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ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF LEAF EXTRACTS OF *ERYTHRINA VARIEGATE*

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ABSTRACT

Erythrina variegate (asclepidaceae) is a plant with a variety of ethnic medicinal uses. Hence an effort has been made to screen the aqueous extract of leaves of *erythrina variegate* for its analagesic and anti-inflammatory activities. Leaves of *erythrina variegate* were extracted with water successively. Preliminary phytoichemical investigation was carried out to identify various phytochemical constituents present in the extract. phytochemical investigation: the aqueous extract of leaves of *Erythrina variegate* showed the presence of alkaloids, carbohydrates, steroids, sapaonins and triterpenes. The toxicity studies; the LD_{50} were found to be aqueous extract: 3162mg/kg, analgesi activity of aqueous extract of leaves of *Erythrina variegate* has reduced the intendity of acetic acid induced writhing and thermally induced pain stimuli using eddy's hot plate method in mice, indicating both peripheral and central analgesic activity. Anti-inflammatory activity of aqueous extract has shown significant activity in carrageenan induced paw oedema model in rats. The effect was observed in middle and final phase of inhibition suggesting that extract can block the mediators like kinins and prostaglandins.

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INTRODUCTION

Inflammation is the response to injury of cells and body tissue through different Factors such as infections, Chemicals, and thermal and mechanical injuries. Most of the anti-inflammatory drugs now available are potential inhibitors of cyclo-oxygenase (COX) pathway of arachidonic acid metabolism which produces prostaglandins. Prostaglandins are hyperalgesic, potent vasodilators and also contribute to erytheme, Edema and pain. Hence for treating inflammatory disease, analgesic and anti-inflammatory agents are required. Non steroidal anti-inflammatory drugs (NSAIDs) are the most clinically important medicine used for the treatment of inflammation-related diseases like arthritis, Asthma, and cardiovascular disease. Non steroidal anti-inflammatory drugs (NSAIDs) are amongst the most widely used medication due to their efficacy for wide range of pain and inflammatory condition. However, the long term administration of NSAID may induce gastro-intestinal ulcers, Bleeding, and renal disorders due to their nonselective inhibition of both constitutive (COX-1) and inducible (COX-2) isoforms of cyclooxygenase enzymes. Therefore, new anti-inflammatory and analgesic drugs lacking those effects are being searched all over the world as alternative to NSAIDs and opiates. Medicinal plants are believed to be important sources of new chemical substance with potential therapeutic effects.

Pain has been defined by International Association for the Study of Pain (IASP) as an unpleasant sensory and emotional experience associated with actual or potential tissue damage [(11)]. Failure to relieve pain is morally and ethically unacceptable. All these drugs carry potential toxic effects. One study suggests that risk of gastrointestinal bleeding was significantly associated with acute use of non-steroidal anti-inflammatory drugs (NSAIDs) like regular-dose aspirin, diclofenac, ketorolac, naproxen or nimesulide. Piroxicam increased the risk of bleeding in both acute and chronic therapy [(12)]. Opioids are the commonly used drugs for the management of acute postoperative pain [(13)].

Plants have been one of the rich and important sources of medicines since the dawn of human civilization. These are the gift of nature to the mankind for treating different types of diseases. Almost from prehistoric period, use of herbal medicine for alleviation of suffering caused by different diseases in human are well documented in India and other countries and even today they are in great use in these countries. There are several beliefs / claims regarding the therapeutic utility of herbs and herbal formulations, they are-

1. The herbal medicines exhibit fewer side effects and are safe.
2. Herbs and herbal formulations are cheaper and easily available.
3. For certain diseases like hepatitis herbs and herbal drugs are the only remedies.
4. Certain chemical constituents from the herbs are serving as prototype molecules for the discovery of more effective drugs than the existing ones.

Considering the importance of plants as source of medicine even today, we have selected a plant Erythrina Variegata (Asclepiadaceae) which has been traditionally used as a folklore medicine in certain regions of India for treatment of bronchial asthma, bronchitis, rheumatism and jaundice. Hence an effort has been made to establish the various pharmacological activities of the extracts of leaves of the Erythrina Variegata (Asclepiadaceae).

AIMS & OBJECTIVES

Since there are no reports available regarding the anti-inflammatory, analgesic, anti-diarrheal and hepatoprotective activity with aqueous extract of leaves of Erythrina Variegata, it is aimed to investigate and validate the ethnical medicinal uses of the plant for activities mentioned above.

