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# *In vivo* anti-inflammatory activity of the essential oil of *zingiber officinale rhizomes* on Wistar rats

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## Abstract

**Background**: *Zingiber officinale* is a spice consumed in Chad and traditionally used against osteoarthritis, migraine and rheumatic pain, but few pharmacological studies of this plant have been carried out. The aim of this study was to assess the in vivo anti-inflammatory activity of Z. officinale rhizomes essential oil (EO).

**Methods**: A preliminary phytochemical screening was carried out on the EO of Z. officinale rhizomes; the anti-inflammatory activity of the given plant extract was then assessed at the concentrations of 0.20%, 1% and 5% on three experimental models: egg yolk-induced oedema, cotton pellet-induced granuloma and formalin. In addition, the essential oil was tested for its in vitro antioxidant capacity using the 2,2-Diphényl-2-picryl-hydrazyle Free Radical Scavenging Test, acide 2,2'-azino-bis 3éthylbenz- thiazoline-6-sulfonique Radical Test and the Ferric reducing antioxidant power Test. The study was extended to the acute toxicity of the EO of the rhizomes of Z. officinale using the sequential method.

**Results**: Qualitative phytochemical screening of the EO of Z. officinale rhizome revealed the presence of tannins, flavonoids, triterpenes, terpenoids and free quinones. In the egg yolk-induced edema model, the EO of Z .officinale rhizomes significantly inhibited ($p < 0.01$) oedema by 25.28%, 31.12% and 68.27% at the concentrations of 0.20%, 1% and 5%, respectively, when compared to controls. In the formalin-induced paw edema model, EO of Z. officinale rhizomes inhibited oedema by 24.27%, 30.14% and 43.14% at the concentrations of 0.20%, 1% and 5%, respectively, when compared to the controls. In the cotton pellet-induced granuloma model, EO of Z. officinale rhizomes induced an anti-granulomatous effect of 32.68%, 38.27% and 41.51% at the concentrations of 0.20%, 1% and 5%, respectively, as compared to controls. Additionally, the EO of the rhizomes of Z. officinale also induced in vitro antioxidant and free radical scavenging activity and iron reduction. Definitely, the LD50 of the EO of Z. officinale rhizomes was estimated as more than 2000 mg/kg b.w.

**Conclusion**: The EO of Z. officinale rhizomes exhibited anti-inflammatory activity. This effect can be explained, at least partly to its chemical content and its antioxidant potential.

**Keywords:** Zingiber; Essential oil; Antioxidant; Anti-inflammatory

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## 1. Introduction

Inflammation refers to a normal reaction of the body in response to a pathogen or trauma (1,2). It is the most common major health problem human's face in their live, and is associated with every infection that occurs in the body. This is the case with illnesses such as influenza or lung infections (3). However, inflammation is not just limited to infections, it is also associated with common diseases such as arteriosclerosis, cardiovascular disease, arthritis, as well as cancerous processes (4). In response to the frequent health problems faced by the body, a complex series of immunological reactions are initiated to neutralize pathogen invasion, repair damaged tissue and promote healing (5,6). However, inflammation can be detrimental due to the aggressiveness of the pathogen, its persistence, the site of inflammation, or abnormalities in the regulation of the inflammatory process (7). According to the World Health Organization, over 35 million people die every year worldwide from inflammation-related diseases (8). The inflammation related-diseases are quite common in Chad, as elsewhere in the world, with prevalence and mortality rates varying between 0.31% and 0.44% (9). Today, inflammatory diseases are increasingly prevalent throughout the world, and current treatments rely on steroidal (e.g. glucocorticoids) and non-steroidal anti-inflammatories (e.g. Diclofenac). Although effective, these molecules are often associate to undesirable side-effects that could hamper their long-term use (10).These side effects include gastrointestinal, metabolic, renal and asthmatic disorders (11) . Accordingly, natural products constitute a leading source of several new drugs and active ingredients in medicines against various communicable and non-communicable diseases. *Z. officinale is* a spice consumed throughout the world and traditionally used in Chad against osteoarthritis, migraine and rheumatic pain (12). Previously, phytochemical studies carried out on the essential oil of *Z. officinale* rhizomes have revealed the presence of bioactive molecules such as sesquiterpenes, flavonoids and polyphenols with healing properties against oxidative damage and numerous inflammatory diseases (13). The current work aimed to evaluate the anti-inflammatory activity of the essential oil of the rhizomes of *Z. officinale* in rats.

