Toxicology International, *30(3) : 409-418; 2023. DOI: 10.18311/ti/2023/v30i3/33434*

Online ISSN : 0976-5131; Print ISSN : 0971-6580

Toxicology International

Contents available at: www.informaticsjournals.com/index.php/toxi



In vivo Acute Toxicity and Anti-inflammatory Activity of *Ferula sulcata* Desf. from Setif, Algeria

Malika Rahmouni[1*], Meriem Hamoudi[2], Randa Mlik[3], Hocine Laouer[2], Seddik Khennouf[2], Saliha Dahamna[2], Nour Elhouda Mekhadmi[4] and Aicha Mouane[4]

[1]*Department of Biology and Vegetal Ecology, Laboratory of Valorization of Natural and Biological Resources, Faculty of Nature and Life Sciences, University Ferhat Abbas, Sétif 19000, Algeria; fekhafadila@yahoo.com*

[2]*Department of Biology and Animals Physiology, Laboratory of Phytotherapy Applied to Chronic Diseases, Faculty of Natural Sciences and Life Sciences, University of Ferhat Abbas, Setif 1, Setif 19000, Algeria*

[3]*National Institute of Agronomic Research of Algeria, Station of Adrar, Adrar, Algeria*

[4]*Department of Biology, Laboratory of Biodiversity and Applications of Biotechnology in the Agriculture Field, University of Chahid Hama Lakhdar, El-Oued, Algeria*

Abstract

Several works are interested in studying the undesirable therapeutic effects of medicinal plants, which must be used with great caution because they can be toxic. Under this objective falls our work, which consists in studying the acute toxicity and anti-inflammatory action *in vivo* of the Essential Oil (EO) of the aerial part (seed) of *Ferula sulcata* Desf. harvested from the region of Setif in western Algeria. Two doses were applied by oral gavage (2000 and 5000 mg/kg body weight). After administration of the oil, the mice were continuously monitored for 14 days. The results obtained showed that there was no mortality or morbidity whereas the animals showed toxic effects e.g., behavioral and morphological changes. On the other hand, a significant increase ($p<0.05$) in body weight was observed. In addition, an important increase was recorded in the relative organ weights compared to the controls, whereas the liver was significantly increased (**$p<0.05$): 6.12±0.19; 6.14±0.31 in females compared to controls. Furthermore, ALT value decreased significantly (**$p<0.05$): 6.83±2.59 in males. In addition, the oral administration of EO at the dose of 600 mg/kg showed an interesting anti-inflammatory activity comparable to indomethacin. Histopathological damage was observed in the liver and kidney tissues of mice treated with EO of this plant. Hence, the acute toxicity and anti-inflammatory activities of EO of *F. sulcata* Desf. are considered the first data.

Keywords: Acute Toxicity, Anti-Inflammatory Activity, Essential oil, *Ferula sulcata*

**Author for correspondence*

Article Received on: 30.03.2023 Revised on: 18.05.2023 Accepted on: 30.05.2023

1. Introduction

Many plants play an important medicinal role and contain a wide variety of phytochemicals with antioxidant properties that minimize the effects of (ROS) in biological systems[1]. Today, consumers prefer to use natural products has led to the exploitation of essential oils and/or extracts of many plants as potent ingredients in the pharmaceutical, cosmetic, and food industries[2]. The toxicity of medicinal plants is generally unknown, but their therapeutic effects have been proven in various laboratories, so evaluating the toxicity of herbal preparations is important to determine the safety of these medicinal products[3]. In this regard, the Apiaceae family is one of the best examples. While the essential oils of this family are widely used for their antibacterial, antifungal, antiviral, antiparasitic, insecticidal and antispasmodic properties[4]. However, since prolonged administration of bioactive molecules in high or low doses has adverse or toxic effects on health. For this, we highlighted to study of one of the species belonging to the family of Apiaceae. This later has about 55 genera and 130 species[5]. The genus *Ferula*, which belongs to the Apiaceae family, contains about 170 species, including 133 species occurring from central Asia westward throughout the Mediterranean to North Africa and 30 species from Iran[6,7]. This genus is represented by six species in Algeria[8]. Ferula spp. are known for their toxicity and pharmacology, whereas Daucane esters of *F. communis* and *F. arrigonii* showed an anti-proliferative effect on human colon cancer lines[9] and ionophoretic and apoptotic calcium effects in the human Jurkat T-cell line[10]. Hence, the current experiment came to study the species of *Ferula sulcata* Desf. Which is an herbaceous plant characterized by certain pharmacologically active compounds such as sesquiterpenes, polyphenols, flavonoids, etc.

