Available online on 15.06.2022 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

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Review Article

Anti-inflammatory, Analgesic, and Antipyretic Potential of *Azadirachta indica*: A Review

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Article Info:

Article History:

Received 21 April 2022
Reviewed 18 May 2022
Accepted 24 May 2022
Published 15 June 2022

Cite this article as:

Oktavia SN, Ifora I, Fauziah F, Anti-inflammatory, Analgesic, and Antipyretic Potential of *Azadirachta indica*: A Review, Journal of Drug Delivery and Therapeutics. 2022; 12(3-S):245-254

DOI: http://dx.doi.org/10.22270/jddt.v12i3-s.5502

Abstract

Inflammation is a protective response to injury or tissue damage and acts by eliminating harmful stimuli such as pathogens cell damage and initiates the healing process. *Azadirachta indica* was known as medicinal plant with various pharmacological activities. This review aims to summarize the scientific activity of *A.indica* as an anti-inflammatory, analgesic, and antipyretic with experimental models either *in vivo* or *in vitro* taken from several databases such as Google Scholar, ScienceDirect, and PubMed over the last ten years (2011- 2021). From the data, *A.indica* was proven to be anti-inflammatory by inhibiting pro-inflammatory cytokines such as IL-6, NFkB, COX2, IL-1, IL-6, TNF-α, IFN- γ, and reducing the volume of edema. Potential analgesic indicated by decreasing stomach writhing and reducing pain sensation in rats. The antipyretic activity was characterized by a decrease in rats' body temperature. This review proves that natural products from *A.indica* have anti-inflammatory, analgesic, and antipyretic potential.

Keywords: Analgesic, Azadirachta, Fever, Inflammation, Neem

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INTRODUCTION

Inflammation is the body's natural response to tissue damage [1]. Characterized by the release of various chemical molecules by macrophages, mast cells, leukocytes, and activation of complement factors that cause extravasation of fluids and proteins and accumulation of leukocytes at the injury site [2]. Mediators and other molecules released during inflammation include vasoactive amines (histamine, serotonin), peptides (bradykinins, eicosanoids (thromboxane, prostaglandins, leukotrienes) [3]. In general, inflammation was divided into two, acute and chronic inflammation [4]. Acute inflammation is a physiological process as a form of body defense. Chronic inflammation is caused by acute inflammation that does not disappear and lasts for weeks to years [5]. Pain is a common and distressing symptom of many diseases; analgesics reduce pain sensation by reducing the sensitivity of pain-sensing systems [6]. Pyrexia or fever increases body temperature triggered by inflammation, graft rejection, malignancy, or tissue damage. By increasing prostaglandin E2 (PGE2), pyrexia can increase the activity of the hypothalamus, which raises the body's temperature [6]. Symptoms of pyrexia include the inability to concentrate, anorexia, and sleepiness. There may also be increased muscle contractions and shivering [7]. Anti-inflammatory drugs are divided into steroids (betamethasone, prednisolone, and dexamethasone) and non-steroidal anti-inflammatory drugs (aspirin, diclofenac, ibuprofen, indomethacin, naproxen, celecoxib) [3]. Inflammation, pain, and fever underlie several pathological conditions [8]. NSAIDs, opioids, and corticosteroids are the most widely used drugs to reduce inflammation, pain, and fever [8,9]. However, the side effects of long-term use cannot be avoided. Using steroids include cardiovascular, endocrine, metabolic, musculoskeletal, and ophthalmological problems, while NSAIDs can cause digestive disorders and bleed [9]. Therefore, many people use traditional plants as alternative medicine because of their safety, economy, and effectiveness [10].

Medicinal plants have long been used for various diseases. One of the plants known claimed and trusted as medicine is neem (*Azadirachta indica* A Juss; Meliaceae), is an evergreen tree originating from India but is also cultivated in South Asia, Africa, Brazil, and even the whole world, especially in tropical and subtropical countries [11,12]. Almost all preclinical studies of *A.indica* and compounds isolated from any part of the plant show therapeutic potential [13]: antioxidant, anticancer, antidiabetic, wound healing effect, hepatoprotective, neuroprotective, immunomodulatory, in dentistry, antimicrobial [11,14], nephrotoxicity [15], anthelmintic [16], and anti-acne [17]. Many compounds have been isolated, but several play an essential role in *A.indica* plants, including azadirachtin, nimbin, nimbolinin, nimbin, and nimbidin nimbidol, sodium nimbinate, gedunin, salannin, quercetin.

In the leaves, there are active compounds such as gedunin, tannin, amino acids, nimbanene, 6-desacetylnimbinene, nimbandiol, ascorbic acid, *n*-hexacosanol, and nimbiol [11,18]. Based on the available data, there is no complete literature

about anti-inflammatory, analgesic and antipyretic on *A.indica*. This review aims to summarize and provide the latest information about the *A. indica* and its activities as an anti-inflammatory, analgesic, and antipyretic in preclinical studies both *in vivo* and *in vitro*. Perhaps, this review can be used as a reference for developing a new drug from the *A.indica* plant.

