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Immunomodulatory and Anti-Arthritic Activities of *Stachys circinata.*

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Abstract

The present study reports on the immunomodulatory and anti-arthritic activity of the dichloromethane extract of *Stachys circinata* L'her dried aerial parts (DMESC). Male *Mus Musculus* Albinos were used in all *in vivo* experiment. The toxicity was determined by the acute oral toxicity test administering DMESC orally, while the effect on phagocytosis was monitored by the blood carbon clearance assay. The formalin-induced arthritis (FIA) approach was used to measure the edema size during a 10 d period and quantify the C-reactive protein (CRP) and anti-cyclic citrullinated peptide (ACCP) at the end of the experiment. DMESC, did not produce visible signs of toxicity nor mortality and the LD resulted > than 2000 mg/kg. Phagocytic activity increased at all tested DMESC concentrations (50, 150 and 200 mg/kg) as evidenced by the half-life of colloidal carbon in the blood, the clearance rate was faster at 150 mg DMESC/kg. Also in the FIA test, DMESC supply at 150 mg/kg, revealed a significant decrease of the edema size, anti-CCP values (P=0.000) and CRP (P<0.05). As conclusion, the results clearly evidence that DMESC owns immune-stimulatory and anti-arthritic activity.

Keywords: *Stachys circinata*, Phagocytic activity, anti-inflammatory, CRP, Anti- CCP

1. Introduction

A large number of plants and their isolated constituents have been shown to potentiate health by exerting anti-inflammatory, anti-stress and anti-cancer effects by modulating the immune function (Bin-Hafeez *et al*., 2003). Macrophage is the first cell to recognize infectious agents and is central to cell-mediated and humoral immunities. It is a specialized phagocytic cell that attacks, destroys, and ingests cancer cells, foreign substances, and infectious microbes, by secreting pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukine-1 (IL-1) (Kim *et al*., 2013).

Rheumatoid arthritis (RA) is a frequent chronic inflammatory disease (Boissier *et al.,* 2012). RA is an unremitting multisystem disease accompanied by immune hyperactivity, persistent synovitis, and synovial hyperplasia along with deposition of autoantibodies to immunoglobulins leading to articular cartilage damage and resorption of osseous matter (Hasan and Alamgeer, 2018).

C-reactive protein (CRP) is an acute phase protein synthesized by hepatocytes in response to proinflammatory cytokines, in particular interleukin (IL)-6. It has been shown to be of great value as an inflammatory marker in RA and has been suggested to mediate part of the complement activation in RA (Singh *et al.,* 2013).

The presence of anti-citrullinated protein/peptide antibodies (ACPAs usually measured as anti-CCP) is highly specific for RA. ACPAs recognize citrullinated peptides found in many matrix proteins such as filaggrin, keratin, fibrinogen, and vimentin and found also in alpha-enolase (Svard *et al*., 2013). Citrulline derives from arginine by post-translational modification by peptidyl arginine deiminases (PADs) (Sakkas *et al.,* 2014). Citrullination of synovial antigens, especially fibrin, is an active process during synovial inflammation that probably allows the induction of anti-CCP antibody in RA patients (Del Val Del Amo *et al*., 2006).

The genus Stachys (Lamiaceae) is widely known in folk medicine and is worldwide distributed accounting for 300 species. In Algeria, this genus is represented by 14 species. Nassar *et al.* (2015) has revealed that three plant extracts belonging to Lamiaceae family exert antioxidant and immunostimulant effects. The study of Laggoune *et al*. (2016) has shown that the n-butanolic extract of the aerial parts of *Stachys mialhesi* exhibited significant antioxidant, antinociceptive and anti-inflammatory effects in laboratory animals. *In vivo* studies have revealed that *Stachys pilifera* possess significant anti-inflammatory effect (Sadeghi *et al.,* 2014), and considerable cytotoxic and anti-proliferative properties on HT-29 colorectal cell line (Panahi Kokhdan *et al*., 2018).

