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Edible vegetable Roktoshirinchi (Achyranthes ferruginea Roxb.) ameliorates pain and inflammation in experimental animal model

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Abstract

Achyranthes ferruginea, a plant commonly used in Bangladeshi traditional medicine, was investigated in this study to uncover new therapeutic applications. The research focused on assessing it's *in vitro* anti-inflammatory properties and *in vivo* analgesic effects using a methanol extract. *In vitro,* the methanol extract of *Achyranthes ferruginea* was examined for its anti-inflammatory effects through membrane stabilization and protein denaturation tests using Human Red Blood Cells (HRBCs). The results indicated a moderate dose-dependent anti-inflammatory effect, with maximum inhibitions of 34.5% (protein denaturation) and 53.68% (membrane stability) at a concentration of 1000 µg/ml For *in vivo* assessments, Swiss albino mice were utilized, with Diclofenac Sodium as a positive control. The extract demonstrated significant dose-dependent analgesic effects in both chemically induced paw licking and writhing tests. Furthermore, molecular docking studies revealed a higher binding affinity of N-trans-feruloyl-4'-O-methyldopamine with COX-1 and COX-2 enzymes. Overall, *Achyranthes ferruginea* methanol extract exhibited moderate anti-inflammatory activity and robust analgesic properties, highlighting its potential therapeutic applications.

Keywords *Achyranthes ferruginea,* Protein denaturation, Membrane stability, *in vivo,* Analgesic

Paper type Research paper

1. Background

Inflammation is a reaction caused by injury to living tissues. In living organisms, inflammatory response acts as a defensive mechanism against infection and herm. The objectives of the inflammatory response are to remove noxious substances that are responsible for tissue damage and initiates healing. During inflammation, the different sequential events occurred such as altered blood flow, permeability of arteries, and movement of body fluid and cellular components including WBC. There are two main types of inflammation; one is acute, which refers to a short-duration of response, whereas chronic inflammation refers to a longer-term response. Drugs

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obtained from natural sources were used for management of 87 percent of all listed human maladies, such as infectious diseases, inflammatory diseases, and immunity problems (Newman & Cragg, 2007). In developing nations, around 80% of the people depends on alternative nature-based ailments (Devine & Furlong, 2007). In Bangladesh, abundant medicinal sources are likely to exist, with roughly 250 of them being used to make alternative therapeutics. However, medicinal plants have yet to be studied chemically, pharmacologically, or toxicologically to determine their bioactive components (Chowdhury, Bhuiyan, & Yusuf, 2008). Bangladeshi plants appear to be an attractive resource for probable lead structures in drug creation, based on historical records and biological variety. Phytochemicals are natural chemical substances found in plants. Some are responsible for the color and others for organoleptic features of foods, such as the deep purple of garlic and its odor. In South Africa, around 115 medicinal plants from 60 different families have been utilized as analgesics in people and animals. Secondary plant metabolites including curcumins, flavonoids, and tannins, saponins, terpenoids, and alkaloids have been isolated from medicinal plants possessing a wide variety of biological activities (Iwalewa, McGaw, Naidoo, & Eloff, 2007). Flavonoids are a class of polyphenols that are considered to decrease the biosynthesis of inflammatory mediators, which are end products of the cyclooxygenase and lipoxygenase pathways (Nijveldt, Van Nood, Van Hoorn, Boelens, Van Norren, & Van Leeuwen, 2001).

To demonstrate the scientific evidence of natural sources for analgesics and anti-inflammatory activities, we have selected *Achyranthes ferruginea (A. ferruginea)*, commonly known as Roktoshirinchi (Reza et al., 2021). It has been used by local folk practitioners for the management of constipation, dropsy, piles, boils, and skin erosion (Garnis, Buys, & Lam, 2004). In addition, the leaves of *A. ferruginea are* used to treat emesis, snake bites, asthma, and hydrophobia (Watt, 1893). Earlier, researchers explored that the *A. ferruginea* has antimicrobial, cytotoxic (Rahman et al., 2007), antidiarrheal (Alam, Rahman, Baki, Rashid, Bhuyan, & Sadik, 2002), sub-acute toxicity (Hasan & Rashid, 2003) and anti-cancer activities (Reza et al., 2021). However, the plant has different *in vitro* cell-free biological activities and is used in traditional medicine for several illnesses. Furthermore, N-trans-feruloyl-4-methyldopamine was previously isolated from the chloroform extract of *A. ferruginea* and demonstrated potential antimicrobial effects (Hasan & Rashid, 2003). Thus, we planned the current study to examine the *in vivo* analgesic and *in vitro* anti-inflammatory activities.

