

EVALUATION OF THE ANTI-INFLAMMATORY ACTIVITY OF COMBINATION OF ETHANOL EXTRACTS OF *AZADIRACHTA INDICA* (NEEM) AND *LAWSONIA INERMIS* (HENNA)

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ABSTRACT

Objective: The aim of the present study was to investigate the *in vitro* anti-inflammatory activity of *Azadirachta indica* (neem) and *Lawsonia inermis* (henna) individual extract and in combination using the same solvent.

Methods: The leaf material of *A. indica* and *L. inermis* was collected from surroundings of Aditya College of Pharmacy, Kakinada, East Godavari. Powdered material was subjected to successive solvent extraction process. The yield was collected and prepared different concentrations (50, 100, and 200 µg/ml) of plant extracts. Diclofenac sodium was used as standard drug. The anti-inflammatory activity was performed by *in vitro* methods such as albumin denaturation method and human red blood cells membrane lysis method.

Results: Denaturation of proteins is a well-documented cause of inflammation. Neem showed a significant membrane stabilizing activity of 46.62% and protein denaturation inhibition activity of 57.32% at concentration of 200 µg/ml. Henna showed a significant membrane stabilizing activity of 39.89% and protein denaturation inhibition activity of 53.75% at 200 µg/ml. In combination, both the extracts showed a significant membrane stabilizing activity of 56.63% and protein denaturation inhibition activity of 67.69% at concentration of 200 µg/ml.

Conclusion: The present study concluded that combination of *A. indica* and *L. inermis* possesses significant anti-inflammatory activity when compared with individual extract.

Keywords: Anti-inflammatory, Human red blood cell, Protein denaturation, *Lawsonia inermis*, *Azadirachta indica*.

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INTRODUCTION

Inflammation is a normal protective response to tissue injury, and it involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown, and repair [1]. When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form of stress. Symptoms of inflammation include redness, swelling and pain, joint stiffness, loss of joint function as a result of infection, irritation, or injury. Plants may become the base for the development of a new medicine, or they may be used as phytomedicine for the treatment of disease [2]. In this growing interest, many of the phytochemical bioactive compounds from medicinal plants have shown many pharmacological activities [3]. Most of the anti-inflammatory drugs available in the market, having a wide range of problems such as efficacy and undesired effects including gastrointestinal tract disorders and other unwanted effects, gastrointestinal disturbances, renal damages, respiratory depression [4]. This situation highlights the need for advent of safe, novel, and effective analgesic and anti-inflammatory compounds [5].

METHODS**Collection of plant material**

The leaf material of *Azadirachta indica* and *Lawsonia inermis* was collected from surroundings of Aditya College of Pharmacy, Kakinada, East Godavari.

Preparation of extract

The plant materials were dried under the shade and ground to fine powder with the help of electrical grinder. The dried powder of leaves of *A. indica* and *L. inermis* was subjected to maceration separately using ethanol for 5 days with intermittent shaking. After 7 days of maceration, the solvents mixture is filtered separately with the help of Whatman

No.1 filter paper. The obtained filtrates are subjected to distillation. The extracts thus obtained are then dried used in the present study.

Assessment of *in vitro* anti-inflammatory activity***Inhibition of protein denaturation***

The anti-inflammatory activity of leaf extracts *A. indica* and *L. inermis* was studied using inhibition of albumin denaturation which was studied according to Mizushima and Kobayashi [6] and Sakat *et al.* [7] followed with minor modifications. Test solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml plant extract (separately) in suitable solvent of different concentrations (50, 100, and 200 µg/ml). Test control solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of distilled water. Product control solution (0.5 ml) consists of 0.45 ml of distilled water and 0.05 ml of plant extract in suitable solvent of different concentrations (50, 100, and 200 µg/ml). Standard solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of different concentrations (50, 100, and 200 µg/ml) of diclofenac sodium. All the above solutions were adjusted to pH 6.3 using 1N hydrochloric acid. The samples were incubated at 37°C for 20 minutes, and the temperature was increased to keep the samples at 57°C for 3 minutes. After cooling, 2.5 ml of phosphate buffer was added to the above solutions. The absorbance was measured using ultraviolet-visible spectrophotometer at 416 nm. The percentage inhibition of protein denaturation was calculated as:

$$\text{Percentage inhibition} = (\text{Abs control} - \text{Abs sample}) \times 100 / \text{Abs control}$$

Membrane stabilization

The principle concerned in this method is stabilization of human red blood cell membrane by hypotonicity-induced membrane lysis. Blood was collected (2 ml) from healthy volunteers and was mixed with equal volume of sterilized Alsever's solution (2% dextrose, 0.8%

