

ANALGESIC AND ANXIOLYTIC PROPERTIES OF AQUEOUS EXTRACT OF BARK FROM TRUNK OF *DIOSPYROS MESPILIFORMIS* (EBENACEAE) ON ARTHRITIS INDUCED IN MICE

Gisèle Atsang à kiki^{1,*}, Finsia Egre¹, Marc Germain Minoué Kuum², Francis Takvou¹, Mathieu Zramah¹, Paul Désiré Dzeufiet Djomeni³

¹ University of Maroua, Faculty of Science, Department of Biological Sciences, Maroua, Cameroon

² Department of Psychology, Faculty of Letters and Social Sciences, P.O. Box 3132, University of Douala, Cameroon.

³ University of Yaounde I, Faculty of Science, Department of Animal Biology, Yaounde, Cameroon

* Corresponding author e-mail: atsanggisele@yahoo.fr

Abstract

Arthritis is an inflammatory disease that affects the joints. Patients suffering from chronic pain are anxious, which contributes to reducing the quality of life. The development of analgesic compounds with anxiolytic properties could prove to be of great interest for the treatment of chronic pain. The objective of the present study was to evaluate the analgesic and anxiolytic properties of the aqueous extract of the bark of the trunk of *Diospyros mespiliformis* in arthritic mice. Arthritis was induced by injection of 1% formaline into the left hind paw of the animals. Inflammatory pain and comorbid anxiety were tested using a hotplate ($55 \pm 0.5^\circ\text{C}$) and labyrinths (Dark and Light and the open arena), respectively. The aqueous extract of *Diospyros mespiliformis* reduced the inflammatory process by inhibiting the edema of the legs of animals to a maximum percentage of 63.63 % (minimum of 8.82%) as well as the significant increase ($p < 0.001$) of the threshold of nociception at the dose of 100 mg/kg. A significant increase ($p < 0.001$) in the time spent in the lighted compartment alongside the decrease in the time spent in the dark environment was observed with the two-compartment maze. In the open arena the time spent in the central plaza significantly ($p < 0.001$) increased compared to the time spent on the edge. In addition, a significant decrease ($p < 0.01$) in the frequency of grooming and training was observed. The aqueous extract of the bark of the trunk of *Diospyros mespiliformis* displayed beneficial effects on pain and anxiety, justifying its traditional use for the management of arthritis.

Keywords: analgesic, anxiolytic, *Diospyros mespiliformis*, arthritis.

Introduction

Arthritis is an inflammatory disease of the joints that can affect any joint in the body (Jennifer 2018). It is a bilateral disease mainly localized in the ankles, characterized by continuous swelling around the joint, pain, synovial hyperplasia, pannus formation and morphological changes. These symptoms can lead to severe disability and a poor, unenviable quality of life (Foyet et al. 2015). Arthritis is a very common disease around the world, with more than 91 million people living with it in the United States (Arthritis Foundation 2019). The main clinical symptom of arthritis is pain leading to limited mobility (Vincent et al. 2010), fatigue, impaired muscle strength, muscle weakness, and a change in gait (Abbott et al. 2017, Rice et al. 2015).

Persistent pain leads to anxiety-type mental disorders in some patients with arthritis (Duica 2016). About 40% of patients with chronic arthritis pain are anxious (Twillman 2007). Anxiety is more common in people with any form of arthritis with increased pain sensitivity (Axford et al. 2019, Sharma et al. 2016). The pain and anxiety caused by arthritis should be considered at the same time (Hermans et al. 2012). Thus to fight against arthritis pain and comorbid mental disorders, drugs such as cyclooxygenase (COX) inhibitors are often used in combination with compounds having anxiolytic effects. But such a combination therapy has limits because of the interaction between pharmacological compounds which constitute this treatment and very considerable side effects such as digestive damage (peptic ulcers, stenosis, perforations), renal insufficiency and hepatitis and even cardiac complications (Yougbaré-ziébrou et al. 2016, Soubrier et al. 2013). However, it is difficult to find conventional medications that work to reduce both pain and anxiety without serious risk. It is therefore important to develop alternative analgesic therapies with anxiolytic properties with a limited side effect profile, beneficial for patients suffering daily from chronic pain and associated psychopathologies, such as anxiety. In recent decades, a lot of research has focused on the valuation of traditional medicine with a view to verify the effectiveness of the substances used and establish scientific rules for their use (Cheriti et al. 2016). *Diospyros mespiliformis* is a plant of the Ebenaceae family, well known in Central Africa for its fruits which are highly appreciated by the population. Commonly known as African ebony, it is registered in the Cameroonian pharmacopoeia and used in traditional medicine for the treatment of arthritis pain and anxiety (Arbonnier 2008). But the population, although they find relief thanks to this treatment, do not know much about the doses and the dosage of this drug, which should not be overlooked when taking drugs. This study was conducted to assess the beneficial effect of different doses of the aqueous extract of the bark of the trunk of *Diospyros mespiliformis* on joint pain and comorbid anxiety disorders.

Material and Methods

Chemicals

To induce peripheral inflammation, mice were injected with formaline (Sigma-Aldrich, St. Louis, MO, USA) subcutaneously under the fascia of animal right paw. To assess changes in paw volume during inflammation, animal were injected in the left hind paw with 0.04 mL of formaline. Control animals received orally distilled water. The anxiolytic and analgesic drugs used were: diazepam, diclofenac (all from Sigma-Aldrich) respectively. Group tested included *Diospyros mespiliformis*.

Phytochemical analyses

Qualitative phytochemical investigations of *Diopyros mespiliformis* aqueous extract were performed for flavonoids, saponins, phenols, lipid, tannins and glycoside cardiac using standard methods previously described Trease and Evans. 1980.

Preparation of plant material

The plant material *Diospyros mespiliformis* collected in the Maroua zone (Region, Far North, Cameroon) (N10°36'45.234" and E14°16'43.08") in June 2020. The plant was authenticated at the herbarium of the School of Fauna of Garoua, Cameroon by a reference sample deposited at number HEFG / 01404.

Diospyros mespiliformis was cut into small pieces and dried in the shade then reduced to a very fine powder. Three hundred grams (300 g) of powder was boiled in 2 liters of distilled water for 15 minutes. The solution was filtered using coffee filter paper (pore diameter 20 µm) from BELLE France (Lyon, France) and then evaporated in an oven at 50°C temperature of evaporation.

Animal and experimental design

Animal material: Adult *Mus musculus* Swiss strain mice of both sexes weighing 25 ± 5 g and aged 10 ± 2 weeks at the start of the experiment were used. The animals were kept in a room at room temperature in cages lined with litter before and during the period of the experiment. The mice had free access to tap water and standard diet.

Thirty-five mice were distributed into 7 groups of 5 mice each without a distinction of sex (Table 1). All treatments were administered orally thirty minutes before formaline induction (day 0), then the animals were treated daily for up to the 10th day.

Table 1. Grouping of animals

Groups	Administered substance	Doses	Route of administration
Normal	Distilled water	10 (mL/kg)	oral
Negative Control	Distilled water + formaline 1 %	10 (mL/kg)	oral
Positive control	Diclofenac+ formaline 1 %	5 (mg/kg)	oral
Positive control	Diazepam	2 (mg/kg)	oral
Treatment	Aqueous extract+ formaline 1 %	100 (mg/kg)	Oral+ subcutaneous
Treatment	Aqueous extract+ formaline 1 %	200 (mg/kg)	Oral+ subcutaneous
Treatment	Aqueous extract+ formaline 1 %	400 (mg/kg)	Oral+ subcutaneous

Induction of arthritis by formaline 1%

To induce inflammatory arthritis we followed the method by injecting a 1% formaline solution (0.04 mL) under the plantar fascia of the left hind paw of the mouse described by Rahmani et al. (2016) after fasting animals for 17 hours with free access to water. The formaline injection (0.04 mL / mouse; 1%) was performed twice, one in the first day and the other in the third day of the experiment. Treatments were started 30 minutes after induction of arthritis and continued throughout the day until the end of the experiment at doses: 100, 200 and 400 mg/kg of AEDM (aqueous extract of *Diospyros mespiliformis*). The negative control group was treated with distilled water (10 mL/kg) while the positive control groups were treated with diclofenac and diazepam respectively for arthritis and anxiety. The animals' body weight was taken daily using an electronic scale.