## 2. Material and methods

### 2.1. Plant material and essential oil preparation

*2.1.1. Species nomencture*

Reign: Plantae
Tracheobionta: Trachéobionta
Division: Angiosperms (or Magnoliophyta)
Class: Liliopsida
Subclass: Zingiberidae
Order: Zingiberales
Family: Zingiberaceae
Subfamily: Zingiberoideae
Genus: Zingiber
Species: *Zingiber officinale*

*2.1.2. Essential oil preparation*

The plant extract used in the present study was prepared in the form of an essential oil, using the hydro-diffusion technique. For this purpose, the rhizomes of *Z. officinale* were treated according to the method previously described by Alexis St-Gelais (14). Briefly, the rhizomes were harvested in the town of Ndjamena (Republic of Chad) in December 2020 underwent a pre-treatment which consists in removing the dirt-covered surface part of the rhizomes. The rhizomes of *Z. officinale* were then washed, rinsed with distilled water, cut and weighed. A 5000 g quantity of cut rhizomes was placed in a column. The steam rising from the column containing the rhizomes of *Z. officinale* finely ground was directed to a condenser and the liquid collected in a graduated burette, then collected from a beaker and let to cool. To eliminate any traces of water in the oil, we removed the oil from the beaker using an adequate pipette, then transferred it to a flask, adding two spatulas of anhydrous copper sulphate. The essential oil of the rhizomes of *Z. officinale* obtained was properly stored for further use.

### 2.2. Phytochemical screening of the essential oil of Z. officinale rhizomes

The essential oil of the rhizomes of *Z. officinale* was subjected to various qualitative tests based on dye reactions and/or differential precipitation: ferric chloride, Shinoda, Mayer, Liebermann-Burchard, Salkowski and soda tests (14,15).

### 2.3. Animals

*Wistar* rats were obtained from the Animal House of the University of Ngaoundere and bred in the Animal House of the Laboratory of Animal Physiology and Pharmacognosy of the University of Maroua. These animals were kept in clean, sterile, polyvinyl cages, with a normal diet of pellets and water ad libitum for the duration of the experiment. They were housed under standard experimental conditions at 25±2 ◦C, and 12-hour light/dark cycle. The rats were acclimatized for a period of 3 weeks prior to the experiment. The study was conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23; revised 1978). All experiments were carried out on adult male and female albino rats weighing between 160-205g. Essential cleanliness and sterile conditions were also adopted in accordance with the requirements of the *specific pathogen free protocol*.

### 2.4. Assessment of the anti-inflammatory activity of the EO of rhizomes Z. officinale

The anti-inflammatory activity the EO of *Z. officinale* rhizomes was assessed against edema induced in the rat paw by formalin injection, paw edema induced by egg white and pouch granuloma induced by cotton pellet. For each model used, animals were divided into 4 groups of 5 rats each and treated as follows: Group 1: negative control receiving distilled water

Group 2: Diclofenac 0.5% ointment by local application (positive control); Group 3: 0.20% EO by local application; Group 4: 1% EO by local application; Group 5: 5% EO by local application.

### 2.5. Formalin-induced paw edema

One hour before the induction of inflammation, the different treatments were administered to the animals in a single dose according to the distribution described above. Subsequently, edema was induced in the rat paw by injecting 20µL of freshly prepared 2.5% formalin in 0.9% NaCl subcutaneously into the rats' right hind paw. Paw thickness was measured before injection ($P_0$), then 4 hours post-injection ($P_t$) to assess the evolution of inflammatory edema.(16) The percentage inhibition (%I) of edema was calculated for each group of treated rats compared with the control group. It was given by the following formula $\%I = (P_t\text{-}P_0)\ x100/P_0$.