MATERIALS AND METHODS

Search strategy for the identification study

PubMed, ScienceDirect, and Google Scholar are the databases used for literature studies reporting on experimental animal experiments *in vivo* or *in vitro* that focuses on the anti-inflammatory, analgesic, and antipyretic activities of *A.indica* for the last ten years (2011-2021). The keywords used in the search engine are (*Azadirachta indica* or *neem*) AND (anti-inflammatory) AND (analgesic) AND (antipyretic).

Inclusion and exclusion criteria

Screening and selection included in this review were preclinical studies *in vivo* and *in vitro* that looked at the activity of a single, or a combination of *A. indica* extracts from various parts of the plant as an anti-inflammatory, analgesic, and antipyretic. The only article that can be accessed and published in both English and Indonesian are used while also limiting publications from the last ten years (2011-2021). The exclusion categories are article review, systematic review, meta-analysis, short communication, newsletter, commercial products with many combinations of plants, expert opinion, phytochemical studies, multiple publications; if the article was published more than once in a search engine, other pharmacological studies.

Data extraction

The information included in the table form is the type of extract, the part of the plant used, the dose/concentration, the experimental model, the type of animal, the route of administration, the pharmacological report (results) of the study, the country and name of the author and the year of publication of the article.

RESULT AND DISCUSSION

Based on the inclusion criteria, 19 articles were included in this review. There were 12 articles with *in vivo* studies and 6 *in vitro* studies with one article combination (in vivo and in vitro). Tables of anti-inflammatory, analgesic and antipyretic are summarized in Tables I, II, and III.

Table I. Data extraction of anti-inflammatory (*in vivo* and *in vitro*)

Type of extract or formulation or product	Plant part used	Dose / concentration	Route of administration	Experimental model	Animal/ disease model/ cell/ specimen	Pharmacological reported	Country	Ref.
Leaf extract	Leaves	62.5, 125, 250, 500 mg/kg	*ip*	Carrageenan induced rat paw edema (*In vivo*)	Albino rats	Aqueous extract from *A. indica* showed anti-inflammatory activity by reducing the volume of edema in rat paws after 4 hours of injection with carrageenan	India	(19)
Petroleum ether, chloroform, methanol, and water	Leaves	1.1g /kg 2,100, 200, 400 mg/kg	Oral	1. Carrageenan induced rat paw edema (*in vivo*) 2.Granuloma cotton pallet test (*In vivo*) 3.COX kits inhibition method (*in vitro*)	Rats, Male Sprague Dawley	All extracts showed anti-inflammatory potency by inhibiting COX-1, COX-2, and cytokines (IL-1 and TNF- *α)*	Malaysia	(20)
Aqueous extract	Leaves	500mg/kg	Oral	Carrageenan induced rat paw edema (*In vivo*)	Wistar Strain Albino rat	Aqueous extract showed anti-inflammatory potential by reducing the volume of edema in rat paws.	India	(21)
Leaf extract	Leaves	100, 200 mg/200g	-	DSS induced in colitis mice (*In-vitro*)	Wistar rats	Leaf extract increases cytokine expression, especially IL -10, and increases the histological score in colitis rats induced with DSS	Indonesia	(22)
Supercritical CO2 neem leaf extract (SCNE)	Leaves	SCNE (20.60 µl/mL) NIM (10, 50µL /mL)	Oral	1.OSCC mouse xenograft models and 4-NQO-1 induced test (*In vitro*)	OSCC cell line (Cal27, SCC4, HSC3)	A pure extract of *A. indica* reduces pro-cancer tumor inflammatory cytokines (host and tumor-secreted) significantly	United States	(23)

