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Anti-inflammatory Properties of *Mitragyna Speciosa* Extract.

R. E. Raja Aziddin, M. R. Mustafa, Z. Mohamed and **M. A. Mohd.**

Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

ABSTRACT The anti-inflammatory property of the alkaloid extract from the leaves of *Mitragyna speciosa* was evaluated on the basis of the inhibitory effect of the extract on carrageenan-induced hind paw edema in rat. This extract when administered by intra peritoneal injection at a concentration of 50 mg/kg produced almost 100% inhibition of the carrageenan-induced paw edema 1 hour after administration. The inhibition was maintained at about 60%, 7 hours after administration. These findings suggest that the alkaloid extracts of the leaves of *Mitragyna speciosa* possess potent anti-inflammatory properties.

Keyword: mitragyna, anti-inflammatory, alkaloids.

INTRODUCTION

In traditional practice, many medicinal plants are used to control inflammation. The severe side-effects of steroidal and non steroidal anti-inflammatory drugs have resulted in an increase in the number of experimental and clinical investigations to search for new anti-inflammatory agents from plant sources.

Mitagyna speciosa, a plant belonging to the Rubiacea family, is found in the northern states of Kedah and Perlis as well as in the East coast states of Malaysia. Over 25 alkaloids, either indole or oxindole alkaloids have been isolated from *Mitragyna speciosa.* The most abundant alkaloids consist of 3 indoles and 2 oxindoles. The 3 indoles are mitragynine, paynanthine and speciogynine, the first two being unique to this species. Oxindoles usually occur in small or trace amounts. The two most abundant oxindoles are mitraphylline and speciofoline.

Other alkaloids present include the indoles and oxindoles such as ajmalicine, corynanthedine, mitraversine, rhychophylline and stipulatine.

Mitragyna speciosa, has many applications in traditional use. Although many studies has shown this plant to have analgesic properties, its anti-inflammatory property has never been reported. In this study the anti-inflammatory activity of *Mirtragyna speciosa* was investigated using the carrageenan-induced edema model. Carrageenan-induced paw edema is a classical model of acute inflammation and is a standard and most commonly used technique to screen for anti-inflammatory activity.

MATERIALS AND METHOD

Plant Material

Extraction of mitragynine and the alkaloid fraction was carried out according to the method described by Ponglux et al. The dried powdered leaves were extracted seven times with hot MeOH and concentrated under reduced pressure. The crude extract was dissolved in 10% acetic acid and basified with sodium carbonate to a pH of 9-12. The aqueous layer was extracted with chloroform. The organic layer was removed, washed with water and dried under pressure to give the alkaloid fraction.

Experimental techniques

Mitragyna speciosa alkaloids were dissolved in propylene glycol. Sprague- Dawley rats age 6-8 weeks were used. The rats were housed in groups of 10 per cage and were on a 12 h light/dark cycle, temperature was maintained at 27°C and animals were acclimatized for 1 week before the experiments with food and water was given *ad libidum.*

Carrageenan-induced paw oedema

Anti-inflammatory activity was evaluated based on the inhibition of the carageenan-induced hind paw edema. The alkaloid extract (50 mg/kg body

weight) in vehicle were given by i.p. to the experimental (n = 6) and control groups (n = 6) respectively, 30 minutes before administration of the edema-inducing agent (0.1 ml of a 1% carrageenan suspension in 0.9% NaCl) Ocete et al, which was injected into the plantar surface of the right hind paw. The left hind paw was injected with 0.9% NaCl. The volumes of the injected paws were measured immediately after and every 30 minutes for 7 hours after treatment by means of volume displacement methods [1, 2] using a Ugo Basile Plethysmometer No. 7140. Edema was expressed as the increase in paw volume due to carrageenan injection. Reference groups were treated with acetylsalicylic acid (20 mg/kg).

The degree of inflammation was indicated by the difference in the swelling between the left paw and right paw. Hind paw swelling was calculated as a percentage [3] as follows:

$$\frac{\text{right paw volume-initial volume}}{\text{right paw initial volume}} - \frac{\text{left paw volume-initial volume}}{\text{left paw initial volume}} \times 100$$

RESULTS

The analgesic activity was expressed as "mean increase in paw volume ± SEM" and percentage inhibition in paw volume.

