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Research Article

ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF *PARTHENIUM HYSTEROPHORUS* LINN.

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ABSTRACT

The present study was conducted to evaluate the antioxidant and anti-inflammatory activity by *Parthenium hysterophorus* Linn. Antioxidant activity of ethanolic extract leaves of *Parthenium hysterophorus* Linn was studied by using *in-vitro* model i.e. DPPH (1, 1–diphenyl picrylhyrazyl) method . DPPH free radical scavenging method was studied by taking various concentrations of test compound (10-100 µg/ml) in the methanol and 1 ml of each concentration was added to 1ml of 0.1mM solution of DPPH and after 30 mins its absorbance was taken at 517 nm. Acute oral toxicity was studied at a dose of 2000 mg/kg body weight (OECD-425), so this dose was considered as a therapeutic dose. The anti-inflammatory activity was done by *in-vivo* model i.e. the carrageenan induced rat paw edema model, this extract reduced carrageenan induced rat paw edema in a dose dependent manner, achieving high degree of antioxidant and anti-inflammatory activity using standard ascorbic acid and Indomethacin. The study shows that *Parthenium hysterophorus* Linn shows a significant antioxidant and anti-inflammatory activity at a dose of 200 mg/kg. It shows potent inhibition in inflammation after 30 min of dosing in rats as compared to control group, and standard group, further increase in dose i.e. 400 mg/kg it shows ulcerogenic responses.

Keywords: anti-inflammatory , ethanolic.

INTRODUCTION

A free radical is defined as any chemical species that contains unpaired electrons. These unpaired electron produces a highly reactive free radical which react with inhaled oxygen in our biological system and produces ROS (reactive oxygen species) and RNS (reactive nitrosative species) which is commonly termed as oxidative stress and nitrosative stress **(Winyard P G, 1988)** .When, there is an imbalance between the production of ROS and antioxidant molecules in the body. However, the excess production of ROS species can damage or inhibit the normal function of lipids, protein and DNA. This effect is observed due to the conversion of oxygen molecule intracellulary into the reactive oxygen species or free radicals (reduction mechanism) which is toxic to the cells and tissues. These reactive oxygen species trigger the production of pro-inflammatory cytokinin and chemokinin mediators **(Nemat Khansari, 2009)**.

The mechanism of inflammation is attributed, to release of ROS from activated neutrophils and macrophages. ROS over production results tissue injury by damaging macromolecules and lipid peroxidation of membranes. In addition, it propagate inflammation by stimulating release of cytokines such as IL-1, TNF- α and interferon-γ which are responsible for the recruitment of additional neutrophils and macrophages. Thus free radicals are important mediators that provoke or sustain inflammatory responses and their neutralization by antioxidants and radical scavengers can reduce inflammation **(Filomena Conforti, 2008)**.

Parthenium hysterophorus Linn also known as carrot grass, congress grass, chatak chandini. This plant was accidently introduced in India during the transportation of cereal. Plant used as a analgesic in neuralgia, antipyretic, tonic, febrifuge and its root decoction was helpful in amoebic dysentery. The whole plant possess the various pharmacological activity such as anticancer activity on A549 cell line **(Siva kumar Ramamurthy, 2011),** skeletal muscle relaxant activity **(Urmilesh Jha, 2011)** and hypoglycemic activity **(Vijay S Patel, 2008)**. The present study reports the Anti-inflammatory and free radical scavenging action of *Parthenium hysterophorus* Linn leaves extract.

MATERIALS AND METHOD

Plant material

The fresh leaves of *Parthenium hysterphorus* Linn was collected from the Meerut (India) in the month of July to August and the plant was identified and authenticated by the research officer of botany at National Bureau of Plant and Genetic Resources (Pusa campus) New Delhi and a voucher specimen had been kept in the department of Pharmacology, Meerut Institute of Engineering and Technology, Meerut. For the preparation of ethanolic extract, the leaves of *Parthenium hysterophorus* Linn was collected ,cleaned, air dried and grinded into coarse powder, then 22 gm powder was packed into the soxhlet extractor with 95% ethanol (170ml) at a temperature 45ºC. The extract was concentrated and dried by using rotary evaporator and the percentage yield was found to be 26.8%, extract was stored in a refrigerator at 5ºC.

