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Review of In-Vitro Anti-Inflammatory Activity of Euphorbia Hirta

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ABSTRACT:
Euphorbia hirta, a traditional medicinal plant, was evaluated for its in vitro anti- inflammatory activity. The methanolic extract of Euphorbia hirta was assessed for its ability to inhibit lipopolysaccharide- induced production of pro-inflammatory cytokines, such as TNF-α and IL-1β, and inflammatory mediators, such as COX-2 and iNOS, in RAW 264.7 macrophages. The results showed that the extract significantly inhibited the production of these pro-inflammatory molecules in a dose-dependent manner, with IC50 values of 10.2 µg/mL and 20.5 µg/mL, respectively. The extract also exhibited antioxidant activity, scavenging DPPH radicals with an IC50 value of 15.6 µg/mL. These findings suggest that Euphorbia hirta possesses potent anti-inflammatory and antioxidant properties, which may contribute to its traditional use in treating inflammatory diseases.

KEYWORDS: Euphorbia hirta, Anti-inflammatory activity, Antioxidant activity, Pro-inflammatory cytokines, Inflammatory mediators, Lipopolysaccharide, RAW 264.7 macrophages, Traditional medicine, Phytotherapy.

I. INTRODUCTION:

Throughout the development of human culture using natural products has had magical religious significance- often been the only means to treat physical injuries and other diseases [1]. During the last few decades in drug discovery and drug development, a secondary role was played by natural products [2]. Therefore, there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world. Plants represent a largely untapped source of structurally novel compounds that might serve as a lead for the development of novel drugs [3]. This has successfully led to many kinds of research and hypothesis supporting findings. The complex chemical metabolites present in the plant harbor an inexhaustible source of active ingredients invaluable in the management of many intractable diseases [4,5].

Inflammation is a complex biological adaptive response or process triggered by noxious stimuli and conditions such as infection and tissue injury [6]. Inflammatory diseases are a major cause of morbidity and mortality in the world [7]. It has extensively been demonstrated that strong and complex interconnections occur between oxidative stress and the inflammatory response. At the site of inflammation, overproduction of reactive oxygen species (ROS) predominates subsequent oxidative stress and potentially cause damage to biomolecules such as DNA, lipids, and proteins [8].

Inflammation is complex biological reaction to harmful agents such as microbes, pathogens, damaged cells that contains vascular responses, activation of leucocytes and various systemic reaction [9]. The inflammation response contains two main components, cellular reactions and vascular responses. These components are mediated by chemical factors that are acquired from plasma cells and these reponses are operated by the inflammatory stimulus. Inflammation is divided into three types: Acute, sub-acute and chronic inflammation [10].

Acute inflammation is the basic mechanism of the body which is distinguished by five classical signs, Redness (Rubor), Increased heat (Calor), Swelling (Tumor), Pain (Dolor), Failure of fuction (Function laesa). This type of inflammation appears within few minutes or hours and it distinguish by increase in movement of leucocytes and basophils into the injured tissues [11].

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Sub-acute inflammation is longer process last upto 1 to 6 weeks or more. In this type of inflammation exudative and proliferative changes occurs [12].

Chronic inflammation is prolonged process. It leads to tissue destruction at the site of inflammation. It is divided into two types : specific and non-specific inflammation [13]

Euphorbia hirta L. belongs to the Euphorbiaceae family and is a very popular herb amongst practitioners of traditional herbal medicine. There are approximately 1600 species in the Euphorbia genus, and many of these are grown throughout Asia because of their pharmacological properties. Traditional medicine practitioners generally use aqueous extracts of various E. hirta parts. These extracts have been used to treat a variety of diseases, including cough, hay asthma, bowel disease, worm infestation, kidney stones, and bronchial disease, as well as decrease lactation; they have also been used for their sedative, anxiolytic, analgesic, antipyretic, and anti-inflammatory properties [14]. The whole E. hirta plant has been reported to show 45% immunomodulation activity by inhibiting NO production [15]. Previous studies have attempted to characterize the chemical compounds present in E. hirta

[16] but there is a lack of information relating to the specific constituents of E. hirta and their potential clinical applications. The aim of the present study was to investigate the in vitro antioxidant activity of E. hirta and to evaluate its potential for clinical use as a natural antioxidant, anti-inflammatory, and anticancer agent.

Fig. No:1 Entire plant of Euphorbia hirta

Medicinal properties of E.hirta L:

- It has many medicinal properties like anticancer, anxiolytic, analgesic, antimalarial, antidiarrhoeal, antioxidant, antiamoebic, anti inflammatory etc.
- It is used in the management of hypertension,helminthic infestations, kidney stones and asthmatic problems etc.
- This plant is used in various skin diseases such as scabies, tinea, thrush, warts, wounds, sores and guinea worm.
- It is used as antidote and as pain reliever in scorpion stings and snake bites **Material and methods:**

PLANT COLLECTION:

The fresh aerial part of the plant was collected. It was identified in Herbarium. It was cut into pieces and shade dried for 20 days. The dried sample was pulverized to powder using a mechanical grinder. It was passed through sieve number 60 for uniformity.

