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Original Research Article

STUDY OF ANTI-INFLAMMATORY, ANTIPYRETIC AND ANALGESIC EFFECTS OF *AZADIRACHTA INDICA* LEAVES EXTRACT IN ALBINO RATS

ALYAS S*[1], ZAHRA N[1], KHADAM A[1], SULTANA R[3], ZAHID B[2], TAQI R[1], AKHTAR R[4], RAFIQUE HM[1], AHMAD S[5]

[1]*Institute of Molecular Biology and Biotechnology (IMBB), University of Lahore, 54000, Punjab, Pakistan.*
[2]*Department of Pathobiology, KBCMA, University of Veterinary and Animal Sciences, Sub campus Narowal, Pakistan.*
[3]*Aditional Director Livestock Office, 16 Cooper Road, Lahore, Pakistan*
[4]*Department of Pathology, University of Veterinary and Animal Sciences, Lahore, Pakistan*
[5]*Department of Poultry Production, Faculty of Animal Production & Technology, University of Veterinary & Animal Sciences, Lahore, Pakistan*
**Correspondence author email address: sobia.alyas@imbb.uol.edu.pk*

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Abstract: *An evergreen tropical tree known as neem (Azadirachta indica) has long been used in traditional medicine to various conditions, including fever, pain and inflammation. The prospective research aims to examine the analgesic, antipyretic and anti-inflammatory properties of the chloroform extract of A. indica. For this investigation, 108 albino rats weighing 160 to 200g each were employed. Carrageenan was used to induce paw edema to test the anti-inflammatory activity. Yeast was used to induce pyrexia to test the anti-pyretic activity. Finally, acetic acid induced abdominal writhing in rats to test the analgesic action. The chloroform extract was injected into the abdominal muscle and intraperitoneal tissue. The largest percentage of anti-inflammatory action that chloroform leaf extract could suppress was 53%. The maximal percentage inhibition of leaf extract against fever in terms of antipyretic efficacy was 90%. The highest percentage of inhibition against pain during analgesic activity was 56%. When compared to the common medications diclofenac and paracetamol substantial outcomes were seen in the anti-inflammatory, antipyretic and analgesic actions at 400 mg/kg, respectively. Fever, pain and inflammation all decreased significantly (p≤ 0.05). As a result, chloroform leaf extract from A. indica can be utilized as a potent medication to treat fever, pain (discomfort) and inflammation. proteins.*

Keywords: *Azadirachta indica*, Anti-inflammatory, antipyretic, analgesic, chloroform, leaf, paracetamol, diclofenac, carrageenan

Introduction

Locally, *Azadirachta indica* is referred to as neem. It belongs to the Meliaceae subfamily of mahogany trees. The genus Azadirachta, it has one or two species. It is indigenous to Pakistan, Bangladesh, Nepal, Thailand and India. It is thriving in tropical and subtropical areas (Hossain *et al*., 2013). It is a tree of fast-growing that rarely attains the height of 35 to 40 meters. Despite being evergreen, it may lose most or almost all of its leaves after a severe drought. The Indian tradition has known for thousands of years that neem has medicinal benefits (Owoyale *et al*., 2019). The adaptable medicinal plant neem is the source of several substances with various chemical structures and biological effects. Neem has several of active phytoconstituents, including steroids, glycosides, alkaloids and tannins. Neem leaves efficiently treat eczema, ringworm and acne have hypoglycemic and anti-inflammatory qualities (Ogidi *et al*., 2021).

Since dawn, man has been looking for treatments for the most prevalent human sufferings, including pain, fever and inflammation. Chronic inflammation concerns in the pathogenesis of different diseases. A clear association has been established for neurodegenerative diseases, obesity, cardiovascular disease, T2DM, metabolic syndrome and cancer (Alyas *et al*., 2020). The most common treatments include analgesics, anti-inflammatory, and antipyretic medications. Various inflammatory illnesses are treated using traditional plant medicines all around the world. One such plant is neem (Indian lilac, *Azadirachta indica*), an inherent of the Indian sub-continent and a highly revered tree for the people in the area. Neem is thought to have antiseptic, anti-

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helminthic, insecticidal, antiseptic, anti-diabetic and anti-hypertensive characteristics. Various inflammatory illnesses are treated using traditional plant medicines worldwide (Kumar *et al*., 2015).

Material and Methods

Sample collection and chloroform extract of *A. indica* leaves

The fully matured leaves of *A. indica* were collected from different botanical areas of Lahore. The staff members of The University of Punjab, Lahore recognized them. Leaves were cleaned with cotton and then dried at room temperature. The cold maceration method was used for leaf extraction (Dhakal *et al*., 2016).

