# Study the Immunomodulatory Effect of Ethanolic Neem Seeds Extract on The Immunologic Response in Mice Vaccinated with *Proteus vulgaris* vaccine

Saif Mezaal Abed- College of Science

Assist. Prof Dr Tariq F.AL Jindeel
College of Veterinary Medicine
AL-Muthanna University

prof. Dr. Turki M.Saad
College of Agriculture
AL-Muthanna University.

## Abstract

This study was carried out to investigate the immunomodulatory effect of the Ethanolic neem seeds extract on the immune response of mice vaccinated with *Proteus vulgaris antigens* and considered as plant immunomodulators. The study included eight groups; the first group (I) was treated with distilled water. II group was mice treated with *Proteus vulgaris* antigens only (III): injected subcutaneously with a dose of (600 ug /Kg) ethanolic neem seed extract, IV group was injected subcutaneously with a dose of (400 ug /Kg) ethanolic neem seed extract only, The V, VI groups were treated with combination of *p. vulgaris* and ethanolic neem seed extract while (VII and VIII) groups 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 Eliza reader and on day I4 for estimation of lymphocyte transformation by MTT index by Eliza 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%). 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 group

At21, 28 days, and the best treatment efficiency was recorded in-group V after 21 days with titer of 512.

## INTRODUCTION

*Proteus vulgaris* is gram-negative facultative anaerobe that has a growth temperature of 37C. *P. vulgaris* occurs naturally in the intestines of humans and a wide variety of animal, manure, soil and polluted waters ,It have been described as opportunistic etiological agents in infections of wounds, burns, skin, eyes, ears, nose, throat and pulmonary system as well as in gastroenteritis from the consumption of contaminated meat or other food (Unachukwu *et al*., 2005). Furthermore the hemolysin that the bacterium secretes is cytotoxic for urinary tract epithelial cells(Struble *et al*., 2009). It has more than four species; most Proteus species known to cause disease in humans are associated with opportunistic infections; Proteus *mirabilis* and *Proteus vulgaris* (O'Hara *et al*., 2000). Virulence factors in Proteus also include resistance to normal human serum (Mishara *et al*., 2001).immunomodulators are plants and plant products, or biological materials that mediate the effectors mechanisms of the immune system through immune stimulation to a given antigen or potentiate the effectiveness of a vaccine (Bose *et at*., 2009.).Materials of fungal and/or plant origins have been the interest of different investigators around the globe with their aims to establish the immunomodulator potentials of these materials. Some risks associated with attenuated or killed whole-organism vaccines can be avoided with vaccines that consist of specific purified macromolecules derived from pathogens or in combination with plant materials (WHO 1998, Aliero *et al*., 2003, Gosh *et al*., 2007, Bose *et at*., 2009, Zahid *et al*., 2013). One of these plants is the Neem tree (*A zadirachta indica* A. Juss.) which is more popular with the name neem, and has the advantage to be a medicinal plant with a wide range of applications in folkloric medicine Coventry *et al*., (2001) .

## MATERIAL AND METHODS

All experiments were done in the laboratories of the college of science in Al_ Muthanna University and research on male and female albino mice (Blab-c), which were supplied by this college their age ranged between 6-8 weeks. They were housed in bio-clean hoods at 20-25ºC with light: dark periods of 14:10 hours. They had free access (*ad libitum*) to food (standard pellets) and water, and their average weight was 22 ± 3 grams at the beginning of the experiments. Before carrying out the experiments, the animals were left in separate cages for one week to experience the acclimatization period. Isolated and identified *P vulgaris* from urines samples that belong to pregnant women, by

biochemical tests and confirmed by Api–20E system, and prepared *P. vulgaris* whole cell antigen according to a method presented by (Motive *et al*., 1992). determined of $LD_{50}$ Neem Table(1)

Table 1 : Doses of Neem that were used in the assessment of LD50.

| | Dose/mouse | Dose/Kg | Number of Animals | Mortality Rate (%) |
|---|---|---|---|---|
| Ethanolic neem extraction | 100 µg | 4 mg | 6 | 0.0 |
| | 200 µg | 8 mg | 6 | 0.0 |
| | 300 µg | 12 mg | 6 | 0.0 |
| | 400 µg | 16 mg | 6 | 0.0 |
| | 500 µg | 20 mg | 6 | 0.0 |
| | 600 µg | 24 mg | 6 | 0.0 |

Blood phagocytic activity for oxidative burst reaction by Nitro Blue Tetrazolium test (NBT) measured by ELIZA (Zakari *et at*., 2011) and the phagocytic activity% was calculate as suggest by (Sanja *et at*., 2009).