The evolution of the edema was followed by measuring the diameter of the edematous paw (mm) of each animal every day throughout the period of the experiment using a digital electronic caliper (precision 0.03 mm). Edema in different groups of animals was determined by the following formula:

$$\Delta E = E_j - E_0$$

ΔE = the difference in edema between j_0 and j_x

E_0 = the initial thickness (mm) of the left paw (before the injection of formaldehyde)

E_j = the thickness of the left paw (mm) at day "j" after the injection of formaldehyde

The percentage of inhibition "% Inh" was calculated by the following

$$\% \text{ Inh} = 100 \left[1 - \frac{\Delta E_t}{\Delta E_c} \right]$$

ΔE_t = represents the difference in edema between j_0 and j_x of the left paw of the treated mouse

ΔE_c = represents the difference in edema between d_0 and j_x of the left paw of the untreated mouse.

At the end of the experiment, all the animals were sacrificed by cervical dislocation. The blood of each animal was collected in an anticoagulant tube (heparin) and then centrifuged at 3000

round/min for 15 minutes at 4 ° C. The sera obtained were collected in microtubes for the assay of the C-reactive protein (CRP). The livers were isolated and then homogenized in a phosphate buffer (0.15M, pH = 7.4) (Zuo et al. 2014).

The supernatant was used for evaluation of some oxidative stress parameters: Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH). The hind legs were removed and fixed in 10 % formalin solution for histological studies.

ANXIETY TEST

Lighted and Dark Compartment Maze Test

The device consisted of a light / dark box (45 x 27 x 27 cm) and composed two chambers connected by an opening (7.5 x 7.5 cm) located at ground level in the center of the wall separating the two chambers.

The small chamber (18 x 27 cm) was painted black (dark room) and the larger room (27 x 27 cm) was painted white (bright room). The parameters taken into account were: latency time, time spent in the lighted compartment, time spent in the dark compartment. Each mouse was placed in the center of the light chamber back to the dark room and allowed to explore both compartments of the device for 5 minutes. After 5 minutes, the animal was removed from the device and the device cleaned with a 70% ethanol solution and allowed to dry between tests.

Open arena test

It is an open space arena, square in shape (72 × 72 cm) and 36 cm high. Visible red lines were drawn on the floor using a marker (Foyet et al. 2012). These lines have the role of delimiting a central (ZC), intermediate (ZI) and peripheral (ZP) zone near the wall. The time spent in the center and on the edge, the training (rearing), the grooming, the number of lines crossed were the parameters taken into account during the tests. After each test, the mouse was removed and returned to its cage. The entire maze floor was cleaned with a 70% ethanol solution after each test and allowed to dry between tests.

Hyperalgesia test

This test consists of a hot plate apparatus maintained at a temperature of 55 ± 0.5 ° C on which the mice were placed for the test (Foyet et al., 2015). The pain threshold was determined by the latency of the nociceptive response (reaction time for the animal to lick the paw or jump off the hotplate) with a maximum cut-off time of 15 s for each animal (Foyet et al. 2015).

Evaluation of the antioxidant activity *in vivo* of the aqueous extract of the bark of *Diospyros mespiliformis*

The antioxidant potential of the extract was assessed by estimating Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) in the liver homogenate of according to the methods of Wilbur et al. in 1949, Misra and Fridovich in 1972, Sinha in 1972 and Ellman in 1959 respectively.

Histological study

For microscopic evaluation, the investigated organs were dehydrated and paraffin-embedded for microscopic examination in accordance with routine laboratory procedures. Paraffin sections of 5 µm were prepared and stained with haematoxylin and eosin for histological examination.

Statistical analyzes

Results were expressed as the mean ± standard error of the mean (ESM) for each group. Number per group = 5. The one way analysis of variance test (Anova) was used followed by the Student Newman Kells post test to compare the values with each other. The results were considered to be significantly different for p <0.05.

Results

Qualitative Phytochemistry

Phytochemical screening of the aqueous extract of the bark of the trunk of *Diospyros mespiliformis* revealed the presence of several bioactive compounds (Table 2).

Table 2. Phytochemical of the aqueous extract of *Diospyros mespiliformis*

Compound class	Alcaloids	Flavonoids	Tanins	Saponins	Terpenoids	Sugar	Quinons	Coumarins
Observation	+	+	+	+	-	+	+	+

+ = presence - = absence

Effect of *Diospyros mespiliformis* aqueous extract on weight gain in mice

A decrease in body weight was noted in all experimental groups except the normal group after the days following formaline injection. This decrease was observed in the negative group from the fourth day and the weight of the animals continued to decrease until the end of the experiment. The difference was only significant ($p < 0.01$) in the negative compared to the normal control. On the other hand, for the treated groups, weight recovery was noted during the last days of the experiment (Figure 1). Treatment of the mice with the aqueous extract of *Diospyros mespiliformis* did not influence the body weight of the animals.

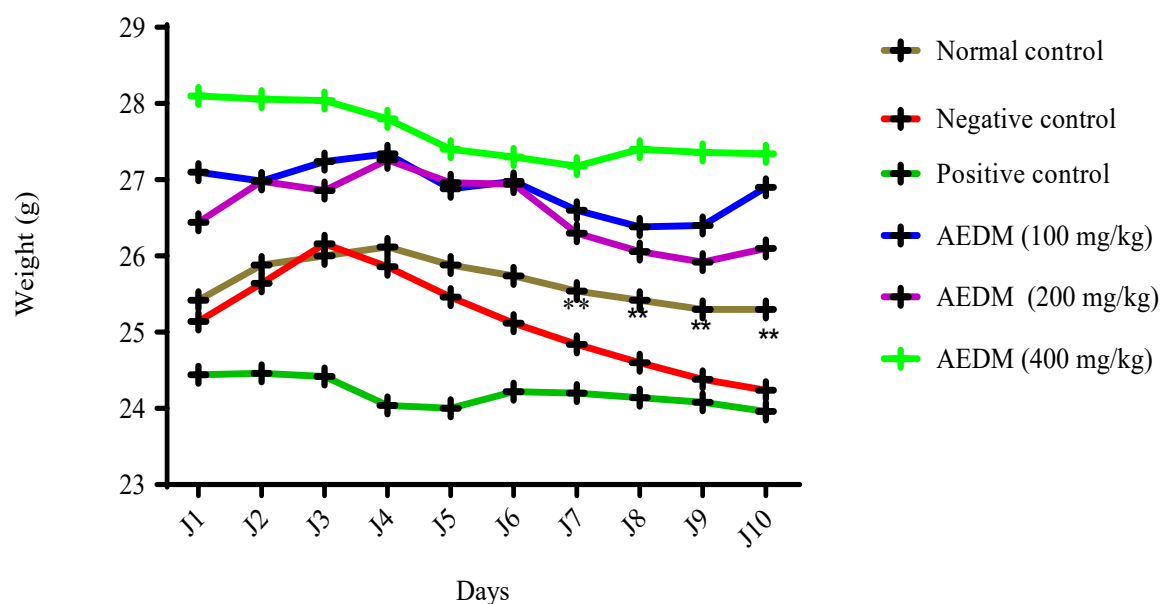


Figure 1. Effect of aqueous extract of *Diospyros mespiliformis* aqueous extract on weight gain in mice

Values represented as means $\pm$ SEM ($n = 5$ for each group). ** $p < 0.01$ for comparison between normal group, one-way analysis of variance (ANOVA) with Newman Kells multiple comparison tests was used.

AEDM: Aqueous extract of *Diospyros mespiliformis*. J = day

Effect of aqueous extract of *Diospyros mespiliformis* on the course of paw edema in animals

The evolution of edema (ΔE) of the inflamed paw during the experimentation period at D2, D4, D6, D8 and D10 is illustrated in Figure 2 below. These data show a reduction in paw edema

represented by the difference between the diameters of the paws - inflamed and non-inflamed (ΔE) in all treated groups compared to the negative control group. Treatment with diclofenac (5 mg / kg) significantly ($p < 0.001$) reduced the edema of the paws of the animals on days (D6, D8, D10) of treatment compared to the negative group. The aqueous extract of *Diospyros mespiliformis* also significantly ($p < 0.001$) reduced paw edema on days D8 and D10 at AEDM doses 100 mg/kg, 200 mg/kg compared to the negative control. On the 6th day (D6) of the experiment, the extract also significantly reduced edema ($p < 0.001$) (AEDM 100 and 200 mg/kg); ($p < 0.01$) (AEDM 400 mg/kg) compared to the negative control.

