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Toxicity of south Morocco *Rosmarinus officinalis* essential oil: antibacterial and histopathological effects

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دراسة الخاصية السمية للزيت الأساسي للأزير المغربي : فعاليتها كمضادات البكتريا ومضادات الأكسدة

الأزير شجيرة عطرية تمتلك خاصيات عديدة وهي معروفة بفعاليتها كمضادات المكروبات ومضادات الأكسدة. الهدف من هذا البحث هو دراسة الخاصيات السمية للزيت الأساسي للأزير و ذلك بواسطة تجريبتين مختلفتين. تم تحليل التركيبة الكيميائية لهذا الزيت بواسطة الإستشراب في الطور الغازي و دراسة نشاط مضاد البكتيريا بتقنية المكرو أطموسفير. تم البحث عن الإصابات النسيجية بواسطة التشريح المرضي النسيجي لأعضاء الفئران (عددها 20) بعد إطعامها بواسطة 50 ميكرولتر في الكيلو غرام من الكتلة الجسمية للحيوان، كمقياس يومي، لمدة سبعة أيام مقارنة مع فئران تم تغذيتها بالماء المقطر بنفس الكمية وخلال نفس المدة. أظهرت النتائج أن المكونات الغالبة للزيت الأساسي للأزير هي سينول (42%)، البنين (11,92%) و الكونفر (13,99%) تتغير الكمية الدنيا للتثبيط عند البكتيريا المجربة من 50 إلى 90 ميكرولتر. أظهرت الدراسة التشريحية المرضية للأنسجة توسع السنخ الرئوي وتضخم خلايا الغدة الكظرية.

الكلمات المفتاحية : الأزير- الزيت الأساسي - نشاط مضاد البكتيريا - التشريح النسيجي - التسمم

Toxicité de l'huile essentielle de *Rosmarinus officinalis* du sud du Maroc: effets antibactérien et histopathologique

Le romarin (*Rosmarinus officinalis*) possède de nombreuses propriétés médicinales. Il est connu pour ses effets antimicrobiens et anti-oxydatifs. Le but de ce travail est d'étudier la toxicité de son huile essentielle (HE) via un test antibactérien selon la technique de micro-atmosphère et un autre histopathologique au niveau des organes de souris (*Swiss albinos*). L'analyse de la composition chimique de cette HE a été effectuée par CPG. Les constituants majoritaires de l'HE de *R. officinalis sont le 1,8-cinéole (42%), l'*alpha-pinène (11,92%) et le camphre (13.99%). Les quantités minimales inhibitrices des souches bactériennes testées varient entre 40 µl et 90 µl. Au plan histopathologique, une dilatation des alvéoles pulmonaires et une hypertrophie des cellules corticales et médullaires des surrénales ont été relevées. En conclusion, l'HE de R. *officinalis ne présente pas de* toxicité à la dose 50 µl/g mais possède des effets stimulants des glandes surrénales et du système respiratoire.

Mots clés: Cytotoxicité - Huile essentielle - *Rosmarinus officinalis* - Activité antibactérienne - Histopathologie - Errachidia - Maroc

Toxicity of south Morocco *Rosmarinus officinalis* essential oil: antibacterial and histopathological effects

Rosemary is an aromatic plant that have several medicinal properties, which is essentially used for its antimicrobial and anti-oxidant effects. The goal of this work is to study the toxicity of south Morocco *Rosmarinus officinalis* essential oil by antibacterial by micro-atmospheric technique and histopathologic tests on various organs of mice (*Swiss albinos*). Essential oil was analyzed by gas chromatography (GC). The main components detected in our essential oil were 1,8-cineole (42%), alpha-pinene (11.92%) and camphre (13.99%). Minimal inhibitory quantities of *R. officinalis* essential oil are ranged from 40 µl to 90 µl. Histopathological study showed a pulmonary alveolar dilation and hypertrophy of cortical and medullary suprarenal gland cells. In conclusion, *R. officinalis* essential oil do not present a serious toxicity at 50 µl/g dose but induce stimulating effects on respiratory system and suprarenal glands.

Key words: Cytotoxicity - Essential oil - *Rosmarinus officinalis* - Antibacterial activity - Histopathology - Errachidia - Morocco

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INTRODUCTION

Rosemary (*Rosmarinus officinalis*), an evergreen shrub, is one of the herb spices of the labiatae family cultivate among the wild world.

