.) leaves. *Food Chemistry*, 102(4): 1233-1240. https://doi.org/10.1016/j.foodchem.2006.07.013

Babu, N.P., Pandikumar, P., and Ignacimuthu, S., 2009. Anti-inflammatory activity of Albizia lebbeck Benth., an ethnomedicinal plant, in acute and chronic animal models of inflammation. *Journal of Ethnopharmacology*, 125(2): 356-360. https://doi.org/10.1016/j.jep.2009.02.041

Barbosa, A.L.R., Pinheiro, C.A., Oliveira, G.J., Moraes, M.O., Ribeiro, R.A., Vale, M.L., and Souza, M.H.L.P., 2009. Tumor bearing decreases systemic acute inflammation in rats–role of mast cell degranulation. *Inflammation Research*, 58(5): 235-240. https://doi.org/10.1007/s00011-008-8226-z

Bayliak, M.M., Sorochynska, O.M., Kuzniak, O.V., Gospodaryov, D.V., Demianchuk, I., Vasylyk, Y.V., Mosiichuk, N.M., Storey, K.B., Garaschuk, O., and Lushchak, V.I., 2021. Middle age as a turning point in mouse cerebral cortex energy and redox metabolism: modulation by every other day fasting. *Experimental Gerontology*, 145(1): 111182. https://doi.org/10.1016/j.exger.2020.111182

Boleto, G., Vieira, M., Desbois, A.C., Saadoun, D., and Cacoub, P., 2020. Emerging molecular targets for the treatment of refractory sarcoidosis. *Frontiers in Medicine*, 7(1): 594133. https://doi.org/10.3389/fmed.2020.594133

Boro, H., Das, S., and Middha, S.K., 2021. The therapeutic potential and the health benefits of Morus indica Linn.: A mini review. *Advances in Traditional Medicine*, 21(1): 241-252. https://doi.org/10.1007/s13596-020-00544-5

Bouaziz, M., Grayer, R.J., Simmonds, M.S., Damak, M., and Sayadi, S., 2005. Identification and antioxidant potential of flavonoids and low molecular weight phenols in olive cultivar Chemlali growing in Tunisia. *Journal of Agricultural and Food Chemistry*, 53(2): 236-241. https://doi.org/10.1021/jf048859d

Channa, S., Dar, A., Anjum, S., and Yaqoob, M., 2006. Anti-inflammatory activity of Bacopa monniera in rodents. *Journal of Ethnopharmacology*, 104(1-2): 286-289. https://doi.org/10.1016/j.jep.2005.10.009

Chauhan, S., Devi, U., Kumar, V.R., Kumar, V., Anwar, F., and Kaithwas, G., 2015. Dual inhibition of arachidonic acid pathway by mulberry leaf extract. *Inflammopharmacology*, 23(1): 65-70. https://doi.org/10.1007/s10787-014-0223-y

Chen, Y.C., Tien, Y.J., Chen, C.H., Beltran, F.N., Amor, E.C., Wang, R.J., Wu, D.J., Mettling, C., Lin, Y.L., and Yang, W.C., 2013. Morus alba and active compound oxyresveratrol exert anti-inflammatory activity via inhibition of leukocyte migration involving MEK/ERK signaling. *BMC Complementary and Alternative Medicine*, 13(1): 1-10. https://doi.org/10.1186/1472-6882-13-45

Christenhusz, M.J., and Byng, J.W., 2016. The number of known plants species in the world and its annual increase. *Phytotaxa*, 261(3): 201-217. https://doi.org/10.11646/phytotaxa.261.3.1

Chung, K.O., Kim, B.Y., Lee, M.H., Kim, Y.R., Chung, H.Y., Park, J.H., and Moon, J.O., 2003. *In-vitro* and *in-vivo* anti-inflammatory effect of oxyresveratrol from *Morus alba* L. *Journal of Pharmacy and Pharmacology*, 55(12): 1695-1700. https://doi.org/10.1211/0022357022313

de Sousa, A.L., de Almeida, C.M.S., Kaplan, M.A.C., and de Oliveira, R.R., 2016. A chemosystematic study of the moraceae family: An analysis of the metabolites from the biosynthetic mixed pathway (Acetate/Shikimate). *International Journal of Sciences*, 5(03): 143-159. https://doi.org/10.18483/ijSci.989

