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Combining extracts of *Zingiber officinale* rhizomes and dried fruits of *Tetrapleura tetraptera* improve oxidative stress and hematological profiles, reduces joint inflammation and hyperalgesia after carrageenan injection in rat joints

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Abstract

Background: *Zingiber officinale* and *Tetrapleura tetraptera* are recognized and widely used for medicinal and nutritional qualities. The study aimed to evaluate the anti-inflammatory properties of the mixture of the aqueous and ethanolic extracts of *Zingiber officinale* and *Tetrapleura tetraptera*.

Methods: Joint inflammation induced by carrageenan (3%), the clinical signs of inflammation (paw edema) and pain (mechanical hyperalgesia and tactile allodynia), biochemical, hematological, oxidative stress, and histological parameters were assessed. Also, the acute toxicity of the mixture of aqueous extracts was evaluated *per os* (2500 and 5000 mg/kg).

Results: Aqueous extract is non-toxic. Extracts mixture (200 mg/kg) inhibited joint edema, mechanical hyperalgesia, and tactile allodynia in a single administration until the 24th hour, then continuous administration until the 10th day of treatment. Extracts did not disrupt the hematological parameters and did not affect organ weights. Both extracts showed significant antioxidant activity on enzymatic and non-enzymatic parameters. Extracts also protected the joint against the deleterious effects and did not affect the liver and kidney tissues.

Conclusion: The activities recorded in this study confirm the traditional use of *Zingiber officinale* and *Tetrapleura tetraptera*. The extracts from these plants are a clear candidate to ensure the prevention and/or treatment of joint pathologies.

Keywords: Joint inflammation; hyperalgesia; *Tetrapleura tetraptera; Zingiber officinale*.

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Background

Edible plants, which are essential in the lives of populations in developing countries for their medical, energy and nutritional benefits, present in the surrounding ecosystems can easily be cultivated for the vast majority [1]. Several works carried out on plant species such as *Elaeis guineensis* (oil palm), *Garcinia kola* (small cola), *Adansonia digitata* (baobab), *Ricinodendron heudelotii* (Njansang), *Tetrapleura tetraptera* (four sides) and *Zingiber officinale* (ginger) have allowed us to highlight the importance of these species for local populations [2,3].

Belonging to the large Fabaceae-Mimosoideae family, *Tetrapleura tetraptera* (*T. tetraptera*), a tree that can reach 20 to 25 meters height, is known for its medicinal, economic and food properties in Africa [4]. *T. tetraptera* fruits (brownish, 15 to 25 cm long) contain aromatic pulps with tiny seeds of around 8 mm in length [5]. The dried fruits of *T. tetraptera* are used in many African countries to generally treat hypertension, leprosy, diabetes, convulsions, rheumatic pain and arthritis [6]; while in particular, the fruits are used in Nigeria to prevent postpartum contractions [4], in Ghana as an ingredient to accompany “fufu” meal [7] and in Cameroon (West region) for making a soup of high cultural value called “yellow soup” [8]. Scientifically, the fruits of this plant have shown anti-inflammatory, antimicrobial, molluscidal, insecticidal, cardiovascular, antiseptic, hypoglycemic, neuromuscular, antiulcer, antiarthritic, healing, contraceptive, anxiolytic, antimalarial, analgesic and anticonvulsant properties [9,10]. Furthermore, *T. tetraptera* is rich in phenolic compounds, flavonoids, saponins, alkaloids and steroids [11,12]. In addition, the presence of compounds such as 7-hydroxy-6-methoxy coumarin, hentriacontane, echinocystic acid, 12(13)-en-28-oic acid, N-acetylglycosides of oleanolic acid, isoliquiritigenin, flavanone-naringenin and sodium echinocystic acid-3-O sulfate have been identified in this plant [13].

Regarding *Zingiber officinale* (*Z. officinale*), it belongs to the Zingiberaceae family. It is an herbaceous perennial plant bearing a rhizome root called ginger which can be consumed fresh, in powder or dried form and having valuable nutritional, medicinal and ethnomedical properties. In several African folk medicines, *Z. officinale* is used for its gastrointestinal, cardiovascular, hepatic protective, immunomodulatory, antimicrobial and thermogenic activities [14,15]. Scientifically, *Z. officinale* has shown on the one hand immunostimulant, anti-hyperglycemic, anti-carcinogenic, antioxidant, anti-apoptotic, anti-ulcer, anti-parasitic, anti-inflammatory, hypolipidemic and antimicrobial activities [16,17]. Indeed, there’s the presence of compounds such as alpha-zingiberene, beta-bisabolene, zingiberol, alpha-faranesene, geraniol, beta-phellandrene, linalool, shogaol, zingiberol, cineole, zingerone, and gingerol [14,15]. Other work has shown that *Z. officinale* can act on the lipid profile (reduce cholesterol and triglyceride levels, then increase HDL cholesterol), protects against peroxidation, promote the release of bile from the gallbladder [18,19], act against arthritis, coughs, colds and sore throats [20].

Knowing that 60 years is the bar set by the WHO as the minimum age for elderly people [21], based on the fact that life expectancy is very low in black Africa in general and particularly in Cameroon (less than 55 years in 2007), the number of elderly people in sub-Saharan Africa will reach 69 million in 2025 and 139 million in 2050 [22]. The fragile state of these elderly people exposes them to the occurrence of numerous medical pathologies and particularly osteoarticular conditions. In fact, more than 1.71 billion people in the world suffer from osteoarticular and muscular conditions, representing the leading factor of disability in many countries. In sub-Saharan Africa, degenerative pathology, chronic inflammatory rheumatism and osteoarticular infections affect respectively 66.90%, 4.64% and 2.78% of the population [23]. In Cameroon, the prevalence of rheumatoid factor in 2015 was approximately 5.4% in the entire population with a female/male ratio of 2.4 [24]. Joint pathologies cause deformities of the hands and feet, as well as mobility impairment; in the vast majority of cases, there also appear pain, stiffness, generalized inflammation accompanied by joint and tissue damage that impairs a person's ability to move. Based on the fact that the fruits of *T. tetraptera* and *Z. officinale*, individually possess important pharmacological and nutritional properties and are very widely used in many cultures for cooking numerous meals, the purpose of this work was to evaluate the effect of the mixture of *T. tetraptera* fruits and *Z. officinale* rhizomes on induced joint inflammation in rats in order to verify whether dietary supplementation of these two elements could be beneficial for people suffering from joint pathologies.