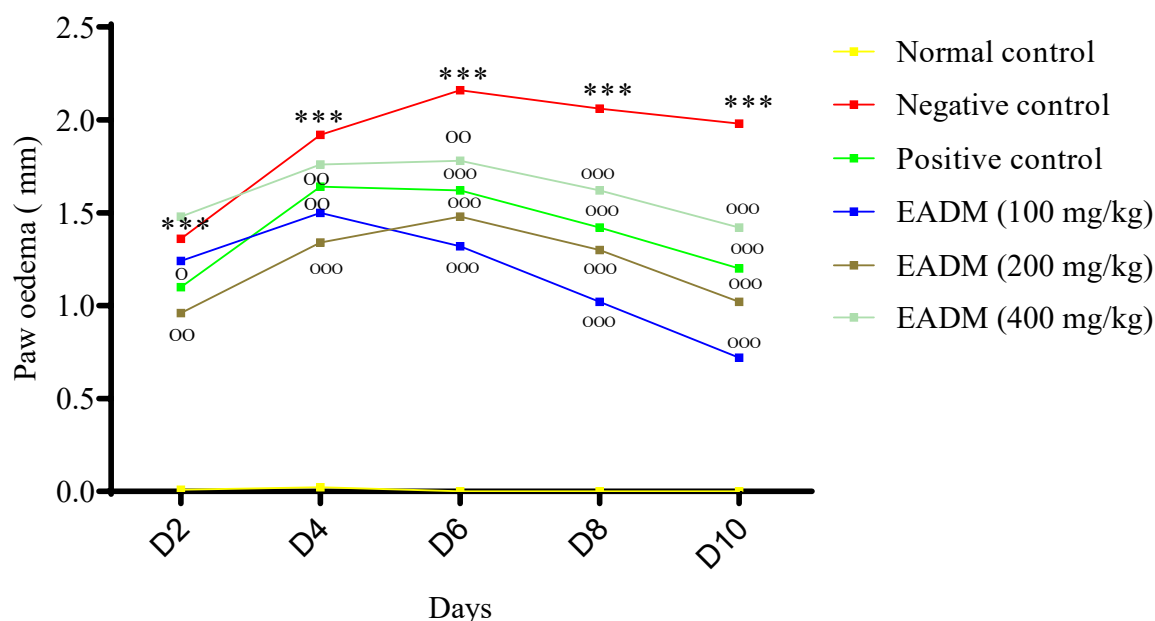


Figure 2. Effect of aqueous extract of *Diospyros mespiliformis* on the course of paw edema in mice

Values represented as means $\pm$ SEM ($n = 5$ for each group). $\Theta\Theta$ $P < 0.01$ $\Theta\Theta\Theta$ $P < 0.001$ and vs control group. For comparison between groups, one-way analysis of variance (ANOVA) with Newman Kells multiple comparison tests was used. **AEDM**: aqueous extract of *Diospyros mespiliformis*

Effect of *Diospyros mespiliformis* aqueous extract on percent inhibition of paw edema in mice

The paw edema of the mice was significantly but unevenly inhibited in the treated groups on the last three days of treatment. The diclofenac group reached only 39.39% on the last day of the experiment. On the other hand, the groups which received the plant extract showed a percentage inhibition of 63.63%, 48.48 and 28.29% respectively at the doses of 100, 200 and 400 mg/kg on the last day of the experiment (Table 3). The maximum inhibition (63.63%) of the diameter of the edematous legs of the animals at the end of the experiment was recorded in the group treated with the plant extract at the dose of 100 mg/kg. This dose is considered the most effective.

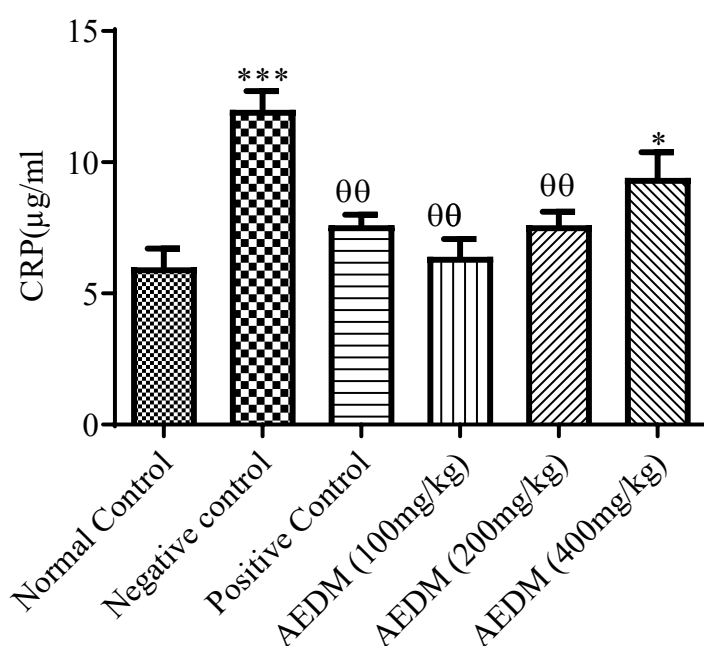
Table 3. Effect of aqueous extract of *Diospyros mespiliformis* on percent inhibition of edema in mouse paws

Group/Dose (mg/kg)	Number	Percentage inhibition (% inh) of paw edema				
		J2	J4	J6	J8	10
DCF 5 mg/kg	5	19.11	14.53	25.00	31.06	39.39
AEDM 100 mg/kg	5	8.82	21.87	38.88	50.48	63.63
AEDM 200 mg/kg	5	29.41	30.20	31.48	36.89	48.48
AEDM 400 mg/kg	5	8.82	8.33	17.59	21.35	28.29

DCF: diclofenac, AEDM: aqueous extract of *Diospyros mespiliformis*

Effect of *Diospyros mespiliformis* extract on plasma concentration of C-reactive protein (CRP)

The results showed a difference in concentration between the mean values of CRP in the different groups of experimental animals. A significant increase ($p < 0.05$, $p < 0.001$, respectively) was observed in the animals treated with the plant extract at the dose of 400 mg / kg and in the negative control group compared to the normal control. Doses 100 and 200 mg / kg of plant extract significantly lowered the plasmatic concentration of CRP ($p < 0.001$; $p < 0.01$, respectively) compared to the negative control group (Figure 3).

**Figure 3.** Effect of aqueous extract of *Diospyros mespiliformis* on plasmatic concentration of C-reactive protein

Values represented as means $\pm$ SEM ($n = 5$ for each group). * $p < 0.05$, *** $p < 0.001$ compared to the normal group; $\theta\theta$ $p < 0.01$; $\theta\theta\theta$ $p < 0.001$ compared to the negative group, one-way analysis of variance (ANOVA) with Newman Kells multiple comparison tests was used.

AEDM: aqueous extract of *Diospyros mespiliformis*

Effect of aqueous extract of *Diospyros mespiliformis* on hyperalgesia activity

The reaction time of the mice to the pain test showed a significant decrease ($p < 0.01$) in the negative control animals compared to the normal control. On the other hand, in the groups treated with the plant extract, we observed a significant increase ($p < 0.001$) in the latency time at doses of 100 and 200 mg / kg. The lag time increased significantly ($p < 0.001$) in animals treated with diclofenac 5 mg / kg compared to the normal control. However, a significant increase ($p < 0.001$) in mouse reaction time was observed at all doses of the extract and diclofenac compared to the negative control (Figure 4).

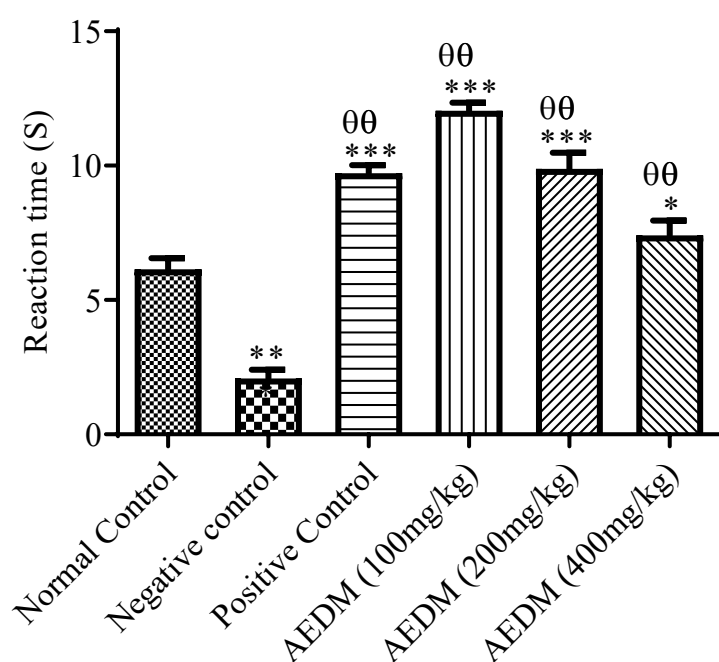


Figure 4. Effect of aqueous extract of *Diospyros mespiliformis* on hyperalgesia activity. Values represented as means $\pm$ SEM ($n = 5$ for each group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to the normal control; θθθ $p < 0.001$ compared to the negative group. One-way analysis of variance (ANOVA) with Newman Kells multiple comparison tests was used. AEDM: aqueous extract of *Diospyros mespiliformis*

Effect of the aqueous extract of *Diospyros mespiliformis* in the light and dark compartment box test

The results showed no significant difference between the negative control and the normal control (Figure 5). In contrast, treatment with diazepam (2 mg / kg) resulted in a significant increase ($p < 0.001$) in latency time compared to normal and negative controls. AEDM was also significantly ($p < 0.001$) increased by normal and negative controls. But at the dose of 400 mg / kg of extract we observe a significant increase ($p < 0.05$) compared to the normal and negative controls.

