Research Article

Acute toxicity and skin tolerance activity of the essential oils from *Rosmarinus officinalis* and *Populus alba*

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Abstract

Objective: Our work aims to study the acute toxicity of the essential oils extracted from two species; *Rosmarinus officinalis* and *Populus alba* and the test of skin tolerance of both essential oils. **Materials and methods:** For this purpose, Wistar rats received different doses of the extract of the essential oil by intra-peritoneal injection. As part of the pre-clinical studies, the test of skin tolerance of both essential oils was conducted by the measuring of primary irritation index. **Results:** The results indicate the absence of severe clinical signs and dead rats during the period of observation. Thus, the essential oil of *Populus alba* and even *Rosmarinus officinalis* administered intraperitoneally has no acute toxicity in rats. In other hand, the essential oil of *R. officinalis* is non-irritating to the skin with an PI equal to 0.45 while the essential oil of *P. alba* is slightly irritating with PI equal to 0.66. **Conclusion:** The evaluation of extract's toxicity and skin irritation potential was essential to ensure the safety of people in contact with substances in pharmaceutical applications.

Keywords: Essential oils, *Rosmarinus officinalis, Populus alba*, Acute toxicity, Irritation

Introduction

For several years, the man who lives side by side with the plants is accustomed to consume for their medicinal and nutritional properties. Natural products are of great interest for the various sectors such as cosmetics, pharmaceuticals, food and industry (Bahorun, 1997). Currently, the World Health Organization (WHO) estimates that about 80% of people use traditional herbal preparations by lack of access to prescription drugs but also because the plants were able to demonstrate effectiveness (W.H.O., 2008). In addition, side effects induced by the drugs concerned users who turn to less aggressive care for the body (Lhuillier, 2007). Indeed, these plants are often characterized by the biosynthesis of odorous molecules which are called "essential oils" (EO) long been known for their therapeutic activities in folk medicine. These bioactive molecules were used given their therapeutic properties in the treatment of several diseases affecting human health (Bensegueni, 2007).

Rosmarinus officinalis L. (Rosemary) belongs to the family *Labiatae* or *Lamiaceae*. It occurs as a shrub, under shrub or herbaceous (Atik bekkara et al., 2007). It is a dense aromatic plant with dark green lavender like leaves, is a native of the Mediterranean region. In traditional medicine, Rosemary is used to treat different diseases including: depression, insomniac and arthritic pains (Zargari, 1995).

Populus alba (White Poplar) is a woody angiosperm higher plant belonging to the family *Salicaceae.* It is a common species of the Mediterranean forests. According to several Mediterranean floras, this species is considered as cultivated or sub-spontaneous around the western Mediterranean Basin (Dickmann and Kuzovkina, 2008). White poplar wood is a very poor fuel, which produces little heat to combustion. From the bark, we extract salicin (Jean-Claude et al., 2008). To avoid any risk of toxicity of these natural products in biological tests, it is necessary to make these toxicity tests.

Our work aims to test the acute toxicity of essential oils of *Rosmarinus officinalis* and *Populus alba*. Then, the test of skin tolerance of both essential oils was conducted by the measuring of primary irritation index.

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Materials and methods

Plant material

It is constituted of aerial parts (leaves and flowers) of the two plant species; *Rosmarinus officinalis* and *Populus alba*. The plants were collected and identified the botanist of the department of Biology during the month of May 2014.

Experimental animals

The Wistar rats used in these experiments were provided by the laboratory of the University of Mascara. Animals were housed at the cage, with water and food *ad libitum*, and the animal room temperature was kept at constant temperature of 20 ± 1 °C on a 12-hour light/12-hour dark cycle. Adequate measures were taken to minimize pain or discomfort of the animals. All experimental procedures were performed in accordance with the ethical guidelines of the Organization for Economic Cooperation and Development (OECD) and after taking the approval of animal ethical committee of the university.

Physicochemical analysis of the plants

Water content: The evaporative drying method was carried out (Audigie et al., 1978).

