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Investigation of antioxidant and anti-inflammatory activity of leaves of *Dalbergia paniculata* (Roxb)

Ganga Rao B[1], Madhu Kiran P[2], Vijaya Raju AD[2*]

[1]*Pharmacognosy and Phytochemistry, A.U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, A.P, India-530 003*
[2]*A.U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, A.P, India-530 003*

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ABSTRACT

Objective: To investigate the antioxidant and anti-inflammatory activity of orally administered methanolic leaf extract of *Dalbergia paniculata* (*D. paniculata*) 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 mg/kg, 400 mg/kg and 800 mg/kg of *D. paniculata* were tested for anti-inflammatory activity in Carrageenan induced rat paw edema model and paw thickness was measured every one hour up to 6 h. **Results:** The methanolic leaf extract of *D. paniculata* 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 edema and 800 mg/kg dose produced maximum percent inhibition of rat paw edema (47.83%) at 3h compared to control group. **Conclusions:** In the present study we found that methanolic leaf extract of *D. paniculata* showed good *in vitro* antioxidant activity and *in vivo* anti-inflammatory activity in rats.

1. Introduction

Dalbergia paniculata (*D. paniculata*) belongs to the family Fabaceae and is commonly called as Padru pachhali. It is annual, prostrate herb, dichotomously branched. It is distributed in India, Mexico, America and in some other tropical regions. The plant is found in many parts of India like Goa, Gujarat, Haryana, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Meghalaya, Orissa, Rajasthan, Tamil Nadu, Uttar Pradesh. Nepal, Andaman and Nicobar, Cambodia, Laos, Myanmar, Vietnam. The plant was claimed to be used traditionally used in the treatment of various ailments including rheumatism[1,2].

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 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.

Previously no study was conducted to evaluate the antioxidant and anti-inflammatory activity of *D. paniculata*. Hence, the present study was done to assess the in vitro antioxidant and in vivo anti-inflammatory activity of orally administered methanolic (using 70% v/v ethanol) leaf extract of *D. paniculata* in Carrageenan induced inflammation in rats.

*Corresponding author: Vijaya Raju A.D, M.Pharm., (Ph.D), A.U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, A.P, India-530 003.
E-mail: addepallivijay5@gmail.com
Tel: +91-9966647666

2. Materials and methods

2.1. Plant material and extraction

D. paniculata was collected from Araku region, Visakhapatnam district of Andhra Pradesh state, India. A voucher specimen (BGR/DP/11/2009) was deposited in college herbarium. The authenticity of the plant was confirmed by Taxonomist Dr. Prayaga Murty Pragada, Andhra University, Visakhapatnam. Freshly collected leaves of *D. paniculata* are dried under shade and pulverized in to coarse powder. The coarse powder was macerated in 70% v/v methanol. The macerate was collected by filtering and it was concentrated under reduced pressure by using rotary evaporator (Buchi R-210) until a soft mass was obtained. This was collected, labeled and stored in dessicator for further investigation.

2.2. Drug and chemicals

Riboflavin, Deoxy ribose, Nitroblue tetrazolium, 2, 2-Diphenyl -1-picrylhydrazyl (DPPH), Indomethacin, Sodium Carboxy Methyl cellulose (Na CMC) and Carrageenan were purchased from Sigma chemicals, USA. All chemicals used were of analytical grade.

2.3. Animals

Adult Wistar rats (National Institute of Nutrition, Hyderabad, India) of either sex weighing 220-250 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 h 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. In vitro antioxidant activity

In a healthy body, reactive oxygen species (ROS) and antioxidants remain in balance. Nevertheless, when this balance is disrupted towards an excess of reactive oxygen species, oxidative stress occurs[3]. Recently an intensive search for novel types of antioxidants has been carried out from numerous plant materials[4, 5]. The methanolic leaf extract of *D. paniculata* was screened for following free radical scavenging activity

2.4.1. Superoxide radical scavenging activity

Superoxide scavenging activity of the plant extract was determined by McCord & Fridovich, 1969 method[6], which depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitroblue tetrazolium.

2.4.2. 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/H_2O_2 system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances[7].

2.4.3. DPPH radical scavenging activity

The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca *et al*., 2003[8]. This assay is based on the measurement of the ability of antioxidants to scavenge the stable radical 2, 2-diphenyl-1-picrylhydrazil (DPPH.). The free radical DPPH is reduced to corresponding hydrazine when it reacts with hydrogen donors.

2.5. Acute inflammation model: carrageenan induced rat paw edema

Five groups of rats were treated orally with 1% Sodium CMC, 5 mg/kg Indomethacin, 200 mg/kg, 400 mg/kg and 800 mg/kg of *D. paniculata* respectively. Sixty minutes later, an injection of 1% carrageenan in normal saline was made into the sub-plantar region of the right hind paw of each rat in each group.

