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RESEARCH ARTICLE

In vivo Evaluation of *Ilex khasiana* for its Analgesic and Anti-inflammatory Activity on Swiss albino Mice Model

Charles Lalnunfela[1], H T Lalremsanga[1], K Lalchhandama[2], P B Lalthanpuii[2], C Lalmuanthanga[3], T C Lalhriatpuii[4]*

1 Department of Zoology, Mizoram University, Tanhril, 796004, Mizoram, India
2 Department of Life Sciences, Pachhunga University College, Aizawl, 796001, Mizoram, India
3 Department of Pharmacology & Toxicology, College of Veterinary Sciences & Animal Husbandry, Central Agriculture University, Aizawl, 796014, Mizoram, India
4 Department of Pharmacy, Regional Institute of Paramedical and Nursing Sciences, Zemabawk, 796017, Mizoram, India

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* **Corresponding author**.

*tclalhriatpuii@gmail.com

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Abstract

Objective: To evaluate the analgesic and anti-inflammatory properties of *I. khasiana* using selected parameters. **Methods:** Acetic acid induced writhing test, Tail emersion test and Hot plate test, Xylene induced ear edema, Formalin induced paw edema and Carrageenan induced paw edema were employed for the studies of anti-inflammatory activity of the plant on Swiss albino mice. **Findings:** In the acetic acid induced abdominal writhing test, the highest percent of inhibition 30.95% was found in 250 mg.kg b.w dose of IKM followed by 25.50% in IKC. In tail emersion test highest analgesic percentage was found at 90 minutes in IKC (250 mg.kg b.w) 68.96, but IKM (250 mg.kg b.w) exhibited the highest inhibition of 50% at 120 minutes. Surprisingly, IKC (500 mg.kg b.w) at 180 minutes gave the highest inhibition percentage of 76.19 but IKM (250 mg.kg b.w) at 180 minutes inhibited 79.36% of the pain which was slightly higher when compared to 60.60% of IKM (500 mg.kg b.w) at the same time point. In xylene induced ear edema test IKM gave 36.84% of inhibition and it was higher than IKC (500 mg/kg b.w) which was found to be 24.73. After 6 hours in formalin induced edema, IKC (250 mg/kg b.w) gave 49.69% of inhibition which was higher than 36.21% inhibition exerted by IKM. In both the paw edema test, at 2 hours after inducing inflammation, the peak was comparatively high and reduced gradually as the time increased. Carrageenan paw edema was measured for 6 hours at hourly intervals. Inhibition of edema increased with time, IKM (250 mg/kg b.w) has a percentage of 36.21% and IKC at the same dose has 28.16% of inhibition. **Novelty:** *Ilex khasiana*, the unexplored yet critically endangered species has a scientific support not only with regards to its protection but its enormous contribution in phytochemistry. **Conclusion**: IKC and IKM showed close efficacy regarding their analgesic and anti-inflammatory activity. Like the other *Ilex* species, *I. khasiana* has valuable medicinal property and is demanding special attention being a critically endangered species in terms of propagation

and protection.
Keywords: *Ilex Khasiana*; Analgesic; Antiinflamatory; IKM; IKC

1 Introduction

Nociception is an irksome sensation caused by physical factors such as injury, temperature, pressure or collisions or chemically induced by cytokines, neutrophins and chemokines. The inflammatory mediators may aid in locating the target source in treating nociception and inflammation. Many series of responses against this painful sensation is mediated by the nervous system [(1)]. Inflammation is a physiological response of a body to neutralize or counteract pathogens, invading organisms as well as a process of tissue repair. It is a complex process that involves numerous mediators and events like chemokines like interferon-α (IFN-α), IFN-γ, interleukin (IL)-1, IL-8, histamine, 5-hydroxytryptamine (5-HT), leukotrienes, prostacyclins, prostaglandins, lymphokines and tumor necrosis factor-α (TNF-α) [(2)]. Inflammation might initiate various diseases like asthma, rheumatoid arthritis, osteoporosis, obesity, cancer, cardiovascular disease and nervous system related diseases like Alzheimer's disease, depression and Parkinson's disease [(3)].

Natural products play a significant role in developing new drugs because of the large varieties of bioactive compounds. The presence of secondary metabolites like polyphenols, flavonoids, carotenoids, coumarins and terpenoids may be associated with the plant's medicinal values. With the advancement in research there are many notable adverse effects in utilizing synthetic drugs like aspirin and other steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) are used extensively for the treatment of inflammation as well as nociception. Agonizingly, these well-known drugs were known to have limitations in curing chronic inflammation and were suspected to induce blood clotting resulting in increased heart attack and stroke [(4)].