The main objective of the current study is to assess the toxicity potential of a medicinal plant by identifying the possible adverse effects associated with its use and determining the exposure threshold at which these effects occur[11].

2. Material and Methods

2.1 Plant Material and Oil Extraction

The aerial parts of *F. sulcata* (seed) were collected in March 2018 from East Algerian region (Setif). The identification was performed by Prof. Laouer Hocine, Laboratory of Natural and Biological Resource Valorization, Department of Biology and Plant Ecology. A voucher specimen (Chifa DZUMCAPPV00027) was deposited in the herbarium of the same laboratory of University Ferhat Abbes, Sétif 1. Essential oils were extracted by hydrodistillation (500 g of plant) using a Clevenger apparatus. The collected oils were stored at 4°C until further analysis.

2.2 Animals

The animal material chosen was male and female albino (Swiss) mice, having the same age (2 months), which were made available by the Pasteur Institute in Algiers. They were used for the study of the acute toxicity of the aerial part (seed) of *F. sulcata* essential oil. Females and males have an average weight of 24.9±0.58 g and 26.86±0.97 g, respectively. Before the experiment, the mice were acclimatized and kept under standard conditions (12 hours light, 12 hours dark) at a temperature of 27±1°C and humidity of 40±5% for two weeks in the animal facility of the Institute of Biology of the University of Ferhat Abbas Setif 1. They fasted 24 hours before the experiment. Commercial food (ONAB, Lekser), which contains cereals, vitamins and minerals, and water were provided ad libitum. Sawdust is used as bedding, which is renewed twice a week to ensure the animals are in good hygienic condition.

Animal experiments are carried out in accordance with the guidelines and the international charter for animal experiments. The animals were handled in accordance with the declaration (from Helsinki 2008). The committee of the Algerian Association of Sciences in Animal Experimentation (http://aasea.asso.dz/articles/) under Law No. 8808/1988, associated with veterinary medical activities and animal health protection (No. JORA: 004/1988) have approved all the studies mentioned.

The essential oil was taken up with water in a constant volume of 10 ml solution per kg mouse body weight.

2.3 Acute Toxicity

To assess the safety of the essential oil, the protocol described in OECD Guideline 423[12] was used. The animals of both sexes were divided into 6 experimental groups (two control groups and 4 test groups) with 6 animals per dose (3 females and 3 males). The animals fasted with free access to water the day before the experiment. EO was dissolved in distilled water. Each

lot received a single dose of EO by gavage, animals in the control group received distilled water by gavage, and animals in the test groups received EO orally at doses of 2000 and 5000 mg/kg.

After the administration of the respective doses, the animals were observed at least once during the first 30 minutes, regularly during the first 24 hours after the treatment with special attention during the first 4 hours and then daily during the 14 days after the treatment with dosing of extracts. This was done to assess the general physical condition of the animals, noting mortality rate, toxicological signs as well as other symptoms such as morphological, physiological and behavioral changes. On the day of sacrifice (day 14), the animals were weighed after the previous day's fast, then sacrificed and their internal organs, including the liver and kidney, were removed for possible histopathological examination. Blood samples were collected from the retro-orbital sinus into tubes containing heparin for analysis of biochemical parameters.

2.4 Analysis of Biochemical Parameters

Plasma was obtained by centrifugation of the heparinized tubes at 3000 rpm for 15 minutes and analyzed using an automated biochemical analyzer (Diatron Pictus 200: Chemistry System) to obtain the concentration of the following parameters: Aspartic Aminotransferase (AST), Alanine Aminotransferase (ALT), creatinine and urea. Relative organ weights were calculated using the following formula:

PRO = [absolute organ weight (g)/body weight of the rat on the day of sacrifice (g)] × 100

2.5 Histopathology Study of the Liver and Kidney

After estimating the weight of the organs (liver, kidney, spleen, testicular, lung, and heart), the liver and kidneys were fixed in 10% formalin, dehydrated in a series of ethanol baths of increasing degree (70-100 %), clarified in xylene, then paraffin blocks were formed. Thin sections 5 µm thick were cut, using a microtome, deparaffinized, and then stained with hematoxylin-eosin. The images of the histological sections of the treated groups and the control were taken at a magnification of 100 using a DFC945-type camera attached to the microscope. They were processed using the LEICA Suite version 3.8.0 application.