Ethanolic extract of *A.indica* and *Lawsonia inermis*	Leaves	50, 100, and 200 µg/mL	-	1. Denaturation of albumin 2.Hypotonicity induces membrane stabilization (*In-vitro*)	1. Protein 2. Human red blood cell	The combination of extracts from *A. indica and Lawsonia inermis* exhibited a membrane-stabilizing effect by inhibiting hypotonicity-induced lysis of erythrocyte membranes and inhibiting protein denaturation. The combination of extracts showed the most significant anti-inflammatory potential compared to individual	India	(24)
Ethanolic Extract	Leaves	1.1, 2 mL 2.1Ml	-	1.Hipotonicity induces membrane stabilization 2. Heat-induced hemolysis test (*In-vitro*)	Human red blood cell	Ethanol extract can maintain membrane stability by inhibiting the hypotonic solution induced in human red blood cells. The heat-induced hemolysis method showed 16.06% inhibition with Na. diclofenac as positive control 58.92%	India	(25)
Fine powder in capsule		0.01 to 10mg/mL	-	LPS induced inflammation in C2C12 cell (*In-vitro*)	C2C12 cell	Herbs from *A.indica* had a strong anti-inflammatory activity with ibuprofen as control positive.	South Korea	(26)
Bark extract	Bark	1, 3, 10, 30, 100 µg/mL	-	LPS to SH-SY5Y cell (*In vitro*)	SH-SY5Y cell	Bark extract showed neuro-inflammatory activity characterized by increased levels of Substance P in concentration 3-100 µg/mL	India	(27)
Hydro-alcoholic extract, Ethyl acetate, and *n*-butanol fractions	Leaves	200,400,600mg/kg	Oral	Carrageenan induced rat paw edema (*In vivo*)	Adult albino rats	Hydro-alcoholic, ethyl acetate, and *n*-butanol fractions showed anti-inflammatory activity by reducing the volume of rat paw edema induced by carrageenan.	India	(28)
Azadirachtin	Leaves	6, 60, 120 mg/kg	Oral	1. Carrageenan induced rat paw edema 2. Fibrovascular tissue growth induced by cotton palate (*In vivo*)	Female swiss mice	Azadirachtin inhibits the proliferative phase of the inflammatory process by reducing the growth of fibrovascular tissue; however, azadirachtin does not can reduce the increase of TNF- α after induced by carrageenan	Brazil	(29)
N-hexane extract (Limonoids)	Seed	1.7 nmol/ear	-	TPA induced rat ear edema	-	Eleven compounds tested showed anti-inflammatory activity and had a strong inhibitory effect with ID50 (50% inhibitory dose.	Japan	(30)
Isolated compound (Total polysaccarides(TPL), Fractioned ion)	Seed	1.TPL (1 mg/kg) 2.FI (0.01, 0.1 01mg/kg)	*iv*	1.Carrageenan, histamine, serotonin, prostaglandin, arginine induced rat paw edema 2.Carrageenan induced peritonitis (*In vivo*)	Wistar rats	TPL showed anti-inflammatory potential by reducing plasma protein leakage, inhibiting PGE2 release in rats. Then FI is effective in inhibiting inflammatory mediators by reducing the number of migration of leukocytes when stimulated with fMLP	Brazil	(31)
Oil	Seed	0.25, 0.5,1, 2 mL	*ip*	Carrageenan induced rat paw edema (*In vivo*)	Albino rats	Flavonoid compounds in *A.indica* showed significant results by reducing edema in the rat by inhibiting prostaglandin and	India	(32)

						endoperoxides synthesis.		
Oil	Seed	-	Spread softly in the rat paw	Carrageenan induced rat paw edema (*In vivo*)	Female wistar albino rats	*A.indica* seed oil exhibits anti-inflammatory potential by reducing swelling in the mouse feet. After giving carrageenan for 4 hours, it did not show a decrease in TNF-*α*	India	(33)
Ethanolic extract	Bark	250,500 mg/kg	Oral	Carrageenan induced rat paw edema (*In vivo*)	Albino rats	Ethanol extract from the bark of *A. indica* showed anti-inflammatory activity by reducing edema in the rat paw. The results obtained were significant with Na. Diclofenac is used as the standard drug.	Bangladesh	(34)
Ethanolic extract	Fruit	1.0, 2.5, 5.0 mg/mL 20µL	*ip*	Carrageenan induced stomach (*In vivo*)	Adult zebrafish	The low concentration was used so that antioxidant compounds such as he isolated phenolic in the ethanolic extract of the fruit did not show potential as an anti-inflammatory.	Brazil	(35)

Anti-inflammatory activity (In-vitro study)

Inflammation responds to the body's defenses to avoid pathological conditions [36]. When the inflammatory process occurs, macrophages in the blood are activated by stimulants or activators such as microbes, injury, and trauma. Activated macrophages release several mediators and cytokines, including interleukin (IL)-1, IL-6, IL-12, tumor necrosis factor-α (TNF-α), and Nitric Oxide (NO) [37] . One of the compounds that play an essential role in inhibiting the mechanism of inflammation is the limonoids contained in *A. indica* [38]. Several studies have reported the activity of limonoids as an anti-inflammatory. One of the compounds that play an essential role in inhibiting the mechanism of inflammation is the limonoids contained in *A. indica*. Several studies have reported the activity of limonoids as an anti-inflammatory [39,40].

