

# Antinociceptive and Anti-inflammatory Activities of Methanol Extract of *Ormosia coccinea* (Aubl.) Jacks *in vivo*

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**Abstract:** The present study was conducted to evaluate the antinociceptive and anti-inflammatory activities of methanol extract of rachis of *Ormosia coccinea* (Aubl.) Jacks (MEOC) using animal models of nociception and inflammation. The antinociceptive activity of the extract was assessed using acetic acid-induced abdominal writhing, hot-plate, and formalin tests. Oral administration of MEOC (500 mg/kg) produced significant ( $p < 0.05$ ) antinociceptive effects when tested in mice using acetic acid-induced abdominal writhing test and on the inflammatory phase of the formalin test. It was also demonstrated that MEOC had no significant effect on the response latency time to the heat stimulus in the thermal model of the hot plate test. The anti-inflammatory activity of the extract was assessed using carrageenan, histamine and serotonin induced oedema in rat paw. The oral administration of MEOC showed maximum inhibition (64.29%) at 1 h on carrageenan edema, but it did not modify the edema induced by histamine and serotonin. The present results suggest that MEOC has a peripheral antinociceptive and anti-inflammatory action.

**Key words:** *Ormosia coccinea*, antinociceptive, anti-inflammatory, fabaceae.

## 1. Introduction

The world is facing an explosive increase in the incidence of many systemic diseases [1]. Pain and inflammation are some of the most common manifestations of many diseases afflicting millions of people worldwide [2]. Inflammatory diseases are currently treated with steroidal and NSAIDs (non-steroidal anti-inflammatory drugs). These drugs are also used to relieve pain which is a major symptom that accompanies several illnesses [3]. Furthermore, long-term treatment with NSAIDs may result in serious side effects, such as gastrointestinal bleeding [4, 5], peptic ulcers [6] and renal morbidity [7]. Consequently

there is a need to develop new anti-inflammatory and analgesic agents with minimum side effects [5]. Plants are an important source of traditional medicine for the treatment of various diseases. It has been estimated that herbal medicines are used by more than 80% of the world's population in developing countries to meet their primary healthcare needs [8]. Plants represent an extraordinary reservoir of novel molecules and currently there is a renewed interest in plant kingdom as a source of novel lead compounds for screening libraries. Panama's flora is one of the richest in the world, whose medical and economic potential has not been fully explored [9]. The plant *Ormosia coccinea* (Aubl.) Jacks, popularly known in Panama as "palo de collar, pernillo, peronil rojo", belongs to the family Fabaceae and grows up to 30 m in height, and it is

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widely distributed from Costa Rica to Brazil. *Ormosia coccinea* is well known in Panama for its ornamental use in floral arrangements and jewelry [10]. However, according to unpublished data, it has been observed that the methanol extract of rachis inflorescence of *Ormosia coccinea* has inhibitory activity against human breast (MCF-7) and prostate cancer (Hs578t) cell lines. The main objective of the present study is to evaluate the antinociceptive and anti-inflammatory activity of the methanol extract of the rachis of *O. coccinea* (Aubl.) Jacks.

## 2. Materials and Methods

### 2.1 Plant Material

The plant material was collected from Coiba National Park (Veraguas Province, Panama) in January of 2012 with the authorization of National Environment Authority (now Ministry of Environment). Its taxonomic identity was established by Alex Espinosa, Taxonomist at the Center for Pharmacognostic Research on Panamanian Flora (CIFLORPAN). A voucher specimen (Florpan 2203) was deposited at the Herbarium of the University of Panama (PMA).

### 2.2 Preparation of Plant Extract

The rachis of *O. coccinea* was air dried and pulverized in a Wiley mill. The powder (100 g) was extracted twice (for 24 hours) by maceration in methanol and concentrated *in vacuo* using rotary evaporator at low temperature (< 40 °C) yielding a brown residue of MEOC (methanol extract of *O. coccinea*).

### 2.3 Experimental Animals

Experiments were carried out using adult male CD1 mice (18-25 g) and adult male Sprague-Dawley rats (150-200 g), obtained from the Animal House of the Faculty of Veterinary Medicine, University of Panama. All animals were kept under standard room conditions (temperature  $22 \pm 2$  °C and relative humidity  $55 \pm 5$  °C with 12 h light-dark cycle for 7 days before the

experiment) with standard rodent diet and water *ad libitum*. When necessary, animals were deprived of food 12 h prior to the experiments. All experimental procedures followed the “Guidelines for the Care and Use of Laboratory Animals” of ILAR (the Institute of Laboratory Animal Resources) of the National Research Council, NIH, USA. Prior authorization for the use of laboratory animals in this study was obtained from the Bioethics Committee of the Pharmacology Department of School of Medicine (CBF-02DEC11).