Ilex, the largest genus of the family Aquifoliaceae is dioecious, evergreen trees or sometimes shrubs without not forming a large homogeneous population. Many of the *Ilex* species are known for their contributions in anticancer, antinociceptive and anti-inflammatory effects. In *I. mamillata* and *I. pubescens* are known for their anti-inflammatory activity which is believed to be associated with saponins [(5)]. The anti-inflammatory mechanism might be associated with the inhibition of proinflammatory cytokines and cyclooxygenase (COX)-2 protein also by increasing IL-4 and IL-10 an anti-inflammatory cytokine. Quercetin and saponins in *I. paraguariensis* collaboratively impede iNOS and COX-2 through NFkB pathways [(6)]. Regular consumption of this yerba mate demonstrated deduction in both lipid peroxidation and free radicals [(7)]. *I. centrochinensis* in China is known to have anti-inflammatory activities. *I. khasianais* an evergreen tree with an average height of 15–20 m and forms the sub-canopy in the humid subtropical forests endemic to Khasi hills, Meghalaya. In this study, *I. khasiana* is scrutinized for its analgesic and anti-inflammatory properties.

2 Methodology

2.1 Chemicals and reagents

Carboxymethyl cellulose, Formalin, Xylene and Acetic Acid were purchased from HiMedia Laboratories Pvt. Ltd. Mumbai, India. Diclofenac sodium standard was procured from Sigma-Aldrich, USA and Morphine Sulphate Injection was obtained from Rusan Healthcare Pvt. Ltd. Mumbai, India .

2.2 Housing and Handling of the Animals

Swiss albino mice (20 — 25g) of both sexes were maintained at Institutional Animal House, RIPANS. The animals were kept in polyvinyl cages under a fully controlled environment of temperature (24-25 °C), 50% humidity and light and dark (12 h each) cycle. The animals were fed with standard food pellets and water *ad libitum* according to OECD guidelines. The Institutional Animal Ethics Committee of Regional Institute of Paramedical and Nursing Sciences approved the entire study vide letter no. **IAEC/RIPANS/25**, Aizawl, India.

2.3 Preparation of Ethanolic Extract

The two semi-solid extracts *I. khasiana* Chloroform Extract (IKC) and *I. khasiana* Methanol Extract (IKM) were dissolved in Carboxymethyl cellulose (CMC) and Normal saline (NS)respectively to obtain a stock concentration of 50mg/ml respectively. The animals were divided into 5 groups consisting of six mice in each group as follows:

1. Group I: Vehicle control
2. Group II: Standard drug
3. Group III: 100 mg/kg b.w of IKC/IKM
4. Group IV: 250 mg/kg b.w of IKC/IKM
5. Group V: 500 mg/kg b.w of IKC/IKM

2.4 Acute Toxicity

The acute toxicity study of different extracts of *Ilex khasiana* was performed according to Organization for Economic Co-operation and Development (OECD) guidelines. The animals of both sexes were used for the study (n=6). The animals were fasted 3-4 hours but water *ad libitum* before giving different extracts of *Ilex khasiana*. The animals were observed for 14 days for any behavioral or physiological changes. If mortality was observed on 2-3 animals, then the treatment was considered as toxic dose.

2.5 Analgesic activity of *Ilex khasiana*

2.5.1 ***Acetic acid writhing test***

Analgesic property of *Ilex khasiana* was assessed by Acetic acid-induced writhing test[8]. Briefly, animals were divided into 5 groups consisting of 6 mice in each group. The first group received morphine (10 mg/kg, i.p.), a standard analgesic drug and the second group was the vehicle control (Normal saline for IKM and CMC for IKC p.o.). The third, fourth and fifth groups received the extracts (IKM and IKC) 100, 250 and 500 mg/kg body weight p.o. respectively. Treatments were given 30 minutes prior to intraperitoneal injection of 0.6%, 10 ml/kg body weight acetic acid (i.e. 0.01 ml/g). Writhes or abdominal constrictions were counted from 5 minutes after the injection up to 20 minutes and the result was expressed as percent protection using the following formula:

Protection (%) = $(N_c - N_t / N_c)$ X 100

Where N_c is number of writhing in control, and N_t is the number of writhing in test animals.

2.5.2 ***Tail immersion test***

Evaluation of *Ilex khasiana* on its analgesic activity was performed as per standard protocols with slide modification[9]. Animals were divided into 5 groups with 6 animals in each group and treatment was given 30 minutes before the test as mentioned in the above experiment. The temperature of the water was maintained at 55 ± 0.5 °C into which 5 cm of the mouse tail was immersed. The reaction time was measured 30 minutes before and after the treatment, denoted by the time at which the mouse withdrew the tail from the water. The cut-off time was fixed at 10 seconds to avoid tissue damage. The test was continued every 30 minutes up to 120 minutes.

Percentage latency period was calculated as follows:

$= (T_n\text{-}T_0)/ (10\text{-}T_0)$ x 100

Where 10 is the cut-off time, T_0 is the latency time before treatment and T_n is latency after treatment.

