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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–Ciocalteu (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) °C 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–Ciocalteu reagent². Folin–Ciocalteu 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 calibration 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 occurs³. 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 Fridovich⁶, 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 Fe²⁺/EDTA/H₂O₂ system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances⁷.

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 Zeitelin's apparatus⁹ 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:

$$\text{Percentage inhibition} = 100 \times [1 - (Y_t / Y_c)]$$

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

$$Y_c = \text{Average increase in paw thickness in control.}$$

2.7. Statistical analysis

Data of paw thickness were analyzed by 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.

3. Results

3.1. Total phenolic content

The phenolic content in alcoholic *E. heyneana* extract was found to be (54.21±2.11 mg/g GAE) by using standard gallic acid calibration curve.

3.2. In-vitro antioxidant activity

3.2.1. Superoxide radical scavenging activity

The alcoholic extract of *E. heyneana* produced dose dependent inhibition of superoxide radicals ranging from (43.17±1.50)% to (91.22±2.12)%. The mean (inhibition concentration) IC₅₀ values for superoxide radical by alcoholic extract of *E. heyneana* and ascorbic acid were found to be 68.11 and 62.27 µg, respectively.

3.2.2. Hydroxyl radical scavenging activity

The alcoholic extract of *E. heyneana* produced dose dependent inhibition of hydroxyl radicals ranging from (32.54±1.23)% to (78.34±2.20)%. In the present study, alcoholic extract of *E. heyneana* was found to possess concentration dependent scavenging activity on hydroxyl radicals. The standard drug ascorbic acid showed better percentage of inhibition of superoxide radicals than alcoholic extract of *E. heyneana*.

Table 1

Effect of alcoholic extract of *E. heyneana* on carrageenan induced rat paw edema (mean±SEM).

Treatments	Mean increase in paw thickness in mm at		Percent inhibition at	
	3 hour	6 hour	3 hour	6 hour
1% Na CMC (1 mL)	6.80±0.12	6.10±0.11	–	–
Indomethacin (5 mg/kg)	3.12±0.22***	3.00±0.12***	54.12	50.82
<i>E. heyneana</i> extract (200 mg/kg)	4.40±0.11***	4.10±0.13***	35.29	32.79
<i>E. heyneana</i> extract (400 mg/kg)	3.80±0.10***	3.70±0.11***	45.59	39.34
<i>E. heyneana</i> extract (800 mg/kg)	3.42±0.21***	3.12±0.13***	47.06	48.85

***: $P < 0.001$ comparing with the control.

4. Discussion

Superoxide anion radical (O₂⁻) is one of the strongest ROS among the free radicals that are generated first after oxygen is taken into living cells[10]. Superoxide anion plays an important role in the formation of more reactive species such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which induce oxidative damage in lipids, proteins, and DNA. Therefore, studying the scavenging activity of plant extracts/compounds on superoxide radical is one of the most important ways of clarifying the mechanism of antioxidant activity.

Among the ROS, the hydroxyl radicals are the most reactive and predominant radicals generated endogenously

The mean IC₅₀ values for hydroxyl radical by alcoholic extract of *E. heyneana* and ascorbic acid were found to be 346.61 and 87.05 µg, respectively.

3.2.3. DPPH radical scavenging activity

The alcoholic extract of *E. heyneana* produced dose dependent inhibition of DPPH radicals ranging from (46.12±1.50)% to (91.03±2.12)%. The data were displayed with mean±SDE of twice replications. The mean IC₅₀ values for hydroxyl radical by alcoholic extract of *E. heyneana* and ascorbic acid were found to be 67.55 and 55.24 µg, respectively.

3.3. Carrageenan induced rat paw oedema

The alcoholic extract of *E. heyneana* at the doses of 200, 400 and 800 mg/kg exhibited significant ($P < 0.05-0.001$) percentage inhibition of paw oedema at 3 hour after carrageenan injection ranging from 35.29%, 45.59%, to 47.06% compared to control group. The standard drug indomethacin at 5 mg/kg dose showed maximum percentage of inhibition of paw oedema 54.12% and among the three doses, 800 mg/kg dose showed maximum percentage inhibition of maximal paw oedema (47.06%) (Table 1).

In the present study we found that alcoholic extract of *E. heyneana* showed concentration dependent free radical scavenging activity and this antioxidant effect may responsible for *in-vivo* anti-inflammatory activity of the alcoholic extract of *E. heyneana*.

during aerobic metabolism. Due to the high reactivity, the radicals have a very short biological half-life. The generated hydroxyl radicals initiate the lipid peroxidation process and/or propagate the chain process *via* decomposition of lipid hydroperoxides. A single hydroxyl radical can result in formation of many molecules of lipid hydroperoxides in the cell membrane, which may severely disrupts its function, and lead to cell death.

The DPPH test provided information on the reactivity of test compounds with a stable free radical. Because of its odd electron, DPPH gives a strong absorption band at 517 nm in visible spectroscopy (deep violet color)[11]. Because of the ease and convenience of this reaction, it now has widespread use in the free radical scavenging activity assessment.

The alcoholic extract of *E. heyneana* was found to possess concentration dependent scavenging activity on superoxide, hydroxyl and DPPH radicals. The standard drug ascorbic acid showed better percentage of inhibition of superoxide radicals than alcoholic extract of *E. heyneana*.

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury^[12–20]. The development of oedema in the paw of the rat after the injection of carrageenan is due to the release of histamine, serotonin, prostaglandin and the like^[21,22]. Acute hind paw oedema is induced in rats by injecting 0.1 mL of 1% v/v carrageenan which reaches a peak edema levels at 3–5 hours after carrageenan injection. Prostaglandin-E2 (PGE2), a powerful vasodilator, synergizes with other inflammatory vasodilators such as histamine and bradykinin and contributes to the redness and increased blood flow in areas of acute inflammation^[23].

In the present study we found that alcoholic extract of *E. heyneana* showed concentration dependent free radical scavenging activity and this antioxidant effect may be responsible for *in-vivo* anti-inflammatory activity of the alcoholic extract of *E. heyneana*.

Conflict of interest statement

We declare that we have no conflict of interest.

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