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IN-VIVO ANTIINFLAMMATORY ACTIVITY OF HYDROALCOHOLIC EXTRACT OF STEM OF *PTEROCARPUS SANTALINUS*

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ABSTRACT

Inflammation is a healing process triggered by the tissue in reaction to an injury caused by infections, irritants, or cell damage. Anti-inflammatory medications utilized routinely have much negative effects.Considering the huge medicinal properties of *P. Santilanus* plant this work is dedicated to elucidate In Vivo anti-inflammatory effect of *Pterocarpus santalinus*. The stem of the plant was collected & subjected to hydroalcoholic extraction. Further qualitative & quantitative estimation of phytochemical was performed by standard procedures. The in vivo anti-inflammatory activity was checked in wistar rats with caragreen induced paw edema. Indomethacin was used as standard drug. Results showed that plant contains phytochemicals like glycoside, flavonoid, saponin, phenolics, protein, carbohydrate, protein. The total phenol & flavonoid content was found to be 0.387 mg/100mg & 0.756 mg/100mg respectively. The groups treated with 100 & 200mg/kg have reduced paw edema of 2.8 ±0.05&1.3 ± 0.04in fourth hour respectively. While using indomethacin as standard drug it was seen that the paw volume was reduced to 0.90±0.05. So, the values indicate that the anti-inflammatory activity of *P. santilanus* is comparable to that of standard drug & reflects its activity in dose dependent manner.

Keywords: Inflammation, Anti-inflammatory drugs, Medicinal plants, Herbal medicines, Phytochemical, *Pterocarpus santalinus*. Carragreen induced paw edema, Indomethacin

INTRODUCTION

Inflammation is the body's strong reaction to any type of injury. Inflammation can be identified by four major symptoms: pain, redness, heat or warmth, and swelling. When any portion of the human body is injured, the arterioles in the surrounding tissue widen. This results in increased blood circulation to the region (redness) Vasoactive drugs also increase arteriole permeability (pore size), allowing blood cells, chemical substances, blood proteins, and fluid to collect in that region. This fluid buildup produces swelling and may compress nerves in the area, causing pain. Furthermore, prostaglandins, which may cause 'irritation' of the nerves and contribute to pain (Medzhitov, 2010; Choy and Panayi, 2001).

Steroids have a significant role in the care of inflammatory diseases, but due to their toxicity, they should only be used for short periods of time or in highly severe conditions where the risks are reasonable. Prolonged use of NSAIDs frequently results in serious adverse effects, particularly gastrointestinal hemorrhage. Following the "golden age" revolution in the 1960s, when almost all classes of essential antibiotics (tetracyclines,

cephalosporins, aminoglycosides, and macrolides) were discovered and the key problems of chemotherapy were solved, history is now repeating itself, and these exciting compounds are in danger of losing their effectiveness due to the rise in microbial resistance. Its current influence on treatment failures related with multidrug-resistant bacteria is significant, and it has become a global public health concern (Li *et al*., 2003; Balouiri *et al*., 2016).

The medicinal herbs have chemical elements that are structurally similar to steroids, and recent clinical research have substantiated their significance as anti-inflammatory agents. The plant biosynthesizes plethora of substances such as alkaloids, glycosides, saponins, steroids, resins, tannins, flavanoids, sesquiterpene lactones, and sesquiterpene lactones that have physiological and medicinal effects. The chemicals found in plants that have therapeutic properties are typically secondary metabolites with a defined chemical structure. These plant metabolites have biological and therapeutic capabilities such as antioxidant, anti-inflammatory, antibacterial, and anticancer activity. Many phenolic substances, including flavonoids, tannins, and curcumins, are hypothesised to function via free radical scavenging or inhibition of pro-inflammatory enzymes such as cyclo-oxygenases (COX) and lipoxygenases (LOX) in inflammatory cascades (Hodzic *et al*., 2009; Sadik *et al*., 2003).

Pterocarpus santalinus L., sometimes known as Res Sanders (RS), is an endemic and endangered wood tree species native to southern India.It is widely dispersed throughout the world's tropical regions, particularly in India, Sri Lanka, Taiwan, and China. Phytochemical analysis of the plant showed the presence of carbohydrates, flavonoids, terpenoids, phenolic compounds, alkaloids, saponins, tannins, and glycoside. It is mentioned in Ayurveda, an Indian system of traditional medicine, that the heartwood of the Externally, the plant is used to treat inflammation, diabetes, headaches, skin problems, and jaundice, as well as in wound healing (Navada and Vittal, 2014; Yadav *et al*., 2019). Considering the huge medicinal properties of this plant this work is dedicated to elucidate *in vivo* anti-inflammatory effect of *Pterocarpus santalinus*.

