ANALGESIC EFFECTS OF *Harungana madagascariensis* STEM BARK EXTRACT USING FOUR EXPERIMENTAL MODELS OF NOCICEPTION.

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ABSTRACT

The analgesic effects of *Harungana madagascariensis* stem-bark ethanolic extract (HME) in mice and rats were studied. The analgesic effects of the extract (HME, 20, 40, 80 mg/kg s.c.) were evaluated by mechanically induced pain through analgesiometer, tail immersion test, hot-plate, and acetic acid-induced analgesic test methods. HME, significantly and dose-dependently produced analgesic effects against thermally- and chemically-induced nociceptive pain in mice, without any effect in mechanically-induced pain through analgesiometer. The opioid antagonist naloxone (2 mg/kg s.c) and acetylsalicylic (100 mg/kg s.c) blocked and potentiated the analgesic effect of *Harungana* extract respectively in various degrees. Our results also tend to suggest that HME possesses centrally- and peripherally-mediated analgesic properties. Although the precise mechanisms of the analgesic actions of HME is established in this study, the findings of this animal study appear to suggest that HME possesses analgesic properties by enhancing Opioidergic neurotransmission and inhibiting COX pathways. These findings, therefore, lend pharmacological credence to the suggested folkloric, ethnomedical uses of the plant as a natural supplementary remedy for the control of pain, as well as for the treatment or management of inflammatory-painful conditions.

Key words: *Harungana madagascariensis* stem bark extract, analgesic effects,

INTRODUCTION

In Africa and the other parts of the world, the extensive use of natural plants as primary health remedies due to their pharmacological properties, is rising (Conco, 1991). In recent years, developing countries seek refuge from natural products extracted from plants, to produce more effective remedies that are affordable by the population especially in infections, inflammation and pain disorders (Farnsworth, 1994). In our laboratory, intensive work has been done in this area of ethnopharmacology (Iwalewa *et al.,* 2003, Omisore *et al.,* 2004, Fadeyi *et al.,* 2004, Okorie *et al.,* 2006, Iwalewa *et al.,* 2006, Idowu *et al.*, 2006). However, *Harungana madagascariensis* Lam. ex Poir, is a very popular and special plant native to Africa and Madagascar (Iwu, 1994). This ornamental garden tree is distributed throughout Africa, from southern Africa to west and east of Africa. Different parts of the plant (leaves, stem bark, roots) are known to possess biological properties, mainly antiprotozoan, antibacterial, antifungal, and antiviral. Other medicinal uses include its use as an abortifacient, to treat anemia, asthma, tuberculosis, fever, angina, diarrhea, dysentery, syphilis, gonorrhea, malaria, parasitic skin diseases and wounds, that may lead to chronic inflammatory pains (Tona, *et al.,* 1998; EMEA, 1999; Lukwa, *et al.,* 2001; Erah, *et al.,* 2003.; Kamanzi Atindehou *et al.,* 2004). Phytochemical studies of this plant have shown that it contains components known for their activities which include anthracenic derivatives, flavonoids, alkaloids, saponins, glycosides, and tannins (Iinuma *et al.,* 1995; Olagunju *et al.,* 2000; Okoli *et al.,* 2002; Capasso *et al.,* 2003) The overall objective of the study is to investigate the analgesic activity of *Harungana madagascariensis* using tail immersion test, hot plate test and acetic acid induced writhing test in mice and mechanically-induced pain through analgesiometer in rats. The study was prompted by the claim that decoctions and infusions of *H. madagascariensis* plant materials (leaves, stem-bark) are effective remedies for the management and control of body pains (EMEA, 1999).

MATERIALS AND METHODS

Plant Materials

Freshly peeled stem-bark of Harungana

madagascariensis_were collected from the main University campus, Ile-Ife, in September 2006. The same *H. madagascariensis* was identified by Mr. O.A Oladele of the Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife and a voucher specimen with voucher number FHI 107392 was kept at the herbarium of the Forestry Research Institute of Nigeria, Ibadan. An ethanolic extract of the plant was used in these experiments.