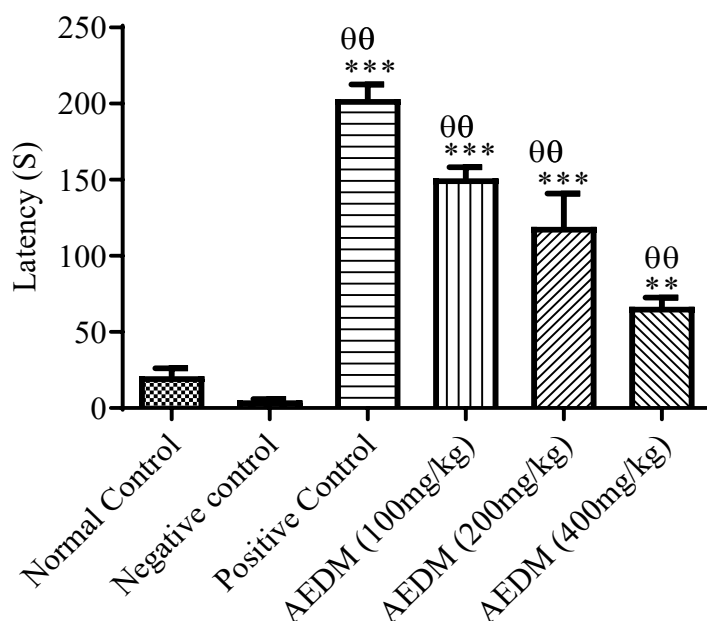


Figure 5. Effect of *Diospyros mespiliformis* on the latency time in the Light and Dark box test. Values represented as means $\pm$ SEM (n=5 for each group). ** $p < 0.01$, *** $p < 0.001$ compared to the normal group; $\theta\theta$ $p < 0.01$, $\theta\theta\theta$ $p < 0.001$ compared to the negative group. One-way analysis of variance (ANOVA) with Newman Kells multiple comparison tests was used. AEDM: aqueous extract of *Diospyros mespiliformis*

Effect of *Diospyros mespiliformis* on the time spent in the dark and lighted compartments of the labyrinth

The results of this test show that the mice in the normal control group and in the negative control group prefer the dark environment of the box. On the other hand, the mice which received different treatment prefer the lighted environment. The mice which received the 2 mg / kg dose of diazepam showed a significant increase ($p < 0.001$) in the time spent in the lighted medium coupled with a significant decrease ($p < 0.001$) in the time spent in the dark medium compared to the normal controls and negative control (Figure 6). The same phenomenon was observed in mice given the plant extract at all doses, but only compared to the negative control. The difference in the time spent in the lighted environment of the labyrinth of mice treated with plant extract compared to the normal control shows a significant dose-dependent variation ($p < 0.001$) (AEDM 100 mg / kg); ($p < 0.01$) (EDM 200 mg / kg); ($p < 0.05$) (AEDM 400 mg / kg). As regards the time spent in the dark medium, a significant decrease ($p < 0.001$) in the time is noted in the treated batches (DZP 2 mg / kg, AEDM 200 mg / kg). At a dose of 400 mg / kg, the extract decreased significantly ($p < 0.05$) compared to the negative control group.

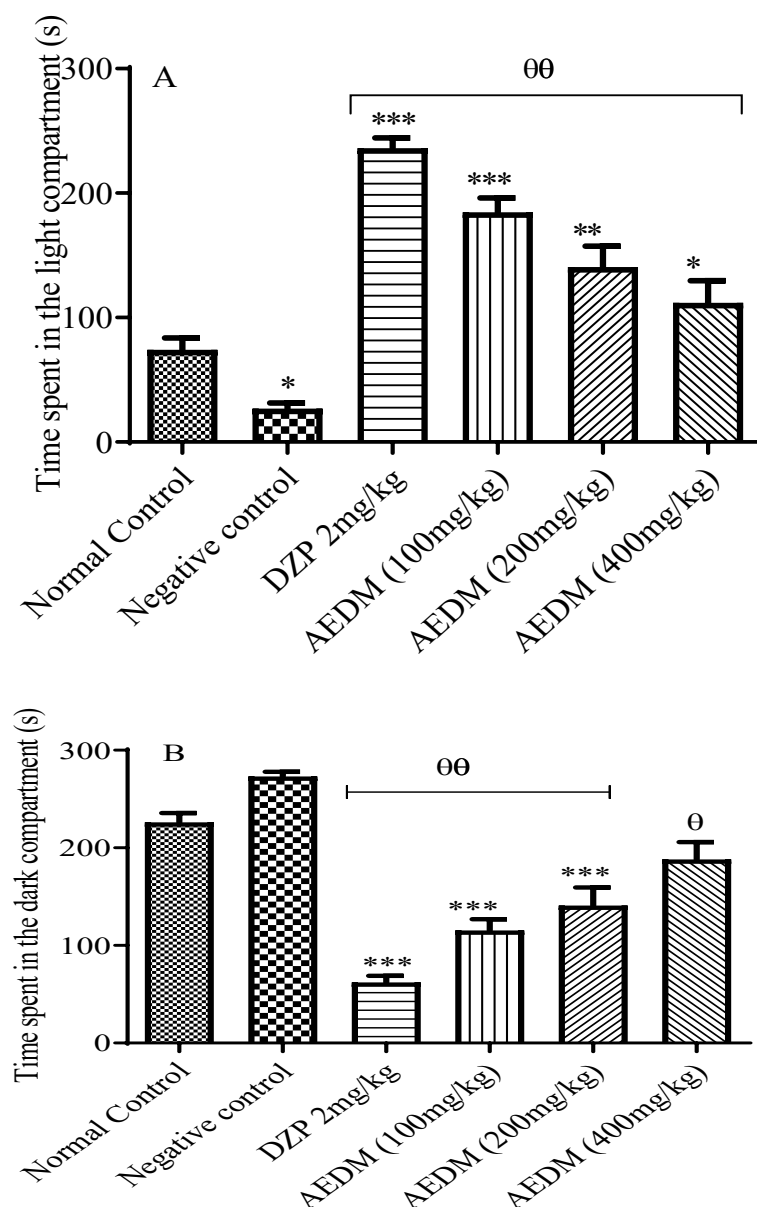


Figure 6. Effect of *Diospyros mespiliformis* on time in the light (a) and dark (b) compartments of the labyrinth

Values represented as means $\pm$ SEM (n=5 for each group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to the normal group; θ $p < 0.05$, $\theta\theta$ $p < 0.001$ compared to the negative group. one-way analysis of variance (ANOVA) with Newman Kells multiple comparison tests was used. **DZP** = diazepam, **AEDM**: aqueous extract of *Diospyros mespiliformis*

Effect of *Diospyros mespiliformis* aqueous extract in the open arena test

The results showed that the animals that received no treatment spent more time at the edge of the maze and little time in the middle but unevenly. Arthritic mice spend more time at the edge compared to normal mice (267.0 ± 5.657 s $>$ 254.2 ± 5.678 s) (Table 4) and less time in the middle place compared to the latter (33.00 ± 5.657 s $<$ 45.80 ± 5.678 s). Time spent at the center was significantly increased ($p < 0.001$) in animals that received diazepam (2 mg / kg) compared to controls. It also increased significantly ($p < 0.001$) (100, 200 mg / kg); ($p < 0.05$) (400 m / kg) in animals treated with aqueous extract of *Diospyros mespiliformis* compared to normal and negative control groups. Time spent on board was significantly reduced in treated animals ($p <$

0.001) (DZP 2mg / kg, AEDM 100 mg / kg); ($p < 0.05$) (200 mg / kg, 400 mg / kg). The locomotor capacity of the mice was evaluated by the number of lines crossed in the field of the labyrinth. The animals of the negative control group show a significant decrease ($p < 0.01$) in the number of crossed lines compared to the normal control. The number of crossed lines increased significantly ($p < 0.001$) (100 mg / kg); ($p < 0.01$) (200 mg / kg) compared to normal control in animals treated with AEDM. In addition, the frequency of grooming and rearing differed in the experimental batches. The negative group showed a significant decrease ($p < 0.05$) in the frequency of grooming compared to the normal control. In the treated batches, the reduction in the frequency of grooming was significant ($p < 0.001$) compared to the untreated controls. For rearing, the extract significantly ($p < 0.01$) reduced its frequency compared to untreated controls. Treatment of mice significantly ($p < 0.05$) (DZP 2 mg / kg, 400 mg / kg) ($p < 0.01$) (100 mg / kg) reduced the defecation spot.

