



## Evaluation of Anti-inflammatory And Analgesic Activities of Methanolic Extract of *Mitragyna Inermis*

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### ABSTRACT

*Inflammation is a pervasive phenomenon elicited by the body in response to obnoxious stimuli as a protective measure. However, a sustained inflammation lead to several diseases including cancer therefore the necessity to neutralize inflammation is paramount. Mitragyna inermis is a medicinal plant traditionally used as a medicine in Ayurveda and other folk systems of medicine. It is commonly used to treat inflammatory diseases including rheumatoid arthritis and asthma. Despite this fact its anti-inflammatory and analgesic effects have not been evaluated scientifically. Therefore, the anti-inflammatory and analgesic activities of M. inermis were studied in Wistar rats by different methods. The hot plate, acetic acid, and tail immersion tests were used to evaluate the analgesic activity whereas paw edema model for acute inflammation using egg albumin were used to study the anti-inflammatory activity. The administration of 250 and 300 mg/kg to rats reduced pain and inflammation indicating that M. inermis possesses analgesic and anti-inflammatory activities. The maximum analgesic and anti-inflammatory activities were observed in rats receiving 300 mg/kg of M. inermis extract. This study indicates that methanolic extract of M. inermis possess both anti-inflammatory and analgesic activities.*

**Keywords:** *Mitragyna inermis*, Analgesic, Anti-inflammatory, Rats

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## INTRODUCTION

Inflammation and pain are common nonspecific manifestations of many diseases; though non-steroidal anti-inflammatory drugs (NSAIDs) and opiates have been used classically in these conditions, some adverse reactions occur with these drugs such as gastrointestinal disturbances, renal damage, respiratory depression, and possible dependence [1-2]. There has been an increasing interest to discover new anti-inflammatory and analgesic drugs from natural sources with fewer side effects. Natural products have a long history of use as a folk remedy for inflammatory conditions including fevers, pain, migraine, and arthritis [3]. In recent times, many diseases are thought to be due to inflammation; therefore, anti-inflammatory agents, anti-inflammatory food and food products are of great interest to contain or reduce inflammation induced health disorders [4].

The genus *Mitragyna* is used in traditional medicine for a wide variety of diseases such as fever, malaria, diarrhea, cough, muscular pains and for the expulsion of worms [5-6]. *Mitragyna speciosa* (Korth.) Havil is specie of medicinal importance known as “Kratom” in Thailand and “Biak- Biak” in Malaysia; the leaves are traditionally used by natives for their opium-like effect and coca-like stimulant effect to combat fatigue and enhance tolerance to hard work under hot weather. It has also been used as a substitute for opium and for weaning addicts off morphine. However, the use of this plant has been banned in those countries because of its narcotic effect [7]. Other species common in Africa include *M. ciliate*, *M. inermis* and *M. stipulosa* are used for inflammation, hypertension, headache, rheumatism, gonorrhoea and bronchopulmonary diseases. *M. africanus* which is one of an African species is used in Nigeria to treat mental illness [8].

From the above it is clear that the systematic evaluation of anti-inflammatory and analgesic

activities of *Mitragyna inermis* is lacking, which stimulated us to obtain an insight into the anti-inflammatory and analgesic activities of this plant in Wistar rats and Swiss albino mice.

## MATERIALS AND METHODS

### Plant collection, identification and preparation

Fresh leaves of *Mitragyna inermis* were collected at University of Abuja premises in Gwagwalada, Abuja Nigeria in the month of June during the peak of the rainy season. Gwagwalada has latitude of 8°56'21.48"N and a longitude of 7°4'43.32"E or 8.9393 and 7.0787 respectively, with raining season that start from April and ends in October. The plant material was air dried for 3 weeks, pulverized and taken to National Institute for Pharmaceutical Research and Development for extraction. The plant was identified by Dr. Ugbabe Grace .E. to be *Mitragyna inermis* *O.kuntze* with the voucher specimen number NIPRD|H|6789 was deposited in the Pharmacology and Toxicology laboratory of the Faculty of Veterinary Medicine, University of Abuja, Nigeria.

