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Effect of Essential Oils of *Rosmarinus Officinalis* and *Populus Alba* in Biomarkers Level in Osteoarthritis Experimental Model

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ABSTRACT

Our work aims to study the effect of essential oils of *Rosmarinus officinalis* and *Populus alba* in biomarkers level in osteoarthritis experimental model**.** The induction of osteoarthritis in an experimental model was carried out by unilateral intra-articular injection of a mono-iodo acetate solution. It was followed by the treatment was given by using doses of the essential oils. The evaluation of biomarkers level was obtained. The results showed a significant difference in the concentrations of biochemical markers between the groups of treated and untreated rats. The essential oil of *Rosmarinus officinalis, Populus alba* may be useful against the osteoarthritis pathology.
KEYWORDS: Essential oils; *Rosmarinus officinalis*; *Populus alba*; Osteoarthritis; biomarkers.

1. INTRODUCTION

Natural products are of great interest for the various sectors such as cosmetics, pharmaceuticals, food and industry [1]. Currently, the World Health Organization (WHO) estimates that about 80% of people use traditional herbal because the plants were able to demonstrate effectiveness. In addition, side effects induced by the drugs concerned users who turn to less aggressive care for the body [2, 3]. 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 [4, 5, 6]. *Rosmarinus officinalis* L. (Lamiaceae) and *Populus alba* L. (Salicaceae) were selected for the present study [7].

Both the plants are rich source of essential oil (EO) and commonly used in traditional system of medicine against inflammatory diseases and other ailments [8, 9]. Nevertheless, phenolic glycosides (salicin and populin) and essential oil of *P. alba* (white poplar) were reported earlier to possess anti-rheumatic properties [10]. The essential oil of *R. officinalis* (rosemary) showed profound antiproliferative, antioxidant, antibacterial, and anti-inflammatory activities [11, 12]. The stability of essential oil and their use in food safety might lead to some serious questions [13]. Interestingly, the EO of *Cymbopogon citratus* and *R. officinalis* demonstrated antimicrobial effect against carbapenems resistant *Klebsiella pneumonia* strains [14].

Basically, OA is a musculoskeletal disease affecting bone structure and stability of the articular cartilage [15, 16]. It is often classified under joint diseases which results from a complex system of mechanical, biological, biochemical or molecular interactions [17]. 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 [18].This degeneration is the cause of the onset of fibrillation, cracks and ulceration. Although cartilage degradation is a characteristic of osteoarthritis, the inflammation of the synovial membrane also significantly participates in the pathology installation [18, 19].

Keeping in view the use of essential oil and other natural remedies used in the folk medicine of Algeria and elsewhere, the present study aims to exploit natural bioactive components from well-chosen plants Rosemary and white poplar. In the present study, initially the organoleptic properties of EO of Rosemary and white poplar were evaluated. In the next step, the effect of treatment on OA induced experimental animals was investigated and the results are presented in current communication.

2. MATERIALS AND METHODS

2.1. Plant material:

The aerial parts including leaves and flowers of two selected plant species namely: Rosemary and white poplar were investigated. The plants were collected in the Mascara region during the month of April-May

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2013.They are identified by the botanist of the Department of Biology, Mascara University (ALGERIA) where the voucher specimens were kept on record.

2.2. The experimental animals:

Wistar rats supplied by the biological laboratory of the University of Mascara were used in all experiments. The animals were randomly assigned to different control and treatment groups and housed in cages, with water and food ad libitum. The animals were kept under standard conditions of temperature 20 ± 1 °C, and 12-hour light/12-hour dark cycle. Adequate measures were taken to minimize pain or discomfort of the animals, and all experimental procedures were performed in accordance with the ethical guidelines of the Organization for Economic Cooperation and Development (OECD).

2.3. Extraction of essential oil (EO):

The extraction of essential oils was carried out by hydrodistillation using Clevenger apparatus. Leaves and flowers (100 g each) were boiled separately. When the temperature stabilized, the distillates in each case were collected in Erlenmeyer collector. Then, sodium chloride was added to the distillates which were stirred until dissolved. An appropriate sized separating funnel was used to achieve three successive washings (10, 10 and 20 ml) with cyclohexane. After agitation, the organic phase was recovered and dried with anhydrous sodium sulfate. The solvent was removed by rotary evaporator and pure essential oil was obtained. The yield was calculated in each case and the EO fractions were stored at +4 ^{o} C.