The current study was designed to evaluate the immunomodulatory and the anti-arthritic effects of the endemic species *S .circinata.*

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2. Materials And Methods

2.1. Plant Collection and authentication

Aerial parts of *S. circinata* L'Her were collected from Djebel El-Ouahch-Constantine (North Eastern Algeria) in April 2013 during the flowering stage. A voucher specimen (LOST SC04/13) has been deposited in the Laboratory of therapeutic substances, University frères Mentouri-Constantine and authenticated by Prof. G. De Belair (University of Annaba, Algeria).

2.2. Preparation of the dichloromethane extract

Air-dried and powdered aerial parts (1kg) of *S. circinata* were macerated three times at room temperature with MeOH-H2O (7:3, v/v) for 24h. After filtration, the filtrate was concentrated and dissolved in water (600 mL). The resulting solution was extracted successively with petroleum ether, CH_2CL_2, EtOAc and *n*-butanol. Concentration in vacuo at room temperature led to the following extracts: petroleum ether (2.3 g), dichlomethane (9 g) EtOAc (5 g) and *n*-butanol (25 g). The resulting dichlomethane extract of *S. circinata* (DMESC) was then used in all experiments.

2.3. Animals

Adult male *Mus Musculus* Albinos mice (2-2.5 mths old) were obtained from central pharmacy Institute, Constantine, Algeria. The animals used in all experiments had a weight range between 26 and 35 g. All the mice were kept under standard laboratory conditions at 24 ± 1°C relative humidity 55% with a 12 h light/dark cycle. They were fed with a stock rodent diet and tap water. The animal studies were conducted after obtaining clearance from Institutional Animal Ethics Committee and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.4. Acute oral toxicity

The present study was conducted according to the guideline proposed by the Organization for Economic Cooperation and Development (OECD) revised up-and-down procedure for acute toxicity testing. This guideline is based on the procedure of Bruce *et al*. 1985.

A 2000 mg/kg dose was used in five adult male mice, the dose was given to a sole mouse, with the aim to monitor mortality and clinical signs (behaviors recorded: unusual aggressiveness, unusual vocalization, restlessness, sedation and somnolence movements, paralysis, convulsion, fasciculation, prostration and unusual locomotion). Observations lasted 48 h and were performed during the first hour and then each 3 h until the end. Upon survival of this mouse, four additional mice were given the same dose sequentially at 48 h intervals and again, clinical signs were monitored. All of the experimental animals were maintained under close observation for 14 d following DMESC administration, and the number of mice that died within the experimental period was noted. The lethal dose 50 (LD50) was established to be above 2000 mg/kg if no health disorders nor death was registered in three or more mice.

2.5. Phagocytosis (carbon clearance method)

Phagocytic activity of reticuloendothelial system (RES) was assayed by carbon clearance test. Phagocytic index was calculated as a rate of carbon elimination of RES by carbon clearance test determined by a reported method (Halpern *et al*., 1953).

Mice were divided into four groups each of 7 animals: group I (Control) received 0.5 mL of a 0.9% NaCl saline solution via intraperitoneal (i.p.) injection; groups II, III and IV were administred by i.p injection with 50, 150 and 200 mg/Kg of DMESC, respectively.

Forty eight hours after the i.p. injection of the treatment, a colloidal carbon ink suspension was injected via tail vein to all groups at a dose of 0.1 mL/10g. The ink suspension consisted of black carbon ink 3 ml, saline 4ml and 3% gelatin solution 4ml. Then, blood samples (≈14 drops or 25µL) were withdrawn from the retro-orbital plexus via heparin glass capillaries at interval of 5 and 15 min after carbon ink injection. Collected blood samples were lysed in a 0.1% sodium carbonate solution (4 mL) and optical density measured spectrophotometrically at 675nm. At the end of the experiment, liver and spleen were removed from each mice, weighted and values used to calculate the phagocytic index K.

Clearance kinetic was expressed by: 1) the phagocytic index K, which follows an exponential function of concentration to time and measures all the RES activity in contact with the circulating blood, and 2) the corrected phagocytic index α, which expresses this activity by, unit of active weight organs (liver and spleen). Finally, the clearance rate was expressed as the half-life period (t½, min) of the carbon ink in the blood. Parameters have been calculated using the following formulas according to Biozzi *et al*., 1970.

$$K = \frac{\text{LogOD1} - \text{LogOD2}}{t2 - t1} \qquad t½ = \frac{0,693}{K}$$

$$\alpha = \sqrt[3]{K}\,\frac{\textbf{Body weight of animal}}{\textbf{Lever + spleen wt}}$$

OD1 and OD2 are the optical densities (at 675nm) recorded at time t1 (5 min) and t2 (15 min), respectively.