Table 3. Effect of *Diospyros mespiliformis* extract on some parameters evaluated in the open arena box

Groups/ Doses (mg/kg)	Time at the center (s)	Time at the edge	Number of line crossed	Frequency of grooming	Frequency of rearing	Spot of defecation
Normal	45.80 ±5.67	254.20 ± 5.67	32.40 ± 4.13	4.40 ± 0.50	5.20 ± 0.66	1.50 ± 0.23
Negative	33.00 ± 5.65	267.00 ± 15.65	15.80 ± 1.35 **	6.60 ± 0.50*	5.60 ± 0.50	2.16 ± 0.31
DZP (2mg/kg)	242.60 ± 16.59*** θθθ	37.40 ± 1.70 *** θθθ	32.40 ± 4.13	1.61 ± 0.51 *** θθθ	2.80 ± 0.86 ^θ	1.00 ± 0.36 ^θ
AEDM (100mg/kg)	190.20 ± 13.84 *** θθθ	133.80 ± 10.29 *** θθθ	63.00 ± 5.06 θθθ	0.80 ± 0.37*** θθθ	1.60 ± 0.50***θθθ	0.67 ± 0.33 θθ
AEDM (200 mg/kg)	139.20 ± 8.73 *** θθθ	174.80 ± 17.88* θ	58.40 ± 5.30 θθ	1.00 ± 0.31 *** θθθ	1.80 ± 0.58***θθ	1.00 ± 0.36 θ
AEDM (400mg/kg)	125.20 ± 27.88 * θθθ	125.20 ± 27.88 * θ	37.20 ± 4.27	1.70 ± 0.35 *** θθθ	2.00± 0.32 **θθ	1.00 ± 0.52

Values represented as means ± SEM (n=5). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to the normal group; ^θ $p < 0.05$, ^{θθ} $p < 0.01$, ^{θθθ} $p < 0.001$ compared to the negative group. One-way analysis of variance (ANOVA) with Newman Kells multiple comparison tests was used. **DZP:** diazepam; **AEDM:** extract aqueous of *Diospyros mespiliformis*

Effect of the aqueous extract of the bark of *Diospyros mespiliformis* on certain oxidative stress parameters

Effects of the aqueous extract of *Diospyros mespiliformis* on the concentration of Malondialdehyde (MDA) in the liver

The level of MDA was significantly increased ($P < 0.01$) in the negative controls compared to the normal control (Figure 7). In the treated batches, the level of MDA decreased significantly ($P < 0.001$) (AEDM 100 mg / kg, AEDM 200 mg / kg 2 mg / kg); ($P < 0.01$) (AEDM 400 mg / kg) compared to the negative control. This decrease was also significant ($P < 0.01$) (EADM 100 mg / kg and 2 mg / kg); ($P < 0.05$) (200 mg / kg).

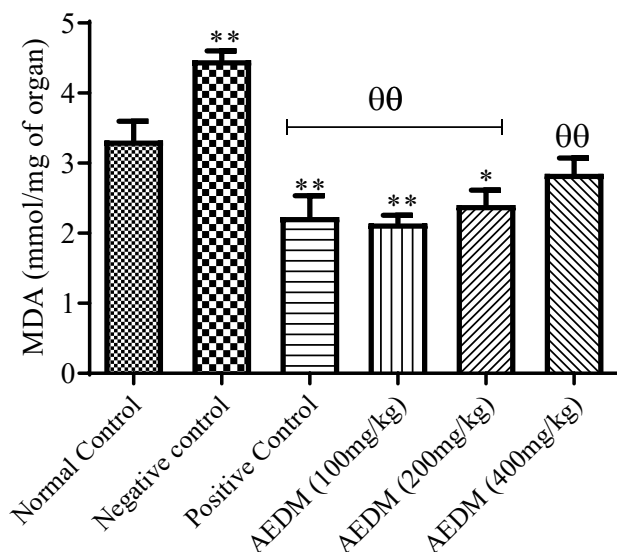


Figure 7. Effects of the aqueous extract of *Diospyros mespiliformis* on the concentration of liver tissue in MDA

Values represented as means $\pm$ SEM (n=5). * $p < 0.05$, ** $p < 0.01$, compared to the normal group; θθ $p < 0.01$, θθθ $p < 0.001$ compared to the negative group. One-way analysis of variance (ANOVA) with Newman Kells multiple comparison tests was used. **AEDM:** extract aqueous of *Diospyros mespiliformis*

Effects of *Diospyros mespiliformis* aqueous extract on superoxydismutase (SOD) activity in the liver

The results showed a significant increase ($p < 0.05$) in the level of SOD in the group treated with diclofenac (5 mg / kg) compared to the normal control. This rate increased significantly ($p < 0.001$) compared to the negative control. Treatment with aqueous extract of *Diospyros mespiliformis* at all doses significantly ($p < 0.05$) increased SOD activity in liver tissue of animals compared to the negative control.

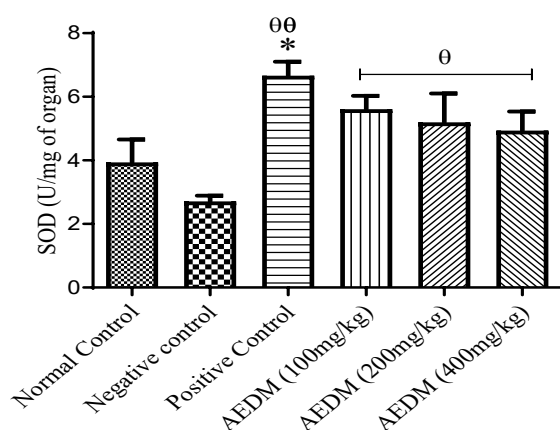


Figure 8. Effects of *Diospyros mespiliformis* aqueous extract on SOD

EADM: extract aqueous of *Diospyros mespiliformis*

Values represented as means $\pm$ SEM (n=5). * $p < 0.05$, compared to the normal group; θ $p < 0.05$, θθθ $p < 0.001$ compared to the negative group. One-way analysis of variance (ANOVA) with Newman Kells multiple comparison tests was used.

Effects of *Diospyros mespiliformis* on catalase activity (CAT)

The level of catalase was significantly ($p < 0.05$) increased in the group treated with diclofenac (5 mg / kg) compared to the negative control. The aqueous extract of *Diospyros mespiliformis* also increased significantly ($p < 0.001$) (AEDM 100 mg / kg); ($p < 0.05$) (AEDM 200 and 400 mg / kg) compared to the negative control.

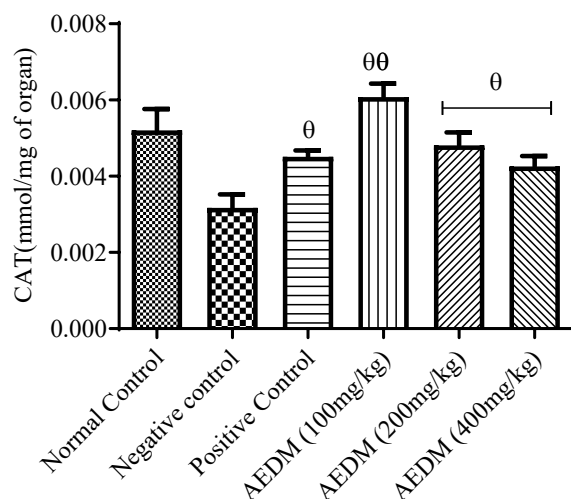


Figure 9. Effects of *Diospyros mespiliformis* on catalase activity

Values represented as means $\pm$ SEM ($n=5$). θ $p < 0.05$, $\theta\theta$ $p < 0.01$, $\theta\theta\theta$ $p < 0.001$ compared to the negative group. One-way analysis of variance (ANOVA) with Newman Kells multiple comparison tests was used. AEDM: extract aqueous of *Diospyros mespiliformis*

Effects of *Diospyros mespiliformis* aqueous extract on hepatic glutathione (GSH) levels

Diospyros mespiliformis aqueous extract significantly increased glutathione ($p < 0.001$) (AEDM 100 mg / kg); ($p < 0.01$) (DCF 5 mg / kg) compared to the negative control. No significant difference was observed in any treated animals compared to the normal control.