2.6 *In vivo* Anti-Inflammatory Activity of Essential Oil

2.6.1 Xylene-Induced Ear Edema

Ear inflammation was induced by xylene; control groups of mice of both sexes received distilled water, while indomethacin is a positive control.

2.7 Data Analysis

Data were expressed as (mean±SEM). One-way Analysis of Variance (ANOVA) was used to test differences between groups. Tukey's multiple comparison tests were used to determine the significance of differences between treatment group means at the p 0.05 level using Graph Pad Prism 7 software.

3. Results and Discussion

3.1 Observation of Behavior and Clinical Symptoms of Animals

At the beginning of the current experiment, females and males had an average weight of 24.9±0.58 g and 26.86±0.97 g, respectively. Oral administration of the EO did not result in the death of mice in all treated lots. On the other hand, clinical observations showed some signs of toxicity during the experiment period. Whereas the animals presented a clinical map characterized by the following symptoms: loss of locomotor activity, and ataxia, while the nervous syndromes were excitability, tremors, accelerated breathing, and protruding eyes. Secondarily, the activity of treated animals decreased, their gait becomes slow, and then gradually they lie down with their hind legs spread. The observed signs disappeared around day 2 and were not seen again for the remaining 12 days. The lethal dose 50 (LD_{50}) was therefore assumed to be higher than the doses tested (Table 1).

Table 1. Acute toxicity results of EO of the aerial part (seeds) of *Ferula sulcata* Desf.

Doses mg/kg)	Nbr of dead	Clinical signs
2000 and 5000	0 and 0	Hair straightening, drowsiness, weakness, labored breathing, rapid heartbeat, movement disorders, and loss of balance. Body tremors, convulsions, asthenia and diarrhea

The current experiment showed us several behaviors and clinical symptoms of animals treated with the EO of *F. sulcata* e.g., immobility, labored breathing, and rapid heartbeat. This can be explained by the presence of alkaloids in *F. sulcata* essential oil, known for their neurological effects, in particular quinolizidine alkaloids, whose effects were linked to various activities such as hypoglycemia, hypotension, and respiratory depression[13,14]. On the other hand, the body weight of these animals change is used as an indicator of the adverse effects of chemical compounds[15]. The variation in body weight was considered a preliminary index that provides information about the toxic side effects of the ingested substance in experimental animals[16].

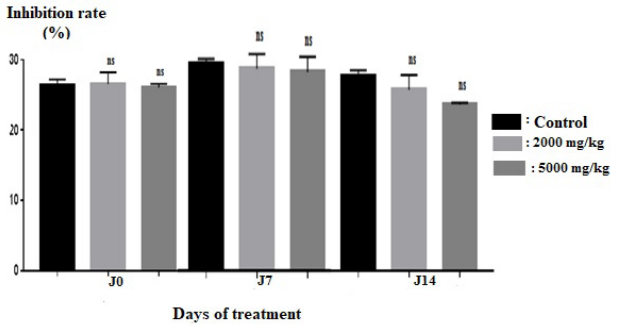


Figure 1. Body weight evolution of male and female mice treated with *F. sulcata* for 14 days.
ns: No significant difference between the treated and non-treated groups of mice

3.2 Body Weight

The evaluation of the acute ingestion of the aerial part of *F. sulcata* essential oil on the body weight of the animals is presented in Figure 1. According to the obtained results, no significant difference ($p<0.05$) was observed between the treated and non-treated groups in mice of both sexes. According to Klaassen *et al.*, and Teo *et al.*, changes in body weight are indicators of the side effects of drugs, chemicals, and bioactive substances[17,18]. The body weight of the female mice then experienced a significant decrease in the 2nd week, marking a recovery for the 3rd and the last week. A decrease or increase in body weight is associated with the toxic effects of chemicals and drugs. However, several researchers confirm that these changes in body weight are accompanied by an accumulation of fat and a physiological adaptive response to plant extracts, and not by the toxic effects of chemicals or drugs that lead to a decrease in appetite by lowering the caloric intake of the animal[19].

