Research Article

BIPHASIC DOSE RESPONSE EFFECT OF *STACHYS OCYMASTRUM* ON THE RETICULOENDOTHELIAL SYSTEM PHAGOCYTIC ACTIVITY

BENMEBAREK A[1]*, ZERIZER S[1], LAKHAL H[2], KABOUCHE Z[2]

[1] Department of Animal Biology, University of Constantine 1, Algeria, Option: Immuno-Oncology, [1] Department of Animal Biology, University of Constantine 1, Algeria, [2] Laboratoire d'obtention de substances thérapeutiques (Lost), University of Constantine 1, Laboratoire d'Obtention de Substances Thérapeutiques (L.O.S.T), Campus Chaabat Ersas, 25000 Constantine, Algeria.
Email: assia.benmebarek@gmail.com

ABSTRACT

Objective: The immunopharmacologic activities of herbal extracts are complex and are still not completely understood. The effects of different compounds of herbal extracts may be antagonistic, in some cases they are immunosupressive, in others immunostimulating.

Methods: The *in vivo* immunomodulatory potential of *S.ocymastrum* on macrophage phagocytosis was evaluated using Carbon Clearance Assay.

Results: *Stachys ocymastrum* extract significantly potentiated the phagocytic activity at 50mg/kg, by stimulating the RES, and thus the clearance rate of carbon was faster after the administration of the plant extract. At 100 and 500mg/kg, the extract decreased the phagocytic activity, and slowed the clearance rate of carbon in a dose dependant manner, by inhibiting the RES. Also, the weights of the liver and spleen expressed in percent body weight were not affected by *S.ocymastrum* extract.

Conclusion: Our results indicate that *S.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. In addition, the increase in the RES phagocytic activity was due to increased RES tissue activity rather than tissue hypertrophy.

Keywords: Biphasic dose response, Phagocytic activity, Carbon Clearance Assay, Reticuloendothelial system, *Stachys ocymastrum*.

INTRODUCTION

The immune system protects against destructive forces either from outside the body (bacteria, viruses, and parasites) or from within (malignant and autoreactive cells). It comprises two functional divisions that work together in a coordinated manner [1]. The innate immune system consists of cellular components, soluble factors, physical barriers and the reticuloendothelial system (RES) [1]. It provides early host defense against infections before the development of an adaptive Immune response [2]. The adaptive immune system produces a specific reaction and immunologic memmory to each pathogen and comprises cellular components and soluble factors [1].

The RES consists of the phagocytic cells such as monocytes and macrophages [3] that kill the invading organism by phagocytosis. Phagocytosis is a multi-step process that begins by engulfing the organism and ends with modification and chemical breakdown of its structural components. Associated with phagocytosis is the oxidative burst during which superoxide anions are produced as toxic oxygen metabolites. A number of chemical reactions may occur in phagocytes including biomolecular breakdown by digestive enzymes and chemical modifications by reactive oxygen species generated in oxidative burst process [2].

Immunomodulation is the regulation and modulation of immunity either by enhancing or by reducing the immune response [4]. An immunomodulator can be defined as a substance, which can influence any constituent or function of the immune system in a specific or nonspecific manner including both innate or adaptive arms of the immune response [5]. It can cause immunostimulation by stimulating effector cells or production of their metabolic inducers or by inhibiting the immunity limiting factors. Immunosuppression can be achieved by stimulating the inhibitor cells and humoral factors, or inhibition of effector cells [6]. A large number of plants and their isolated constituents have been shown to have potential immunity. Some medicinal plants have been shown to exert immunomodulatory and anti-cancer activity [7, 8, 9]. *Stachys* genus (Lamiaceae) has shown various activities such as anti-inflammatory [10], antimicrobial [11], and antioxidant [12] activities. [13] reported that the administration by i.p injection of the ethanolic extract *S. mialhesi* in mice at different concentration increased the phagocytic activity. Because of the various biological interests in the secondary metabolites (flavonoids, diterpenes, phenylethanoid glycosides) of *Stachys* genus [14], our main objective was to investigate the effect of *Stachys ocymastrum* on the the phagocytic activity of the reticuloendothelial system in mice.