2.5.3 ***Hot Plate Test***

The hot plate test was used to calculate analgesic activity using the method explained by Eddy and Leimbach[10] with minor modifications. In this test, mice were placed on a hot plate having a temperature of 55 ± 1 °C (UGO BASILE, 35100, Italy)

before the treatment and at 30, 60, 90, 120 and 180 minutes after the administration of the treatment. The time taken by the mouse for licking or jumping was recorded in order to analyze the analgesic effect of IKC and IKM compared to Morphine, the standard drug for electrical heat-induced pain.

The percentage protection against thermal pain stimulus was calculated according to the following formula:

Protection (%) against thermal stimulus

= {Test mean (T_a) − Control mean (T_b)}/Control mean (T_b) x 100

2.6 Anti-inflammatory activity of *Ilex khasiana*

2.6.1 *Xylene induced ear edema model*

The mice were divided into 5 groups (6 mice in each group) and xylene induced ear edema model was used to determine the anti-inflammatory activity of both IKC and IKM[11]. Each group received treatment saline (p.o.), the IKM or IKC (100, 250 and 500 mg/kg, p.o.), diclofenac sodium (20 mg/kg, i.p.) 30 minutes before topical administration of 30 μl of xylene in the posterior and anterior surfaces of the right ear. The left ear served as control. After 15 minutes, the animals were euthanized and circular sections (7 mm) were taken using a cork-borer and the weight for both sections. The weight difference was the edematous response. The percentage inhibition was calculated as

Inhibition (%) = [1−E_t / E_c] x100

where E_t and E_c are the average weight of the edemas in the sample-treated and control groups, respectively.

2.6.2 *Formalin-induced paw edema test*

The anti-inflammatory activity of *Ilex khasiana* extracts (IKC and IKM) was performed by using formalin–induced paw edema test following the standard protocol with minor modification[12]. Treatments with saline (p.o.), the IKM or IKC (100, 250 and 500 mg/kg, p.o.), diclofenac sodium (20 mg/kg, i.p.) were given 1 hour prior to formalin injection (n = 6 per group). The paw thickness was measured bilaterally before injecting formalin. Subcutaneously, the paw was injected with 20 μl/paw of formalin ((2.5 % in 0.9 % sterile saline). Paw thickness was re-measured at 1, 2, 3, 4, 5 and 6 hours after formalin injection to determine the edema using Plethysmometer (UGO BASILE, 7140, Italy).

The difference in paw diameters was taken as inflammatory response.

% Inhibition = $\{(V_t - V_o)_{control} - (V_t - V_o)_{treated} / (V_t - V_o)_{control}\} \times 100$

where $(V_t - V_o)_{control}$ is the difference in the volume displacement in Plethysmometer at different hours in a control group, $(V_t - V_o)_{treated}$ is the difference in the volume displacement in Plethysmometer at different hours in treated group.

2.6.3 *Carrageenan-induced paw edema test*

Carrageenan-induced paw edema in mice a well-known method for screening the anti-inflammatory potential of bioactive compounds. The anti-inflammatory activity of IKC and IKM was observed by following a standard protocol with slight modification[13]. The mice were pretreated 1 hour before administration of 50 μl of 1% λ carrageenan (in 0.9% saline) with diclofenac sodium (20 mg/kg), normal saline and 100, 250 and 500 mg/kg b.w of both IKC and IKM separately. The paw edema was measured at hourly intervals at 1, 2, 3, 4, 5, and 6 hours using vernier caliper. The paw size before the treatment served as the initial paw size/control. The inhibitory effect was determined by using the following formula:

% Inhibition = $\{(P_t - P_o)_{control} - (P_t - P_o)_{treated} / (P_t - P_o)_{control}\} \times 100$

where $(P_t - P_o)_{control}$ is the difference in the paw size at different hours in control group, $(P_t - P_o)_{treated}$ is the difference in the paw size at different hours in treated group.

2.7 Data Analysis

Data were analyzed using the statistical software GraphPad Prism version 8.0.2 One-way analysis of variance (ANOVA) test was used to ascertain the significance of variations followed by Tukey HSD post hoc test. Data are shown as mean ± S.E.M. All data were considered significant at $P < 0.05$.

3 Results and discussion

3.1 Acute Toxicity

At the dose of 2000 mg/kg b.w, the extract did not show any sign of lethality and the animal behavior did not display any sign of discomfort.

3.2 Acetic acid Writhing test

Acetic acid-induced abdominal constriction is a method that involves prostaglandin pathway-mediated local peritoneal cells to analyze peripherally acting analgesics. The analgesic activity that was found in both IKM and IKC extracts might be the result of prostaglandin pathways acting mechanism of the extracts. Significant inhibition of abdominal nociception was observed upon oral treatment of IKC and IKM (**Figure 1**). At 250 mg/kg b.w, IKC showed 25.50% inhibition and IKM showed maximum inhibition of 30.95%. Among the treatment groups in both the extracts which is comparatively lower than the inhibition induced by morphine (56.47%). The mean difference of each treated group in comparison with the standard drug showed that only IKM at 250 mg/kg b.w was found to have significant variation compared with the control while all the other treatment groups in IKC and IKM were statistically significant when compared with the standard drug, Morphine.

