

SCREENING OF THE ANTI-INFLAMMATORY ACTIVITY OF SIMAROUBA GLAUCA EXTRACT AGAINST FORMALDEHYDE INDUCED INFLAMMATION IN RATS

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Article Received on
06 June 2018,

Revised on 26 June 2018,
Accepted on 17 July 2018,

DOI: 10.20959/wjpr201815-12963

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ABSTRACT

Objective: The aim of the current study was to evaluate in-vivo Anti-inflammatory activity of *Simarouba glauca* against formaldehyde induced inflammation in Wistar albino Rats. **Methods:** The methanolic extract of *Simarouba glauca* was observed in formaldehyde (acute)-induced hind paw oedema test in rats. The *Simarouba glauca* were dried under shade and then powdered, and extracted with 90% methanol by reflux. Preliminary phytochemical studies and acute toxicity studies were also carried out. Inflammation was induced in the left hind paw of Wistar rats by injecting 0.1 ml of 4% formaldehyde solution below the plantar aponeurosis of the hind paw of the rats in the last day of the experiment. Group 1 served as normal control while

group 2 was considered as disease control. Group 3 was standard receiving Diclofenac 10 mg/kg and Groups 4 and 5 disease animals were treated with *Simarouba glauca* (250 mg/kg and 500 mg/kg respectively). The parameters included in this study was to observe the inflammation of the paw edema of rats. **Results:** The present study demonstrated that *Simarouba glauca* extract has an anti-inflammatory action against the acute phase of inflammation using the Plethysmometer. **Interpretation and conclusion:** Significant Anti-inflammatory activity of *Simarouba glauca* observed in the present investigation could be the result of reducing the inflammation. Methanolic extract of *Simarouba glauca* also showed improvisation in reducing the inflammation and may have protective effect in Anti-inflammatory related complications.

KEYWORDS: *Simarouba glauca*, Anti-inflammation, Formaldehyde, Plethysmometer.

INTRODUCTION

Inflammation is a reaction of the body against an aggressive agent, characterized by vasodilatation and access of fluid and cells to the target tissue. One of the major signs of inflammation is the pain that can be triggered by direct stimulation of nociceptors or by the action of inflammatory mediators.^[1] Inflammation is fundamentally a protective response the ultimate goal of which is to help the organism get rid of both, the initial cause of injury (e.g. microbes, toxins) and the consequences of such injury (e.g. necrotic cells and tissues).^[2]

The traditionally anti-inflammatory such as antihistamines, steroids and other nonsteroidal drugs are available associated with some side effects including immunosuppression, gastrointestinal disturbances.^[3] It has prompted a search for new, effective, and safe anti-inflammatory agents.

Further, a large proportion of plant based compounds are used as lead molecules in drug discovery to produce synthetic molecular analogs, implying that phytochemicals play a critical role in diversity oriented synthesis of natural product.^[4] *Simarouba glauca* (Family: Simaroubaceae) is a medium-sized tree that grows up to 20 m high, with a trunk 50 to 80 cm in diameter. It produces bright green leaves 20 to 50 cm in length, small white flowers, and small red fruits. This tree species is a native of Central and South America and found under a wide range of conditions and at low to medium elevation from Southern Florida to Costa Rica, Caribbean islands, Bahamas, Jamaica, Cuba, Hispaniola, Puerto Rico, Nicaragua, Mexico, El Salvador etc. This plant was introduced in India during 1960.^[5]

The main active groups of chemicals in *Simarouba* are called quassinoids, which belong to the main plant chemicals in *S. glauca*.

The previous study of this plant shows that it has antibacterial, antioxidant, hemolytic, thrombolytic activities.^[6]

MATERIALS AND METHODS

Collection and authentication of plant material

The fresh leaves of *Simarouba glauca* were collected and authenticated by Botanist, Bangalore.

Preparation of 90% v/v methanolic extract of *Simarouba glauca*^[7]

The leaves of *Simarouba glauca* was chopped into small pieces and dried under shade at room temperature for seven days. The dried leaves were powdered and passed through the sieve (coarse10/40). The powder was used for the preparation of methanolic extract. Dried and powdered leaves of *Simarouba glauca* (each 1.0 kg) were extracted with boiling 90% MeOH in a reflux condition. After filtration, the solution was concentrated under a vacuum.

Phytochemical analysis of *Simarouba glauca*

Preliminary qualitative analysis of *Simarouba glauca* was analysed qualitatively.