PLAN OF WORK

The genus *Erythrina* comprises of about 110 species of trees and shrubs. The name "coral tree" is used as a collective term for these plants. Coral tree is indigenous to the Old World tropics, possibly originally from India to Malaysia, but is native of ancient westward to Zanzibar and eastward to eastern Polynesia (the Marquesas). It is typically found on sandy soil in littoral forest, and sometimes in coastal forest up to 250m (800ft) in elevation. The coral tree is cultivated particularly as an ornamental tree and as a shade and soil improvement tree (it fixes nitrogen) for other tree crops such as coffee and cacao. The most attractive type, var. *variegata*, is grown for its variegated leaves, as well as its seasonal showy red flowers.[14-16] This fast-growing, 50-60 feet tall and wide deciduous tree with green and yellow-variegated, 6-inch-long leaves creates a broad canopy but has spiny branches. In spring, before the leaves appear, coral tree is decorated with showy red blossoms, each flower 2.5 inches long and arranged in dense, six-inch-long racemes. These blooms are followed by 12-inch-long, red/brown seedpods which contain poisonous seeds.[17] Studies on phytochemical *of Erythrina variegata* species have demonstrated alkaloids and flavonoids as major constituents. Different parts of *E. Variegata* have used in traditional medicine as nervine sedative, febrifuge, anti-asthmatic and antiepileptic.[18] In the some experiments, it has potential effects for treatment of some diseases like convulsion, fever, inflammation, bacterial infection, insomnia, helminthiasis, cough, cuts and wounds.[19-22].

TAXONOMY

Kingdom : Plantae – Plants
Division : Magnoliophyta - Flowering plants
Class : Magnoliopsida - Dicotyledons
Family : Fabaceae (Legume family)
Subfamily : Papilionoideae
Genus : *Erythrina L.* - Coral Tree
Species : *E. variegata L.*

CHEMICAL CONSTITUTENTS:

Alkaloids:

The plant is a rich source of alkaloids (2.5%). (+) - 3 - Demethoxyerythratidinone, (+) - erythraline, (+) - erythramine, (+) - erythrinine, (+) -erythratidinone, (+) - erysonine, (+)-erysotine, (+)-erysodine, (+)-erysovine, (+)-ll-hydroxy-epi-erythratidine, (+)-erythratidine, *(+)-epi*-erythratidine, (+)-erysodienone, (+)-erysotrine, (+)-erysopitine, (+)-ll-p-hydroxyerysotrine[(+)-erythrartine] are the tetracyclic alkaloids isolated from the various parts of plants and scoulerine, (+) coreximine, 1-reticuline, and erybidine isolated from leaves.[23] The ethanolic extract yielded chloroform-soluble and water-soluble bases, identified as erysovine and stachydrineJ[24]^ Eight spiroamine alkaloids along with three carboxylated indole-3-alkylmamines - hypaphorine, its methyl ester *andN*, A'-dimethyltryptophan isolated from various parts of plant. Presences of isoquinoline (erythritol) and isococcolinine alkaloids are also reported in the studies.[25]

Flavonoids:

Flavonoids are chemical phenylbenzopyrones, which, usually conjugated with sugars, are present in all vascular plants.(26) Isoflavonoids are reported to be the major phytoconstituents of *E. Variegctta.* It contains mainly erythrinins A, B, and C, osajin and alpinum isoflavone, in addition to the styrene oxyresveratrol and dihydrostilbene dihydroxyresveratrol. Linear pyranoisoflavones, robustone and 4-0-methylalpinum isoflavone are also isolated from the plant.(27,28) The previous studies that reported erycricstagallin, orientanol B, erystagallin A, stigmasterol, campesterol, stigmoidins A, B, and C, phaseollin, 3-P-acetoxy-B-norcholest-5-ene, docosanoic methyl ester, 29-norcycloartenol, p-sitosterol and its archidate, and capric acid as main components refuted by recent well-documented and reliable G1PS College of Pharmacy investigations. Presence of flavonoid abyssinone V, erycricstagallin and 4-hydroxy-6, 3 triprenylisoflavonone was confirmed in other studies. In recent studies two diphenylpropan-l,2-diols, eryvarinols A and B, three new isoflavonoids, eryvarins M-O, new 2-arylbenzofurans, eryvarins P and Q and a 3-aryl-2,3-dihydrobenzofuran, eryvari were isolated from the roots of *E. variegata* and their structures were elucidated on the bas spectroscopic and chemical evidence. Bioassay-directed fractionation of the stem bark ex *ofE. variegata has* resulted in the isolation of three new isoflavones: 5, 4'-dihydroxy-8-i dimethylallyl) -2 "ethoxyisopropylfurano [4, 5:6, 7] isoflavone, 5,7,4'-trihydroxy-6-dimethylallyloxiranylmetyl) isoflavone, 5,4'-dihydroxy-8-(3,3 dimethylallyl hydroxymethyl-2"methylpyranol [5, 6:6, 7] isoflavone and a new isoflavanone, 5,4'dihydi 2"methoxy-8-(3,3-dimethylallyl)-2",2"-dimethylpyranol [5, 6:6, 7] isoflavanone, together seven known compounds, euchrenone bio, isoerysenegalensein E, wighteone, labun lupiwighteone, erythrodiol, and oleanolic acid