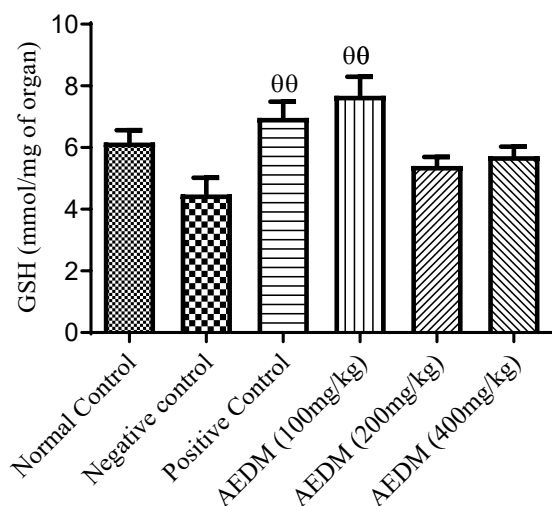


Figure 10. Effects of the aqueous extract of *Diospyros mespiliformis* on the hepatic level of glutathione (GSH)

Values represented as means $\pm$ SEM ($n=5$). $\theta\theta$ $p < 0.01$, $\theta\theta\theta$ $p < 0.001$ compared to the negative group. One-way analysis of variance (ANOVA) with Newman Kells multiple comparison tests was used. AEDM: extract aqueous of *Diospyros mespiliformis*

Effect of the aqueous extract of the bark of *Diospyros mespiliformis* on the histology of the legs

Photomicrographs of the paw of the animals show normal structure of the epidermis and dermis in a normal control group. The negative control animals showed in comparison with the normal control, inflammation and thickening of the epidermis and dermis. An improvement in the structure of the dermis compared to the negative control was observed in the batches treated with the aqueous extract of *Diospyros mespiliformis* and with diclofenac.

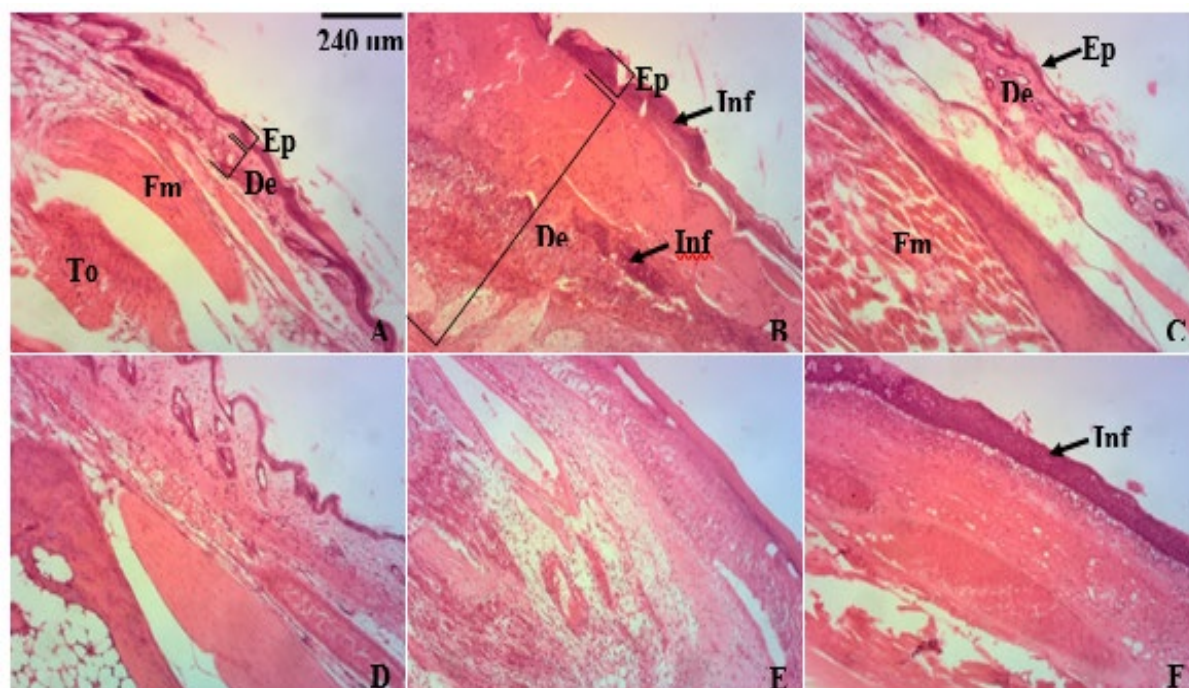


Figure 11. Photomicrograph of the arch of the foot (Hematoxylin-eosin X 40). A: Normal control; B: Negative control; C: diclofenac 5 mg / kg; D: *Diospyros mespiliformis* 100 mg / kg; E: *Diospyros mespiliformis* 200 mg / kg; F: *Diospyros mespiliformis* 400 mg / kg; Ep: epidermis; De: Derma; Fm: Skeletal striated muscle fibers; Inf: Inflammation.

Discussion

The analgesic and anxiolytic properties of the aqueous extract of the bark of the trunk of *Diospyros mespiliformis* were evaluated in arthritis in mice. Injection of a formaline solution into the fascia of the left hind paw of each mouse caused swelling of the paws of the mice. The increased leg size of mice is a sign of a formalin-induced inflammatory reaction leading to edema (Rahmani et al. 2016). This inflammatory response is biphasic, the first phase is due to the release of histamine and during the second phase there is release of serotonin, bradykinin, prostaglandins (Atsang et al. 2014). These inflammation mediators are responsible for vasodilation and increased vascular permeability (Gao et al. 2009, Foughalia 2017). The exudate consequently escapes from the bloodstream to the intestinal medium causing edema and pain (Mansour. 2015). The nociceptive response during inflammation has two phases, the first phase mediated peripherally by the release of chemical mediators, the second neurogen mediated centrally with direct stimulation of the C fiber and the release of substance P (Atsang et al. 2014). Diclofenac is a drug known for its ability to counter the symptoms of the inflammatory reaction (Bektas et al. 2012). It is prescribed for the treatment of arthritis and muscle pain (Akinawo et al. 2017). The administration of this drug inhibits cyclooxygenase 1 and 2 (cox-1 and cox-2) which leads to a decrease in prostaglandins and thromboxanes thus