Methods

Collection of plant and extraction

The fresh *Z. officinale* rhizomes and the dried fruits of *T. tetraptera* were purchased from Mendong market in Yaoundé (Center region, Cameroon) in July 2023. The two plants were identified in comparison with the original material of the Cameroon National Herbarium preserved under the numbers 43143/HNC and 2316/SRFCam respectively for *Z. officinale* and *T. tetraptera*. Both were washed separately with clean water, cut into small pieces, shade dried and crushed into a fine powder. For aqueous extraction, 500 grams of each powder were macerated separately in 3 L of distilled water for 48 hours and stirred regularly. Thus, the macerate obtained was filtered using Whatman No.4 filter paper and evaporated (40 °C) in an oven, giving 7g with extraction yield of 3.5% and 6g (3% yield) for *Z. officinale* and *T. tetraptera* extracts respectively. Concerning ethanolic extraction, 250g of each powder was macerated in 2 L of 95° ethanol for 72 hours. The macerates obtained were filtered with a Whatman No.4 filter paper and then concentrated using a rotary evaporator (40 °C) under reduced pressure. 9g (3.6% yield) and 8g (3.2% yields) were obtained for *Z. officinale* and *T. tetraptera*, respectively. Each extract from water and ethanol extraction were separately kept in a dark and sterile container and stored in a fridge at 4°C. The mixture preparation was administered to the animals at 50/50 proportion.

Animals

Wistar rats (*Ratus ratus*) of both sexes (1.5 months old, average weight 120 g) and adult female rats (3 months old, average weight 160 g) were used for toxicity study and for pharmacological tests respectively. These animals, obtained from Animal house of Laboratory of Animal Physiology (University of Yaoundé I), were acclimatized for a period of 10 days before the experiments. Animals with free access (water and food) were housed in an animal room with a controlled environment room (50 % -80 %; 25°C; 12h light-dark cycle). All experiments were validated by the Joint Institutional Review Board for Animal & Human Bioethics (JIRB) with Ethical Clearance Reference No: BTC-JIRB2022-014.

Carrageenan-induced joint inflammation

Distribution and treatment of animals

Animals were divided into 7 groups (n=5). Naive and negative control groups received *per os* 10 mg/kg of saline solution (0.9 %), positive control group received *per os* 5 mg/kg of diclofenac, and treated groups received *per os* 100 and 200 mg/kg of aqueous or ethanol extract of the mixture of *Z. officinale* rhizomes and *T. tetraptera* fruits.

Induction of inflammation

One hour after administration of each treatment, 3% of carrageenan solution was injected (0.1 mL) into the ankle joint (left hind paw) of each animal using an insulin syringe (1 ml) except the animals of Naive group [25].

Evaluation of paw edema effect

Before carrageenan injection, the volume of the injected paw was measured three times, then the average volume (V_0) of the paw was obtained from the average of the 3 readings. After injection of the phlogistic agent, the volumes (V_t) were measured at 1, 2, 4, 6 and 24 hours, using a water plethysmometer.

After this first measurement, the treatment of the animals resumed after 24 hours and continued for 10 consecutive days. During that period, paw volume (V_t) was measured on days 2, 4, 6, 8 and 10. The percentage of inhibition (I%) was calculated [26]:

$$I\% = \frac{(V_t - V_0)_{Control} - (V_t - V_0)_{Test}}{(V_t - V_0)_{Control}} X \ 100$$

Evaluation of mechanical hyperalgesia effect

This test was conducted by applying an increased pressure to the paw of rats using an analgesic (Ugo Basile, type 7200), the force with which each animal withdrawal the paw was recorded and considered as an indication of pain [27]. The reaction force of each rat was determined before any treatment (T_0), then 1, 2, 4, 6 and 24 hours after a single treatment (T_t). Thereafter, the treatment was given daily, and the same parameter was measured on days 3, 4, 5, 6, 7, 8, 9 and 10. The inhibition percentage (I %) was determined using the formula below.

$$I\% = \frac{(T_t - T_0)_{Control} - (T_t - T_0)_{Test}}{(T_t - T_0)_{Control}} X \ 100$$

Collecting samples

On day 11, 50 mg/kg of thiopental were injected (intraperitoneally) into each rat, blood was collected (abdominal artery catheterization) and distributed into a dry tube then centrifuged (4 °C, 3000 rpm, 15 minutes), serum obtained was used to evaluate oxidative stress parameters (superoxide dismutase, glutathione, catalase, malondialdehyde and nitric oxide) and nitrite oxide. Subsequently, the liver was removed, cleaned and ground in PBS (0.1 g of organ/ml of PBS). After grinding, the homogenates were centrifuged (Eppendorf 5804R, 4 °C, 15 minutes, 3000 rpm, Hamburg), and the supernatants were collected (Eppendorf tubes) and stored (-20 °C), and used for the measurement of parameters mentioned above. The synovial fluid was also collected by washing (NaCl) and preserved in Eppendorf tubes for the evaluation of the same oxidative stress parameters. At the end, the joint was preserved (10% formalin buffered, PBS) for histological analysis.

Toxicity Study

Three (3) groups (6 young rats each) were used. Control group received saline solution (10 ml/kg, *per os*), while the aqueous extract of the mixture of *Z. officinale* rhizomes and fruits of *T. tetraptera* was administered at the doses of 2500 and 5000 mg/kg. After extract administration, animals were observed (4 hours post-gavage), then for 7 days following treatment [28], parameters such as mortality, food intake, water consumption, stool status, locomotion, urination, were recorded [28,29].