3.3 Organ Weights

Macroscopic examination of various organs after 2 weeks of force-feeding revealed a morphological change in the organs of the mice treated with EO at doses of 2000 mg/kg and 5000 mg/kg compared to the organs of the untreated mice. A significant increase (**$p<0.05$) was observed in the relative weight of the liver at both doses (2000 and 5000 mg/kg) in female mice with 6.12±0.11 g and 6.07±0.15 g, respectively.

The increase recorded in the weight of organs suggests that the essential oil administration had an impact on their normal growth. The increase in liver weight may be related to the congestion from blood reserves in the liver[20]. The liver is the first target of toxicity and the first organ exposed to anything that is absorbed in the small intestine and metabolizes foreign substances into compounds that may be hepatotoxic[21]. The liver works with the kidneys to remove toxic substances from the blood[22]. Any necrosis of liver cells leads to a significant increase in AST, ALT and LDH enzymes in blood serum[23]. However, these results require further analysis to determine the effect of the extract on these organs. According to Lullmann-Rauch[24], relative organ weight is considered to be a relatively sensitive indicator in toxicity studies. As well as Ozer *et al.*, mentioned that hepatocytes play an important role in detoxification in this phenomenon, while the kidney has the role of blood purification and waste elimination[25]. Thus, the analysis of kidney and liver function is very important in evaluating the toxicity of drugs and plant extracts, since they are necessary for the survival of an organism[26]. This justifies the concern of performing biochemical analyzes to evaluate any liver and kidney damage caused by ingesting the extracts. Several toxic plant compounds accumulate in the liver, where they are detoxified[27]. According to Xin *et al.*, an increase in relative organ mass indicates either congestion, edema, or hypertrophy, while a decrease in relative organ mass indicates organ atrophy and other degenerative changes[28]. In addition, when the plasma membrane of hepatocytes is damaged, a variety of enzymes normally found in the cytosol are released into the blood, their evaluation in serum is a useful marker of the extent and type of hepatocellular damage[29]. On the other hand, kidney dysfunction can be assessed by simultaneous measurements of urea, creatinine, and uric acid, and their normal values reflect a reduced likelihood of kidney problems[30].

Table 2. Organ weights of untreated and treated mice with *Ferula sulcata* seed essential oil

	Males			Females		
Organ	**Control**	**2000 mg**	**5000 mg**	**Control**	**2000 mg**	**5000 mg**
Liver	5.64±0.47	7.5±0.7^{ns}	6.92±0.25^{ns}	5.17±0.27	6.12±0.11**	6.07±0.15**
Kidney	1.45±0.11	2.28±0.16^{ns}	1.95±0.03^{ns}	1.02±0.06	1.54±0.07^{ns}	1.47±0.07^{ns}
Heart	0.48±0.05	0.51±0.02^{ns}	0.61±0.03^{ns}	0.49±0.01	0.49±0.04^{ns}	0.42±0.02^{ns}
Spleen	0,72±0,00	1.47±0.45^{ns}	1.85±0.06*	0.88±0.12	0.84±0.04^{ns}	1.11±0.11^{ns}
Lung	0.63±0.05	0.88±0.07^{ns}	0.86±0.03^{ns}	0.44±0.06	0.61±0.07^{ns}	0.77±0.22^{ns}
Testicles	0.66±0.03	0.66±0.08^{ns}	0.77±0.04^{ns}			

Table 3. Biochemical parameters of treated and untreated mice with the essential oil of *Ferula sulcata* seeds

	Males			Females		
Parameters	**Control**	**2000 mg**	**5000 mg**	**Control**	**2000 mg**	**5000 mg**
ASAT	119,63±0,46	107,06±11,82^{ns}	129.53±0.38^{ns}	134.70±17.59	104.65±10.25^{ns}	130.29±1.84^{ns}
Creat	4.92±0.09	6.95±0.3**	7.17±0.09**	6.40±0.33	7.76±0.37^{ns}	8,25±0,75^{ns}
ALT	10.96±0.0	6.83±1.49**	8.03±0.02^{ns}	7.09±0.57	6.69±0.26^{ns}	6.67±0.32^{ns}
Urea	0.32±0.02	0.28±0.01^{ns}	0.257±0.01 ^{ns}	0.32±0.01	0.31±0.01^{ns}	0.35±0.01^{ns}

In toxicity studies, the relative weight of the organs is a very important indicator of the physiological and pathological conditions of the animals and for confirming whether these organs have been damaged or not. Noting that the liver is the target organ of vertebrates and plays a major role in metabolism. It is most exposed to the toxicity of chemicals, especially when they are absorbed at the level of the small intestine. The liver could convert these foreign substances into hepatotoxic compounds[21].