Three hundred and fifty (350 g) grams of the air-dried leaves were pulverized to a coarse powder and soaked in 2 liters of 80 % methanol using a Soxhlet apparatus for 18 hours. The extract was collected and concentrated *in vacuo* using a rotary evaporator at 40°C to dryness and stored at 4°C until used.

The percentage yield was calculated using:

$$\frac{\text{Weight of extract}}{\text{Weight of pulverized plant material}} \times 100$$

### Determination of the yield of the extract

An empty clean and dry beaker was weighed. The extract was poured into it and the beaker was weighed again after the extract had dried

into a solid mass.

The weight of the extract was determined as follows:

$$\begin{aligned} \text{Percentage yield of the extract \% (w/w)} \\ = \frac{\text{Weight of beaker and extract} - \text{Weight of empty beaker}}{\text{Weight of pulverized sample}} \times 100 \\ = \frac{148 - 130}{350} \times \frac{100}{1} \\ = \frac{18}{350} \times \frac{100}{1} \\ = 5.1\% \end{aligned}$$

The extract obtained was sticky and dark brown in colour with a pungent smell.

### Experimental Animals

Forty Wistar albino rats weighing between 75 – 120 g and 20 mice weighing between 19 – 34 g were used for this study. The animals were kept in well ventilated cages under normal environmental condition (12 hrs light and 12 hrs dark cycles) and ambient temperature of 37 °C at the Veterinary Teaching Hospital, University of Abuja for two weeks to acclimatize before the commencement of the experiments. The animals were fed with Grower's mash from Grand cereals® and water provided *ad libitum*.

### Acute toxicity study

The acute toxicity study was conducted in accordance with Lorke's method with modifications [9]. The study was conducted in two phases using a total of fifteen rats. In the first phase, nine rats were divided into 3 groups of 3 rats each. Groups 1, 2 and 3 animals were given 10, 100 and 1000 mg/kg of the extract respectively, to establish the range of doses producing any toxic effect. In addition, a fourth group of three rats was set up as control group and animals in the group were administered equal volume of distilled water. In the second phase of the experiment, further specific doses of 1600, 2900 and 5000 mg/kg of the extract

was administered to three rats (one rat per dose) to further determine the correct LD<sub>50</sub> value. All animals were observed frequently on the day of treatment and were monitored for 72 hours for signs of acute toxicity.

### Anti-inflammatory study

The anti-inflammatory activity was evaluated using rat paw edema model for acute inflammation induced in Wistar rats by egg albumin [10]. Rats were divided into 5 groups. The base-line reading of the rats paw was taken and recorded before administering any drug. The first group served as the negative control and were administered only egg albumin sub-plantarily. Rats in the second, third and fourth groups were administered 100 mg/kg, 200 mg/kg and 400 mg/kg of the extract respectively, through intra-peritoneal route, 30 minutes later, edema was induced by injecting 0.2 ml egg albumin into the rat hind paws. The fifth group served as the positive control and rats in this group were administered aspirin at 80 mg/kg orally and egg albumin sub-plantarily after 30 minutes.

The edema of the rats paw was measured using black thread and a white calibrated ruler after 20, 40, 60, 80, 100, and 120 minutes. The volume of edema was expressed in mean ± SEM [10]. Protection Against inflammation is expressed as inhibition of edema 20, 40, 60, 80, 100, and 120 minutes after egg albumin injection in comparison with the control group.

### Analgesia study

Acetic acid-induced writhes in mice method as described Koster 1959 [11] was used with some modifications. Twenty (20) mice were divided into 5 groups with four animals each, the first group served as the negative control and were administered only the vehicle (distilled water) intra-peritoneally. The second, third, and fourth group were administered the extract intra-peritoneally, after 30 minutes, 1% acetic acid solution (0.1 ml/kg) was administered intra-peritoneally. The number of writhes (each of

which is characterized by a wave of contraction of abdominal musculature followed by extension of the hind limb) was counted and recorded 5 minutes after acetic acid injection for a period of 10 minutes. The fifth group served as the positive control which was administered aspirin orally and after 30 minutes acetic acid was also administered via the intra peritoneal route.