2.4. Organoleptic characters:

According to AFNOR NF ISO 280: 1999, essential oils must respond to analytical characteristics that are established by international committees of experts. To know the quality of the EO of *R. officinalis* and *P. alba*, standard organoleptic tests like color and odor were performed [20].

2.5. Acute toxicity test:

Acute toxicity was estimated by adopting the procedure of Tahraoui et al., (2010) [21]. To assess the acute toxic effects of the EO and the lethal dose LD_{50} was calculated using albino rats (5 rats in each group) of either sex which were kept segregated in different cages. The average weight of female rats used was 150 ± 5 g whereas average weight of male rat was 220 ± 8 g. The rats were distributed in five groups: each group with 10 rats; 5 males and 5 females. The animals in each group received a single dose (i.p. injection) of 0.4 – 1 mg/kg of the EO obtained from each plant while the control received 9% NaCl. After administration of the 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.

2.6. The anti-inflammatory activity of essential oils:

2.6.1. Preparation of rats:

The operations were conducted in accordance with the Welfare of Animal protocol, excluding any stress and nervousness which might interfere with the results. A total of 25 male Wistar rats weighing 190 - 260 g were used in the present study to evaluate the activity of essential oils (EO) extracted from Rosemary and White Poplar.

OA disorder was created by using mono-iodo acetate (MIA). The rats were placed in five cages, with free access to a standard food (corn, soy, salt, amino acid, phosphate, antioxidants, multivitamin and growth factors) and water. The experiments were initiated after acclimatization of animals. 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 [22].

2.6.2. Induction of osteoarthritis:

The choice of the model of OA turned to unilateral intra-articular injection of MIA (0.3 mg) solution prepared in saline. Under anesthesia, the femorotibial joint was immobilized and a needle was inserted inside of the articular capsule through the patellar ligament. 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. The basal readings were established with a group of rats that were injected with saline in their knees [23]. 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 [24].

2.6.3. Treatment:

The development of a treatment must be considered that the signs and symptoms vary according to the affected joint and by stage of disease progression [25]. When the condition is already present, the therapies are directed to symptoms such as pain, instability, joint weakness and decreased function of the joint. The NSAIDs are the most common analgesics to treat pain associated with osteoarthritis [26]. To highlight the effect of EO against this model of osteoarthritis, treatment given to induced rats consists of a dose of 50 µl of each EO (Rosemary and White Poplar). The control group was treated with a standard NSAID drug Voltum (Diclofenac).

The drug dose was given via muscular route with an interval of 2 days to avoid any adverse drug reactions of Voltum.

2.6.4. Body weight of rats:

The body weight of rats was measured using a Sartorius balance (BP 610, precision: 0.01 g). Taking the body weight of the rats was carried out 7 days after the MIA injection. Then, the growth of rats was monitored every fifth day during treatment period with EO and Voltum.

2.6.5. Determination of biomarkers:

The blood samples were collected in tubes (containing EDTA anticoagulant) done on 7th day after treatment. The blood samples were centrifuged at 4000 tour for 10 min. and thus obtained serum were stored at -20 °C until determination of the biochemical parameters which could act as biomarkers for inflammation. The selected parameters were as follows: the C-reactive protein; alkaline phosphatase (ALP), serum calcium, creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) [27].

2.7. Statistical analysis:

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

3. RESULTS AND DISCUSSION

3.1. Extraction yields:

The EO contents, obtained from the aerial parts (leaves + flowers) were found to be: 1.29 ± 0.3% for the Rosemary and 0.9 ± 0.08% for white poplar. It is worth mentioning that EO contents were found to vary depending on different factors described by earlier researchers [28, 29, 30].

3.2. Organoleptic characters:

The organoleptic properties such as appearance, color and smells were used to define the quality of EO by using standard AFNOR method. The EO of rosemary appeared as a yellowish oily liquid with characteristic smell. However, EO of white poplar was oily and pale yellow in color. EO of both plant species were confirmed to be of good quality [31].

3.3. Acute toxicity:

After administration of EO with gradual doses, the rats were followed by observations over a period of experimentation. It showed no severe clinical symptoms of pain, despite some common signs seen as anorexia, hypoactivity, which are reversible and have appeared in rats for a short time and then they returned to their activity. The absence of mortality and clinical signs therefore indicates that the EO of *R. officinalis* and *P. alba* are devoid of acute toxicity in rats in the given dose levels.