3.4 Biochemical Analysis

The treatment with EO caused significant changes in ALT and creatinine levels at the dose of 2000 mg/kg in male mice compared to controls. A significant increase in creatinine (**p<0.05) was observed in the male mice of the group receiving EO at a dose of 2000 mg/kg. In contrast to the ALT enzyme, which decreased significantly (**p<0.05) in the group of mice receiving EO at a dose of 2000 mg/kg compared to the control group (Table 3).

Biochemical analysis is another sensitive indicator of the toxicological effects that can be induced after substance administration. The liver is the main organ responsible for the biotransformation of xenobiotics in the body and is therefore considered the main target of toxic substances[31]. According to Vysakh *et al.*, certain phytochemicals in plants are responsible for inducing hepatotoxicity[32]. This leads to high plasma levels of indicators of liver damage. With this in mind, measuring plasma biomarkers of liver damage such as AST and ALT is essential to detect potential liver damage by the essential oil. The results of the present study showed that acute oral treatment with the essential oil significantly altered the levels of hepatic biomarkers, suggesting that the essential oil is a hepatotoxic extract. On the other hand, urea, creatinine, and uric acid are important indicators of kidney function[33]. The lack of change in renal biomarkers indicates that the essential oil did not cause nephrotoxicity at the doses tested, suggesting that the phyto-components of the essential oil have no adverse effects on renal physiology. In general, an increase in the transaminases in the blood is an indication of damage to the parenchymal cells of the liver[25].

Furthermore, monitoring serum liver enzyme levels (ALT, AST, and ALP) is very important to detect liver damage and injury[34,35]. Alanine Aminotransferase (ALT) is a cytoplasmic enzyme found in very high concentrations in the liver, and a significant serum elevation of this enzyme is indicative of hepatocellular damage. Aspartate Aminotransferase (AST) is found in the cytoplasm and mitochondria of various tissues of the liver, heart, muscle, kidney, and brain[35,36].

In addition, urea is the product of protein metabolism and its concentration is affected by the rate of excretion, it is freely filtered by the glomerulus and is theoretically not reabsorbed, synthesized, or metabolized in the kidney[37]. It is also a more accurate marker of kidney function than urea, and any elevation in creatinine levels indicates marked damage to nephron function[38]. The significant increase in urea and a non-significant reduction in creatinine in treated mice indicate that the extract had no deleterious effect on the kidneys[39].

3.5 Histopathological Examination of Organs

A comparison of the organs, liver, and kidney, of the mice treated with doses of 2000 and 5000 mg/kg with those of the healthy control showed a preserved liver and kidney architecture, with no signs of cytolysis, indicating possible toxicity of the extract inherent. The effect of acute administration of EO from *F. sulcata* at both doses on the histology of the liver and kidney of male and female rats is shown in Figure 2 of both sexes treated with 2000 and 5000 mg/kg. The EO showed the presence of some modifications when compared to their respective controls. On the renal level: Extensive renal parenchymal suffering with partial lysis and necrosis, glomerular and tubular dystrophy, the glomeruli are of variable size, extensive cytolysis and sometimes necrosis of the retracted renal tubes, significant congestion. No dye uptake (non-functional kidney).

On the hepatic level, we noted clear congestion of the sinusoid capillaries, the Centro lobular veins, and the portal spaces. As well as, multiple karyorrhexis nuclei with numerous foci of poorly limited hepatocyte necrosis, the hepatocytes are often clarified. Hepatocyte cytolysis and necrosis are significant with the persistence of rare subcapsular, centro and perilobular dystrophic hepatocytes that have taken up the dye weakly, a discreet inflammatory infiltrate have also been observed. The information drawn from all the results presented in this work on experimental data of acute toxicity in female and male Albino Wistar mice suggest that EO can cause symptoms of toxicity when administered orally, the congestion of certain vessels may be due to the vasoconstrictor effects of the extract's phytochemicals on the wall of blood vessels[40].