Preparation of Ethanolic Extract of *Harungana madagascariensis* Stem-bark

The ethanolic extraction of *H. madagascariensis* was prepared by soaking 325 g of powdered stem bark in 50:50 ethanol: water for 24 h. After maceration the extract was evaporated in vacuo on a rotary evaporator to dryness. Weight of crude extract obtained is 9 g (% yield =2.8%)

Animals

Mice and rats of either sex, weighing between 18 – 24 g and 150-200 g respectively were used. The animals were maintained at 25 $\pm$1°C under natural 12 h daylight/night conditions for at least 5 days before the experimental procedures. All the animals were fed with standard diet in the Department of Pharmacology Animal House and water was given *ad libitum*. The "principle of laboratory animal care" (NIH publication No. 85-23) guidelines and procedures were followed in this study (NIH publication revised, 1985).

Drugs

The following drugs were used during the experiment: Carragenan (Sigma), Naloxone (Sigma, St. Louis, USA), Disprin(R) Acetylsalicylic acid (Reckiti-Benckiser), Indomethacin (Sigma), Ethanol 99% (Analar Grade), Glacial acetic acid (BDH).

Analgesic Activity

Tail Immersion Test Method

Six groups of mice, each group containing 5 mice were used in this method. Group I served as the control that received 0.5 ml of normal saline s.c., Groups II-IV were given the ethanolic extract at doses of HM 20, 40, and 80 mg/kg s.c. while groups V and VI as positive controls received ASA (100 mg/kg) and naloxone (2 mg/kg). In another six groups, combined studies were done for extracts and the positive controls. 3 groups received three doses of the extract and ASA, while the other 3 groups got the extract and naloxone. After 1 h of agents' administration, mice tails were placed in a water bath (50 ^{0}C) and observed for the reaction time to the thermal stimulus. The reaction time was measured at 30, 60, and 90 minutes (T_{30}, T_{60}, and T_{90}). The reaction time was taken as the time when the animals withdrew their tails completely from the hot water in the bath (Parimaladeri *et al.,* 2003).

Hot Plate Test in Mice

Six groups of mice (n = 5) were also used in this method. Group I serves as the control that received 0.5 ml of normal saline s.c., Groups II-IV were given the ethanolic extract at doses of 20, 40, and 80 mg/kg s.c. while groups V and VI as positive controls received ASA (100 mg/kg) and naloxone (2 mg/kg). In another six groups, combined studies were done for extracts and the positive controls. 3 groups received the extract and ASA, while the other 3 groups got the extract and naloxone. After 1 h of agents' administration, mice were placed on a test hot plate (55 ^{0}C) and observed for the reaction time to the thermal stimulus according to the method described by Woolfe and MacDonald (1944). The time that elapsed until the occurrence of either a hind paw licking or a jump off the surface was recorded as the hot plate latency. Effects of the groups treated with ethanolic extract at the doses of 20, 40, 80 mg/kg s.c were measured at 30, 60 and 90 minutes (T_{30}, T_{60}, and T_{90}) respectively.

Acetic Acid Induced Writhing Method

Six groups of mice (n = 5) were used in this method. Group I served as the control that received 0.5ml of normal saline s.c., Groups II-IV were given the ethanolic extract at doses of 20, 40, and 80 mg/kg s.c. while groups V and VI as positive controls received ASA (100 mg/kg) and naloxone (2 mg/kg). In another six groups, combined studies were done for extracts and the positive controls. 3 groups received the extract and ASA, while the other 3 groups got the extract and naloxone. After 1 h of agents' subcutaneous administration of the plant extract or the combination of the extract with either naloxone or acetylsalicylic acid, 0.1 ml of 3% acetic acid solution was injected to each of the test mice s.c

(Koster *et al.,* 1995). The number of abdominal contractions that occurred within the next 20 minutes following active acetic acid administration were counted and recorded. A significant reduction in the number of acetic acid-induced abdominal contractions of the treated mice, compared to the contractions in the untreated control mice was taken as an indication of analgesic activity.