Animals: *Wistar rats* weighing 150-200g were used for the experiment. They were acclimatized for one week prior to experiment. Animals were caged in fully ventilated room, were maintained in 12:12 h light and dark cycle and were housed at temperature of $25 \pm 2^\circ\text{C}$. They had free access to a standard chow diet and water *ad libitum*. All the experiments conducted on the animals were in accordance with the standards set for the use of the laboratory animal use and the experimental protocols were duly approved by the IAEC (Institutional Animal Ethical Committee) of Karnataka College of pharmacy, Bangalore.

Acute Oral Toxicity Study

The acute oral toxicity study was performed according to the OECD guidelines no. 425.

Experimental protocol: Inflammatory reaction is readily produced in rats in form of paw edema in rats with help of irritation, substances like carrageenan, formaldehyde, bradykinin, histamine, 5-hydroxy tryptamine, mustard; when injected in the dorsum of the foot of rats produce acute paw edema within few minutes of injection. Formaldehyde-induced paw edema is one of the most used methods in experimental pharmacology. Animals were weighted and numbered and the hind paws were marked (left and right) just beyond the tibio-torsal junction, so that every time the paw is dipped in mercury up to the fixed mark. The initial volume for both right and left hind paws was marked for each rat by mercury displacement method using Plethysmometer and animals were divided into 5 groups each comprising of 2 rats. Inflammation was induced in the left hind paw of *Wistar rats* by injecting 0.1 ml of 4% formaldehyde solution below the plantar aponeurosis of the hind paw of the rats. Two acute doses (250 and 500 mg/kg) of the methanolic extract of *Simarouba glauca* were administered orally for 7 days. Standard drug Diclofenac was administered 1 hr prior to formaldehyde injection in standard drug group. The paw volume was recorded

immediately prior to compound administration (0 min) and then 15, 30, 60 and 120 min after formaldehyde injection.

Table 1: Experimental protocol.

Group-1	Normal Group	Rats were injected with normal saline
Group-2	Control Group	Rats were injected with 0.1 ml of 4% Formaldehyde solution
Group-3	Standard Group	Rats were treated with Diclofenac 10 mg/kg I.P + 0.1 ml of 4% Formaldehyde solution after 1 hr on 7 th day.
Group-4	Low Dose S.G	Rats were treated with <i>Simarouba glauca</i> methanolic extract dissolved in water as solvent orally, low dose (250 mg/kg for 7 days) + 0.1 ml of 4% Formaldehyde solution on 7 th day
Group-5	High Dose S.G	Rats were treated with <i>Simarouba glauca</i> methanolic extract dissolved in water as solvent orally, High dose (500 mg/kg for 7 days) + 0.1 ml of 4% Formaldehyde solution on 7 th day.

RESULTS

Phytochemical screening

The qualitative analysis of extracts of *Simarouba glauca* were carried out and extracts showed the presence of various chemical constituents such as carbohydrates, saponins, terpenoids, glycosides, phenolics, flavonoids, and tannins. The results are shown in Table 2.

Table 2: Preliminary phytochemical screening.

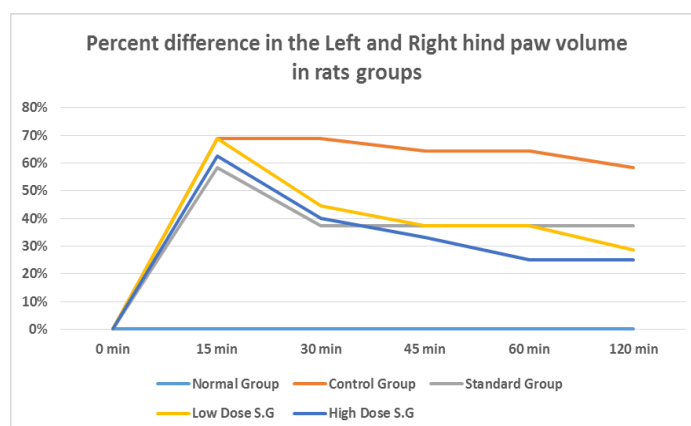
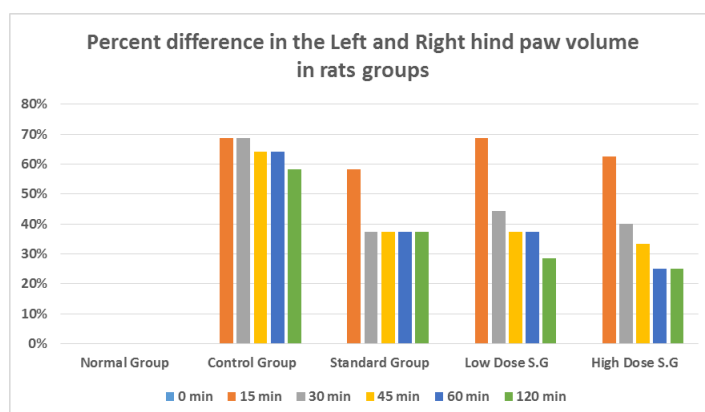
SI. No.	Tests	Crude ethanol extract of <i>Simarouba glauca</i>
1	Alkaloids	+
2	Carbohydrates	+
3	Saponins	+
4	Terpenoids	+
5	Flavanoids	+
6	Proteins	-
7	Glycosides	+
8	Tannins	+

