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IN VIVO EVALUATION OF ANTI-INFLAMMATORY AND ANTIOXIDANT POTENTIAL OF AQUEOUS EXTRACT OF ASTRAEUS HYGROMETRICUS ON ALBINO RATS

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ABSTRACT

Background and aims - Medicinal plants have been tested for their potential therapeutic effects. This study was designed to screen the in vivo anti-inflammatory activity of Astraeus hygrometricus in acute inflammation. Materials and methods – Anti-inflammatory activity was assessed by Carrageenan induced rat paw edema method. Albino rats of either sex were divided in 5 groups (N=6) [Group-I – saline control; Group- II, III and IV aqueous extracts of 200, 400, 600 mg/kg, P.O. respectively and Group- V Diclofenac1mg/kg, P.O. The paw volume was measured initially at 0, 1, 2 and 3hr after carrageenan injection using digital plethysmometer. Results - Astraeus hygrometricus showed significant anti-inflammatory activity at doses 200, 400 and 600 mg/kg compared to the control group ($p < 0.05$). On superoxide free radicals extracts showed radical scavenging at all concentrations, which is lower than used standard ascorbic acid. The extract showed highest activity at concentration 250µg/ml. Extracts showed highest scavenging activity on H_2O_2 at 200µg/ml concentration. Conclusions – The aqueous extract of Astraeus hygrometricus has a good anti-inflammatory activity and considerable antioxidant potential.

KEYWORDS: Astraeus hygrometricus, anti-inflammatory, carrageenan, antioxidant

INTRODUCTION

It is realized that lots of today's diseases are owing to the "oxidative stress". There are considerable evidences which showed that cellular damage caused by reactive oxygen species (ROS), sometimes partly, in the etiology and pathophysiology of human diseases such as neurodegenerative disorders (e.g. Alzheimer disease, Parkinson disease, multiple sclerosis, Down's syndrome), inflammation, viral infections, autoimmune pathologies, and digestive system disorders such as gastrointestinal inflammation and ulcer.[1, 2] Medicinal plants are used as an important source of new chemical substances with potential therapeutic effects.[3, 4] Over the past two decades, an expanding body of evidence from epidemiological and laboratory studies have demonstrated that some edible plants as a whole, or their identified ingredients with antioxidant properties have substantial protective effects on human carcinogenesis. Similar evidence also exist to demonstrate the chemopreventive capacities of ethnobotanicals and components of vegetable diets with free-radical scavenging potential on ulcers, diabetes, memory and cognitive function, Alzheimer's disease, age-related neurological dysfunction, cardiovascular and renal disorders and several other human ailments.

The research with various plants with alleged folkloric use as pain relievers, anti-inflammatory agents, should therefore be considered as a fruitful and logical research strategy in finding new analgesic and anti-inflammatory drugs.[5]

Astraeus hygrometricus, commonly known as the hygroscopic earthstar, the barometer earthstar, or the false earthstar, is a species of fungus in the Diplocystaceae family.[6] They are regularly consumed in Asia, including Nepal and South Bengal.[7] The sample of this plant extract has exhibited potential anti-inflammatory activity both in vitro and in vivo.[8] This study was designed to screen the in vivo anti-inflammatory activity of Astraeus hygrometricus in acute inflammation.

The purpose of our study was to estimate the antioxidant potential and free radical scavenging activity of water extract of Astraeus hygrometricus.

MATERIALS AND METHODS

All chemicals were commercially purchased and were of AR grade and used without any further purification. Astraeus hygrometricus were purchased from the market. Carrageenan, 2, 2-diphenylpicryl- 1-picryl-hydrazyl

(DPPH), Nitroblue tetrazolium (NBT), NADH, Phenazine methosulphate (PMS), Hydrogen peroxide (H_2O_2)was purchased from Himedia Labs, India. Throughout the experiments double distilled water was used.

Preparation of Astraeus hygrometricus extract

Commercially available Astraeus hygrometricus was collected, grinded and shade dried to remove the moisture. From this 20 g of sieved powder was extracted in 100 ml sterile distilled water. The extract was centrifuged and supernatant was preserved at 4ºC for the study.

Animals

Adult albino rats of both sexes weighing between 150-200 gm were used for the experiment. The animals are kept in standard conditions with 12 hours light and dark cycle. They were allowed to take foods in the form of dry pellet and water ad libitum.

Anti-inflammatory activity by Carrageenan induced rat paw edema method

Anti-inflammatory activity was assessed by Carrageenan induced rat paw edema method.[9] Albino rats of either sex weighing 200 – 250 g were divided in 5 groups (N=6).