It have been used in cosmetics and in folk medicine as an antispasmodic of renal colic, for relieving respiratory disorders or to stimulate hair growth (Lemonica *et al.*, 1996; Al-Sereiti *et al.*, 1999; Munne-Bosch *et al.*, 2001). It have a potential therapeutic to prevention and treatment of bronchial asthma, inflammatory diseases, atherosclerosis and cancer (Offord *et al.*, 1995; Hui-Hui *et al.*, 2001; Sotelo-felix *et al.*, 2002; Mimica-Durck *et al.*, 2003).

The rosemary anti-oxidant properties is attributed to its diterpenoids, lavonoids, triterpenoids and phenolic acids constituents (Calabrese *et al.*, 2001; Munne *et al.*, 2001; Kim *et al.*, 2003; Ponce *et al.*, 2004). Volatile compound essential oil of secondary metabolism plant may act as phytoprorective agents defending some species of conifer from herbivore and pathogen attack (Gijzen *et al.*, 1991; Faleiro *et al.*, 1999).

Their bioactive components have recently gained momentum in many pharmaceutical and food processing applications (Cowan., 1999; Silva *et al.*, 2003; Vadar-Unlu *et al.*, 2003). They have insecticide, antifungal and antibacterial activities (Pattanaik *et al.*, 1996; Dorman *et al.*, 2000; Benkeblia, 2004).

Several studies have proved that rosemary essential oil possess anti-oxidative and antimicrobial activities which are using for food preservation and human microbial diseases control (Lis-Balchin, 1997; Mondello1 e*t al.*, 2003).

The aim of this work was to determine the composition of Moroccan *Rosmarinus officinalis* essential oil and to study its toxicity by antibacterial and histopathological tests.

MATERIAL & METHODS

1. Plant material

Rosemary (*Rosmarinus officinalis*) samples were collected from Errachidia, Morocco in May 2002. The specimen identification was realized in Biochemistrical Laboratory of the "Institut Agronomique et Vétérinaire Hassan II".

2. Essential oil extraction

Rosemary was submitted for 3 hours to hydrodistillation using a Clevenger-type apparatus. The essential oil was dissolved in n-hexane (10% v/v) before gas chromatography (GC) analysis.

3. Gas chromatography Analysis

The essential oil was analyzed by capillary gas chromatography (Chromapack Cp 9001) equipped with a SE54DF (30 m x 0.25 mm) capillary column. The column temperature was programmed initially at +50°C (isothermal for 5 min), then gradually increased to +230°C (isothermal for 10 min) at +4°C/min rate. A flame ionization detector (FID) was used for routine quantitative analysis. Detector and injector temperature were at respectively +235°C and +240°C. The nitrogen carried gas was adjusted at a flow rate of 1 ml/min.

4. Antibacterial assay: micro-atmosphere method

The micro-organisms used were *Escherichia coli* CIP54127, *Proteus vulgaris* CIP5860T and *Klebsiella pneumoniae* CIP8291T which were a gift from a Pasteur Institute. We have also used *Escherichia coli*, *Proteus vulgaris* and *Salmonella enteritidis* strains which were isolated from patients in Pasteur Institute biological center. These bacteria were selected because they are frequently reported in human infection and are multiresistant to several antibiotics. Strains were maintained in Kligler agar at +4°C.

Bacteria inoculate were prepared by growing cells in Triptic Soy Broth for 24 h at +37°C. The cell suspensions were diluted with peptone water to provide initial cell counts of about 10^7 to 10^8 colony forming unit (CFU)/ml.

A suspension of the tested micro-organism (5 µl at 10^8 cells/ml) was distributed on the C.E.L.D. agar (Cystine-Lactose-Electrolyte-Déficient) surface. Filter paper discs (20 mm diameter) were impregnated with various quantities of the essential oils (from 0 µl to 100 µl with 5 µl of increment), placed in the cover of the Petri box then incubated at +37°C for 24h. All the tests were performed induplicate and repeated triplicate.

5. Histopathological study

5.1. Animals

Swiss albino mice, six weeks old, purchased from Sciences Faculty, University Hassan II Ain Chock Casablanca, were housed in plastic cages in a conditioned air room (+22 ± 2°C, humidity 55 ± 10%) and given food and water freely.

5.2. Gavage

Experimental group of mice (10 males and 10 females) were fed with 50 µl/g of *Rosmarinus officinalis* essential oil during 7 days and the control group (5 males and 5 females), received the buffer water during the same period.

5.3. Secondary effects

The observation of the general state and the mice mortality has been followed during the 7 days of treatment. Every day, all animals have been weighted and their water and food consumption has been evaluated.

5.4. Microscopic analysis

The mice were sacrified and the following organs: liver, kidney, brain, spleen, lung, bowel, stomach, heart, testicular, suprarenal glands were removed, fixed in Bouin and embedded in paraffin. 4-5 µm sections were stained with hematein-eosin, then examinated under light microscopy (Olympus-BH-2).