Experimental rats

Albino rats of either sex female or male (160- 200g) were used for anti-inflammatory antpyretic and analgesic activity. Rats were kept in the animal house of The University of Lahore in polyprophylene cages. Before experimental work, rats were kept in a fasting condition. After that, they were given distilled water and balanced feed.

Drugs used

Normal saline 10ml/kg, Acetic acid induce pain 400mg/kg, Paracetamol 100mg/kg, Yeast 400mg/kg, Diclofenac 100mg/kg, Carrageenan 100mg/kg, Leaves extract (50,100,200,400mg/kg).

Anti-inflammatory activity model

For this activity model, 36 rats were alienated into 3 groups.

Carrageenan induce oedema

Initially, all groups of rats were treated with carrageenan in which 0.1 ml of 1%carrageenan was induced in the left hind paw. The volume of the paws was measured with a Vernier calliper before and after the injection. Anti-inflammatory activity was calculated by given formula:

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

The rats in the control group were given an injection of normal saline10ml/kg in the sub-planter region of the hind paw. The rats in the standard group were given a dose of diclofenac 100mg/kg in the sub-planter region of the hind paw. After carrageenan induction, the paw size was increased in the experimental group. After six hours, the chloroform extract of *A. indica* was injected into group III at doses of 50 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg correspondingly.

Yeast induce fever

All the groups were injected with yeast below the nape of the neck to induce fever. After injecting yeast, the fever developed after 21 hours and the highest temperature was 101.54 Fahrenheit. In group I, rats were injected with normal saline (10ml/kg) below the nape of the neck. In group II, the rats were treated with a dose of paracetamol 10ml/kg. While in group III the rats were treated with different concentrations of chloroform extract of *A.indica* at dose of 50, 100, 200 and 400mg/kg. After 1, 2, 3 and 4 hours the body temperature (rectum) of rats was measured with a digital thermometer.

For calculating the anti-pyretic activity, the formula is given below:

$$\text{Inhibition} = \frac{B-CB}{B-A} \times 100$$

B = temperature after fever induction

A = original body temperatureCB = temperature after 1, 2 and 3 and 4 hours

Writhing induced by Acetic acid

With the injection of acetic-acid, the writhing process began in rats. It was injected to determine the potential of chloroform extract of *A. indica*in pain process. But before 1 hour of the experiment, rats were injected with normal saline (10ml/kg) intraperitoneally in group I. In group II, the rats were treated with a dose of diclofenac10ml/kg. While in group III rats were treated with different concentrations of chloroform extract of *A. indica*at doses of 50, 100, 200 and 400mg/kg. For counting the writhes, the stop watch was used. The rats were placed into different cages during the activity.

Analgesic activity was calculated by the given formula:

$$\text{Analgesic activity} = \frac{Nc-Nt}{Nc} \times 100$$

Where,

Nt = treated group writhe

Nc = control group writhe

Statistical analysis

Collected data were analyzed through on-way ANOVA technique followed by Duncan's multiple range test using SAS software (version 9.1), the significance level was considered as $p \leq 0.05$.

Results

Edema induced by carrageenan

In this activity, group I remained untreated after carrageenan injection, hence, there was 0%inhibition activity. While, group III at different doses showed 40%, 47%, 49% and 53%inhibition of edema accordingly. Group II and Group III of *A.indica* showed significant ($p<0.05$) % inhibition in carrageenan-induced paw edema of rats after 1-5hours as compared to group I (Table 4.1).

Pyrexia test

In this activity, group III of *A. indica* leaf and the standard drug paracetamol showed significant **Table 4.1:** %inhibition carrageenan induced paw edema of rats by group I, II and III (chloroform extract of *A.indica*)