2. Extract preparation

In April 2018, *A. ferruginea* whole plant was collected from Rajshahi University Campus, Bangladesh. The plant was identified by a taxonomist from the University of Rajshahi's Department of Botany, and a voucher specimen from this collection is housed at the Bangladesh National Herbarium (BNH) under the accession number DACB-29533 (Reza et al., 2021). In an amber-colored extraction bottle, 450 g grinded plants were macerated in 1.5 L 80% methanol. The enclosed containers were kept for 7 days, shaking and stirring occasionally. The extraction was then followed by cotton filtration and filtration with Whatman No. 1 filter paper. The extract was then concentrated using a rotary evaporator (Bibby Sterilin Ltd., Staffordshire, UK) at 50 °C under lower pressure to obtain 30 g crude extracts (CME).

3. *In vitro* anti-inflammatory activity of CME by protein denaturation method

3.1. The anti-inflammatory effect of CME extract was investigated by the methods described by Ansari (Ansari, et al, 2017)

3.2. Membrane stabilization activity of CME

The membrane stabilization activity of CME was performed by methods described by Shinde and Ansari (Ansari, et al., 2017; Shinde, Phadke, Nair, Mungantiwar, Dikshit, & Saraf, 1999).

4. *In vivo* analgesic activity of CME

A variety of tests are used to assess analgesic potential. Methods for evaluating *in vivo* analgesic activity of test materials may be classified into two groups based on the mechanism (Uddin et al., 2018).

4.1. Acetic acid induced writhing test

The acetic acid induced pain test was performed by the method described by Koster (Koster, Anderson, & De Beer, 1959).

4.2. Formalin induced paw licking test

Formalin induced biphasic method was applied in an animal model to evaluate the analgesic activity of CME and the method is described previously by Burgos (Burgos, Pascual, Martín, & Goicoechea, 2010).

4.3. *In silico* Molecular Docking

The 3D crystal structure of Cyclooxygenase-1 (COX 1, PDB id: 2OYE), cyclooxygenase-2 (COX 2, PDB id: 6COX) were downloaded in PDB format from the protein data bank (Berman et al., 2002). Target compounds N-trans-feruloyl-4'-O-methyldopamine were retrieved from Pubchem databases. The preparation of protein, ligand preparation, receptor grid

generation and glide standard precision (SP) ligand docking were performed (Uddin et al., 2018).

5. Statistical analysis

All data are presented as mean ± standard deviation (SD). The data for significant differences between the test and control groups was illustrated using a one-way analysis of variance (ANOVA), followed by Dunnett's test.

6. Results and discussion

6.1. Determination of in vitro protein denaturation activity

Phytochemical investigation of the CME indicates the presence of secondary metabolites including total phenols, flavonoids and tanins (Reza et al., 2021). The CME inhibited protein denaturation at 62.5 µg/ml by 22.19% compared to 31.02 % of diclofenac sodium (DS) at the same concentration. Diclofenac Sodium inhibited 82.56 % at 1000 µg/ml compared to the maximum % of inhibition of crude extract 34.5 % at 1000µg/ml as shown in (Figure 1). The damaged protein articulate antigens associated to Type III hyper-sensitive reaction which are correlated to diseases such as serum sickness, glomerulonephritis etc (Hossain et al., 2020). Thus protein denaturation method is a convenient method to prove the anti-inflammatory activity. The current results of CME revealed that the plant has anti-inflammatory potential compared with reference drugs.

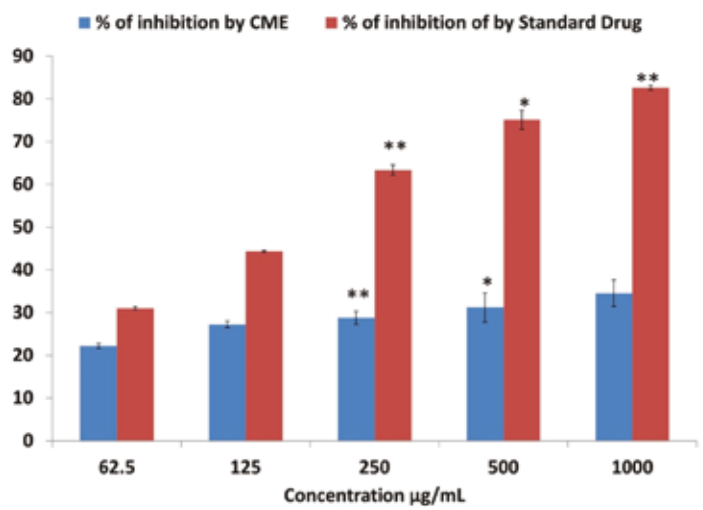

Figure 1

*Determination of in vitro anti-inflammatory activity of CME using protein denaturation method. Values are mean ± S.E.M. *p<0.05 and **p<0.01, significantly different from control; ANOVA followed Dunnett's test. CME: crude methanol extract of A. ferruginea.*