RESULTS

1. Chemical composition of essential oil

Hydrodistillation of *Rosmarinus officinalis* dried plant yielded 0.85% (volume/weight) of essential oil (calculated per weight of dried material). GC analysis of the crude oil resulted in the identification of seventeen components representing 95.75% of the total components of essential oil from *Rosmarinus officinalis* (Table 1).

2. Antibacterial activity

Results of antibacterial activity of *Rosmarinus officinalis* essential oil against bacteria is presented in table 2. Minimal inhibitory quantities are ranged from 40 µl to 90 µl for all strains.

Table 1. Main components (%) of *Rosmarinus officinalis* essential oil. Compounds listed in order of elution. R_t: retention time (as minutes)

	Components	Rt	Percentage
1	α-pinene	7.55	11.92
2	camphene	8.09	4.55
3	β-pinene	9.22	7.71
4	β-myrcene	9.94	1.41
5	p-cymene	11.12	0.93
6	1,8-cineole	11.49	42.00
7	γ-terpinene	12.66	0.86
8	terpinolene	13.85	0.39
9	linalol	14.39	0.88
10	camphor	15.98	13.99
11	borneol	16.81	3.57
12	1-terpinen-4-ol	17.35	0.81
13	α-terpineol	17.86	2.40
14	berny-acetate	21.44	0.73
15	β-caryophyllen	26.07	3.60

Table 2. Minimal inhibitory quantities of *Rosmarinus officinalis* essential oil obtained using micro-atmospheric technique

Strains	Minimal inhibitory quantities (µl)
Escherichia coli CIP54127	65
Proteus vulgaris CIP5860T	40
klebsiella pneumoniae CIP8291T	90
Proteus vulgaris	40
Salmonella enteritidis	60
Escherichia coli	70

3. Histopathological study

Mice treated by *Rosmarinus officinalis* essential oil showed scratching of the muzzle and bewilderment. These signs disappear three hours after the gavage. In the fifth day of treatment, we noted a somnolence and a reduction of the locomotors and the escarping activity.

However, mice do not present any respiratory difficulty during the treatment and no mortality has been observed during all the study.

Weight evaluation and consumption of food and water did not present a very noticed variations (Figures 1, 2 & 3).

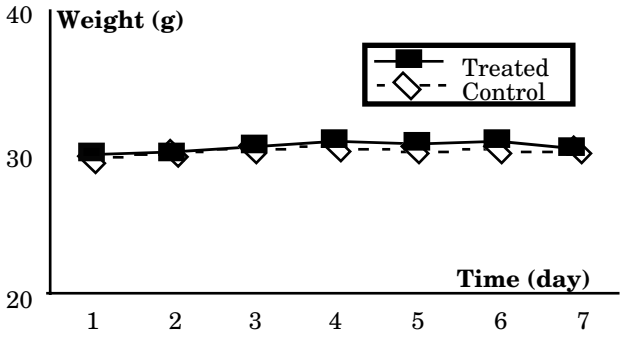

Figure 1. Weight variation of the Swiss albinos mice (n=30) during daily treatment (7 days) with 50 µl/g body weight of *Rosmarinus officinalis* essential oil

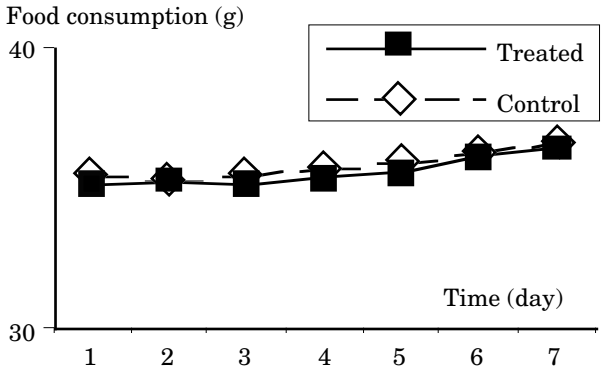

Figure 2. Variation of food consumption of the *Swiss albinos* mice (n=30) during daily treatment (7 days) with 50 µl/g body weight of *Rosmarinus officinalis* essential oil