Denny, C., Melo, P.S., Franchin, M., Massarioli, A.P., Bergamaschi, K.B., de Alencar, S.M., and Rosalen, P.L., 2013. Guava pomace: A new source of anti-inflammatory and analgesic bioactives. *BMC Complementary and Alternative Medicine*, 13(1): 1-7. https://doi.org/10.1186/1472-6882-13-235

Deshpande, R., and Zou, C., 2020. Pseudomonas aeruginosa induced cell death in acute lung injury and acute respiratory distress syndrome. *International Journal of Molecular Sciences*, 21(15): 5356. https://doi.org/10.3390/ijms21155356

Dou, B., Li, Y., Ma, J., Xu, Z., Fan, W., Tian, L., Chen, Z., Li, N., Gong, Y., Lyu, Z., Fang, Y., and Lin, X., 2021. Role of neuroimmune crosstalk in mediating the anti-inflammatory and analgesic effects of acupuncture on inflammatory pain. *Frontiers in Neuroscience*, 15(1): 695670. https://doi.org/10.3389/fnins.2021.695670

Duarte, I.D., Nakamura, M., and Ferreira, S.H., 1988. Participation of the sympathetic system in acetic acid-induced writhing in mice. *Brazilian Journal of Medical and Biological Research= Revista brasileira de pesquisas Medicas e Biologicas*, 21(2): 341-343.

Erarslan, Z.B., and Kültür, Ş., 2019. Ethnoveterinary medicine in Turkey: A comprehensive review. *Turkish Journal of Veterinary and Animal Sciences*, 43(5): 55-582. https://doi.org/10.3906/vet-1904-8

Eric, H.T., and Dick, G.R., 1996. Text book of therapeutics: Drugs and disease management. *Baltimore: Williams and Wilkins Company*, pp. 579-595.

Eruygur, N., and Dural, E., 2019. Determination of 1-deoxynojirimycin by a developed and validated HPLC-FLD method and assessment of *in-vitro* antioxidant, α-amylase and α-glucosidase inhibitory activity in mulberry varieties from Turkey. *Phytomedicine*, 53(1): 234-242. https://doi.org/10.1016/j.phymed.2018.09.016

Fanelli, A., Romualdi, P., Viganò, R., Lora Aprile, P., Gensini, G., and Fanelli, G. 2013. Non-selective non-steroidal anti-inflammatory drugs (NSAIDs) and cardiovascular risk. Acta

Biomed, 84(1): 5-11.

Ferdous, M., Rouf, R., Shilpi, J.A., and Uddin, S.J., 2008. Antinociceptive activity of the ethanolic extract of *Ficus racemosa* Lin.(Moraceae). *Advances in Traditional Medicine*, 8(1): 93-96. https://doi.org/10.3742/OPEM.2008.8.1.093

Franceschi, C., and Campisi, J., 2014. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*, 69(Suppl_1): S4-S9. https://doi.org/10.1093/gerona/glu057

Ginwala, R., Bhavsar, R., Chigbu, D.G.I., Jain, P., and Khan, Z.K., 2019. Potential role of flavonoids in treating chronic inflammatory diseases with a special focus on the anti-inflammatory activity of apigenin. *Antioxidants*, 8(2): 35. https://doi.org/10.3390/antiox8020035

Goldring, M.B., Otero, M., Plumb, D.A., Dragomir, C., Favero, M., El-Hachem, K., Hashimoto, K., Roach, H.I., Olivotto, E., Borzì, R.M., and Marcu, K.B., 2011. Roles of inflammatory and anabolic cytokines in cartilage metabolism: Signals and multiple effectors converge upon MMP-13 regulation in osteoarthritis. *European Cells and Materials*, 21(1): 202. https://doi.org/10.22203/eCM.v021a16

Gupta, A.K., Parasar, D., Sagar, A., Choudhary, V., Chopra, B.S., Garg, R., and Khatri, N., 2015. Analgesic and anti-inflammatory properties of gelsolin in acetic acid induced writhing, tail immersion and carrageenan induced paw edema in mice. *PLoS One*, 10(8): e0135558. https://doi.org/10.1371/journal.pone.0135558

Hameed, A., Galli, M., Adamska-Patruno, E., Krętowski, A., and Ciborowski, M., 2020. Select polyphenol-rich berry consumption to defer or deter diabetes and diabetes-related complications. *Nutrients*, 12(9): 2538. https://doi.org/10.3390/nu12092538