Subsequently, 25 rats were divided into 5 groups of 5 animals each and treated for 10 days (Naive groups and negative control groups received *per os* 10 mg/kg of saline solution (0.9 %), positive control group received *per os* 5 mg/kg of diclofenac, and treated groups received *per os* 200 mg/kg of aqueous or ethanol extract of the mixture of *Z. officinale* rhizomes and *T. tetraptera* fruits). On the 11th, after having anesthetized (50 mg/kg of thiopental) animals, blood was collected (abdominal artery catheterization) and distribute into EDTA tubes for hematological analysis (automatic hematological analyzer, Sysmex pocH-100i); and in a dry tube then centrifuged (4 °C, 3000 rpm, 15 minutes), the serum obtained was used to evaluate biochemical parameters (total protein, creatinine, alanine and aminotransferase). Subsequently, organs such as the liver and kidneys were removed, cleaned, weighed and preserved (10% formalin buffered, PBS) for histological analysis.

Histological analysis

After dehydration of joints, liver and kidney fixed in formalin (10%), inclusion and section were done. Hematoxylin/eosin staining was performed followed by mounting and observation using a Leica DM500 microscope equipped with a Canon Power shot SX620 digital camera for image acquisition [30].

Statistical analysis

The results expressed as mean accompanied by the standard error of the mean (SEM), were analyzed with one-way and two-way ANOVA followed respectively by the Tukey and Bonferroni post-test. The software used for data analysis was GraphPad software (Instant Biostatistic) version 3.0. The significance was fixed at $p<0.05$.

Results

Effect of the mixtures on paw edema

Figure 1 shows that the presence of carrageenan in the joint led to a significant increase in joint edema and a reduction in the pain threshold from the first hour until the 10th day in all animals in the negative control group receiving saline. However, the administration of the mixtures (aqueous and ethanolic) as well as diclofenac significantly reduced ($p<0.001$) edema compared to the negative control group. The extract mixture and diclofenac caused a significant decrease ($p<0.001$) in paw volume from day 4 to day 10 compared to the negative control group.

Effect of the mixture on mechanical hyperalgesia

In Figure 2, it can be noted that the administration of different extracts significantly reduced the thickness of the joint from day 2 until the end of the treatment compared to the animals in the negative control group. Aqueous mixture significantly increased ($p<0.001$) the pain threshold of the animals from day 5 to day 10 compared to the negative control group. Ethanolic mixture significantly ($p<0.001$) inhibited pain from day 3 to day 10 compared to animals in the negative control group.

Effect of the mixture on oxidative stress parameters

Concerning reduced glutathione, it appears that the injection of carrageenan did not cause any significant variation in the serum of animals treated with the mixtures or diclofenac (Figure 3). While in the liver and synovial fluid, the mixtures caused a significant reduction ($p<0.001$) in glutathione levels. Regarding malondialdehyde, oral administration of both mixtures significantly ($p<0.001$) decreased their levels in serum and liver. Both mixtures induced a significant increase ($p<0.001$) in SOD activity in serum, liver and synovial fluid. For catalase activity, extracts and diclofenac significantly ($p<0.001$) increased its activity only in serum.

Effect of the mixture on nitrite oxide

In Figure 4, we observed a significant increase ($p<0.001$) in the level of nitric oxide in the serum, liver and synovial fluid in the animals in the negative control group compared to the Naive group. Extracts reduced serum, liver and synovial fluid level of nitrite oxide compared to the negative control group. This reduction is significantly ($p<0.001$) with ethanolic extract at 200 mg/kg.

Effect of the mixture on joint tissues

Histological analysis revealed in the normal group, normal architecture of joint. Compared to the Naive group, histopathological changes in the tibia-tarsal junction (loss of the cartilaginous matrix marking a weakening of the articular cartilage) were observed in the joint of animals treated with carrageenan. Animals treated with different extracts, as well as those treated with reference substances, showed joint restoration (Figure 5).

Toxicity study of the mixture

As shown on Table 1, mixture didn't exhibit any sign of toxicity and mortality even at 5000 mg/kg. Therefore, the LD_{50} was greater than 5000 mg/kg.

It appears from Table 2 that the levels of white blood cells, granulocytes, monocytes, lymphocytes increased significantly ($p<0.01$) in the negative control group compared to those in the Naive group. The levels of hematocrit, red blood cells, hemoglobin, blood platelets, procalcitonin and the average hemoglobin content of corpuscles decreased significantly ($p<0.001$) in the negative control group compared to animals in the Naive group.

Figure 6 shows a significant decrease ($p<0.001$) in liver mass in animals in the negative control group. However, the administration of the different extracts or diclofenac resulted in a significant reduction ($p<0.001$) in liver mass compared to animals in the negative control group. Concerning kidney mass, no significant difference was observed.

Table 3 revealed that carrageenan injection in rats caused a significant decrease ($p<0.01$) in alanine aminotransferase activity and total protein level in the negative control group. However, administration of both mixtures and diclofenac significantly ($p<0.01$) increased alanine aminotransferase activity and total protein levels compared to the negative control group. Regarding aspartate aminotransferase activity and creatinine levels, no significant variations were observed.

Histological analysis revealed in the Naive group, normal architecture of the liver and kidney (Figure 7 and Figure 8). Compared to naive, histopathological changes in the liver (hepatic cytolysis) were observed in the negative control group. The treated group with the different extracts showed no hepatic disorder.

Discussion

This study aimed to evaluate the anti-inflammatory and antihyperalgesic properties of the mixture of aqueous and ethanolic extracts from *Z. officinale* rhizomes and dried fruits of *T. tetraptera* on a model of joint inflammation induced by carrageenan (joint injection, 0.1 ml, 3%) but also to assess acute toxicity and possible treatment-related intoxication. Results showed that both extract mixtures significantly inhibit joint edema and decrease pain sensitivity, improve oxidative stress and hematological profiles; the mixture of aqueous extracts administered to the animals showed no signs of toxicity.