Miscellaneous phytoconstituents:

Various other constituents, which have been reported from *E. variegata* in erythrabyssin II, dihydrofolinin,[29] octacosyl ferulate, wax alcohol, wax acids, alkyl fen and alkyl phenolates. Seeds content moisture (3.8%), crude protein (31.2%), pentosan (11 and water-soluble gum (1.6%). The amino acid composition of seed protein is as fo alanine (7.2), arginine (3.4), aspartic acid (12.9), glutamic acid (13.4), glycine (7.6), hi; (3.9), isoleucine (3.6), leucine (7.1), lysine (5.1), methionine (0.5), phenylalanine (3.3), { (4.7), serine (7.1), threonine (5.7), tyrosine (2.2), and valine (4.8) g/lOOg. The seec contain isolecitins (EVLI, EVLII and EVLIII), the kuntz-type trypsin inhibitors (ET ETIb) chymotrypsin inhibitor (ECI)

PHARMACOLOGICAL ASPECTS:

Antibacterial/dental caries prevention:

Isoflavonoids isolated from *E. variegata* has been screened for antibacterial activity against methicillin-resistant *Staphylococcus aureus* and various other strains. Of the active compounds, erycristagallin and orientanol B showed the highest antibacterial activity. The antibacterial effect of erycristagallin to mutans streptococci was based on a bactericidal action. Erycristagallin (6.25ng/ml: MIC) completely inhibited incorporation of radio-labeled thymidine into *Streptococcus* mutans cells. Incorporation of radio-labeled glucose into bacterial cells was also strongly inhibited at MIC, and 1/2 MIC of the compound reduced the incorporation.

Antioxidant:

The generation of free radicals and other reactive oxygen species in the body is compensated by an elaborate endogenous antioxidant system. However, due to many environmental, lifestyle, and pathological situations, excess radicals can accumulate, resulting in oxidative stress. The potential value of antioxidants in eradicating oxidative stress has provoked researchers to investigate for natural compounds with potent anti-oxidative activity but low cytotoxocity. Crude extract obtained from the *E. variegata* evaluated for their radical scavenging properties and assessed that it could be rich source of natural oxidants for applications.

Analgesic and anti-inflammatory:

The alkaloids extracted from the leaves of E. *variegata* are reported to have anti-inflammatory activity. The leaves and barks are also used in fever and rheumatism. It has been reported that in acetic acid induced writhing model the methanolic extract of the leaf of *E. variegata* at a dose of 500 mg/kg showed significant antinociceptive activity with 49.03% inhibition of writhing response. The results were statistically significant ($P < 0.01$) in omparison to the control. In radiant heat tail-flick model, the extract also showed significant ncrease in the tail flick latency at a dose of 500mg/kg body weight with 36.02% elongation of tail flick time.

Cardiovascular effects:

Despite improved pharmacotherapies and mechanical treatments, cardiovascular disease remains a principal cause of morbidity and mortality worldwide, with every chance that "this burden will increase. The intravenous administration of the *E. variegata* seed extract at a dose, varying from 0.1 to 0.4 mg/kg produced a sharp and short-lived fall in BP, both in cats and rats in acute experiments. On the isolated frog hearts, the extract has no action in smaller dose but at a dose of 5 mg resulted a complete but reversible block of the heart.[29]

CNS effects:

In the study total alkaloid fraction from the bark showed several characteristic pharmacological effects: neuromuscular blocking, CNS depressant, and anticonvulsant Effects which are consistent with the reported uses of the plant extracts in the indigenous system of medicine. *E. variegata* also causes passivity and decreases spontaneous activity with positive grip strength. This indicates CNS relaxant activity (anxiolytic) of this plant. The current therapeutic treatment of epilepsy with modern antiepileptic drugs (AEDs) is associated with side-effects, dose-related teratogenic effects, and approximately 30% of the patients continue to have seizures with current AEDs therapy. Natural products from folk remedies have contributed significantly in the discovery of modern drugs and can be an alternative source for the discovery of AEDs with novel structures and better safety and efficacy profiles. Evidence for anticonvulsant activity of *E. variegata* in the clonic seizure of pentylenetetrazole model has been tested in mice. As the protective effects of *E. variegata* in clonic seizure, it suggests that it could be useful for treatment of absence seizure [30]

Smooth muscle relaxant:

Total alkaloidal fraction from bark caused smooth muscle relaxation of isolated rabbit ileum and inhibited spontaneous rhythmic contraction of isolated rat uterus in concentration of 0.5-2.0mg/mL. *E. variegata* behaves like a spasmolytic agent due to its relaxing activity; therefore, it can play an important role in conditions like diarrhea or spasm or colic pain.