MATERIALS AND METHODS

Chemical and reagent

Potassium Mercuric Iodide, Potassium Iodide, Iodine, Ferric chloride, Lead acetate, Nitric acid, Copper acetate, Aluminum chloride Potassium Bismuth Iodide, Picric acid, Sodium nitropruside and Sodium hydroxide obtained from Loba Chemical Pvt Ltd (Mumbai, India). Hydrochloric acid, methanol and ethanol were obtained from Merck Ltd, Mumbai, India. All solvents and reagents were of analytical grade.

Collection of plant material

Fresh & healthy stem of *Pterocarpus santalinus* free from diseases were collected from Shubham nursery of Bhopal (M.P.) in the month of January, 2023.

Extraction by soxhlet extraction process

Defatting of plant material

42gram of *Pterocarpus santalinus* shade dried plant material were coarsely powdered and subjected to extraction with petroleum ether

using soxhlet extraction method. The extraction was continued till the defatting of the material had taken place.

Defatted plant materials of *Pterocarpus santalinus* were exhaustively extracted with hydroalcoholic solvent (Ethanol: aqueous: 80:20v/v) by soxhlet extraction method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts (Mukherjee *et al.*, 1998).

In vivo anti-inflammatory activity of extract of *Pterocarpus santalinus*

Animals

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 ℃, 55–65%). Rats received standard rodent chow and water *ad libitum* (Meshram *et al.*, 2016). Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. A separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by the Ministry of Environment and Forests, Government of India, New Delhi, India.

Carrageenan induced hind paw oedema

The antiinflammatory activity was measured using carrageenan-induced rat paw oedema assay. The rats were divided into four groups of 6 animals each (plant extract was dissolved and administered per oral at different dose levels).

Group I: was treated as control (0.1 ml of 1% (w/v) of was treated with carragenan (1%w/v) in saline in the subplanter region of the right hind paw),

Group II: Carragenan+ Stem extract of *Pterocarpus santalinus* -100 mg/kg.

Group III: Carragenan + Stem extract of *Pterocarpus santalinus* -100 mg/kg.

Group IV: Carragenan + Indomethacin (10 mg/kg bw). Oedema was induced by injecting 0.1 ml. of a 1% solution of carrageenan in saline into the sub plantar region of the right hind paw of the rats. The volumes of oedema of the injected and the contralateral paws were measured after the induction of inflammation using a plethysomgraph.

Statistical analysis

All analysis was performed using graph pad prism for Windows. All statistical analysis is expressed as mean ± standard error of the mean (SEM). Data were analyzed by one-way ANOVA, where applicable $p<0.05$ was considered statistically significant, compared with vehicle followed by Dunnett's test.

Table 1: Result of phytochemical screening of extract of *Pterocarpus santalinus*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids A) Hager's Test	 -Ve
2.	Glycosides A) Legal's Test:	 +Ve
3.	Flavonoids A) Lead acetate Test: B) Alkaline Reagent Test:	 +Ve +Ve
4.	Saponins A) Froth Test:	 +Ve
5.	Phenolics A) Ferric Chloride Test:	 +Ve
6.	Proteins A) Xanthoproteic Test:	 +Ve
7.	Carbohydrate A) Fehling's Test:	 +Ve
8.	Diterpenes A) Copper acetate Test:	 +Ve

Table 2: Estimation of total phenolic and flavonoids content of *Pterocarpus santalinus*

S. No.	Hydroalcoholic extract	Total phenol content	Total flavonoids content
1.	*Pterocarpus santalinus*	0.387 mg/100mg	0.756 mg/100mg

Table 3: Effect of *extract of Pterocarpus santalinus* on paw edema induced by carrageenan in rats by different timelines

Groups	Dose (mg/kg)	0 hr	30 min	1 hr	2 hr	4 hr
Group-I	0.1 ml of 1% (w/v)	3.7±0.07	4.1 ±0.04	4.4 ±0.02	4.6 ±0.04	5.0 ±0.04
Group-II	100 mg/kg	2.4±0.06	2.5 ±0.05	2.6 ±0.12	2.7 ±0.15	2.8±0.05
Group-III	200 mg/mg	1.8 ±0.10	1.7 ±0.05	1.5 ±0.5	1.4 ±0.15*	1.3 ±0.04*
Group-IV	10 mg/kg	1.15 ±0.03	1.10 ±0.04	1.00±0.06	0.95±0.03*	0.90±0.05**

Values are expressed as mean ± SD.

*P < 0.05-significant compared to carragenan treated group.

