Research Article

Anti-Inflammatory Effects of Essential Oils from *Rosmarinus officinalis* and *Populus alba* on Experimental Models of Acute and Chronic Inflammation in Rats

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ABSTRACT

Objective / Purpose: Essential oils (EOs) distelled form aromatic plants have a wide range of uses because of their rich pharmacological activities including, antiinflammatory, antitumor, antimicrobial, antioxidant, antidiabetic and hepatoprotective. The present research aimed to investigate *in-vivo* anti-inflammatory effects of EOs from *Rosmarinus officinalis* and *Populus alba* as biomarker levels in well-defined acute and chronic inflammation models. **Material and Methods:** The anti-inflammatory activity of EOs of *R. officinalis* and *P. alba* was carried out using two model, one of them is acute inflammation that induction by dextran 1% at the rat paw[1] and another is chronic inflammation that induction by monoiodoacetic solution.[2] To evaluate the anti-inflammatory properties of EOs, a measure of paw thickness was carried out with the calculation of percent inhibition. Thus, monitoring of some biomarkers will be paramount. **Results / Discussion:** The results indicate the absence of severe clinical signs or dead in rats during the observation period. Therefore, the EOs of *R. officinalis* and *P. alba* are devoid of acute toxicity in rats. For treatment with the EOs of *R. officinalis*, *P. alba* and Diclofenac®, the results showed a significant reduction for responses induced by dextran. Treatments produced reductions in inflammation ranging from 2.19 to 15.15%. Treatment with EOs showed a recovery of values of biochemical markers during the experimental period. **Conclusion:** The EOs of *R. officinalis* and *P. alba* demonstrate anti-inflammatory effect on both acute and chronic inflammation models, thus they could be used for pharmacological purposes as anti-inflammatory agents.

Keywords: Anti-inflammatory, Essential oils, Experimental model, *Rosmarinus officinalis*, *Populus alba.*

INTRODUCTION

Currently, the side effects induced by the drugs concerned users who turn to less aggressive care for the body.[1] Indeed, several of the medicinal plants grown worldwide are well known for their essential oil (EO) which are aromatic, antimicrobial and possess curative potential against different disease states including osteoarthritis (OA) and other inflammatory conditions.[2,3,4] *Rosmarinus officinalis* L. (*Lamiaceae*) and *Populus alba* L. (*Salicaceae*) were selected for the present study. Both the plants are rich source of EO, and commonly used in traditional system of medicine against inflammatory diseases and other ailments.[5,6] Nevertheless, phenolic glycosides (salicin and populin) and EO of *P. alba* (white poplar) were reported earlier to possess anti-rheumatic properties.[7] The EO of *R. officinalis* (rosemary) showed profound antiproliferative, antioxidant, antibacterial, and anti-inflammatory activities.[8,9] The stability of essential oil and their use in food safety might lead to some serious questions.[10] Interestingly, the EO of *R. officinalis* demonstrated antimicrobial effect against resistant *Klebsiella pneumonia* strains.[11]

DOI: 10.5530ijper.51.3s.8
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Osteoarthritis (OA) is a musculoskeletal disease affecting bone structure and stability of the articular cartilage.[12,13] It is often classified under joint diseases which results from a complex system of mechanical, biological, biochemical or molecular interactions.[14] The degeneration of joint cartilage originating from the destruction of the extracellular matrix of chondrocytes despite the repair with targeting the recovery of the homeostatic balance between synthesis and degradation of matrix components.[15] In the present study we aimed to investigate the effects of natural bioactive components from rosemary and white poplar on OA induced experimental animals.

MATERIALS AND METHODS

Plant material

The aerial parts (leaves and flowers) of rosemary and white poplar were collected and identified in the Mascara region during the month of May 2013.

The experimental animals

Male Wistar rats of approximately two months of age were housed in standard environmental conditions of temperature (22 ± 3°C), humidity (60 ± 5%) and a 12h light/dark cycle. They were fed with granules food and water *ad-libitum*. This work was carried out with respect for the welfare of animals, as recommended by WHO.[16]

Extraction of essential oil (EO)

The extraction of EOs was carried out by hydrodistillation using Clevenger apparatus. The pure EO was obtained and stored at +4°C after the yield calculating.