Analgesiometer Method: Pain Threshold of Rats

The oedematous right hind paws induced by 0.1ml of 1% carrageenan were subjected to an increasing force (pressure) according to the method of Randall and Sellito (1957). One hour following the administration of ethanolic extract of *H. madagascariensis* (20, 40 and 80 mg/kg i.p), or IND (10 mg/kg i.p.) into all the test animals in groups 2-5, and 0.3 ml/kg i.p normal saline into the control animals, the pain threshold was measured mechanically using Ugo Basile Analgesiometer – Model 09380, Milan, Italy) at 0, 0.5, 1, 2, 3, 4 h after drug administration. Squealing of the animals as a consequence of application of continuous pressure to their paw was taken as the reaction time of the animals. Thereafter, the pressure (force) stimulus was terminated, and the pain threshold was read off from the scale.

Statistical Analysis

Values were expressed as mean $\pm$ S.E.M. Statistical significance was determined using the student's t-test. Values with $p < 0.001$, 0.005 and 0.05 were considered significant.

RESULTS

Analgesic Effects of Stem-bark Extract (HME) on Thermally-chemically-and Mechanically-induced Nociceptive Pain

In the experimental animal used, *H. madagascariensis* stem-bark extract (HME, 20–80 mg/kg s.c.) produced dose-related and significant ($p<0.05$–0.005) analgesic effects against thermally- and chemically-induced nociceptive pain (Tables & Figures 3.1, 3.2, 3.3). *H. madagascariensis* stem-bark extract (HME, 20–80 mg/kg s.c..) dose-dependently and significantly delayed ($p<0.05$–0.001) the reaction times of the mice used in the tail immersion test and hot-plate analgesic test methods (Tables & Figures 3.1, 3.2). In the same analgesic test methods, acetylsalicylic acid (ASA, 100 mg/kg s.c.) and naloxone (2 mg/kg s.c.) also profoundly delayed the reaction times of the animals. Moreover, the plant extract (HME, 20–80 mg/kg s.c.) dose-dependently and significantly inhibited ($p<0.05$–0.001) acetic acid-induced writhes in mice (Table & Figure 3.3). Similarly, acetylsalicylic acid (ASA, 100 mg/kg s.c.) and naloxone (2 mg/kg s.c.) markedly and significantly reduced ($p<0.05$–0.005) acetic acid-induced writhes in the mice. However, when compared with the normal control, the treatment with *H. madagascariensis* extract produced no significant response of rats to the mechanical pain induced by the use of the analgesiometer, while indomethacin showed an increase in pain threshold significantly at 3 h (Table. 3.4)

Table 1. Effects of Different Doses of the Ethanolic Extract of *H. madagasceriensis* (HME) Alone and with Concomitant Administration of Naloxone (2 mg/kg) or Acetysalicylic acid (100 mg/kg) on Mice Subjected to Tail Immersion Test.

Group/Dose (mg / kg)	Reaction time T_{30}	Reaction time T_{60}	Reaction time T_{90}
Normal Saline (Control)			
0.5ml	1.37 ± 0.30	1.27 ± 0.20	1.38 ± 0.20
HME			
20	1.40 ± 0.20	1.50 ± 0.04	1.75 ± 0.10
40	2.33 ± 0.01*	2.00 ± 0.13*	2.17 ± 0.20*
80	2.45 ± 0.30*	2.00 ± 0.20*	3.36 ± 0.60*
Naloxone (2 mg/kg)/HME			
20	1.40 ± 0.2	1.33 ± 0.10	1.41 ± 0.10
40	1.38 ± 0.1	1.30 ± 0.40	1.50 ± 0.20
80	1.50 ± 0.14	1.80 ± 0.13*	1.80 ± 0.11
ASA (100 mg/kg)/HME			
20	1.60 ± 0.30	1.30 ± 0.10	2.10 ± 0.50
40	2.40 ± 0.40	2.80 ± 1.70	2.40 ± 0.70
80	4.70 ± 0.40***	5.70 ± 0.30***	4.80 ± 0.90*
ASA			
100	1.90 ± 0.50	3.40 ± 0.50**	4.80 ± 0.10*
Naloxone			
2	1.69 ± 0.10*	1.84 ± 0.10*	1.44 ± 0.40

Significantly Different at $p < 0.05$*, 0.005** and 0.001*** compared to Control.

