

Research Article



Anti-inflammatory and Immunomodulatory Activities of Argan oil (*Argania Spinosa. L*) and Sodium Selenite after Exposure to Mercuric Chloride in Rats

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ABSTRACT

Immunomodulatory activity of Argan oil and sodium selenite was evaluated on phagocytic activity by carbon clearance test and serum pro-inflammatory cytokine after exposure to mercuric chloride. Adult *Albinos Wistar* rats were exposed to mercuric chloride (0.25 mg/kg b.w intraperitoneal injection), or argan oil (5ml/kg b.w by gavage), or sodium selenite (0.25 mg/kg b.w by gavage), or a combination of both. Change in phagocytic activity was determined after 48h injection of carbon ink suspension, serum IL1, IL6 and TNF α levels was determined after 3weeks. Exposure of rats to mercuric chloride caused a significant decrease in phagocytic activity as compared to control. The levels of IL1, IL6 and TNF α increased by mercuric chloride exposure rat. Supplementation of argan oil or sodium selenite or combination of argan oil and sodium selenite with mercuric chloride exhibited significantly phagocytic index and restored the level of IL1, IL6 and TNF α near normal compared to control. Indicating stimulation of the reticulo-endothelial system. Present study thus reveals that argan oil and sodium selenite holds promise as immunomodulatory agent, which act by stimulating phagocytic function.

Keywords: Argan oil, Immunomodulatory, Phagocytic activity, pro-inflammatory cytokine, Rat, Sodium selenite.

INTRODUCTION

Mercury is able to suppress cell mediated immunity¹, it impairs mitogen responsiveness of T lymphocytes², NK cell cytotoxicity³, and Th1 cytokine production⁴, it tilts the Th1/Th2 balance through high IL6 pro-inflammatory cytokine production, inducing chronic inflammation and depressing cell mediated immunity.^{5,6}

Recent biochemical studies have shown that fatty acids could modify immune responses. Indeed, lymphocyte proliferation, lymphocyte-derived cytokine production, or cell-mediated immunity can be influenced by dietary lipids. The effect of dietary argan oil on the immune system has been evaluated on rats. Those studies have shown that argan oil effect on immune cells is similar to that of olive oil, widely consumed oil, and that argan oil has no marked effects on immune cell function.⁷

Pharmacological studies have confirmed that *Argania spinosa* have several biological effects including: antiproliferative⁸⁻¹⁰, Hypolipidemic, hypocholesterolemic, antiatherogenic, antiradical and anti-inflammatory activities.^{11,12}

Selenium behaves as a modulator of the production of inflammatory mediators. When deficiencies, production of prostacyclin vasodilator and antiaggregatory 35diminuée is in favor of thromboxane A2 proagregant and vasoconstrictor. Selenium supplementation reversed this trend.¹³ When vitamin E deficiency and selenium supplementation these two elements (one and / or the other) causes a potentiation of the antibody response. Furthermore, selenium is involved in immunity by

stimulating the migration of phagocytic cells, phagocytosis and lymphocyte reactivity.¹⁴

The present investigation was undertaken to evaluate the immunostimulatory effect of argan oil and sodium selenite against mercuric chloride using phagocytic activity by carbon clearance test and pro-inflammatory cytokine *in vivo* experimental model rat.

MATERIALS AND METHODS

The Argan oil used in this work originated from Tindouf (south-west of Algeria), It was extracted by a traditional method. Animals *Albinos Wistar* rats were housed under hygienic conditions in the departmental animal house. Animals were housed under standard conditions of temperature (21 \pm 1°C), and up to 12h of light daily, fed with standard pellet diet, and had free access to water. All the experiments were performed in accordance with the institutional animal ethics committee.

Phagocytic activity

Phagocytic activity index was determined as per the method reported by Cheng *et al.*, 2005.¹⁵ Phagocytic activity of reticulo-endothelial system was assayed by carbon clearance test. Phagocytic index was calculated as a rate of carbon elimination of reticulo-endothelial system by clearance test. They were divided into eight experimental groups; each consists of eight rats. The first group was served as the control, whereas the remaining groups were respectively treated with argan oil (5ml/kg body weight), sodium selenite (0.25mg/kg body weight), argan oil and sodium selenite, mercuric chloride (0.25mg/kg body weight), argan oil and mercuric chloride, sodium selenite and mercuric chloride, and mercuric chloride + argan oil + sodium selenite.



