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**ORIGINAL ARTICLE**

**ANTIARTHRITIC EFFECT OF AQUEOUS EXTRACT OF *LAWSONIA INERMIS.L* - AN  
INVITRO STUDY**

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**ABSTRACT**

Rheumatic arthritis (RA) is a severe, inflammatory autoimmune illness that affects the joints. The *Lawsonia inermis.L* (*henna leaves*) has been used as a traditional Indian herbal medicine. The present study was designed to assess the antiarthritic potential of aqueous extract of *Lawsonia inermis.L*. The various concentrations (50, 100, 250, 500, 1000, 2000) of the extract were tested for antiarthritic potential by percentage inhibition of protein denaturation and membrane lysis method. The anti-arthritis activity of *Lawsonia inermis. L* extracts was compared with standard drug diclofenac sodium. The aqueous extract of *Lawsonia inermis.L* revealed antiarthritic activity in a dose dependent method. The aqueous extract of *Lawsonia inermis. L* showed substantial antiarthritic activity that was similar to that of diclofenac sodium statistically. The present study suggested that the protective activity of *Lawsonia inermis. L* was efficient against arthritis and may perhaps due to the presence of biologically active constituents.

**Keywords:** *Lawsonia inermis. L*, Rheumatoid arthritis, Diclofenac sodium, Protein denaturation, Membrane lysis.

**1.INTRODUCTION**

Rheumatoid arthritis (RA) is a systemic autoimmune disease which is interrelated with progressive, disability, complications, socioeconomic costs and early death (Firestein, 2003). The number of people affected by the RA and the social impact of diagnosed arthritis is projected to increase by 40 % over the next 25 years (Hootman and Helmick, 2006). Even though the accurate cause of RA remains unknown (Smolen and Steiner, 2003). Autoimmunity plays a crucial role in both its chronicity and progression. In addition, it affects the tissues surrounding the joints such as blood vessels, muscles and skin (Asolkar *et al.*, 1992).

Rheumatoid arthritis involves a composite interplay among genotype, environmental triggers, and chance. The Pathology of RA consists of the new blood vessel formation, proliferation of synovial joints and inflammatory cell infiltration. The symptom of RA includes redness, warmth, pain, swelling, morning stiffness, and restrictions on the functions of the joints (Firestein, 2001). The inflammatory process in RA primarily affects the synovial membrane lining, but can affect other organs also. The inflamed

synovium leads to the aggressive cartilage destruction and advanced bony erosions. The disease is consistently progressive and results in pain, stiffness, and swelling of joints. In late stages, deformity and ankylosis develop (Stephen and William, 2006).

Even though a variety of drugs have been used to control RA, there are diverse reports regarding the side effects of these drugs. As a consequence, researchers are now searching for alternative therapeutics. As part of this search, significant attention has been paid to the plant-based drugs that are used in traditional medicine because these drugs elicit few side effects and are inexpensive (Dharamsiri *et al.*, 2003). The modern drugs, both steroidal and non-steroidal anti-inflammatory drugs are used for the amelioration of the symptoms of the disease. However, they offer only temporary relief and also produce severe side effects (Hazeena and Sadique, 1988) . Ancient history of India describes its diverse uses and also plays an appreciable role in Ayurvedic or natural herbal medicines (Lavhate and Mishra, 2007).

*Lawsonia inermis. L* is a member of the family Lythraceae which consists of about 500 species, widely spread in tropical regions with relatively few species in temperate regions (Jones and Luchainger , 1979). The useful parts of the plant are leaves, flower, bark, seed and root. The

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principle coloring substance of henna is a red-orange color molecular formula (Lawsonia, 2-hydroxy-1,4 naphthaquinone) having molecular formula,  $C_6H_6O_3$  and melting point of  $190^\circ C$ , present in dried leaves in a concentration of 1-1.4 % w/w (Jain *et al.*, 2010; Chaudhary *et al.*, 2010). Henna leaves contain an important pigment called "lawsone." It contains tannic acid, gallic acid mucilage and naphthaquinone. Henna leaves, flowers, seeds, stem bark and roots are used in traditional medicine to treat a variety of ailments as headache, ulcers, diarrhea, leprosy, fever, leucorrhoea, diabetes, cardiac disease, hepatoprotective and coloring agent (Chetty, 2008; Chopra, 1956; Reddy, 1988). As far as we know, there have been no reports on the anti arthritic activity of this plant so far. Hence, the present attempt is to evaluate the anti arthritic effect of *Lawsonia inermis*. L.