A.indica leaves

A study conducted by Ruslie *et al.* looked at anti-inflammatory cytokine (IL-10) activity in colitis rats divided into two phases. The first phase, colitis in 7 rats, was induced by dextran sodium sulfate (case group) and then seven mice without treatment (control group). In the second phase, 84 rats were divided into groups 1 receiving 7.8mg/kg, and 2 and 3 receiving *A.indica* leaf extract 100mg and 200/200g twice a day, respectively. All rats were sacrificed for their intestines. Immunohistochemical analysis and histopathological scores showed that the IL-10 expression of the case group was higher than that of the control group. IL-10 expression was observed on days 7, 14, 21, and 28. Groups 1 and 2 showed significant similarities on day 28, while in groups 1 and 3, IL-10 expression was significant from days 7 to 28. The histological scores showed differences, which were significant in groups 1 and 2 for 28 days, and significant in groups 1 and 3 on days 21 and 28. It proves that *A.indica* at a dose of 200mg/200g is comparable to mesalazine, the same as increasing IL-10 expression and histologic scores in dextran sodium sulfate-induced colitis rats [22].

Another study was also reported by Morris *et al.* that Super Critical Neem Extract (SCNE) and Nimbolide (NIM) compounds effectively suppress pro-inflammatory cytokines. In SCC4 cell lines, SCNE or NIM can suppress COX-2 after 8 hours and the HSC3 cells line. Mouse tumors induced with 4-Nitroquinoline-1oxide (4-NQO-1) were able to increase TNF- α and IL-1β levels compared to healthy mice. They also demonstrated that SCNE and NIM were able to suppress pro-cancer inflammatory cytokines such as IL-6, IFN- γ TNF –α [23].

Another fact was reported by Annavarapu *et al.,* who tested a combination of *A.indica* and *Lawsonia inermis* on albumin denaturation and hypotonicity-induced membrane stabilization in human red blood cells (HRBC). It can be concluded that the combination of extracts has greater inhibitory power than single extracts by inhibiting hypotonicity-induced lysis of erythrocytes and stabilizing the lysosomal membrane. Lysosomal membrane stabilization has an essential role in inflammation because it prevents the activation of neutrophils such as bactericidal and protease enzymes. The maximum percentage inhibition of denaturing and membrane-stabilizing proteins was found at a concentration of 200 µg/ml (67.69% and 56.63%) with the percentage of diclofenac (90.40) [24] .

Another study of ethanolic leaf extract was conducted by Naidu *et al*., with a stabilization test of human red blood cell membranes, with the standard drug used was diclofenac, testing of *A.indica* with several concentrations and using 10% DMSO with a 1:1 dilution showed 98.63 and a dilution of 1:2 18.33% in hypotonicity-induced membrane maintenance. It can be concluded that the ethanolic extract of *A.indica* can be developed as a pain reliever drug in the future [25].

A study conducted by Kang *et al*. on *A. indica* showed an anti-inflammatory effect on C2C12 cells. The anti-inflammatory effect at the cellular level is associated with an increase in dose resulting in an increase in TNF-α and Monocyte Chemoattractant Protein-1 (MCP-1) [26], as well as cytokines (IL-10 and 12), chemokines, lymphocyte mitogens, and Nitric Oxide (NO) [41,42].

Another *in vitro* study conducted by Umar *et al.* stated that three subfractions (SF) of *n*-hexane-chloroform tested on the COX kit significantly inhibited the activity of COX-1 and COX-2 enzymes at a concentration of 200 µg/mL maximum percentage of 53.14% and 67.81% (SF-1), respectively. The synergistic effect of the multipolar *A.indica* compound was analyzed by GC-MS (Gas Chromatography and Mass Spectroscopy). They stated that compounds in SF-1 have the potential to inhibit the interaction of pathogen-associated molecular patterns (PAMPs) and Toll-like receptors (TLRs),

both of which can trigger induction via intracellular signals on antigen-presenting cells (APCs) or macrophages, thereby increasing pro-inflammatory cytokines such as TNF-α and IL-1 [20]. The fraction can also inhibit the COX-1 and COX-2 enzymes, where these enzymes play a role in synthesizing prostaglandins [43].

A. indica bark

A study by Reddy *et al.* revealed that short-term exposure of differentiated SH-SY5Y neuronal cells to LPS 1µg/mL increased substance P levels in the LPS-only group. The mechanism that occurs maybe its action on central opioid receptors or through the release of endogenous peptides by inhibiting the transmission of impulses in the dorsal horn. However, on the other hand, neem root bark has weak activity as an analgesic due to the lack of compounds contained in the extract from the neem root bark. Although the root has weak activity, this study concluded that substance P has the potential for inflammation and pain relief with morphine as the standard drug [27].

Anti-inflammatory activity (In vivo study)

The most common anti-inflammatory assay method is the carrageenan-induced mouse paw method. Carrageenan-induced rat paw edema can induce inflammation without causing injury or damage to the inflamed paw [44,45]. This model has two phases: an initial phase that begins 6 hours after carrageenan injection and ends 72 hours later, and a subsequent phase that begins 6 hours after carrageenan injection and ends 72 hours later. Between 48 and 72 hours, inflammation reaches its peak [46]. Histamine, serotonin, and bradykinin were the first mediators detected when rat paw was induced by carrageenan; Prostaglandins (PGs) increase vascular permeability and are detected later. Local/systemic inflammation is associated with increased pro-inflammatory cytokines TNF- α, IL-1, and IL-6 [45].