Determination of lymphocyte transformation by MTT index by Eliza reader (Farid *et al*., 2003)

Quantitative Determination of anti- *Proteus vulagis* antibodies serum Level at 21 and 28 days were carried out by using a mouse anti rabbit antibodies kit (company), which is an indirect Immunoflourescent assay (WHO 1998). The Statistical Analysis values of the investigated parameters were given in terms of means ± standard errors (S.E.), and differences between means were assessed by analysis of variance (ANOVA), least significant difference (LSD) test, using the computer programmer SPSS version 7.5. The differences were considered significant when the probability value was equal or less than 0.01. Further estimations were also given; they were treated efficiently (Perez-Serrano *et at*., 1997 ), which were calculated according to the following equation

$$\text{Phagocytic activity percentage (\%)} = \left(\frac{A - B}{B}\right) \times 100$$

A = Treated groups; B = Negative control group

## RESULTS AND DISCUSSION

The results of NBT index were given in table 2 . All groups of mice showed different significant increases in the NBT index which represented the phagocytic activity% ( 27%,10% , 5%, 57% ,50% , 44%, and 27% respectively) as compared to group I (0%), which was injected with deionized distilled water (control group). The best NBT index was recorded in group 5 (57%), which included mice that were treated with 600 µg/ kg of ethanolic neem extraction prior to the vaccination with *P.vulgaris* antigen while lowest NBT index was recorded in group 4 (5%) included mice that were treated 400 µg/ kg of ethanolic neem extraction only .

| Groups | (mean ± S.E). | Phagocytic activity% |
|---|---|---|
| I | $1.29\pm0.12^{c}$ | 0% |
| II | $1.64\pm0.20^{bc}$ | 27% |

Table 2: Nitro blue tetrazolium (NBT) index in mice vaccinated with *P. vulgaris* vaccine and treated with ethanolic seed neem extract

| | | |
|---|---|---|
| III | $1.39\pm0.14^{c}$ | 10% |
| IV | $1.36\pm0.14^{c}$ | 5% |
| V | $2.03\pm0.22^{a}$ | 57% |
| VI | $1.44\pm0.22^{c}$ | 50% |
| VII | $1.86\pm0.18^{b}$ | 44% |
| VIII | $1.64\pm0.20^{bc}$ | 27% |

***The different letters denoted that significant differences among the groups <p≤0.01***

Results of NBT index, which showed a significantly increased percentage in treated mice, are also in favour of such agreement. The results obtained in the present study are identical with those obtained by (Kawarada *et al*., (2001); Biswas K, *et al*., (2002); Hague ,(2006) ; Ghosh *et al* .,(2007).

Phagocytic activity by reduction of (NBT) to insoluble blue Formozan granules occurred during the stimulus-induced respiratory burst of mature granulocytes, Nitro blue tetrazolium (NBT) test addition of the yellow NBT dye to plasma results in the formation of a NBT–heparin or NBT–fibrinogen complex, which may be phagocytosis by neutrophils (Kumar *et al*., 2008). Antigenic components from *P.vulgaris* bacteria were compared to intact cells for their ability to induce natural cytotoxic immunoeffectors and intracellular conversion of nitro blue tetrazolium (NBT) to formazan by immunomodulator was used to measure the generation of reactive oxygen species and the amount of formazan formed was measured by Eliza reader (Angelica *et al*., 2001) Nitro blue tetrazolium reduction by polymorphonuclear cells may require oxidative metabolism by the hexose monophosphate shunt, and is impermeable to cell membrane, but it entered the cell during the process of phagocytosis, and it is reduced by diphorase activity within phagosome Stimulated and, after neutrophils incorporated the dye complex into phagosome. Balwinder *et al*., (2005). lysosomal fusion, intracellular reduction results in the formation of blue insoluble crystals of The percentage of phagocytic cells activity may be quantified spectrophotometrically after formazan. alkaline DMSO, which reacts with NBT to produce coloured diformazan, formed dioxin extraction