For many years, the complexity of understanding the different pathological mechanisms of inflammatory arthritis has led scientists to search for new anti-inflammatory agents of natural origin [31]. *Z. officinale* and *T. tetraptera* are widely consumed traditionally and known for their richness in secondary metabolites and their anti-inflammatory, antioxidant and analgesic activities [32]. To evaluate the preventive or curative effect of the mixture on joint inflammation, the carrageenan inflammatory model was assessed by injecting carrageenan into the rat joint, since this substance induces several types of inflammation, acute monoarthritis and paw edema [33]. The injection of carrageenan causes a local action with the release of numerous pro-inflammatory mediators leading to edema and joint hypersensitivity (hyperalgesia and allodynia) similar to the symptoms of human arthritis [34]. Worsening of arthritis is caused by an overproduction of chemokines, pro-inflammatory cytokines and growth factors resulting in a vasodilation of blood vessels which increases the movement of immune cells and the amount of nutrients entering the synovial tissue, in the inflammatory zone [35]. In this study, oral administration of the mixture acutely or chronically significantly reduced the allodynia and hyperalgesia caused by the injection of carrageenan; in addition, the mixture significantly reduced joint edema at all the doses. It is known that *Z. officinale* rhizomes and *T. tetraptera*, individually are capable of significantly inhibiting the production of many inflammatory mediators as well as mediators of angiogenesis (IL-8) [36,37]. PGE_2 is a potent mediator of inflammatory pain [38], IL-8 is a key component of angiogenesis [39], and pathological angiogenesis is the critical process that leads to worsening of arthritis [40]. Thus, the effect of the mixture could result in an inhibition of angiogenesis leading to an inhibition of the abnormal growth of inflammation, synovial tissue and a reduction in hyperalgesia and allodynia observed.

Although the *Z. officinale* rhizomes, like the dried fruits of *T. tetraptera*, have been widely used for years by populations, and considering that toxicity is dose-dependent, we wanted to evaluate the toxicity (acute) of the mixture of these two elements in this study. Since the WHO recommends studying any product consumed not only for pharmacological properties, but above all for safety [41]. However, it is known that, for any pharmacological

substance evaluated during the study of acute toxicity in oral treatment having a LD_{50} (lethal dose 50) greater than 5000 mg/kg, this substance is considered non-toxic [42]. In this study, the mixture of aqueous extracts from *Z. officinale* rhizomes and dried fruits of *T. tetraptera* administered (2500 and 5000 mg/kg) did not cause any deaths and showed no signs of toxicity in all treated animals. These results suggest that the consumption of the mixture is safe for populations.

During the inflammatory process, many enzymatic systems such as transaminases are affected using anti-inflammatory substances, which subsequently cause the increase production of polypeptide-kinins and a significant reduction in the synthesis of mucopolysaccharides, which greatly worsen the inflammation [43,44]. In addition, chronic use of non-steroidal anti-inflammatory drugs can seriously alter the kidney function and increase the level of creatinine in the blood circulation. The significant reduction in serum transaminase activities and serum creatinine levels in the animals treated in this study confirms not only the antiproliferative activity but the protective effect on renal function of the mixture. Results show a significant drop in red blood cell count, hemoglobin, hematocrit and platelet levels, and then a significant increase in white blood cells in animals in the negative control group. This suggests the progressive establishment of anemia resulting from the production of cellular necrosis factors, excessive stimulation of the immune system against the antigens present in carrageenan [45] but also from the production of interleukin which is responsible for the production of macrophages and granulocytes [46]. The mixture induced an increase in the levels of hemoglobin and red blood cells, then a decrease in the level of white blood cells, patently reflecting its anti-anemic and immunomodulatory activities. In the inflammatory process, free radicals play a predominant role, depicting why oxidative stress is considered a risk factor for many inflammatory pathologies [47]. In the present study, the mixture significantly increased the activities of catalase, glutathione, and superoxide dismutase, then significantly decreased the levels of malondialdehyde and nitric oxide in the animal's serum.

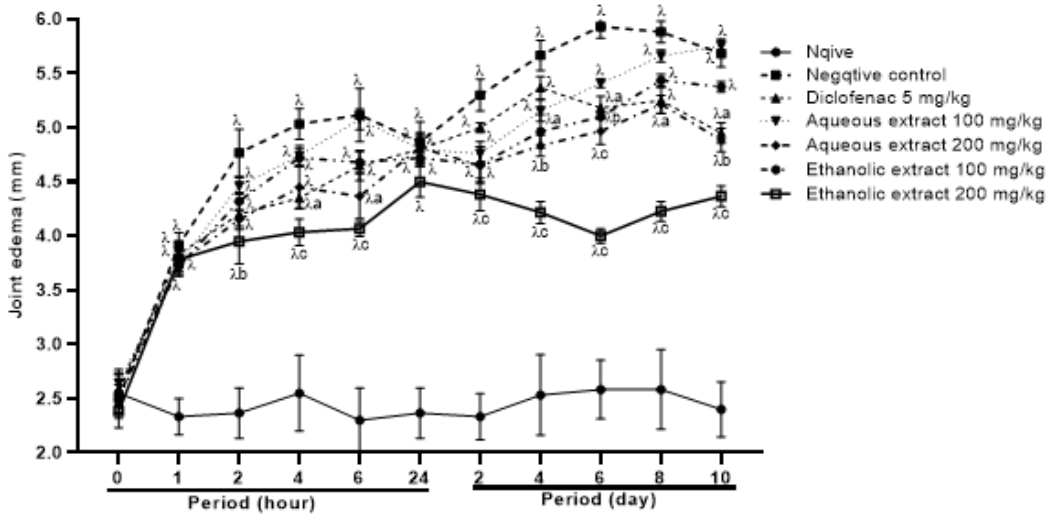

Figure 1. Effects of mixture of aqueous and of ethanolic extracts from *Zingiber officinale* rhizomes and *Tetrapleura tetraptera* dried fruits on the paw edema after injection of carrageenan on the joint.

Each value represents the mean ± SEM of 5 animals; [a]p<0.05, [b]p<0.01, and [c]p<0.001 are significantly different from negative control; [^]p<0.001 are statistically significant compared to the Naive group.