A. indica leaves extract

A study was conducted by Dinda A. *et al.,* using hydro-alcoholic extract, ethyl acetate, and an n-butanol fraction of *A.indica*. Each extract showed the percentage of inhibition after 3 hours of being induced with carrageenan subcutaneously at a dose of 200 400 600 mg/kg was 38.29, 62.76, 69.14%, and indomethacin 70.2%. Several isolated compounds in hydro-alcoholic extract include alkaloids, triterpenoids, tannins, and flavonoids. It is known that flavonoids have an essential role in anti-inflammatory by inhibiting the cyclooxygenase enzyme, which is responsible for synthesizing prostaglandins [28]. Flavonoids that play a role, such as gallic and ferulic acid, are proven in Xanthine Oxidase (XO) and cyclooxygenase activity [47].

Buchineni *et al.* also reported a model of acute inflammation of the paw of rats induced with carrageenan and celecoxib (positive control) that aqueous extract at a dose of 500 mg/kg exhibited anti-inflammatory activity by reducing the volume of edema after 3 hours of carrageenan administration with a maximum percentage of 16.27% while celecoxib 34.16%. Isolated compounds such as alkaloids, tannins, flavonoids, and tetranortriterpenes (nimbin, nimbinin, nimbidinin, nimbolide, and nimbidic acid) allow inhibition of prostaglandin synthesis [21].

The same thing was also reported by Kumar *et al*., with aspirin 200 mg/kg as a comparison. Neem leaf extract with different doses (62.5, 125, 250, and 500 mg/kg). Aspirin inhibits edema from one to 4 hours with a maximum percentage of 72.05%, while aqueous extract of *A.indica* at a dose of 250 and 500mg/kg reduced the edema volume from two to 6 hours 52.32 and 63.01%, respectively. It proves that aqueous extract *of A.indica* has anti-inflammatory effects at doses of 250 and 500mg/kg [19].

The activity of azadirachtin (a limonoid compound) was reported by Soares *et al*., the method used is carrageenan which induces edema and formation of fibrovascular tissue induced by the cotton pellet. Dexamethasone was used as a positive control. Only the highest dose (120 mg/kg) had the potential to reduce edema on the soles of the rats. It was also characterized by inhibiting the proliferative phase in which the growth of fibrovascular tissue was reduced. Single or repeated administration of azadirachtin reduces carrageenan-induced edema and the prolonged proliferative response induced by cotton pellets, respectively, and exhibits specific inhibitory effects on other inflammatory molecules such as interleukins and TNF- α [29].

Umar *et al.* reported on the anti-inflammatory assay of Male Sprague-Dawley rats 200-250g by carrageenan inducing rat paw and cotton pellet-induced fibrovascular tissue formation. Indomethacin and dexamethasone were used as positive controls. The chloroform extract had a high percentage of inhibition, namely 53.25%, while in the fraction, the highest inhibition percentage was at F2 (*n*-hexane-chloroform) 51.02% at a dose of 400 mg/kg. In granuloma tissue, F2 (400mg/kg) was able to inhibit the production of IL-1 (34.38%) and TNF-α (23%). This study also proved that non-polar extracts were more effective than polar extracts. These extracts together have anti-inflammatory action by suppressing COX production and the production of pro-inflammatory cytokines, such as IL-1 and TNF-α [20].

A.Indica seeds

Akihisa *et al.* also revealed 17 isolated limonoid compounds, of which 11 had anti-inflammatory activity. The isolated compounds were 17 Epiazadiradione, 17 Hydroxy azadiradione, Aadiradionolid, 1,3 Diacetylvilasinin, 6 Deacetylnimbin, 6 Acetylnimbandiol, 28 Deoxonimbolide, Ohcininacetate, Salannin, 2'3' Dihydrosalannin, 3 Deacetylsalannin. TPA (12 O-tetradecanoylphorbol-13-acetate) that induces inflammation in rat ears was evaluated by ID50 (50% inhibitory dose). The percentage inhibition of indomethacin inhibition (positive control) was 0.91 mol/ear. 6-Deacetylnimbin has a strong percentage of inhibition of 0.75 mol/ear, and salanine had a weak anti-inflammatory potency of 0.22 mol/ear. This proves that limonoids from *A.indica* have anti-inflammatory [30].