Hao, J., Gao, Y., Xue, J., Yang, Y., Yin, J., Wu, T., and Zhang, M., 2022. Phytochemicals, pharmacological effects and molecular mechanisms of mulberry. *Foods*, 11(8): 1170. https://doi.org/10.3390/foods11081170

Hasriadi, Wasana, P.W.D., Sritularak, B., Vajragupta, O., Rojsitthisak, P., and Towiwat, P., 2022. Batatasin III, a constituent of dendrobium scabrilingue, improves murine pain-like behaviors with a favorable CNS safety profile. *Journal of Natural Products*, 85(7): 1816-1825. https://doi.org/10.1021/acs.jnatprod.2c00376

Henriques, M.G., Silva, P.M., Martins, M.A., Flores, C.A., Cunha, F.Q., Assreuy-Filho, J., and Cordeiro, R.S., 1987. Mouse paw edema. A new model for inflammation. *Brazilian Journal of Medical and Biological Research*, 20(2): 243-249.

Hu, C., Zhang, X., Zhang, N., Wei, W.Y., Li, L.L., Ma, Z.G., and Tang, Q.Z., 2020. Osteocrin attenuates inflammation, oxidative stress, apoptosis, and cardiac dysfunction in doxorubicin-induced cardiotoxicity. *Clinical and Translational Medicine*, 10(3): e124. https://doi.org/10.1002/ctm2.124

Hunskaar, S., and Hole, K., 1987. The formalin test in mice: Dissociation between inflammatory and non-inflammatory pain. *Pain*, 30(1): 103-114. https://doi.org/10.1016/0304-3959(87)90088-1

Hvattum, E., and Ekeberg, D., 2003. Study of the collision-induced radical cleavage of flavonoid glycosides using negative electrospray ionization tandem quadrupole mass spectrometry. *Journal of the American Society for Mass Spectrometry*, 38(1): 43-49. https://doi.org/10.1002/jms.398

Ignat, M.V., Coldea, T.E., Salanță, L.C., and Mudura, E., 2021. Plants of the spontaneous flora with beneficial action in the management of diabetes, hepatic disorders, and cardiovascular disease. *Plants*, 10(2): 216. https://doi.org/10.3390/plants10020216

Ionica, M.E., Nour, V., and Trandafir, I., 2017. Bioactive compounds and antioxidant capacity of some Morus species. *South-Western Journal of Horticulture, Biology and Environment*, 8(1): 79-88.

Jaiswal, K.K., Banerjee, I., and Mayookha, V.P., 2021. Recent trends in the development and diversification of sericulture natural products for innovative and sustainable applications. *Bioresource Technology Reports*, 13(1): 100614. https://doi.org/10.1016/j.biteb.2020.100614

Jan, B., Parveen, R., Zahiruddin, S., Khan, M.U., Mohapatra, S., and Ahmad, S., 2021. Nutritional constituents of mulberry and their potential applications in food and pharmaceuticals: A review. *Saudi Journal of Biological Sciences*, 28(7): 3909-3921. https://doi.org/10.1016/j.sjbs.2021.03.056

John, N.A.A., and Shobana, G., 2012. Antiinflammatory activity of of *Talinum fruticosum* L. on formalin induced paw edema in albino rats. *Journal of Applied Pharmaceutical Science*, 2(1): 123.

Katsube, T., Tsurunaga, Y., Sugiyama, M., Furuno, T., and Yamasaki, Y., 2009. Effect of air-drying temperature on antioxidant capacity and stability of polyphenolic compounds in mulberry (*Morus alba* L.) leaves. *Food Chemistry*, 113(4): 964-969. https://doi.org/10.1016/j.foodchem.2008.08.041

Kotas, M.E., and Medzhitov, R., 2015. Homeostasis, inflammation, and disease susceptibility. *Cell*, 160(5): 816-827. https://doi.org/10.1016/j.cell.2015.02.010

Logie, E., and Vanden, B.W., 2020. Tackling chronic inflammation with withanolide phytochemicals. A withaferin A perspective. *Antioxidants*, 9(11): 1107. https://doi.org/10.3390/antiox9111107

Luo, J., Wang, Y., and Zhao, A.Z., 2019. The complete chloroplast genome of Morus alba (Moraceae: Morus), the herbal medicine species in China. *Mitochondrial DNA Part B*, 4(2): 2467-2468. https://doi.org/10.1080/23802359.2019.1638328

Ma, Y., Li, Y., Li, X., and Wu, Y., 2013. Anti-inflammatory effects of 4-methylcyclopentadecanone on edema models in mice. *International Journal of Molecular Sciences*, 14(12): 23980-23992. https://doi.org/10.3390/ijms141223980