### Statistical analysis

Differences between the control and the treated groups was analysed using Student's t-test for the methanol extract and one way analysis of variance (ANOVA) and was expressed as Mean  $\pm$  Standard Error of Mean (SEM) using the SPSS version 17. A  $P < 0.05$  was considered statistically significant [12].

## RESULTS

### Acute toxicity

No sign of toxicity was observed neither was mortality recorded in any of the tested doses of 10, 100, 1000, 1600, 2900 and 5000 mg/kg, even after 72 hours of administration of the extract

### Anti-inflammatory study

The result of methanol extract of *Mitragyna inermis* leaf on egg albumin induced edema in rats showed that there was a significant difference ( $p < 0.05$ ) between groups treated with *Mitragyna inermis* extract at 100 and 200 mg/kg, while there was no significant ( $p > 0.05$ ) difference between the fourth group at 400 mg/kg and the fifth group which were administered aspirin orally, more so, there was a significant difference ( $p < 0.05$ ) between the negative control group and the other groups. However, the anti-inflammatory effect of the extract was dose dependent.

**Table.1 Results of the anti-inflammatory study**

Group	Weight(g) (mean $\pm$ SEM)	Baseline reading(cm) (mean $\pm$ SEM)	Time(min)(mean $\pm$ SEM)					
			20	40	60	80	100	120
1	47.05 $\pm$ 1.27 <sup>a</sup>	1.73 $\pm$ 0.09 <sup>a</sup>	3.18 $\pm$ 0.63	3.18 $\pm$ 0.48	3.05 $\pm$ 0.06	3.03 $\pm$ 0.05	2.85 $\pm$ 0.03	1.73 $\pm$ 0.09 <sup>a</sup>
2	52.95 $\pm$ 0.95 <sup>b</sup>	1.98 $\pm$ 0.07 <sup>a</sup>	2.82 $\pm$ 0.15	2.63 $\pm$ 0.13	1.98 $\pm$ 0.07 <sup>b</sup>	1.98 $\pm$ 0.04	1.90 $\pm$ 0.00	1.88 $\pm$ 0.03
3	61.65 $\pm$ 3.26 <sup>c</sup>	1.68 $\pm$ 0.08 <sup>a</sup>	2.55 $\pm$ 0.05	2.40 $\pm$ 0.41	2.10 $\pm$ 0.06	1.78 $\pm$ 0.05	1.68 $\pm$ 0.05 <sup>c</sup>	1.68 $\pm$ 0.05
4	59.98 $\pm$ 2.87 <sup>d</sup>	1.85 $\pm$ 0.03 <sup>a</sup>	2.78 $\pm$ 0.63	2.33 $\pm$ 0.12	1.95 $\pm$ 0.03	1.85 $\pm$ 0.03 <sup>c</sup>	1.85 $\pm$ 0.03	1.85 $\pm$ 0.03
5	64.68 $\pm$ 3.36 <sup>c</sup>	2.05 $\pm$ 0.16 <sup>a</sup>	2.78 $\pm$ 0.16	2.35 $\pm$ 0.24	2.05 $\pm$ 0.16	2.05 $\pm$ 0.16 <sup>b</sup>	2.05 $\pm$ 0.16	2.05 $\pm$ 0.16

n= 4, N= 20, Mean  $\pm$ SEM along

the same vertical column with different superscript show

Statistical difference at  $p < 0.05$

Key: Group 1 = Negative control (egg albumin only).

Group 2 = Positive control (ASA + egg albumin).