Calcium homeostasis:

E. variegata extracts were evaluated on calcium homeostasis in overiectomized rats and the regulation on gene expression in duodenum and kidney. It improve the serum Ca level and inhibit the urinary Ca excretion in OVX rats, and this might be due to the upregulation of *E. variegata* on VDR mRNA expression in duodenum and CaBP-9k mRNA expression in kidney.

Antiosteoporotic effect:

Study showed that *E. variegata* could suppress the high rate of bone turnover induced by estrogen deficiency, inhibit bone loss and improve the biomechanical properties of bone in the lab rats.

Trypsin/proteinase inhibitors:

Study indicates that erythrina kunitz proteinase inhibitors possess different potency towards serine proteinases in the blood coagulation and fibrinolytic systems, in spite of their high similarity in amino acid sequence.

Cytotoxicity:

The lethality of the n-hexane, carbon tetrachloride, chloroform, and aqueous soluble fractions of the methanolic extract to brine shrimp was evaluated on *Artemia salina.* The LC50 were found to be 36.68, 4.67, 7.733, and 14.289 ug/mL for n-hexane, carbon tetrachloride, chloroform, and aqueous fractions, respectively. In comparison with the positive control (vincristine sulphate), the cytotoxicity exhibited by the carbon tetrachloride and chloroform-soluble fractions of the methanolic extract was significant. This clearly indicated the presence of potent bioactive principles in these extractives, which might be very useful as antiproliferative, antitumor, pesticidal, and other bioactive agents.

MATERIALS & METHODS

METHODOLOGY

The plant *Erythrina Variegata,* collected from the surrounding fields of Narsapur forest and was identified by Botanist. The leaves collected in the month of January were dried in shade at room temperature and size reduced to coarse powder.

PREPARATION OF DIFFERENT EXTRACTS:

Preparation of Aqueous extract:

The marc about l00g was taken in a round bottom flask (2000ml) and macerated with 500ml of distilled water and 10ml of chloroform (preservative) for seven days with occasional shaking for every one hour. Then the marc was removed by filtering the extract, and it was concentrated on a water bath at 50° C to get a semi solid mass. These extract was stored in an airtight container below 10° C in a refrigerator. Their percentage yield was calculated with reference to air dried sample.

Investigation of *Erythrina Variegata* extracts:

Aqueous extract of *Erythrina Variegata* were subjected to the following investigations:

PRELIMINARY PHYTOCHEMICAL SCREENING

The preliminary phytochemical investigations were carried out with aqueous extracts of leaves of Erythrina Variegata for qualitative identification of phytochemical constituents. Phytochemical tests were carried out by standard methods all the chemicals and reagents used were of analytical grade.

i. Test for alkaloids:

a) **Preliminary test:**

A 100 mg of an extract was dissolved in dilute hydrochloric acid. Solution was clarified by filtration. Filtrate was tested with Dragendroff s and Mayer's reagents. The treated solutions were observed for any precipitation.

b) **Confirmatory test:**

Five grams of the extract was treated with 40% calcium hydroxide solution until the extract was distinctly alkaline to litmus paper, and then extracted twice with 10 ml portions of chloroform. Chloroform extracts were combined and concentrated *in vacua* to about 5 ml. Chloroform extract was then spotted on thin layer plates. Solvent system (n-hexane-ethyl acetate, 4:1) was used to develop chromatograms and detected by spraying the chromatograms with freshly prepared Dragendroff s spray reagent. An orange or dark colored spots against a pale yellow background was confirmatory evidence for presence of alkaloids.

ii. Test for steroidal compounds:

a) Salkowski's test:

0.5 g of the extract was dissolved in 2 ml chloroform in a test tube. Concentrated sulfuric acid was carefully added on the wall of the test tube to form a lower layer. A reddish brown colour at the interface indicated the presence of a steroid ring (i.e. the aglycone portion of the glycoside).

b) Lieberman's test:

0.5 g of the extract was dissolved in 2 ml of acetic anhydride and cooled well in an ice-bath. Concentrated sulfuric acid was then carefully added. A colour change from purple to blue to green indicated the presence of a steroid nucleus i.e. aglycone portion of the Cardiac glycosides.

iii. Test for phenolic compounds:

a) To 2 ml of filtered solution of the aqueous macerate of the plant material, 3 drops of a freshly prepared mixture of 1 ml of 1% ferric chloride and 1 ml of potassium ferro cyanide was added to detect phenolic compounds. Formation of bluish-green color was taken as positive.

b) The dried alcoholic extract (100 mg) was dissolved in water. Few crystals of ferric sulfate were added to the mixture. Formation of dark-violet color indicated the presence of phenolic compounds.

iv. Flavonoids:

a) Test for free flavonoids:

Five milliliters of ethyl acetate was added to a solution of 0.5 g of the extract in water. The mixture was shaken, allowed to settle and inspected for the production of yellow colour in the organic layer which is taken as positive for free flavonoids.

b) Lead acetate test:

To a solution of 0.5 g of the extract in water about 1 ml of 10% lead acetate solution was added. Production of yellow precipitate is considered as positive for flavonoids.

c) Reaction with sodium hydroxide:

Dilute sodium hydroxide solution was added to a solution of 0.5 g of the extract in water. The mixture was inspected for the production of yellow color which considered as positive test for flavonoids.