MATERIALS AND METHOD

Plant material and extraction

Aerial parts of *Stachys ocymastrum* were collected from Djebel El-Ouahch-Constantine (North Eastern Algeria) in June 2005 during the flowering stage. A voucher specimen has been deposited in the Herbarium of the Department of Chemistry, University Mentouri-Constantine, and authenticated by Prof. G. De Belair (University of Annaba, Algeria). Air-dried and powdered aerial parts (890 g) of *Stachys ocymastrum* were macerated in a methanolic solution (70%) at room temperature. The extract was concentrated under low pressure, diluted, and filtered, then successively extracted with petroleum ether, dichloromethane, ethyl acetate, and *n*-butanol.

Animals

Adult male *Mus Musculus* mice (2.5- 3 month old) from central pharmacy Algeria, weighing (16-23g), were used for determination of the phagocytic activity. The animals were kept under standard laboratory conditions of humidity, temperature (25± 1°C) and up to 12h of light daily. The mice were allowed free access to food and 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 Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Macrophage Phagocytosis by Carbon Clearance Assay

The clearance rate of carbon was measured by the method of [15]. Mice were divided into four groups, consisting of 7 mice in Group III, 6 mice in Groups I and IV, and 5 mice in Group II. Group I (Control) was given 0.9% NaCl (0.5 ml/mouse, i.p), Groups II, III and IV were administered by i.p injection with different concentrations of S. ocymastrum extract (50, 100 and 500 mg/kg) which was dissolved

in 0.9% NaCl. After 48h of i.p injection, the mice were administered with carbon ink suspension at a dose of 0.1ml/10g through the tail vein; the mixture consisted of black carbon ink 3 ml, saline 4ml and 3% gelatin solution 4 ml.

Blood samples were taken by retro orbital bleeding using glass capillaries, at an interval of 5 min (t_1) and 15 min (t_2). Blood sample drops (14) were mixed with 0.1% sodium carbonate solution (4ml) for the lysis of erythrocytes. Absorbance of these samples were measured at 675 nm using a spectrophotometer. Then the liver and spleen of individual mice were culled and weighed immediately. The phagocytic activity is expressed by the phagocytic index K which measures all the RES function in the contact with the circulating blood, and by the corrected phagocytic index α which expresses this activity by unit of weight of active organs: liver and spleen.

The clearance rate is expressed as the half-life period of the carbon in the blood ($t_{1/2}$, min). These are calculated by means of the following equations:

$$K = \frac{\ln OD_1 - \ln OD_2}{t_2 - t_1}$$

$$\propto = \sqrt[3]{K} \left(\frac{Body\ wt}{Liver\ wt + Spleen\ wt} \right)$$

$$t_{1/2} = \frac{0.693}{K}$$

Where OD_1 and OD_2 are the optical densities at times t_1 and t_2 respectively.

Statistical analysis

Data were analyzed for differences between the groups across dietary treatments by one –way ANOVA test and Tukey's multiple comparison tests (SPSS version 22). P values less than 0.05 were considered statistically significant.

RESULTS

The present data shows that there is a difference in the means for the phagocytic index (K) between groups (GI, GII, GIII and GIV) P=0,053. Figure 1 demonstrates that the phagocytic index in GII (0.062 ±0.03) increased significantly when compared with the control group GI (0.027 ±0.014) P= 0,032. Then, the phagocytic index decreased in GIII (0.036 ±0.023) and decreased significantly in GIV (0.026 ±0.018), when compared with GII (0.062 ±0.03) P= 0,118, and P= 0,039 respectively.