Table 2: Effects of Different Doses of the Ethanolic Extract of *H. madagasceriensis* Alone and with Concomitant Administration of Naloxone (2 mg/kg) or Acetysalicylic acid (100 mg/kg) on Mice Subjected to Hot Plate Test.

Group/Dose (mg / kg)	Reaction time T_{30}	Reaction time T_{60}	Reaction time T_{90}
Normal Saline (Control)			
0.5ml	8.6 ± 1.4	8.5 ± 1.8	9.8 ± 1.3
Harungana			
20	12.45 ± 0.5*	8.7 ± 0.7	9.65 ± 0.7
40	14.33 ± 0.8**	15.97 ± 0.9**	10.70 ± 0.1
80	21.13 ± 2.5***	22.93 ± 3.6***	14.37 ± 0.6*
Naloxone (2 mg/kg)/Harungana			
20	9.5 ± 0.1	13.9 ± 3.8	20.3 ± 2.8*
40	10.4 ± 2.0*	14.3 ± 0.9*	17.7 ± 2.9*
80	9.2 ± 0.4	16.6 ± 1.8*	15.7 ± 3.5
ASA (100 mg/kg)/Harungana			
20	13.4 ± 1.8	13.1 ± 1.9	12.6 ± 1.4
40	16.9 ± 1.7***	17.0 ± 2.4*	18.7 ± 2.8*
80	25.2 ± 0.5**	23.1 ± 2.5***	16.0 ± 2.5
ASA			
100	13.7 ± 1.2*	15.2 ± 2.1*	19.0 ± 2.3*
Naloxone			
2	12.3 ± 2.2	12.8 ± 2.5	15.5 ± 2.0

Significantly different at $p < 0.05$*, 0.001** and 0.005*** compared to Control.

Table 3: Effects of Different Doses of the Ethanolic Extract of *H. madagasceriensis* Alone and When Pretreated with Naloxone (2 mg/kg) and Acetysalicylic acid (100 mg/kg) on Mice Subjected to Acetic Acid Induced Writhing.

Group/Dose (mg / kg)	Average Number of Contraction/ ± SEM
Acetic acid (Control) 0.1ml	82.6 ± 4.6
Harungana	
20	69.3 ± 3.5*
40	60.3 ± 7.1*
80	48.8 ± 3.5***
Naloxone (2 mg/kg)/Harungana 20	64.7 ± 5.8*
40	57.0 ± 8.23*
80	37.0 ± 11.1**
ASA (100 mg/kg)/Harungana 20	27.7 ± 3.6***
40	15.3 ± 2.9***
80	15.7 ± 2.8***
ASA 100	3.4± 0.8**
Naloxone 2	15.3 ± 2.6***

Significantly different at $p < 0.05$*, 0.001** and 0.005*** compared to Control.