6.2. Determination of in vitro membrane stabilization activity

The CME demonstrated moderate anti-inflammatory activity in membrane stabilization methods when compared to the reference drug (Figure 2). The results showed that the highest % inhibition of CME membrane stabilization was 53.68 % at 1000 µg/ml when compared to the standard Diclofenac Sodium which was found to be 92.31% at a dose of 1000 µg/ml. The lysosomal enzymes are produced when inflammation occurs and responsible for tissue damage and associated with diseases like pain and inflammation (Ackerman & Beebe, 1974). The NSAIDs act against lysosomal components and protect against cell damage (Hossain et al., 2020). The inhibition of cell damage and or lysis initiated by hypotonicity is considered as a mechanism of anti-inflammatory activity (Mounnissamy, Kavimani, Balu, & Quine, 2007). The CME showed effective red blood cell stabilization potentials with good protection against hypotonic solution.

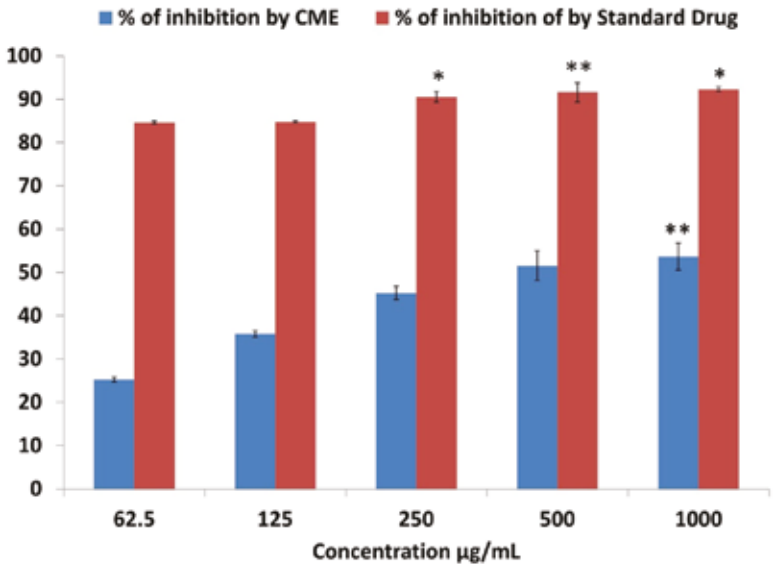

Figure 2

*Determination of in vitro anti-inflammatory activity of CME using membrane stabilization method. Values are mean ± S.E.M. *p<0.05 and **p<0.01, significantly different from control; ANOVA followed Dunnett's test. CME: crude methanol extract of A. ferruginea.*

6.3. Determination of acetic acid induced writhing (AAIW) in mice

The CME considerably and dose dependently decreased the occurrence of acetic acid produced writhing in mice. The CME displayed 30.41 % writhing inhibition at 200 mg/kg b.w and 59.82 % writhing inhibition at 400 mg/kg

b.w, as indicated in (Figure 3). At a dosage of 10 mg/kg b.w, the reference drug Diclofenac sodium reduced 63.74 % writhing. In AAIW test, firstly anti-nociceptive potentials of CME are characterized by abdominal tightening, activities of the whole body and contraction and or relaxation of dorsal-abdominal muscles. This non-specific pain evaluating method is suitable for evaluation of any substances having weak analgesic activity. In this model, pain is produced by bradykinin and serotonin, responsible for stimulating neurons associated with pain (Sakiyama, Sujaku, & Furuta, 2008). The pain sensation in AAIW response is demonstrated by the release of free arachidonic acid from tissue phospholipids via COX-1 and COX-2 and PGE2 and PGF2 biosynthesis (Adzu, Amos, Kapu, & Gamaniel, 2003). Our findings substantiate strong peripheral analgesic activity of CME, which is mediated by inhibition of COX-1 and COX-2 activity or PGE2 and PGF2 synthesis.