The results indicate that *S.ocymastrum* extract enhanced the phagocytic activity at 50mg/kg by stimulating the RES, and then at 100 and 500mg/kg, the extract decreased the phagocytic activity, in a dose dependant manner, by inhibiting the RES.
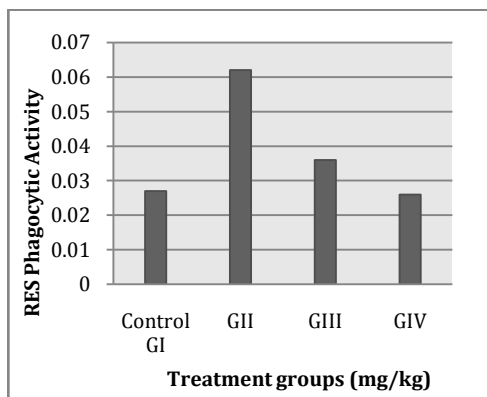

Fig. 1: The phagocytic activity of mice treated with *S. ocymastrum* extract

The following data shows that there is a difference in the means but not significantly for the Carbon clearance rate between groups (GI, GII, GIII and GIV) P=0,184. Figure 2 demonstrates

that the clearance rate of carbon was significantly faster at 50mg/kg, in GII (14.41 ±9.38) when compared with the control group GI (31.66 ±14.17) P= 0,046. Then, the clearance of carbon was slow in GIII (28.73 ±18.86) and significantly slow in GIV (35.02 ±16.73), when compared with GII (14.40 ±9.38) P= 0,152, and P= 0,037 respectively.

This indicates that *S.ocymastrum* extract showed carbon clearance enhancing activity at 50mg/kg, which affirms that it enhanced the phagocytic activity. Then, *S.ocymastrum* extract reduced the phagocytic activity at 100 and 500mg/kg.
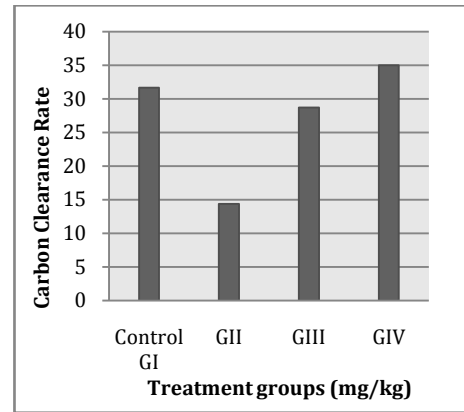

Fig. 2: The Carbon Clearance Rate of mice treated with *S.ocymastrum* extract

The following data shows that there is a difference in the means for the corrected phagocytic index α between groups (GI, GII, GIII and GIV) P=0,051. Figure 3 demonstrated that the corrected phagocytic index α increased in group GII (7.18 ± 1.81), but not significantly when it is compared with the control group GI (5.49 ±1.77) P=0,130. However, the corrected phagocytic index α decreased significantly in GIII (5.38 ±1.38) and the decrease was highly significant in GIV (4.67 ±0.83), when it is compared with GII (7.44 ± 2.11) P= 0,068, and P= 0,016 respectively.
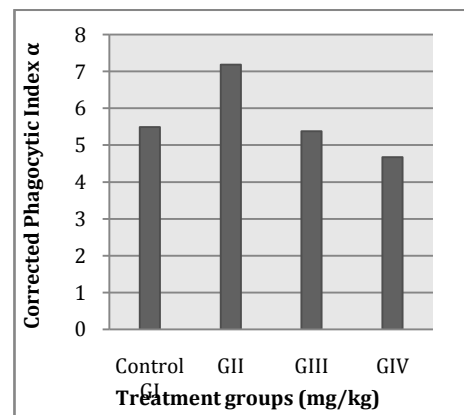

Fig.3: The corrected phagocytic index α of mice treated with *S.ocymastrum* extract

Figure 4 shows that the weights of the liver and spleen of mice expressed in percent body weight did not produce any significant difference when compared with the control GI, P>0.05. This indicates that the weights of the two phagocytic organs (Liver and Spleen) were not affected by *S.ocymastrum* extract.

