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Original Article

IMMUNOMODULATORY ROLE OF HONEY AND PROPOLIS ON CARBON TETRACHLORIDE (CCl_4) INJECTED RATS

KADRY A. ELBAKRY[1], CAMELIA A. ABDEL MALAK[2], MAHMOUD M. HOWAS[3]

[1,3]Department of Biochemistry, Faculty of Science, Damietta University, New Damietta city, Egypt, [2]Department of Zoology, Faculty of Science, Damietta University, New Damietta City, Egypt
Email: mahmudhowas@yahoo.com

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ABSTRACT

Objective: To evaluate the role of some inflammatory cytokines in hepatic injury induced by carbon tetrachloride (CCl_4). Also, the effects of honey bee and propolis extract to modulate the role of these cytokines.

Methods*:* Fifty female Wister rats were divided into five groups, 10 per each group. After 6 w of all treatments, rats were sacrificed, whole blood and serum were collected to determine the number of white blood cells (WBCs), percentage of granulocytes, percentage of lymphocytes and the level of some cytokines such as gamma interferon(INF-γ), tumor necrosis factor(TNF-α) and interleukin-2(IL-2).

Results: CCl_4 administration resulted in significant increase in the number of WBCs and percentage of granulocytes ($P<0.05$ and $P<0.01$ respectively). After treatment with honey and propolis, the number of WBCs was decreased significantly in the case of propolis treatment ($P<0.05$) compared with CCl_4 group. The levels of TNF-α and INF-γ cytokines were significantly decreased ($P<0.05$) after treatment with honey or propolis compared with CCl_4 group. However, there is no significant change in the level of IL-2 after treatment of CCl_4 administered rats with honey or propolis.

Conclusion: The present study suggested that honey and propolis can improve the immune disorders induced by CCl_4 in rats by modulating the levels of inflammatory cytokines, TNF-α, and IFN-γ.

Keywords: Carbon tetrachloride, Cytokines, IFN-γ, TNF-α, IL-2, Propolis and honey bees.

INTRODUCTION

The model of carbon tetrachloride (CCl_4) induced hepatic injury has been extensively used in animals to evaluate the therapeutic effects of drugs and dietary antioxidants [1]. It is well known that carbon tetrachloride (CCl_4) intoxication causes free radical generation in many tissues such as liver, kidney, heart, lung, testis, brain and blood [2-4].

Superoxide anion, arising either through metabolic processes or following oxygen "activation" by physical irradiation, is considered the "primary" ROS, and can further interact with other molecules to generate "secondary" ROS, either directly or prevalently through enzyme-or metal-catalyzed processes [5].

Honey bee is used in almost all cultures and traditions as a food and medicinal product [6]. Honey is characterized by some nutritional [7] and biological effects [8] such as antibacterial [9], antioxidant [10], antiviral [11], antiparasitic [12], anti-inflammatory [13], anticancer [14], and immunosuppressive [15] activities.

Propolis is an extremely complicated mixture of substances found in plants, and it contains more than 300 ingredients. Propolis has some pharmacologic activities such as antibacterial, antifungal, antiviral [16], anti-inflammatory [17], vasorelaxant [18], and antioxidant [19] activities.

Hepatocellular damage and inflammatory cell infiltration can leads to fibrosis and cirrhosis [20]. One of the inflammatory mediator is the tumor necrosis factor-alpha (TNF-α). It is a pleiotropic inflammatory cytokine involved in systemic inflammation that stimulates the acute phase reaction. [21]. TNF-α has been known to contribute to chronic inflammation and promote tumor formation, growth and metastasis [22]. Gamma interferon (IFN)-γ is an inflammatory cytokine recognized for its antiviral and immune-modulatory properties. Also known as type II interferon, IFN-γ is secreted primarily by activated T cells and natural killer (NK) cells [23]. IFN-γ is a potent stimulus for macrophage activation, and activated macrophages produce an abundance of the other cytokines, such as TNF-α, which may also modulate hepatocyte function [23]. Interleukin-2 (IL-2) is a pleiotropic cytokine produced after antigen activation that plays pivotal roles in the immune response [24]. IL-2 was first discovered over 35 y ago as an activity present in supernatants of activated human T cells that mediates T cell growth and proliferation [25]. The present study was conducted to investigate the role of inflammatory cytokines in liver injury induced by CCl_4 and the immunomodulatory role of honey and propolis against CCl_4toxicity.

MATERIALS AND METHODS

Animals

The present study was conducted on fifty female albino Wister rats, obtained from Helwan animal station, Ministry of Health, Egypt. Animals were housed in the animal house of Zoology Department of Damietta faculty of Science, Damietta University, Egypt and allowed to adapt for two weeks.