### 2.6. Egg white-induced paw edema

One hour before the induction of inflammation, the different treatments were administered to the animals in a single dose according to the distribution described above. Inflammatory edema was induced in the rat paw by injecting 20µL of hen egg white into the dorsal part of the rat paw. The thickness of the paw was measured before injection ( $P_0$), every 30 minutes up to 5 hours post-injection ($P_t$) to assess the evolution of inflammatory edema. (16) The percentage inhibition (%I) of edema was calculated for each group of treated rats compared with the control group. It was given by the following formula $\%I = (P_t\text{-}P_0)\ x100/P_0$.

### 2.7. Cotton pellet-induced granuloma

After anesthetizing the animals with Ketamine Hypochloride (25mg/kg, i.m) combined with Valium (5mg/kg, i.m), a small incision was made in the axilla of the foreleg using a sterilized scalpel blade. Next, 20 mg of cotton was weighed and soaked in 70° alcohol, then inserted into the pocket under the armpit using forceps. The pouch was then sutured with sterile surgical thread (Agary Pharmaceutical Ltd) as previously described (17) . Treatments were administered to the animals for 7 days, then on the eighth day, the animals were sacrificed by excess anesthetic and the cotton pellets removed. The percentage inhibition) $\%inh = \frac{(Pt-P0)x100}{P0}$

### 2.8. Evaluation of in vitro antioxidant activity of the EO of *Z. officinale rhizomes*

*2.8.1. DPPH (2, 2-Diphenyl-2-picrylhydrazyl) free radical scavenging test.*

This method is based on measuring the ability of antioxidants to trap the DPPH radical by Stephane. (18).The assay was consisting in taking a volume of 0.2mL of the EO of the rhizomes of *Z. officinale* or standard solution with 2mL of DPPH solution, shaking the mixture after 5 min and reading at absorbance 517 nm. Inhibition percentages were calculated using the following formula % inh = ((Ac-At)/Ac) X 100.

With Ac : absorbance of the negative; At : absorbance of the test performed.

*2.8.2. ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging test*

Free radical scavenging activity of the EO of EO of *Z. officinale* rhizomes was also assessed by the $ABTS^{+}$ cation radical decolorization test using the technique described by khan (19) **.** Abts was dissolved in distilled water at a concentration of 7nm. The $ABTS^{\pm}$ cation radical solution was obtained by incubating for 12 to 16 h in the dark at room temperature a mixture of equal volumes of the abts stock solution with a solution of potassium persulfate at 2.45 nm. The $ABTS^{\pm}$ solution was diluted with ethanol to an absorbance of 734 nm before use. Next, 1.5 ml of the $ABTS^{\pm}$ solution was mixed with 50 µl of HE or reference (ascorbic acid). Absorbances were measured at 734 nm after a 10-minute incubation in the dark at room temperature. The inhibition percentages were calculated using the following formula: % inh = ((Ac-At)/Ac) X 100

with AC : absorbance of the negative; At : absorbance of the test performed.

### 2.9. Ferric iron reduction assay (FRAP)

This technique was performed to measure the capacity of the EO of the rhizomes of *Z. officinale* to reduce ferric iron (Fe3+) present in the TPTZ complex (Fe(III)-2, 4,6-Tri (2-pyridyl) -striazine) to ferrous iron (Fe2+).The absorbance of the reaction medium was determined at 593 nm. The assay consisted in taking a volume of 0.1 mL of the EO or standard solution with 1mL of Fe(III)-TPTZ solution (acetate/TPTZ/FeCl3 buffer=10 :1 :1), shaking the mixture after 5 min, then reading the absorbance at 593 nm. The reducing power of the sample was determined from a calibration range established with vitamin C (0-125µg/mL). (20)

### 2.10. Acute oral toxicity of the EO of the rhizomes of Z. officinale

This study was carried out in accordance with OECD Sequential Test Guideline No. 423(21). Nine (09) rats were randomly selected, weighed and divided into 3 groups of 3 rats each to determine the $LD_{50}$ of the EO of *Z. officinale* rhizomes. Prior to the start of the test, all rats were fasted for 12 hours. On the first day of the experiment, rats in the normal control group were given distilled water (10 mL/kg) via oral gavage. Those of the first test group received a single oral dose of 2000 mg/kg b.w. of the EO of the rhizomes of *Z. officinale*. Three (03) other rats, considered as the second test group (confirmation group), were also fasted for 12 hours and then orally given the same single dose of 2000 mg/kg b.w. of the rhizomes of *Z. officinale*. The animals' clinical parameters were observed for the first 30 minutes, then periodically (with particular attention to the first 4 hours) for 24 hours, 48 hours and daily for 14 days .(22).