3.6 Xylene-Induced Ear Edema

The results of this experiment are shown in Figure 3. EO showed a better anti-edematous effect with inhibition percentages of 85.74% and 45.77% for the 300 and 600 mg/kg doses, respectively. The effect of EO at 300 mg/kg is almost double (84.50%) that of indomethacin (45.77%). On the other hand, the effect of EO at 600 mg/kg is comparable to that of indomethacin (43.19%).

Concerning the anti-inflammatory effect, xylene-induced ear edema in mice, which is a model of acute inflammation, was used to evaluate the anti-edematous effect of *F. sulcata* essential oil. Xylene-induced ear edema is a reproducible experimental model and provides good predictive value for anti-inflammatory drug screening. Application of xylene to the ear induces fluid accumulation leading to the formation of edema characteristic of acute inflammation[41]. The thickness of the edema reached its maximum 30 minutes after the application of xylene[42]. The difference in ear thickness of mice before and half an hour after topical application of xylene was used in this publication to assess the anti-edematous effect of EO from *F. sulcata*.

The oral pretreatment of mice with indomethacin significantly reduced the development of edema with 44% inhibition. Our findings were relatively similar to those found by Li *et al.*[43] reported that the effect of essential oil inhibition was 51%. The anti-inflammatory mechanism of action of indomethacin is based on the inhibition of the synthesis of pro-inflammatory prostaglandins. Likewise, pretreatment of mice with EO led to significant inhibition of edema development. This inhibition is almost similar to that exerted by the reference anti-inflammatory agent. This inhibition may be due to the reduction in substance P released or to the antagonism of its action[44]. The anti-inflammatory effect of EO is probably due to the presence of lipophilic substances that can cross the skin barrier and are endowed with anti-inflammatory activities[45]. Noting that indomethacin, a potent NSAID, reduces edema formation by inhibiting cyclooxygenase activity and therefore prostaglandin synthesis[46]. Our results showed that the essential oil effectively reduced the development of ear edema, indicating that *F. sulcata* oil is a cyclooxygenase inhibitor. Depending to Mouffouk et al.[47], the edema has a biphasic character, caused during the first phase (0-1h) by the release of histamine or serotonin, while during the second phase (1h to 4h) it