### 2.4 Drug and Chemicals

Acetylsalicylic acid, tramadol, acetic acid, formalin,  $\lambda$ -carrageenan, histamine, serotonin, indomethacin, loratadine and cyproheptadine were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All drugs and MEOC were suspended in 2% (w/v) sodium CMC (carboxymethyl Cellulose), except indomethacin which was suspended in 0.5% CMC. All treatments were administered orally, except tramadol that was administered subcutaneously. Acetic acid, formalin,  $\lambda$ -carrageenan, histamine and serotonin were dissolved and diluted in saline (0.9% NaCl) prior to using.

### 2.5 Acute Toxicity Study

This study was performed according to the OECD guideline; rats were divided into three groups of eight animals each. Different doses (500, 1000 and 2000 mg/kg) of methanol extract were administered by oral gavage. Then the animals were observed for 24 hours (0.5, 1, 3, 6, and 12 h) and daily until day 14 after dosing. The body weight of the rats was measured on days 1, 7, and 14 (OECD 420, 2002) [11]. At the end of the experiment, biochemical data were collected and all animals were euthanized by exsanguination under light anesthesia, and their organs were extirpated and examined macroscopically.

### 2.6 Assessments of the Antinociceptive Activity

2.6.1. Acetic Acid-Induced Abdominal Writhing Test  
The method described by Koster et al. [12], was used

to evaluate antinociceptive effects of the methanolic extract of *O. occinea* rachis. Briefly, three groups of mice (n=6) were pretreated with MEOC (500 mg/kg), acetylsalicylic acid (200 mg/kg) or CMC (200 mg/kg). Thirty-five minutes later each mouse was given intraperitoneally 1% aqueous solution of acetic acid (10 mL/kg body weight), and then was placed in the individual observation boxes. Five minutes after the injection of acetic acid, the number of writhing responses per mouse was counted for 30 minutes during acetic acid-induced abdominal writhings. The percentage of the analgesic activity was calculated as following Eq. (1):

$$\text{Inhibition(\%)} = \frac{N_c - N_t}{N_c} \times 100 \quad (1)$$

In which  $N_c$  = mean number of writhing in control group;  $N_t$  = mean number of writhing in treated group.

#### 2.6.2 Formalin Test

Formalin-induced tonic pain was carried out in a similar manner to the method previously described by Hunskaar and Hole [13]. In this test mice were pretreated with MEOC (500 mg/kg; p.o), acetylsalicylic acid (200 mg/kg; p.o), tramadol (20 mg/kg; s.c) or CMC (200 mg/kg; p.o). Thirty minutes later, each mouse received an intra-plantar injection of 20  $\mu$ L of 1.4% formalin in the sub-plantar space of the right-hind paw and the mice were individually placed in a transparent Plexiglas cage and observed. The duration of paw licking was recorded at the early phase or neurogenic pain (1-5 min) and late phase or inflammatory pain (15-30 min) after formalin injection. The percentage of analgesic activity at each phase was calculated using the following Eq. (2):

$$\text{Inhibition(\%)} = \frac{C - T}{C} \times 100 \quad (2)$$

In which  $C$  = mean time in control group for each phase and  $T$  = mean time in treated group for each phase.

#### 2.6.3 Hot Plate Test

We introduced a slight modification to the hot plate

test described by Langers et al. [14]. In a preselection test, mice were screened by placing the animals onto hot plate (Socrel<sup>®</sup> DS-37) setting at  $55 \pm 0.2$  °C and those who failed to lick their hind paw or jump (nociceptive responses) within 10 s were discarded. An average of the two readings was obtained as the initial reaction time. Eligible animals were divided into three different groups (n = 6) and pretreated with MEOC (500 mg/kg, p.o), tramadol (20 mg/kg, s.c.) or CMC 2% (0.1 mL/10 g, p.o.), using each animal as its own control. Thirty minutes after treatment, mice were placed individually on the hot plate and the reaction time was again recorded at 0.5, 1 and 2 h after administration of different treatments. In order to minimize damage to the animal's paw, the cut-off time for latency of response was taken as 20 s [14]. The percentage of analgesic activity was calculated as Eq. (3):

$$\text{Inhibition(\%)} = \frac{(Pt - Po)_{test} - (Pt - Po)_{control}}{(Pt - Po)_{control}} \times 100 \quad (3)$$

In which  $Po$  = threshold value before administration of drug and  $Pt$  = threshold value at time  $t$  after administration of drugs.