leading to a decrease in inflammatory effects (Marchlewicz et al. 2016). The aqueous extract of *Diospyros mespiliformis* significantly ($p < 0.001$) reduced inflammatory edema compared to the negative control with a percent inhibition of 63.63, 48.48 and 28.29% respectively at doses of 100, 200 and 400 mg / kg. This reduction was coupled with a significant ($p < 0.001$) decrease in plasma C-reactive protein (CRP) concentration. CRP is a hepatic protein released during the inflammatory response. It is an early, sensitive and specific marker of the inflammatory reaction increasing in proportion to its intensity (Povoa 2002). EADM also significantly increased the pain threshold. The aqueous extract of *Diospyros mespiliformis* may have an inhibitory effect on cyclooxygenase and lead to a decrease in prostaglandins. These effects are thought to be due to the presence of bioactive compounds such as alkaloids, flavonoids, tannins, coumarins and glucosides which are endowed with significant analgesic and anti-inflammatory properties (Tunalier et al. 2007, Batista et al. 2009). This is because flavonoids, coumarins and tannins are phenolic compounds that act on inflammation by inhibiting enzymes involved in the arachidonic acid mechanism and enzymes that generate reactive oxygen species as well as inhibition of NF κ B transcription factors. Alkaloids, for their part, have anti-inflammatory properties by direct inhibition of phospholipase A2 (Lamnaouer 2008). On the other hand, people with persistent arthritis pain exhibit similar patterns of avoidance activity and anxiety which are a very common comorbidity (LE Simons et al. 2012). This worrying clinical situation makes it difficult for the attending physician to select the pharmacological approach to be favored for effective treatment (Rabenda et al. 2005). However, in addition to the analgesic effect, the anxiolytic activity of AEDM was evaluated. The test in the two-compartment labyrinth provides information on the emotional state of rodents according to their preference for the dark compartment considered as non-anxiety-inducing and the illuminated anxiety-producing compartment (Ramos et al. 2008). The aqueous extract of *Diospyros mespiliformis* reduced the time spent in the dark compartment compared to untreated controls at doses of 100, 200 and 400 mg / kg. Treatment with diazepam (2 mg / kg) significantly ($p < 0.001$) increased the time spent in the lighted compartment compared to untreated controls. Likewise, AEDM significantly increased ($p < 0.001$) at all doses the time spent in the illuminated compartment compared to the negative control. But to degrees of dose-dependent significance ($p < 0.05$) (400 mg / kg); ($p < 0.01$) (200 mg / kg); ($p < 0.001$) (100 mg / kg) compared to the normal control. Alongside the time spent in the two compartments of the box, the normal control animals spontaneously enter the dark compartment in order to take refuge in the dark. Diazepam (2 mg / kg) significantly ($p < 0.001$) increased latency compared to two controls. AEDM also increased significantly ($p < 0.001$) (100 and 200 mg / kg); ($p < 0.01$) (400 mg / kg) compared to the negative and normal control. Anxious people are generally driven by a feeling of fear, doubt and avoidance (Sandeep et al. 2017). In fact, the more time the animal spends in the dark environment, the more anxious it is and the more it spends in the lighted environment, the less anxious it is. AEDM could have an anxiolytic effect given the behavior of the animals observed in the maze. The effect of the plant was confirmed by a second test in the open field box "The Open field" which made it possible to assess the level of anxiety in the animals by comparing the time spent in the central place and in the middle edge of the box, the number of lines crossed, the frequency of training and grooming and the defecation spot in the different groups. The increase in locomotor capacity and general activity in the central zone is interpreted as an anxiolytic effect (Prut et al. 2003). The time spent in the central place by animals treated with diazepam (2 mg / kg) increased significantly ($p < 0.001$) compared to control mice. The time spent in the central plaza by the animals treated with the aqueous extract of *Diospyros mespiliformis* also increased significantly ($p < 0.001$) at the doses of 100, 200 and 400 m / kg ($p < 0.05$). AEDM significantly improved locomotor capacity in animals ($p < 0.001$) at the dose of 100 mg / kg. The frequencies of grooming and rearing also express a state of anxiety. However, *Diospyros mespiliformis* extract significantly reduced ($p < 0.001$) the number of

grooming and rearing compared to control animals. These results suggest that the aqueous extract of *Diospyros mespiliformis* improves the state of anxiety. This action could be linked to the presence in the extract of phenolic compounds including flavonoids, tannins known for their ability to improve mood and the disorder that occurs in the central nervous system (Bibi et al. 2017). Patients with rheumatoid arthritis show significant variations in liver enzyme activity (Bihani et al. 2014) with liver and kidney damage. The inflammatory reaction increases free radicals such as O_2^- (superoxide anion) hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) responsible for oxidative stress and which can damage DNA, proteins and lipids (Vital et al. 2013). In the body, SOD prevents the accumulation of O_2^- and transforms it into H_2O_2 and O_2 by catalase (Rabaud et al. 1997). Determination of the oxidative stress parameters revealed a significant increase ($p < 0.001$) in the level of MDA in the negative control group compared to the normal control group. MDA is a biomarker of lipid peroxidation of membranes (Foyet et al. 2019). The increased concentration of MDA in liver tissue in the negative control group suggests that formaline induced lipid peroxidation in these animals. AEDM significantly ($p < 0.001$) reduced MDA levels compared to the negative control at 100 mg / kg and 200 mg / kg and at the same time significantly increased SOD ($p < 0, 05$) at all doses of the extract compared to the negative control. AEDM also significantly ($p < 0.001$) increased catalase and glutathione levels. In fact, superoxide dismutase is an enzyme that has the ability to catalyze the superoxide anion into less toxic hydrogen peroxide (Pincemail 2005). Catalase and glutathione have a similar action to catalyze the decrease in hydrogen peroxide (H_2O_2) (Guimard et al. 2007). The increase in these parameters suggests that the aqueous extract of *Diospyros mespiliformis* may have antioxidant power. This effect could be due to the presence in this extract of phenolic compounds such as flavonoids, tannins and alkaloids which have well-known antioxidant powers (Mzid et al. 2017). Histological study of the legs of the animals reveals that formaline caused an alteration in the structure of the dermis and epidermis of the arch of the foot shown in the photomicrograph. Animals treated with the aqueous extract of the plant showed normal structure compared to the negative control. These results suggest that the extract limited the destruction of paw structures in animals. Limiting the destruction of these structures, the extract would certainly have reduced the inflammatory process in the legs of animals and consequently the pain.

Conclusions

The present study proved the properties of the aqueous extract of the bark of the trunk of *Diospyros mespiliformis* on the pain and anxiety of induced arthritis in mice. The aqueous extract of *Diospyros mespiliformis* reduced formaline-induced edema and increased the threshold of nociception in mice. It also improved the state of anxiety in animals. Our plant would therefore have an anti-inflammatory, antioxidant effect and would fight against anxiety

Ethical considerations

The study was approved by ethic Committee of the Faculty of Sciences of the University of Maroua (Ref N°14/0261/Uma/D/FS/VD-RC), according to the guidelines of Cameroonian bioethics committee (Reg N.° FWA-IRB00001954).

References

Abbott JH, Usiskin IM, Wilson R, Hansen P, Losina E. 2017. The quality-of-life burden of knee osteoarthritis in New Zealand adults: A mode l based evaluation. PLoS ONE, 12: 185-676.

- Akinnawo, Omowumi O, God'swill N, Anyasor, Osilesi, O. 2017. Aqueous fraction of *Alstonia boonei* de Wild leaves suppressed inflammatory responses in carrageenan and formaldehyde induced arthritic rats. *Biomedicine et Pharmacotherapy*, 86: 95-101.
- Arbonnier M, Ligneux du Sahel, v 1.0. CIRAD, Montpellier; Museum national d'histoire naturelle, Paris. 2008. 574.
- Arthritis Foundation. 2019. Arthritis by the Numbers, v3; 4100.17.10445.
- Atsang A G, Dzeufiet D PD, Foyet H S, Dimo T, Kamtchouing P. 2014. Analgesic and Anti-inflammatory Effect of the Aqueous Extract of *Dichrostachys glomerata* (Forssk.) Hutch Fruits. *European Journal of Medicinal Plants*, 4(8): 964-978.
- Axford J, Butt A, Heron C, Hammond J, Morgan J, Alavi A, Bolton J, Bland M. 2019. Prevalence of anxiety and depression in osteoarthritis: Use of the Hospital Anxiety and Depression Scale as a screening tool. *Clinical. Rheumatology*, 29: 1277-1283.
- Batista, Kelly SDL, Guilherme E ND, Anderson LF, Alba RMSB. 2009. Flavonoids with gastrprotective activity. *Molecules*, 14(3): 979-1012 P 36.
- Bektas N, Arslan R, Goger F, Kirimer N, Ozturk Y. 2012. Investigation for anti-inflammatory and anti-thrombotic activities of methanol extract of *Capparisovata* buds and fruits. *Journal of Ethnopharmacology*, 2(3): 052-054.
- Bibi MR, Naser Z, Hossein H. 2017. Anti-anxiety and hypnotic effects of ethanolic and aqueous extracts of *Lippia citriodora* leaves and verbascoside in mice. *Journal of Phytomedicine*, 7(4): 353-365.
- Bihani GV, Rojatkhar SR, Bodhankar SL. 2014. Anti-arthritis activity of methanol extract of *Cyathocline purpurea* (whole plant) in Freund's complete adjuvant-induced arthritis in rats. *Biomed Aging Pathol*. 4: 197-206.
- Cheriti, Abdelkrim, Rahmani, Smahia, Belboukhari et Nasser. 2016. Évaluation de l'activité anti-inflammatoire d'extraits aqueux de feuilles *Limoniastrum feei*. *Algerian Journal of Arid Environment*, 6(1): 80-86.
- Duica L. 2016. Depression_multiple psychopathological facets, Mental Health-actual views in psychology, medicine and anthropology, sep, p 20-24.
- Dupuy AM, Bdiou BD S, Cristol JP. 2000. Dosage immuno turbidimetrique de la Crp et de la Crp haute sensibilisé sur le serum et plasma hépariné: Apport des réactifs Olympus. *Spectra biologie*, 22(132): 44-46.
- Ellman GL. 1959. Tissue sulfhydryl groups, *Archives of Biochemistry and Biophysics*, 82: 70-77.
- Foughalia A. 2017. Évaluation de l'activité anti-inflammatoire de l'extrait brut de la graisse de la bosse de *Camelus dromedarius* sur un modèle murin d'arthrite expérimentale. Mémoire de Master II, Université des Frères Mentouri Constantine1, p. 54.
- Foyet HS, Tsala D E, Zogo EBJC, Azanfack NC, Toussoumna LH1, Eyong KO. 2015. Anti-inflammatory and anti-arthritis activity of a methanol extract from *Vitellaria paradoxa* stem bark. *Pharmacognosy Research*, 4(7): 4-12.
- Foyet HS, Tsala DE, Armand A and Lucian H. 2012. Anxiolytic and Antidepressant-Like Effects of the Aqueous Extract of *Alafia multiflora* Stem Barks in Rodents. *Advances in Pharmacological Sciences*: 1-8.
- Foyet HS, Wado, EK, Abaïssou HHN, Assongalem EH, Eyong OK. 2019. Anticholinesterase and Antioxidant Potential of Hydromethanolic Extract of *Ziziphus mucronata* (Rhamnaceae) Leaves on Scopolamine-Induced Memory and Cognitive Dysfunctions in Mice. *Complementary and Alternative Médecine*, 2019: 1-14.
- Gao G, Li C, Hu X, M FuB. 2009. Anti-nociceptive and anti-inflammatory activity of sophocarpine. *Journal of Ethnopharmacology*, 125: 324-329.
- Guimard NK, Gomez N, Schmidt CE. 2007. Conduction polymer in biomedical engineering. *Progress in polymer Science*, 32: p876.

