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(RESEARCH ARTICLE)



Pharmacological evaluation of the anti-ulcer and anti-arthritic potential of ethanolic extract of acalypha indica linn

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Abstract

The objective of present study to evaluate the anti-ulcer and anti-arthritic activity of ethanolic leaf extract of Acalypha indica. The anti-ulcer activity of ethanolic extract of Acalypha indica was investigated by pylorous ligation and indomethacin induced gastric ulcer in rats. The cause of ulceration in patients is mainly due to hyper secretion of gastric acid and pepsin. Acalypha indica plant extract some of the most attractive sources of new drugs and have been shown to produce promising results in the treatment of gastric ulcers. Rheumatoid arthritis is a systemic auto immune disease characterized by articular inflammation that eventually leads to the destructions of joints. The present work is aimed to evaluate the anti-arthritic effect of plant Acalypha indica extract by Freund's Complete Adjuvant (FCA) induced arthritis in rats. Medicinal plants possess numerous active phyto constituents that are responsible for several biological activities. Acalypha indica is very important medicinal plant, to possess anti-ulcer and anti-arthritic activity.

Keywords: Indomethacin; Pylorous Ligation; Acalypha Indica; Freund's Complete Adjuvant; Anti Arthritic; Ethanolic Extract; Helicobacter Pylori

1. Introduction

Peptic ulcer is a gastro intestinal disorder due to an imbalance between the aggressive factors like acid, pepsin, *Helicobacter pylori* and defensive factors like bicarbonate secretion, prostaglandins, gastric mucus, innate resistance of the mucosal cell factors [1]. Normally peptic ulcers develops when aggressive factors overcome the defensive factors are *Helicobacter pylori*, acid pepsin hyper secretion, non-steroidal anti-inflammatory drugs, sometimes idiopathic due to usage of tobacco, psychological stress, rapid gastric emptying and zollinger-ulcer Ellisson syndrome where there is a high and uncontrollable production of acid also leads to ulcer formation [2,3,4,5]. Synthetic drugs such as proton pumb inhibitors, H₂ receptors, cytoprotectants, demulcents, anti cholinergies, antacids and prostaglandin analogues are used for the treatment of ulceration but these drugs produce several side effects. So herbal medicines are considered as better alternatives for the treatment of peptic ulcer [6]. For example (Omeprazole, lansoprazole) may cause nausea, abdominal pain, constipation, diarrhoea and H₂ receptor antagonists (cimetidine) may cause gynaecomastia, loss of libido. Due to the occurrence of many side effects by use of synthetic drugs for many diseases, medicinal plants are considered as the main source of new drugs as they have less or no side effects. As herbal medicines are considered as safe for the treatment of ulcers with lesser adverse effects, economical, effective, relatively less toxic, extensive research is carried out in search for potent anti-ulcer agents of plant orgin[7,8].

Rheumatoid arthritis is an immune mediated inflammatory disease. Rheumatoid arthritis occurs when our immune system attacks the tissues near joints, this is due to release certain chemicals and enzymes that begin to eat away the cartilage and bones. Rheumatoid arthritis affects all the joints in the body, some forms of arthritis can also affect the

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body's internal organs. The symptoms of Rheumatoid arthritis include inflammation, pain, swelling and stiffness of the joints, it can also lead to deformity and disability of the joint in sever cases[9]. These are several causes for Rheumatoid arthritis these causes are unknown but some include genetic factor, family history, age, environmental factors, hormones, smoking etc[10,11]. The medicinal plants with phyto constituents which can be used in the treatment of Rheumatoid arthritis[12].

2. Materials And Method

2.1. Chemicals

All chemicals used including the solvents were analytical grade. Folin-ciocalteu phenol reagent 1,1-diphenyl-z-picryl hydrazyl (DPPH), polyvinyl polypyrro lidone (PVPP), ammonium acetate and 2,4,6 – tripyridyl-s-triazine (TPTZ) were purchased from merch co. (Germany).

2.2. Preparation of Plant Extract

The *Acalypha indica* plant were shade dried at room temperature. The dried material was then homogenized to obtain coarse powder and stored in air-tight bottles for further analysis. The shade dried, powdered leaves were extracted with ethanol by hot extraction using soxhlet apparatus, collected and stored in a vial for further analysis.