### 2.11. Statistical analysis EO of Z. officinale rhizomes

For for in vivo anti-inflammatory tests, results were expressed as mean ± SEM (Standard Error on the Mean) and were analyzed using Graph Pad prism software, version 5.00. The analysis of variance (ANOVA) was used, followed by Tukey's post-test, to compare batch means with each other. Values of $p < 0.05$ were considered significant.

## 3. Results

### 3.1. Qualitative phytochemical composition of the EO of Z. officinale rhizomes

**Table 1** Phytochemical composition of the *EO of Z. officinale rhizomes*

| Phytochemical class | Observation |
|---|---|
| Tannins | + |
| Flavonoids | + |
| Alkaloids | - |
| Triterpenes | + |
| Terpenoids | + |
| Saponins | - |
| Free Quinones | + |

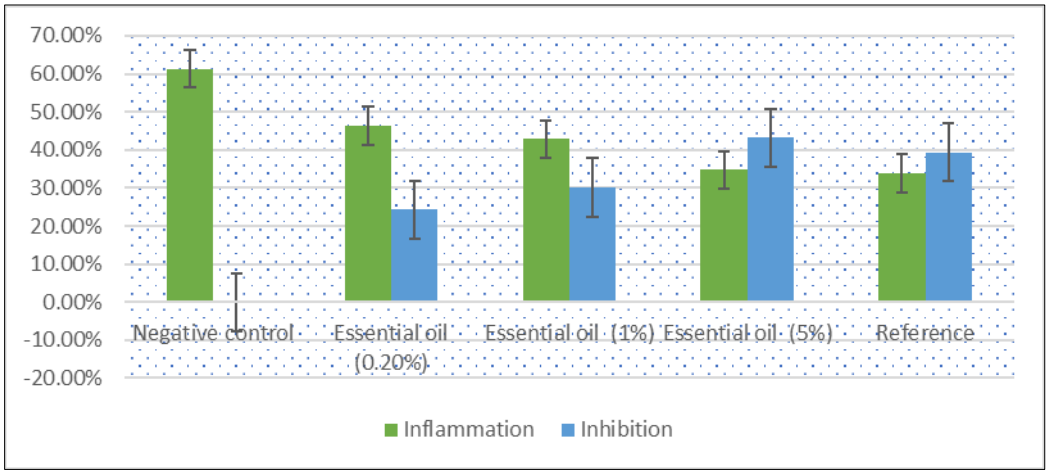
(+) : Présent ; (-) : Absent

Qualitative phytochemical screening of the EO of the rhizomes of *Z. officinale* (**Table 1**) revealed the presence of certain classes of bioactive metabolites such as tannins, flavonoids, triterpenes and free quinones. Saponins and alkaloids were not detected in the EO of *Z. officinale* rhizomes

### 3.2. Anti-inflammatory activity of the EO of *Z. officinale rhizomes*

*3.2.1. Effect of the EO of the rhizomes of Z. officinale on the formalin-induced paw edema*