Pereira *et al*. stated that polysaccharides isolated from the seed tegument exhibited anti-inflammatory activity. Before the rat's paws were stimulated, TPL (1mg/kg) or FI (0.01, 0.1, 1 mg/kg) were administered *i.v.* methods of peritonitis induced by carrageenan (500mg/kg) and *N*-formyl-methionyl-leucil-phenylalanine (fMLP) (50mg/kg) *i.p*. TPI and FI showed significant anti-inflammatory properties. TPL 1mg/kg showed 17% inhibition percentage of dextran-induced anti-inflammatory, 55% carrageenan-induced. FI showed 77% inhibition induced by carrageenan, serotonin (54%), PGE2 (69%), and nitric oxide (73%). Peritonitis induced with carrageenan and fMLP was 48% and 67%, respectively. TPL and FI fractionated from the tegument of *A. indica* seeds exhibit potent anti-inflammatory activity on vascular and cellular inflammatory events involving serotonin, PGE2, and NO [31].

A study conducted by Naik *et al*. stated that doses of 0.25mg/kg up to 2 mg/kg showed anti-inflammatory potential induced by carrageenan, maximum inhibition at a dose of 2 mg/kg after 4 hours of carrageenan administration (53.14%), This is significant when compared with 200mg/kg aspirin (70%). Carrageenan induction consists of two phases; the first phase, after 0-2 hours of being induced by carrageenan, was

the release of 5-HT, histamine, and bradykinin from mast cells; after 3 hours, the release of kinins, and the final phase 4 hours after administration of carrageenan causing the synthesis of prostaglandins, proteases, and lysosomes. The compounds contained in *A. indica,* such as flavonoids, triterpenes, tannins, saponins, and nimbidins, play an essential role in inhibiting the synthesis process of prostaglandins [32].

Another study revealed a topical formulation of *A.indica* by Banerjee *et al.* by applying the seed oil on the sub-plantar foot using diclofenac gel as a comparison. The anti-inflammatory activity showed 39-60% for 30-180 minutes [33]. This topical drug capable of reducing inflammation may not be related to the TNF-α pathway but due to the involvement of other pro-inflammatory enzymes such as (COX) or lipoxygenase (LOX) in reducing inflammation [48].

A.indica bark

Emran TB *et al.* examined the tree bark of *A. indica* at different doses (250, 500 mg/kg BW) with carrageenan-induced rat paws. The edema volume was measured with a plethysmometer at 30-8 hours after carrageenan administration. After 3 hours of giving carrageenan at a dose of 250, 500 mg/kg, the percentage of inhibition was 32.18%, 54.17%, and Na. Diclofenac 60.27% showed that *A.indica* bark ethanol has significant anti-inflammatory results by reducing edema volume. It can be concluded that the ethanolic extract of the bark of *A.indica* has anti-inflammatory activity (250 and 500mg/kg) [34].

Fruit anti-inflammatory activity of neem

Batista *et al.* demonstrated that an ethanolic extract of *A.indica* on the abdomen of adult zebrafish induced by carrageenan with different concentrations of 1.0, 2.5, 5.0 mg/mL observed for 4 hours did not reduce inflammation in the abdomen of adult zebrafish, this is due to the low phenolic compounds found in ethanol fruit extract [35]. Several studies have revealed that zebrafish can be used as an alternative in models of acute inflammation other than rodents. Carrageenan-induced adult zebrafish can significantly increase abdominal edema by the intraperitoneal route [49].

From the available data, it can be concluded that carrageenan-induced rat paw is the most widely used method with various extracts. Polar and non-polar extracts showed anti-inflammatory potential from the leaves and root bark. Isolated compounds to the total polysaccharide in *A.indica* reduced edema volume in rats and adult zebrafish. The higher the percentage of inflammation, the higher the dose/concentration given. However, an aqueous extract shows little potential. At a dose of 500mg/kg with 16.27% inhibition and at a maximum dose of 1g/kg, it only showed 26.18%. The seed oil of *A. indica* also exhibited anti-inflammatory potential either by intraperitoneal administration or applied to carrageenan-induced rat paws. Unfortunately, in adult zebrafish, the ethanolic extract of *A. indica does* not show any anti-inflammatory potential. This is due to the lack of antioxidants contained in the extract. Further *in vitro* studies on other parts need to be reviewed, not only on the leaves.

Analgesic activity (In vivo study)

Table II. Data extraction of analgesic activity (*In vivo study*)

Type of extract or formulation or product	Plant part used	Dose/ concentration	Route of administration	Experimental model	Animal/ disease model/ cell/ specimen	Pharmacological reported	Country	Ref.
Leaf extract	Leaves	62.5, 125, 250, 500 mg/ kg	*ip*	Tail flick method	Albino rats	Aqueous extract from *A.indica* leaves shows analgesic activity through its effect, as seen from the wagging of rat tails.	India	(19)
Aqueous extract	Leaves	500mg/kg	Oral	Hot plate method	Wistar stain albino rats	Aqueous extract from *A.indica* can reduce jumping in mice. This is comparable to celecoxib which is used as the standard	India	(21)
Hydro-alcoholic extract, Ethyl acetate, and n-butanol fractions	Leaves	1,100,100,100 mg/kg 2,200,400,600 mg/kg 3,200,200,200 mg/kg	1.Oral 2. Oral 3.Intraperitoneal (*ip*)	1. Writhing method induced by acetic acid 2. Hot plate method 3. Tail flick method	Albino mice	All extracts have analgesic activity by inhibiting prostaglandin synthesis and are significant for indomethacin which is used as a standard	India	(28)
Azadirachtin	Leaves	6, 60, 120 mg/kg	*po (oral)*	1. Hot plate method 2. Writhing method induced by zymosan	Female Swiss mice	The limonoid compounds of azadirachtin exhibit analgesic activity by inhibiting the activation of the endogenous opioid pathway	Brazil	(29)