Ash content: The calcination mineralization method was performed (Pinta et al., 1980; AOAC, 1980).

Extraction of essential oils

The extraction of essential oils from the two plants (*R. officinalis* and *P. alba*) was conducted in the laboratory of Mascara University. The extraction of essential oils was carried out by hydrodistillation in a Clevenger apparatus. 100 g of leaves and flowers of each plant was boiled. When the temperature stabilizes, the distillate was collected in an Erlenmeyer. 18 g of sodium chloride (NaCl) was added to the distillate. After stirring, the mixture was placed in a separating funnel and three successive washes (10, 10, 20 ml) of cyclohexane were achieved. After agitation, the organic phase was recovered. The product was dried with anhydrous sodium sulfate. After the concentration with rotary evaporator, the obtained essential oil was stored at + 4 °C after the calculation of the yield of extraction.

Acute toxicity test

Acute toxicity was estimated by adopting the procedure of Tahraoui et al., (2010). To assess the acute toxic effects of the EO, the lethal dose LD_{50} was calculated using 50 albino rats. The rats were distributed in five groups: each group with 10 rats; 5 males and 5 females. The average weight of female rats used was 150 ± 5 g whereas average weight of male rat was 220 ± 8 g.

The rats were received a single dose (intra-peritoneal injection) of 0.4, 0.6, 0.8 and 1 ml / kg of the EO of *P. alba* and from 1, 3, 5 and 7 ml / kg of the EO of *R. officinalis* while the control group was received the saline (9‰ NaCl). After administration of the essential oil (EO), the rats were monitored for different signs of toxicity and death during the first, 6th and 24 hours. All signs and symptoms were compared with the control group.

Skin tolerance test (PI)

The skin tolerance test aims to predict from the knowledge of the toxicity on a living organism, the toxicological risk in humans. The index of primary skin irritation (PI) of the extracts was determined by official method published in the Official Journal of the French Republic on 21 February 1982. The method was based on the observation of skin reactions caused by the applying of principle extract.

Protocol

The method was performed on 06 Wistar rats of body weight between 280-350 g. Rats were anaesthetized by ketamine (40 mg/kg, i.m). The back and sides were shaved to clear an area of approximately 5 cm x 5 cm; operated with caution so as to avoid irritation. Performed the right of the vertebral axis, using a sterile scalpel blade, three parallel scarifications over a length of about 2.5 cm, spaced approximately 0.5 cm. Apply the product on the skin (scarified areas and not scarified) at 0.5 g. Place the protective compress. Remove the bandage 24 h after the applying.

The reading was based on the recording of erythema and edema according to the numerical scale of Draize (1959). To calculate the index of skin irritation (PI), the figures of erythema and edema was recorded at each reading time (24 and 72 h) on the six areas scarified and six unscarified. Then, the average was calculated to be classified according to this formula:

$$\mathbf{PI = (edema + erythema)_{side\ scarified} + (edema + erythema)_{intactside} / 24}$$

With: 24 = number of rats (6) x number of tested areas (intact and scarified = 2) x number of type of irritation (erythema and edema = 2).

The rating system was used to classify the product in 4 categories (Cohen and Pradeau, 1992):

- Action non-irritating, PI less than 0.5: PI <0.5
- Action slightly irritating, PI between 0.5 to 2: 0.5 < PI <2
- Action moderately irritating, PI between 2 and 5: 2 < PI <5
- Severely irritating Action, PI between 5 and 8: 5 < PI <8

Statistical analysis

The values were expressed as mean ± standard deviation (mean ± SD). The results of the different tests were

analyzed by ANOVA single factor for multiple comparisons. The P values less than 0.05 ($p < 0.05$) were considered statistically significant.

Results and discussions

Physicochemical analysis of the plants

Water content and Ash content

Fresh plants were rich in water and contained from 60 to 80% of water (Paris and Moyse, 1965). The weight method was used for determining the water content in the leaves of our plants. It was the determination of the mass loss by drying in an oven.