2.3. Animals

The experiment was carried out by male wister albino rats weighing (150-175g) and were procured from small animals breeding station, Mannuthy, Kerala, India. The animals were housed under standard conditions of temperature (23 \pm 1 $^{\circ}$ C), relative humidity (55 \pm 1 $^{\circ}$ C), 12h /12h light / dark cycle and fed with standard pellet diet (Pranav Agro Industries Ltd., Sangli, India) and water *ad libitum*. Animals described as tested were deprived of food for atleast 18h. but allowed free access to water. All the experimental procedures and used in the study were received by the Institutional Animals Ethics Committee (Reg.No.688/2/C-CPCSEA) and were in accordance with the guildelines of the CPCSEA.

3. Antiulcer activity

3.1. Sample Preparation

Coarse powder from the shade dried plant material was exhaustively extracted with ethanol to yield a dark greenish semisolid residue. The dried extract was dissolved in distilled water right before use.

3.2. Indomethacin (IND) Induced Ulcers

The experiment was performed according to the method of Djahanguiri Four groups of male wister rats (n=4) were fasted overnight prior to the start of the experiment and water *ad libitum*. The first group received distilled water, which the second group was treated with omeprazole (10mg kg⁻¹ day⁻¹ P.O.). Where as third to fourth groups were administrated with the ethanolic extract of present study plants (200 and 400 mg kg⁻¹ day⁻¹ P.O. respectively). On day third after 30 min of omeprazole and plant extract treatments, indomethacin (50 mg kg⁻¹) suspended in 0.5% carboxymethy cellulose was given as a single oral dose to groups 2-4 to induce gastric ulcers. After 5h, the animals were sacrified with over dose of diethylether and each stomach was examined for ulcer index[13].

3.3. Experimental Design

- Group I: Control which received distilled water orally.
- Group II: Omeprezole (10 mg kg⁻¹ day⁻¹ P.O.)
- Group III: Served as test sample which received ethanolic leaf extract of 200 mg kg⁻¹ P.O. of *Acalypha indica*.
- Group IV: Served as test sample which received ethanolic leaf extract of 400 mg kg⁻¹ P.O. of *Acalypha indica*.

3.4. Ulcer Index

The stomach were removed and opened along the greater curvature, washed gently in normal saline and the mean ulcer index was calculated. The number of ulcer lesion were counted using a magnifying glass and the diameter of the ulcer was measured using a vernier calliper. Ulcer index was determined by following the scoring method [14].

- Score 1: Maximal diameter of 1mm
- Score 2: Maximal diameter of 1-2 mm
- Score 3: Maximal diameter of 2-3 mm
- Score 4: Maximal diameter of 3-4 mm
- Score 5: Maximal diameter of 4-5 mm
- Score 10: Maximal diameter of 5 mm and above
- Score 25: A per forated ulcer

The sum of the length (mm) of all lesions for each stomach was used as the ulcer index (UI), and the protection percentage was calculated from the following formula.

$$\frac{Percentage}{Protection} = \frac{\textit{UI Control} - \textit{UI Treated}}{\textit{UI Control}} \times 100$$

3.5. Anti-Arthritis

3.5.1. Procedure

Wister Albino Male rats (150-200g) were divided into 5 groups of six animals each (n=6). Group I served as control. Arthritis was induced in rats by injecting 0.1 ml of 0.1% Freund's Complete Adjuvant (FCA), (Sigma Aldrich, USA) into the sub planter region in the right hint paw of Group II-V was administrated with 200 mg kg⁻¹ day⁻¹ P.O. and 400 mg kg⁻¹ day⁻¹ P.O. ethanolic leaf extract of *Acalypha indica* daily for 15 days [15].

3.5.2. Experimental Design

The plant extract treatment was administrated as follows for 5 days.

- Group I: Served as Control
- Group II: Freund's Complete Adjuvant (FCA) into the sub planter region in right hind paw.
- Group III: Administered with indomethacin (10 mg kg⁻¹ day⁻¹ P.O.) daily
- Group IV: 200 mg kg⁻¹ day⁻¹ P.O. of Acalypha indica
- Group V: 400 mg kg-1 day-1 P.O. of Acalypha indica

The increase in joint diameter was measured daily starting from day 1, by using vernier calibre. Percentage protection rendered by the plant extract is calculated using the formulate,

Percentage Protection = Difference in Paw volume of induced- $\frac{\text{Difference in Paw Volume of Standard / or treated}}{\text{Difference in Paw Volume of induced}} \times 100$

3.6. Statistical Analysis

The data presented here are means \pm SD of 6 rats in each group. The results were analysed using one-way analysis of variance (ANOVA) and the group means were compared by Duncan's Multiple Range Test (DMRT) using Statistical Program For Social Sciences (SPSS Version 16.0) software for windows. The findings were considered statistically significant at P < 0.05[16].