2.6. Formalin-induced arthritis

To perform this test, mice (20-30 g) were divided into four groups (F, FF, SC, D) of five animals each. Group 'F' remained untreated (negative control); group 'FF' (positive control) was subjected to the sole formalin treatment; group 'SC' to formalin + DMESC at 150 mg/kg and group 'D' to formalin + the anti-inflammatory standard drug (diclofenac of sodium) at 10 mg/kg. The administration was done orally by mixing the plant extract or diclofenac of the treated groups into a flour balls. The delivery of DMESC or diclofenac of sodium was carried out while maintaining the standard diet. In this experiment, the concentration with the highest efficacy of DMESC (150 mg/kg) was employed according to the results attained in the phagocytosis experiment.

According to the protocol of Mazumder *et al.* (2012), Formalin treatments were performed by injecting into the sub-plantar of the right hind paw 100 µL of formalin (2%) on the 1st and 3rd day of the experiment. Then, diclofenac and the DMESC were daily administered until the end of the experiment. During the 10 d experiment, a daily

measurement of the edema size was realized with a digital caliper.

2.7. Blood investigation

At the end of the experiments, animals from the entire groups were water-fasted overnight before collecting the blood samples. Blood samples were withdrawn as reported in the phagocytosis paragraph. The separated plasma was assayed for hs-C-reactive protein by an immunoturbidimetric method on a Cobas integra 400 plus analyser (Roche) and anti-CCP was measured by Stratec Biomedical Systems Gemini 6280 Automated Compact Microplate Processor.

2.8. Statistical analysis

The data are reported as mean ± SEM (standard error of the mean). Statistical analyses of the results were performed using one–way ANOVA test and Tukey's multiple comparison tests (SPSS version 20). The values of, ***P<0.001, **P< 0.01and *P< 0.05 were considered to indicate the significant levels.

3. Results

3.1. Acute toxicity study

According to the preliminary toxicity test, the DMESC was found to be safe up to 2000 mg/kg. Indeed, during the 14 day-assessment time, mice were not affected by the amendment of floor balls with 2000 mg/kg of DMESC and they remained healthy and with no visible signs of toxicity nor mortality. This result stand up for an LD50 higher than 2000 mg/kg.

3.2. Phagocytic activity

The results show a significant increase of K index mean values in mice belonging to DMESC supplied groups if compared to the control (NaCl group) with *P*= 0.001 (Fig. 1A). Index values for the DMESC administered groups were: 0.031 ± 0.004; 0.038 ± 0.005 and 0.035 ± 0.007 with 50, 150 and 200 mg/kg, respectively. The NaCl group attained a mean index value of 0.017 ± 0.005. The highest activity was monitored in the group of mice fed with 150 mg DMESC /kg (65.3% increase), but difference among DMESC doses was not significant. This indicates that DMESC enhanced the phagocytic activity by stimulating the RES. and according to the results, it seems that the tested concentrations lower or higher than 150 mg/kg do not improve the phagocytic index value.

DMESC supply to mice influenced significantly the calculated half time (t½) of colloidal carbon clearance which decreased by nearly 50% compared to the control (NaCl). Among DMESC supplied groups the probability was *P*= 0.01 with a t½ of 23.01 ± 3.14 min; 18.65 ± 2.52 min and 20.56 ± 2.83 min with 50, 150 and 200 mg/kg, respectively (Fig. 1B). The NaCl group owned a t½ of 40.62 ± 20.35 min. Compared to control, the clearance rate with150 mg DMESC/kg was lowered more than twice.