Meacock, S.C.R., and Ann Kitchen, E., 1976. Some effects of non-steroidal anti-inflammatory drugs on leucocyte migration. *Agents and Actions*, 6(1-3): 320-325. https://doi.org/10.1007/BF01972249

Miranda, H.F., Noriega, V., Moreno, F., Sierralta, F., Sotomayor-Zárate, R., and Prieto, J.C., 2022. Morphine at inflammatory experimental pain: A review. *World Journal of Advanced Research and Reviews*, 15(1): 116-121. https://doi.org/10.30574/wjarr.2022.15.1.0675

Mo, Q., Zhang, W., Zhu, A., Backman, L.J., and Chen, J., 2022. Regulation of osteogenic differentiation by the pro-inflammatory cytokines IL-1β and TNF-α: Current conclusions and controversies. *Human Cell*, 35(4): 957-971. https://doi.org/10.1007/s13577-022-00711-7

Mohammadifar, M., Tamtaji, O.R., Behnam, M., Taghizadeh, M., and Talaei, S.A., 2016. Analgesic effect of alcoholic extract of *Morus alba* L. leaf on male rats. *Internal Medicine Today*, 22(2): 151-158. https://doi.org/10.18869/acadpub.hms.22.2.151

Mutebi, C.M., 2022. Mulberry (*Morus* spp.) as a plant for building the resilience of smallholder farmers during climate change and COVID 19 pandemic. *International Journal of Horticultural Science and Technology*, 9(1): 73-83.

Nigussie, D., Makonnen, E., Tufa, T.B., Brewster, M., Legesse, B.A., Fekadu, A., and Davey, G., 2021. Systematic review of Ethiopian medicinal plants used for their anti-inflammatory and wound healing activities. *Journal of Ethnopharmacology*, 276(1): 114179. https://doi.org/10.1016/j.jep.2021.114179

Okaiyeto, K., and Oguntibeju, O.O., 2021. African herbal medicines: Adverse effects and cytotoxic potentials with different therapeutic applications. *International Journal of Environmental Research and Public Health*, 18(11): 5988. https://doi.org/10.3390/ijerph18115988

Parr, A.J., and Bolwell, G.P., 2000. Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *Journal of the Science of Food and Agriculture*, 80(7): 985-1012. https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<985::AID-JSFA572>3.0.CO;2-7

Pathak, P., Shukla, P.K., Naumovich, V., Grishina, M., Verma, A., and Potemkin, V., 2020. 1, 2, 4-Triazole-conjugated 1, 3, 4-thiadiazole hybrid scaffolds: A potent ameliorant of carrageenan-induced inflammation by lessening proinflammatory mediators. *Archiv der Pharmazie*, 353(1): 1900233. https://doi.org/10.1002/ardp.201900233

Patil, K.R., Mahajan, U.B., Unger, B.S., Goyal, S.N., Belemkar, S., Surana, S.J., Ojha, S., and Patil, C.R., 2019. Animal models of inflammation for screening of anti-inflammatory drugs: Implications for the discovery and development of phytopharmaceuticals. *International Journal of Molecular Sciences*, 20(18): 4367. https://doi.org/10.3390/ijms20184367

Patil, K.S., and Suryavanshi, J., 2007. Effect of *Celastrus paniculatus* willd seed on adjuvant induced arthritis in rats. *Pharmacognosy Magazine*, 3(11): 177.

Pei, J., Palanisamy, C.P., Alugoju, P., Anthikapalli, N.V.A., Natarajan, P.M., Umapathy, V.R., Swamikannu, B., Jayaraman, S., Rajagopal, P., and Poompradub, S., 2023. A comprehensive review on bio-based materials for chronic diabetic wounds. *Molecules*, 28(2): 604. https://doi.org/10.3390/molecules28020604

Pinho-Ribeiro, F.A., Verri Jr, W.A., and Chiu, I.M., 2017. Nociceptor sensory neuron–immune interactions in pain and inflammation. *Trends in Immunology*, 38(1): 5-19. https://doi.org/10.1016/j.it.2016.10.001

Placha, D., and Jampilek, J., 2021. Chronic inflammatory diseases, anti-inflammatory agents and their delivery nanosystems. *Pharmaceutics*, 13(1): 64. https://doi.org/10.3390/pharmaceutics13010064