Carbon ink suspension was injected via the tail vein to each rat 48 hours, at a dose of 0.1 ml/10g. After 48h of i.p injection, the rat were administered with carbon, the mixture consisted of black carbon ink 3ml, saline 4ml and a 3% gelatine solution 4ml. Blood samples were taken from the retro orbital vein by using glass capillaries, at 5 and 15 min. Blood sample drops were mixed with 0.1% sodium carbonate solution (4ml) for the lysis of erythrocytes and the absorbance measured at 675 nm using a spectrophotometer.

The phagocytic activity is expressed by the phagocytic index K which measures all the reticulo endothelial system function in the contact with the circulating blood. 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 equations¹⁶:

$$K = \frac{\ln OD_1 - \ln OD_2}{t_2 - t_1}, \quad t_{1/2} = \frac{0.963}{k}$$

Where OD1 and OD2 are the optical densities at times t1 and t2 respectively.

Pro-inflammatory cytokine

The rats were divided into eight experimental groups; each consists of eight rats. The first group was served as the control, whereas the remaining groups were respectively treated with argan oil (5ml/kg body weight), sodium selenite (0.25mg/kg body weight), argan oil and sodium selenite, mercuric chloride (0.25mg/kg body weight), argan oil and mercuric chloride, sodium selenite and mercuric chloride, and mercuric chloride + argan oil + sodium selenite. The treatment of all groups was lasted for 3 consecutive weeks.

Twenty four hours after the last administration, blood was collected by retro- orbital sinus puncture from each anesthetized rat. After centrifugation at 3000 rpm for 10min, the serum was separated immediately and stored at -20°C until determination of: IL1, IL6 and TNF α .

Statistical analysis

The data were subjected to student *t* test for comparison between groups. The values are expressed as mean \pm SEM. Significance level was set at $P < 0.05$, $P < 0.01$, $P < 0.001$.

RESULTS

Effects of treatments on phagocytic activity

Treatment with HgCl₂ caused a significant decrease in the phagocytic activity as compared to control. Only argan oil and sodium selenite treatment no significant increase in the phagocytic activity as compared to control. However, the combined treatment of argan oil or sodium selenite with mercuric chloride results in gradual recovery in phagocytic activity as compared to control (Figure 1).

Effects of treatments on half-time $t_{1/2}$ of carbon in blood

Mercuric chloride exposure a significant increase in half-time of carbon in blood as compared to control. Only argan oil and sodium selenite treatment no significant decrease in half time of carbon in blood. However, the combined treatment of argan oil or sodium selenite with mercuric chloride results in gradual recovery in phagocytic activity as compared to control (Figure 2).

Effects of treatments on pro-inflammatory cytokine

A very highly significant increase in IL1 level in mercuric chloride treatment group. Argan oil or sodium selenite or combined treatment of argan oil and sodium selenite alone treatment did not show any significant decline. A significant decline in argan oil with mercuric chloride in IL1 level. But no significant decreased in combined treatment of sodium selenite or argan oil and sodium selenite with mercuric chloride was noticed with compared to control.

A significant increase in IL6 level in mercuric chloride treatment group. Argan oil or sodium selenite or combined treatment of argan oil and sodium selenite alone treatment did not show any significant decline. no significant decline in combined treatment of argan oil or sodium selenite or argan oil and sodium selenite with mercuric chloride in IL1 level was noticed with compared to control.

A highly significant increase in TNF α level in mercuric chloride treatment group. Argan oil or sodium selenite or combined treatment of argan oil and sodium selenite alone treatment did not show any significant decline. a significant increase in combined treatment of argan oil or sodium selenite or argan oil and sodium selenite with mercuric chloride in TNF α level was noticed with compared to control (figure 3).

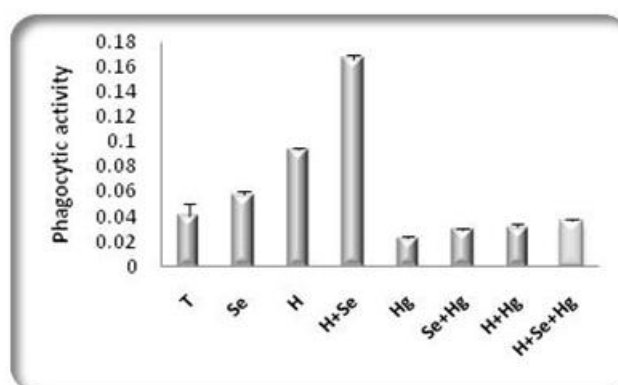


Figure 1: Effect of Argan oil on phagocytic activity

DISCUSSION

The reticulo-endothelial system (R.E.S) consist of the spleen, thymus and other lymphoid tissues, together with cells lining the sinuses of the spleen, bone marrow, and lymph nodes and capillary endothelium of the liver (kuppfers cells), and of the adrenal and pituitary glands,

these comprise the sessile or fixed macrophage, are transported by the body fluids or wander through the tissues. The RES is the best defined functionally by its ability to scavenge debris or other foreign matter and forms first line of defense.