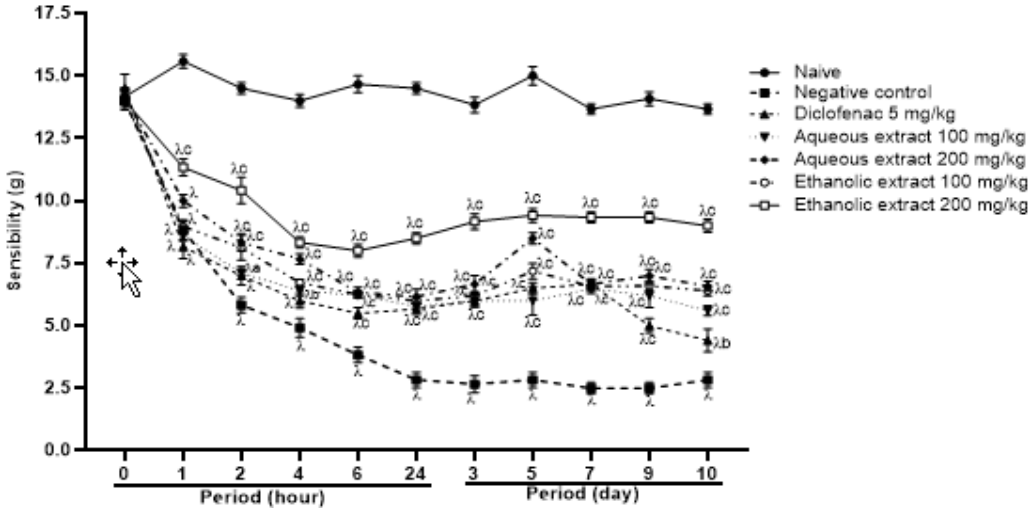

Figure 2. Effects of mixture of aqueous and of ethanolic extracts from *Zingiber officinale* rhizomes and *Tetrapleura tetraptera* dried fruits on mechanical hyperalgesia after injection of carrageenan on the joint.

Each value represents the mean ± SEM of 5 animals; [a]p<0.05, [b]p<0.01, and [c]p<0.001 are significantly different from negative control; [^]p<0.001 are statistically significant compared to the Naive group.

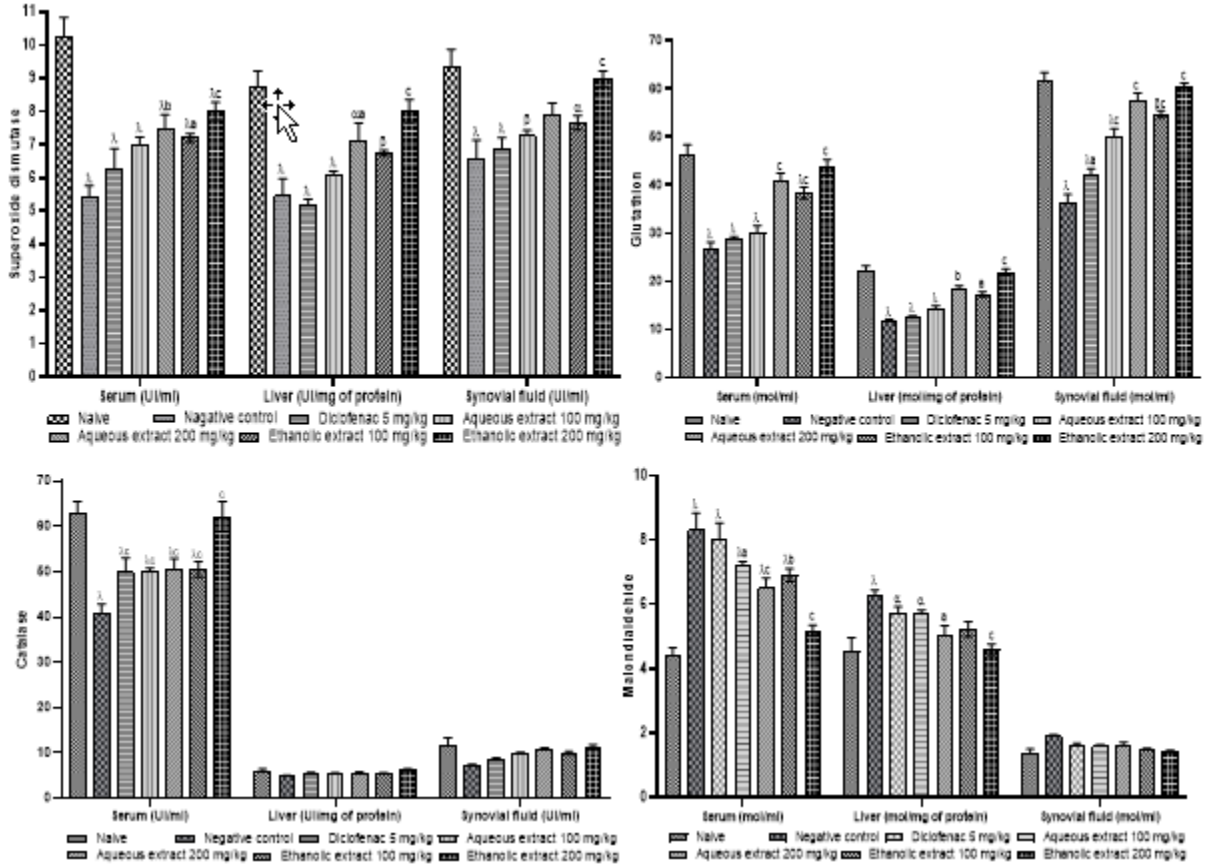

Figure 3. Effect of mixture of aqueous and ethanolic extracts from *Zingiber officinale* rhizomes and *Tetrapleura tetraptera* dried fruits on oxidative stress parameters in serum, liver and synovial fluid.

Each histogram represents the mean ± SEM, n = 5; $^{a}p<0.05$, $^{b}p<0.01$, and $^{c}p<0.001$ are significantly different compared to negative control; $^{\alpha}p<0.05$, $^{\beta}p<0.01$ and $^{\Lambda}p<0.001$ are statistically signi6cant compared to the Naive.