Test for saponins:

Froth test: 0.5 g of the extract was dissolved in 10 ml of distilled water in a test tube. The test tube is stoppered and shaken vigorously for about 30 seconds. The test tube was allowed to stand in a vertical position and observed over a 30 minute period of time. If a "honey comb" froth aove the surface of liquid persists after 30 min. the sample is suspected to contain Saponins

Test For tannins:

Ferric chloride test:

A portion of the extract was dissolved in water. The solution was clarified by filtration. 10% ferric chloride solution was added to the clear filtrate. This was observed for a change in colour to bluish black.

Formaldehyde test:

To a solution of about 0.5 g of the extract in 5ml water, 3 drops of formalehyde and 6 drops of dilute hydrochloric acid were added. The resulting mixture was heated to boiling for 1 minute and cooled. The precipitate formed (if any) was washed with hot water, warm alcohol and warm 5% potassium hydroxide successively. A bulky precipitate, which leaves a colored residue after washing, indicated the presence of phlobatannins.

Test for Phlobatannins:

Deposition of a red precipitate when an aqueous extract of the plant part was .boiled with 1% aqueous hydrochloric acid was taken as an evidence for thepresence of phlobatannins.

Modified iron complex test:

To a solution of 0.5 g of the plant extract in five milliliter of Water drop of 33% acetic acid and 1 g sodium potassium tartarate was added. The mixture warmed and filtered to remove any precipitate. A 0.25% solution of ferric ammonium citrate was added to the filtrate until no further intensification of colour is obtained and then boiled. Purple or blackish precipitates which is insoluble in hot water; alcohol or dilute ammonia denotes pyrogallol tannin present.

Vii. Test for Anthraquinones:

a) Test for free anthraquinones (Borntrager's test)

The hydro-alcoholic extract of the plant material (equivalent to 100 mg) was shaken vigorously with 10 ml of benzene, filtered and 5 ml of 10% ammonia solution added to the filtrate. Shake the mixture and the presence of a pink, red or violet color in the ammonia (lower) phase indicated the presence of free anthraquinones.

b) Test for O-anthraquinone glycosides (Modified Borntrager's test):

For combined anthraquinones, 5 g of the plant extract was boiled with 10 ml 5% sulphuric acid for 1 hour and filtered while hot. The filtrate was shaken with 5 ml benzene; the benzene layer separated and half its own volume of 10% ammonia solution added. The formation of a pink, red or violet color in the ammonia phase (lower layer) indicated the presence of anthraquinone derivatives in the extract.

Viii. Test for Carbohydrates:

The extracts were treated with 3 ml of alpha naphthol in alcohol and Conc.Sulphuric acid was carefully added to side of the test tubes. Formation of a violet ring at the junction of two liquids indicates presence of carbohydrates.

i) **Fehling's Test:** To the sample Fehling's solution A and B was added and heated for two minutes. Appearance of reddish brown color indicates presence of reducing sugars.
ii) **Benedict's Test:** To the sample benedict's solution was added and heated, appearance of reddish orange precipitate indicates presence of reducing sugars.
iii) **Barfoed's Test:** The sample were, treated with Barfoed's reagent and heated, appearance of reddish orange precipitate indicates presence of reducing sugars.

Test for Proteins:

Biuret's Test: To the extracts copper sulphate solution followed by sodium hydroxide solution, a violet color precipitates indicates presence of proteins.

Million's Test: To the extracts million's reagent was added, appearance of pink color indicates presence of proteins. **.**

Test for Gums and Mucilage:

The extracts were treated with 25 ml absolute alcohol and then the solution was filtered. The filtrate was examined for its swelling properties.

Test for Glycosides:

A pinch of the extract were dissolved in glacial acetic acid and few drops of ferric chloride solution was added followed by the addition of Cone.Sulphuric acid, formation of red ring at the junction of the two liquids indicates presence of glycosides.

Test for Terpenes:

The extracts were treated with tin and thionyl chloride, appearance of pink color indicates presence of terpenes.

Pharmacological activities:

1. Toxicity studies (LD)
2. Analgesic activity
3. Anti-inflammatory activity

Pharmacological activities:

Animals; Albino mice (20-30g) and Albino rats (150-200g) procured from Shri Venkateswara Enterprises Bangalore for experimental purpose. The animals were housed into groups of 6-8 per cage at a temperature of 25°±1°C and 45-50% relative humidity. A 12:12 dark:light cycle was followed during the experiments. Animals had free access to food and water ad libitum. However food, but not water was withdrawn 18hr before and during the experiments The studies were approved by the CPCSEA and the institutional ethics committee.