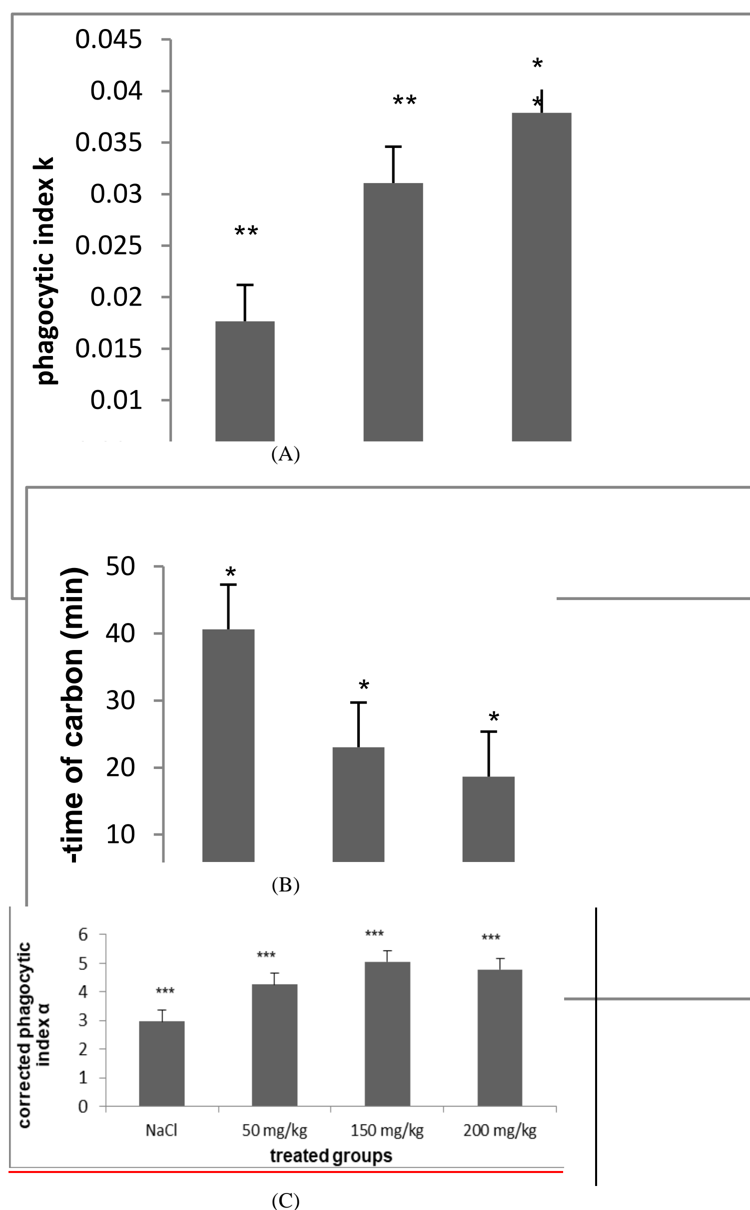

Fig. 1. Effect of dichloromethane extract *S. circinata* of aerial parts on phagocytic activity expressed as: (A) index phagcytic K; (B) half-time t1/2 of carbon in the blood; (C) corrected phagocytic index α. Values are mean ± SEM (n=7) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

GI: Control group received NaCl; **GII:** group received dichloromethane extract of *S. circinata* at dose 50mg/kg; **GIII:** group received dichloromethane extract of *S. circinata* at dose 150mg/kg; **GIV:** group received dichloromethane extract of *S. circinata* at dose 200mg/kg.

In addition to the results reported in Fig.1A, B a significant increase in the corrected phagocytic index α occurred between DMESC groups *P*=0.000 (α= 4.26 ± 0.74; 5.05 ± 0.57; 4.78 ± 0.60 with 50, 150 and 200 mg/kg, respectively) and the control group (α= 2.97 ± 0.50) (Fig. 1C).

3.3. Formalin induced arthritis

The results evidenced a significant inhibition in the edema size in group 'SC' and 'D' (*P*=0.000) while, on the other hand a significant increase of the size occurred in

group 'FF' (*P*=0.000) in comparison to the negative control, group 'F' (*P*=0.000) (Fig. 2).

One day following the first injection of formalin, the edema size enlarged significantly in groups 'FF', 'SC' and 'D' (3.24 ± 0.37; 3.26 ± 0.08 and 3.10 ± 0.24 mm, respectively) in comparison to the negative control group 'F' (1.71 ± 0.016 mm), while, after the second injection of formalin, size increase on the 3rd day was negligible.