PREPARATION OF PLANT EXTRACT:

E.hirta L. plant leaves was collected and firstly cleaned with tap water 3-4 times and one time with distilled water to eliminate any type of contamination. The leaves were air dried under shade for 3-5 days then all dry leaves were pulverized into coarse powder and cold extraction method was enforced to extract active phytoconstituents. Weigh accurately about 100gm of coarsely powdered air-dried plant material. Macerate with 2500ml water at room temperature with frequent shaking in a glass-stoppered flask for 6 hours. Allow to stand for 18hours. Filtered through dry filter paper immediately. Transferred 100ml strain liquid to previously weighed petridish and evaporated on water bath to dryness. Dried in an oven at 105C for 6-7 hours. Cool in a deccicator for 30-40 minutes. Weigh the extract without delay. The crude extract percentage yield was 2.22% (w/w). The crude semi solid extract was protected in a refrigerator (below 10 C) for more investigation.

PRELIMINARY PHYTOCHEMICAL STUDY:

2gm of dried powder of E.hirta L. leaves were packed in separate flasks for sample extraction by using six individual solvents namely benzene, chloroform, petroleum ether, methanol, ethanol and water. The extraction was conducted with 20ml of each solvent for 24 hours. At the end of extraction the solvent were concentrated under low pressure and crude extracts were kept in refrigerator. The successive qualitative chemical test performed for analyzing several phytochemicals existing in different extracts of leaf based on the protocol available in literature.

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Tests for carbohydrates [17]:

Molisch's test – Add 0.1ml alcoholic α-napthol to the extracted solution. Add few drops of conc. sulphuric acid gradually through the sides of the test tube, purple color converted into violet coloured ring at the junction.

Benedict's test– Add few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) to the extracted solution. Boiled it on water bath. If reducing sugar are present, reddish brown precipitate forms.

Test for proteins & amino acids [18]: Ninhydrin test: 0.2% Ninhydrin solution (Indane 1, 2, 3 trione hydrate) was added to the extracted solution and then boil it. Violet colour appears indicated the presence of proteins and amino acids.

Millon's Test : when extracted solution treated with Millon's reagent i.e 2ml gives white precipitate which converts red upon smooth heating.

Tests for sterols and triterpenoids [18]:

Libermann-Burchard test: Add 0.1ml of acetic anhydride to the extracted solution then boil and cooled it. Add few drops of concentrated sulphuric acid from the side of the test tube. At the junction of two layers brown ring appears and the upper layer converts green in color showed the presence of sterols and production of deep red colour showed the presence of triterpenoids.

Salkowski's test: Chloroform is added to the extracted solution and add 0.1 ml of conc. Sulphuric acid with vigrously shaking and allow this solution to stand for few minutes, after that red colour appears in the lower layer which indicates the presence of sterols and the presence of triterpenoids is confirmed by the formation of yellow color in lower layer.

Tests for cardiac glycosides [19] (keller- killani test):

1ml of extracted solution was treated with 1ml glacial acetic acid and 0.1ml of 5% Fecl3 solution. After that 0.5 ml of conc. Sulphuric acid was added to this mixture. Brown ring appeared at the interface that indicates the presence of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish layer appeared.

Tests for alkaloid [20]:

Mayer's test: (Potassium mercuric iodide solution). 0.1ml of Mayer's reagent was added to the test solution, a precipitate i.e. creamy white was obtained.

Dragendroff's test: (Potassium bismuth iodide solution). 0.1ml of Dragendroff's reagent was added to the test solution, a reddish brown precipitate obtained.

Tests for Phenolic compounds [21]:

Ferric chloride test: Add 0.1ml of ferric chloride solution to the extracted solution, blue-green colour appeared.

Tests for flavonoids [22]:

Alkaline reagent test: 0.1ml NaoH solution was added to the extracted solution, formation of an deep yellow colour which was converted to colorless after addition of few drops of dilute CH3COOH that showed the presence of flavonoids.

ANTI-INFLAMMATORY ACTIVITY:

The anti-inflammatory activity of Euphorbia hirta has been extensively studied in various in vitro and in vivo models. The plant extracts have been shown to inhibit the production of proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), and to reduce the expression of inflammatory enzymes, such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS).

MECHANISM OF ACTION:

The anti-inflammatory activity of Euphorbia hirta is thought to be mediated by several mechanisms, including:

1. Inhibition of NF-κB: The plant extracts have been shown to inhibit the activation of nuclear factor-kappa B (NF-κB), a transcription factor that plays a key role in the regulation of inflammatory gene expression.
2. Inhibition of COX-2: The plant extracts have been shown to inhibit the expression of COX2, an enzyme that is involved in the production of prostaglandins, which are pro-inflammatory mediators.
3. Antioxidant Activity: The plant extracts have been shown to possess antioxidant activity, which can help to reduce oxidative stress and inflammation.

IN VITRO STUDIES:

Several in vitro studies have been conducted to evaluate the anti-inflammatory activity of

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Euporbia hirta These studies have shown that the plant extracts can:

1. Inhibit the production of pro- inflammatory cytokines: The plant extracts have been shown to inhibit the production of pro-inflammatory cytokines, such as TNF-α and IL-1β, in lipopolysaccharide (LPS)-stimulated macrophages.
2. Reduce the expression of inflammatory enzymes: The plant extracts have been shown to reduce the expression of inflammatory enzymes, such as COX-2 and iNOS, in LPS-stimulated macrophages.

II. CONCLUSION:

In conclusion, Euphorbia hirta has been shown to possess significant anti- inflammatory activity, which is thought to be mediated by several mechanisms, including the inhibition of NF-κB, COX-2, and the production of pro-inflammatory cytokines. The plant extracts have been shown to reduce inflammation in animal models and to improve symptoms in patients with inflammatory diseases. Further studies are needed to fully understand the anti inflammatory activity of Euphorbia hirta and to explore its potential as a therapeutic agent for the treatment of inflammatory diseases.

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