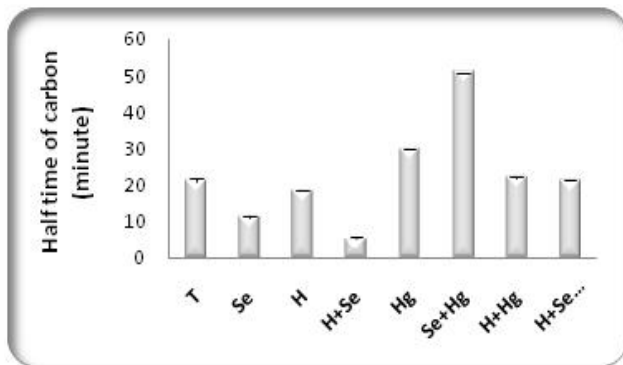


Figure 2: Effect of Argan oil on half time $t_{1/2}$ of carbon in blood

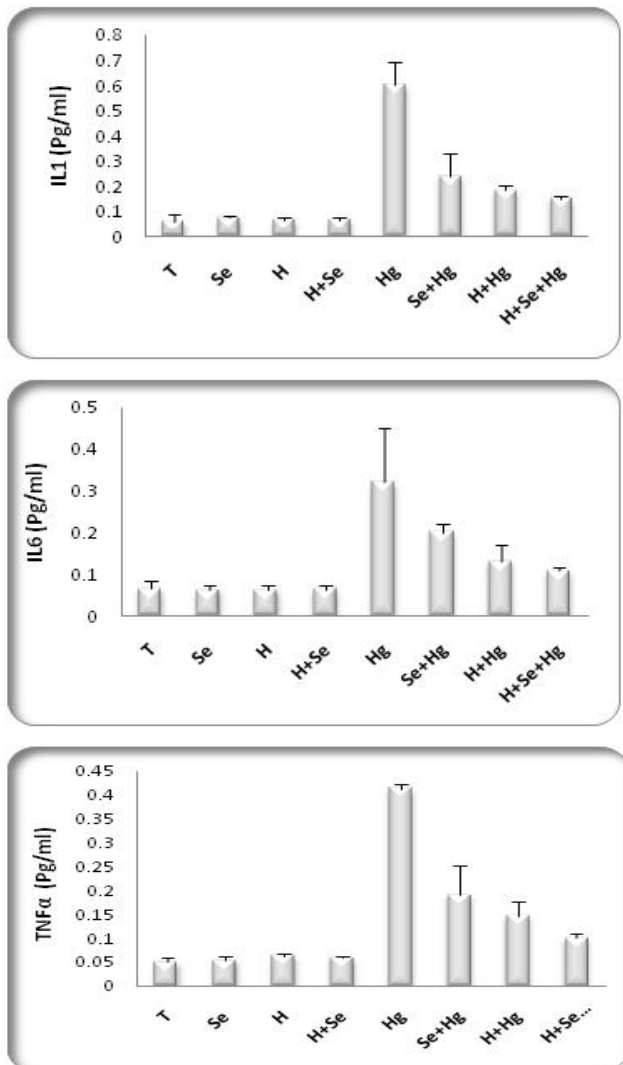


Figure 3: IL1(Pg/ml), IL6(Pg/ml) and TNFα (Pg/ml) levels in serum of control and rats treated with Se, H, H/Se, Hg, Se/Hg, H/Hg and combined treatment of H+Se/Hg after 21 days of treatment. Values are given as mean \pm SEM for group of 8 animals each significant difference: * compared to controls (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$).

The rate of removal of carbon particles, by the sessile intravascular phagocytes in the liver and spleen, from the blood stream is a measure of reticulo-endothelial phagocytic activity.

Our results show that the rats via IP injection at a dose of mercury from 0.25 mg / kg, causes a diminution in phagocytic activity after 48 hours of injection and the increase in the profile of pro-inflammatory cytokines after 21 days of treatment.