### 2.7 Assessments of the Anti-inflammatory Activity.

#### 2.7.1. Carrageenan Induced Oedema

Carrageenan-induced paw inflammation was performed according to the method described by Winter et al. [15]. Male rats of 150-200 g body weight were randomly divided into three groups (n = 6 per group) and treated by oral gavage with MEOC (500 mg/kg), indomethacin (10 mg/kg) or CMC 2% (0.1 mL/100 g).  $\lambda$ -Carrageenan [(0.1 mL, 1%, w/v in normal saline solution (NSS))] was injected intradermally into the plantar side of the right hind paw 1 h after (preventive effect) or 1 h before (curative effect) of the different treatment administration. Paw volumes were measured using a plethysmometer (Panlab Harvard Apparatus<sup>®</sup> LE7500) before and at 0.5, 1, 2, 3, 4, 5 and 6 h post carrageenan injection in

preventive test or 4, 4.5, 5, 6 and 24 h after carrageenan in curative model [3]. The oedema was expressed as an increase in the volume of paw ( $\Delta V$ ), and the percentage of inhibition ( $I\%$ ) for each treatment was obtained as following Eq. (4):

$$\Delta V = \Delta t - V_0$$

$$I(\%) = \frac{\Delta V_c - \Delta V_{tr}}{\Delta V_c} \times 100 \quad (4)$$

In which  $\Delta V_{tr}$  = right hind paw average increased volume in treated group and  $\Delta V_c$  = right hind paw average increase in control groups.

### 2.7.2 Histamine and Serotonin Induced Paw Oedema

The anti-inflammatory activity of the MEOC was evaluated according to the method previously described by Singh et al. [16], with minor modifications. The paw oedema was induced in the rats by sub-plantar administration of 0.1 mL of freshly prepared solutions of histamine (0.5%) or serotonin (0.5%). The paw volumes were recorded at 0, 0.5, 1 and 2 h after inflammatory agent. Rats were divided into three groups (n=6) and pretreated orally with MEOC (500 mg/kg), indomethacin (10 mg/kg) or CMC 2% (0.1 mL/100 g), one hour before eliciting paw oedema. Loratadine (10 mg/kg) and cyproheptadine (10 mg/kg) were used as standard drugs against histamine and serotonin induced oedema, respectively. The percentage inhibition induced by each drug was calculated as was previously described in carrageenan test.

### 2.8 Statistical Analysis

Data obtained from animal experiments were expressed as the mean  $\pm$  SEM (standard error of the mean). Statistical differences between the treated and the control groups were analyzed statistically by one-way ANOVA followed by the Dunnet's post test or two-way ANOVA followed by Bonferroni post test. All data were processed with GraphPad prism 5.01 Software. The value of  $p < 0.05$  was considered as indicative of significance.

## 3. Results

### 3.1 Acute Toxicity

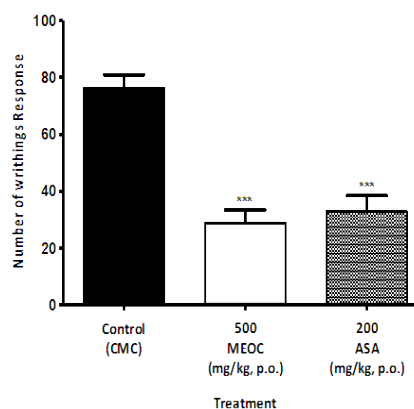
In the acute toxicity study the MEOC did not produce mortality including the highest dose (2,000 mg/kg) during 48 hours of observation. The animals showed a slight restlessness few minutes after administration but normal activity was completely recovered a few hours later.

The highest dose (2,000 mg/kg) administrated did not produce mortality. The animals manifested restlessness, however there were no sign of toxicity or biochemical changes observed after 24 h of treatment. Under these observations, we considered that the methanolic extract of *O. coccinea* was safe for rats. Thus, a dose of 500 mg/kg of extract was selected for further studies.

