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Study the Immunomodulatory Effect of Ethanolic *Azadirachta indica* Seeds Extract on the Immunologic Response in Mice Vaccinated with *Proteus vulgaris*

A Thesis

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Summary

A total of 100 urine samples were collected from pregnant women who attended to Children and delivery's hospital in Samawah city, during the period extended from November to January 2012. The study is carried out for bacteriological isolation and identification of *P. vulagris*, antibacterial susceptibility testing and to investigate the immunomodulatory effect of the Ethanolic neem seeds extract on the immune response of mice vaccinated with *Proteus vulgaris* antigens. The study included eight groups; the first group (I) was treated with distilled water. group II included mice treated with *Proteus vulgaris* antigens only (III): injected mice subcutaneously with a dose of (600 µg /Kg) ethanolic neem seed extract, IV group included injected subcutaneously with a dose of (400 µg /Kg) ethanolic neem seed extract only, The V, VI groups mice were treated with combination of *p.vulgaris* and ethanolic neem seed extract while (VII and VIII) groups mice were injected with the immunosuppressive drug prednisolone prior to the forthcoming treatment 5 days. All these treatments were carried out at day 1, and then the mice were sacrificed at day 8 to estimate phagocytic activity index by ELISA reader and on day I4 for estimation of lymphocyte transformation by MTT index by ELISA reader and for delayed-type hypersensitivity reaction test at 24 , 48 and 72 hours after proteus vulgaris antigens injection, at day 21 and 28 for (anti-*proteus vulgaris* antibody titer) by indirect immunoflourescent assay and for serum electrophoresis to estimate the serum fraction.In this regard, all groups of mice showed different significant increases($P \leq 0.01$) in the NBT index which represent the phagocytic activity% as compared to group I (0%), which was injected with distilled water (negative control group). The best treatment efficiency was recorded in-group V (57%), while the lowest treatment efficiency was recorded in-group IV (5%). The results of lymphocyte transformation index in mice of all groups showed different significant increases by MTT assay which represent the lymphocyte transformation index as compared to group I (0%). The best treatment efficiency was recorded in-group V (264%), while the lowest treatment efficiency was recorded in-group VI (17%).Delayed type hypersensitivity (DTH) index estimated by skin test in footpad of the mice that immunized with *P vulgaris* antigens and treated with ethanolic neem seed extract and the high value in the group V after 24 hours. The best delayed type hypersensitivity

(DTH) index was recorded in group V. The anti-Proteus antibodies assessed by indirect immunoflourescent test also showed a significant increase titer in immunomodulator-treated and -vaccinated mice in comparison with negative and positive groups . At 21, 28 days, and the best treatment efficiency was recorded in-group V after 21 days with titer of 512. In all mice a significant increase in the value of gamma globulin fraction and Alpha-1 serum fraction and Alpha-2 Fraction and Beta serum fraction were observed in group V (19.24 ± 0.80، 8.68 ± 0.23، 27.31 ± 0.06، 34.02 ± 0.21) respectively after 21 days of immunization, and also The highest values of gamma globulin fraction and Alpha-1 serum fraction and Alpha-2 Fraction and Beta serum fraction were observed in V (19.26 ± 0.8 , 8.17 ± 0.38, 27.43 ± 0.12, 34.10 ± 0.06) respectively after 28 days of vaccination of mice. The results demonstrated a clear immunomodulatory effect of the ethanolic neem seed extract (improvement of non-specific, and cellular immune response) of the treated mice immunized with *proteus vulgaris* .

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