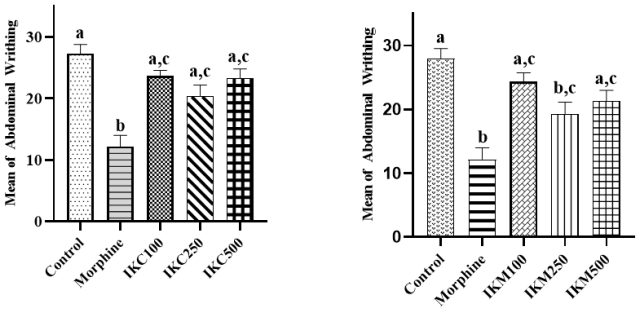

Fig 1. Analgesic effect of IKC and IKM on acetic acid induced writhing test. Values are expressed as Mean ± SEM. Different letters indicate significant variation at P<0.05 (ANOVA followed by Tukey multiple comparison test)

3.3 Tail immersion test

The tail emersion test was employed to determine the antinociceptive activity of *I. khasiana*. The reaction time was measured for all the untreated control groups, morphine and groups that received 100, 250 and 500 mg/kg b.w of IKC/IKM. Plots of changes in latency at different time intervals (30, 60, 90 and 120 minutes) for both the extract was given inFigure 2. The results showed dose-dependent inhibition**.** Among the two extracts, IKC (250 mg/kg b.w) showed the highest inhibition 68.96 at 90 minutes but IKM showed the highest inhibition 50% at 120 minutes after administration of the extracts.

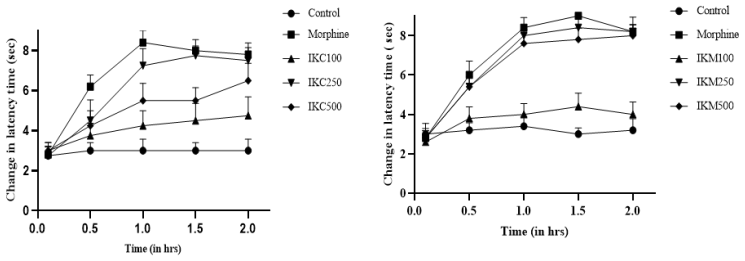

Fig 2. Analgesic effect of IKC and IKM on latency time in Tail emersion test. Values are expressed as Mean ± SEM. Different letters indicate significant variation at P<0.05 (ANOVA followed by Tukey multiple comparison test). (IKC= *Ilex khasiana* chloroform extract, IKM= *Ilex khasiana* methanol extract)

3.4 Hot Plate Test

The latency time in the Hot plate test showed dose-dependent increase throughout the test time points **(Figure 3)**. IKC and IKM were selected for the analgesic activity analysis of *I. khasiana* at three different doses (100, 250 and 500 mg/kg b.w). There were significant variations from the control group at 250 and 500 mg/kg b.w after 90 minutes of the treatment for both extracts. Maximum inhibition for IKC (76.19%) was given by 500 mg/kg b.w at 180 minutes after the oral administration, while 250

mg/kg b.w of IKM exhibited maximum inhibition of 79.36% at 180 minutes after the treatment. Comparatively, IKM showed better inhibitory effect than IKC in the hot plate test.

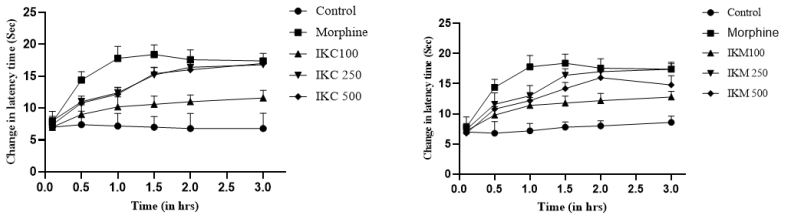

Fig 3. Analgesic effect of IKC and IKM on latency time in Hot plate test. Values are expressed as Mean ± SEM. Different letters indicate significant variation at P<0.05 (ANOVA followed by Tukey multiple comparison test). (IKC= *Ilex khasiana* chloroform extract, IKM= *Ilex khasiana* methanol extract)

3.5 Xylene induced Ear Edema

The anti-inflammatory activity of IKC and IKM were shown inFigure 4. The vehicle control group receiving CMC showed an increase in ear weight up to 7.75 while the lowest mean of weight was observed in 500 mg/kg. Diclofenac gave the highest inhibition of 50.97% and oral treatment of IKC showed 9.68, 19.35 and 24.75 % at 100, 250 and 500 mg/kg respectively. While groups that received IKM exhibited inhibition percentages of 18.42, 36.84 and 31.58 at 100, 250 and 500 mg/kg respectively. Notably, 250 mg/kg dose has a higher inhibitory effect than the higher dose 500 mg/kg b.w in IKM treated group. On both the extracts at different doses, only IKM 250 mg/kg showed significant variation from the groups that received vehicle control.