### 3.2 Assessments of the Antinociceptive Activity

#### 3.2.1 Acetic Acid-Induced Abdominal Writhing Test

Fig. 1 demonstrates that MEOC significantly reduced the number of abdominal writhings induced by intraperitoneal injection of acetic acid 1% in mice as compared with CMC control group. This protective



**Fig. 1** Effect of methanol extract of *Ormosia coccinea* (MEOC) on acetic acid-induced abdominal writhings in mice.

Each column represents the mean  $\pm$  SEM of 6 mice; \*\*\*  $p < 0.001$  statistically significant compared to the control group.

effect reached an inhibition of 61.98% ( $p < 0.001$ ) at the dose of 500 mg/kg. At this dose, the extract showed an antinociceptive activity comparable to 200 mg/kg acetylsalicylic acid (% inhibition of 56.73,  $p < 0.001$ ), an established antinociceptive drug.

### 3.2.2 Formalin Test

As shown in Fig. 2, compared to the control group, MEOC (500 mg/kg) and acetylsalicylic acid significantly reduced the time the animals' licking and biting the injected paws in the second phase (15-30 minutes after injection) ( $p < 0.001$ ). However, nociception during the first phase appeared to be unaffected by MEOC or acetylsalicylic acid. In contrast, tramadol produced powerful inhibition of responses to formalin in both phases ( $p < 0.01$  for first phase;  $p < 0.001$  for second phase).

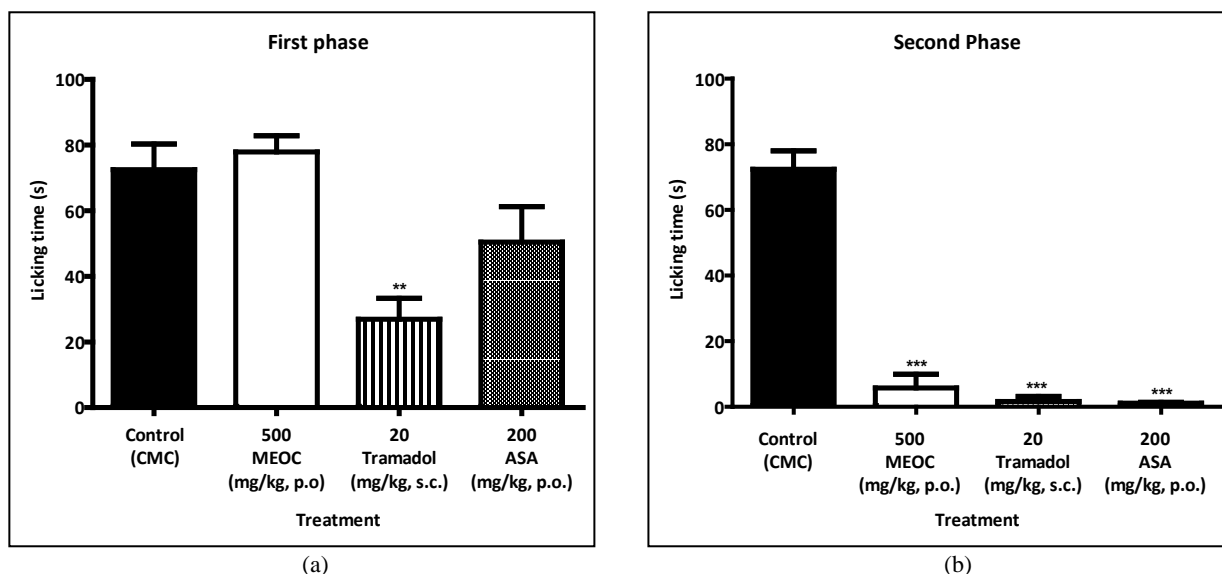
### 3.2.3 Hot Plate Test

The results in Table 1 shows that treatment with tramadol (20 mg/kg s.c.) increased the latency response in the hot plate test at 0.5, 1 y 2 h after treatment ( $p < 0.01$  at 0.5 and 2 h;  $p < 0.001$  at 1 h). On the other hand, MEOC at a dose of 500 mg/kg did not influence significantly the reaction time of the animals in any of analyzed periods ( $p > 0.05$ ).

### 3.3 Assessments of the Anti-inflammatory Activity

#### 3.3.1 Carrageenan Induced Edema

The anti-inflammatory effects of the MEOC on carrageenan induced edema in rat's hind paws are presented in Table 2. In the control CMC-group we observed a gradual increase in edema paw volume of rats. However, in the test groups, both the extract and



**Fig. 2** Effect of methanolic extract of *Ormosia coccinea* (MEOC) in formalin induced paw licking test (first phase (a) and second phase (b)) in mice.