4. Results and discussion

Gastric lesions were induced in rats by oral administration of Indomethacin (50 mg kg⁻¹). Oral administration of ethanolic leaf extract of *Acalypha indica* registered a significant dose dependent decrease in the extent of gastric mucosal damage in indomethacin induced ulcer models (Figure I). The protective effect of the *Acalypha indica* extract at a dose level 400 mg kg⁻¹ b.w. (Ulcer Score 13.95; 67.72% Protection) was slightly lower than that of the standard drug omeprazole (Ulcer Score 11.37; 72.44% Protection). Indomethacin induced depletion of gastric wall mucous formation has been significantly reduced by ethanolic leaf extract of *Acalypha indica* at the dose levels of 200 and 400 mg kg⁻¹ b.w. Showing protection percentage as 48.13 and 67.72 respectively (Table 1 and Fig 1).

The anti-arthritic activity of the ethanolic leaf extract of *Acalypha indica* (Tables 2 and 3) studied against FCA – induced arthritis is exhibited in Figure II. The tested plant drug extracts of *Acalypha indica* at two different concentrations (200 and 400 mg kg⁻¹ b.w. P.O.) were administrated orally and the activity was compared with the standard drug Indomethacin (10 mg kg⁻¹ day⁻¹ P.O.). Sub planter injection of FCA (Freund's Complete Adjuvent) in the rat hind paw led

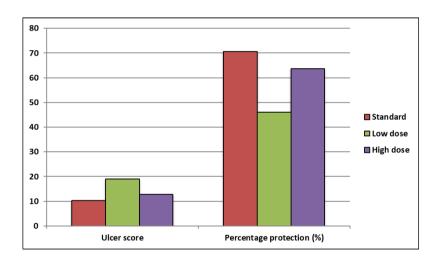
to the development of arthritis which reached a peak edema on the 10^{th} day of injection. The standard indomethacin seemed to inhibit this edema to an extent of 64.14%. Among the plant drugs used in the present study, percentage protection of *Acalypha indica* at a high dose was slightly higher (71.22%) and it was very close to the standard (72.20%) on the 15^{th} day (Table 3 and Fig 2).

Ethanolic extract of *Acalypha indica* found to contain alkaloids, glycosides, flavonoids, saponins, phenolic compounds and tannins as their phytoconstituents. These secondary metabolites play an important role to act as an anti-microbial, anti-oxidant, anti-inflammatory, anti-ulcer and anti-arthritic activity in the presently investigated plant in *Acalypha indica*.

Table 1 effect of ethanolic extract of acalyphaindica leaves on ulcer index of indomethacin induced gastric ulcer in rats^A

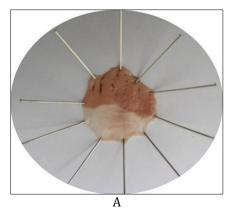
| Group | Ulcer Score | Percentage Protection (%) |
|----------------------------------------|---------------------------|---------------------------|
| Induced | $36.17^{\alpha} \pm 0.98$ | - |
| Standard | 11.37d ± 0.72 | 72.44 |
| Low dose (200mg kg ⁻¹ b. w) | 19.87 ^b ± 0.36 | 48.13 |
| High dose (400mg kg-1b. w) | 13.95° ± 0.38 | 67.72 |

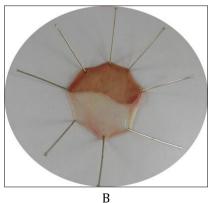
 a Values are expressed as mean \pm SD for 4 animals (n=4) significant at P < 0.05 level. One way ANOVA Followed by DUNCAN'S Multiple Range Test.



ACALYPHA INDICA

Figure 1 percentage protection of ethanolic extract of *acalypha indica* leaves on ulcer index of indomethac in induced gastric ulcer in rats





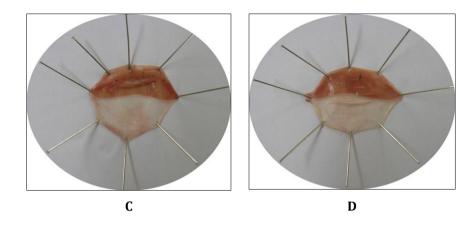


Figure 2 Effect of ethanolic leaf extract of *acalypha indica* leaves on ulcer index of indomethacin induced gastric ulcer in rats