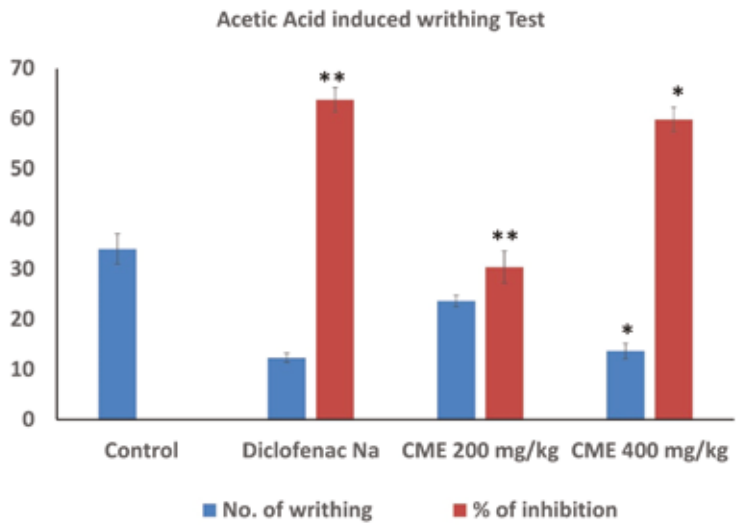

Figure 3

*Effect of methanol crude extract of the A. ferruginea, diclofenac sodium (10 mg/kg) on acetic acid induced writhing test. Values are mean ± S.E.M. *p<0.05 and **p<0.01, significantly different from control; ANOVA followed Dunnett's test (n = 6, per group). CME: crude methanol extract of A. ferruginea.*

6.4. Determination of formalin induced paw licking in (FIPL) mice

The CME dose dependently inhibited the frequency of FIPL in mice. At 200 mg/kg b.w., the CME inhibited 35.53 % in the early stage and 36.09 % in the late phase. At 400 mg/kg b.w., the CME inhibited licking time by 59.045 % in the early phase and 63.16% in the late phase (Figure 4A and 4B). At a

dosage of 10 mg/kg b.w., the reference drug Diclofenac Na suppressed 68.68 % and 63.16 % early and late phase licking time, respectively. The FIPL test is the common method for evaluating pain mechanism (Khan, Saeed, Khan, Dar, & Khan, 2010). Using these pain models researchers can differentiate between central and peripheral analgesics. The biphasic model is characterized by two phases one is neurogenic (1-5 min) and another is inflammatory pain (15-30 min) (Dallel, Raboisson, Clavelou, Saade, & Woda, 1995). The neurogenic pain, expresses an acute response initiated just after the administration of a stimulant. Whereas inflammatory pain is characterized by the release of inflammatory markers such as histamine, serotonin, prostaglandins and bradykinin, and commencement of the neurons in the dorsal horns of the spinal cord (Clavelou, Dallel, Orliaguet, Woda, & Raboisson, 1995). The increased percentages of liking time in late phase indicate that CME strongly inhibited the peripheral pain.

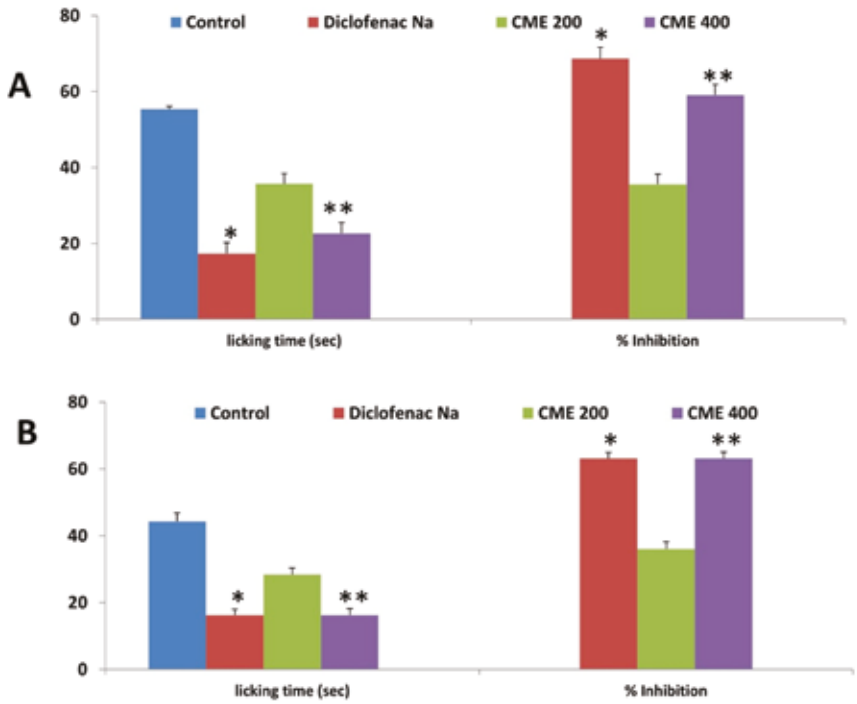

Figure 4

*Effect of methanol crude extract of the A. ferruginea, diclofenac sodium (10 mg/ kg) on formalin test (A. first phase and B. second phase). Values are mean ± S.E.M. *p<0.05 and **p<0.01, significantly different from control; ANOVA followed Dunnett's test (n = 6, per group). CME: crude methanol extract of A. ferruginea*

