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Evaluation of antioxidant and anti-inflammatory activity of *Euphorbia heyneana* Spreng

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ABSTRACT

Objective: To assess the antioxidant and anti-inflammatory activities of the alcoholic extract of *Euphorbia heyneana* (*E. heyneana*) in carrageenan induced inflammation in rats. **Methods:** *In vitro* antioxidant activity was evaluated for superoxide radical, hydroxyl radical and DPPH radical scavenging activity. Three doses 200, 400 and 800 mg/kg were tested for anti-inflammatory activity in carrageenan induced rat paw oedema model and paw thickness was measured every one hour up to 6 hours. **Results:** The alcoholic extract of *E. heyneana* produced dose dependent inhibition of superoxide radical, hydroxyl radical and DPPH radicals. In carrageenan induced inflammation model, all three doses produced significant percentage inhibition of rat paw oedema and 800 mg/kg dose produced maximum percent inhibition of rat paw oedema (47.06%) among the three doses compared to control group. **Conclusions:** It can be concluded that alcoholic extract of *E. heyneana* shows good *in vitro* antioxidant and *in-vivo* anti-inflammatory activities in rats.

1. Introduction

Free radicals are unstable, highly reactive molecules that lose an electron as a result of this activity. Since electrons come in pairs, when molecules lose an electron, they "steal" electrons from other molecules. These molecules then "steal" electrons from other molecules, thus starting a dangerous chain reaction called "free radical damage." Reactive oxygen species (ROS) are widely believed to be involved in the etiology of many diseases including inflammation as indicated by the signs of oxidative stress seen in those diseases. Inflammation is our body's natural reaction to invasion by an infectious agent, toxin or physical, chemical or traumatic damage. One purpose of inflammation is to protect the site of an injury.

Euphorbia heyneana (*E. heyneana*) belongs to family Euphorbiaceae. In Telugu language it is called Alumu. It is an annual, prostrate herb, dichotomously branched. It is distributed India, Pakistan, Bangladesh, Myanmar, and Indonesia. In India it is distributed in Andhra Pradesh, Tamil Nadu, Karnataka and Bihar states. The plant extract is beneficial in jaundice. It is able to normalize the level of lipid accumulation induced by carbon tetrachloride in liver of rats. It is also able to lower the elevated levels of serum bilirubin[1].

There has been no study to evaluate the antioxidant and anti-inflammatory activity of orally administered whole plant alcoholic extract of *E. heyneana*. Hence, the present study was done to assess the *in vitro* antioxidant and *in vivo* anti-inflammatory activities of orally administered alcoholic (using 70% v/v ethanol) extract of *E. heyneana* in carrageenan induced inflammation in rats.

2. Materials and methods

2.1. Plant material and extraction

E. heyneana was collected from Rajahmundry region of Andhra Pradesh state, India. A voucher specimen was deposited in our laboratory. The authenticity of the plant

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was confirmed by Taxonomist Dr. Prayaga Murty Pragada, Department of Botany, Andhra University, Visakhapatnam. Freshly collected *E. heyneana* whole plant was dried under shade and was made into coarse powder. The coarse powdered aerial part was macerated in 70% v/v ethanol. The liquid extract was collected and evaporated under reduced pressure by using rotary evaporator (Buchi R-210) until a soft mass obtained and used for investigation.

2.2. Drugs and chemicals

Folin-Ciocalteau (FC) reagent, riboflavin, deoxyribose, nitroblue tetrazolium, 2,2-diphenyl-1-picrylhydrazyl (DPPH), indomethacin, sodium carboxy methyl cellulose (Na CMC) and carrageenan were purchased from Sigma Chemicals, USA. All other chemicals used were of analytical grade.

2.3. Animals

Adult Wistar rats (National Institute of Nutrition, Hyderabad, India) of either sex weighing (200-250 g) were used in the studies. The animals were maintained under standard laboratory conditions at an ambient temperature of (23±2) ℃ having (50±5)% relative humidity with 12-hour light and dark cycle. The use and care of the animals in the experimental protocol has been approved by the Local Institutional Animal Ethics Committee (Regd. No. 516/01/A/CPCSEA) following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

2.4. Determination of total phenolic content

Total phenolic content was determined using the Folin-Ciocalteau reagent[2]. Folin-Ciocalteau colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue absorption with a maximum at 765 nm. The intensity of the light absorption at that wave length is proportional to the concentration of phenols. Gallic acid was used as standard for the calibraion curve. The total phenolic content was expressed as mg gallic acid equivalents (GAE) per gram of sample (mg/g).

2.5. In-vitro antioxidant activity

In a healthy body, ROS and antioxidants remain in balance. Nevertheless, when this balance is disrupted towards an excess of ROS, oxidative stress occurs[3]. Recently an intensive search for novel types of antioxidants has been carried out from numerous plant materials[4,5]. The alcoholic extract of *E. heyneana* selected was screened for the following free radical scavenging activities.

2.5.1. Superoxide radical scavenging activity

Superoxide scavenging activity of the plant extract was determined by the method of McCord and Fridovich[6], which depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitroblue tetrazolium.

2.5.2. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe^{2+}/EDTA/H_2O_2 system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances[7].

2.5.3. DPPH radical scavenging activity

The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca *et al*[8]. This assay is based on the measurement of the ability of antioxidants to scavenge the stable radical DPPH. The free radical DPPH is reduced to the corresponding hydrazine when it reacts with hydrogen donors.

2.6. Acute inflammation model: carrageenan induced rat paw oedema

Five groups of rats were treated orally with 1% Na CMC, 5 mg/kg indomethacin, 200, 400 and 800 mg/kg of alcoholic extract of *E. heyneana*, respectively. Sixty minutes later, an injection of 1% carrageenan in normal saline was made into the subplantar region of the right hind paw of each rat in each group.

Before induction of oedema, the dorsiventral thickness of both the paws of each was measured using Zeitlin's apparatus[9] which consists of a graduated micrometer, combined with a constant loaded lever system to magnify the small changes in the paw thickness during the course of experiment. The measurements were taken at 1 hour intervals after induction of oedema for up to 6 hours. Oedema was monitored as the percentage increase in paw thickness in the carrageenan injected paw. To assess the oedema in control paw (right) saline was injected subcutaneously.

The percent inhibition of paw thickness is calculated using the formula:

Percentage inhibition = $100\times[1-(Yt / Yc)]$

Yt = Average increase in paw thickness in groups tested with test compounds.

Yc= Average increase in paw thickness in control.

2.7. Statistical analysis

Data of paw thickness were analyzed buy using one-way ANOVA followed by *post hoc* test Dunnett's test using Graph pad Prism-5 software. The results were expressed as Mean± SEM. $P<0.05$ was considered as significant.