Animal

The Wistar albino male rat of body weight 150-200 gm was used for screening of both pharmacological activity. They were kept in Propylene cages with relative humidity of 40-50% under 12 hr light and dark cycle. All animals were kept in quarantine area for a week before use. They were feed with standard diet and water *ad libitum*. The test and standard drug were taken with1% solution of carboxy methyl cellulose. All the pharmacological experimental protocols were approved by the Institutional animal ethics committee (Sanction No: CPCSEA/a/02/711 and 29-oct-2002.

ACUTE ORAL TOXICITY

According to the OECD Guideline 425 the female mice of body weight 27.3gm was selected and administered a dose of 2000mg/kg by oral route and observed for 72hr. No change in behavior, no mortality and no sign of toxicity were observed. When no sign of toxicity was observed then a dose 5000mg/kg was administered. At this dose, the mortality was observed then again 2000mg/kg dose was administered to 6 mice. But no mortality had been observed. So the 1/ 10 of this dose i.e. 2000 mg/kg were selected for pharmacological screening. The dose of study is 200mg/kg and 100mg/kg **(Lorke D, 1983)**.

ANTIOXIDANT ACTIVITY

DPPH Free Radical Scavenging Method

A stock solution of 100µg/ml was prepared of *Parthenium hysterophorus* Linn as well as of standard ascorbic acid. Different concentrations were made of 10, 20, 30, 40, 50 and 100 µg/ml from stock solutions using methanol and 0.1mM solution of DPPH in methanol was prepared in a volumetric flask which was completely kept away from light. Then 1ml of all concentration of test and standards were mixed with 1ml of DPPH solution. This solution was kept for 30 min in dark. Only methanol with DPPH was used as

control. Absorbance of all the samples was taken on UV-spectrophotometer, 1700 Shimadzu at a λmax of 517nm.

$$\textbf{(\% Inhibition} = [\{A_{cont} - A_{test/std}\} / A_{cont}] \times 100\textbf{)}$$

$A_{test/std}$ = Absorbance of test or standard

A_{cont} = Absorbance of control

IC_{50} values were calculated from linear regression by plotting the graph between concentration and % inhibitions (Bandgar, B.P, 2009). IC_{50} resembles that it was that concentration of the sample which is required to scavenge 50% of DPPH free radicals .

Compounds	% Scavenging						IC_{50}
	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50 µg/ml	100µg/ml	
Standard(Ascorbic acid)	17.627	35.118	57.355	80.5932	88.135	92.203	40.01
Parthenium hysterophorus Linn	12.203	28.034	59.562	69.324	85.813	93.737	52.02

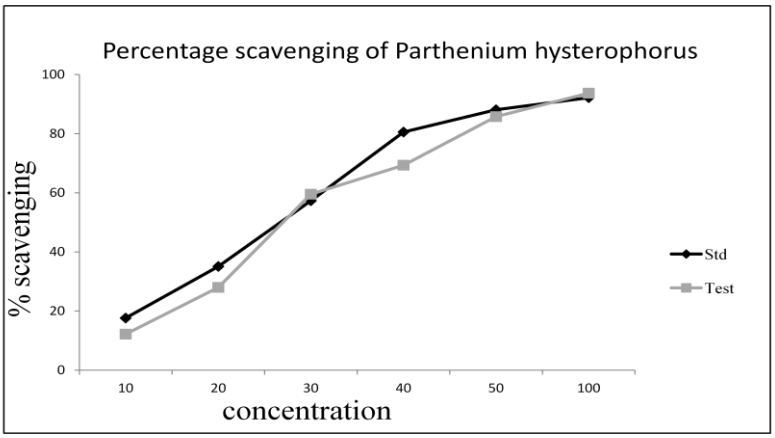


Figure 1: Comparative free radical scavenging activity of *Parthenium hysterophorus* Linn at a concentration of 10-100 µg/ml incubated for 30 minute with DPPH (0.1mM) at 517 nm as compared to standard Ascorbic acid.