Acute inflammation (Dextran model)

For acute inflammation model, 20 albino rats of body weight of 200 ± 10 g were used. The different treatments (essential oil, Ibuprofen) were administered orally for 1h, before the injection of dextran. After 1h treatment, the rats received 0.1 ml of a 1% dextran solution in saline in the sub-plantar region of the right hind paw.[17] The thickness of the paw was measured both before and 1, 2, 4 and 6h after the injection of dextran using a caliper.

Chronic inflammation (MIA model)

25 male Wistar rats weighing 190 - 260 g were used for chronic inflammation model. OA disorder was created by using mono-iodo acetate (MIA). The experimental groups were as follows: Group 1 (untreated normal rats), Group 2 (vehicle), Group 3 (MIA induced+ treatment with EO of Rosemary), Group 4 (MIA induced rats + treated with EO of white poplar), Group 5 (MIA induced rats + treated with Voltum). All experimental procedures were performed in accordance with the ethical guidelines of the Council Directive of the European Communities 86/609 / EEC. For the induction of osteoarthritis, the rats were anesthetized with isoflurane.[18] In all cases, MIA was dissolved in water and administered (50 µl) to each rat. In control group, the left knee was injected with physiological saline water.[19] MIA is known to disrupt glycolysis by inhibiting the activity of dehydrogenase glyceraldehyde 3-phosphate enzyme, resulting in a decrease in metabolic synthesis of cells and optionally lead to necrosis.[20] Treatment given to induced rats consisted of a dose of 50 µl of each essential oil (rosemary and white poplar) and a standard drug Voltum (Diclofenac). The body weight of rats was measured 5 days after the MIA injection. The blood samples were collected in tubes (containing EDTA anticoagulant). The selected parameters were as follows: the C-reactive protein, alkaline phosphatase (ALP) and Calcium.[21]

Statistical analysis

The values are expressed as mean ± SEM. The results of different tests were analyzed by ANOVA. The P values less than 0.05 ($p < 0.05$) are considered as statistically significant.

RESULTS AND DISCUSSION

Acute inflammation (Dextran model)

The essential oils of *R. officinalis* and *P. alba* showed significant anti-inflammatory activity to different degrees in the acute inflammation model of dextran in rats (Figure 1). Significant changes were observed between the rats treated with the extract of *R. officinalis, P. alba* and Ibuprofen compared to control rats. Acute pain can be effectively relieved by nonsteroidal anti-inflammatory drugs, which inhibited cyclooxygenase (COX-1 and COX-2).[22]

Chronic inflammation (MIA model)

Body weight of rats

The results of present study showed a significant decrease in body weight in rats injected with MIA as compared to the control group (Figure 2). This could be explained by the direct effect of unconscionable injury and pathophysiological factors of osteoarthritis process. In several earlier studies, the body weight changes were used as a parameter of toxicity evaluation. Hence, the observed reduction in body weight of animals in the present study may be associated with the decrease in daily food in-take and toxicity of MIA treatment.[22] Initially there was a statistically significant change ($p < 0.05$)

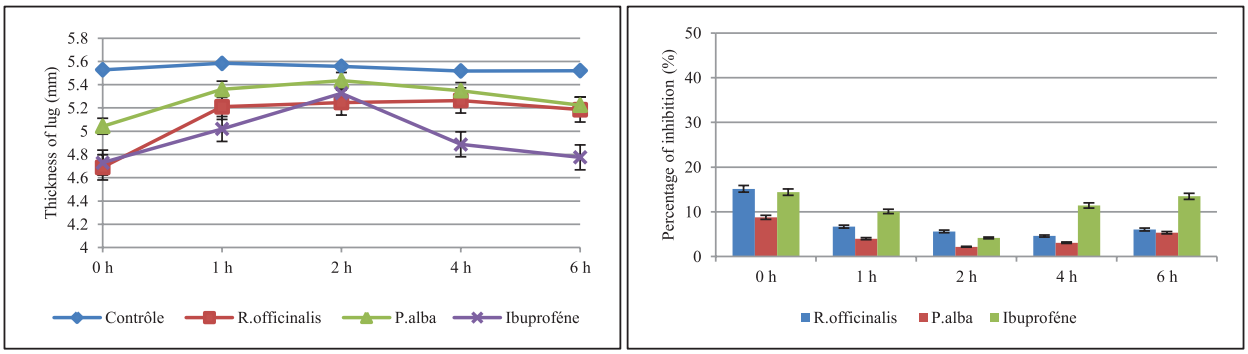

Figure 1: Evolution of paw thickness and percentage of inhibition in control and treated groups.