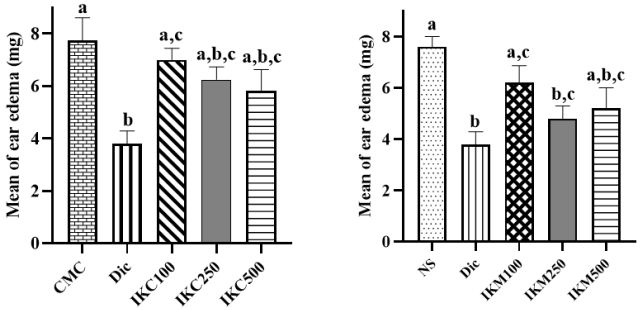

Fig 4. Anti-inflammatory effect of IKC and IKM in Xylene induced ear edema. Values are expressed as Mean ± SEM. Different letters indicate significant variation at P<0.05 (ANOVA followed by Tukey multiple comparison test). (IKC= *Ilex khasiana* chloroform extract, IKM= *Ilex khasiana* methanol extract)

3.6 Carrageenan induced paw edema

The inhibition potency of *I. khasiana* on carrageenan-induced paw edema at different time points were given in Figure 5 for IKC and IKM. At a dose of 250 mg/kg b.w, IKC and IKM showed significant variation at 5 hours after the treatment compared to the control group. IKC and IKM (250 mg/kg b.w) exhibited the highest inhibition percentage of 28.16% and 36.21 at 6 hours respectively which was slightly lower when compared to the reference drug diclofenac sodium 47.41%.

3.7 Formalin induced Mice Paw Edema

The anti-inflammatory activity of *I. khasiana* was determined using Formalin-induced paw edema. Different doses (100, 250 and 500mg/kg) of IKC and IKM were administered orally. The mean volume change in the paw edema was displayed in Figure 6 for both extracts. The highest peak was observed at 2 hours after formalin administration and declined gradually in time. Diclofenac sodium (20mg/kg b.w) gave the highest inhibitory effect compared to the groups that received both IKC and IKM. Animals that

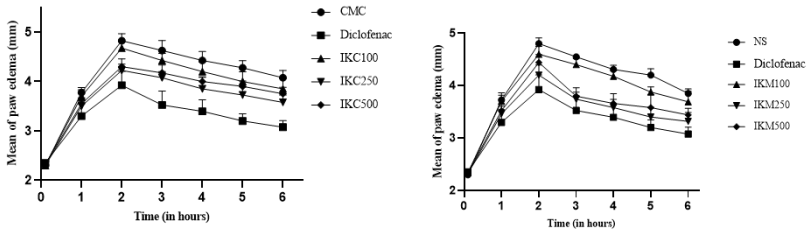

Fig 5. Anti-inflammatory effect of IKC and IKM in Carrageenan induced paw edema. Values are expressed as Mean ± SEM. Different letters indicate significant variation at P<0.05 (ANOVA followed by Tukey multiple comparison test). (IKC= *Ilex khasiana* chloroform extract, IKM= *Ilex khasiana* methanol extract)**.**

were treated with IKC revealed statistical differences at 250 and 500 mg/kg b.w at 4, 5 and 6 hours after sub-planter injection of formalin. In groups that received IKM, 250 mg/kg b.w showed statistical significance. The IKC extract (250 mg/kg b.w) revealed highest inhibitory percentage of 49.69 at 6 hours while 36.21% was the highest inhibition given by IKM (250 mg/kg b.w) at the same time point.

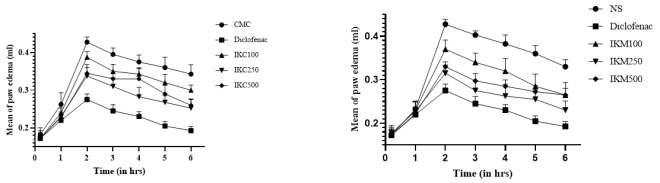

Fig 6. Anti-inflammatory effect of IKC and IKM in Formalin-induced paw edema. Values are expressed as Mean ± SEM. Different letters indicate significant variation at P<0.05 (ANOVA followed by Tukey multiple comparison test). (IKC= *Ilex khasiana* chloroform extract, IKM= *Ilex khasiana* methanol extract)

3.8 Discussions

Inflammation results when the body is invaded by harmful foreign bodies or suffered tissue injuries to protect, repair, prevent and heal the body from damages. It showed symptoms like swelling, redness and severe heat which give rise to an unpleasant sensation called pain[14]. Pro-inflammatory mediators like COX-2 and iNOS increase upon inflammation, which led to the rise in the level of cytokines such as TNF-α, IL1β, and IL-6 as well as PGE2 and NO too. This in turn played a crucial role in pathogenesis such as Parkinson's and Alzheimer's disease[15]. Thus, blocking the pro-inflammatory mediators using bioactive compounds is a long-time target, in which natural products like phenols are known to inhibit inflammation.