Table 2 antiarthritis activity of ethanolic leaf extract of acalypha indicaa

| Paw volume(mm) | | | | | |
|----------------|-----------------|----------------------------|-------------------------|-------------------------|--------------------------|
| Days | Control | Induced | Standard | Low dose | High dose |
| 0 | 3.28 ± 0.16 | 3.15° ± 0.12 | $3.40^{p} \pm 0.08$ | $3.34^p \pm 0.11$ | 3.37 ^p ± 0.14 |
| 1 | 3.28 ± 0.16 | $6.96^{\text{n}} \pm 0.24$ | $6.60^{i} \pm 0.21$ | $7.23^{i} \pm 0.43$ | 7.25 ^a ± 0.47 |
| 2 | 3.28 ± 0.16 | $7.45^{\rm m} \pm 0.23$ | $7.27^{\rm f} \pm 0.14$ | $7.29^{g} \pm 0.40$ | $6.89^{b} \pm 0.55$ |
| 3 | 3.28 ± 0.16 | $7.95^1 \pm 0.02$ | $7.83^{d} \pm 0.06$ | $7.47^{d} \pm 0.39$ | 6.67° ± 0.42 |
| 4 | 3.28 ± 0.16 | $8.60^{k} \pm 0.09$ | 8.38a ± 0.09 | $7.53^{\circ} \pm 0.38$ | $6.07^{d} \pm 0.28$ |
| 5 | 3.28 ± 0.16 | $9.32^{j} \pm 0.05$ | $8.16^{b} \pm 0.04$ | 7.66a ± 0.35 | 5.96f ± 0.25 |
| 6 | 3.28 ± 0.16 | 9.92 ^h ± 0.04 | $7.89^{\circ} \pm 0.05$ | $7.60^{b} \pm 0.33$ | $5.87^{g} \pm 0.26$ |
| 7 | 3.28 ± 0.16 | $10.25^{\rm f} \pm 0.09$ | 7.73e ± 0.11 | $7.48^{e} \pm 0.32$ | $5.71^{h} \pm 0.17$ |
| 8 | 3.28 ± 0.16 | $10.77^{d} \pm 0.04$ | $7.23^{g} \pm 0.06$ | $7.37^{\rm f} \pm 0.32$ | $5.60^{i} \pm 0.16$ |
| 9 | 3.28 ± 0.16 | $11.44^{\rm b} \pm 0.08$ | $6.89^{h} \pm 0.10$ | $7.27^{\rm h} \pm 0.33$ | $5.48^{j} \pm 0.13$ |
| 10 | 3.28 ± 0.16 | 11.85a ± 0.06 | $6.52^{j} \pm 0.12$ | $7.18^{j} \pm 0.32$ | 5.99e ± 0.11 |
| 11 | 3.28 ± 0.16 | 11.23° ± 0.07 | $6.15^{k} \pm 0.05$ | $6.98^{k} \pm 0.34$ | 5.31 ^k ± 0.12 |
| 12 | 3.28 ± 0.16 | 10.73° ± 0.06 | $5.73^1 \pm 0.06$ | $6.77^1 \pm 0.33$ | $5.24^1 \pm 0.13$ |
| 13 | 3.28 ± 0.16 | 10.11g ± 0.04 | $5.43^{m} \pm 0.06$ | $6.59^{m} \pm 0.35$ | $5.22^{m} \pm 0.10$ |
| 14 | 3.28 ± 0.16 | 9.85 ⁱ ± 0.08 | $5.25^{\rm n} \pm 0.08$ | $6.40^{\rm n} \pm 0.36$ | 5.20 ⁿ ± 0.09 |
| 15 | 3.28 ± 0.16 | $9.30^{k} \pm 0.08$ | 5.11° ± 0.04 | 6.20° ± 0.36 | 5.14° ± 0.08 |

 $^{^{\}rm a}$ Values are expressed as mean $\pm {\rm SD}$ for 6 animals (n=6) significant at p<0.05 level. One way ANOVA followed by Duncan's Multiple Range Test.

Table 3 percentage protection of acalypha indica leaf extract against FCA induced arthritis at different days intervalsa

| Days | Group | Initial paw volume(mm) | Final paw volume(mm) | Difference(mm) | Percentage protection (%) |
|----------|----------|------------------------|----------------------|----------------|---------------------------|
| 5th day | Control | 3.28d ± 0.16 | 3.28e ± 0.16 | - | - |
| | Induced | 3.15e ± 0.12 | 9.32a ± 0.05 | 6.17a ± | - |
| | Standard | 3.40a ± 0.08 | 8.16b ± 0.04 | 4.76b ± | 22.85 |
| | Low | 3.34b ± 0.11 | 7.66c ± 0.35 | 4.32c ± | 29.98 |
| | High | 3.37c ± 0.14 | 5.96d ± 0.25 | 2.59d ± | 58.02 |
| 10th day | Control | 3.28d ± 0.16 | 3.28e ± 0.16 | - | - |