Poulsen, N.B., Lambert, M.N.T., and Jeppesen, P.B., 2020. The effect of plant derived bioactive compounds on inflammation: A systematic review and meta-analysis. *Molecular Nutrition and Food Research*, 64(18): 2000473. https://doi.org/10.1002/mnfr.202000473

Rached, I.C.F.S., Castro, F.M., Guzzo, M.L., and de Mello, S.B.V., 2010. Anti-inflammatory effect of bee venom on antigen-induced arthritis in rabbits: Influence of endogenous glucocorticoids. *Journal of Ethnopharmacology*, 130(1): 175-178. https://doi.org/10.1016/j.jep.2010.04.015

Rezagholizadeh, L., Aghamohammadian, M., Oloumi, M., Banaei, S., Mazani, M., and Ojarudi, M., 2022. Inhibitory effects of Ficus carica and Olea europaea on pro-inflammatory cytokines: A review. *Iranian Journal of Basic Medical Sciences*, 25(3): 268.

Rohela, G.K., Shukla, P., Kumar, R., and Chowdhury, S.R., 2020. Mulberry (*Morus* spp.): An ideal plant for sustainable development. *Trees, Forests and People*, 2(1): 100011. https://doi.org/10.1016/j.tfp.2020.100011

Rosland, J.H., Tjølsen, A., Mæhle, B., and Hole, K., 1990. The formalin test in mice: Effect of formalin concentration. *Pain*, 42(2): 235-242. https://doi.org/10.1016/0304-3959(90)91167-H

Sadeghi, H., Hajhashemi, V., Minaiyan, M., Movahedian, A., and Talebi, A., 2013. Further studies on anti-inflammatory activity of maprotiline in carrageenan-induced paw edema in rat. *International Immunopharmacology*, 15(3): 505-510. https://doi.org/10.1016/j.intimp.2013.01.018

Salehi, B., Capanoglu, E., Adrar, N., Catalkaya, G., Shaheen, S., Jaffer, M., Giri, L., Suyal, R., Jugran, A.K., Calina, D., Docea, A.O., and Capasso, R., 2019. Cucurbits plants: A key emphasis to its pharmacological potential. *Molecules*, 24(10): 1854. https://doi.org/10.3390/molecules24101854

Salehi, B., Gültekin-Özgüven, M., Kırkın, C., Özçelik, B., Flaviana Bezerra Morais-Braga, M., Nalyda Pereira Carneiro, J., Fonseca Bezerra, C., Gonçalves da Silva, T., Douglas Melo Coutinho, H., Amina, B., Armstrong, L.,C., and Cho, W., 2019. Anacardium plants: Chemical, nutritional composition and biotechnological applications. *Biomolecules*, 9(9): 465. https://doi.org/10.3390/biom9090465

Salehi, B., Gültekin-Özgüven, M., Kirkin, C., Özçelik, B., Morais-Braga, M.F.B., Carneiro, J.N. P., Bezerra, C.F., Silva, T.G.D., Coutinho, H.D.M., Amina, B., Armstrong, L., and Martins, N., 2020. Antioxidant, antimicrobial, and anticancer effects of anacardium plants: An ethnopharmacological perspective. *Frontiers in Endocrinology*, 11(1): 295. https://doi.org/10.3389/fendo.2020.00295

Sadeghi, H., Hajhashemi, V., Minaiyan, M., Movahedian, A., and Talebi, A., 2013. Further studies on anti-inflammatory activity of maprotiline in carrageenan-induced paw edema in rat. *International Immunopharmacology*, 15(3): 505-510. https://doi.org/10.1016/j.intimp.2013.01.018

Sarkhel, S., Manvi, D., and Ramachandra, C.T., 2020. Nutrition importance and health benefits of mulberry leaf extract: A review. *Journal of Pharmacognosy and Phytochemistry*, 9(5): 689-695.

Selamoglu, Z., 2017. Polyphenolic compounds in human health with pharmacological properties. *Journal of Traditional Medicine and Clinical Naturopathy*, 6(4): 138. https://doi.org/10.4172/2573-4555.1000e138

Shafeghat, M., Kazemian, S., Aminorroaya, A., Aryan, Z., and Rezaei, N., 2022. Toll-like receptor 7 regulates cardiovascular diseases. *International Immunopharmacology*, 113(A): 109390. https://doi.org/10.1016/j.intimp.2022.109390

Shah, A., Sarvat, R., Shoaib, S., Ayodele, A.E., Nadeem, M., Qureshi, T.M., and Abbas, A., 2018. An ethnobotanical survey of medicinal plants used for the treatment of snakebite and scorpion sting among the people of Namal valley, Mianwali district, Punjab. Pakistan. *Applied Ecology and Environmental Research*, 16(1): 111-143. https://doi.org/10.15666/aeer/1601_111143

Sharma, P., Sharma, A., Thakur, J., Murali, S., and Bali, K., 2020. Mulberry as a life savior. A review. *Journal of Pharmacognosy and Phytochemistry*, 9(2): 2445-2451.