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Treatment Groups	1-hour	2-hour	3-hour	4-hour	5-hour
Control	46.17^{a}±1.64 0%	49.83^{a}±1.99 0%	57.67^{a}±2.54 0%	64.67^{a}±2.11 0%	62.00^{a}±2.08 0%
Standard	34.17^{d}±1.33 26%	36.33^{c}±0.95 28%	36.67^{b}±1.45 36%	30.67^{d}±1.52 53%	26.17^{e}±0.83 58%
50mg/kg	39.33^{b}±1.12 15%	40.50^{b}±0.67 19%	41.33^{b}±1.36 28%	38.17^{b}±0.31 38%	37.17^{b}±0.31 40%
100mg/kg	37.83^{bc}±0.79 18%	37.67^{bc}±0.88 24%	39.67^{b}±0.80 31%	33.67^{cd}±0.33 48%	33.00^{c}±0.52 47%
200mg/kg	34.67^{cd}±0.84 25%	36.00^{c}±1.13 28%	40.33^{b}±1.15 30%	37.00^{bc}±0.86 43%	31.33^{cd}±0.95 49%
400mg/kg	34.33^{d}±0.67 26%	35.83^{c}±1.01 28%	39.50^{b}±1.06 32%	32.50^{d}±0.72 50%	29.17^{de}±0.83 53%
p-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Superscripts on different means within column differ significantly at $p \leq 0.05$

Pyrexia test

In this activity, group III of *A. indica* leaf and the standard drug paracetamol showed significant ($p<0.05$) % inhibition in yeast- induced pyrexia of rats after 1-4 hours as compared to group I (Table 4.2).

Table 4.2: %inhibition of yeast induced pyrexia of rats by group I, II and III (chloroform extract of *A.indica*)

Treatment	Initial Temp	Temp at 21 hour	1 hour	2 hour	3 hour	4 hour
Control	97.92±0.38	101.93±0.24	101.55±0.13 9%	101.40^{a}±0.10 13%	101.20^{a}±0.09 18%	101.07^{a}±0.08 21%
Standard	97.18±0.22	101.73±0.10	100.70 ±0.37 22%	99.90^{b}±0.54 40%	98.57^{b}±0.43 69%	97.18^{c}±0.24 100%
50mg/kg	97.17±0.19	101.98±0.21	102.85±1.40 19%	101.25^{a}±0.13 14%	100.82^{a}±0.14 23%	100.48^{ab}±0.17 30%
100mg/kg	97.17±0.30	101.50 ±0.26	101.30 ±0.19 8%	101.08^{a}±0.20 12%	100.92^{a}±0.20 17%	100.67^{a}±0.15 22%
200mg/kg	97.50 ±0.35	101.95±0.15	101.62±0.14 11%	101.08^{a}±0.33 20%	100.35^{a}±0.42 36%	99.80^{b}±0.33 48%
400mg/kg	97.18±0.24	101.93±0.27	100.85±0.2 22%	100.13^{b}±0.21 38%	98.77^{b}±0.21 68%	97.68^{c}±0.38 90%
p-value	0.3784	0.5868	0.1886	0.0031	< 0.0001	<0.0001

Superscripts on different means within column differ significantly at $p \leq 0.05$

Writhing test

The maximum percentage inhibition of leaf extract was shown at the dose of 400mg/kg which was 56% as compared to standard and control which was 66% and 0% respectively (Table 4.3).

Table 4.3: %inhibition of Acetic acid induced writhing of rats by group I, II and III (chloroform extract of *A.indica*)

Treatment	Analgesic activity
50mg/kg	20.67^{a}±0.715%
100mg/kg	16.50^{b}±0.5624%
200mg	13.17^{c}±0.7540%
400mg	9.50^{d}±0.56 56%
Control	21.83^{a}±0.790%
Standard	7.33^{e}±0.8866%
p-value	< 0.0001

Superscripts on different means within column differ significantly at $p \leq 0.05$, means within column differ significantly at $p \leq 0.05$

Discussion

A. indica leaf chloroform extract is used to assess the analgesic, antiinflammatory, and anti-pyretic

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properties. In terms of anal-gesic, anti-inflammatory, and antipyretic drug usage, the world today has few options; they include mostly narcotics (morphine) and non-narcotics such salicylates and corticosteroids. Even though most of us are aware of the negative side effects and high cost of these medications, we nevertheless hopelessly rely on them without any better options (Bhattacharya et al., 2014).

The body uses the inflammation as a physiological defence mechanism to protect itself against allergens, harmful substances, blisters, infections and other unpleasant stimuli (Ghauri*et al*., 2021). Carrageenan is frequently used to simulate inflammation in lab to identify substances with anti-inflammatory properties. Yeast causes pyrexia in rats, and acetic acids used to assess the writhing test (Alyas *et al*., 2022). Serotonin and histamine are thought to be released in the first phase of carrageenan-induced edema whereas, prostaglandins, protease and lysosomes are thought to be released in the second phase (Bokanisereme and Okechukwu, 2013). The active ingredients in *A. indica* are thought to exert their anti-inflammatory effects, preventing the production, discharge or activity of inflammatory mediators such as serotonin, prostaglandins and histamine (Mahabub-Uz-Zaman *et al*., 2009).