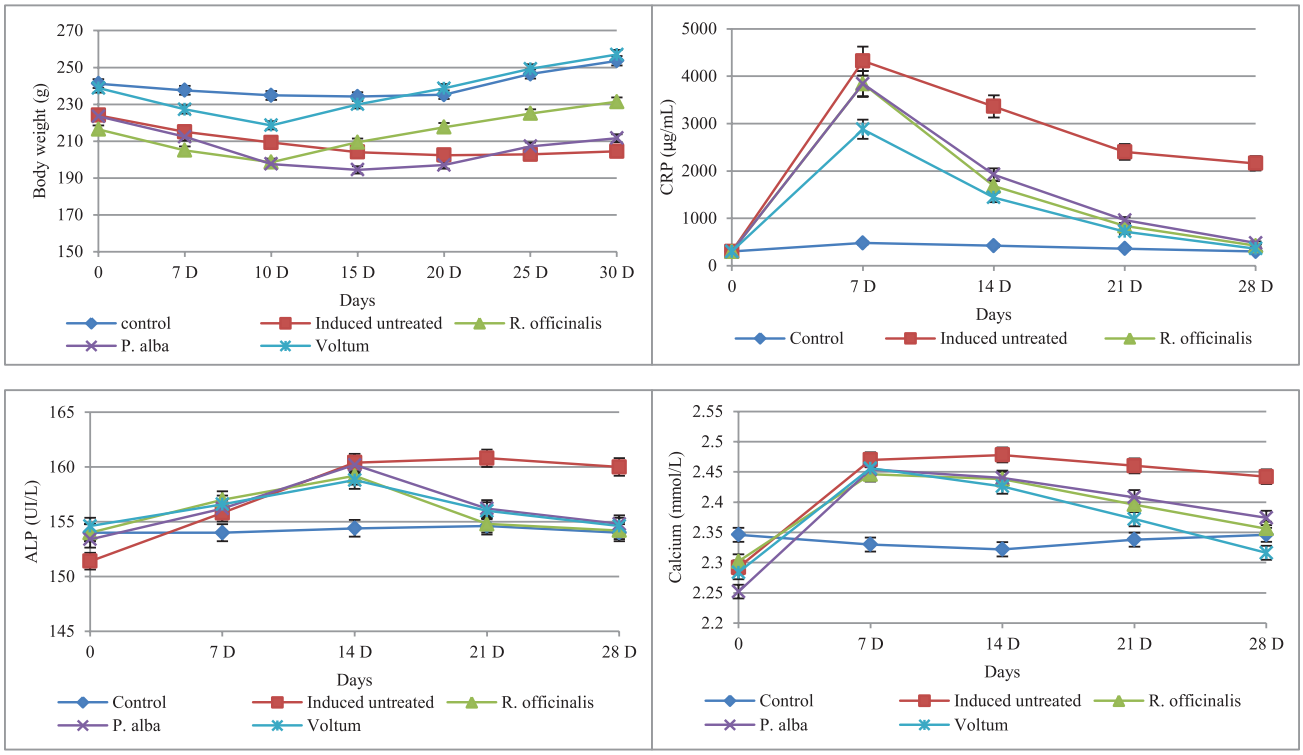

Figure 2: Evolution of body weight and biochemical parameters in control and treated groups.

in body weight of rats of group 03 and 05 as compared to other group of rats. Similarly, weight reduction was recorded in rats of group 04 after 15 days of experimentation.