On the 4th d, size decreased significantly in group 'SC' and 'D' (2.98 ± 0.13 and 3.04 ± 0.11 mm, respectively) compared to the group 'FF', positive control, where the edema size increased until 5 days after the 2nd formalin injection (3.83 ± 0.28 mm).

From the 5th d until the end of the experiment, the sizes in group 'SC' and 'D' decreased slightly and mean values were almost identical, whereas a significant decrease occurred in the positive control group 'FF' (Fig. 2).By comparing the edema size of each group during the experimental period, the size of the negative control group 'F' remained nearly stable throughout the experiment with an average of 1.7 mm, while compared to the edema size in group 'FF' a decrease of about 77,6 and 79,2% was attained in treated groups 'SC' and 'D', respectively.

It is noteworthy to evidence that the edema development in group 'FF' progressed differently from group 'SC' and 'D' following the 2nd injection of formalin at the 3rd day. Indeed, in group 'FF' size continue to enlarge until day 8 and then decreased till 10th d (3.27 ± 0,44 mm). On the other hand, starting from day 4, edema in group 'SC' and 'D' slowly underwent a similar decease evidencing clearly a comparable anti-inflammatory effect of DMESC and diclofenac of sodium.

The C-reactive protein (CRP) concentration in mice blood following formalin injection decreased by supplying mice with 150 mg/kg DMESC (group 'SC') or with 10 mg/kg diclofenac of sodium (group 'D') and values were about 1.03 ± 0.74 and 0.68 ± 0.26 mg/L, respectively. However, the mean value of CRP (1.62 ± 0.82 mg/L) in group FF was increased but not significantly when it's compared to group 'SC' and 'D' (Fig. 3A).

The ACCP values were also influenced by treatments and had a similar trend to those of CRP but differences resulted a significantly lower (*P* = 0.000) in blood of mice supplied with DMESC or diclofenac of sodium. The concentration of ACCP is decreased in the groups 'SC' and 'D' (2.14 ± 0.38 and 1.57 ± 0.56 UI/mL, respectively) when it's compared to the group 'FF' (positive control) which had the highest ACCP values (3.13 ± 0.96 UI/mL) (*P* = 0.000), while the group 'F' (negative control) had the lowest (0.62 ± 0.32 UI/mL) values as shown in Fig. 3B.

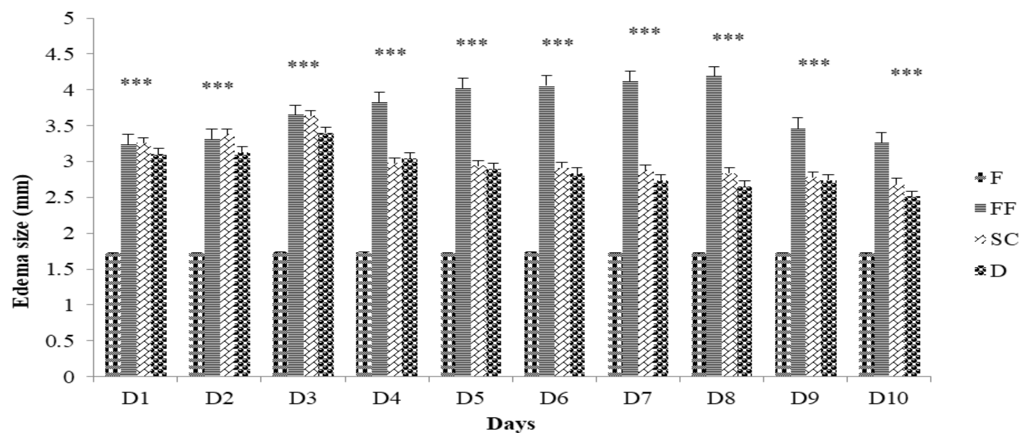

Figure 2: Anti-inflammatory effect dichloromethane extract of *S. circinata* aerial parts on the formalin induced mice hind paw edema during a 10 day experimental period. Values are mean ± SEM (n=8) and significant difference from the control group is shown as *p<0.05, **p<0.01, ****p*<0.001. Treatments: F= untreated (negative control); FF= (positive control) sole formalin treatment; SC= formalin + DMESC (150 mg/kg); D= formalin + diclofenac of sodium (10 mg/kg).