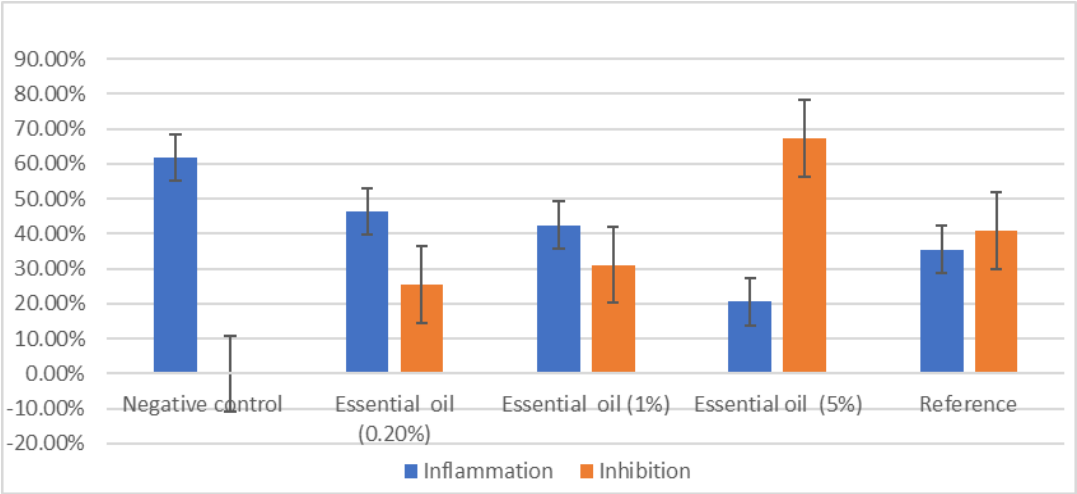
The Figure 1 depicts the results of the effect of the EO of Z. officinale rhizomes on the formalin-induced paw edema. The administration of formalin in rats resulted in a significant ($p< 0.01$) increase in paw volume (61.31%) in control animals. The positive control (Dichlofenac) caused a significant inhibition ($p< 0.05$) of the volume of the edema by 39.40%. Treatment with the EO of *Z. officinale* rhizomes at concentrations of 0.20%, 1% and 5% caused a significant inhibition ($p< 0.05$) of the paws edema up to 24, 27%; 30.14% and 43.14% respectively, as compared to control animals.




**X axes**: test group; **Y axes:** percentage

**Figure 1** Effect of the EO of Z. officinale rhizomes on the formalin-induced paw edema.

Each value represents the mean ± ESM, n= 0.05: statistically significant compared to the negative control group (distilled water).The essential oil of rhizomes of Z. officinale. *Effect of the EO of Z. officinale rhizomes on the egg white-induced paw edema*




**X axes**: test group; **Y axes:** percentage; Each value represents the mean ± ESM, n= 0.05: statistically significant compared to the negative control group (distilled water).The essential oil of rhizomes of Z. officinale.

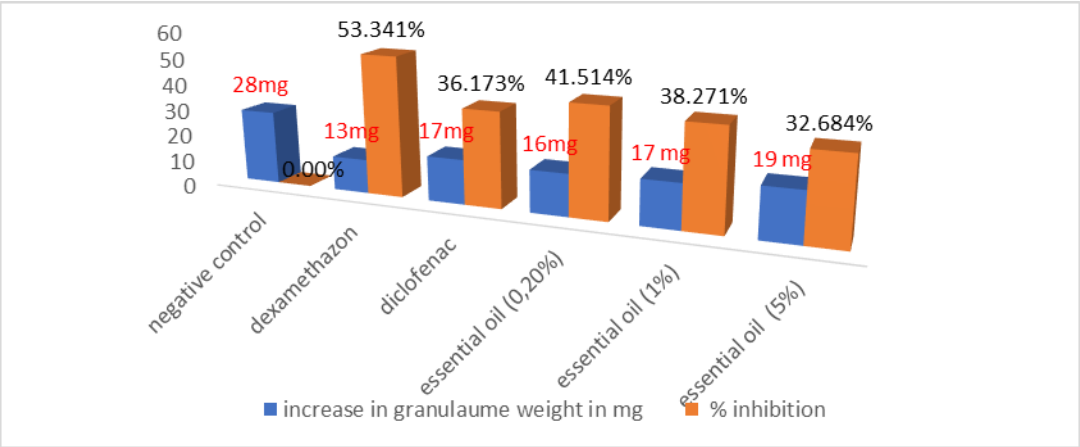
**Figure 2** Effect of the EO of *Z. officinale* rhizomes on the egg white-induced paw edema

The Figure 2 depicts the results of the effect of the EO of Z. officinale rhizomes on the egg white-induced paw edema. Injection of egg albumin into the paws of the animals resulted in a significant ($p< 0.01$) increase in paw volume of

61.76%. In addition, administration of dichlofenac caused a significant inhibition ($p< 0.05$) of 39.40% of the induced edema. Treatment with the EO of *Z. officinale* rhizomes at concentrations of 0.20%, 1% and 5% caused 25%, 38%, 31.12% and 67.29% inhibition of the paw edema respectively.