A Determination of acute toxicity (LD):

Method:

The acute toxicity of aqueous extract of leaves of Erythrina Variegata were determined by using albino mice of either sex (20-30g) maintained under standard husbandry conditions. The animals were fasted overnight prior to the experiment and fixed dose (OECD guideline No. 425) method of CPCSEA was adopted for toxicity studies. 175^{th} dose of the lethal dose was taken as effective dose ED50 (Therapeutic dose) for the present study.

Analgesic activity: -

Hot Plate method:

In this study Eddy's hot plate method was used. Here, for screening of central analgesic property, heat is used as a source of pain. Animals were placed individually on the hot plate, maintained at constant temperature (55° C ± 1° C). The reaction time i.e., time taken by the animal for licking its hind paws or to leap out of the plate after placing it on the hot plate was taken as the reaction of painful stimuli and end point. Analgesics should increase the reaction

Requirements:

1. Animal: Albino mice
2. Instrument: Eddy's hot plate
3. Drugs: Pentazocine (75mg/kg), Aqueous extract (200,400mg/kg)

Procedure:

Albino mice of either six were selected and divided into four groups, containing six animals in each group. These animals were fasted for 24 hrs, prior to the experiment.

Group A - Normal Control (treated with 0.2 ml of 2 %Tween -80 p.o.)
Group B - Pentazocine (75 mg / kg, p.o.)
Group C - Aqueous extract (200mg / kg, p.o.)
Group D - Aqueous extract (400mg / kg, p.o.)

The reaction time for each mouse was recorded at 30, 60, 90, 120, 180 and 240 minutes after the administration of the substances by using Eddy's hot plate.

Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Dunnet's test, and in all cases results are expressed as mean ± SEM.

Acetic acid induced writhing model:

Albino mice of either sex, weighing between 20-30 g were selected. They were maintained on standard pellet diet and free access to water.

The animals were divided into six groups of each having six animals. The various groups were treated as follows:

Group A - Normal Control (treated with 0.2 ml of 2 %Tween -80p.o.)
Group B - Diclofenac sodium (20 mg / kg, p.o.)
Group C - Aqueous extract (200mg / kg, p.o.)
Group D - Aqueous extract (400mg / kg, p.o.)

Analgesic activity was evaluated in mice by using the writhing test. After an over night fasting, mice were distributed into six group of each group containing six animals One hour after the oral administration of vehicle, standard and drug extracts, the mice were given with intraperitoneal injection of 0.7 %(V/V) acetic acid solution (0.1 ml/1 Og body weight). The number of writhings produced in these animals was counted for 20min.

The results were subjected for the statistical analysis by ANOVA followed by Dunnet's test to note the significance of result.

Anti-inflammatory activity:

Carrageenan induced rat paw oedema model:

Albino rats of either sex weighing 150 - 200 g were selected. They were maintained on standard pellet diet and free access to water. The animals were divided into 6 groups of each containing six animals. The various groups treated were as follows:

Group A - Normal Control (treated with 0.2 ml of 2 %Tween -80 p.o.)
Group B - Diclofenac sodium (20 mg / kg, p.o.)
Group C - Aqueous extract (200mg / kg, p.o.)
Group D - Aqueous extract (400mg / kg, p.o.)

The normal control, Diclofenac sodium and test extracts were administered to the rats 30 minutes before the injection of O.lml of 1% carrageen and suspension in normal saline at subplantar region of the left hind paw, and the right hind paw served as reference. Immediately thereafter the oedema volume of the injected paws was measured plethysmographically by mercury displacement method. For comparison purpose, the volume of oedema at various prefixed time intervals (0, 1,2,3,4 hours) were measured. The difference between paw volumes of the treated animals was measured and the mean oedema volume was calculated. Percentage reduction in oedema volume was calculated by using the formula-

$$\% \text{ reduction} = \frac{Vo - Vt}{Vo} \times 100$$

Where,
Vo = Volume of the paw of control at time't'
Vt = Volume of the paw of drug treated at time't'.

From the data, the mean oedema *volume*, standard deviation (S.D.), standard error (SEM) and percentage reduction in oedema were calculated.

The results of all the experiments conducted were subjected to one way analysis of variance (ANOVA) and Dunnet's test.

RESULTS

Chemical investigation:-

Preliminary phytochemical investigation was done for alkaloids, carbohydrates steroids, saponins, tannins, triterpenes, flavonoids, proteins and aminoacids, glycosides, fixed oils and fats. It was found that-

Table No l: Preliminary phytochemical test for *E.Variegata* Linn.