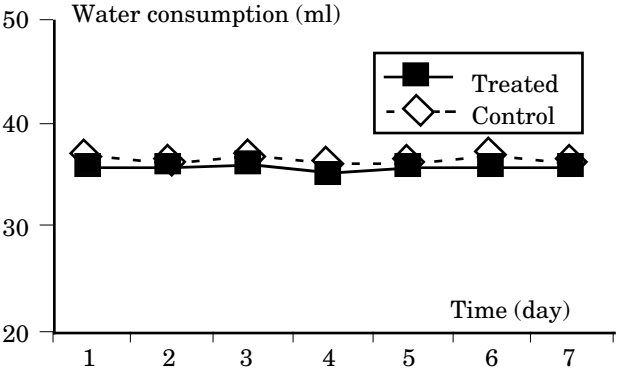

Figure 3. Variation of water consumption of the *Swiss albinos* mice (n=30) during daily treatment (7 days) with 50 µl/g body weight of *Rosmarinus officinalis* essential oil

The macroscopic analysis of the removed organs during the dissection revealed that the treated lungs were more red then the controls. All the other organs have a normal aspect.

The microscopic analysis of the treated organs showed clearly a pulmonary alveolar dilation (Figure 4) and a cortical and a medullary suprarenal cells hypertrophy (Figure 5).

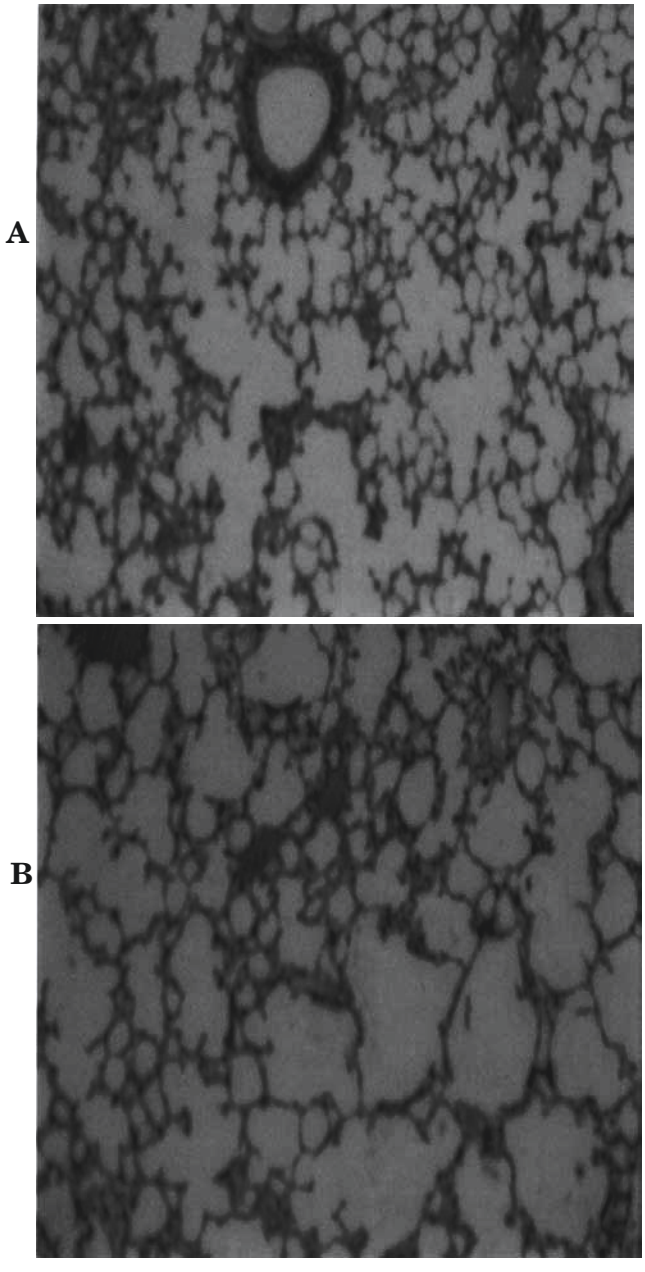

Figure 4. Photomicrographs (Gx40) of the *Swiss albinos* lung (n=30). Section from control (A) and mice treated with 50 µl/g body weight of *Rosmarinus officinalis* essential oil (B), were stained with hematein-eosine