*3.2.2. Effect of the EO of Z. officinale rhizomes cotton pellet-induced granuloma*

The Figure 3 depicts the results of the effect of the EO of *Z. officinale* rhizomes on the cotton pellet-induced granuloma**.** Eight days after administering the various treatments to the animals, the cotton pellets were removed and dried. Diclofenac prevented granuloma formation by 36.17% compared with control rats. The weight of the granuloma thus obtained was 28 mg in the controls. The various doses of essential oil extract also inhibited ($p<0.05$) granuloma formation by 41.51%, 38.27% and 32.684% respectively.




**X axes**: test group; **Y axes:** percentage; Each value represents the mean ± ESM, n= 0.05: statistically significant compared to the negative control group (distilled water).The essential oil of rhizomes of Z. officinale.

**Figure 3** Effect of the EO of Z. officinale rhizomes cotton pellet-induced granuloma.

*3.2.3. In vitro antioxidant activity of the EO of Z. officinale rhizomes*

The following assays were used to demonstrate the antioxidant activity of *the EO of Z. officinale* rhizomes : DPPH (2,2-Diphenyl-2-picrylhydrazyl) free radical scavenging test, ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging test and ferric iron reduction assay (FRAP).

The results shown in **Figure 4** summarise the *in vitro* antioxidant effect of the *EO of Z. officinale* rhizomes. This figure shows that the *EO of Z. officinale* rhizomes has an ABTS and DPPH scavenging capacity of 77.77% and 68.57% respectively, as well as an iron reduction capacity of 58.33%.

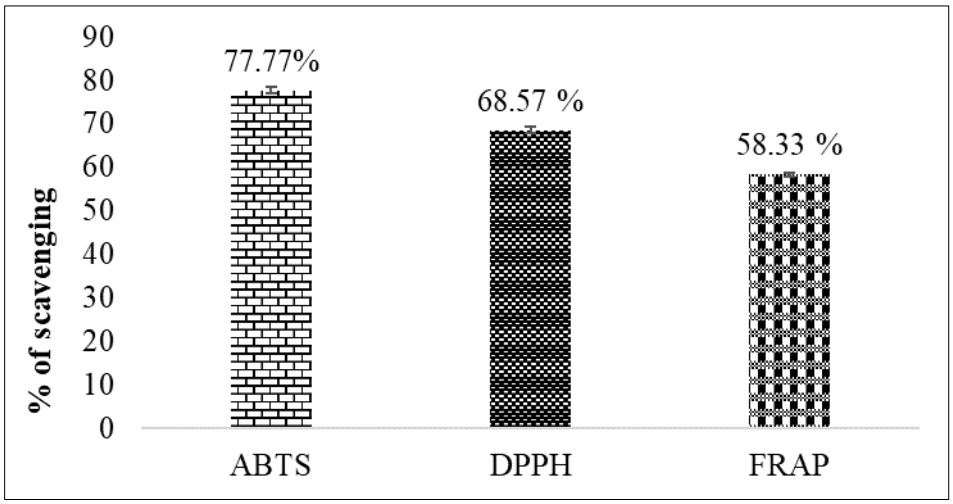



**Figure 4** Antioxidant activity of the EO of Z. *officinale* rhizomes

*3.2.4. Acute oral toxicity of the EO of Z. officinale rhizomes*

Administration of the EO of *Z. officinale* rhizomes *at* the single dose of 2000 mg/kg b.w. by gavage did not cause any mortality in the animals within 24 hours of administration, and even less during the 14 days of observation that followed. As a result, the mean lethal dose ($LD_{50}$) of the essential oil of *Z. officinale* was considered to be greater than 2000 mg/kg b.w. in rats.

## 4. Discussion

Most parts of plants have been used as extracts and may possess anti-inflammatory and antioxidant properties related to diseases such as diabetes, atherosclerosis, neurodegenerative, or cancer. In the current study, we investigated the anti-inflammatory and antioxidant activity of the essential oil of the rhizomes of *Z. officinale*; the study was extended to the chemical content and the acute toxicity screening.