## 2.MATERIALS AND METHODS

### Plant samples and extraction procedure

*Lawsonia inermis*.L was collected from gardens. The leaves of *Lawsonia inermis*.L (authentication no. 289) were authenticated by Botanist, Department of Botany, Annamalai University, Chidambaram. The collected leaves were left to dry at room temperature for 24 h. The dried leaves were ground to a powder and were kept in dry containers. The extracts were prepared in the present study: water-based extracts. The extract was prepared by mixing 30 g of dried leaf powder of *Lawsonia inermis*. L with 500 ml of water for 24 h. This mixture was filtered by funneling and filter paper (Wattman No.1). The solvents were then removed by air drying using oven over 3 days at  $40^\circ C$  to obtain a crude extract, which were stored at  $4^\circ C$  in dark vials (Muhammad and Muhammad, 2003).

### Inhibition of Protein Denaturation

The following procedure was followed for evaluating the percentage of inhibition of protein denaturation

1. Test solution (0.5 ml) consists of 0.45 ml of Bovine serum albumin (5 w/v aqueous solutions) and 0.05 ml of test solution.
2. Test control solution (0.5 ml) consist of 4.5 ml of Bovine serum albumin 5 % w/v aqueous solution) and 0.5 ml of distilled water.
3. Product control (0.5 ml) consists of 0.45 ml of distilled water and 0.05 ml of test solution.
4. Standard solution (0.5 ml) consists of 0.45 ml of Bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of Diclofenac sodium.

Various concentrations (50, 100, 250, 500, 1000, 2000  $\mu g/ml$ ) of protein extracts (test solution) and diclofenac sodium (standard) of were taken respectively. All the above solutions were adjusted to pH 6.3 using 1N hydrochloric acid. The samples were incubated at  $37^\circ C$  for 20 minutes and the temperature was increased to keep the samples at  $57^\circ C$  for 3minutes. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 416 nm.

The control represents 100% protein denaturation. The results were compared with Diclofenac sodium. The

percentage inhibition of protein denaturation can be calculated as

$$\% \text{ of Inhibition} = \frac{[100 - (\text{O.D of test solution} - \text{O.D of product control})]}{\text{O.D of test control}} \times 100$$

### Effect of membrane stabilization / inhibition of membrane lysis

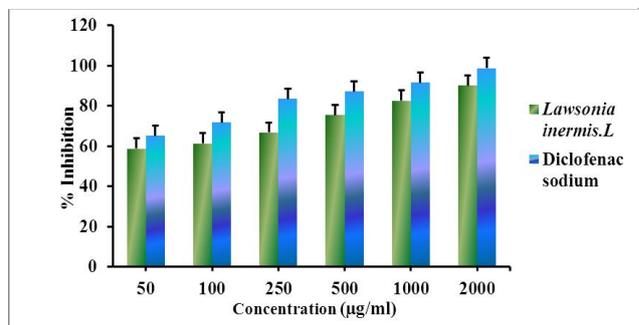
The principle involved here is stabilization of human red blood cells (HRBC) membrane by hypotonicity induced membrane lysis. The assay mixture contains 1ml of phosphate buffer {pH 7.4, 0.15 M}, 2ml hypo saline {0.36 %}, 0.5 ml of HRBC suspension {10 % v/v} with 0.5ml of plant extracts and standard drug diclofenac sodium of various concentrations (50, 100, 250, 500, 1000, 2000  $\mu g/ml$ ) and control (distilled water instead of hyposaline to produce 100 % hemolysis) were incubated at  $37^\circ C$  for 30 min and centrifuged respectively. The haemoglobin content in the suspension was estimated using spectrophotometer at 560 nm. The percentage inhibition of membrane stabilization can be calculated as

$$\% \text{ of Inhibition} = \frac{[100 - (\text{O.D of test solution} - \text{O.D of product control})]}{\text{O.D of test control}} \times 100$$

## 3.RESULTS

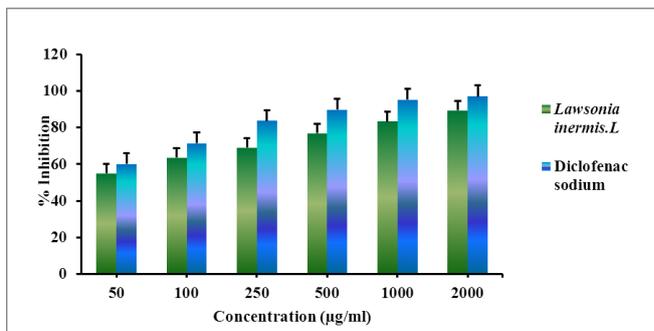
The results of the effect of *Lawsonia inermis*.L and Diclofenac sodium on inhibition of protein denaturation and membrane stabilization were shown in the **figure 1 & 2**. The aqueous extract of *Lawsonia inermis*.L showed concentration dependent anti-arthritic activity. All the results were compared with the standard drug Diclofenac sodium.