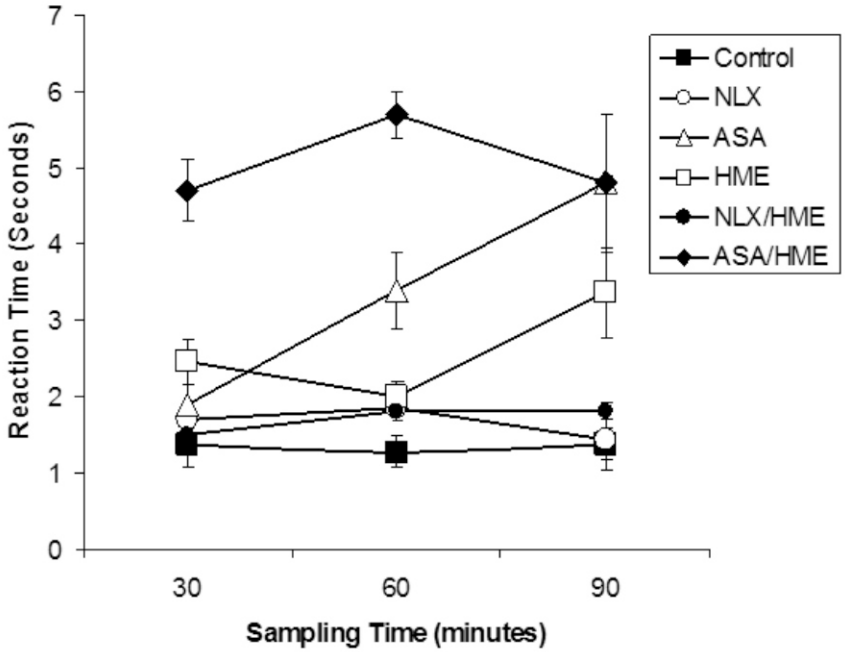


Figure 1: The Effects of Subcutaneous Administration of 80 mg/kg Ethanolic Extract of *Harungana madagascariensis* (HME) Alone and After Pretreatment with 2 mg/kg Naloxone (NLX/HME) and 100 mg/kg Acetylsalicylic Acid (ASA/HME) on Mice Subjected to Tail Immersion Test.

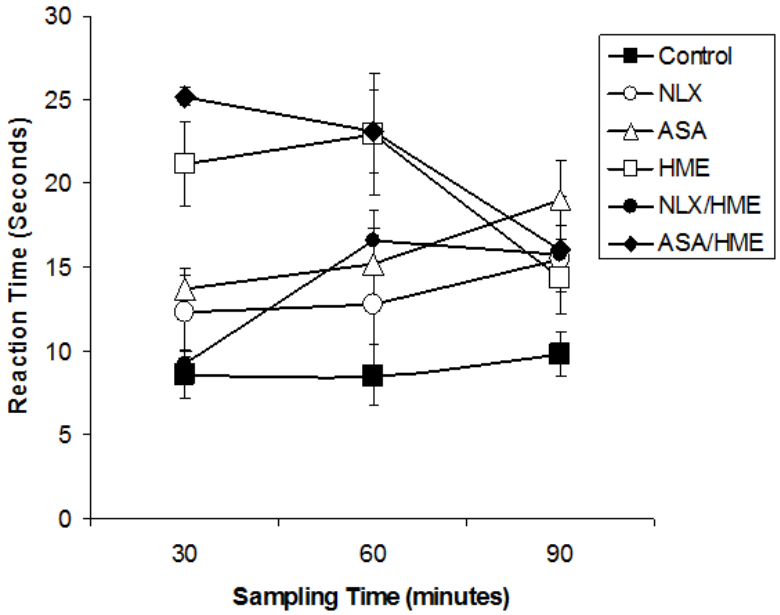
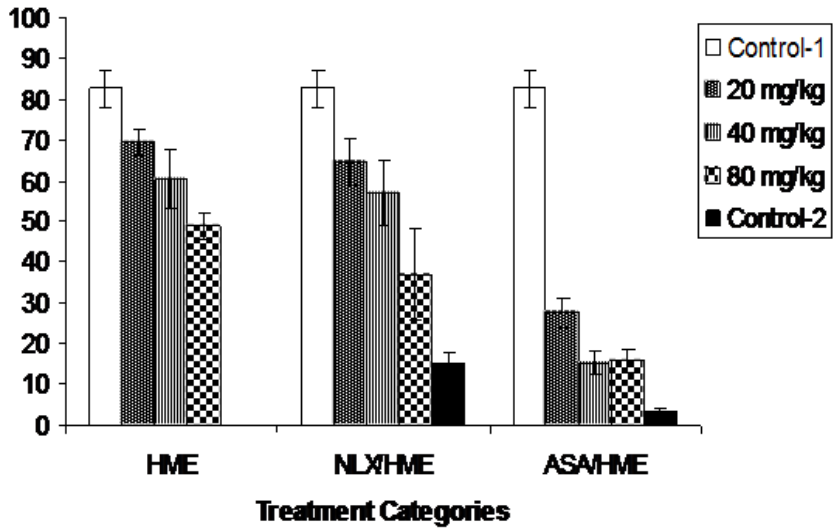