3.4. The anti-inflammatory activity of essential oils:

3.4.1. Body weight of rats:

The evolution of weight curve of rats in different groups given same food was given due consideration and all animals were weighed on the same scale. Regular monitoring of body weight of normal rats and the rats treatment groups (treated with the essential oil of *R. officinalis, P. alba* and Voltum) were analyzed and the results are presented in Figure 1.

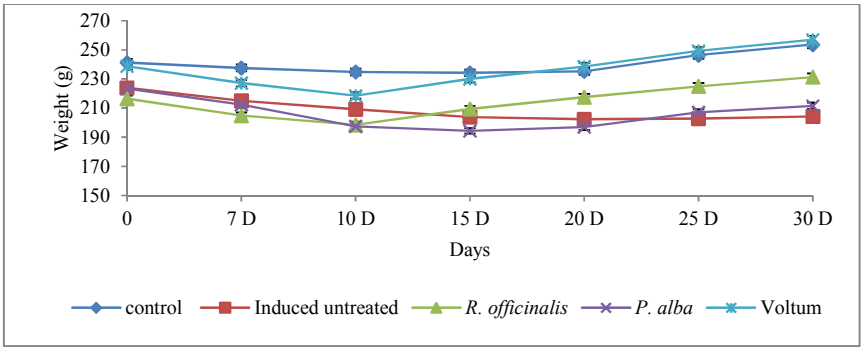

Figure 1: The body weight (g) of normal rats and treated with essential oil of *R. officinalis, P. alba* and Voltum.

The results of present study showed a significant decrease in body weight in rats injected with MIA as compared to the control group. This could be explained by the direct effect of unconscionable injury and pathophysiological factors of osteoarthritis process. Thus, the changes in body weight growth of rats is due to the process of defending the body against the trauma of osteoarthritis that require the intervention of several organic and biological systems and functions such as protein synthesis (eg collagen). There is also the division and

growth of different cell strains participant and / or involved in post-traumatic pathophysiological process [32]. Body weight reduction was used as an indicator of the deterioration of the general health of rats in different groups. 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 effect of MIA treatment [33, 34]. It was noted that the diet consumed by the rats is decreased during the induction period.

Initially, there was a statistically significant change ($P < 0.05$) 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. On the contrary, body weight decreased along with more precisely at the joint in the rats of group 02 (MIA induced).

3.4.2. Biomarkers:

3.4.2.1. The C - reactive protein (CRP):

According to the results, the CRP values in rats of 04 lots (Induced untreated, *R. officinalis, P. alba*, Voltum) 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) [35]. While the administration of various treatments (essential oil of *R. officinalis, P. alba*, Voltum) by intramuscular injection will decrease gradually to these values that CRP take its normal value to the 28 th day (figure 2). There were higher mean CRP concentrations in group 2 after 7 days.

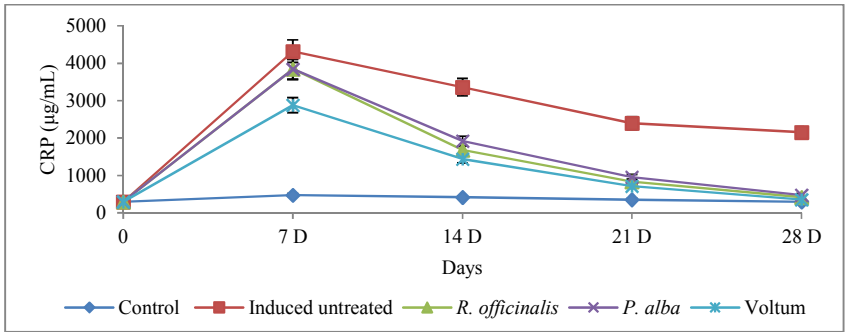

Figure 2: CRP values (µg / mL) of normal rats and treated with the essential oil of *R. officinalis, P. alba* and Voltum.

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 [36]. Though, C-RP concentrations were related to reports of knee joint pain and knee injury [37]. The concentration of CRP does not only reflect the activity of the disease but also the joint destruction [38].

Given the known mechanistic relationship between CRP and IL-6, this suggests that IL-6 produced in the affected joint may in part be responsible for the elevations in systemic CRP seen in this population of patients [39]. Another potential stimulus of CRP production by hepatocytes is IL-1. This cytokine appears to work synergistically with IL-6 to promote CRP production [40].

3.4.2.2. Alkaline phosphatase (ALP):

Our results showed a statistically significant increase of the enzymatic activity of alkaline phosphatase (ALP) in rats receiving the MIA when compared to normal rats. While it recorded a recovery by a statistically significant decrease to take its normal value in treated groups opposed to induced untreated lot where the value of the ALP still increasing (figure 3).