In this study, edema was induced in animals, first using formalin which causes local inflammation when injected into the fascia of the foot (24,25). The tissue lesion thus leads to inflammation, and tissue damage is known to induce the synthesis of histamine, prostaglandins, leukotrienes (26), PAF (platelet activating factor), cytokines and NO (nitric oxide) (27). These mediators increase the permeability of the capillaries in the damaged region. Treatment of the animals suffering from formalin-induced edema effect of the rhizomes of the EO of Z. *officinale*, significantly reduce the volume of the edema in animals. The present study did not investigate the exact mechanisms of formalin-induced oedema inhibition; but suggest aprobable effect in the sense of reducing inflammatory mediators such as leucocyte and prostaglandin levels (28).

egg yolk edema is also one of the most widely used methods for studying the anti-inflammatory potential of active substances (30). Egg white has been taken as a prototype for the exudative phase of acute inflammation. The development of edema following an injection of egg white into the paw of a rat is attributed to the release of histamine, serotonin, kinins and prostaglandins. These events take place in 3 distinct phases in time and the mediators involved (31). Evaluation of the percentage inhibition of inflammation shows that the EO of the rhizomes of Z. *officinale* acts in the same way as the reference anti-inflammatory, but with less efficacy.

The cotton pellet-induced granuloma model is widely used to assess the transudative and proliferative components of chronic inflammation (29). The repair phase of the inflammatory process begins with the proliferation of fibroblasts and the multiplication of capillaries (neo-vascularisation).

The EO of *Z. officinale rhizomes* of effectively reduced the granuloma mass induced by cotton pellets, suggesting its activity in the proliferation phase of inflammation.

There is a relationship between Oxidative stress and inflammation. Inflammatory process induces oxidative stress and reduces cellular antioxidant capacity (23). Oxidative stress is viewed as an imbalance between the production of reactive oxygen species (ROS) and their elimination by protective mechanisms, which can lead to chronic inflammation. Oxidative stress can also activate a variety of transcription factors, which lead to the differential expression of some genes involved in inflammatory pathways. The inflammation triggered by oxidative stress is the cause of many chronic diseases ( (32). In the current work, the EO of the rhizomes of *Z. officinale* displayed an ABTS and DPPH scavenging capacity of 77.7% and 68.57% respectively, as well as an iron reduction capacity of 58.33%. The antioxidant power of this extract is therefore prone to potentiate the anti-inflammatory activity.

Also, it is well known that the pharmacological efficiency of plant extracts is due to their bioactive phytochemicals (33). The phytochemical analysis of the EO of the rhizomes of *Z. officinale* revealed the presence of tannins, flavonoids, triterpenes and free quinones. These secondary metabolites are hence responsible of its observed pharmacology activity. In fact, tannins possess anti-inflammatory effects which are positively associated with their antioxidant activities as described previously (34). Recent studies have demonstrated that flavonoids can inhibit regulatory enzymes or transcription factors important for controlling mediators involved in inflammation. Flavonoids are also known as potent antioxidants with the potential to attenuate tissue damage or fibrosis (35). In addition, the in *vitro* anti-inflammatory activity of terpenes via suppression of superoxide and nitric oxide generation and the NF-κB signalling pathway has been also demonstrated. The presence of these compounds justifies the pharmacological activity of the extract (32).

In the present study, the acute toxicological study performed on the EO of the rhizomes of *Z. officinale* rhizomes revealed that this plant extract was almost non-toxic with an $LD_{50}$ estimated to be greater than 2000 mg/kg b.w. This result confirms that of a previous study wich estimated that the $LD_{50}$ of the ethanolic extract of rhizomes of *Z. officinale* is greater than 5000mg/kg of body weight.(22)

## 5. Conclusion

The present study revealed that the EO of Z*. officinale* rhizomes showed dose-dependent anti-inflammatory activity in some models of acute and chronic inflammation. Moreover, the EO of *Z. officinale* rhizomes displayed anti-oxidant activity. This plant extract is therefore a potential non-toxic source of ant-inflammatory and anti-oxidant phytomedicines.

## Compliance with ethical standards

*Disclosure of conflict of interest*

The authors declare no conflict of interest

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