Figure 5, 6 and 7 display an inverted U- and U-shaped curves. These curves have typically been viewed as models of a biphasic dose response (Hormesis) [16]. As seen in Figure 6, the U-shaped response reflects a decrease in the half-life period of the carbon in the blood at low dose and its increase at higher dose. In Figure 5 and 7, the inverted U-shaped response represents the enhancement of phagocytic activity and the corrected phagocytic index α at low dose but their reduction at higher dose.
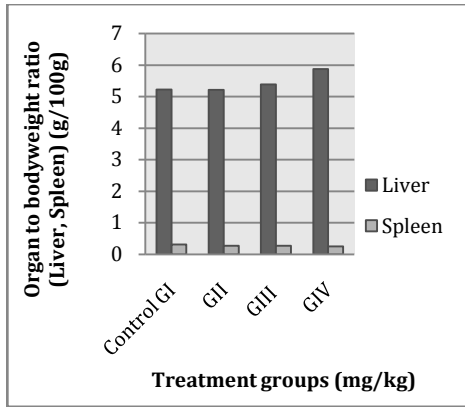
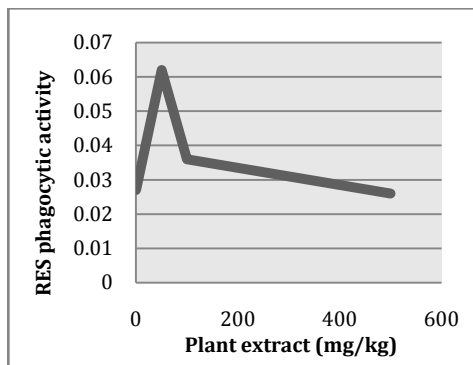**Fig.4: Organ weight to body weight ratio (Liver, Spleen) of mice treated with *S.ocymastrum* extract**

Fig.5: Biphasic dose-response effect of *S.ocymastrum* on the phagocytic activity of the reticuloendothelial system
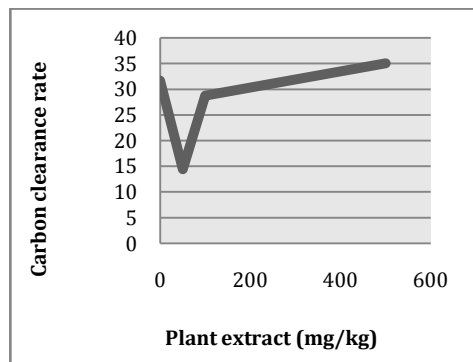

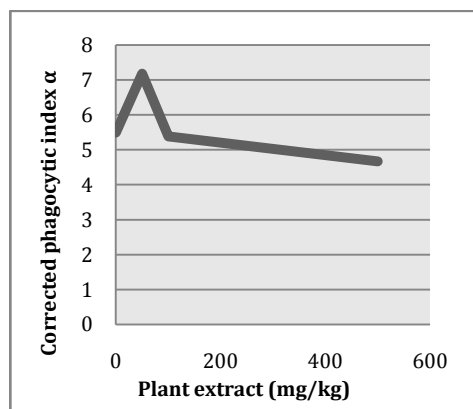**Fig.6: Biphasic dose-response effect of *S.ocymastrum* on the carbon clearance rate**

Fig.7: Biphasic dose-response effect of *S.ocymastrum* on the on the corrected phagocytic index α

DISCUSSION

The immunopharmacologic activities of herbal extracts are complex and are still not completely understood. Findings made *in vitro* not always agree with *in vivo* observations. Moreover, the effects of different compounds of herbal extracts may be antagonistic, in some cases they are immunosupressive, in others immunostimulating [17].

In this study, our results demonstrated that animals administered with *S.ocymastrum* extract had a stimulated phagocytic activity at 50mg/kg. Then, this activity decreased in a dose dependant manner at 100 and 500mg/kg. These results could be explained by the hormesis concept. The hormetic dose response may be reliably described as being a low-dose stimulatory and a high-dose inhibitory response [18].

The magnitude of the stimulatory response at maximum is typically modest, being only about 30–60% above that of the control response [18] which corresponds to our results expressed in (Figure 5, 6 and 7). The strong majority of stimulatory responses are less than twice the control value. This is the most distinguishing characteristic of the hormetic dose response, being its most consistent and reliable feature [18].