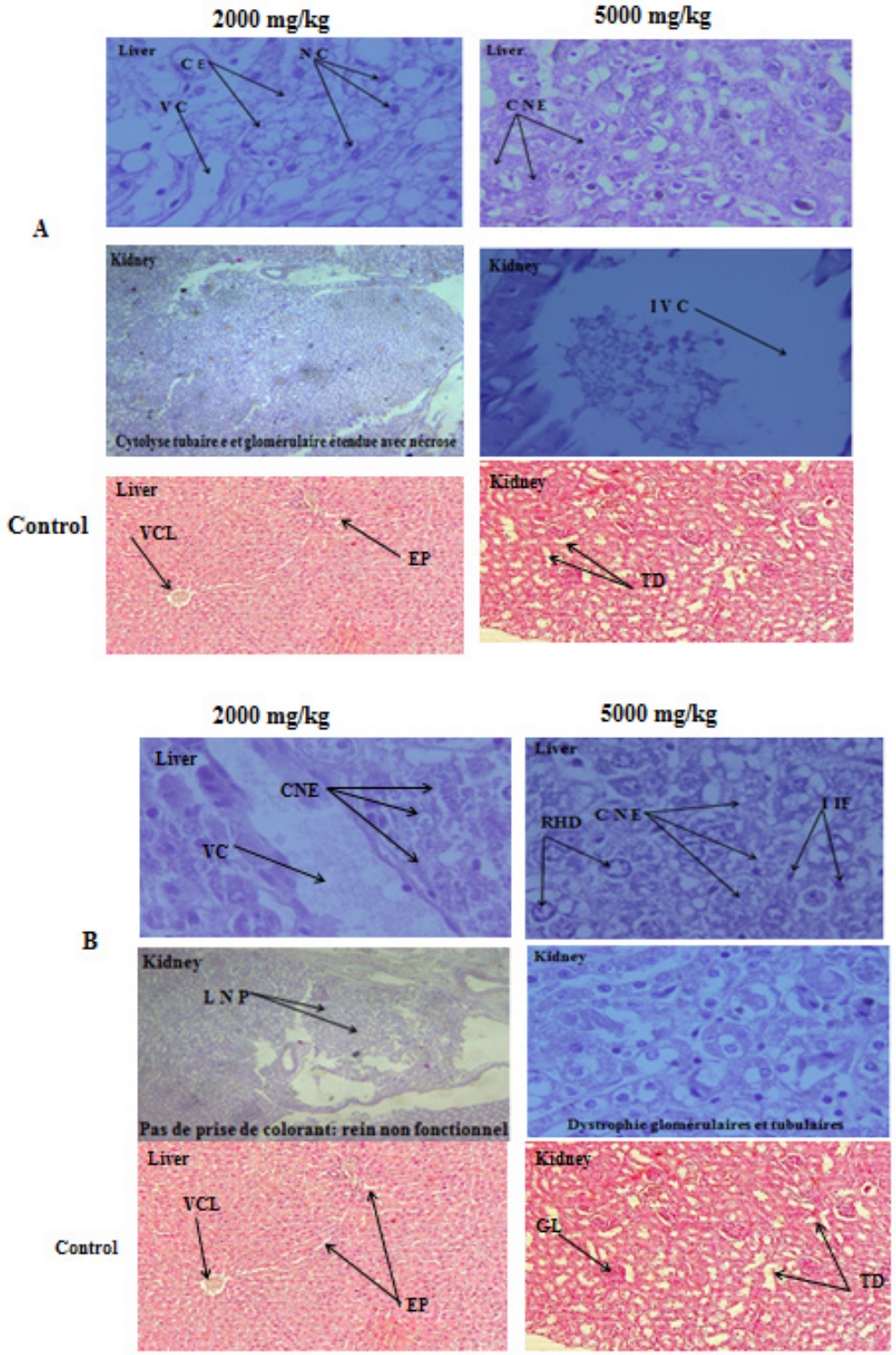


Figure 2. Histological sections of livers and kidney of male **(A)** and female **(B)** mice treated with *F. sulcata* during 28 days of treatment (x 4), (x 40) (hematoxylin-eosin stain).

VC: Vascular Congestion; **CLV:** Centro-lobular vein, **PS:** portal spaces; **DT:** distal tubes, **ENC:** Extensive Necrosis Cytolysis; **NC:** Necrosis and Cytolysis; **SVC:** Significant Vascular Congestion; **CHN:** Cytolysis and Hepatocyte Necrosis; **RDH:** Rare Dystrophic Hepatocytes

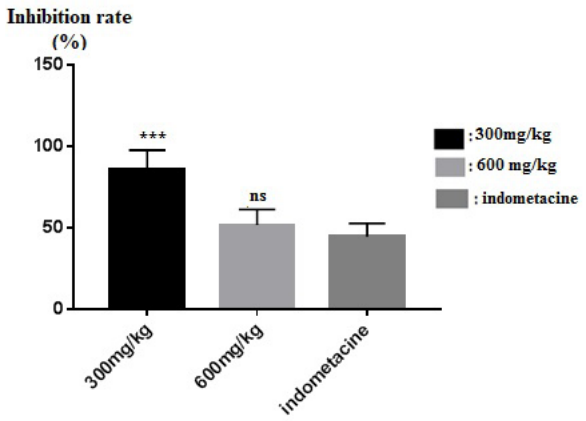


Figure 3. Anti-inflammatory effect of EO of *F. sulcata* on xylene-induced edema in mice.

is mediated by the releasing metabolites of arachidonic acid (prostaglandins), protease and lysosomes.

4. Conclusion

The oral administration of the essential oil extracted from the seeds of *Ferula sulcata* Desf., has shown acute toxicity in mice at both doses studied. The histological examination of the organs (liver and kidneys) showed no signs of essential oil toxicity, there were no organic lesions or fibrosis at the kidney or liver level. On the other hand, the analysis of the anti-inflammatory activity indicated that the essential oil contains phytochemicals that may be responsible for this pharmacological activity.

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