Each column represents the mean  $\pm$  SEM of 6 mice. The mice were pretreated with CMC (control), MEOC, tramadol, or ASA (acetylsalicylic acid), 30 min before subplantar injection of formalin; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$  statistically significant compared to the control group.

**Table 1** Effect of methanol extract of *Ormosia coccinea* on pain induced by hot plate method.

Group	Dose (mg/kg)	Reaction time (s)			
		0	0.5	1	2
CMC	0.1 mL/10 g	5.51 $\pm$ 0.43	5.86 $\pm$ 0.82	4.76 $\pm$ 0.67	6.94 $\pm$ 1.47
MEOC	500	5.59 $\pm$ 0.75	7.12 $\pm$ 0.73	6.86 $\pm$ 1.18	6.56 $\pm$ 1.69
Tramadol	20	5.72 $\pm$ 0.81	14.30 $\pm$ 1.89**	14.35 $\pm$ 2.21***	15.03 $\pm$ 1.90**

Results are expressed as the mean  $\pm$  SEM, n = 6 animals in each group; \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  compared with the control group.

the reference substance showed a significant decrease in the edema paw volume. Our results show that oral administration of methanol extracts of *O. coccinea* at 500 mg / kg p.o. 1 h before carrageenan exhibits a maximal inhibition of hind paw edema between 0.5 and 1 h (58.64 and 64.29% respectively). Additionally, this antiinflammatory effect induced by treatment was sustained over 24 h. On the other hand, indomethacin as reference drug (10 mg/kg orally) produced a significant inhibitory effect that was not comparable to the extract in the initial observations. As shown in Table 2, maximal inhibition of hind paw edema exhibited by positive control was observed from 2 to 6 h (46.73 to 37.71%,  $p < 0.05$ ). After carrageenan administration MEOC and Indomethacin exhibited 64.29 and 46.73 % as maximal inhibitory effect of edema formation at 1 and 2 h, respectively.

Modified edema test was conducted to quantify

curative anti-inflammatory effects of the MEOC. The percentage protection of inflammation is presented in Table 3. The injection of the carrageenan in paw after CMC treatment created an inflammatory edema, which decreased gradually. The inflammatory edema induced by carrageenan in this model was not inhibited by MEOC. However, indomethacin caused significant reduction of the hind paw edema at 4, 5 and 6 h ( $p < 0.01$  at 4, 4.5 and 5;  $p < 0.05$  at 6 h).

### 3.3.2 Histamine and Serotonin Induced Paw Edema

As shown in Tables 4 and 5, loratadine and cyproheptadine were used as reference drugs for histamine and serotonin edema, respectively. They significantly decreased paw edema inflammation at all time points studied, ( $p < 0.001$ ). Compared to two reference groups, MEOC and CMC control group, had no significant effects on edema induced by histamine or serotonin.

**Table 2 Preventive effect of methanol extract of *Ormosia coccinea* on rat paw edema induced by carrageenan.**

Group	Dose (mg/kg)	Duration of study (h)						
		0.5	1	2	3	4	5	6
CMC	0.1 mL/100 g	0.27 ± 0.05	0.33 ± 0.06	0.88 ± 0.15	1.13 ± 0.11	1.26 ± 0.15	1.27 ± 0.11	1.06 ± 0.13
MEOC	500	0.14 ± 0.05	0.15 ± 0.05	0.58 ± 0.10	0.99 ± 0.15	1.09 ± 0.12	1.03 ± 0.09	0.71 ± 0.08
Indomethacin	20	0.19 ± 0.06	0.24 ± 0.07	0.47 ± 0.13*	0.66 ± 0.15*	0.71 ± 0.16**	0.79 ± 0.17**	0.66 ± 0.13*

Results are expressed as the mean ± SEM, n = 6 animals in each group; \*  $p < 0.05$ , \*\*  $p < 0.01$  compared with the control group.