Super oxide free radical. The methanolic extract of *Portulaca oleracea* scavenges superoxide radical and thus inhibits formazan formation. Sanja *et al*., (2009). Although macrophages and monocyte possess killing mechanisms in the resting state, these mechanisms can be enhanced, and new mechanisms can be expressed when they are activated. Activation can occur through exposure to microbial products (i.e. *P.vulgaris* components antigens) and/or materials extracted from plants (i.e. ethanol extracts of neem seeds). Such picture is enhanced by the findings of the present study and confirmed by other (Kawarada *et al*., (2001); Biswas *et al*., (2002);Hague ,2006; Ghosh *et al*., (2007) ; Thakurataap *et al.*, (2007); Khan & Aslam, (2008). Such immunomodulators can cause a direct activation of phagocytes, or an indirect activation through triggering cytokine release from them. Once the organism is internalized, it is exposed to an array of killing mechanisms; oxygen-dependent killing mechanisms (this pathway is also called reactive oxygen intermediates( ROIs) and reactive nitrogen intermediates (RNI) (Kubby 2007). It is expected that the ethanolic seed extract is effective immunomodulators. These are in agreement with this conclusion, several researchers suggested the potentianal use of in this line of experimental immunology by using different laboratory approaches and animals The concluded that neem oil acts as a non-specific immunostimulant and that it selectively activates the cell-mediated immune mechanisms to elicit an enhanced response to subsequent mitogenic or antigenic challenges (Hague *et al*., (2006). This study attempted to use an immunostimulatory neem (Azadirachta indica) leaf preparation (NLP) to prevent the cyclophosphamide (CYP) induced reduction in the WBC count. Pretreatment of mice with NLP reduced the extent of leucopenia and neutropenia in normal and tumor bearing CYP treated mice( Gosh *et al* .,(2006) .The results of lymphocyte transformation index in treated mice measured by MTT test can be found in table 4-6. mice showed different significant increases in the Lymphocyte transformation index which represent the lymphocyte transformation index % (52%, 23%,17% , 264%, 82 % ,64% and 23%, respectively) as compared to group I ( 0% ) which was injected with deionized distilled water (control group). The best Lymphocyte transformation index was recorded in-group V (264%), which included mice that were treated with combination of 600 µg/ kg ethanolic neem extract and *P. vulgaris* antigens while the lowest index was recorded in -group IV (17%) included mice that were treated with 400 µg/ kg of ethanolic neem extract only The best Lymphocyte transformation index was recorded in-group V (264%), which included mice that were treated with combination of 600 µg/ kg ethanolic neem extract and *P. vulgaris* antigens while the lowest index was recorded in -group IV (17%) included mice that were treated with 400 µg/ kg of ethanolic neem extract only .

Table 3: Lymphocyte transformation index in mice vaccinated with *P. vulgar*is vaccine and treated

with *ethanolic neem ex*tract..

| Groups | (mean ± S.E.) | Phagocytic activity % |
|---|---|---|
| I | $0.11\pm0.001^{c}$ | 0% |
| II | $0.12\pm0.003^{c}$ | 23% |
| III | $0.26 \pm 0.004^{b}$ | 52% |
| IV | $0.20\pm0.0026^{b}$ | 17 % |
| V | $0.62\pm0.01^{a}$ | 264% |
| VI | $2.80\pm0.004^{c}$ | 82% |
| VII | $3.56\pm0.006^{b}$ | 64% |
| VIII | $3.13\pm0.005^{b}$ | 23% |