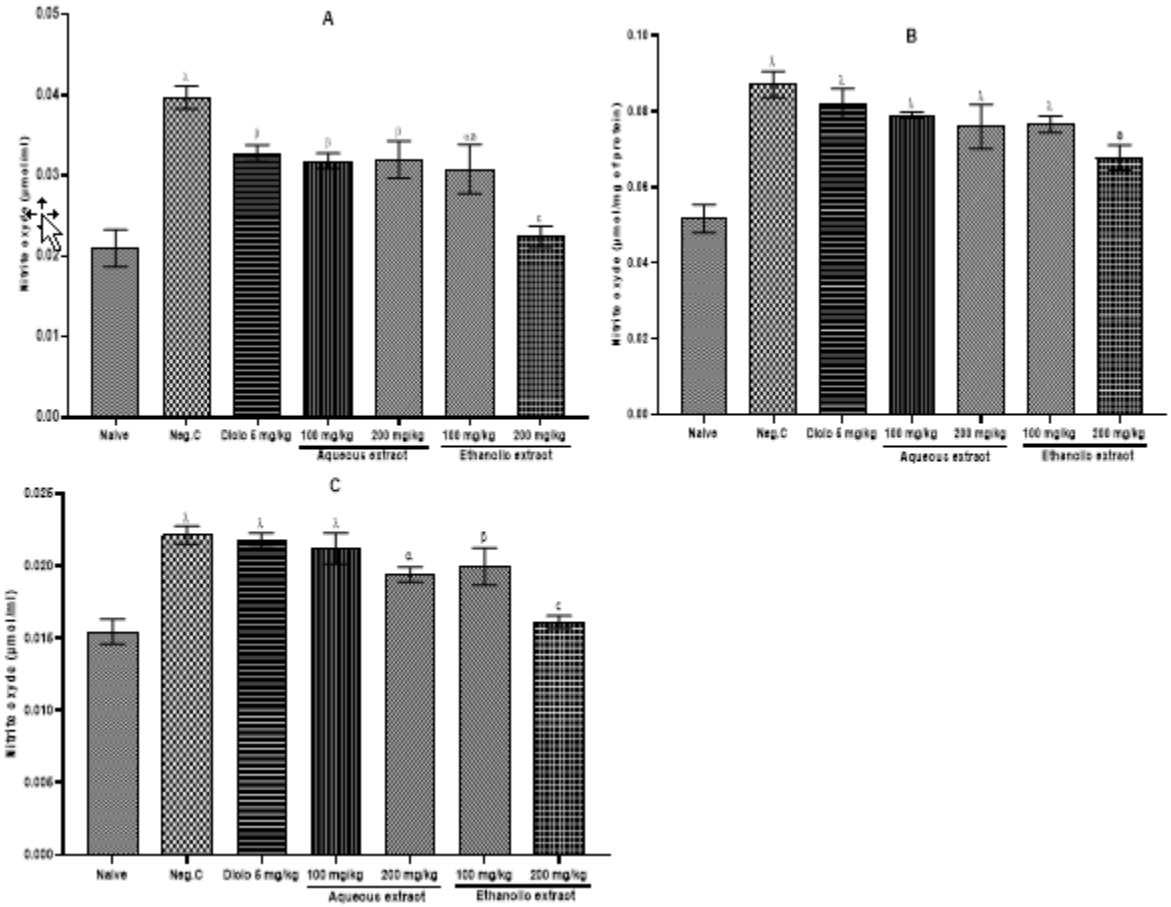

Figure 4. Effect of mixture of aqueous and of ethanolic extracts from *Zingiber officinale* rhizomes and *Tetrapleura tetraptera* dried fruits on nitrite oxyde in serum (A), liver (B) and synovial fluid (C).

Each histogram represents the mean ± SEM, n = 5; $^{a}p<0.05$, and $^{c}p<0.001$ are significantly different compared to negative control; $^{\alpha}p<0.05$, $^{\beta}p<0.01$ and $^{\lambda}p<0.001$ are statistically signi6cant compared to the Naive.

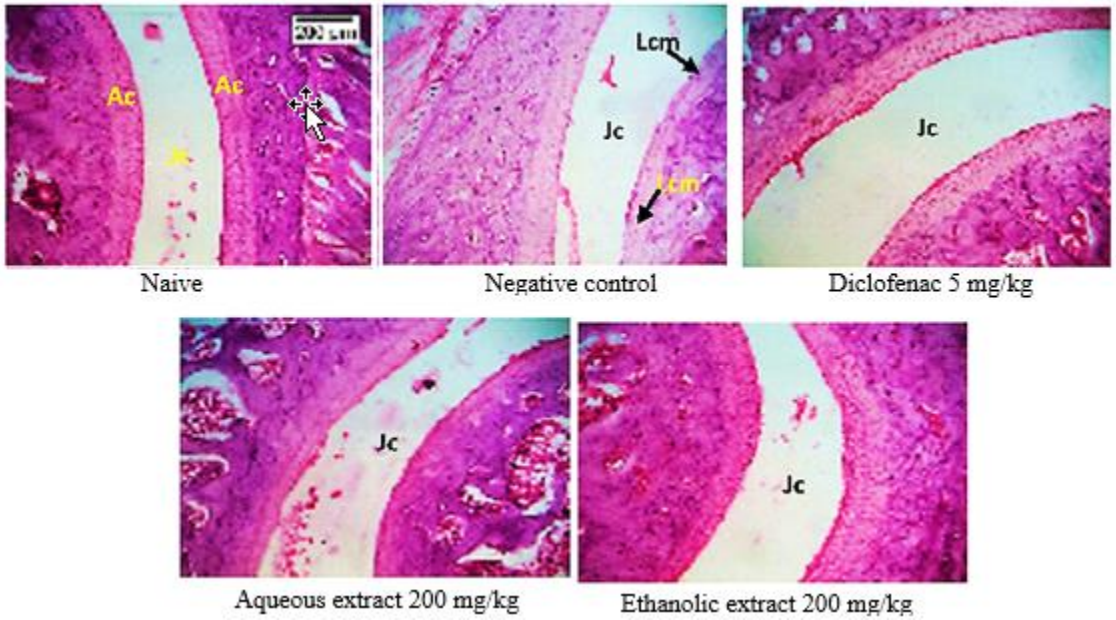

Figure 5. Micrograph of the tibiotarsal joint (hematoxylin-eosin X 100)

Jc: Joint cavity; Ac: Articular cartilage; Lcm: Loss of the cartilaginous matrix.