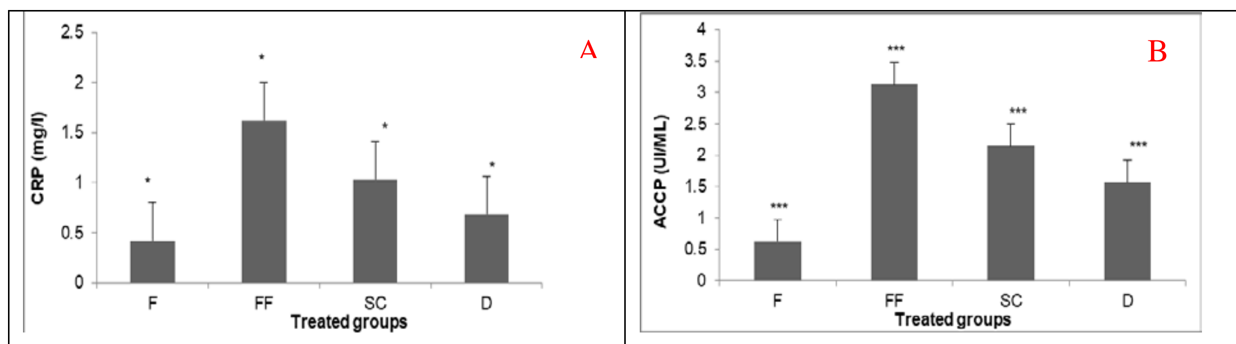

Figure 3: Effect of dichloromethane extract of *Stachys circinata* on serum parameters in Formalin-induced arthritis in mice. (A) Blood levels of C-Reactive Protein (CRP) in mice following formalin-induced arthritis as influenced by the dichloromethane extract of *S. circinata* aerial parts (DMESC) and diclofenac of sodium; (B) Anti-cyclic citrullinate peptide (ACCP) levels in mice blood following formalin-induced arthritis as influenced by the dichloromethane extract of *S. circinata* aerial parts (DMESC) and diclofenac of sodium. Values are mean ± SEM (n=7) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001. Group F: untreated (negative control); Group FF: (positive control) formalin inflammation; Group SC: formalin inflammation+ DMESC (150 mg/kg); Group D: formalin inflammation+ diclofenac of sodium (10 mg/kg).

4. Discussion

The use of herbal medicines as alternative or adjuvant treatment has been increasing worldwide and gaining popularity in developing countries where ethnobotanical practices are still very popular. Although medicinal plants may have biological activities that are beneficial to humans, the potential toxicity of these bioactive substances has not been well established. Thus, the safety and efficacy of these plants must be studied thoroughly to maximize their benefits for mankind (Elsnoussi *et al*., 2011).

In our study, we have demonstrated that the dichloromethane extract of *S. circinata* is not toxic by oral administration in mice up to 2000 mg/kg, similar toxicological concentrations were achieved for plant extracts of *Argania spinosa, Citrullus colocynthis* and *Boswellia serrata* by Aribi, 2015.

The evaluation of the immunomodulatory effect of DMESC took into account that the immune cells and mediators are directly involved in the processing of antigens, removal of microorganisms by, phagocytosis, lysis of bacteria, viruses or tumor cells. Many malignant diseases are caused by, a decreased number or function of immune competent cells. Hence, modification of immune response either through suppression or stimulation may be helpful in avoiding diseases related to the immune system (Sharma *et al*., 2012).

Currently several of the available therapeutic drugs have potential side effects. Thus, medicinal plants and their active components as a source of immunomodulatory agents are gaining importance (Sharma *et al*., 2012).