**** Different letters: Significant difference means of the same column. (P≤0.01) between***

the An in vitro test which have lymphocytes, by a certain been sensitized antigen, transform into blasts and proliferate when they are again exposed to this antigen. This proliferation is determined by MTT (3-[4, 5-dimethyl-2-thiazolyl] -2, 5-diphenyl -2H- tetrazolium bromide)-reduction method measured by ELIZA reader. The test has the advantage over skin tests of avoiding re-exposure of individuals to the same antigen, and it was, therefore, hoped that it might also help to as an invitro test for evaluation of cell mediated immunity (Khosravi *et al.,(* 2006). However, the LTT measures only the sensitization of lymphocytes, but not the effector reaction. The principle of the LTT is based on the fact that lymphocytes, which have been sensitized by a certain antigen (memory cells), transform into blasts and proliferate when they are again exposed to this antigen ( Moragues *et al.*, 2003). Moragues *et al* ., (2003) , also used the MTT-reduction to investigate the effects of anti- *Candida* monoclonal antibodies in vitro; that appears to be the only report based on the use of MTT for this purpose. The tests were based on the capacity of viable cells

to reduce MTT to formazan that was assayed by spectrophotometric quantitation of optical density (OD) after its extraction with acid-propanol, with the OD taken as a measure of the metabolic status and the total, viable mass of the *Candida* cells and effects of some Iranian herbal essences have been evaluated on the function of immune system using experimental animals. .

Gosh *et al* .,(2006); Haque *et al* .,(2006); Khosravi *et al*., (2007) ;Nielubowicz *et al*., (2008) in agreement with our results, The results of their studied were indicate that neem oil acts as a non-specific immunostimulant and that it selectively activates the cell mediated immune mechanism.. Haque *et al* .,(2006) observed that number of splenic T lymphocytes (CD4+ and CD8+) and NK cells were also to be increased in mice injected with 0.5 units and 1 unit of NLP(neem leaf product).However, NLP dose of 2 units could not exhibit such immunostimulatory changes . on using neem as a method of birth control indicates that neem initially stimulates TH1 cells and macrophages, and then causes an elevation of both immunoreactive and bioactive TNF-alpha and gammainterferon in serum and mesenteric lymph nodes (Makeri *et at*., 2007). A cording to our results the fact that ethanolic neem seed extract affects the cell-mediated immune system is particularly important to most people .Neem also boosts the body's macrophage response, which stimulates the lymphocytic system, and boosts production of white blood cells. The immunobiology mechanism may be due to increase of T-lymphocytes CD receptors; MHC 1 and enhance cytokines production result in stimulates TH1 cells and macrophages, and then causes an elevation of both immunoreactive and bioactive TNF-alpha and gamma interferon in serum and mesenteric lymph nodes.

.

The sera of treated mice showed some variations. Groups I, III, IV, showed no anti-*P. vulgaris* antibodies at the start titer 1:16 after 21 days, while the other groups VI, VIII, VII and showed a higher positive immunoflourescent reaction at the titer 1:128,1:64 and 1:32 respectively while the group V showed a highest positive immunoflourescent reaction at the titer 1:256. These results are given in (Table 4). The sera of mice in groups I, III, and IV showed no anti-*P. vulgaris* antibodies and at the start titer 1:32 in groups II, VIII, VII after 28 days. While the highest anti *P.vulgaris* antibodies titer was recorded in mice of group V, VI at the titer I: 256 and at I: 128 respectively. These results were given in (Table 5).

Table 4: Anti-*Proteus vulgaris* antibody titer in sera of treated mice after 21 days.

| | Anti-*Proteus vulgaris* antibodies Titer after 21 days | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 16 | 32 | 64 | 128 | 256 | 512 | 1024 | 2048 | 4096 |
| I | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| II | 16 | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| III | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| IV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| V | 16 | 32 | 64 | 128 | 256 | 512 | 0 | 0 | 0 |
| VI | 16 | 32 | 64 | 128 | 0 | 0 | 0 | 0 | 0 |
| VII | 16 | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| VIII | 16 | 32 | 64 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 5: Anti-*Proteus vulgaris* antibody titer in sera of treated mice after 28 days

| Groups | Anti-*Proteus vulgaris* antibodies Titer after 28 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 16 | 32 | 64 | 128 | 256 | 512 | 1024 | 2048 | 4096 |
| I | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| II | 16 | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| III | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| IV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| V | 16 | 32 | 64 | 128 | 256 | 0 | 0 | 0 | 0 |
| VI | 16 | 32 | 64 | 128 | 0 | 0 | 0 | 0 | 0 |
| VII | 16 | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| VIII | 16 | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

.