Ethanolic extract	Bark	250, 500mg/kg	Oral	1. Hot plate method 2. Writhing method induced by acetic acid	Swiss albino mice	Bark extract *A.indica* showed analgesic activity by inhibiting the synthesis of arachidonic acid	Bangladesh	(34)
Ethanolic extract	Fruit	All methods with the same dose (1.0, 2.5, 5.0 mg/mL 20 µL)	*ip*	Pain induced nociceptive, formalin, cinnamaldehyde, capsaicin, menthol, acid-ic saline	Adult zebrafish	All methods used showed results in behavioral changes and had analgesic potency from *A. indica*	Brazil	(35)
Ethanolic extract of *A. indica* and *Momardica charantia*	Leaves	F1 : *Momardica charantia* (300 mg/kg) *A.indica* (200 mg/kg) F2 : *A.indica* (500mg/kg) *Momardica charantia* (200mg/kg)	Oral	1.Tail flick method 2.Hot plate method	Wistar albino rats	Second, the formulation of the ethanolic extracts of *A. indica* and *Momardica charantia* showed antinociceptive activity by inhibiting the cyclooxygenase enzyme and acting as a central opioid receptor	India	(50)

A.indica fruits

An experiment conducted by Batista *et al.* examining the antinociceptive activity of A.indica fruit in adult zebrafish (*Danio rerio*) induced by formalin (2.5, 5mg/mL), glutamic acid (1.0, 2.5, 5.0 mg /mL), acid saline (5.0 mg/mL), capsaicin, menthol, and cinnaldehyde, showed significant results with morphine used as a comparison. The antinociceptive effect is inhibited by naloxone, ketamine, and amiloride. The pharmacological properties of the ethanolic extract of the neem fruit allow it to treat acute pain and are modulated by opioids, NMDA receptors, and acid-sensitive ion channels (ASICs). ASICs are one of the receptors that can detect changes in pH in the body because of their involvement in body physiology [35].

A.indica leaves

The aqueous extract was also reported by Buchineni *et al.* by induced hyperalgesia in rats using the hot plate method; rats' responses such as jumping or licking were recorded for 1 hour to 3 hours. Aqueous extract at a 500mg/kg dose showed significant results with celecoxib as a positive control. The maximum inhibition showed 2 hours after hyperalgesia was induced 218.65%. The analgesic effect can be seen in the reduced frequency of jumping and licking the soles of the paws in rats. This proves that the aqueous extract of *A.indica* has an analgesic effect (500mg/kg) [21].

Another study of aqueous extract of *A.indica* investigated by Kumar *et al.* was done with the tail-flick response to thermal stimulation measured by tail-flick latency of an analgesiometer. The standard drugs used were morphine and *A.indica* doses of 62.5, 125, 250, and 500 mg/kg. Maximum inhibition was shown at doses of 250 and 500 mg/kg with a percent inhibition of 70% after 1 hour of administration, analgesic effect, and tail-flick latency decreased after 90 minutes of extract administration. This proves that *A.indica* has analgesic activity at 250 and 500 mg/kg [19].

In another study, the hydro-alcoholic extract of ethyl acetate extract and the *n*-butanol fraction of *A.indica* evaluated by Dinda A *et al*., with acetic acid-induced writhing method showing the maximum inhibition percentage after 30 minutes in the *n*-butanol fraction (100mg/kg) 49.92% this reduced the amount of writhing in mice by inhibiting prostaglandin synthesis. Indomethacin is positive control (62.5%). The hot plate method showed that the maximum percentage of *n*-butanol fraction (600mg/kg) was 83.17%. The tail-flick method showed the analgesic effect after 30 minutes after administration of the hydro-alcoholic extract. Ethyl acetate and *n*-butanol were 6.5, 6.8, and 8.5, respectively [28].

The combined ethanol extract of *Momardica charantia* and *A.indica* evaluated by Gomase *et al*., hot plate, and heat conduction methods were used in this study. The first formula, namely *Momardica charantia* and *A.indica* (200mg/kg), and the second formulation at a 500mg/kg dose for both extracts, where Na.diclofenac was used as a positive control. Both formulations have the same analgesic potential by inhibiting the cyclooxygenase enzyme or acting on opioid receptors, but the first formulation is more effective than the second formulation [50].