The results of the present study showed that DMESC may stimulate cell mediated immunity as shown by an increase in macrophage induced phagocytosis in carbon clearance test. When ink containing colloidal carbon is injected into the systemic circulation, the macrophages engulf the carbon particles of the ink and the rate of clearance of ink from blood is known as phagocytic index (George *et al*., 2014). DMESC stimulated the RES by, a high significant increase in the phagocytic index. Our results are in agreement with those of Benmebarek *et al*. (2014) who indicated that *Stachys ocymastrum* extract appears immune stimulatory at low concentrations and immunosuppressive at high concentrations as it exhibited a biphasic effect on the phagocytic activity of the RES and with those of Nassar *et al.* (2015) who reported that the *n* butnolic extract of *Stachys circinata* increased the phagocytic index at 150 mg/kg.

The improvement in phagocytic function by DMESC may be due to a number of actions of different effector components of the phagocytes. However, the most important mechanism is the up-regulation of receptors that are required to interact with the pathogens which include mannose and toll like receptors. It may also be due to the increased opsonization of carbon particles by complement proteins and immunoglobulins. So, the immunostimulate activity of the DMESC which acted by activating the function of the RES is due to the fact that it contains natural physiologically active substances such as terpenoids, phenolic compounds and flavonoids (Laggoune et al., 2016), which increase the humoral response, by stimulating the macrophages and B-lymphocytes subsets involved in antibody synthesis (Dash *et al*., 2006).

In our work we are searching for a new therapeutic drugs for arthritis because synthetic drugs, in recent years, are accompanied by numerous unwanted side effects, such as the Non Steroidal Anti-Inflammatory Drugs (NSAIDs) that produce gastric ulcer, and as major side effects glucocorticoids are associated with adrenal suppression (Boddawar *et al*., 2016). In addition, their use cannot halt the development of rheumatoid arthritis and Disease Modifying Antireheumatic Drugs (DMARDs) have been impeded by their potential of long-term side effects, toxicity and immunosuppression (Tag *et al.,* 2014). So, it is very important to search for new therapeutic drugs from a natural source with greater efficiency and lower toxicity.

Findings of the present study have revealed that DMESC treatment exerts anti-arthritic effect. It decreased the inflammation compared to the control group as observed by the decrease in the edema size, the concentration of ACCP and CRP values. These results agree with those of Mazumder *et al.* (2012) who reported that in the formaldehyde induced arthritis inflammation test (FIA), the methanol extract of *Barleria lupulina* owned a significant inhibition of the edema formation during the experimental period of 10 days. Our results are in agreement with those of Kehili *et al.* (2016) who reported that ACCP and CRP are decreased in mice injected with formalin and treated with Algerian *Phoenix dactylifera* fruit. Benmebarek *et al*. (2013) reported a decrease of hs-CRP when mice was treated with extracts of *S. mialhesi* following an inflammation induction by hyperhomocysteinemia.

FAI is one of most commonly used acute models for assessing anti-arthritic potential of plant extract (Kore *et al*., 2011). Injection of formalin into hind paw produces localized pain and inflammation, which is biphasic response, an early neurogenic component followed by a later tissue-mediated response (Shastry *et al*., 2011).

The initial phase of the edema is due to the release of histamine and serotonin and the edema is maintained during the plateau phase by kinin like substance and the second accelerating phase of swelling due to the release of prostaglandin like substances (Manguesh *et al*., 2010); this phase seems to be an inflammatory response with inflammatory pain that can be inhibited by anti-inflammatory drugs (Vasudevan *et al.,* 2006).

The anti-inflammatory activities of many plants have been attributed to their saponin, terpenoids, flavonoids and steroids contents (Shastry *et al*., 2011). Flavonoids were considered to be the active components responsible for the biological actions of the genus *Stachys.* In addition, this genus has been shown to possess various biological properties related to antioxidant, anti-nociceptive and anti-inflammatory mechanisms by targeting reactive oxygen species and prostaglandins which are involved in the late phase of acute inflammation and pain perception (Kehili *et al*., 2016; Laggoune *et al*., 2016). These results are compatible with those of Peng *et al*. (2016) who reported that the flavonoids and alkaloids in the ethanol extract of the roots of *Caragana pruinosa* might be responsible for its anti-arthritic activity. In our research, we evidence that DMESC had accelerated the anti-inflammatory activity in a similar pathway as diclofenac and this is remarkable by lower volume of edema, CRP and Anti-CCP values.