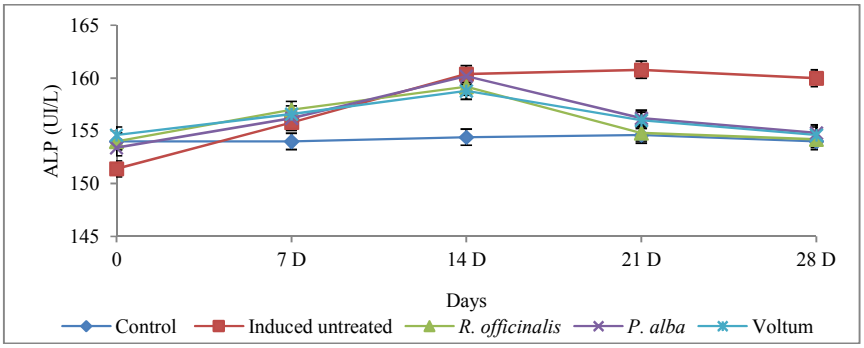

Figure 3: ALP values (UI/L) of normal rats and treated with the essential oil of *R. officinalis, P. alba* and Voltum.

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. Interference with these enzymes leads to biochemical disturbances, tissue damage and loss of cell function. In addition, alkaline phosphatase (ALP) is used as an indicator of the state of absorption and transport channels in the cell membrane [41].

3.4.2.3. Serum calcium:

There was an increase in calcium levels of the group of rats injected with MIA after 7 days of intra-articular administration (Figure 4). 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) [42]. 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 [43].

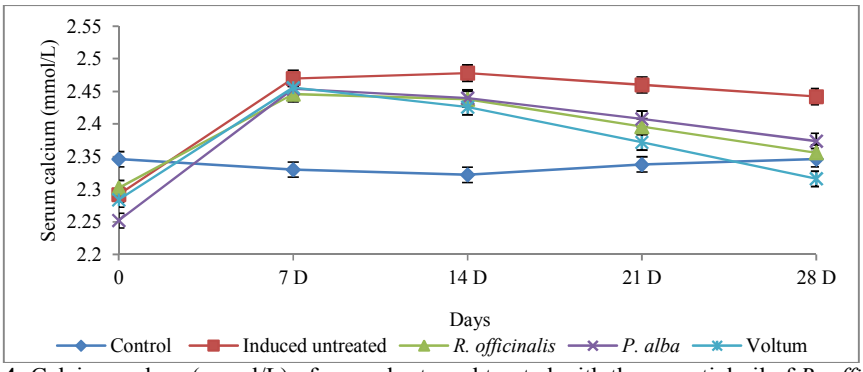

Figure 4: Calcium values (mmol/L) of normal rats and treated with the essential oil of *R. officinalis, P. alba* and Voltum.

While the administration of extracts of the essential oil from *R. officinalis, P. alba* and even drug Voltum promotes a decrease in the mean concentration of calcium. This decrease is observed after a period of 14 days until it reaches values that indicate the homeostatic balance of calcium at the end of the experiment.
The homeostasis of calcium is controlled and coordinated by hormones, growth factors and cytokines [44].

3.4.2.4. Creatinine:

A week after MIA injection in rats, the biochemical assay showed an increase of creatinine values as compared to the control group (Figure 5). In addition, there was an increase in plasma creatinine which indicated decreased ability of kidneys to filter waste from blood and excrete it in urine [45]. However, on the contrary, subsequent measures following the intramuscular injection of the extracts in the group 03, 04 and 05 revealed the recovery of creatinine values.

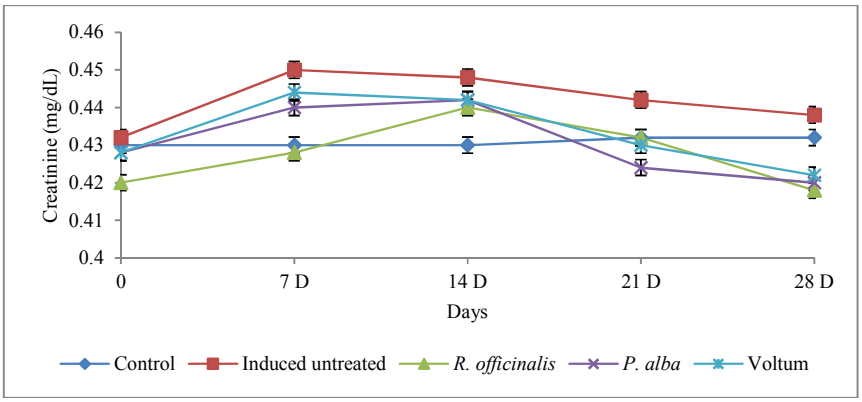

Figure 5: Creatinine values (mg/dL) of normal rats and treated with the essential oil of *R. officinalis, P. alba* and Voltum.