- Hermans J, Koopmanschap MA, BiermaZeinstra SMA. 2012. Productivity costs and medical costs among working patients with knee osteoarthritis. *Arthritis Care and Research*, 64: 853-861.
- Jennifer G. 2018. L'expérience psychosociale de l'arthrite vécue par les jeunes étudiants post secondaires du nord-est de l'ontario : une étude photovoix. Thèse de doctorat, université de laurientienne. P35
- LE Simons B, Sieberg LC. 2012. Anxiety and functional disability in a large sample of children and adolescents with chronic pain. *Pain Res Manage*, 17(2): 93-97.
- Mansour S. 201). Evaluation de l'effet anti inflammatoire de trois plantes médicinales: *Artemisia absinthium* L, *Artemisia herba* et *Hypericum scarboides*: Etude in vivo-. Thèse de Doctorat. Université des Sciences et de la Technologie Mohamed BOUDIAF Oran.
- Marchlewicz A, Domaradzka D, Guzik U, Wojcies Zynska D. 2015. *Bacillus thurin giensis* is a gram- positive bacteria able to degrade naproxen and ibuprofen. *Water Air Soil pollt*: 227-197.
- Megarbane B, Gueye P, Baud F. 2003. "[Interactions between benzodiazépines and opioids]." *Ann Med Interne (Paris)* 154 Spec No 2: S64-72.
- Meyer-Rosberg K, Kvarnstrom A, Kinnman E, Gordh T, Nordfors LO, Kristofferson A. 2001. "Peripheral neuropathic pain-a multidimensional burden forpatients." *Eur T Pain*, 5(4): 379-389.
- Misra H, Fridovich. 1972. The role of superoxide anion in auto oxydation epinephrine to adrenochrome and simple assay for superoxide dismutase, *Journal of biology chemistry*, 24(7): 3170-3175.
- Mzid M, Ben Khedir S, Ben Salem M, Regaieg W, Rebai T. 2017. Antioxidant and antimicrobialactivities of ethanol and aqueous extracts from *Urticaurens*. *Pharmaceutical biology*, 55(1): 775-781.
- Pincemail J, Bonjean K, Cayeux K, Defraigne JO. 2005. Physiological action of Antioxidant défenses, *Nutritionclinique et métabolisme*, 16: 233-239.
- Povoa Pedro. 2002. C-reactive Protein: evaluate marker of Sepsis. *Intensive Care medecine*, 28(3): 235-243.
- Prut, Belzung. 2003. Use of the Open Field Maze to Measure Locomotor and Anxiety-like Behavior in Mice. *Journal of Visualized Experiments*, 96: 524-534.
- Rabaud Ch, Tronel H, Fremont S, May T, Canton P, Nicolas JP. 1997. Radicaux libres et infection par le VIH. *Annales de Biologie Clinique*, 55(6): 565-71.
- Rabenda V N, burllet O, Ethgen F, Raeman J, Belaiche, Reginsetr. 2005. A naturalistic study of the determinants of health-related quality of life improvement in arthritic patients treated with on specific on- steroidal anti-inflammatory drugs. *Ann Rheum Dis*. 64: 688-693.
- Rahmani, Smahia, belboukhari, Nasser, cheriti, Abdelkrim. 2016. Evaluation de l'activité anti-inflammatoire d'extraits aqueux de feuilles de *Limoniastrum feei* (Plumbaginacea). *Algerian Journal of Arid Environment*, 6(1): 80-8.
- Ramos A, Pereira E, Martins GC, Wehrmeister TD, Izídio GS. 2008. Integrating the open field, elevated plus maze and light/dark box to assess different types of emotional behaviors in one single trial. *Behav Brain Res*. 193(2): 277-288.
- Reamongkol W, TYoppapan T, Subhadhirasakul S. 2009. Antynocptive, antipyretic and anti-inflammatory activities of putanjiva roxburghii Wall leaf extract in experimental animals. *Journal of natural medecines*, 63(3): 290-296.
- Rice DA, McNair PJ, Lewis GN, Mannion J. 2015. Experimental knee pain impairs submaximal force steadiness in isometric, eccentric, and concentric muscle actions. *Arthritis Res. Ther*. 1: 1-6.

- Sandeep K, Rajmeet S. 2017. Role of different neurotransmitters in anxiety: a systemic review. International Journal of Pharmaceutical Sciences and Research, 8(2): 411-421.
- Sharma A, Kudesia P, Shi Q, Gandhi R. 2016. Anxiety and depression in patients with osteoarthritis: impact and management challenges. Open Access Rheumatol. 8: 103-113.
- Sinha A K. 1972. Colorimetric assay of catalase. Analytical Biochemistry, 47(2): 389-394.
- Soubrier M, Rosenbaum D, Tatar Z. 2013. Antiinflammatoires non stéroïdiens et vaisseaux. Revue du rhumatisme, 80(3): 204-208.
- Trease GE, Evans WC. 1983. "Pharmacognosy". Ballière Tindall Press. London. pp. 309. 706.
- Tunali Z, Kosar M, Kupeli E, Calis I, Can Baser KH. 2007. Antioxidant, anti-inflammatory, anti-nociceptive activities and composition of *Lythrum salicaria* L. extracts. Journal of Ethnopharmacology, 110: 539-547.
- Twilman RK. 2007. "Mental disorders in chronic pain patients." J Pain Palliat Care Pharmacother. 21(41): 13-19.
- Vetal S. 2013. Anti-inflammatory and anti-arthritis activity of type-A procyanidine polyphenols from bark of *Cinnamomum zeylanicum* in rat. Science Direct 2: 59-67.
- Vincent HK, Lamb KM, Day TI, Tillman SM, George SZ, Morbid. 2010. Obesity Is Associated with Fear of Movement and Lower Quality of Life in Patients with Pain Related Diagnoses. PmR, 6(2): 713-722.
- Wilbur KM, Bernheim, Shapiro OW. 1949. Determination of lipid peroxidation, Archives of Biochemistry and Biophysics, 24: 305-310.
- Yougbaré-ziébrou, Ouédraogo L. 2016. Activités anti-inflammatoire, analgésique et antioxydante de l'extrait aqueux des tiges feuillées de *Saba senegalensis* Pichon (Apocynaceae). Phytothérapie, 14(4): 213-219.
- Zuo J, Xia Y, Li X, Chen JW. 2014. Therapeutic effects of dichloromethane fraction of *Securidacina appendiculata* on adjuvant induced arthritis in rat. J Ethnopharmacol. 153: 352-358.