Table 1: *In vitro* anti-inflammatory activity of neem

Treatment	Concentration (µg/ml)	Percentage Inhibition of protein denaturation	Percentage of membrane stabilization
Neem extract	50	32.02	21.30
	100	47.21	32.45
	200	57.32	46.62
Diclofenac sodium	50	94.62	73.43
	100	96.97	82.14
	200	99.03	90.40

Table 2: *In vitro* anti-inflammatory activity of henna

Treatment	Concentration (µg/ml)	Percentage of protein denaturation	Percentage of membrane stabilization
Henna extract	50	26.01	19.78
	100	38.42	24.60
	200	53.75	39.89
Diclofenac sodium	50	94.62	73.43
	100	98.97	82.14
	200	99.03	90.40

Table 3: *In vitro* anti-inflammatory activity of neem and henna

Treatment	Concentration (µg/ml)	Percentage of protein denaturation	Percentage of membrane stabilization
Neem and henna	50	34.34	22.45
	100	52.12	49.42
	200	67.69	56.63
Diclofenac sodium	50	94.62	76.05
	100	98.97	82.14
	200	99.03	90.40

sodium citrate, 0.5% citric acid, and 0.42% NaCl in distilled water) and centrifuged at 3000 rpm. The packed cells were washed with isosaline solution, and a 10% v/v suspension was prepared with normal saline. Different concentrations of each plant extract and in combination (50, 100, 200 µg/ml), diclofenac sodium (50, 100, 200 µg/ml) as standard and control (distilled water instead of hyposaline to produce 100% hemolysis) were separately mixed with 1 ml of phosphate buffer, 2 ml hyposaline solution, and 0.5 ml of 10% human red blood cell (HRBC) suspension was added to prepared reaction mixture. All the assay mixtures were incubated at 37°C for 30 minutes and centrifuged at 3000 rpm for 20 minutes, and hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm [8]. The percentage of HRBC membrane stabilization or protection was calculated using the formula:

$$\text{Percentage inhibition} = (\text{Abs control} - \text{Abs sample}) \times 100 / \text{Abs control}$$

RESULTS

The ethanol extracts of the leaves of *A. indica* and *L. inermis* was studied for anti-inflammatory activity by protein denaturation and HRBC membrane stabilization. The anti inflammatory activity is concentration dependent, with increase in concentration the activity also increases. The anti inflammatory activity of ethanol extract of neem with reference to diclofenac sodium was illustrated in (fig. 1 and fig. 2). The percentage of inhibition of protein denaturation and percentage of