According to our findings, anti-inflammatory efficacy was dose-dependent. Group III showed less notable inhibition at the 50 mg/kg and 100 mg/kg doses, respectively. At a dose of 400 mg/kg, it demonstrated the most notable edoema inhibition as compared to group II. At 500 mg/kg b.w, ethanol extract of the *A. indica* showed a significant dose-dependent decrease. However, at eight hours into the research at a dose of 500 mg/kg b.w, the maximum edema inhibition was reported to be 22.22 percent (Emran *et al*., 2015). Numerous biological activities, including anti-allergic and anti-inflammatory actions, have been linked to flavonoids. They accomplish this by inhibiting the activity of the enzymes cyclooxygenase and lipoxygenase, lipid peroxidation, capillary permeability and platelet aggregation (Avior *et al*., 2013).

Pathogenic fever, which is caused by injecting yeast suspension into albino rats' nape of the neck to induce pyrexia, is linked to an increase in prostaglandin synthesis. Antipyretic drugs can lower elevated body temperature in specific clinical circumstances. The plant extract inhibits the enzyme cyclooxygenase, which reduces the release of prostaglandins and gives rise to the antipyretic characteristics of the substance (Safari *et al*., 2016). Similar to how paracetamol affects cyclo-oxygenase enzyme activity, antipyretic effectiveness may be mediated by suppressing prostaglandin synthesis. Neem leaf extract may lessen pyrexia by lowering hypothalamic PGE2 levels, blocking the processes that link peripheral inflammation to the formation of PGE2 or both (Santra *et al*., 2014).

In this study, the experimental group receiving *A. indica* leaf chloroform extract experienced a marked reduction in fever. Flavonoids are recognized to target prostaglandins, which are intricate in pyrexia. Therefore, flavonoids in the chloroform leaf extract of *A. indica* may help explain its anti-pyretic properties. By blocking the synthesis of prostaglandins, paracetamol also lessens fever brought on by yeast (Saini and Singha, 2012). Neem leaves extract has flavonoids, tannins, alkaloids, nimbin, nimbinin, nimbolide and nimbidic acid which may be the cause of its anti-inflammatory, analgesic and anti-pyretic properties (Timothy *et al*., 2011).

The well-proposed acetic acid-induced writhing is a useful tool for assessing the analgesic potential of drugs. In the acetic acid-induced writhing paradigm, the discharge of free arachidonic acid from tissue phospholipids via COX results in a localized inflammatory response, and prostaglandins production, notably PGE2 and PGE2 and an increase in the level of lipoxygenase products in peritoneal fluid (Afsar *et al*., 2015). These prostaglandins and lipoxygenase products increase capillary permeability, releasing endogenous chemicals that excite pain nerve endings, resulting in swelling and anguish. NSAIDs inhibit the COX enzyme in peripheral tissues and impact the major afferent nociceptors' transduction mechanisms (Shukla and Mehta, 2015).

The abdominal muscle contracts, the hind limbs extend, and the affected body part lengthens. This characteristic of pain activity caused by intraperitoneal injection of acetic acid is assumed to be mediated via the local peritoneal receptor. The number of writhes decreased, indicating an analgesic effect. Abdominal constriction brought on by acetic acid is a susceptible method for analyzing peripherally acting analgesics. When acetic acid is subjected to a painful stimulus, endogenous chemicals such bradykinins, serotonin, progesterone, histamine and substance P are produced (Subedi *et al*., 2016). Reference medication diclofenac, reduced pain by preventing the synthesis of pain mediators in peripheral tissues (Alyas *et al*., 2020). In the current investigation, the 400 mg/kg dose of chloroform leaf extract from *A. indica* significantly reduced pain in the experimental group. The experimental group showed significant ($p>0.05$) analgesic potential in a dose-dependent manner. Alkaloids are necessary for the analgesic effect (Emran *et al*., 2015).

Conclusion

From the overall results, we deduced that the occurrence of phytoconstitutes for instance flavonoids, alkaloids, tannin, sponins, terpenoids, glycosides, phenolics, and steroids in the chloroform extract of *A. indica* contributed to its considerable

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analgesic, anti-pyretic and anti-inflammatory effects. Our research supports the neem plant's traditional usage as medicine. Future anticipated phytochemicals will aid in identifying clinically useful molecules for the occurrence of their primary and secondary metabolites. Flavonoids may be account for the effects mentioned above.

Conflict of interest

The authors declared absence of conflict of interest.

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