Preparation of aqueous extract of propolis

The method was done according to [26]. Briefly, under sterile conditions 16.8 mg of the brown powder of propolis was dissolved in 10 ml distilled water and mixed vigorously for 10 min. Finally, this suspension was centrifuged at 1000 rpm for 10 min in room temperature. The supernatant was collected and stored under freezing condition at-20 ° C until used.

Experimental design

Fifty rats were divided into five groups each of 10 rats as follows: control group fed on normal basic diet and water. Olive oil group: injected ip with olive oil three times per week (0.5 ml/kg b. wt.). CCl_4 group: injected ip with CCl_4 (0.5 ml/kg b. wt), mixed with olive oil (v/v) for 6 w three times per week. Honey group: injected with CCl_4 (0.5 ml/kg b. wt.), mixed with olive oil (v/v) and treated with 10% honey in drinking water daily for 6 w. Propolis group: injected with CCl_4 (0.5 ml/kg b. wt.), mixed with olive oil (v/v) and treated with aqueous extract of propolis (200 mg/kg b. wt.) orally by gastric tube once daily for 6 w. At the end of the 6th week, rats were anesthetized using chloroform, and then, blood samples were collected from the heart. Blood was collected in EDTA tube for WBCs counting. Also

sera were obtained and stored at-20°C until used for cytokines determination.

Hematological studies

Complete blood count (CBC) was done by Automated Hematology Analyzer (Diff3) Mek-641/Mek-6420.

Immunological studies

Interferon factors-gamma (INF-γ), tumor necrosis factor (TNF-α) and Interleukin–2 (IL–2) levels were determined by sandwich ELISA on sera of all groups according to manufacturer instructions of Boster Biological Technology Co., Ltd, California, USA.

Statistical analysis

Student T-test was performed using the statistical program package, SPSS, version 14. Degrees of significance were as follow P<0.05 significant, P<0.01 highly significant and P<0.001 extremely significant.

RESULTS

White blood cell count

WBCs count and the percentage of granulocytes were significantly increased (P<0.001& P<0.01 respectively) in CCl_4 group compared with normal control group. On the other hand, the percentage of lymphocytes was significantly decreased (P<0.05) in CCl_4 group compared with normal group. After treatment with honey, the percentage of granulocytes was decreased significantly (P<0.05) compared with CCl_4 group. Treatment with propolis causes a significant decrease of WBCs count and %GRA (P<0.01). Otherwise, treatment with propolis leads to a significant increase lymphocytes percentage (P<0.01) (table, 1).

Table 1: Effect of honey bee and propolis treatment on CBC of rats injected with CCl_4

Groups	WBCs (10^3/ml)	% of lymphocytes	% of granulocytes
Control	6.75±0.48	74.51±3.81	17.21±1.90
Olive oil	10.48±2.10$^{£}$	75.55±3.34	19.10±2.60
CCl_4	14.43±0.62$^{♠}$	65.70±7.40$^{♥}$	25.0±4.61$^{£}$
Honey	13.35±1.48$^{£}$	73.50±10.6	18.0±7.0 $^{■}$
Propolis	6.88±2.00$^{♯}$	73.17±3.05$^{♯}$	15.42±6.0$^{♯}$

♥, £, ♠ significant at levels 0.05, 0.01 and 0.001 respectively as compared with control group; ■, ♯ significant at levels, 0.05 and 0.01 respectively as compared with CCl_4 group. Data were expressed as mean±standard deviation.

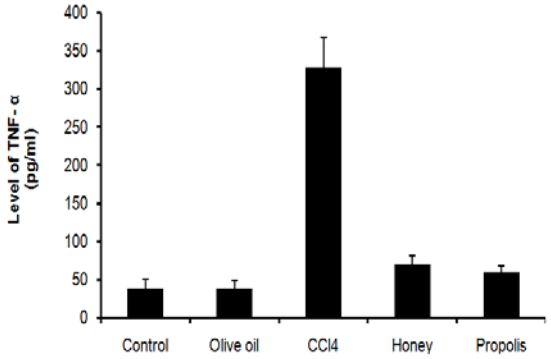

Fig. 1: Effect of honey bee and propolis treatment on TNF-α of rats injected with CCl_4. Each bar represents the mean±SD of ten rats per group. The level of TNF-α was increased in CCl_4 group (P<0.001) but after treatment with honey and propolis, the levels were decreased significantly compared with CCl_4 group (P<0.001)