Inflammation is initiated with the upregulation of enzymes like iNOS and cyclooxygenase-2 (COX-2). Therefore, inhibition of COX pathway of arachidonic acid metabolism which produces prostaglandins is required for anti-inflammatory activity[16]. Besides its role in causing edema and erythema, prostaglandins are known to be hyperalgesic and have vasodilating activity. Thus, the anti-inflammatory drug must have analgesic activity as well. Many side effects are reported in using Nonsteroidal anti-inflammatory drugs (NSAIDs) like ulcers, and renal ailments due to their non-specific inhibition of COX-1 and COX-2 isoforms in spite of its celebrated anti-inflammatory and analgesic activity[17].

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most prominent clusters of drugs that are widely consumed because of their antipyretic, analgesic and anti-inflammatory properties. In 1982, Nobel Prize in Medicine was awarded to John R. Vane for describing the working mechanism of Acetylsalicylic acid (ASA) which was introduced under the brand name 'Aspirin'. The blooming of NSAID drugs continues to rise with the discovery of ibuprofen and indomethacin in 1969 and 1964. Globally, 2.5% of the total dollar spent on prescriptions was on NSAID drugs serving people having arthritis Reiter's syndrome, systemic lupus erythematosus**,** thrombosis, pericarditis, gout, gouty arthritis, ankylosing spondylitis, patent ductus arteriosus and Kawasaki disease[18].

In spite of their medical importance, NSAIDs are known to have renal, gastrointestinal and cardiovascular side effects. Short-term and reversible side effects of NSAIDs on GI was commonly reported which include dyspepsia, heartburn and nausea[19]. The side effects found in GI are usually in the upper GI and lower GI side effects are not thoroughly studied.

Among the commonly available polyphenols – quercetin, hesperidin and rutin are confirmed to have significant anti-inflammatory activity[20]. Thus, phenolic compounds not only exhibited antioxidant properties but also anti-inflammatory activity by modulating the signaling pathway of inflammation[21].

Many *Ilex* species are known to have analgesic and anti-inflammatory properties. *I. paraguariensis* is known as Yerba mate, a common beverage in Southern Larin America is known to have bioactive compounds like saponins, methylxanthines and phenolic compounds. These bioactive compounds contributed enormously to the plants' anti-inflammatory activity. *I. paraguariensis* reduced Adenosine deaminase (ADA) enzymes, an anti-inflammatory mediator besides down-regulating NF-κB activation and p65 phosphorylation[22].

I. cornuta leaves inhibited Nitric oxide synthase and cyclooxygenase-2 attenuating the production of NO and PGE2, inflammation mediators. Studies have shown that there are eight responsible compounds for its anti-inflammatory activity. Among them kaempferol and isoquercetin play a major role in suppressing IL-6, IL-1b, and PGE2 production synergistically, as there is no report on the anti-inflammatory effect of these individual compounds[23]. *I. vomitoria*, yaupon holly is known to contain quercetin and kaempferol 3-rutinosides besides other flavonols and caffeoylquinic acid derivatives. It exhibited anti-inflammatory activity by up-regulation of microRNA-146a (miR-146a) which down-regulates pro-inflammatory NF-κB activation.

The two extracts IKC and IKM exerted notable analgesic as well as an anti-inflammatory activity when compared with the reference drugs morphine and diclofenac respectively. Among the two, IKM showed slightly higher activity in both cases, which showed that methanol is a more suitable solvent for extracting the compounds that are responsible for its anti-inflammatory and antinociceptive activity. In many situations, in both IKC and IKM, the treatment dose of 250 mg/kg b.w is found to be the most suitable dose as the efficacy of the plant did not increase upon increasing concentration. So, *I. khasiana* is expected to be loaded with phytochemical compounds like phenols, saponin and phytosterols like those that are found in other *Ilex* species.