**Table 3 Curative effect of methanol extract of *Ormosia coccinea* on rat paw edema induced by carrageenan.**

Group	Dose (mg/kg)	Duration of study (h)				
		4	4.5	5	6	24
CMC	0.1 mL/100 g	1.75 ± 0.09	1.67 ± 0.09	1.58 ± 0.11	1.53 ± 0.07	0.74 ± 0.08
MEOC	500	1.32 ± 0.14	1.23 ± 0.14	1.25 ± 0.14	1.14 ± 0.12	0.45 ± 0.06
Indomethacin	20	1.16 ± 0.16**	1.13 ± 0.16**	1.01 ± 0.14**	1.03 ± 0.15*	0.46 ± 0.07

Results are expressed as the mean ± SEM, n = 6 animals in each group; \*  $p < 0.05$ , \*\*  $p < 0.01$  compared with the control group.

**Table 4 Effect of methanol extract of *O. coccinea* on paw edema induced by histamine in rat.**

Group	Dose (mg/kg)	Duration of study (h)		
		0.5	1	2
CMC	0.1 mL/100 g	0.77 ± 0.07	0.73 ± 0.10	0.74 ± 0.10
MEOC	500	0.65 ± 0.04	0.62 ± 0.05	0.55 ± 0.03
Loratadine	10	0.78 ± 0.04***	0.80 ± 0.04***	0.61 ± 0.06***
Indomethacin	20	0.31 ± 0.05	0.22 ± 0.04	1.20 ± 0.04

Results are expressed as the mean ± SEM, n = 6 animals in each group; \*\*\*  $p < 0.001$  compared with the control group (one-way ANOVA followed by Dunnett's post test).

**Table 5** Effect of methanol extract of *O. coccinea* on paw edema induced by serotonin in rat.

Group	Dose (mg/kg)	Duration of study (h)		
		0.5	1	2
CMC	0.1 mL/100 g	0.63 ± 0.06	0.65 ± 0.06	0.76 ± 0.07
MEOC	500	0.60 ± 0.06	0.63 ± 0.05	0.71 ± 0.09
Cyproheptadine	10	0.27 ± 0.05***	0.32 ± 0.04***	0.25 ± 0.06***
Indomethacin	20	0.53 ± 0.04	0.58 ± 0.05	0.73 ± 0.06

Results are expressed as the mean ± SEM, n = 6 animals in each group. \*\*\*  $p < 0.001$  compared with the control group (one-way ANOVA followed by Dunnet's post test).

#### 4. Discussion

*Ormosia coccinea* is a species of the family Fabaceae for which there are no ethnobotanical uses described, perhaps because of the toxic effects that are linked to eating their seeds. Other species of this genus are used in traditional medicine [17, 18]. *Ormosia coccinea* grows in Central America: Costa Rica, Dominican Republic, Cuba, Honduras, Nicaragua, Panama, and in most of South America. The MEOC for which the antinociceptive and anti-inflammatory properties were evaluated, was selected for bioprospecting studies in antiproliferative assays. The antinociceptive activity of MEOC was studied using three important experimental models, which allowed assessment of responses to two different types of noxious stimuli: a thermal stimulus and chemically-induced pain stimulus.

The acetic acid- induced abdominal writhing test is a peritoneo visceral inflammatory pain model [16] widely used to evaluate peripheral antinociceptive activity [19, 20]. Because this model is highly sensitive and also has the ability to detect antinociceptive effects of compounds at dose levels that may appear inactive in other models [20, 21]. The intraperitoneal administration of agents that irritate serous membranes provokes a stereotyped behaviour in the mice which is characterized by abdominal contractions, movements of the body as a whole and twisting of the dorso-abdominal muscles [22] and a reduction in motor activity and motor incoordination [23]. It has been suggested that acetic acid injection into peritoneal cavity leads to increased levels of cyclooxygenases

(COX) and lipoxygenase [24] and indirectly leads to the release of endogenous nociceptive mediators such as PGE<sub>2</sub> and PGF<sub>2α</sub> [5, 20, 25-30], serotonin [22, 25, 26, 29, 30], histamine [22, 26, 30, 31], bradykinin [21, 22, 24], substance P [24, 25], cytokines (TNF-α, IL-1β, IL-8) [21, 24, 25, 29] and lipoxygenase products [20], which eventually excites the primary afferent nociceptors [25] that contribute to the development of inflammatory pain [22]. The data presented in Fig. 1 indicate that MEOC significantly reduced acetic acid induced abdominal writhing in mice. These results support the hypothesis that MEOC may act by inhibiting prostaglandin synthesis because of the nociceptive mechanism of abdominal writhing induced by acetic acid metabolites via cyclooxygenase and prostaglandin biosynthesis [22, 32]. However, an important disadvantage of the acetic acid-induced abdominal writhing test model is that other classes of drugs, including adrenergic receptor antagonists, antihistamines, central nervous system stimulants, monoamine oxidase inhibitors, serotonin antagonists, muscle relaxants, and neuroleptics, can also inhibit abdominal writhing, favoring possible false-positive result [22, 28, 32]. Due to the lack of specificity, positive results in the abdominal writhing test should be recognized in the context of results obtained in other experimental models. For this reason, the formalin test was employed to confirm a possible antinociceptive action of the extract. The formalin test is a tonic model of continuous pain resulting from formalin-induced tissue injury [30]. It is a widely used model, particularly for the screening of novel compounds, since it encompasses inflammatory, neurogenic, and