It appears that the MIA injection caused an increase of creatinine in rats which may justify the risk of renal impairment and deterioration of endogenous metabolism of rats. According Phillipe (1983) [46], the plasma

creatinine level provides information on the endogenous metabolism and the rate is proportional to the muscle mass of the body [47].

Transaminases:

MIA injection in rats induced an increase in liver enzymes (ALT, AST) which might be due to stress during the installation of osteoarthritis. On the other side, the treatment of rats of group 03, 04 and 05 showed a reduction in the enzymatic activity of alanine aminotransferase (ALT) observed on the 7th day after treatment. However, the activity of aspartate aminotransferase (AST) was decreased on the 14th day. Being from the group of NSAIDs, Voltum treatment group also showed increase in enzyme activity (Figure 6). The results showed that the treatment with EO of rosemary was appeared closer than the Voltum treatment.

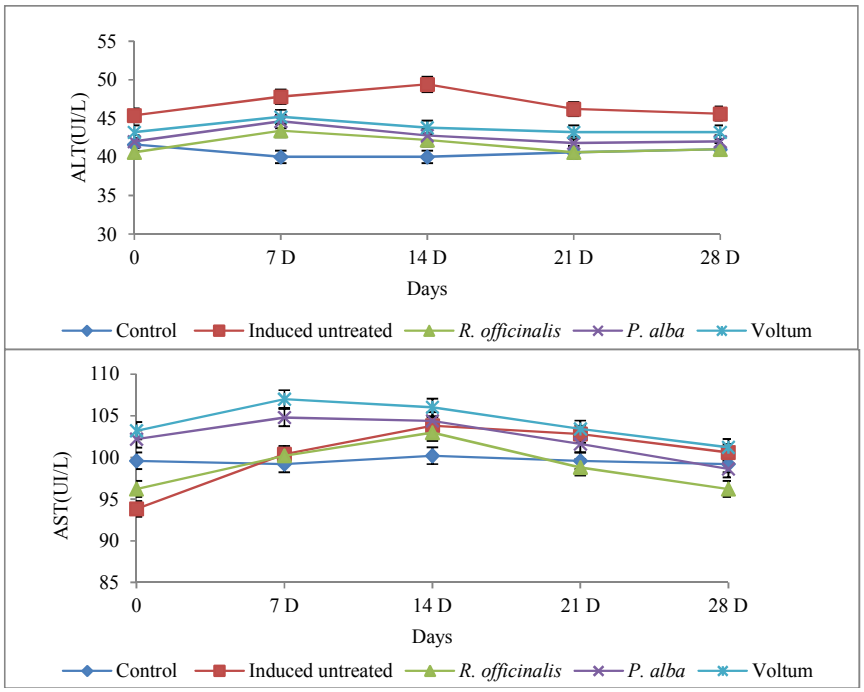

Figure 6: Values of ALT and AST (UI/L) of normal rats and treated with the essential oil of *R. officinalis, P. alba* and Voltum.

Transaminases are enzymes having a high metabolic activity within the cells. They are involved in some energy reactions. The increase in serum transaminases reflects cell injury, especially in the liver and certain cardiac cells [48]. In addition, the level of plasma ALT is also useful in indicating the existence of liver disease, since this enzyme is present in large amounts in the liver. It increases in plasma when degeneration or cell destruction is produced in this organ [49].

Conclusion:

The result from this study showed a significant difference in the concentrations of biochemical markers between the groups of rats treated and untreated. The effect of the essential oil of *R. officinalis* appeared closer than the Voltum drug that shows the best recovery of biochemical parameters. So, we conclude that the essential oil of *R. officinalis, P. alba* may be useful in preventing the destruction of joint architecture in the osteoarthritis pathology. Despite the availability of modern osteoarthritis treatment methods, man still opts for natural healing method to avoid side effects and use less expensive products with high efficiency. Therefore, natural products can give us this opportunity.

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