The decrease in phagocytic activity could be explained by the effect of mercury immunosuppressor. These results are in agreement with the work of Necib et al (2013h)¹⁷, who found that the mercury IP injection at a dose 0.25 mg / kg in mice causes significant Rating Decrease in phagocytic activity after 48 hours of injection. Increased cytokine profile proinflammatory after 21 days of treatment with mercury, can be explained by the immunomodulating effect of mercury, mercury inhibit cellular mediators of the immune system by the cell-specific mitogenic effect on T cells, NK (the cytotoxic cells) and stimulated Th1 (the cells responsible for the secretion of proinflammatory cytokines), all these disruption leads to the occurrence of an imbalance in the balance Th1 / Th2 to production of pro-inflammatory cytokines causing chronic inflammation and thus the decrease in the production of cellular immune mediator system¹⁸. The mercury also a toxic effect on the efficiency of thymic from previous work that is performed on the atrophy of thymic cortex in mice after exposure to mercury. The molecular mechanisms by which the mercury affect both the thymus and peripheral immune efficiency are not fully understood. In addition, mercury inhibits protein synthesis stronger than puromycin and cycloheximide. Mercury can also inhibit the proteins belonging to the family which causes INF in the synthesis of cellular mediators. The immunomodulatory effect of mercury immunosuppression results in an inhibition of lymphocyte proliferation and increased production of proinflammatory cytokines. Moreover, for some authors, mercury can induce an increase in free radicals in the cells causing intracellular oxidative stress¹⁸. In vitro study of santarelli et al (2006)¹⁹ in the thymus cell culture exposed mercury from mercury demonstrate immunomodulatory by inhibiting cellular proliferation and activity of mediators and NK cell as CD4 + and CD 8 + and increases on the one hand the profile of pro-inflammatory cytokines on the other of Another study by Kim et al (2002)¹⁸ show that in vitro exposure to a mercury dose 20 .mu.m in macrophage cell culture inhibit nitric oxide that is an antioxidant against the mercury and causes in the activation of proinflammatory cytokines -inflammatoire or concentration becomes higher appearing to witness. Another in vivo study santarelli et al (2006)¹⁹ demonstrate that administration to mice of a mercury dose of 1 mg / kg for 1 month in the causes inhibition of the activity by thymulin, decreased NO production, alteration cellular mediators of NK however, increases the profile of pro-inflammatory cytokines.

Selenium supplementation to rats treated with mercury causes an increase of the phagocytic activity and reduction in the profile of pro-inflammatory cytokines. This result can be explained by the ability of selenium to regulate the expression of receptors of various cytokines, the interaction between interleukins and their receptors on lymphocytes is essential to enable them clonal expansion and differentiation, the results of the activity phagocytic in this study are in agreement with the study of Beck et al (2005)²⁰ showed that phagocytosis of *E. coli* in calves Butcher increased following supplementation of selenium in cows.

Recent studies revealed that argan oil has an immune effect. It stimulates lymphocyte proliferation, and the phagocytic activity decreases the production of pro-inflammatory cytokines. The decrease in production of pro-inflammatory cytokines can be explained by the action of the compounds of argan oil on the immune system, recent studies have shown that saponins of *Argania Spinosa* have an anti-inflammatory to dose 500 mg / kg orally in vivo, these can be explained by the action of saponins on the profiles of pro-inflammatory cytokines. Our results are in agreement with the work Necib et al (2013f)²¹ which have demonstrated that olive oil reduces the profile of pro-inflammatory cytokines in rats treated with 15 days of mercury laying treatment, they explain this decrease by the presence of the antioxidant and anti-inflammatory compounds in olive oil. Two recent studies Necib et al (2013d, h)^{17,22} demonstrate that argan oil increases the phagocytic activity in rats treated with doses: 2.5 ml / kg, 5 ml / kg, 10 ml / kg. The second study by the same author reveals that the administration of the argan-oil combined with mercury, stimulates phagocytic activity in mice. Our results also agree with recent work Benzaria et al (2006)²³ showing that the effect of argan oil on the immune system is similar as olive oil.

CONCLUSION

Present study thus reveals that argan oil and sodium selenite holds promise as immunomodulatory agent against mercuric chloride toxicity, which act by stimulating phagocytic function measured in terms of phagocytic index and reduction the profile of pro-inflammatory cytokine.

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