central mechanisms of nociception [33]. Intraplantar injection of formalin has been reported to produce a distinct biphasic nociceptive response [12, 21], termed first and second phases [20]. The first phase, commonly denominated early or neurogenic phase (from 0 to 5 min after injection formalin) results from a direct stimulation of nociceptors (predominantly C-fibres) [22, 23, 27, 28, 31, 32, 34]. Substance P, glutamate and bradykinin are thought to participate in this phase, which is believed to be non-inflammatory pain [28]. The second phase, commonly denominated late or inflammatory phase (from 15 a 30 min) [29] is associated with the release of local endogenous mediators (histamine, serotonin, prostaglandins and bradykinin) responsible for sensitization of primary and spinal sensory neurons and subsequent activation of the nociceptor [20, 25, 34]. It is well established that both phases of the formalin test can be inhibited by centrally acting drug, such as narcotics, whereas peripherally acting drugs, such as acetylsalicylic acid, only inhibit the second phase [21, 22, 29, 30]. As presented in Fig. 2, the MEOC and acetylsalicylic acid decreased the licking time only in the second phase pain, and showed no significant effects on the first phase. The effect of MEOC on the second phase indicates that the extract has a possible peripheral analgesic activity.

To provide a confirmation of the central antinociceptive activity of MEOC, we used the hot plate test, since this model is sensitive and specific for strong analgesics (opioids), while peripherally acting analgesics are inactive [23, 26, 28, 35, 36]. This model of nociception, predominantly a spinal reflex, is thought to involve supraspinal nociceptive processing [21, 27] and has often been used to assess central antinociceptive activity [25, 28]. The central analgesics activate the release of endogenous peptides via the periaqueductal gray matter (PAG), which is then carried to the spinal cord to inhibit the pain impulse transmission within the dorsal horn [25]. As shown in Table 1, MEOC showed no significant effect on pain

latency compared to the control group. In contrast, tramadol, a centrally acting analgesic that is believed to exert an action on opioid receptors, was associated with a significant antinociceptive effect.

The results obtained in evaluating the extract in nociception test, where we observed inflammatory phases related effects led us to conduct further studies to evaluate the effect of MEOC on inflammation induced by different agents. The carrageenan-induced paw oedema test is a well-researched and highly reproducible model, and is therefore frequently chosen for evaluating the acute anti-inflammatory actions of natural products [15, 28, 29, 37]. In general, the development of oedema in the rat hind paw following injection of carrageenan has been described as a biphasic event [5, 28, 38-40], in which various mediators operate in sequence to produce this inflammatory response [28]. In this model, MEOC showed a significant inhibitory effect of carrageenan-induced oedema, showing them more effective during the first determinations. It has been described that the initial phase (0 to 2 h after injection of carrageenan) mainly is due to the release of pro-inflammatory agents, such as histamine, 5-hydroxytryptamine and bradykinin, from damaged surrounding tissues. These observations validate the use of variations to plantar edema test in which it replaces the carrageenan by histamine or serotonin. MEOC administration did not modify edema intensity induced by autacoids agents. These results obtained in antinociception assays and carrageenan test make an antiinflammatory activity for MEOC, but these properties do not appear to be mediated by inhibition of serotonin or histamine activity.

## 5. Conclusions

The results obtained in this study reveal that the MEOC has peripheral antinociceptive activity as demonstrated by writhings and formalin tests, and this effect could be related to the anti-inflammatory properties exhibited in carrageenan-induced rat paw



edema model. This is the first report on the pharmacological properties of *Ormosia coccinea*. However, further studies are required to isolate the bioactive compounds presented in the methanolic extract and to elucidate the mechanism of action of anti-inflammatory activity.

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