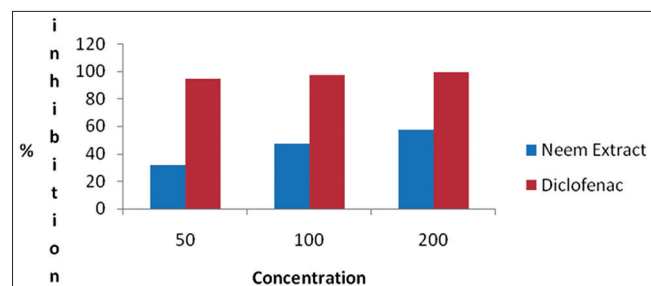


Fig. 1: Percentage inhibition of neem by protein denaturation

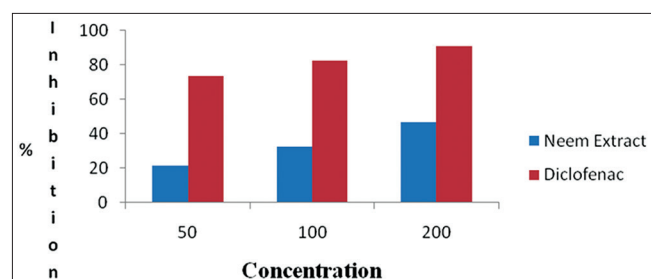


Fig. 2: Percentage inhibition of neem by membrane stabilization

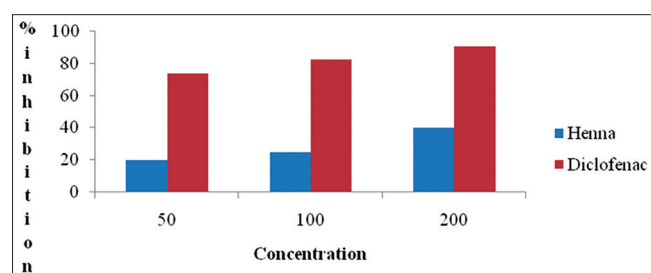


Fig. 3: Percentage inhibition by henna by membrane stabilization

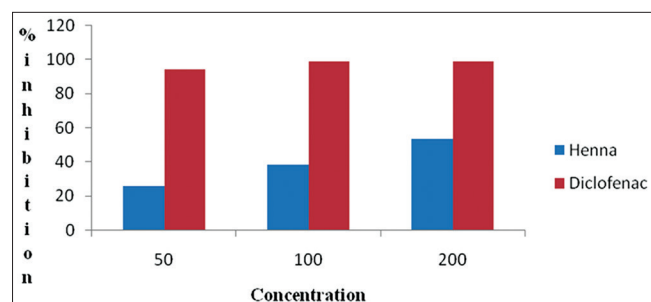


Fig. 4: Percentage inhibition of henna by protein denaturation

membrane stabilization for ethanolic extracts and Diclofenac sodium were done at 50, 100 and 200 µg/ml. It shows anti inflammatory activity at concentration 200 µg/ml shows 57.32% (inhibit protein denaturation) and 46.62% (protection membrane stabilization).

From the Table 2, the ethanolic extract of henna shows anti-inflammatory activity at concentration of 200 µg/ml shows 53.75% (inhibition of protein denaturation) and 39.89% (protection membrane stabilization). With the increasing concentration protein denaturation is decreased as shown in Fig. 4 and membrane stabilization/protection is increased as shown in Fig. 3.

Combination of ethanolic extracts of neem and henna was studied for anti-inflammatory activity and it shows increased activity at 200 µg/ml is 67.69% (inhibition of protein denaturation method) and 56.63% (membrane stabilization method) illustrated in Fig. 5 and Fig. 6.

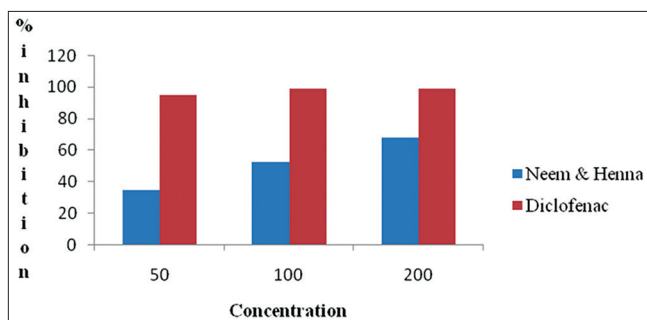


Fig. 5: Percentage inhibition of neem and henna by protein denaturation

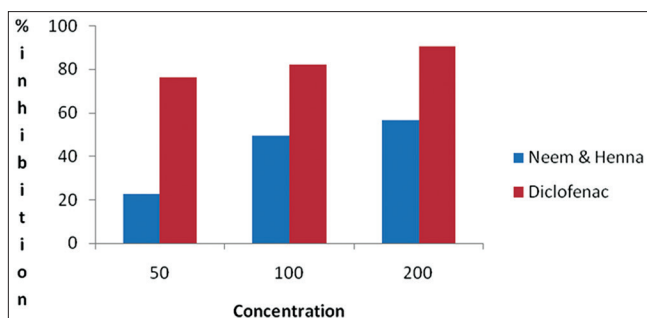


Fig. 6: Percentage inhibition of neem and henna by membrane stabilization

DISCUSSION

From the results, it is concluded that the combination of ethanolic extracts of neem (*A. indica*) and henna (*L. inermis*) possess greater anti-inflammatory activity than individual plant extract. Here, the anti-inflammatory activity was assessed by *in vitro* screening methods such as protein denaturation and HRBC method. Denaturation of proteins is a well-documented cause of inflammation. Most biological proteins lose their biological functions when denatured, and production of autoantigen in certain arthritic disease is due to denaturation of the protein [9]. The mechanism of denaturation involves alteration in electrostatic hydrogen, hydrophobic, and disulfide bonding [10]. The inhibition of protein denaturation with neem extract was 57.32%, with henna extract was 53.75%, and in combination was 67.69% at a concentration of 200 µg/ml. During inflammation, lysosomal hydrolytic enzymes are released which causes damages of the surrounding organelles and tissues with variety of disorders. The erythrocyte membrane is analogous to the lysosomal membrane, [11] and its stabilization implies that the extract may as well stabilize lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases which cause further tissue inflammation and

damage upon extracellular release [12]. It is reported that the ethanolic extract of *A. indica* showed 46.62% and *L. inermis* showed 39.89%, and in combination, it showed 56.63% protection at a concentration of 200 µg/ml.

CONCLUSION

In the present investigation, the results indicate that the ethanolic leaf extracts of *A. indica* and *L. inermis* possess anti-inflammatory activity properties. The protective effect against protein denaturation and membrane stabilization is known to be a good index of anti-inflammatory activity. From the present study, it is concluded that combination of *A. indica* and *L. inermis* possesses the highest anti-inflammatory activity when compared with individual extract.

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