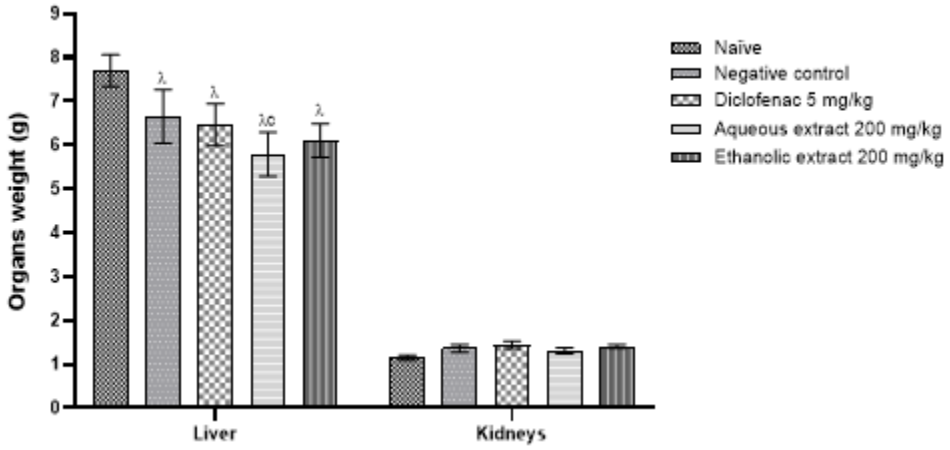

Figure 6. Effect of mixture of aqueous and of ethanolic extracts from *Zingiber officinale* rhizomes and *Tetrapleura tetraptera* dried fruits on organs weight.
Each value represents the mean ± SEM of 5 animals. $^{c}p<0.001$ are statistically signi6cant compared to negative control; $^{\lambda}p<0.001$ are statistically signi6cant compared to the naive.

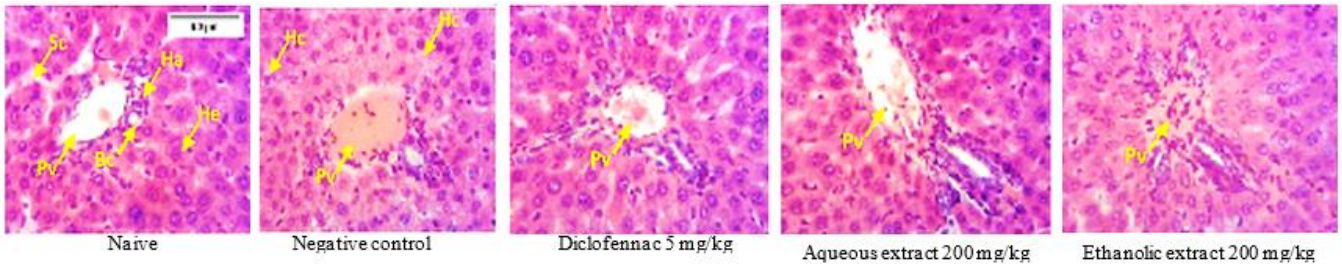

Figure 7. Micrograph of the liver (hematoxylin-eosin X 100):
Pv = Portal vein; Ha = Hepatic artery; Sc = Sinusoid capillary; He = hepatocyte; Bc = Bile canaliculus; Hc = Hepatic cytolysis.

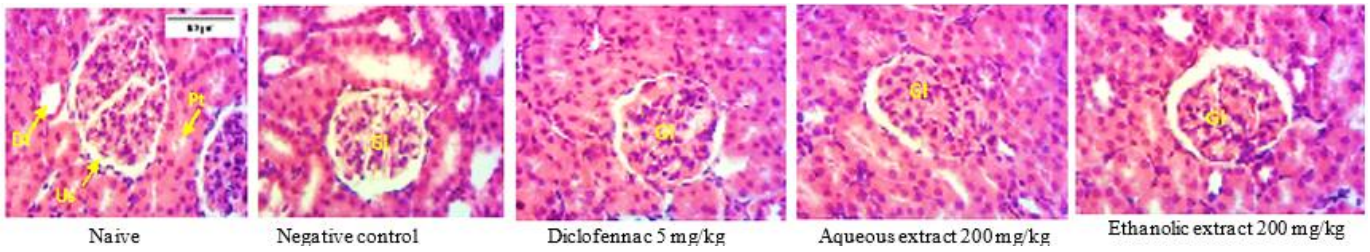

Figure 8. Micrograph of the kidney (hematoxylin-eosin X 100):
Gl = Glomerulus; Us = Urinary space; Dt = Distal tubule; Tp = Proximal tubule.

Table 1. General appearance and behavioral observations of acute toxicity study after extract administration.

Observation	NaCl	After 4 hours		After 7 days	
		2500 mg/kg	5000 mg/kg	2500 mg/kg	5000 mg/kg
Alertness	Normal	Normal	Normal	Normal	Normal
Body weight	Normal	Not change	Not change	Not change	Not change
Grooming	Absent	Absent	Absent	Absent	Absent
Temperature	Normal	Normal	Normal	Normal	Normal
Restlessness	Absent	Absent	Absent	Absent	Absent
Touch response	Normal	Normal	Normal	Normal	Normal
Food intake	Normal	Normal	Normal	Normal	Normal
Pain response	Normal	Normal	Normal	Normal	Normal
Urination	Normal	Normal	Normal	Normal	Normal
Water intake	Normal	Normal	Normal	Normal	Normal
Righting reflex	Normal	Normal	Normal	Normal	Normal
Tremor	Absent	Absent	Absent	Absent	Absent
Breathing	Normal	Normal	Normal	Normal	Normal
Convulsion	Absent	Absent	Absent	Absent	Absent
Corneal re0ex	Present	Present	Present	Present	Present
Lacrimation	Normal	Normal	Normal	Normal	Normal
Eye color	Normal	Normal	Normal	Normal	Normal
Diarrhea	Absent	Absent	Absent	Absent	Absent
General appearance	Normal	Normal	Normal	Normal	Normal
Mortality	Nil	Nil	Nil	Nil	Nil
Drowsiness	Absent	Absent	Absent	Absent	Absent
Body color	Normal	Normal	Normal	Normal	Normal