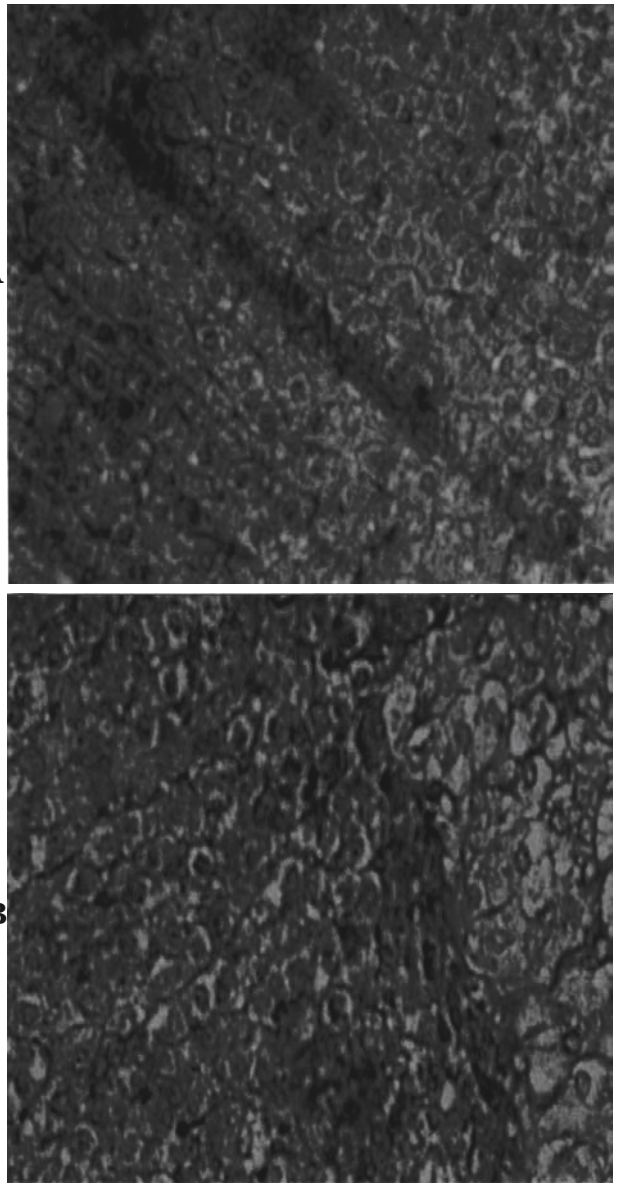

Figure 5. Photomicrographs (Gx40) of the *Swiss albinos* suprarenal glands (n=30). Section from control (C) and mice treated with 50 µl/g body weight of *Rosmarinus officinalis* essential oil (D), were stained with hematein-eosine

The results of histopathologic analysis of the other organs revealed no considerable abnormality.

DISCUSSION

Chemical composition of Moroccan *Rosmarinus officinalis* essential oil is in accordance with some published data (Chalchat *et al.*, 1993; Fechtal *et al.*, 2000). The 1,8 cineole (42%), the α-pinene (11.92%) and the camphre (13.99%) were the main components which presented 67.91% of total essential oil. Among the world, the 1,8-cineole chemotype characterises the *Rosmarinus officinalis* essential oil. It concentration was ranged from 41 to 63% (Chalchat *et al.*, 1993; Fechtal *et al.*, 2001).

Chemical composition of the Portugal *Rosmarinus officinalis* essential oil is different of the Marroccan one collected in the same period (Faleiro *et al.*, 1999). This could be due to several factors particularly to the geographic region or environmental conditions (Cavaleino *et al.*, 2001; Angioni *et al.*, 2003).

The tested bacteria were all sensitive to *R. officinalis* essential oil in steam phase using the micro-atmospheric method. *P. vulgaris* was the most sensitive however *K. pneumonia* was the most resistant bacteria. It is interesting to note that *Rosmarinus officinalis* essential oil manifested important antibacterial activity against *E. coli* and *S. enteritidis*, which are known to be very resistant bacteria to synthetic drugs (Mimica *et al.*, 2003). Several components of the essential oils seem to contribute to the antimicrobial activity and there is no major component solely responsible for a such property (Faleiro *et al.*, 2003).

Prolonged treatment of mice allowed us to determine the functional and anatomopathological changes consecutive to the repeated administration of rosemary essential oil. The weight evolution and consumption of food and water showed absence of difference at the treated mice and witnesses during the treatment. We would conclude the absence of toxicity by Moroccan *Rosmarinus officinalis* essential oil during the treatment by oral administration. This in agrees with Lemonica *et al.* (1996).

The red color of lungs would indicate a better blood irrigation, probably by stimulation of the arteriolo-capillary system and that could be the cause of the alveolar dilation observed in lung. In fact, several studies showed that rosemary intervenes in relieving respiratory disorders and in prevention of asthma and cardio-vascular diseases (Nasel *et al.*, 1994; Al-Sereiti *et al.*, 1999; Aghel *et al.*, 2004). It is also a stimulant of suprarenal glands, what explain the hypertrophy of cells of suprarenal glands observed in our study. This histopathological investigations are the originality of the present paper.

We have conclude that *Rosmarinus officinalis* essential oil present no toxicity at 50 µl/g dose and possess stimulating effects of the suprarenal glands and of the respiratory system.

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