Research Article

Immunomodulatory Effect of Argan oil (*Argania spinosa. L*) After Exposure to Mercuric Chloride in Mice

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ABSTRACT

Immunomodulatory activity of Argan oil was evaluated on phagocytic activity by carbon clearance test after mercuric chloride exposure to mice. Male Albinos randomly divided into four groups, were the first was served as a control, while the remaining groups respectively treated with: argan oil (0.5 ml/kg b.w. by gavage), mercuric chloride (0.25 mk/kg b.w. IP) and combination of argan oil and $HgCl_2$. Change in phagocytic activity was determined after 48 h injection of carbon ink suspension. Exposure of mice to mercuric chloride caused a significant decrease in phagocytic activity as compared to control. Supplementation of argan oil, exhibited significantly phagocytic index as compared to control, indicating stimulation of the reticulo-endothelial system. Present study thus reveals that argan oil holds promise as immunomodulatory agent, which acts by stimulating phagocytic function.

Keywords: Immunomodulatory, Phagocytic activity, mercury, Carbon Clearance rate, Argan oil.

INTRODUCTION

Mercury is able to suppress cell mediated immunity[1], it impairs mitogen responsiveness of T lymphocytes[2], NK cell cytotoxicity[3], and Th1 cytokine production[4], 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, a 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: antiproliferative[8-10], Hypolipidemic, hypocholesterolemic, antiatherogenic, antiradical and anti-inflammatory activities[11,12].

The present investigation was undertaken to evaluate the immunostimulatory effect of argan oil against mercuric chloride using phagocytic activity by carbon clearance test *in vivo* experimental model mice.

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* was housed under hygienic conditions in the departmental animal house. Animals were housed under standard conditions of temperature (21±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[13]. 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. In this test four groups of animals were used. Group I was kept as a control, the second group was given argan oil at dose of: 0.5 ml/kg b.w 10 days before carbon ink suspension injection. While the third group was intraperitoneally given mercuric chloride at dose of 0.25 mg/kg b.w, finally, the fourth group, argan oil orally was given(0.5ml/kg b.w) 10 days before $HgCl_2$ (0.25mg/kg b.w). Carbon ink suspension was injected via the tail vein to each rat 48 hours after the 10 days treatment, at a dose of 0.1 ml/10g. After 48h of i.p injection, the mice were administered with carbon, the mixture consisted of black carbon ink 3ml, saline 4ml and a3% gelatine solution 4ml. Blood samples were taken from the retro orbital vein by using glass capillaries, at 5 and 10 min. Blood sample drops[14] 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 reticuloendothelial 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 equations[14]:

$K = \frac{lnOD1 - lnOD2}{t2 - t1}$, $t_{1/2} = \frac{0.963}{k}$

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

Statistical analysis

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

RESULTS

Effects of treatments on phagocytic activity

Treatment with $HgCl_2$ caused a significant decrease in the phagocytic activity as compared to control. Only argan oil treatment a highly significant increase in the phagocytic activity as compared to control. However, the combined treatment of argan oil 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 highly significant increase in half-time of carbon in blood as compared to control. Only argan oil treatment a significant decrease in half time of carbon in blood. However, the combined treatment of argan oil with mercuric chloride results in gradual recovery in phagocytic activity as compared to control (Fig.2).

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 enthelium 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. 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.

In the present study, decrease in activity of phagocytic in mice treated with mercuric chloride with compared to control, probable due that mercuric chloride has a toxic effect on peripherial immune efficiency, this indicates inhibition of the reticulo-endothelial system by mercuric chloride, more recent in vitro and in vivo studies have shown that exposure to Hg^{+2} leads to decreased numbers of CD^{+}_{4} and CD^{+}_{8}.[15] Co-administration of argan oil with mercuric chloride, exhibited significantly high phagocytic index.[16] This indicates stimulation of the reticulo-endothelial system by drug treatment. It may be possible that the argan oil influence the mechanism of phagocytosis, largely distributed monocytes macrophages or R.E.S which result in significant increase in the phagocytic index with carbon clearance test[17]. The presence of some constituents in argan oil (polyphenols, b-carotene, oleic acid, vitamin E) are reported for its antioxidants, which indicate the probable role of argan oil for stimulant activity on macrophage.