The water content of our samples was of the order of 67.6% for rosemary and 73.4% for the white poplar (Figure 1). Albu and colleagues (2004) found that in the fresh leaves of rosemary, the water content was 40%. This content differed remarkably with our results. However, it was noticed that the two plants showed slightly difference in ash content. Variations encountered in water and ash content of our samples compared to some earlier work may be due to some environmental factor like age of the plant, the period of the growing season, or even genetic factors (Laurent, 1991).

Table 1. Extraction yields

Plant material	Mass of plant (g)	Mass of extract (g)	Aspect	Color	Y (%)
R. officinalis	850	11	oily	yellow	1.29 ± 0.03
P. alba	850	7,8	oily	pale yellow	0.9 ± 0.08

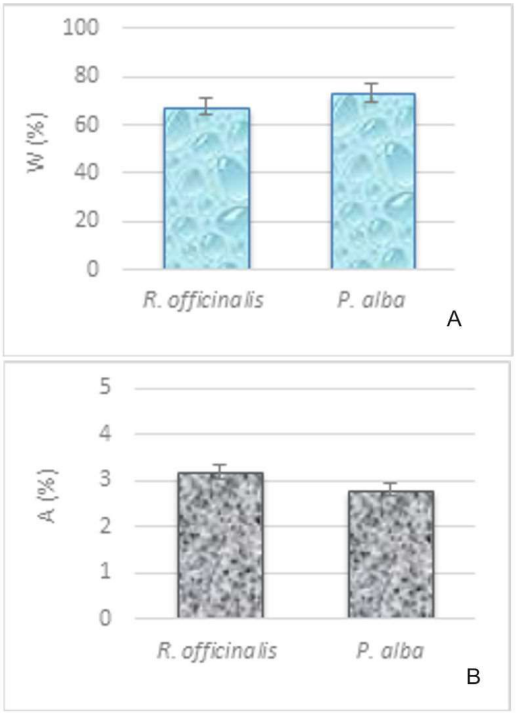

Figure 1. (A)The water content (W %) and (B) the ash content (A %) of the plants

Analysis of essential oils

Extraction yields

The essential oil content obtained from the aerial parts (leaves + flowers) was 1.29 ± 0.03% for the Rosemary and 0.9 ± 0.08% for white poplar. The yields of essential oils were widely variable (Table 01). The yield of essential oil of *Rosmarinus officinalis* was higher than that quoted by Atik Bekkara et al. (2007) and those of Rouabeh (2010) where the quantities obtained by these two works were respectively 0.8% and 0.9%. Indeed, the extraction yield, as the quality of EO, was influenced by the type of soil on which the planting was done, the material of the equipment used, the cleanliness of the equipment, the operating pressure, regularity the heating, the cooling of the distillate, method and distillation time (Brulé and Pecout, 1995).

Acute toxicity

After administration of rosemary oil and white poplar with gradual doses, observations over a period of 14 days showed no severe clinical symptoms of pain, despite some common signs seen as anorexia, hypoactivity, which were reversible and have appeared in rats for a short time and then they returned to their activity.

Measurement of the weight of the rats

The variation of the weight of the rats was a very important parameter. Regular monitoring of rats during the 14 days period has led us to obtain the values for the figure 2. A significant decrease ($p < 0.05$) was observed in rats treated with both essential oils compared to control rats. Then a body weight recovery was recorded after the 4 th day after treatment with essential oils.

The absence of mortality and clinical signs therefore indicated that the essential oil of *R. officinalis* and *P. alba* was devoid of acute toxicity in rats.

Test of skin tolerance (PI)

The evaluation of skin irritation potential was essential to ensure the safety of people in contact with substances in pharmaceutical applications (Goossens and Lepoittevin, 2003). Based on the results, the index of primary skin irritation of *R. officinalis* essential oil was equal to 0.45 while that of the essential oil of *P. alba* was equal to 0.66. According to numerical Draize scale, it was concluded that the essential oil of *R. officinalis* was non-irritating to the skin ($PI < 0.5$), while the essential oil of *P. alba* was slightly irritating ($0.5 < PI < 2$).