6.5. In silico Molecular Docking

To evaluate the potential analgesic molecule, we have undertaken the docking analysis of the isolated active compounds of *A. ferruginea* to the active site cyclooxygenase enzymes viz. COX-1 and COX-2. For studying the interaction of the compounds N-trans-feruloyl-4'-O-methyldopamine with 2OYE and 6COX. The glide docking analysis was performed by Schrodinger suite v10.1, where the compound N-trans-feruloyl-4'-O-methyldopamine shows highest docking score shown in Table 1 and Figure 5. The low and negative free energy values for binding indicate a strong favorable bond between 2OYE and 6COX (Uddin et al., 2018). The N-trans-feruloyl-4'-O-methyldopamine shows highest negative and low score (-7.948) while interacting with COX-1, whereas interacting with COX-2 the compounds displayed score (-7.191). Docking score suggests N-trans-feruloyl-4'-O-methyldopamine might be the responsible compound for potential analgesic activity.

Table 1

Molecular docking study of N-trans-feruloyl-4'-O-methyldopamine and standard drug diclofenac sodium for analgesic activity

Compound	Docking score (kcal/mol)	
	COX-1 (PDB:2OYE)	COX-2 (PDB: 6COX)
N-trans-feruloyl-4'-O-methyldopamine	-7.948	-7.191
Diclofenac sodium	-7.42	-7.567

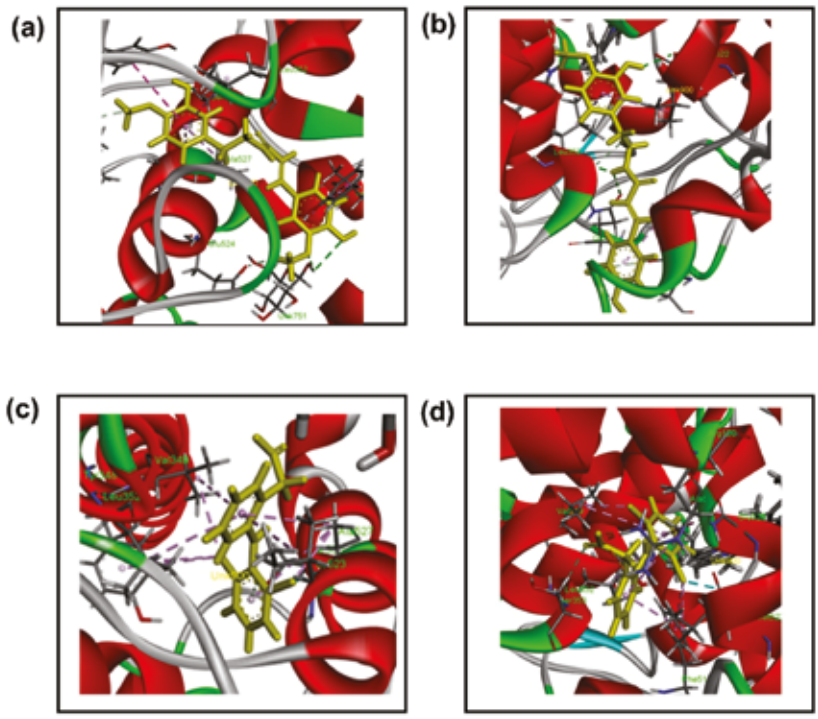

Figure 5
Docking result of: (a) N-trans-feruloyl-4'-O-methyldopamine with COX-1; (b) N-trans-feruloyl-4'-O-methyldopamine with COX-2; (c) Diclofenac sodium with COX-1; (d) Diclofenac sodium with COX-2

7. Conclusions

In this study, we found that the methanolic extract of this plant showed anti-inflammatory and significant dose-dependent antinociceptive activities. The secondary metabolites present in the CME might be responsible for anti-inflammatory and analgesic activities. Mechanistic study is required to establish A. ferruginea as alternative therapeutics for the management of pain and inflammation. Moreover, the molecular docking study demonstrated higher binding affinity of N-trans-feruloyl-4'-O-methyldopamine with COX-1, COX-2.

Disclosure statement

The author declares that he has no conflict of interests. The author read and approved to submit of the manuscript for this journal.

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