- Group-I received 0.9% NaCl solution (control),
- Group- II, III and IV received aqueous extract (200, 400, 600 mg/kg, P.O. respectively) of Astraeus hygrometricus respectively.
- Group- V received Diclofenac (reference standard 1mg/kg, P.O).[10]

Animals were treated with drugs by oral route and subsequently 1 h after treatment; 0.1ml of 1% suspension of carrageenan in normal saline was injected into the sub planter region of left hind paw to induce edema. The paw volume was measured initially at 0, 1, 2 and 3hr after carrageenan injection using digital plethysmometer. The difference between the initial and subsequent values gave the actual edema volume which was compared with control.

ANTIOXIDANT ACTIVITY BY DIFFERENT SCAVENGING METHODS

DPPH Free Radical Scavenging

The DPPH radical scavenging activity of extract was measured to evaluate the antioxidant potentiality. Phosphate buffer (2 ml, pH 6.86), freshly prepared DPPH in ethanol solution (2 ml, 0.5 mM) and samples (0.5 ml) with different concentrations were mixed. The mixture was shaken and incubated at room temperature for 30 min. The reduction of the DPPH radical was measured by the absorbance at 517 nm.[11,12]

Superoxide anion scavenging

Scavenging activity by superoxide anion using the extract was measured by mixing1ml NBT solution, 1ml NADH solution and 0.1 ml extract. The reaction was stimulated by adding 100µl PMS solution to the mixture. The reaction mixture was incubated at 25^{o}C for 5 minutes and absorbance at 560 nm was measured.[13]

Hydrogen peroxide radical scavenging

Scavenging activity of Hydrogen of peroxide radical was seen using the extract by mixing with 2 ml of 20 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm.[14]

Statistical analysis

Data analysis was carried out using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. $P < 0.05$ was considered statistically significant. IBM SPSS 22 was used for statistical calculation.

RESULTS

The effect of aqueous extract of Astraeus hygrometricus on carrageenan induced paw edema in rats is shown in table 1. It showed significant anti-inflammatory activity at doses 200, 400 and 600 mg/kg compared to the control group. Table 2 shows percentage inhibition of paw edema in each group.

Table: 1 Effect of aqueous extract of Astraeus hygrometricus on carrageenan induced paw edema in rats

Treatment groups (n=6)	Dose (mg/kg BW)	Edema volume(ml)			
		1 hour	2 hour	3 hour	4 hour
Normal saline (I)	10 ml/kg	2.84 ± 0.31	3.50 ± 0.15	3.61 ± 0.22	3.67 ±0.20
Aqueous extract (II)	200	2.72±0.35[b]	2.63±0.24[a]	2.58 ± 0.254[a]	2.51 ± 0.33[a]
Aqueous extract (III)	400	2.60 ± 0.236[b]	2.46 ± 0.148[a]	2.35 ± 0.172[a]	2.27 ± 0.204[a]
Aqueous extract (IV)	600	2.58 ± 0.284[a]	2.18 ± 0.188[a]	1.98 ± 0.138[a]	1.841 ± 0.156[a]
Diclofenac (V)	1 mg/kg	2.62 ± 0.23[b]	2.55 ± 0.19[a]	2.40±0.19[a]	2.28±0.18[a]

Each value is in mean ± 2SD (N=6 rats); a $P < 0.01$; b $P < 0.05$; One way ANOVA followed by Dunnett's multiple comparison test

Table: 2 Percentage inhibition of paw edema exhibited by aqueous extract of Astraeus hygrometricus

Treatment groups	Dose (mg/kg)	Percent inhibition (%)			
		1 hour	2 hour	3 hour	4 hour
Aqueous extract	200	4.16	24.70	28.38	31.82
Aqueous extract	400	8.27	29.61	34.67	38.24
Aqueous extract	600	8.30	37.62	44.96	49.91
Diclofenac	1 mg/kg	7.55	26.96	33.24	38.01

The effect of aqueous extract of Astraeus hygrometricus on DPPH radical scavenging, superoxide radical scavenging and hydrogen peroxide radical scavenging was carried out by using different concentration of extract as 50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml, 250µg/ml, 300 µg/ml. Ascorbic acid with same concentration used as reaction standard. (Figures 1,2 and 3)