4 Conclusion

Therefore, the present studies deal with the analgesic and anti-inflammatory properties if *I. khasiana*. Dolefully, there is no record of any kind regarding the plant's medicinal values and phytochemical compositions. In this study, the two extracts IKC and IKM showed astonishing analgesic and anti-inflammatory properties. In the acetic acid induced abdominal writhing test, the highest percentage of inhibition 30.95% was found in 250 mg.kg b.w dose of IKM followed by 25.50% in IKC at the same dose among the extract treatment groups. In the tail emersion test highest analgesic percentage was found at 90 minutes in IKC (250 mg.kg b.w) 68.96, but IKM (250 mg.kg b.w) exhibited the highest inhibition of 50% at 120 minutes. Surprisingly, IKC (500 mg.kg b.w) at 180 minutes gave the highest inhibition percentage of 76.19 but IKM (250 mg.kg b.w) at 180 minutes inhibited 79.36% of the pain which was slightly higher when compared to 60.60% of IKM (500 mg.kg b.w) at the same time point. The anti-inflammatory properties of IKC and IKM were determined by Xylene induced ear edema, formalin induced paw edema and carrageenan induced paw edema. As usual, the treatment dose of 250 mg/kg b.w seemed to be the best dose in both extracts in all the three parameters. In xylene induced ear edema test IKM gave 36.84% of inhibition and it was higher than IKC (500 mg/kg b.w) which was found to be 24.73. After 6 hours in formalin induced edema, IKC (250 mg/kg b.w) gave 49.69% of inhibition which was higher than 36.21% inhibition exerted by IKM. In both the paw edema test, at 2 hours after inducing inflammation, the peak was comparatively high and reduced gradually as the time increased. Carrageenan paw edema was measured for 6 hours at hourly interval. Inhibition of edema increased with time, IKM (250 mg/kg b.w) has a percentage of 36.21% and IKC at the same dose has 28.16% of inhibition. From the results stated above, *I. khasiana* being a critically endangered species must get special attention and exploration of its medicinal values must be done extensively. The study of the plant extracts must lean towards characterization and chromatographic study of the bioactive compounds using LC-MS. The efficacy and nature of the responsible compounds must be analyze using molecular simulation using appropriate docking software.

5 Declaration

Presented in 4th Mizoram Science Congress (MSC 2022) during 20th & 21st October 2022, organized by Mizoram Science, Technology and Innovation Council (MISTIC), Directorate of Science and Technology (DST) Mizoram, Govt. of Mizoram in collaboration with science NGOs in Mizoram such as Mizo Academy of Sciences (MAS), Mizoram Science Society (MSS), Science Teachers' Association, Mizoram (STAM), Geological Society of Mizoram (GSM), Mizoram Mathematics Society (MMS), Biodiversity and Nature Conservation Network (BIOCONE) and Mizoram Information & Technology Society (MITS). The Organizers claim the peer review responsibility