The initiation of RA involves the activation of auto-reactive T cells and the recruitment of these T cells along

with other leukocytes into the joints. These leukocytes produce a variety of mediators of inflammation. Prominent among these mediators are arachidonic acid metabolites, pro-inflammatory cytokines, free radicals and matrix-degrading enzymes. These mediators modulate the processes relating to cell migration into the joints as well as angiogenesis and degradation of the extracellular matrix within the joints, leading to the arthritic inflammation (Venkatesha *et al*., 2011).

Naderi *et al*.(2016) reported that phenolic compounds in the Ginger decrease the pro-inflammatory factors of TNF-α and IL-1β. Both mediators induce NF-kB, which is a ubiquitous eukaryotic transcription factor with a pivotal role in inflammatory pathways. In addition, these crucial compounds suppress the synthesis of prostaglandin and leukotriene by inhibiting the COX-2 and lipoxygenase pathways and also inflammation-involved pathways diminishing the inflammation. Therefore the active compounds obtained by Lggoune *et al*.(2016), could play same role for inhibition of TNF-α and IL-1β.

Serum CRP, a surrogate marker of disease severity that correlates with final outcome of arthritis is also a potent endogenous ligand for TLR-2 present on the surfaces of synovial fibroblasts, PMNs and macrophages and its transcription is regulated by pro-inflammatory cytokines including IL-6 (Adhikary *et al*., 2016).Serum concentration of CRP, the most common type of acute phase proteins was tested in our experiments and was found to be significantly attenuated in case of FAI in mice treated with DMESC. This explains a protective role of DMESC against liver damage and inflammatory reactions in hepatic tissues during pathogenesis of FIA.

Immune complexes (IC) deposited into the synovial joints elevate pro-inflammatory cytokines in serum, through induction of mononuclear cells, these immune complexes can stimulate PMNs and macrophages to secrete pro-inflammatory cytokines, like TNF-α. In both ways, there is activation of synovial macrophages which ultimately results in increased production of TNF-α, IL-1β and other pro- inflammatory cytokines like IL-6, IL-12 and IL-15 which are involved in RA pathogenesis (Adhikary *et al*., 2016). However, IC containing citrullinated fibrinogen have been detected in the peripheral blood of anti-citrullinated protein/peptide antibody (ACPA) -positive RA patients and also in synovial pannus (Fisher, 2014). These IC stimulate macrophage TNFα production and the accumulation of multiple ACPA specificities is correlated with preclinical inflammation (elevation of TNF-α, IL-6, and IFN-γ) preceding clinical arthritis (Sakkas *et al*., 2014).

A considerable improvement in the management of RA has been obtained since the advent of biological agents such as (TNF)-α inhibitors (adalimumab, certolizumab, etanercept, golimumab or infliximab) anti-B cell agent (rituximab), anti-IL-6 receptor inhibitor (tocilizumab), T cell modulator (abatacept) (Atzeni *et al*., 2013). A problem facing the practicing physician is to prescribe the most appropriate biological agent to individual patient, in other words, to match a biological agent with a patient profile, given the high cost of biological (Boissier *et al*., 2012). The presence of ACPAs was associated with reduced response to anti-TNFα agents (Potter *et al*., 2009).

From the DMESC, a variety of secondary metabolites were isolated and fifteen known compounds have been identified, among them flavonoids such as luteolin, apigenin, isorhamnetin, triterpenoids such as betulinic acid, ursolic acid and olealonic acid, sterols such as stigmasterol and β-sitosterol (Lggoune *et al*., 2016). However the effect shown by DMESC that ameliorates FIA induced inflammation and progressive bone damage is an outcome of a cumulative effect of all these bioactive compounds; therefore, further studies are warranted on these chemical constituents from DMESC after purifying them and administering them separately in animal models with respect to formalin-induced arthritis.

5. Conclusion

These findings provide a basis for the therapeutic potential of *S. circinata* for the control and management of diseases in which the immune system needs to be stimulated and for inflammatory conditions like rheumatoid arthritis.

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Conflicts Of Interest

All authors report no conflicts of interest regarding this manuscript.

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