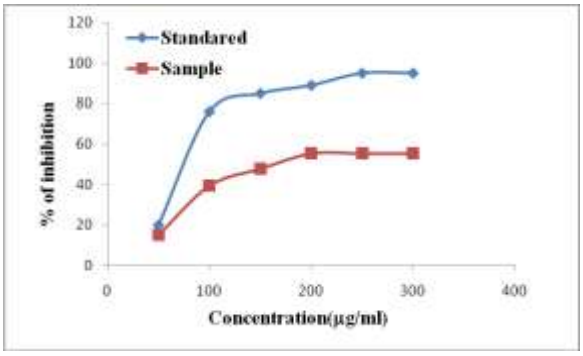

Figure 1: DPPH radical scavenging, Ascorbic acid used as standard

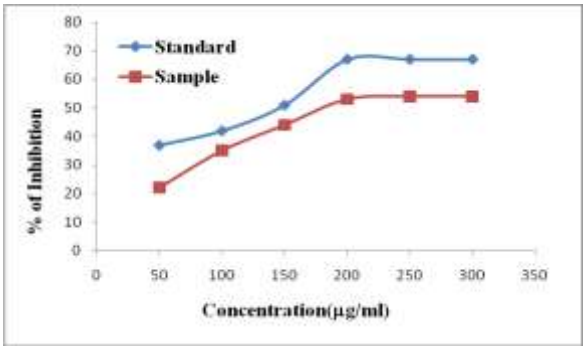

Figure 2: Superoxide free radical scavenging, Ascorbic acid used as standard.

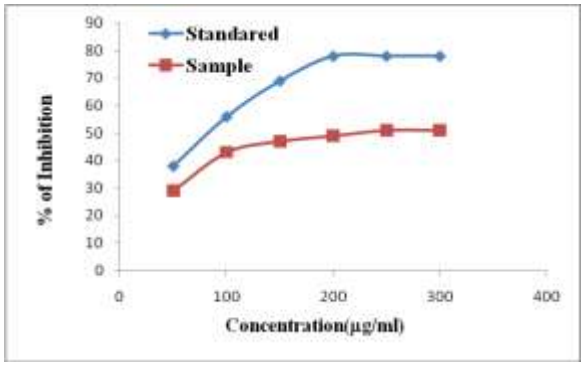

Figure 3: Hydrogen peroxide radical scavenging, Ascorbic acid used as standard.

DISCUSSION

The most commonly used primary experiment to screen herbal products for their anti-inflammatory activity is to measure the ability of a compound to reduce local edema induced in the rat paw by injection of an irritant agent.[9] Carrageenan induced rat paw edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1–2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the inflamed tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages.[15,16]

The significant anti-inflammatory activity shown by the Astraeus hygrometricus extract (200, 400, and 600 mg/kg) over a period of 4 hours in the animal model was quite similar to the result exhibited by the group treated with the standard drug i.e. diclofenac sodium. The highest percentage inhibition activity was seen in the dose of 600 mg/kg with the mean percentage inhibition of 49.91% after 4 hours of administration.

Previous studies with some other plants like Solanum trilobatum,[17,18] Plumeria acuminate[16] and Thesium chinense[19] also showed similar effect in this model. These results indicate that the extract acts in later phases in dose dependent manner, probably involving arachidonic acid metabolites, which produce an edema dependent on neutrophils mobilization.[20] Flavonoids present in this mushroom may have played the key role in its anti-inflammatory activity observed.

Antioxidant compound founds in mushrooms have diverse polarities; various solvents are used to isolate antioxidants. In this study water was preferred as solvents for the extraction.

DPPH radical scavenging, superoxide radical scavenging and hydrogen peroxide radical scavenging are extensively used for the analysis of antioxidant activity.[14] Among all the DPPH method is preferable method for its reliable and accuracy to analysis. Due to the DPPH radical's capacity to join H, it is measured to contain a radical scavenging property. The color changes from violet to yellow due to reduction. In this study ascorbic acid used as standard and Astraeus hygrometricus as a sample with different concentration and showed highest activity at concentration 200µg/ml.

On superoxide free radicals extracts showed radical scavenging at all concentrations, which is lower than used standard ascorbic acid. The extract showed highest activity at concentration 250µg/ml.[12]

H_2O_2 scavenging by extracts may possibly contribute electrons to H_2O_2, thus neutralizing it. Extracts showed highest scavenging activity on H_2O_2 at 200µg/ml concentration (Figure 3). Although hydrogen peroxide itself is not very reactive, it can sometimes motive cytotoxicity by giving hydroxyl radicals in the cell. Thus, removing H_2O_2 is important in food systems.[21,22]

CONCLUSION

It may be concluded from the results observed in the experiment that the aqueous extract of Astraeus hygrometricus has a good anti-inflammatory activity and considerable antioxidant potential. Further investigations should be carried out to explore the biologically active compounds present in it.

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