Sl.No	Phytochemical Tests	Results
1	Test for Alkaloids	+Ve
2	Test for Carbohydrates	-Ve
3	Test for Proteins	-Ve
4	Test for Steroids	+Ve
5	Test for Sterols	+Ve
6	Test for Phenols	-Ve
7	Test for Flavonoids	+Ve
8	Test for Gums and mucilage	-Ve
9	Test for Glycosides	-Ve
10	Test for Saponins	-Ve
11	Test for Terpenes	+Ve

+ve: indicates the presence of compounds
-ve: indicates the absence of compounds

Pharmacological investigations:-

Toxicity studies:-

In the present study the aqueous extract of leaves of *Erythrina Variegata* were subjected for toxicity studies.

Acute toxicity studies of aqueous extract of leaves of *Erythrina Variegata* were determined in mice, as per OECD guide lines 425.

For the LD50 dose extract was administered up to a limit dose of 5g/kg. The aqueous extract produced mortality at 5g/kg, thus l/5th, l/10th, 1725th ,1750th dose of maximum dose tested were selected for the present study .

LD50 of both extract of *Erythrina Variegata* were calculated and found to be as follows:

Aqueous extract -3162mg/kg

Analgesic activity:

1. Hot- plate method:

The anti-nociceptive activity was expressed as mean basal reaction in seconds The activity of aqueous (200,400mg/kg) extract and standard drug Pentazocine were tested by using Eddy's hot plate with rats at different time intervals i.e.,0,30,60,90,120,150,180,210,240 min.

Statistical analysis by ANOVA indicated a significant difference among the groups after 30, 60, 90,120,150,180,210 and 240minutes.

Dunnets't' test indicated that 200 and 400mg/kg dose of both extracts showed significant activity after 30 minutes when compared to the normal control group.

Table 2: Analgesic effect of aqueous extract *ofE.variegata* on Eddy's hot plate method.

TREATMENT GROUPS	DOSE (mg/kg)	BASAL REACTION TIME			
		0	30	60	120
Control	-	2.0±0.1	2.2±0.2	2.33±0.21	2.28±0.1
Pentazocine	75(mg/kg)	3.1±0.2	7.5±0.3	10.1±0.3**	12.8±0.80**
E.variegata	200(mg/kg)	2.5±0.3	2.8±0.1	4.33±0.3333	4.8±0.7*
E.variegata	400(mg/kg)	2.2±0.5	4.7±0.2*	7.33±0.3333**	8.8±0.5**

All values are given in mean±SD, (n=6) ANOVA *p<0.05, **p< 0.01, ***p<0.001, when compared to control group

2. Acetic acid induced writhing model:

The antinociceptive activity was expressed as mean number of writhings in mice observed for 20 minutes after injection of 0.7 % V/V acetic acid. Diclofenac sodium showed 76.68% protection of writhings, where as aqueous extract with doses 200 and 400mg/kg showed 49.79%, 60.72% after 20 minutes of treatment. =*

ANOVA studies indicated a significant different among the groups after 20mins. Dunnet's test indicates more significant antinociceptive activity with alcoholic extract than aqueous extract.

Table 3: Effects of crude extract on acetic acid induced writhing response in mice.

GROUP	DOSE (mg/kg)	WRITHING"	% INHIBITION
Control -	-	17.30 ±1.34	-
E.variegata	200(mg/kg)	10.41 ±0.74**	39.90
	400(mg/kg)	8.25 ±0.63**	52.40
Aminopyrine	50(mg/kg)	7.16 ±0.76**	58.65
One-way ANOVA	F	25.2	-
	Df	3,20	-
	P	<0.001	-

a) lhr after treatment, mice were injected i.p. with 0.7%(v/v) acetic acid (0.1 ml/1 Og); 10 minutes after the injection, the number writhing was counted for 10 min.

b) Values are mean ± SEM (n = 6); One-way ANOVA; **P<0.001, compared to control.

3. Anti-inflammatory activity:

The two extracts of leaves of *Erythrina Variegata* at dose of 400 and 800 mg / kg exhibited significant anti-inflammatory activity in acute paw oedema model. 800mg/kg dose of Alcoholic extract exhibited maximum inhibition of 90.90% and 400mg/kg dose of alcoholic extract showed maximum inhibition of 81.81% whereas 800mg/kg dose of aqueous extract shown 81.81% and 400mg/kg dose of aqueous extract shown 72.72% respectively, the Diclofenac sodium has shown reduction in oedema volume by 90.90% in carrageenan induced rat hind paw oedema model.

Table 4: Anti-inflammatory activity of crude extract of *E.variegata* by carrageenan induced rat paw edema.