Immunofluorescence is the visualization of antigens within cells using antibodies as fluorescent probes. The benefits of immunofluorescence are numerous, and the technique has proven to be a powerful tool for determining the cellular distribution of known antigens in the frozen tissues or in the localization of specific DNA sequences on chromosomes.. Anti-*Proteus vulgaris* antibodies showed an increased titer in all immunized groups treated with the ethanolic seed neem extract used in the study, especially groups V and IV as compared to the control group (I) that received vaccine only. Such observation suggests that the immunomodulatory effect of neem seed also involved the humoral immune response, although the pathway may be through the modulation of macrophages and T lymphocytes as both types of cells are required to enhance the B-lymphocytes to produce immunoglobulin (Takahashi 2003). .

The results of the present study were in accordance with Ray *et al*., (1996) they were observed neem modulations in mice on their humoral and cell mediated immune responses when those were treated with neem meal (100 mg neem leaf extract/kg diet). The mice showed higher levels of IgM and IgG along with increased antiovalbumin antibody titer. The results of present study revealed that neem leaf meal had good immunomodulatory effects against ND and IBD as indicated by the serum antibody titers.. The findings of the present study were also in alignment with the work of (Durrani *et al*., (2008). They concluded that 4% neem leaves *(A. indica)* infusion at 50 ml/ltr of fresh drinking water successfully improved antibody titer against IBD, growth performance and lowered the mortality. .

The results of the present study were also in accordance to the outcome of a work done by Landy *et al*., (2011) who conducted the experiment to see the effects of different levels of neem in combination with an antibiotic (Flavofosfolipol) on humoral immune response of broiler chicks. Neem at 7gm/kg in diet led to the highest antibody titers a gains Newcastle disease virus. These are in agreement with our conclusion with Zahid *et al* .,(2013) study they were concluded that addition of neem leaves in broiler feed has better effects on antibody production against new castle and infectious bursal disease viruses.

**Conclusions**

The results of present study revealed that ethanolic seed extract neem had good immunomodulatory effects against immune response in mice immunized with whole cell antigen of *P.vulgaris* against. The results of nitro blue tetrazolium index, lymphocyte transformation, anti-*proteus vulgaris* antibody titer, strongly support such conclusions. The results of this study indicate that ethanolic neem extract seed acts as a non-specific immunostimulant and that it selectively activates the cell mediated immune (CMI) mechanism and it was dose dependant.'

# Reference

Balish, E.; Warner, T. F.; Nicholas, P. J.; Paulling, E. E.; Westwater, C. and Schofield, D. A. (2005). Susceptibility of Germfree Phagocyte Oxidase- and Nitric Oxide Synthase 2-Deficient Mice, Defective in the Production of Reactive Metabolites of Both Oxygen and Nitrogen, to Mucosal and Systemic Candidiasis of Endogenous Origin. Infect. Immun. 73: 1313-1320.

Biswas, K., Chattopadhyay, I., Banerjee, R.K., Bandopadhyay, U. 2002.Biolog-ical activities and medicinal properties of neem (Azadirachta Indica). *Curr Sci* 82**:** 1336-1345.

Bose, A., Chakraborty, K ., Sarkar, K., Goswami, S., Haque, E.,Chakraborty, T., Ghosh, D., Roy, S., Laskar, S. and Baral, R. (2009*). Neem (AzadirachtaIndica)* leaf preparation prevents leukocyte apoptosis mediated by cisplatin plus 5-fluorouracil treatment in Swiss mice. *Source* 55:137-144.

Coventry, E. and Allan, EJ. (2001).Microbiological and chemical analysis of neem (Azadirachta indica) extracts; new data on antimicrobial activity. Phytoparasitica, 5: 1-2.

Durrani, F.R., Sultan, A., Akhtar, S., Jan. M., Chand, N., Durrani, Z. (2008). Immunomodulatory and growth promoting effects of neem leaves infusion in broiler chicks. *Sarhad j. Agric* 24(4):655-659.

Farid , A. B., Bortros, R. M., Gala, T. M., and Mohamed, M.A. (2003). Immunomodulatory Triterpenoids from the oleogum Resin of Boswellia carterii bird wood. *Z.Naturforsch+-9* .58:505-516.