Biochemical parameters

The C - reactive protein (CRP)

The CRP values in rats of treated groups were remarkably high after 07 days of intra-articular injection of MIA, since they exceed the reference values in the normal rats (< 300 µg/mL).[23] The administration of treatments (EOs of *R. officinalis, P. alba*, Voltum) will decrease gradually to these values that CRP take its normal value to the 28th day (Figure 2). It also notes that the normal control group rats presented a stability of CRP values throughout the experiment. The assay of C-reactive protein (CRP) has shown some potential to predict the evolution of knee osteoarthritis.[24] A serum concentration of CRP does not only reflect the activity of the disease but also the joint destruction.[25]

Alkaline phosphatase (ALP)

The results showed a significant increase of alkaline phosphatase (ALP) in rats receiving the MIA compared

to normal rats (Figure 2). The recovery of ALP values can be explained by the action of the essential oil of *R. officinalis, P. alba* and even Voltum drug with some difference in the degree of influence. Phosphatases are critical enzymes in the biological system, responsible for the metabolism, detoxification, and the biosynthesis of energetic macromolecules for different physiological functions. In addition, alkaline phosphatase (ALP) is used as an indicator of the state of absorption and transport channels in the cell membrane.[26]

Calcium

There was an increase in calcium levels of the group of rats injected with MIA after 7 days of intra-articular administration (Figure 2). The average calcium concentration in rats of group 01 remains as increasing as and greater than the reference value obtained in the literature (2.35 ± 0.05).[27] Our results are in agreement with the earlier reports on hypercalcemia which might occur due to malignancies, bone metastasis, primary hyperthyroidism, vitamin D intoxication, kidney failure, hypoparathyroidism and in hypomagnesaemia.[28]

CONCLUSION

The result showed that the EOs of *R. officinalis, P. alba* can be useful in preventing the destruction of joint architecture in the osteoarthritis pathology. Despite the availability of modern OA treatment methods, natural healing methods are preferred in order to avoid side effects and use less expensive products with high efficiency. Thus, natural products including EOs are a great potential resource as anti-inflammatory agents.

ACKNOWLEDGMENTS

The authors would like to express their sincere gratitude to all staff of the directorate of Education, Mascara and even the Laboratory of Molecular Biology, Gaziantep for their technical support.

CONFLICT OF INTERESTS

The authors have declared that no conflict of interest exists.

ABBREVIATIONS

ALP: alkaline phosphatase; **CRP:** C-reactive protein; **EDTA:** ethylenediaminetetraacetic acid; **EOs:** essential oils; **MIA:** mono-iodoacetate; **OA:** osteoarthritis; **WHO:** world health organization.

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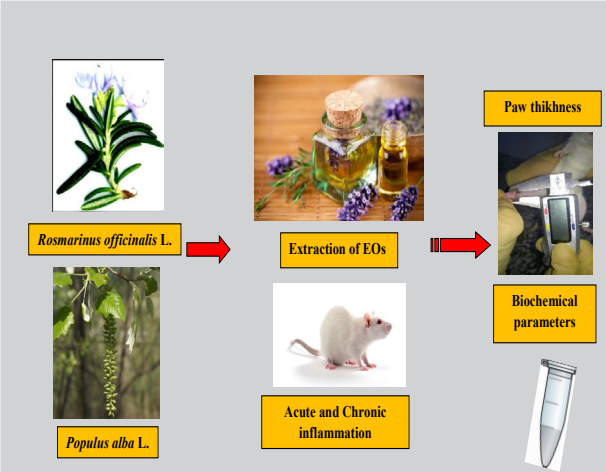
PICTORIAL ABSTRACT



SUMMARY

- Several MAPs grown worldwide are well known for their EOs which are aromatic, antimicrobial and possess curative potential against different disease including osteoarthritis (OA) and other inflammatory conditions.
- *In-vivo* anti-inflammatory effects of EOs from R. officinalis and P. alba and biomarker levels in acute and chronic inflammation models were evaluated in this research.
- As a result, both of the EOs showed a significant reduction for responses induced by dextran.
- Treatment with EOs showed a recovery of values of biochemical markers in osteoarthritis model during the experimental period.
- This research clearly showed these oils have significant potential for developing therapeutic drugs in pharmaceutical industry especially in anti-inflammatory sector.

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Cite this article: Belkhodja H, Meddah B, Gezici S. Anti-Inflammatory Effects of Essential Oils from *Rosmarinus officinalis* and *Populus alba* on Experimental Models of Acute and Chronic Inflammation in Rats. Indian J of Pharmaceutical Education and Research. 2017;51(3)Suppl:S180-84.