From the results obtained, the observed phenomena are only erythema with a varying degree but a decrease was noted after 72 hours. While edema are totally absent in rats

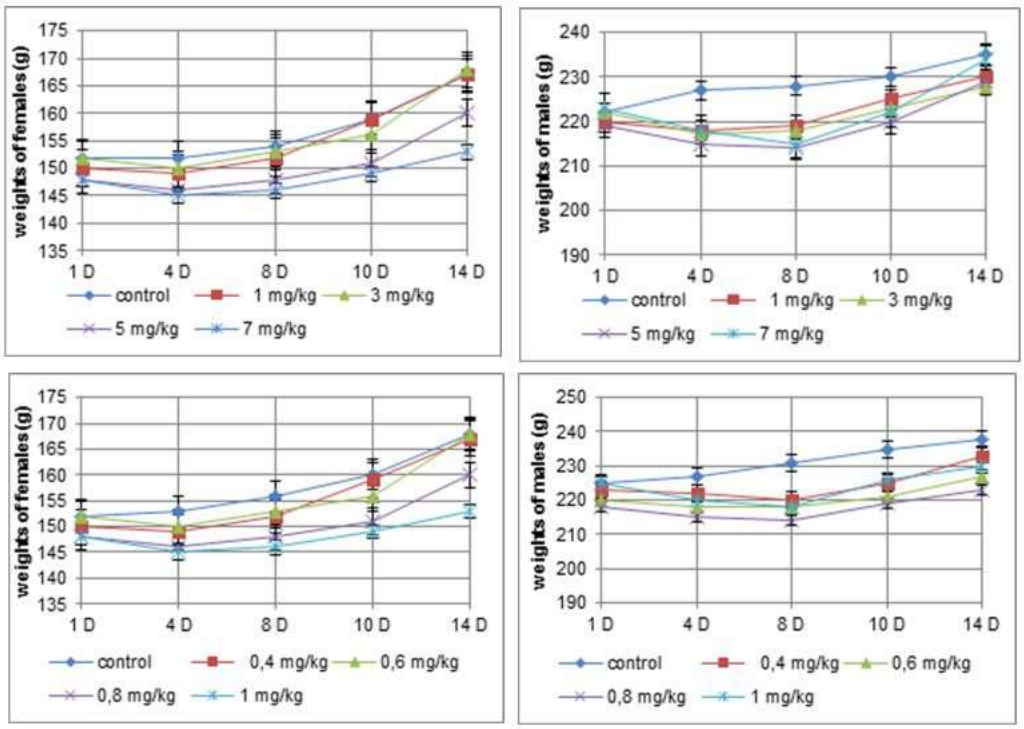

Figure 2. Evolution of the weight of the treated rats during the period of observation

treated with the essential oil of *R. officinalis* but for the essential oil of *P. alba,* a slight appearance of edema during 24 h will be completely disappeared after 72 hours. The appearance of erythema in a few rats can due to penetration of the constituents of the oil in the epidermis (Goossens and Lepoittevin, 2003). Thus, the appearance is quite noticeable on scarified flanks, which may be due to the scarification, which reaches the dermis. Some factors can affect the erythema phenomena such as rats friction between them during the period of accommodation and even hypersensitivity rats with oil constituents (Lis-Balchin, 2005).

Conclusion

All the results obtained in this study allowed us to show that the essential oils of the two plants (*R. officinalis, P. alba*) was devoid of acute toxicity in Wistar rats. The skin tolerance test on rats has allowed us to classify our essential oil of rosemary as non-irritating (IP = 0.45), while that based on white poplar was seen as a product to slightly irritating action to the skin (IP = 0.66).

Conflict of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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