ANTI-INFLAMMATORY ACTIVITY

Carrageenan-induced rat paw edema

Four group of six rat were taken. Group I (control) was treated with carboxymethylcellulose (10ml/kg) which is used as a vehicle. Group II (Standard) was treated with 10mg/kg of Indomethacin by oral route. Group III and Group IV (test) was treated with 200 mg/kg and 100mg/kg of *Parthenium hysterophorus* Linn by oral route. After 30 mins of test drug administration, 0.1% of carrageenan (0.1ml) was administered into the sub plantar tissue of right hind paw (Raji Y, 2002). After a duration of 30min, 1 hr, 2hr, 3hr and 4hr, the paw volume was measured by the digital plethylsmograph. The percentage of edema inhibition was calculated by the formula.

$$\text{Percentage inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

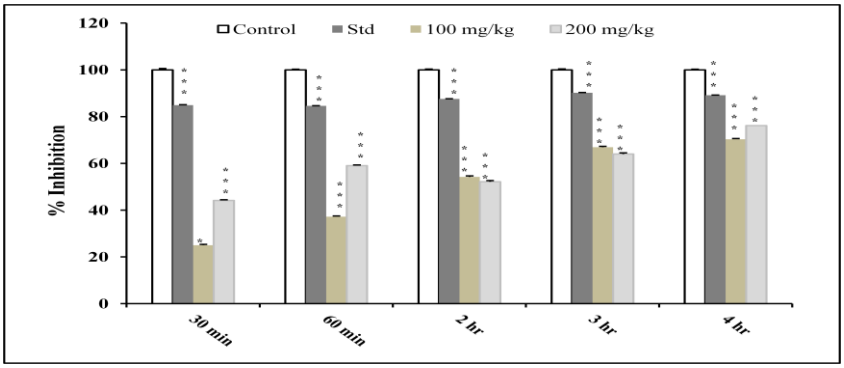
Where

V_c = Mean increase in paw volume of control group.

V_t = Mean increase in paw volume of treated group/standard group

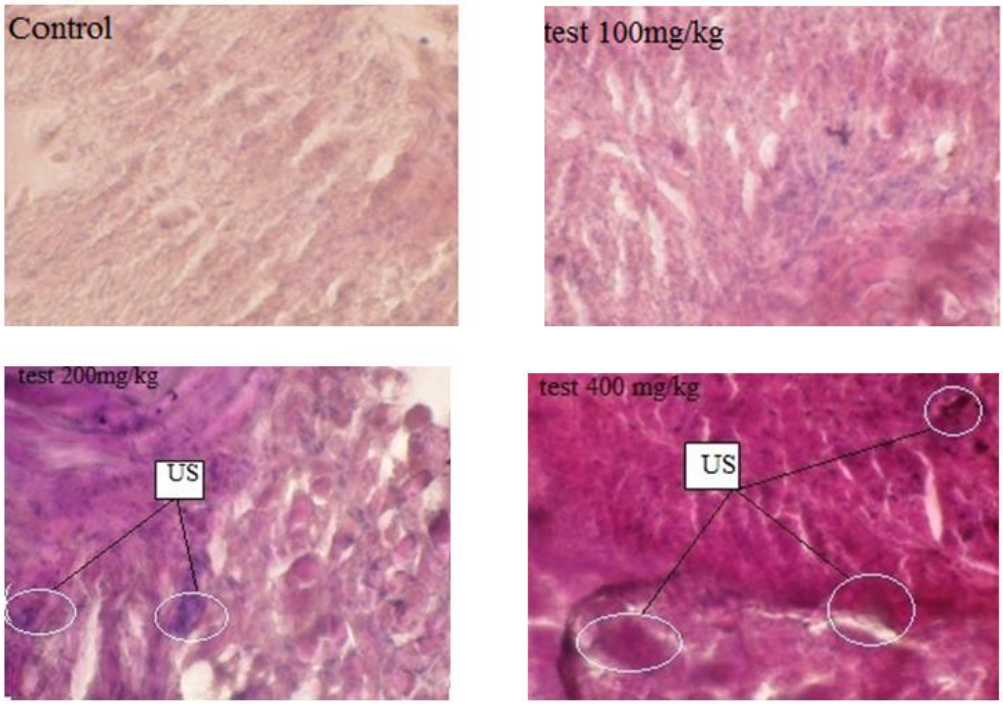
Group	Dose	Increase in Paw edema				
		30 min	60 min	2hr	3hr	4hr
Control	10ml/kg	4.51±0.44	4.71±0.16	5.10±0.20	5.52±0.28	6.08±0.15
Standard (Indomethacin)	10mg/kg	0.68±0.18*** (84.92%)	0.725±0.06*** (84.6%)	0.63±0.08*** (87.58%)	0.54±0.11*** (90.21%)	0.66±0.06*** (89%)
Test (Parthenium)	200mg/kg	2.52±0.31*** (44.12%)	1.93±0.30*** (59.02%	2.44±0.48*** (52.15%	1.98±0.48*** (63.98%)	1.45±0.25*** (76.25%)
Parthenium	100mg/kg	3.36±0.315* (25%)	3.02±0.32*** (37.18%)	2.68±0.42*** (54.18%)	2.02±0.41*** (66.88%)	1.72±0.26*** (70.34%)