Figure 2: The effects of Subcutaneous Administration of 80 mg/kg Ethanolic Extract of *Harungana madagascariensis* (HME) Alone and After Pretreatment with 2 mg/kg Naloxone (NLX/HME) and 100 mg.kg Acetylsalicylic acid (ASA/HME) on Mice Subjected to Hot Plate Test.



(Control-1 is Normal saline while Control-2 represents NLX alone in NLX/HME and ASA alone in ASA/HME group). Dose (mg/kg) in the X axis and (%) percentage inhibitions or number of writes in the Y axis

Figure 3: The effects of Ethanolic Extract of *Harungana madagascariensis* (HME) Alone and After Pretreatment with Naloxone (NLX - 2 mg/kg) and Acetylsalicylic acid (ASA – 100 mg/kg) on Mice Subjected to Acetic Acid Writhing Test.

Table 4: The Effects of *Harungana madagascariensis* Stem Bark Extract, on Pain Response Induced by Analgesiometer in Rats. (Mean± SEM, n=5)

Treatment Groups	Dose of Extract/ Standard Drugs (mg/kg)	No of Rats	Pain Threshold (Mean +/- SEM) (ml)					
			0	0.5hr	1hr	2hr	3hr	4hr
Normal Control	-	5	235.7 ± 31.6	201.4 ±16.9	197.1 ±21.5	171.4 ±16.9	154.3 ±39.9[a]	150.0 ±33.3
1	20	5	232.5 ±33.3	225.0 ±8.7	202.5 ±14.4	187.5 ±18.9	187.5 ±22.5	172.5 ±37.5
2	40	5	210.0 ±27.4	180.0 ±24.5	165.0 ±28.7	172.5 ±18.9	217.5 ±7.5	255.0 ±28.7
3	80	5	240.0 ±47.4	180.0 ±27.4	202.5 ±57.9	165.0 ±25.9	127.5 ±18.9	150.0 ±12.2
(IND) Indomethacin	10	5	187.5 ±33.3	225.0 ±35.7	247.5 ±51.1	277.5 ±51.1	307.5 ±7.5 [ab]	270.0 ±36.7

DISCUSSION

Pain in man is an unpleasant sensory and emotional experience associated with actual or potential tissue damage (IASP, 1979). It is also an aversive sensory experience that elicits protective motor actions, results in learned avoidance and may modify species-specific traits of behaviour, including social behaviour (Zimmerman, 1986). In Africa, malaria and other infections results in pain and febrile conditions among children and adults alike especially in the rural communities. Some of these conditions often result into death. Although there are a number of synthetic analgesic drugs currently available for use in the management and control of inflammatory pain, most of these synthetic analgesics and antiinflammatory drugs are not only inaccessible and unaffordable in the rural setting, but they also possess many toxic adverse effects. There is, therefore, a dire need for the discovery of cheap, effective and safe analgesic agents from plants and other natural sources. In our laboratory, work has been geared towards this area of ethnopharmacology (Iwalewa *et al.,* 2003, Omisore *et al.,* 2004, Fadeyi *et al.,* 2004, Okorie *et al.,* 2006, Iwalewa *et al.,* 2006, Idowu *et al.,* 2006).