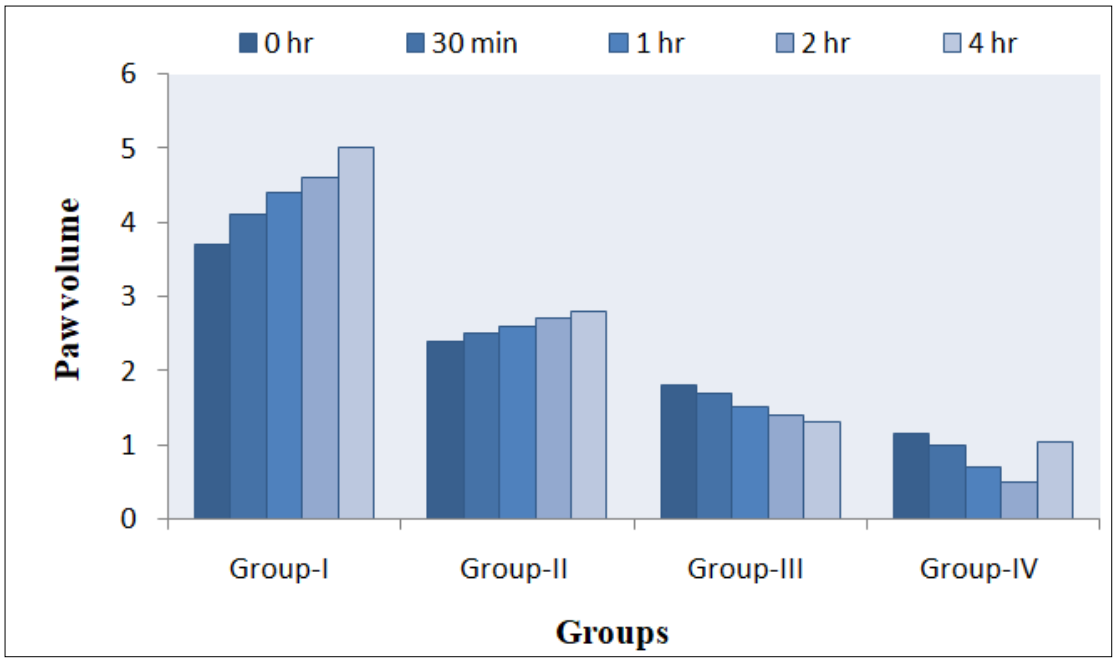

Figure 1: Effect of *extract of Pterocarpus santalinus* on paw oedema induced by carrageenan in rats

RESULTS AND DISCUSSION

The phytochemical screening revealed the presence of glycoside, flavonoid, saponin, phenolics, protein, carbohydrate, protein. The total phenol & flavonoid content was found to be 0.387 mg/100mg &0.756 mg/100mg respectively. The paw edema measurements were taken at different time points after administering the treatments, such as 0 minutes, 30 minutes, 1 hour, 2 hours, and 4 hours, and the values were recorded using techniques such as calipers or plethysmometry.

The effect of an extract of *Pterocarpus santalinus* on paw edema induced by carrageenan in rats was evaluated at different time intervals. The study involved four groups of rats, each receiving a different dose of the extract. The paw edema was measured at 0 hours, 30 minutes, 1 hour, 2 hours, and 4 hours after carrageenan administration.

In Group-I, which served as the control group and received 0.1 ml of 1% (w/v) solution, there was a gradual increase in paw edema over time, indicating the induction of inflammation by carrageenan.

In Group-II, treated with 100 mg/kg of the *Pterocarpus santalinus* extract, there was a mild decrease in paw edema compared to the control group at all time intervals. In this group, the edema was founds to be reduced by 2.8 ±0.05. This suggests a potential anti-inflammatory effect of the extract.

In Group-III, treated with 200 mg/kg of the extract, a significant reduction in paw edema was observed at 2 hours and 4 hours compared to the control group. In fourth hour, the size of paw edema reduced to 1.3± 0.04. This indicates a dose-dependent effect of the extract on reducing inflammation.

In Group-IV, treated with 10 mg/kg of the extract, there was a noticeable decrease in paw edema at all time intervals, with the most significant reduction seen at 2 hours and 4 hours. While using indomethacin as standard drug it was seen that the paw volume was reduced to 0.90±0.05. This further supports the anti-inflammatory activity of the extract, even at a lower dose.

CONCLUSION

Our findings reveal that hydroalcoholic extract has strong anti-inflammatory effect, with maximum inhibition of edoema similar to the standard medicine indomethacin (10 mg/kg) on carrageen induced paw edoema and experimental trauma induced rat paw edoema in a dose dependent fashion. This anti-inflammatory activity may be attributed to the presence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenols. The extract fractions act as free radical inhibitors or scavengers or possibly as primary oxidants, inhibiting heat-induced albumin denaturation, proteinase activity, and stabilising the Red Blood Cells membrane. *Pterocarpus santilanus* hydroalcoholic extracts demonstrated minor anti-inflammatory efficacy. This research implies that *Pterocarpus santilanus* hydroalcoholic could be a promising option for the discovery of novel anti-inflammatory medicines. Purification of each bioactive ingredient is required, and this purified form of the compound can be employed, potentially resulting in greater activity.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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