All values are significant and are express in mean ± SEM (n=6). ****p ≤ 0.001,P ≤ 0.01,* * p≤0.05 indicates the level of statistical significance as compared with control.**



***Parthenium hysterophorus* Linn shows significant anti-inflammatory activity at 100 mg/kg and 200 mg/kg in compared to control. * p≤0.05 less significant, ** p≤0.01 significant, *** p≤0.001 highly significant.**

Ulcerogenic response of *Parthenium hysterophorus* Linn:



US= Ulcer Spot

S.no	Group	Dose	No of ulcer spot
1	Control	10 ml/kg	0.5±0.05
2	*Parthenium hysterophorus* Linn	100mg/kg	0.16±0.17
		200 mg/kg	2.5±0.42**
		400 mg/kg	3.66±0.33***

All values are expressed as mean ± SEM (n=6) and ***$p \leq 0.001$,$P \leq 0.01$, * $p \leq 0.05$ indicates the level of statistical significance as compared with control .**

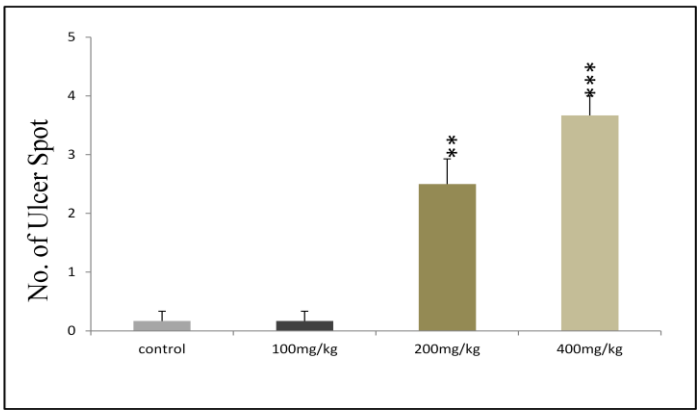


Figure 2: *Parthenium hysterophorus* Linn shows significant ulcerogenic activity in rats at a dose of 100, 200 and 400 mg/kg as compared to control and ***$p \leq 0.001$,$P \leq 0.01$, *$p \leq 0.05$ indicates the level of statistical significance as compared with control.**

STATISTICAL ANALYSIS

All data was expressed as mean ± SEM. It was analysed using one way anova, turkey test and the results were considered as highly significant, moderate significant and less significant when p valve is $p \leq 0.001$, $p \leq 0.01$ and $p \leq 0.05$ respectively.

RESULT AND DISCUSSION

Antioxidant activity

The scavenging activity of *Parthenium hysterophorus* Linn was evaluated by taking various concentration (10, 20, 30, 40, 50, 100µg/ml) of test and standard was prepared in methanol and 1 ml of above concentration was added in 0.1mM solution of DPPH radical. However, the *Parthenium hysterophorus* Linn exhibits 50-60% free radical scavenging activity at a concentration of 100 µg/ml. The Profile of the scavenging effect of *Parthenium hsterophorus* Linn are compared to ascorbic acid which is used as a reference compound.

Anti-inflammatory activity

The *in-vivo* anti-inflammatory activity was performed by the carrageenan induced rat paw edema. *Parthenium hysterophorus* Linn shows highly significant anti-inflammatory activity at dose of 200mg/kg and a lesser effect was observed at a dose of 100mg/kg. The percentage change in paw volume was observed at 30min, 1 hr, 2hr, 3hr and 4hr.

CONCLUSION

The ethanolic extract of *Parthenium hysterophorus* Linn was screened for their *in-vitro* antioxidant and *in-vivo* anti-inflammatory activity. This Plant shows potent antioxidant activity as compared to the ascorbic acid. It also shows highly potent anti-inflammatory activity at a dose of 200mg/kg and a lesser effect at 100mg/kg as compared to control. For the future aspect this plant at a dose of 200mg/kg is used to reduce the inflammation. So this Plant has a beneficial effect in the field of allopathic medicine.

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