Ghosh, D., Bose, A., Haque, E., Baral, R.N. 2006.Pretreatment with neem (Azadirachta indica) leaf preparation in Swiss mice diminishes leu-kopenia and enhances anti-tumor activity of cyclophosphamide. *Phytother Res* 20**:** 814-818.

Ghosh, I.M., Chattopadhyay, U. and Rathin, D.(2007). Neem leaf preparation enhances Th1 type immune response and anti-tumor immunity against breast tumor associated antigen. *Cancer Immunity* 7: 8 -9.

Haque, E., Mandal, I., Pal, S., Baral, R.N., 2006. Prophylactic dose of neem (Azadirachta indica) leaf preparation restricting murine tumor growth is nontoxic, hematostimulatory and immunostimulatory. *Immunopharmacol Immunotoxicol* 28**:** 33-50.

Kawarada, Y, Ganss, R., Garbi, N., Sacher, T., Arnold, B., Hammerling, G.J. 2001.NK- and CD8(+) T cell mediated eradication of established tumors by peritumoral injection of CpG-containing oligodeoxynu-cleotides. *J Immunol* **167:** 5247-5253.

Khan, S. and Aslam, J. (2008). Study on the effect of neem (*Azadirachtaindica*) Leaves smoke in controlling airborne bacteria in residential premises. *Curr Res in bacterial* 1:64-66.

Khosravi, A.R., Franco, M., Shokri, H., Yahyaraeyat, R. (2007). Evaluation of the effects of *Zatariamultiflora*, *Geranium pelargonium*, *Myrth*and *Lemon* essences on immune system function in experimental animals. *J.Vet.Res*. 62 (4): 119-123.

Kubby, J. (2007) .*Immunology Text book*,3rd Ed W.H freeman and Company USA New York.

Kumar, R.V., Gupta, V.K., (2002) Thrust on neem is need of today. In: *Employment news*, July 20-26. New Delhi, India.

Kuwahara, H., Y. Miyamoto, T. Akaike, T. Kubota, T. Sawa ,S. Okamoto, and H. Maeda. 2000. Helicobacter pylori urease suppresses bactericidal activity of peroxynitrite via carbon dioxide production. *Infect. Immun.*68: 4378-4383.

Landy, N., Ghalamkari, G., Toghiani, M., Yazdi, F.F (2011). Humoral immune responses of broiler chickens fed with antibiotic and neem fruit powder (*Azadirachta indica*) as feed additive supplemented diet. *IPCBEE* 3:153-155.

Makeri, H.K., Maikai, V.A. and Nok, J.A.(2007). Effect of topical application of neem seed (*Azadirachta indica*) extract on sheep infested with *Amblyomma variegatum*. *African Journal of Biotechnology* 6(20):2324-2327.

Mizutani, S., Endo, M., Toshiaki, M., Yokouno, H., Ikunoshin, K. and Takesako, K. (2000). Immunization with the *Candida albicans* membrane fraction and in combination with fluconazole protects against systemic fungal infections. Antimicrob Agents chemother 44:243-247.

Moragues, M, Moralejo, M.A., Villegas, D., Royo, C. (2003). Genetic and agronomic variability of durum wheat within the Mediterranean region. In: Maggioni L, Faberova I, Blanc AL, Lipman E (eds) Report on Working Group on Wheat, European Cooperative Programme for Crop Genetic Resources Networks(ECPGR). *International Plant Genetic Resource Institute (IPGRI), Prague-Ruzyne, Czech Republic* 116–118.

Motive, I., Denchen, V. and Linde, K. (1992). Humoral and cell mediated live oral vaccines of ***Salmonella typhimurium*** auxotrophic mutants with two attenuating markers. Vaccine. 10: 61-66

Nielubowicz, Greta. R., Sara, N., Smith, Harry., L.T. Mobley. (2008). Outer membrane antigens of the uropathogen *Proteus mirabilis* recognized by the humoral response during experimental murine urinary tract infection. *Infection and Immunity*. 76(9): 4222-31.

O'Hara, C. M., Brenner, F. W., and Miller, J. M. (2000) Classification, identification, and clinical significance of Proteus, Providencia, and Morganella. *Clinical Microbiology Reviews* 13: 534–546.