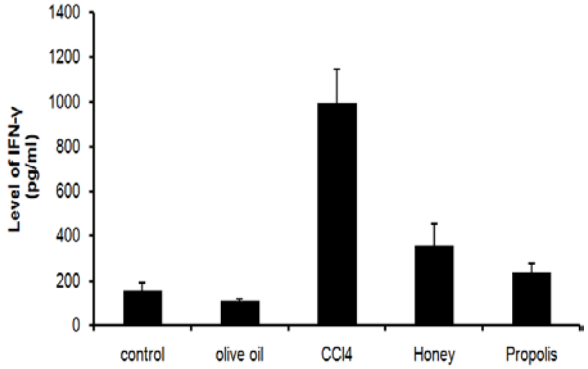

Fig. 2: Effect of honey bee and propolis treatment on IFN-γ of rats injected with CCl_4. Each bar represents the mean±SD of ten rats. The level of on IFN-γ was increased after CCl_4 injection (P<0.001). After treatment with honey and propolis, the levels were decreased significantly (P<0.001)

Inflammatory cytokines

Fig. (1) and (2), Indicate that, there is a significant increase in the mean of TNF-α and INF-γ of CCl_4 group compared with the normal group(P<0.001). However, fig. (3) Showed that there is a significant decrease in the mean of IL-2 of CCl_4 group compared with the normal group (P<0.05). After treatment with honey and propolis, TNF-α and INF-γ levels were significantly decreased (P<0.001 and P<0.01 respectively) compared with CCl_4 group. On the other hand, treatment with propolis resulted in a non-significant decrease in the mean of IL-2 compared with CCl_4 group (P>0.05).

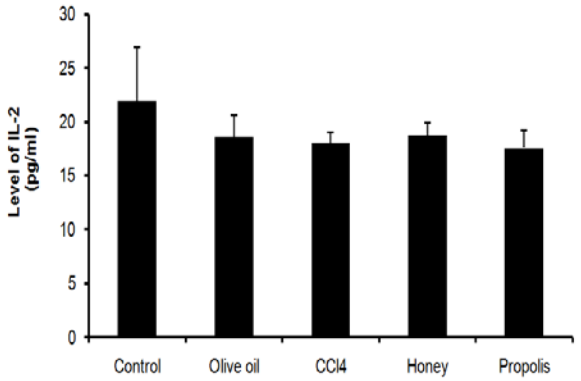

Fig. 3: Effect of honey bee and propolis treatment on IL-2 of rats injected with CCl_4. Each bar represents the mean±SD of ten rats. A significant decrease was observed in CCl_4 group compared with the normal control group (P<0.05). After treatment of CCl_4 injected rats with honey and propolis, no significant differences were observed

DISCUSSION

The human body is exposed to increasing attacks by toxic compounds in polluted air, industrially processed foods, and alcohol and drug consumption that increase liver toxicity, leading to more and more severe cases of hepatic disorders [27].

In the present study, i. p. injection of rats with CCl_4 showed reduction of lymphocytes population in blood. This result was explained by the decline of immune activity by long-term liver damage [28]. On the other hand, injection of CCl_4 for six weeks increases the number of WBCs count. This may be attributed to the defensive mechanism of immune system as reported by [29]. The ability of free radicals to increase WBCs count indicates that these free radicals can affect the defense mechanism of CCl_4 injected rats and mediate inflammation [30].

In consistence to the increased number of WBCs, the levels of tumor necrosis factor-α (TNF-α) and interferon (INF-γ) were increased significantly after injection of rats with CCl_4. TNF-*α* is a proinflammatory cytokine that is elevated in acute and chronic diseases. In addition, TNF-*α* stimulates the release of cytokines from macrophages and induces phagocyte oxidative metabolism [31]. CCl_4 activate several inflammatory mediators such as IFN-γ and TNF-α by kupffer cell [32, 33]. They were reported to play a key role in the pathogenesis of various liver diseases. Following its release from activated Kupffer cells, TNF- α worsens both oxidative stress and inflammatory responses in the liver [34].

The anti-inflammatory activity of honey was evaluated *in vivo* by measuring the release of some inflammatory markers that are controlled by NF-*κ*B like IL-2, INF-γ, and TNF-*α*. Honey stimulates monocytes in cell cultures to release the cytokines, IL-1 and IL-6, the cell messengers that activate many of the immune response to infection [35]. Some photochemical and others have been shown to inhibit inflammation by blocking inflammatory pathways downstream of proinflammatory factors [36].

Propolis has been used as a medicine all over the world for various purposes. It has been shown to possess anti-inflammatory, immunostimulatory, antiviral, antibacterial and hepatoprotective activities [37].

On the contrary, to CCl_4 group, treatment of CCl_4 injected with honey or propolis significantly reduces TNF-α, INF-γ levels to nearly the normal group. This reduction in the levels is due to the ability of honey or propolis to prevent the formation of free radicals, enhance the endogenous antioxidant activity beyond its free radical scavenging property as well as the anti-inflammatory effect of honey or propolis [38, 39].

CONCLUSION

The present study suggested that TNF-α and IFN-γ plays an obvious role in liver injury induced by $CCl_4$and treatment with honey and propolis improve the levels of these cytokines that may lead to decrease such injury.

CONFLICT OF INTERESTS

Declared none

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