Group 3 = 100 mg/kg B.W of the extract + egg albumin

Group 4 = 200 mg/kg B.W of the extract + egg albumin

Group 5 = 400 mg/kg B.W of the extract + egg albumin

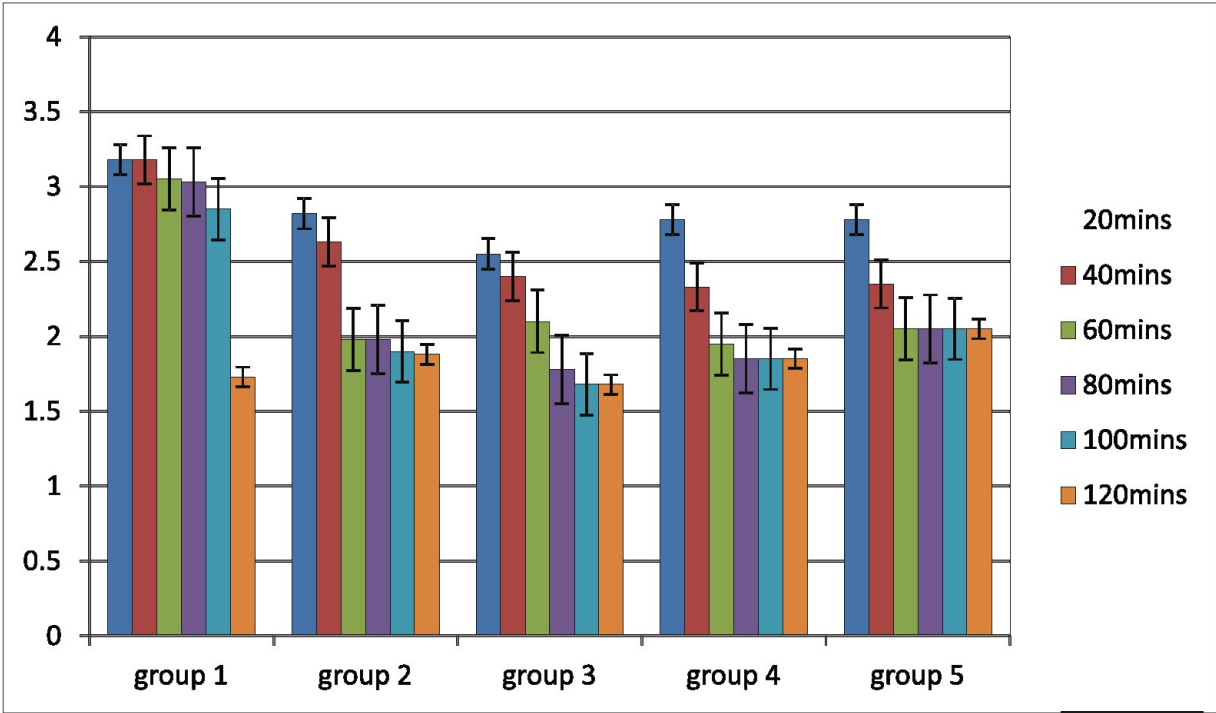


Figure 2: Comparison between minutes per group

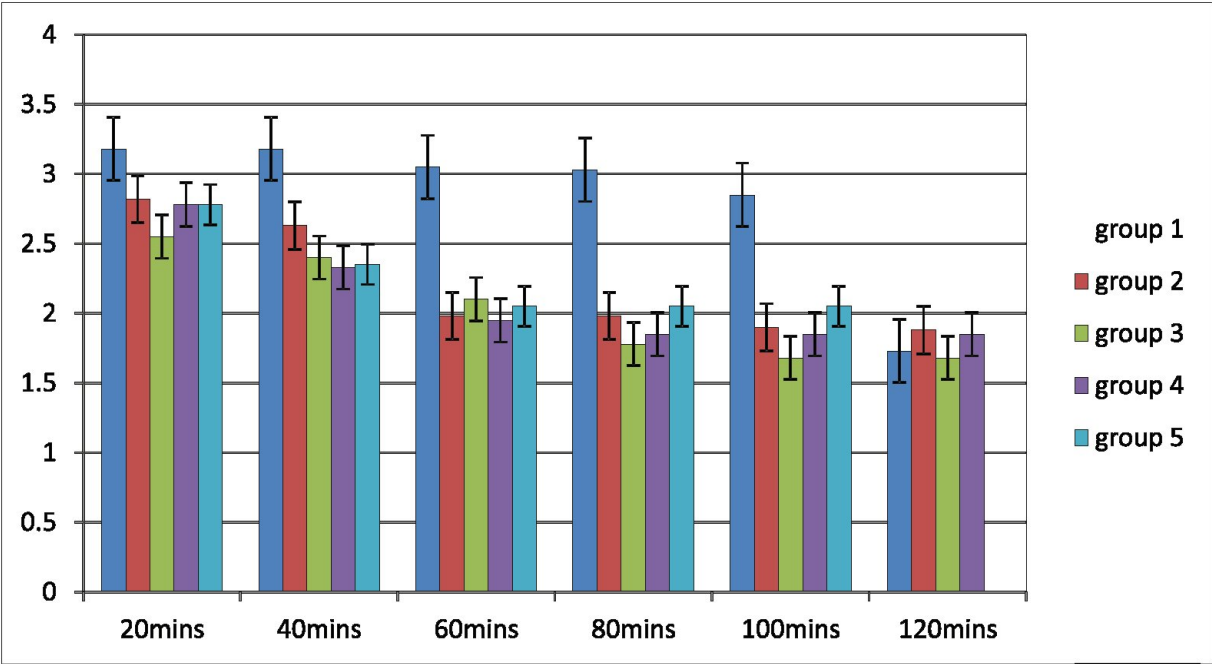


Figure 3: Comparison between groups per minutes

**Table 2: Results of phytochemical screening of *Mitragyna inermis* methanol extract**

PHYTOCHEMICAL CONSTITUENTS	STATUS
Carbohydrate	-
Cardiac glycosides	+
Phenol	+
Cadenolides	+
Balsam	-
Terpenoids	-
Alkaloid	+
Tanins	+
Glycosides	+
Saponins	+
Steriods	+
Resin	-
Phenols	+
Volatile oil	+
Flavonoids	+
Phlobatannoids	-
Triterpenoids	-

Key: + = present and - = absent

### **Analgesia study**

The result of methanol extract of *M. inermis* leaf in acetic acid-induced writhes in mice showed that there was significant difference ( $p < 0.05$ ) between group 1 which was administered the extract at 100 mg/kg, group 2 which was administered the extract at 200 mg/kg and group 4 which was the positive control group and was administered aspirin at 19.2 mg/kg after 30 minutes. However, there was no significant difference ( $p > 0.05$ ) in group

3 which was administered the extract at 400 mg/kg after 30 minutes when compared with the positive control group which was group 4 and was administered aspirin. More so, after 60 minutes there was no significant difference ( $p > 0.05$ ) between group 1, 2, and 3 when compared with the positive control group. Therefore, the analgesic effect of *M. inermis* leaf is both dose dependent and time dependent.

**Table 3: Result of the analgesia study**

GROUPS	No. of writhes in 30 mins (Mean $\pm$ SEM)	No. of writhes in 60 mins (Mean $\pm$ 0.05)
1	7.50 $\pm$ 0.87 <sup>a</sup>	0.75 $\pm$ 0.25 <sup>a</sup>
2	5.75 $\pm$ 0.48 <sup>b</sup>	0.75 $\pm$ 0.25 <sup>a</sup>
3	2.75 $\pm$ 0.50 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>a</sup>
4	3.00 $\pm$ 0.82 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>a</sup>