Perez-Serrano, J.; Denegri, G. ,Casado, N., Rodrigue, Z. , Caaberio, F. (1997 ). In vivo effect of oral albendazole sulphoxide on development of secondary echinococcosis in mice . *Int J parasitol* (27): 1341-1345.

Ray, A., Banerjee, B,D. and Sen, P. (1996). Neem Kernel oil induces secretion of gamma--interferon and tumor necrotic factor-alpha and enhanced level of IgG, Igm, IgA. *Curr. Opin. Immunol* 8:9.

Sadekar, R.D., Kolte, A.Y., Barmase, B.S., Desai, V.F (1998). Immunopotentiating effects of *Azadirachta indica* (Neem) dry leaves powder in broilers, naturally infected with IBD virus. *Indian. J. Exp. Biol*. 36(11):1151-3.

Sanja, S.D. ; Sheth,N.R.; Patel N.K.; Dhaval, P. and Biraju, P. (2009).Characterization and evaluation of antioxidant activity of Portulaca oleracea International Journal of pharmcy and pharmaceutical Science .74-84.

Subapriya, R., Nagini, S., (2005). Medicinal properties of neem leaves: a review. *Curr Med Chem Anticancer Agents* **5**: 149-156.

Swierzko,A.S. , Kirikae,T. , Kirikae,F.. (2000).Biological activities of lipopolysaccharides of *Proteus Spp.*and their interactions with polymyxin-B. ; and 18-KDa cataionic antimicrobiol protein (cap 18) derived peptide.*J.Med.Microbiol*. 49:127-138.

Takahashi, H. (2003).Antigen presentation in vaccine development. *Comp. immunol. Microbiolo. Infection Dis* 5(6):309-328.

Thakurataap, B., Mukherjees, H., Patra, A., Bag, P.K. (2007).Antibacterial, an secretory and antihemorrhagic activity of Azadirachtaindica used to treat cholera and diarrhea in India. *J Ethanopharmacol* 21: 37.

Threadgill,D.S., McCormick,L.L.,McCool, T. L., Greenspan ,N.S. and Schreiber,J.R.(1998).Mitogenic Synthetic polynuclerotides suppress the antibody response to bacterial polysaccharide. *Vaccine* 16:76-82.

Unachukwu, C.N., Obunge, O.K., Odia, O.J. (2005). The Bacteriology of Diabetic Foot Ulceration in Port Harcourt, Nigeria. Nigeria *J. Med* 14:173-176.

World Health Organization (WHO) .(1998).The development of new/improved Brucellosis vaccines: report of a WHO meeting 11-12 December 1997 Geneva WHO Geneva 19-21.

Zahid ,J., Muhammad ,Y., Mutti-ur-Rehman1, Azhar, M., Rashad, M., Khushi, M., Roshan ,, and Izhar, H.( 2013). Effect of neem leaves (*Azadirachta indica)* on immunity of commercial broilers against new castle disease and infectious bursal disease. *African Journal of Agricultural Research* 8(37):4596-4603.

Zakaria, Z. A. ; Rofiee M. S. ; Teh L. K. ; Salleh M. Z.; Sulaiman M. R. and M. N. Somchit1(2011). Bauhinia purpurea leaves' extracts exhibited in vitroantiproliferative and antioxidant activities. *African Journal of Biotechnology* Vol. 10(1):65-74. .

# دراسة تأثير المحورات المناعية للمستخلص الأيثانولي لبذور نبات النيم على الاستجابة المناعية للفئران الملقحة بلقاح بجرثومة *Proteus vulgaris*