| | Induced | 3.15e ± 0.12 | 11.85a ± 0.06 | 8.70a ± | - |
|----------|----------|------------------|------------------|--------------|-------|
| | Standard | 3.40a ± 0.08 | 6.52c ± 0.12 | 3.12c ± | 64.14 |
| | Low | $3.34c \pm 0.11$ | 7.18b ± 0.32 | 3.84b ± | 55.86 |
| | High | 3.37b ± 0.14 | 5.99d ± 0.11 | 2.62d ± | 69.89 |
| 15th day | Control | 3.28d ± 0.16 | 3.28e ± 0.16 | - | - |
| | Induced | 3.15e ± 0.12 | 9.30a ± 0.08 | 6.15a ± 0.19 | - |
| | Standard | $3.40a \pm 0.08$ | 5.11d ± 0.05 | 1.71d ± 0.09 | 72.20 |
| | Low | $3.21c \pm 0.13$ | 5.64b ± 0.51 | 2.86b ± 0.33 | 53.50 |
| | High | 3.23b ± 0.11 | $5.13c \pm 0.36$ | 1.77c ± 0.18 | 71.22 |

a Values are expressed as mean ±SD for 6 animals (n=6) significant at p<0.05 level.

One way ANOVA followed by Duncan's Multiple Range Test.

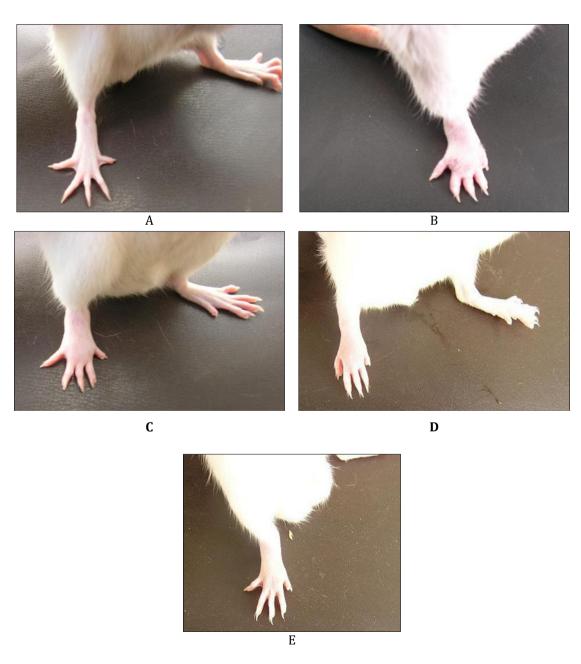
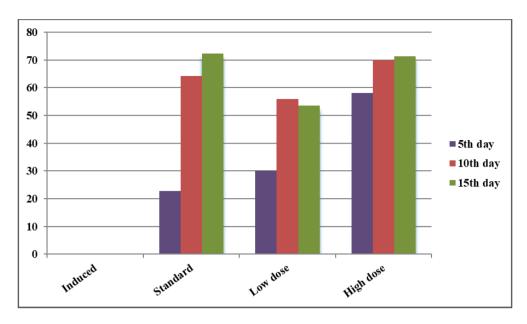


Figure 3 percentage protection of acalypha indica leaf extract against FCA induced arthritis at different day intervals



Acalypha Indica

Figure 4 Percentage protection of *acalyphaindicaleaves* extract against FCA induced arthritis at different days intervals

5. Conclusion

Herbal medicinal products have a wide scope of assorted variety of multi-dimensional synthetic strctures in the on going occasions, the utility of normal items as natural capacity modifiers has impressive considerations. The botanical study evaluated the identification of 2,50,000 to 3,50,000 plant species over the plant. The curative properties of medicinal plant are due to presence of major group of active components which are mainly alkaloids, phenols, flavonoids, steroids, saponins, terpenoids, tannins and glycosides. Plants are served as major structural resources for traditional as well as modern medicinal system all over the world. The therapeutic potential of plants and plant products can be treated back to thousands of years ago. *Acalypha indica* possess the antimicrobial, anti-oxidant, anti-inflammatory, anti ulcer and anti arthritic activities. These findings justify the usefulness of these plant in the management and treatment of inflammation associated diseases like ulcer and arthritis.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

If studies involve use of animal/human subject, authors must give appropriate statement of ethical approval. If not applicable then mention 'The present research work does not contain any studies performed on animals/humans subjects by any of the authors'.

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