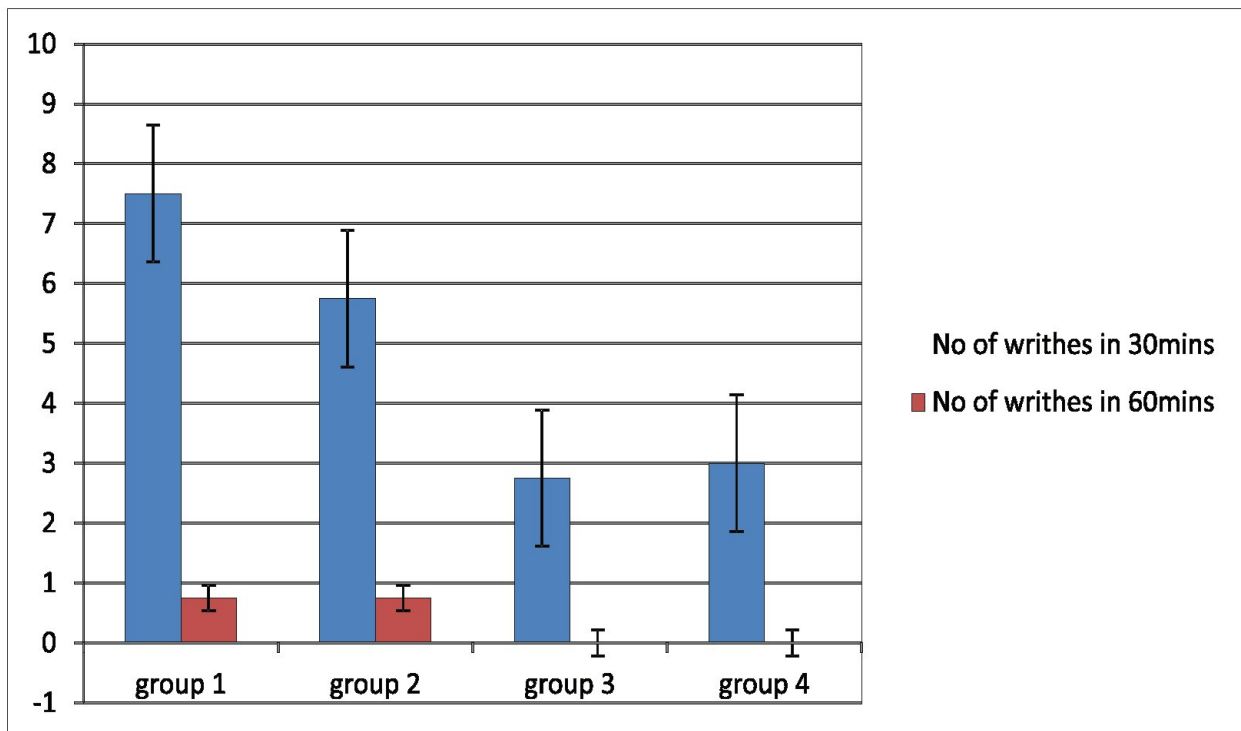
N = 20, n = 4, Mean  $\pm$  SEM along the same vertical column with different superscript are significantly different at P < 0.05

Key: Group 1 = 0.01 ml of extract at 100 mg/kg

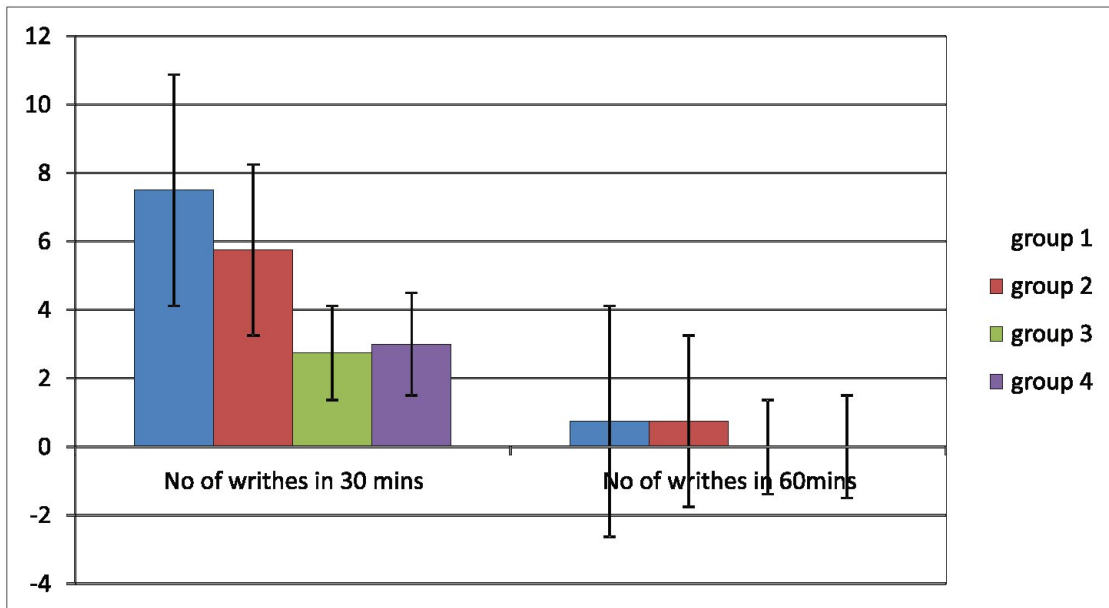
Group 2 = 0.02 ml of extract at 200 mg/kg