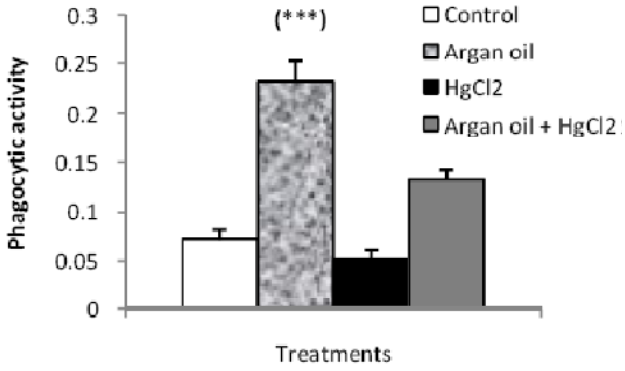

Figure 1: Effects of treatments on phagocytic activity

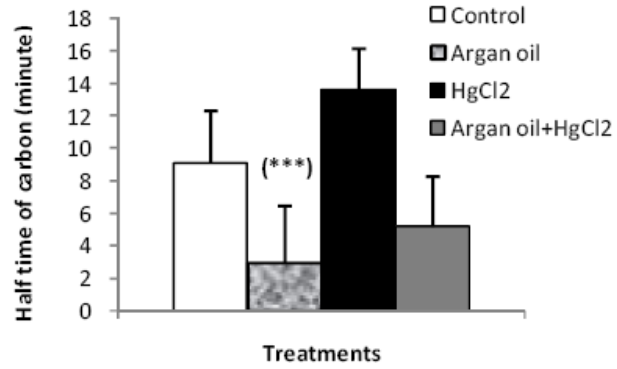

Figure 2: Effect of treatments on half time $t_{1/2}$ of carbon in blood.

CONCLUSION

Present study thus reveals that argan oil holds promise as immunomodulatory agent against mercuric chloride, which act by stimulating phagocytic function measured in terms of phagocytic index and this could be attributed to its natural components.

REFERENCES

1. Pollard Km, Hultman P. Effect of mercury on the immune system. Met Ions Biol Syst. 34, 1997, 421-40.
2. Gaworski CL, Sharma RP. The effect of heavy metals on thymidine uptake in lymphocytes. Toxicol Appl Pharmacol. 46, 1978, 305-13.
3. IIback NG, Sundberg J, Oskarsson A. methyl mercury exposure via placenta and milk impairs natural killer(NK) cell function in newborn rats. Toxicol Lett. 58, 1991, 149-58.
4. Kim SH, Johnson VJ, Sharma RP. Oral exposure to inorganic mercury alters T lymphocyte phenotypes and cytokine expression in BALB/c mice. Arch Toxicol. 77, 2003, 613-20.
5. Dubey C, Bellon B, Druet P. mercury inhibits nitric oxide production but activates pro-inflammatory cytokine expression in murine macrophage, differential modulation

of NF-kappaB and p38 MAPK signaling pathways. Nitric Oxide. 7, 2002, 67-74.

6. Kim SH, Johnson VJ, Sharma RP. Mercury inhibits nitric oxide production but activates pro-inflammatory cytokine expression in murine macrophage, differential modulation of NF-kappa B and p38 MAPK signaling pathways. Nitric Oxide. 7, 2002, 67-74.
7. Benzaria, A. Effect of dietary argan oil on fatty acid composition, proliferation, and phospholipase D activity of rat thymocytes. Nut. 22, 2006, 628-637.
8. Drissi A, Bennani H, Giton F, Charrouf Z, Fiet J , and Adlouni A . Tocopherols and saponins derived from Argania spinosa exert an antiproliferative effect on human prostate cancer. Cancer Invest. 24, 2006, 588-592.
9. Samane S, Noel J , Charrouf Z, Amarouch H , and Haddad P S. Insulin sensitizing and Anti-proliferative Effects of Argania spinosa Seed Extracts. Evid Based Complement Alternat Med. 3, 2006, 317-327.
10. Bennani H, Drissi A, Giton F, Kheuang L, Fiet J, Adlouni A. Antiproliferative effect of polyphenols and sterols of virgin argan oil on human prostate cancer cell lines. Cancer Detect Prev. 31, 2006, 64-69.
11. Necib Y, Bahi A, and Zerizer S. Argan oil (Argania spinosa L) Provides protection against mercuric chloride induced oxidative stress in rat Albinos Wistar. Inter J of Bas and App Scie, 2, 2013, 73-80.
12. Amzal H, Alaoui K, Tok S, Errachidi A, Charof R, Cherrah Y, Benjouad A. Protective effect of saponins from Argania spinosa against free radical-induced oxidative haemolysis. Fitoterapia. 79(5), 2008, 337-44.
13. Cheng W, Li J, You T, Hu C. Anti-inflammatory and immunomodulatory activities of the extracts from the inflorescence of Chrysanthemum indicum Linné. Journal of Ethnopharmacol. 101(1-3), 2005, 334-7.
14. Shah AS, Wakade AS, Juvekar AR. Immunomodulatory activity of methanolic extract of Murraya koenigii (L) Spreng leaves. Ind J of Exp Biol. 46(7), 2008, 505-9.
15. Thuvander A, Sundberg J, Oskarsson A. immunomodulating effects after perinatal exposure to methylmercury in mice. Toxicology, 114, 1996, 163-75.
16. Stuart, A.E., I.A. Habshaw, A. Davidson, Phagocytes *in vitro*, In: Handbook of Experimental Immunology. Weter D.M., Blackwell Sciences Publication, 1973.
17. Goldsby R., Kindt TJ, Osborne BA, Kuby J. Immunology, WH. Freeman and Co, New York. 2003, 1-25.

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