**سيف مزعل عبد**
**كلية العلوم _جامعة المثنى**

**ا.م د طارق فعل الجنديل**
**كلية الطب البيطري- جامعة المثنى**

**ا. د. تركي مفتن سعد**
**كلية الزراعة- جامعة المثنى**

## الخلاصة

تضمنت الدراسة تأثير المحورات المناعية المتمثلة بالمحلول الأيثانولي لنبات النيم على الاستجابة المناعية للفئران الملقحة بلقاح بكتريا البر وتيس *Proteus vulgaris*.تضمنت الدراسة ثمانية مجموعات ( 40 فأر في كل مجموعة) وتمت معاملة المجاميع كما يلي (I: معاملة بالماء المقطر، II: معاملة بمستضد بكتريا البر وتيس فلكارس فقط ، III: معاملة بالمحلول الأيثانولي لنبات النيم بجرعة مقدارها 600 ملغم لكل كغم ، IV: معاملة بالمحلول الأيثانولي لنبات النيم بجرعة مقدارها 400 ملغم لكل كغم ة ، V: معاملة بالمحلول الأيثانولي لنبات النيم بجرعة مقدارها 600 ملغم لكل كغم ومستضد بكتريا البر وتيس فلكارس معا ،VI: معاملة بالمحلول الأيثانولي لنبات النيم بجرعة مقدارها 400 ملغم لكل كغم و مستضد بكتريا البر وتيس فلكارس ، VII : معاملة بالمحلول الأيثانولي لنبات النيم بجرعة مقدارها 600 ملغم لكل كغم ومستضد بكتريا البر وتيس فلكارس معا، VIII: معاملة بالمحلول الأيثانولي لنبات النيم بجرعة مقدارها 400 ملغم لكل كغم و مستضد بكتريا البر وتيس فلكارس وتم حقن المجموعتين الأخيرتين بمقدار 5 ملغم لكل كغم من وزن الجسم من مادة البر وسلون (Prednisolone) المثبطة للمناعة قبل 5 أيام من أجراء المعاملة . أجريت جميع هذه المعاملات في اليوم الأول في اليوم الرابع وضحي بالحيوانات في اليوم 8 (معامل اختزال ملون ;Nitro blue tetrazolium NBT) مقاسا بجهاز الاليزا ، في اليوم 14 (معامل تفاعلات فرط الحساسية المتأخرة ومعامل تحول الخلايا اللمفاوية المقاسة بطريقة الاليزا )في اليوم 21 و 28 (عيارية أضداد البر وتيس والهجرة الكهربائية لبروتينات المصل). أظهرت النتائج تأثيرات واضحة للمحورات المناعية المستخدمة في الدراسة وفي المجاميع الممنعة بمستضد البر وتيس فلكارس ومن خلال تحسن الاستجابة المناعية غير النوعية والمناعة الخلوية الخلطية مقارنة مع المجاميع غير المعاملة بالمحورات المناعية، وأشارت النتائج إلى ارتفاع ملحوظ في نسبة الخلايا الموجبة لفحص NBT في المجاميع الممنعة والمعاملة بالمحورات المناعية وبفوارق إحصائية معنوية بالمقارنة بمجاميع السيطرة السالبة (معاملة بالماء المقطر فقط) والسيطرة الموجبة المعاملة بالمحورات المناعية أو اللقاح فقط . وقد ظهر ارتفاع في النسبة المئوية لمعامل الخلايا الموجبة لفحص NBT في كل المجاميع وسجلت المجموعة V أعلى نسبة مئوية للخلايا الموجبة لفحص NBT ( ٥٧% ) وبفوارق إحصائية معنوية ($P\leq 0.01$) وسجلت المجموعة IV ادنى نسبة مئوية (5%) مقارنة بمجموعة السيطرة السالبة I ( 0% )، كما لوحظ ارتفاع واضح في النسبة المئوية لمعامل التحول للخلايا اللمفية في الدم المحيطي ، حيث أعطت جميع الحيوانات الممنعة والمعاملة بالمحورات المناعية نتائج أعلى من تلك الممنعة والغير معاملة بالمحورات المناعية وبفوارق إحصائية معنوية وسجلت المجموعة V أعلى مستوى للنسبة المئوية لمعامل تحول الخلايا اللمفاوي في الدم (264%) ، بينما أعطت المجموعة IV ادنى نسبة وهي ( 17% ) . وعند قياس عيارية أضداد anti *proteus vulgaris* بواسطة الوميض الإشعاعي غير المباشر، أظهرت المجاميع الممنعة والمعاملة بالمحورات المناعية ارتفاع مستوى هذه الأضداد مقارنة بمجاميع السيطرة السالبة والموجبة

وبفوارق إحصائية معنوية بمستوى ($P \leq 0.01$) وسجلت اعلى معيار حجمي لتلك الأضداد بمستوى 512بعد 21يوم التمنيع