Table 2. Effect of mixture of aqueous and of ethanolic extracts from *Zingiber officinale* rhizomes and *Tetrapleura tetraptera* dried fruits on hematology

	Dose (mg/kg)	WBC (10^3/µL)	Lympho-cytes (10^3/µL)	Monocytes (10^3/µL)	Granulocytes (10^3/µL)	RBC (10^6/µL)	Hemo-globins (g/dl)	Hemato-crits (%)	Platelets (10^3/µL)	Procal-citonin (%)	MCHCt (pg)	MCHC (g/dl)
Naive	-	5.47 ± 0.29	3.97 ± 0.38	0.70 ± 0.06	0.47 ± 0.07	5.58 ± 0.15	12.03 ± 0.39	29.20 ± 0.60	516.3 ± 21.54	0.34 ± 0.02	21.53 ± 0.15	41.17 ± 0.55
Negative control	-	8.57 ± 0.49$^{\beta}$	6.97 ± 0.68$^{\alpha}$	1.17 ± 0.12	0.87 ± 0.07$^{\alpha}$	3.74 ± 0.35$^{\beta}$	8.73 ± 0.19$^{\lambda}$	22.87 ± 0.38$^{\beta}$	351.3 ± 23.10$^{\alpha}$	0.23 ± 0.01$^{\alpha}$	14.77 ± 0.43$^{\lambda}$	40.03 ± 0.34
Diclofenac	5	6.67 ± 0.58^{a}	4.90 ± 0.15	1.03 ± 0.15	0.73 ± 0.03	5.09 ± 0.15^{a}	10.43 ± 0.22$^{\beta b}$	25.70 ± 0.40	461.7 ± 39.10	0.31 ± 0.02	20.47 ± 0.47^{c}	40.57 ± 0.35
Aqueous extract	200	7.17 ± 0.24	5.30 ± 0.65	0.93 ± 0.09	0.70 ± 0.10	4.96 ± 0.22^{a}	10.60 ± 0.15$^{\alpha b}$	25.90 ± 0.96	451.0 ± 11.93	0.30 ± 0.01	19.63 ± 0.42$^{\beta c}$	40.23 ± 0.38
Ethanolic extract	200	5.80 ± 0.31^{b}	4.43 ± 0.43^{a}	0.67 ± 0.07^{a}	0.50 ± 0.06^{a}	5.42 ± 0.26^{b}	11.27 ± 0.19^{c}	28.87 ± 1.26^{b}	531.7 ± 31.25^{b}	0.40 ± 0.03^{c}	21.03 ± 0.22^{c}	40.93 ± 0.50

Table 3. Effect of mixture of aqueous and of ethanolic extracts from *Zingiber officinale* rhizomes and *Tetrapleura tetraptera* dried fruits on some serum parameters

	Dose (mg/kg)	ALAT (UI)	ASAT (UI)	Creatinine (mg/l)	Proteins (mg)
Naive	-	112.53 ± 3.44	224.10 ± 9.46	3.94 ± 0.31	0.80 ± 0.05
Negative control	-	195.89 ± 3.86$^{\lambda}$	243.40 ± 7.48	5.34 ± 0.11$^{\alpha}$	0.69 ± 0.16
Diclofenac	5	168.12 ± 1.68$^{\beta a}$	230.70 ± 14.65	5.22 ± 0.21$^{\alpha}$	0.66 ± 0.03
Aqueous extract	200	139.12 ± 3.61$^{\alpha c}$	229.70 ± 7.23	4.89 ± 0.49	0.71 ± 0.03
Ethanolic extract	200	129.89 ± 4.62^{c}	226.50 ± 4.02	4.15 ± 0.10	0.79 ± 0.02

Each value represents the mean ± SEM of 5 animals. AST: aspartate aminotransférase; ALT: alanine aminotransferase. $^{a}p<0.05$ and $^{c}p<0.001$ are statistically signi6cant compared to negative control; $^{\alpha}p<0.05$, $^{\beta}p<0.01$, and $^{\lambda}p<0.001$ are statistically signi6cant compared to the naive.

Conclusion

As conclusion, this work demonstrates that aqueous and ethanol extracts of the mixture of *Z. officinale* rhizomes and dried fruits of *T. tetraptera* possessed anti-inflammatory, antihyperalgesic, antioxidant and hepato-nephroprotective properties. These beneficial properties justify the traditional use of *Z. officinale* rhizomes and dried fruits of *T. tetraptera*. Thus, the mixture would be a clear candidate to ensure prevention and/or treatment of joint pathologies.

Abbreviations

T. tetraptera : Tetrapleura tetraptera
Z. officinale : Zingiber officinale
HDL : high density lipoprotein
WHO: world health organisation
PBS: phosphate buffered saline
NaCl: Sodium clhoride
EDTA: Ethylene diamine tetra acetic acid
SEM: standard error of the mean

ANOVA: analysis of variance
SOD: superoxide dismutase
LD_{50}: lethal dose 50

Authors' Contribution

DBM, MM and DDPD are involved in the study's conceptualization and design, data collection and analytics supervision, and manuscript revision for essential intellectual content. DBM, MM, DDPD, BDC, YNW, BTFD and AAD involved in the conceptualization and design of the study, data gathering and analysis, and manuscript drafting. DBM, MM, DDPD, DNSF, FYMO, BJD and KMF contributed to the data analysis and interpretation, as well as to the manuscript's scientific quality. MM, DDPD and AG helped with data collection and analysis as well as manuscript review. The final document for submission was read and approved by all authors.

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Conflict of interest

The authors declare no conflict of interest

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