The results of the present study provide evidence in favour of the analgesic activity of *Harungana madagascariensis* stem-bark extract in the experimental animal models used. In all the models, except the mechanically induced pain, HME showed a dose- and time-dependent analgesic actions, which were either blocked by the pre-treatment of naloxone (an opioids receptor antagonist) or potentiated by ASA (an inhibitor of COX enzymes). The results obtained appear to suggest that *H. madagascariensis* stem-bark extract possesses centrally- and peripherally-mediated analgesic properties. The central analgesic action of the plant's extract may be mediated via blockade of central opioids pain receptors, as shown in tail immersion test, while the peripheral analgesic effect of the extract may be mediated through both peripheral opioids pain receptors in the GIT and inhibition of cyclo-oxygenases and/or lipoxygenases (and other inflammatory mediators) in hot plate and acetic acid-induced nociceptionsm. This hypothesis is in agreement with those of Koster *et al.* (1959), Collier *et al.* (1968), Williamson *et al.* (1996) and Martins *et al.* (2004) who postulated that acetic acid-induced writhing and hot-plate test methods are useful techniques for the evaluation of peripherally- and centrally-acting analgesic drugs, respectively.

The result obtained for the study of analgesic activity of the plant extract in mechanically induced pain (Table 3.4) shows that the ethanolic stem bark extract of the *H. madagascariensis* has no analgesic activity. When compared to the normal

control result, no significant change in response to pain threshold was observed at all dose levels of the extract tested. The reason for non-activity through analgesiometer could not be ascertained here knowing fully well that the mechanism of carrageenan induced-oedema formation is by the production of mediators (histamine, serotonin, kinnin and prostaglandins), which are responsible for pain at the hind limbs (DiRosa, *et al.,* 1971). The biosynthesis and release of nitric oxide (NO) and prostaglandins (PGs) share a number of similarities.

Three major forms of nitric-oxide synthase (constitutive, inducible and endothelial NOS) and cyclooxygenase (COX) enzymes have been identified to date (Mollace *et al.,* 2005). Recently, investigators have studied the effects of selective inhibitors of the different COX and NOS isoforms on nociceptive processing. Moore *et al.,* 1993a, 1993b found that intraperitoneal (i.p.) injections of the nNOS inhibitor, 7-nitroindazole (7-NI), significantly reduced hindpaw-licking behaviours due to formalin. Meller *et al.* (1994b) demonstrated that i.t. administration of the iNOS-selective inhibitor, aminoguanidine (AG), was able to inhibit thermal, but not mechanical hyperalgesia in the zymosan inflammatory model. In the carrageenan inflammation model, Lawand et al. (1997) found that intra-articular administration of 7-NI, after carrageenan-induced joint inflammation, attenuated thermal hyperalgesia for approximately 1 h, while Handy and Moore (1998b) also found that 7-NI, (i.p.), inhibited thermal hyperalgesia due to carrageenan-induced hindpaw inflammation. This suggests that NO and COX contributes to nociception after peripheral injury; however, since these studies have used different nociceptive models and different routes of administration for their inhibitors, it is difficult to establish the relative role of the different enzymes isoforms in either peripheral or spinal nociceptive mechanisms. In our study therefore, a different COX enzyme isoforms totally different from COX-1 and -2 is suggested or could be involved since the extract and indomethacin could hardly produce any significant change in pain threshold.

The data obtained in the present study allow us to draw a crude conclusion on the mechanisms of action of *H. madagascariensis* stem-bark extract in the experimental animal used. These actions could be linked to the involvement of COX and opioids receptors activation. In addition to this, a number of investigators have shown that tannins and other polyphenolic compounds (e. g., coumarins), flavonoids, triterpenoids, and a host of other secondary plant metabolites possess analgesic, anti-inflammatory properties in various experimental animal models (Jäger, *et al.,* 1996; Adzu *et al.,* 2003; Dongmo *et al.,* 2003; Iwalewa *et al.,* 2003; Taesotiku *et al.,* 2003; Asongalem *et al.,* 2004). *H. madagascariensis* plant has been shown to contain constituents that include anthracenic derivatives, flavonoids, alkaloids, saponins, glycosides, and tannins (Iinuma *et al.,* 1995; Olagunju *et al.,* 2000; Okoli *et al.,* 2002; Capasso *et al.,* 2003), it is therefore reasonable to suggest that some of these polyphenolic compounds and flavonoids are probably responsible for the observed analgesic effects of the plant's extract.

In conclusion, therefore, this pharmacological action of HME provides some rational explanations and justifications for the ethnomedical uses of the plant in African traditional medicine.

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