A. indica barks

Ethanol extract of *A.indica* studied by Emran *et al.*. Potential analgesics were evaluated by the hot plate method and induced acetic acid in rats. *A.indica* extract at doses of 250 and 500mg/kg was significant to Na.diclofenac in reducing latency time in rats. The acetic acid-induced writhing test showed significant results comparable to ketorolac, which was used as a positive control. The acetic acid-induced writhing test increased the levels of PGE2 and PGE2-α in the peritoneal fluid and lipoxygenase enzymes. It proves that the ethanolic extract of the bark of the *A.indica* has an analgesic effect [34].

On the other hand, isolated compounds such as azadirachtin with an acute inflammation model were evaluated by Soares *et al*., in the hot plate method, the dose of azadirachtin 120 mg/kg and dipyrone 500 mg/kg as a comparison administered *ip* able to reduce latency for nociceptive responses to heat. The zymosan-induced writhing method showed results where the highest dose of 120 mg/kg was potentially antinociceptive by reducing writhing in rats, where diclofenac sodium was used as a comparison (10mg/kg BW). It can be concluded that *A.indica* has analgesic activity at a dose of 120mg/kg [29].

The writhing, tail-flick, and hot plate methods are the most common and simple methods for analgesic testing. Aqueous extracts showed weak potency compared to non-polar extracts. This potency is related to the increase in the dose or

concentration of the extract. The combination of extracts from *A.indica* has a higher mechanism than single extracts. On rodents, analgesic tests were also carried out on adult zebrafish with several inducers.

Antipyretic activity (In vivo study)

Table III. Data extraction of antipyretic activities (*In vivo*)

Type of extract or formulation or product	Plant part used	Dose or concentration	Route of administration	Experimental model	Animal/ disease model/ cell/ specimen	Pharmacological reported	Country	Ref.
Leaf extract	Leaves	62.5, 125, 250, 500 mg/kg	*ip*	Fever induced by baking yeast	Albino rats	Aqueous extract from *A.indica* reduces fever in rats after 4 hours of administration of	India	(19)
Ethanolic extract	Leaves	100,200,300mg/kg	Oral	Fever induced by baking yeast	Wistar rats	The ethanol extract was able to reduce the rectal temperature in rats induced by fever with	India	(51)

Antipyretics are drugs used to lower body temperature. Bread yeast is a pathogenic agent that can cause fever in test animals.

A.indica leaves

Another study by Kumar *et al.* demonstrated the antipyretic potential of *A.indica*. Fever induced by yeast (20 mg/kg) and paracetamol 100mg/kg compared with intraperitoneal administration. The study showed that at a dose of 62.5, it did not show antipyretic activity, while a dose of 125mg/kg BW lowered the basal temperature of rats after 6 hours, doses of 250 and 500mg/kg BW decreased the basal temperature at 4 hours. This proves that the extract of *A.indica* has antipyretic activity by lowering basal body temperature in rats (250 and 500 mg/kg [19].

A study conducted by Vijayalakshmi. Showed that *A. indica* proved to be an antipyretic in which the fever was given by baker's yeast on the back of the rat's ear. Then after 18 hours, the rectal temperature was rechecked. The extract was given at different doses of 100, 200, and 300 mg/kg. The study results concluded that *A. indica* leaves act as an antipyretic by lowering the rectal temperature in rats. It may be due to the flavonoid compounds in *A. indica* that work by inhibiting the activity of prostaglandins in the hypothalamus [51].

Antipyretic tests generally use baker's yeast, which can cause fever in mice. In the data we gathered, the antipyretic test only used *A.indica* leaves. It needs to be followed up on whether other plant parts also have the same potential. This antipyretic activity was achieved at 100-500mg/kg, where the higher the dose given, the higher the effect.

CONCLUSION

Medicinal plants have an essential role in developing new drugs, including anti-inflammatory, analgesic, and antipyretic. The review proves that *Azadirachta indica,* both *in vivo* and *in vitro,* shows its activity on inflammatory stimuli by inhibiting and reducing the production of pro-inflammatory cytokines such as IL-6, TNF-α, NFkB, COX2, IL-1, IL-6, IFN-γ, Xanthine Oxidase (XO), Monocyte Chemoattractant Protein-1 (MCP-1), lymphocyte mitogens, and Nitric Oxide (NO), chemokines, and also suppresses leukocyte migration and inhibits prostaglandin synthesis and can reduce edema in experimental animals. The analgesic effect *of A.indica* was seen by reducing the amount of wriggling in rats and decreasing pain response. The antipyretic activity of *A.indica* was significant in lowering rats' body temperature after several hours of extract administration. Natural products from *A.indica* have potential effects depending on the dose and concentration given. However, further observations are needed to develop *A.indica* as a new drug.

CONFLICT OF INTEREST

The authors declare no conflicts of interest

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