The intra-plantar injection of carrageenan into the hind paw induced a progressive edema reaching a maximum after 4 hrs. Animals treated with the alkaloid extracts of *Mitragyna speciosa* showed a maximum anti-inflammatory effect 1 hour after carrageenan administration with 100% inhibition of edema for the 50 mg/kg dose. The inhibitory effect was significantly maintained at about 60% up to 7 hours of the edematous agent. The anti-inflammatory effect of the reference (positive) group progressively increased and reached a maximum at 2.5 hours. Following this, there was a progressive decrease but edema inhibition was maintained at about 44.74% at 7 hours following carrageenan administration. In this study, the results time course of anti-inflammatory trend for the reference drug is similar to those reported in other studies [4].

DISCUSSION

The anti-inflammatory response in the alkaloid of *Mitragyna speciosa* was significant during the first phase of edema development suggesting an inhibitory effect on the release of active pain substances such as histamine, serotonin or polypeptides. This response was significantly maintained during the second and third phases of edema development suggesting an inhibition of the release of kinins or cycloxygenase, one of the enzymes involved in the formation of prostaglandins that induced the inflammation process.

Anti-inflammatory effect of the alkaloid extract of *Mitragyna speciosa* and Acetylsalicylic acid on carrageenan-induced rat paw edema

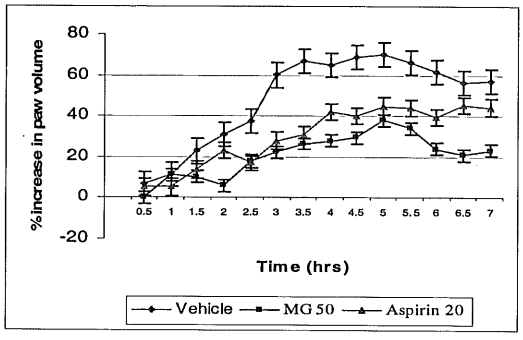

Figure 1. Carrageenan-induced paw volume change with time (h). Effect of alkaloid extract from leaves of *Mitragyna speciosa*, 50 mg.kg (MG 50) and Acetylsalicylic acid 20 mg/kg (Aspirin20). All points represent the mean±SEM of six animals.

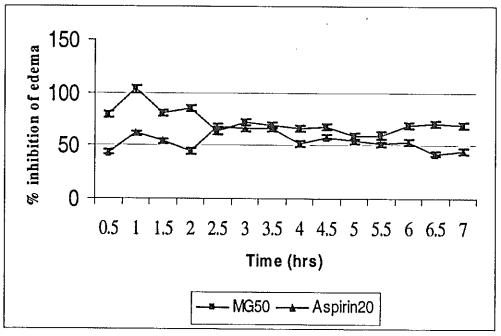

Figure 2. Inhibitory effect of alkaloid extract from leaves of *Mitragyna speciosa*, 50 mg.kg (MG50) and Acetylsalicylic acid 20 mg/kg (Aspirin20) on Carrageenan-induced paw edema. All points represent the mean±SEM of six animals.

Table 1. Mean hind paw volume at different time intervals of carrageenan injection after the administration of the alkaloid extract of *Mitragyna speciosa* and Acetylsalicylic acid.