Souza, G.R., Oliveira-Junior, R.G., Diniz, T.C., Branco, A., Lima-Saraiva, S.R.G., Guimarães, A.L., Oliveira, A.P., Pacheco, A.G.M., Silva, M.G., Moraes-Filho, M.O.D., and Costa, M.P., 2018. Assessment of the antibacterial, cytotoxic and antioxidant activities of *Morus nigra* L. (Moraceae). *Brazilian Journal of Biology*, 78(1): 248–254. https://doi.org/10.1590/1519-6984.05316

Sriramula, S., 2020. Kinin B1 receptor: A target for neuroinflammation in hypertension. *Pharmacological Research*, 155(1): 104715. https://doi.org/10.1016/j.phrs.2020.104715

Straat, M., van Bruggen, R., de Korte, D., and Juffermans, N.P., 2012. Red blood cell clearance in inflammation. *Transfusion Medicine and Hemotherapy*, 39(5): 353-360. https://doi.org/10.1159/000342229

Süntar, I., 2020. Importance of ethnopharmacological studies in drug discovery: Role of medicinal plants. *Phytochemistry Reviews*, 19(5): 1199-1209. https://doi.org/10.1007/s11101-019-09629-9

Thaipitakwong, T., Numhom, S., and Aramwit, P., 2018. Mulberry leaves and their potential effects against cardiometabolic risks: A review of chemical compositions, biological properties and clinical efficacy. *Pharmaceutical Biology*, 56(1): 109-118. https://doi.org/10.1080/13880209.2018.1424210

Vaisbuch, E., Levy, R., and Hagay, Z., 2002. The effect of betamethasone administration to pregnant women on maternal serum indicators of infection. *Journal of Perinatal Medicine*, 30(4): 287-291. https://doi.org/10.1515/JPM.2002.041

Vanderlei, E.D.S.O., de Araújo, I.W.F., Quinderé, A.L.G., Fontes, B.P., Eloy, Y.R.G., Rodrigues, J.A.G., and Evangelista, J.S.A.M., 2011. The involvement of the HO-1 pathway in the anti-inflammatory action of a sulfated polysaccharide isolated from the red seaweed Gracilaria birdiae. *Inflammation Research*, 60(12): 1121-1130. https://doi.org/10.1007/s00011-011-0376-8

Warren, J.S., 1990. Interleukins and tumor necrosis factor in inflammation. *Critical Reviews in Clinical Laboratory Sciences*, 28(1): 37-59. https://doi.org/10.3109/10408369009105897

Weng, G., Duan, Y., Zhong, Y., Song, B., Zheng, J., Zhang, S., Yin, Y., and Deng, J., 2021. Plant extracts in obesity: A role of gut microbiota. *Frontiers in Nutrition*, 8(1): 727951. https://doi.org/10.3389/fnut.2021.727951

Winter, C.A., Risley, E.A., and Nuss, G.W., 1962. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Proceedings of the Society for Experimental Biology and Medicine*, 111(3): 544-547. https://doi.org/10.3181/00379727-111-27849

Yuan, Q., and Zhao, L., 2017. The mulberry (*Morus alba* L.) fruit a review of characteristic components and health benefits. *Journal of Agricultural and Food Chemistry*, 65(48): 10383-10394. https://doi.org/10.1021/acs.jafc.7b03614

Zhang, R., Zhang, Q., Zhu, S., Liu, B., Liu, F., and Xu, Y., 2022. Mulberry leaf (*Morus alba* L.): A review of its potential influences in mechanisms of action on metabolic diseases. *Pharmacological Research*, 175(1): 106029. https://doi.org/10.1016/j.phrs.2021.106029

Zhang, Z., and Kurashima, Y., 2021. Two sides of the coin: Mast cells as a key regulator of allergy and acute/chronic inflammation. *Cells*, 10(7): 1615. https://doi.org/10.3390/cells10071615