Before induction of edema, the dorsiventral thickness of both the paws of each was measured using Zeitlin's apparatus[9, 10] 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 edema for up to 6 h. Edema was monitored as the percentage increase in paw thickness in the Carrageenan injected paw. To assess the edema in control paw (right) saline was injected subcutaneously.

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

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

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

Y_c= Average increase in paw thickness in control.

2.6. Statistical analysis

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

3. Results

3.1. In vitro antioxidant activity

3.1.1. Superoxide radical scavenging activity

The *D. paniculata* produced dose dependent inhibition of superoxide radicals ranging from (45.07±1.40) % to (79.57±3.20)%. In the present study, the *D. paniculata* was found to possess concentration dependent scavenging activity on superoxide radicals. The standard drug ascorbic acid showed better percentage of inhibition of superoxide radicals than *D. paniculata*. The mean (Inhibition concentration) IC_{50} values for superoxide radical by *D. paniculata* and Ascorbic acid were found to be 69.3 μg and 80.2 μg respectively.

3.1.2. Hydroxyl radical scavenging activity

The *D. paniculata* produced dose dependent inhibition of hydroxyl radicals ranging from (26.07±1.20)% to (77.01±2.40)%. In the present study *D. paniculata* was found to possess concentration dependent scavenging activity on hydroxyl radicals. The standard drug Ascorbic acid showed better percentage of inhibition of hydroxyl radicals than *D. paniculata*. The mean IC_{50} values for hydroxyl radical of *D. paniculata* and ascorbic acid were found to be 112 μg and 190.2 μg respectively.

3.1.3. DPPH radical scavenging activity

The *D. paniculata* produced dose dependent inhibition of DPPH radicals ranging from (44.95±1.20) to (85.02±1.60). The data are displayed with Mea ± SEM error mean of twice replications. The mean IC_{50} values for DPPH radicals by *D. paniculata* and ascorbic acid were found to be 70.6 μg and 60.24 μg respectively

3.2. Carrageenan induced rat paw edema

The methanolic leaf extract of *D. paniculata* at the dose of 200 mg/kg, 400 mg/kg and 800 mg/kg exhibited significant (P<0.05-0.001) percentage inhibition of paw edema at 3 h after carrageenan injection ranging from 33.34% , 40.58%, 47.83% compared to control group. The Standard drug Indomethacin 5 mg/kg dose showed maximum percentage of inhibition of paw edema 55.1%

Table 1
Effect of methanolic leaf extract of *D. paniculata* on Carrageenan induced rat paw edema.

Group/Drug	Dose	Mean increase in paw thickness (mm)	
		3h	6h
Group I/1%Na CMC	1 mL	6.90±0.14	6.30±0.11
Group II/Indomethacin	5 mg/kg	3.20 ±0.27*	3.10±0.9*
Group III/ DPLE	200 mg/kg	4.60±0.18*	4.30±0.15*
Group IV/ DPLE	400 mg/kg	4.10±0.11*	3.90±0.8*
Group V/ DPLE	800 mg/kg	3.60±0.14*	3.20±0.11*

* Significance at $P < 0.001$ compared with control group, DPLE- methanolic leaf extract of *D. paniculata*. Values are expressed as Mean ± SEM.

4. Discussion

Superoxide anion radical (O_2^-) is one of the strongest reactive oxygen species among the free radicals that are generated first after oxygen is taken into living cells[11]. 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 reactive oxygen species, the hydroxyl radicals are the most reactive and predominant radicals generated endogenously 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, 2, 2-Diphenyl-Picryl Hydrazyl radical (DPPH) gives a strong absorption band at 517 nm in visible spectroscopy (deep violet color)[12]. Because of the ease and convenience of this reaction it now has widespread use in the free radical-scavenging activity assessment.

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury[13]. The development of edema in the paw of the rat after the injection of Carrageenan is due to the release of histamine, serotonin, prostaglandin and the like[14, 15]. Acute hind paw edema 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[16].

In the present study, the *in vitro* antioxidant study of the methanolic leaf extract of *D. paniculata* was done against

superoxide, hydroxyl and DPPH radical scavenging activity. The DPLE showed good free radical scavenging activity in a dose dependent manner and in turn the results were comparable to the free radical scavenging activity of the standard drug Ascorbic acid.

Carragenan induced rat paw edema model is the most commonly used screening model for testing the anti-inflammatory activity of the plant extracts. The methanolic leaf extract of *D. paniculata* was given in three doses of 200 mg/kg, 400 mg/kg and 800 mg/kg. The dose 800 mg/kg showed maximum percentage inhibition (47.83%) of paw edema at 3 h after carrageenan injection. We found that methanolic leaf extract of *D. paniculata* showed concentration dependent free radical scavenging activity and this antioxidant nature might be responsible for its *in vivo* anti-inflammatory activity[17-22].

It can be concluded that methanolic leaf extract of *D. paniculata* possess in vitro antioxidant activity and *in vivo* anti-inflammatory activity against carrageenan induced rat paw edema model.

Conflict of interest

The authors declare that there are no conflicts of interest.

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