Treatment by *S.ocymastrum* extract enhanced also the rate of carbon clearance from the blood at 50mg/kg and decreased it in a dose dependant manner at 100 and 500mg/kg. These results could be explained by the enhancement of the phagocytic activity of phagocytes and non specific immunity, which includes opsonisation of the foreign particulate matter with antibodies and complement C3b, leading to a more rapid clearance of foreign particulate matters from the blood [19].

Then, our results showed that the clearance of carbon from the blood was slower at 100 and 500mg/kg. These findings could be explained by the presence of two receptor subtypes affecting cell regulation, one with high and the other with low affinity for the agonist but with notably more capacity (i.e. more receptors) [20]. This may lead to the biphasic dose response, with the high-affinity receptor activated at low concentrations, which stimulates DNA synthesis and cellular proliferation; and the low affinity/high-capacity receptor becoming dominant at higher concentrations decreasing the cell proliferative response [21]. This pharmacological mechanism may explain the biphasic dose response effect of *S.ocymastrum* extract.

Phagocytosis is activated by attachment to Pathogen-associated molecular patterns (PAMPS), which leads to NF-κB activation [22]. Recent findings have elucidated the cellular signaling pathways and molecular mechanisms that mediate hormetic responses which typically involve transcription factors such as Nrf-2 and NF-κB. As a result, cells increase their production of cytoprotective and restorative proteins. including growth factors, phase 2 and antioxidant enzymes, and protein chaperones [23]. In the fields of biology and medicine, hormesis is defined as an adaptive response of cells and organisms to a moderate stress. Examples include exposures to low doses of certain phytochemicals [23]. In fact, micromolar concentrations of vitamin E and numerous polyphenols can protect a variety of cells against oxidative stress in cell culture models of cancer, atherosclerosis and neurodegenerative disorders [24, 25, 26]. However, clinical trials and primary prevention studies of high doses of such antioxidants in humans have been disappointing at best [27].

In addition, the metabolic activation of phagocytes during phagocytosis causes the activation of NADPH oxidase: a complex enzymatic system that catalyses NADPH oxidation to produce a superoxide radical and other reactive products of oxygen [28]. The anti-inflammatory action of many, but not all flavonoids, appears to be largely based on their antioxidant effect. They can scavenge active oxygen species including superoxide radicals, hydrogen peroxide and hydroxyl radicals [29]. However, oxygen radical formation by peripheral blood monocytes is suppressed by the flavonoid catechin [30]. Flavonoids like fisetin and quercetin, have been shown to inhibit oxidative modification of LDL by macrophages [31]. The

activation of NF-κB is critical for the production of pro-inflammatory cytokines. Flavonoids and related compounds are reported to repress NF-κB dependant gene expression [32]. These findings can also explain the mecanism by which *S.ocymastrum* extract acted in a biphasic manner, attributed to its phenolic and flavonoid components [33].

On analysis of the weights of the liver and spleen, the corrected phagocytic index α of GII treated with 50mg/kg of plant extract was also found to be higher than that of the control GI, and the corrected phagocytic index α of GIII and GIV treated with 100mg/kg and 500mg/kg of plant extract was lower than that of the GII, while the weights of the liver and spleen expressed in percent body weight were not affected by *S.ocymastrum* extract (Figure 4). The increased phagocytic indices (both K and α values) suggested that most of the increase in RES phagocytic activity was due to increased RES tissue activity rather than tissue hypertrophy. These results agree with those of [34] who reported that the chronic exposure of rats to a simulated high altitude stimulated RES phagocytic activity and that the stimulation is due to increased RES tissue activity per unit mass of tissue rather than tissue hypertrophy.

CONCLUSION

Considering the results of the present study, we can conclude that *S.ocymastrum* induced a biphasic dose response, and that the hormetic dose response is an important feature by which *S.ocymastrum* may act. It can also be confirmed that the property of the herbal extract *S.ocymastrum* in exhibiting a stimulatory effect on the RES depends on the dose of the extract. *S.ocymastrum* induced a biphasic dose response, since it appears immunostimulatory at low concentrations and immunosuppressive at high concentrations. In addition, the increase in RES phagocytic activity was due to increased RES tissue activity rather than tissue hypertrophy.

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