Treatment	Mean increase in right paw volume ± SEM (ml)						
	0.5hr	1 hr	1.5 hr	2 hr	2.5 hrs	3 hrs	3.5 hrs
Control gp (vehicle)	.05±0.14	0.16±0.22	0.51±0.21	0.74±0.42	1.05±0.22	1.61±0.16	1.94±0.23
Test gp (*M. speciosa* extraxt 50mg/kg)	.02±0.05	0.16±0.14	0.19±0.10	0.15±0.11	0.33±0.08*	0.40±0.16***	0.58±0.13***
Positive control gp (Acetylsalicylic acid 20 mg/kg)	.08±0.12	0.13±0.13	0.13±0.14	0.45±0.18	0.32±0.18*	0.56±0.22**	0.65±0.25**
Treatment	Mean increase in right paw volume ± SEM (ml)						
	4 hrs	4.5 hrs	5 hrs	5.5 hr	6 hrs	6.5 hrs	7 hrs
Control gp (vehicle)	2.09±0.24	1.87±0.4	1.7±0.27	1.61±0.33	1.69±0.26	1.63±0.26	1.91±0.26
Test gp (*M.speciosa* extract 50mg/kg)	0.68±0.13***	0.71±0.16*	0.69±0.21*	0.76±0.10*	0.56±0.15**	0.46±0.12**	0.74±0.13**
Positive control gp (Acetylsalicylic acid 20 mg/kg)	0.76±0.22**	0.83±0.23*	0.84±0.18*	0.83±0.20*	0.85±0.16*	0.93±0.18	1.24±0.15*

Edema is expressed as increase in paw volume±SEM. n=6
* P<0.05 : statistically significant relative to control
** P<0.01 : statistically significant relative to control
***P<0.001: statistically significant relative to control

Table 2. The inhibition of inflammation of the hind paw at different time intervals of carrageenan injection after the administration of the alkaloid extract of *Mitragyna speciosa* and Acetylsalicylic acid in comparison to control.

Treatment		Time (hrs)				
		0.5hr	1 hr	1.5 hr	2 hr	2.5 hrs
Control	Mean	0.21±0.17	0.36±0.28	0.72±0.32	0.99±0.26	1.22±0.40
Test gp (*M. speciosa* extraxt 50mg/kg)	Mean	0.43±0.09	-0.01±0.18	0.14±0.15*	0.15±0.14**	0.44±0.12***
	%Inhibition	79.00	103.24	80.41	85..33	64.1
Positive control gp (Acetylsalicylic acid 20 mg/kg)	Mean	0.12±0.10	0.14±0.13	0.33±0.18	0.55±0.21	0.39±0.25*
%Inhibition		43.65	61.57	54.15	44.69	68.08

Treatment		Time (hrs)				
		3 hrs	3.5 hrs	4 hrs	4.5 hrs	5 hrs
Control	Mean	1.93±0.4	0.76±0.31	2.11±0.42	2.22±0.44	2.24±0.34
Test gp (*M. speciosa* extraxt 50mg/kg)	Mean	0.55±0.19***	0.66±0.17***	0.71±0.16***	0.72±0.09***	0.92±0.16***
	%Inhibition	71.61	69.01	66.3	67.49	58.91
Positive control gp (Acetylsalicylic acid 20 mg/kg)	Mean	0.65±0.21**	0.72±0.26**	1.01±0.3*	0.93±0.28**	1.02±0.24**
	%Inhibition	66.52	65.77	52.01	58.11	54.36

Treatment		Time(hrs)			
		5.5 hr	6 hrs	6.5 hrs	7 hrs
Control	Mean	2.12±0.47	1.99±0.45	1.81±0.44	1.85±0.28
Test gp (*M. speciosa* extraxt 50mg/kg)	Mean	0.85±0.12***	0.60±0.16***	0.54±0.15**	0.56±0.24**
	%Inhibition	59.81	69.71	70.36	69.52
Positive control gp (Acetylsalicylic acid 20 mg/kg)	Mean	1.03±0.24**	0.93±0.23**	1.05±0.23*	1.02±0.19**
%Inhibition		51.58	53.36	42.11	44.74

Edema is expressed as increase in paw volume±SEM. n=6
* P<0.05 : statistically significant relative to control
** P<0.01 : statistically significant relative to control
***P<0.001: statistically significant relative to control

CONCLUSION

These findings suggest that the alkaloid extracts from *Mitragyna speciosa* possess very potent anti-inflammatory effects. As this plant comprise of many alkaloids, further investigations is needed to determine the exact alkaloid that exerts this effect as the extract used were comprised of mixture of alkaloids. Further work is also needed to understand the exact mechanism of action in the anti-inflammatory activities of the extract.

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