GROUP	*% INCREASE IN RAW VOLUMES (ML X 1000)± SEM (PERCENT INHIBITION)*			
	1HR	2HR	3HR	4HR
Control	70.7 ± 2.06	92.8 ±1.19	107.2 ± 2.27	114 .5 ± 3. 47 1
E.Variegata (200m g/kg)	58.2 ± 1.14** (17.69)	70.3 ±1.91** (24.24)	71.2 ±3.44** (28.77)	83.0 ±2.50** (27.51)
E.variegata (400mg/ kg)	50.3 ± 2.68** (28.77)	63.2 ±1.74** (31.96)	69.2 ± 2.98** (35.46)	72.5 ±2.92** (36.68)
Diclofenac sodium (20mg/kg,p.o)	47.3 ± 1.48** (33.02)	57.7 ±2.64** (37.88)	61.3 ±1.58** (38.72)	71. 7 ±3.04** (37.41)

*probability values (calculated as compared to control using one way-ANOVA followed by Dunnet's Test): **P<0.001 .All values are means of individual data obtained from six rats (n =6)

DISCUSSION

Analgesic Activity:

Analgesic effect of leaf extracts of *Erythrina variegata* was tested in two different models of analgesia. Hot plate method is commonly used to assess analgesia with narcotic drugs. Drugs that act centrally inhibit pain produced by thermal stimulus. Alcoholic and aqueous extracts of leaves of *Erythrina variegata* have produced anti-nociceptive effects against thermally induced pain stimulus in mice in hot plate method at various points after the post treatment. The peak effect was observed at 120min. after drug administration.

In our study's it was found that alcoholic and aqueous extracts of leaves *of Erythrina variegata* had retarded the intensity of acetic acid induced writhing in mice. Acetic acid causes increase in peritoneal fluid level of prostaglandins involved in port peritoneal receptor and inflammatory pain by capillary action Although the test is non specific (eg. anticholinergics and antihistaminics shows the activity in this test) but it is widely used for screening of analgesics that predominantly involve the induction of prostaglandins. The mechanism of analgesic action of both the extracts of *Erythrina variegata* is probably due to blockade of capillary permeability or release of endogenous substances like prostaglandins.

The results with hot plate and writhing model suggest that alcoholic and aqueous extracts of leaves of *Erythrina variegata* inhibits both central and peripheral mechanism of nociception and the alcoholic extract was more potent in blocking the nociceptive mechanism than aqueous extract.

The hot plate methods have been found to be suitable for evaluation of centrally acting analgesics. The nociceptors seem to be sensitized by sensory nerves. The involvement of endogenous substances such as PGs may be minimized in this model. In centrally acting analgesic methods, the drug in200 mg/kg and 400 mg/kg doses was found to be significantly effective. The 30 mg/kg dose was found to be ineffective in this analgesic model for evaluating centrally acting drugs. However, all the three doses were found to be effective in acetic acid-induced abdominal constriction method, that is used to evaluate peripherally acting analgesics In the acetic acid-induced abdominal constriction method, pain is generated indirectly via endogenous mediators like prostaglandin, which stimulates peripheral nociceptive neurons. These neuronal fibers are sensitive to both narcotics and non steroidal anti-inflammatory drugs. The Aqueous extract of *Erythrina variegate* inhibited the acetic acid-induced pain with potency compared to the aspirin. The standard drug Diclofenac sodium which inhibit the peripheral pain induced by direct action of acetic acid in the abdomen by inhibiting the prostaglandin secretion.

Anti - inflammatory activity:

The carrageenan-induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti inflammatory agents, which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis[32] Carrageenan-induced hind paw edema is the standard experimental model of acute-inflammation. The time course of edema development in carrageenan-induced paw edema model in rats is generally represented by a biphasic curve [33]. The first phase of inflammation occurs within an hour of carrageenan injection and is partly attributed to trauma of injection and also to histamine, and serotonin components [34]. The second phase is associated with the production of bradykinin, protease, prostaglandin, and lysosome[35]. Prostaglandins (PCs) play a major role in the development of the second phase of inflammatory reaction which is measured at +3 h[36]

The doses 200 mg/kg and 400 mg/kg of alcoholic extract of *Erythrina variegata* produced a significant inhibition of carrageenan induced paw edema at +3h and +6h. Therefore, it can be inferred that the inhibitory effect of alcoholic extracts of *Erythrina variegata* on carrageenan induced inflammation could be due to inhibition of the enzyme cyclooxygenase and subsequent inhibition of prostaglandin synthesis. Significant inhibition of paw edema in the early hours of study by *Erythrina variegata* could be attributed to the inhibition of histamine [32] and/or serotonin. The decrease in paw edema inhibition at +6h may be attributed to the termination of test drug action.

CONCLUSION

On the basis of these findings, it may be inferred that aqueous extract of Erythrina *variegata* has analgesic and anti-inflammatory activities. These activities were related to the dose and these results corroborate the potential traditional use of the plant in folk medicine. At present, there are no reports on investigation to identify the active components present in ethanolic extract of *Erythrina variegata.* Further investigations are anticipated to identify the active components and lead to their further clinical use.

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