**Figure 1. Effect of *Lawsonia inermis*. L and diclofenac sodium on inhibition of protein denaturation.**



Values represented as mean  $\pm$  S.D. of six samples of each. Significance at the level of  $p < 0.05$

**Figure 2. Effect of *Lawsonia inermis*. L and diclofenac sodium on inhibition of membrane stabilization.**



Values represented as mean  $\pm$  S.D. of six samples of each. Significance at the level of  $p < 0.05$

It was found that the aqueous extract of *Lawsonia inermis*.L exhibits utmost percentage inhibition of protein denaturation and membrane stabilization (which showed  $90.11 \pm 0.68$  &  $89.38 \pm 1.33$  respectively) at the concentration of 2000 µg/ml when compared to the standard drug Diclofenac sodium (which showed  $98.69 \pm 0.61$  &  $97.10 \pm 0.81$  respectively).

#### 4.DISCUSSION

It is well known that plants have been used in traditional herbal medicine for many years. In some parts of the world, plants and herbs are still the prime medicines used in medical treatment (Natarajan *et al.*, 2005; Hemem, 2002). The extensive survey of literature revealed that *Lawsonia inermis*. L is highly regarded as a universal panacea in the herbal medicine with diverse pharmacological activity spectrum.

This versatile medicinal plant is the unique source of various types of chemical compounds including lawsone, isoplumbagin lawsoniaside, lalioside, lawsoniaside B, syringinoside, daphneside, daphnorin, agrimonolide, 6-O-β-D-glucopyranoside, (+)-syringaresinol, O-β-D-glucopyranoside, (+)-pinosresinol di-O-β-D-glucopyranoside, syringaresinol di-O-β-D-glucopyranoside, isoscutellarin-3-β, hennadiol, (2S)-3-β, 30-dihydroxylupane, lawnermis acid, 3-methylnonacosan-1-ol, laxanthones I, II, III and lacoumarin etc., which are responsible of the various activities of the plant. Major active constituents of henna leaf are flavonoids (Rastogi and Mehrota, 1993) which have been shown to possess various biological properties related to antioxidant mechanisms (Latha, 2003; Shankar *et al.*, 2005).

Previous study reported that the development and conformation of arthritis in this model by the measurement of arthritic index and markers of arthritis. The extract SA (*Semecarpus anacardium*) contains a number of minerals and vitamins. Analysis of SA revealed the presence of calcium, iron, copper, sodium and aluminium (Ramprasath *et al.*, 2006). It was also found to be significantly recouped to near normal conditions on SA treatment. SA stimulates bone formation by enhancing the absorption of calcium from intestine and averted bone loss by improving the balance of bone formation and resorption. On comparison of SA with methotrexate (MTX) from the available literature, it is evident that SA is more active than methotrexate. Bendele *et al.* have demonstrated that MTX caused a 47 % inhibition in paw swelling and 58 % inhibition in histological variations (Bendele *et al.*, 1999).

In the current study, the aqueous extract of *Lawsonia inermis*. L showed the activity of inhibition of protein denaturation and membrane stabilization compared to the effect of standard drug Diclofenac sodium, and this may be due to the presence of large quantity of active substances such as phenols, sterols, xanthone, terpenoids, etc. In our results, we observed that the *Lawsonia inermis*. L shows 65% inhibition of protein denaturation and membrane stabilization when compared to the inhibitory effect of drug Diclofenac sodium. It may be due to the presence of

abundant phytoconstituents which are responsible for antiarthritic activity.

#### 5.CONCLUSION

The results of the current study contribute towards corroborate the traditional use of this multi-herbal formulation in the treatment of rheumatoid arthritis. Our findings clearly suggested that the aqueous extract of *Lawsonia inermis*. L has significant antiarthritic activity in a dose-dependent manner when compared with standard drug Diclofenac sodium. It can be concluded that the active constituents responsible for antiarthritic activity present in the aqueous extract of *Lawsonia inermis*. L. Hence, this study is further extended to recognize and discriminate the accurate active constituents in *Lawsonia inermis*. L and to elucidate this precise mechanism of action, which is responsible for the observed significant antiarthritic activity.

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