Group 3 = 0.06 ml of extract at 400 mg/kg

Group 4 = 0.08 ml of ASA at 19.2 mg/kg



**Figure 4:** Comparison between minutes per group



**Figure 5:** Comparisons between groups per minute.

## DISCUSSION

In the acute toxicity study, the absence of toxicity signs and mortality in all the doses administered (10, 100, 1000, 1600, 2900, and 5000 mg/kg) show that the median lethal dose is  $\geq 5000$  mg/kg. This suggests that the extract is relatively safe at that dose. The result of methanol extract of *M. inermis* leaf on egg albumin induced edema in rats showed that there was a significant difference ( $P < 0.05$ ) between the groups treated with *M. inermis* extract at 100 and 200 mg/kg, while there was no significant ( $p > 0.05$ ) difference between the fourth group at 400 mg/kg and the fifth group which were administered the extract orally. There was also a significant difference ( $p < 0.05$ ) between the negative control group and the other groups. However, the anti-inflammatory effect of the extract was dose dependent.

The result of methanol extract of *M. inermis* leaf in acetic acid-induced writhes in mice showed that there was significant difference

( $p < 0.05$ ) between group 1 which was administered the extract at 100 mg/kg, group 2 which was administered the extract at 200 mg/kg and group 4 which was the positive control group and was administered aspirin at 19.2 mg/kg after 30 minutes. However, there was no significant difference ( $p > 0.05$ ) in group 3 which was administered the extract at 400 mg/kg after 30 minutes when compared with the positive control group which was group 4 and was administered aspirin. More so, after 60 minutes there was no significant difference ( $p > 0.05$ ) between group 1, 2, and 3 when compared with the positive control group.

Therefore, the analgesic effect of *M. inermis* leaf is both dose dependent and time dependent. The actual mechanism of action of methanol extract of *M. inermis* is not fully understood but it could be due to the inhibition of cyclooxygenase leading to the inhibition of prostaglandin synthesis which results due to the combination of inhibition of pro-inflammatory



mediators release and vascular permeability in addition to enhanced immunity, stimulation of tissue repair and healing process [13]. Steroids and flavonoids are the active constituents of *M. inermis*, which has been found to possess anti-inflammatory and analgesic properties [14]. The combination of steroids and flavonoids could make it a very effective drug in the management of inflammation and pain.

### Conclusion

Based on the results of the current study, methanol leaf extract of *M. inermis* has been found to possess anti-inflammatory and analgesic effect in egg albumin induced oedema in Wistar rats and acetic acid-induced writhes in mice respectively. The anti-inflammatory and analgesic activities of the extract at 100, 200 and 400 mg/kg are comparable to that of aspirin. *Mitragyna inermis* therefore, has a potential for the management of inflammation and pain. This however is a preliminary study and it is recommended that further studies on isolation of the active principle and the precise mechanism of action of *M. inermis* leaf extract in anti-inflammation be carried out. This study also authenticates the folkloric use of *M. inermis* in the management of pain and inflammation.

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