References

1) Yao M, Xie J, Du H, Mcclements DJ, Xiao H, Li L. Progress in microencapsulation of probiotics: A review. *Comprehensive Reviews in Food Science and Food Safety*. 2020;19(2):857–874. Available from: https://doi.org/10.1111/1541-4337.12532.
2) Chazaud B. Inflammation and Skeletal Muscle Regeneration: Leave It to the Macrophages! *Trends in Immunology*. 2020;41(6):481–492. Available from: https://doi.org/10.1016/j.it.2020.04.006.
3) Mahnashi MH, Alyami BA, Alqahtani YS, Jan MS, Rashid U, Sadiq A, et al. Phytochemical profiling of bioactive compounds, anti-inflammatory and analgesic potentials of Habenaria digitata Lindl.: Molecular docking based synergistic effect of the identified compounds. *Journal of Ethnopharmacology*. 2021;273:113976–113976. Available from: https://doi.org/10.1016/j.jep.2021.113976.
4) Cordaro M, Siracusa R, Fusco R, D'amico R, Peritore AF, Gugliandolo E, et al. Cashew (Anacardium occidentale L.) Nuts Counteract Oxidative Stress and Inflammation in an Acute Experimental Model of Carrageenan-Induced Paw Edema. *Antioxidants*. 2020;9(8):1–19. Available from: https://doi.org/10.3390/antiox9080660.
5) Pachura N, Kupczyński R, Sycz J, Kuklińska A, Zwyrzykowska-Wodzińska A, Wińska K, et al. Biological Potential and Chemical Profile of European Varieties of Ilex. *Foods*. 2022;11(1):1–28. Available from: https://doi.org/10.3390/foods11010047.
6) Alizadeh SR, Ebrahimzadeh MA. Quercetin derivatives: Drug design, development, and biological activities, a review. *European Journal of Medicinal Chemistry*. 2022;229:114068–114068. Available from: https://doi.org/10.1016/j.ejmech.2021.114068.
7) Zapata FJ, Rebollo-Hernanz M, Novakofski JE, Nakamura MT, De Mejia EG. Caffeine, but not other phytochemicals, in mate tea (Ilex paraguariensis St. Hilaire) attenuates high-fat-high-sucrose-diet-driven lipogenesis and body fat accumulation. *Journal of Functional Foods*. 2020;64:1–13. Available from: https://doi.org/10.1016/j.jff.2019.103646.
8) Petrou A, Fesatidou M, Geronikaki A. Thiazole Ring - A Biologically Active Scaffold. *Molecules*. 2021;26(11):1–75. Available from: https://doi.org/10.3390/molecules26113166.
9) Sharma A, Cooper R, Bhardwaj G, Cannoo DS. The genus Nepeta: Traditional uses, phytochemicals and pharmacological properties. *Journal of Ethnopharmacology*. 2021;268:113679–113679. Available from: https://doi.org/10.1016/j.jep.2020.113679.
10) Verbitsky A, Dopfel D, Zhang N. Rodent models of post-traumatic stress disorder: behavioral assessment. *Translational Psychiatry*. 2020;10(132):1–28. Available from: https://www.nature.com/articles/s41398-020-0806-x.
11) Lemoui R, Cheriet T, Kahlouche F, Noman L, Seghiri R, Abudunia A. LC-MS profile, in vitro acetylcholinesterase inhibitory, antibacterial and hemostatic properties of Ranunculus bullatus extract. *Natural Product Research*. 2022;p. 1–5. Available from: https://doi.org/10.1080/14786419.2022.2136661.
12) Tjølsen A, Berge OGG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. *Pain*. 1992;51(1):5–17. Available from: https://doi.org/10.1016/0304-3959(92)90003-T.
13) Winter CA, Risley EA, Nuss GW. Carrageenin-Induced Edema in Hind Paw of the Rat as an Assay for Antiinflammatory Drugs. *Experimental Biology and Medicine*. 1962;111(3):544–547. Available from: https://doi.org/10.3181/00379727-111-27849.
14) Stewart AG, Beart PM. Inflammation: maladies, models, mechanisms and molecules. *British Journal of Pharmacology*. 2016;173(4):631–634. Available from: https://doi.org/10.1111/bph.13389.
15) Shao J, Li Y, Wang Z, Xiao M, Yin P, Lu Y, et al. 7b, a novel naphthalimide derivative, exhibited anti-inflammatory effects via targeted-inhibiting TAK1 following down-regulation of ERK1/2- and p38 MAPK-mediated activation of NF-κB in LPS-stimulated RAW264.7 macrophages. *International Immunopharmacology*. 2013;17(2):216–228. Available from: https://doi.org/10.1016/j.intimp.2013.06.008.
16) Oyedapo OA, Adewunmi CO, Iwalewa EO, Makanju VO. Analgesic, antioxidant and anti-inflammatory related activities of 21-hydroxy-2, 41-dimethoxychalcone and 4-hydroxychalcone in mice. *Journal of Biological Sciences*. 2008;8(1):131–136. Available from: https://doi.org/10.3923/jbs.2008.131.136.
17) Dziedziński M, Kobus-Cisowska J, Stachowiak B. Pinus Species as Prospective Reserves of Bioactive Compounds with Potential Use in Functional Food—Current State of Knowledge. *Plants*. 2021;10(7):1–28. Available from: https://doi.org/10.3390/plants10071306.
18) Rao S, Li Y, Ramakrishnan R, Hassaine A, Canoy D, Cleland J, et al. An Explainable Transformer-Based Deep Learning Model for the Prediction of Incident Heart Failure. *IEEE Journal of Biomedical and Health Informatics*. 2022;26(7):3362–3372. Available from: https://doi.org/10.1109/JBHI.2022.3148820.
19) Wong RSY. Role of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) in Cancer Prevention and Cancer Promotion. *Advances in Pharmacological Sciences*. 2019;2019:1–11. Available from: https://doi.org/10.1155/2019/3418975.
20) Nunes CDR, Arantes MB, Pereira SMDF, Cruz LLD, Passos MDS, De Moraes LP, et al. Plants as Sources of Anti-Inflammatory Agents. *Molecules*. 2020;25(16):1–22. Available from: https://doi.org/10.3390/molecules25163726.
21) Peng Y, Yan Y, Wan P, Dong W, Huang K, Ran L, et al. Effects of long-term intake of anthocyanins from Lycium ruthenicum Murray on the organism health and gut microbiota in vivo. *Food Research International*. 2020;130:108952–108952. Available from: https://doi.org/10.1016/j.foodres.2019.108952.
22) Bandeira M, Possan AL, Pavin SS, Raota CS, Vebber MC, Giovanela M, et al. Mechanism of formation, characterization and cytotoxicity of green synthesized zinc oxide nanoparticles obtained from Ilex paraguariensis leaves extract. *Nano-Structures & Nano-Objects*. 2020;24:100532–100532. Available from: https://doi.org/10.1016/j.nanoso.2020.100532.
23) Oh KKK, Adnan MD, Cho DHH. Network Pharmacology Study to Interpret Signaling Pathways of Ilex cornuta Leaves against Obesity. *Processes*. 2021;9(7):1–24. Available from: https://doi.org/10.3390/pr9071106.