(+) indicates present, (-) indicates absent

Table 3: Observations of the anti-inflammatory action of *Simarouba glauca* methanolic extract in formaldehyde-induced rat paw edema.

Group	Sample	Weight	0 min		15 min		30 min		45 min		60 min		120 min	
			L	R	L	R	L	R	L	R	L	R	L	R
Normal Group	1	150	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	2	165	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Mean			0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
% Protection			0%		0%		0%		0%		0%		0%	
Control	3	180	0.3	0.3	1	0.3	0.8	0.3	0.8	0.3	0.8	0.3	0.6	0.3

Group	4	120	0.2	0.2	0.6	0.2	0.8	0.2	0.6	0.2	0.6	0.2	0.6	0.2
Mean			0.25	0.25	0.8	0.25	0.8	0.25	0.7	0.25	0.7	0.25	0.6	0.25
% Protection			0%		68.75%		68.75%		64.28%		64.28%		58.33%	
Standard Group	5	200	0.3	0.3	0.6	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.4	0.3
Group	6	110	0.2	0.2	0.6	0.2	0.4	0.2	0.4	0.2	0.4	0.2	0.4	0.2
Mean			0.25	0.25	0.6	0.25	0.4	0.25	0.4	0.25	0.4	0.25	0.4	0.25
% Protection			0%		58.33%		37.5%		37.5%		37.5%		37.5%	
Low Dose S.G	7	170	0.2	0.2	0.8	0.2	0.4	0.2	0.4	0.2	0.4	0.2	0.3	0.2
Group	8	180	0.3	0.3	0.8	0.3	0.5	0.3	0.4	0.3	0.4	0.3	0.4	0.3
Mean			0.25	0.25	0.8	0.25	0.45	0.25	0.4	0.25	0.4	0.25	0.35	0.25
% Protection			0%		68.75%		44.44%		37.5%		37.5%		28.57%	
High Dose S.G	9	140	0.3	0.3	0.8	0.3	0.5	0.3	0.5	0.3	0.4	0.3	0.4	0.3
Group	10	175	0.3	0.3	0.8	0.3	0.5	0.3	0.4	0.3	0.4	0.3	0.4	0.3
Mean			0.3	0.3	0.8	0.3	0.5	0.3	0.45	0.3	0.4	0.3	0.4	0.3
% Protection			0%		62.5%		40%		33.3%		25%		25%	



DISCUSSION

Simarouba glauca methanolic extract showed anti-inflammatory action against formaldehyde-induced paw edema in rats. Inflammation is part of biological response of body tissue to harmful stimuli, such as pathogens, damaged cells or irritants. Inflammation caused by release of chemicals from tissues and migrating cells. Most strongly implicated are the prostaglandins (PGs), leukotriens(LTs), histamine, bradykinin and platelet activating

factor(PAF) and interleukin- 1. Arachidonic acid (AA) is metabolized into various mediators that induce the formation of edema, such as PGE₂, LTC₄ and LTD₄. The plasma membrane epidermal cells produce AA, which is oxidized to form prostaglandins, leukotrienes, and thromboxanes, responsible for inflammation. As part of the immune response elicited by antigens such as phospholipase A₂ (PLA₂). Thus, it is possible to identify, in this model compounds that inhibit AA metabolism into prostaglandins (PG) and leukotrienes. The nonsteroidal anti-inflammatory drugs (NSAID) inhibit the COX pathway thereby impeding the synthesis of PG.^[8]

In-vivo anti-inflammatory activity was done for the extracts of *Simarouba glauca* by using formaldehyde induced method. The methanolic extract showed activity by inhibition if edema and its effect was compared with standard drug Diclofenac.

CONCLUSION

It is concluded that the methanolic extract of *Simarouba glauca* possess significant anti-inflammatory activity may be due to the presence of falvonoids, tannins, glycosides, saponins, diterpenoids and triterpenoids proving the validity of its activity.

ACKNOWLEDGEMENT

It is a matter of great pleasure for us that we got an opportunity to complete our project work under the supervision of Dr. Raju Koneri and the management of Karnataka Education Trust.

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