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In Vivo Pathogenicity of Actinomycetes *(Nocardiopsis dassonvillei)* spores on male albino rats.

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Abstracts: *Nocardiopsis dassonvillei* was isolated from the **i**ndoor air of critical areas of hospitals and its pathogenicity was evaluated in vivo. Male albino rats were injected I.P with *Nocardiopsis dassonvillei* spores (1×10^7/rat) for 2 weeks. Different changes in haematological and biochemical parameters after one and two weeks of actinomycete spores injection were assessed. Nocardiopsis infection caused RBCs count decrease and significantly decline Hb concentration compared to control group, while significant increase in WBCs, Neutrophil, Lymphocytes and Platelets were observed. Hepatotoxicity of Nocardiopsis was also investigated through severe drop in the activity of liver function markers (ALT, AST) after 2 weeks. Moreover, it induced hepatic apoptosis which is detected by caspase 3. It can be concluded that exposure to *Nocardiopsis dassonvillei* spores may cause haematological , biochemical disorders and heptotoxicity,

Keywords: Nocardiopsis, Indoor air, Hospital critical areas, haematology and pathogenicity

I. Introduction

Biological agents in indoor air are known to cause three types of human disease: infections, where pathogens invade human tissues; hypersensitivity diseases, where specific activation of the immune system causes disease; and toxicosis, where biologically produced chemical toxins cause direct toxic effects. In addition, exposure to conditions conducive to biological contamination (e.g., dampness, water damage) has been related to nonspecific upper and lower respiratory symptoms (Burge and Harriet, 1990). The presence of spore-forming actinobacteria in indoor environments may indicate a risk to human health but do not specify any genera or species (Anonymous, 1997).

Nocardiopsis like most other clinically important actinomycetes, their natural habitat are the soil and it is opportunistic rather than invasive pathogens. They have been especially implicated in cutaneous and subcutaneous and also in respiratory diseases (McCarthy, 1989; Bowden & Goodfellow, 1990). *Nocardia* are primary soil organisms and most live as saprophytes. Infections with pathogenic *Nocardia* do occur, and these can affect many different sites, including the brain, lung and other epithelial cell-lined organs (Beaman & Beaman, 1994).

Manifestations of nocardiosis vary depending on the infecting bacterial species and the organ related. In the lung, illness occurs due to infiltration of responding inflammatory cells, leading to pneumonia or abscess formation. In addition, granulomas can be formed as seen in tuberculosis. In the brain, infections are usually associated with meningitis or abscess formation, and may manifest as a variety of neurology disorders (Beaman & Beaman, 1994).

Some species, especially *Nocardia brasilliensis* can cause mycetomas, chronic infections initiated at the site of an infected puncture wound that can spread through and destroy surrounding muscle and bone (Salinas 2000). The mechanism by which *Nocardia* cause disease is not been studied in depth. The bacterial cell surface appears to play a role in its pathogenesis. The composition of the cell envelope varies depending on the stage of growth, and these differences correlate with bacterial virulence (Beaman & Moring, 1988). Pathogenic *Nocardia* also possess counter measures to evade the host immune system. These bacteria produce a superoxide dismutase that allows them to withstand the oxidative burst in phagocytes (Beaman *et al.,* 1985; Beaman & Beaman, 1990). In addition, some strains can inhibit phagosome –lysosome fusion and block the functions of these compartments (Davis & Beman, 1980; Black *et al.,* 1986).

The production of toxin –like substances may also play some part in pathogenesis, as it has also been reported in some strains of *Nocardia* (Emeruwa, 1986; Mikami *et al.,* 1990). These findings suggested that *Nocardia* were directly causing the death of eukaryotic cells, potentially by apoptosis.Therefore this study was designed to illustrate the microbial air quality within the special units of the hospitals and investigate the possible *in vivo* toxicity of the hospital air borne actinomycetes on male albino rats.

II. Materials And Methods

A- Source of the isolate

The isolate used in this study was isolated from the intensive care unit (ICU) of a private-sector hospital and identity was confirmed as *Nocardiopsis dassonvillei* H1(*N. dassonvillei*), based on 16Sr DNA sequencing (El-Shatoury *et al*., 2012).

B- **Experimental Design:**

Male albino rats weighting 100-120 g were obtained from the Egypt Cancer Institute. After an adaptation period of two weeks, the animals were divided into two groups comprising of (5-8) animals. The 1st group was injected intraperitoneally (I.P) with normal saline solution (0.9%) and represented the control group (n=5). The 2nd group was injected I.P with (1×10^7/rat) *Nocardiopsis dassonvillei* spores (n=8) according to (Popov *et al*., 2004). The two groups were kept in separate cages with continuous supply of food and water *ad libithum*. The animals were examined daily for 2 weeks where they were kept under observation for clinical and pathological changes. All animals were weighed before and after the injection every week.

C- Hematological Assay:

Blood samples were taken after one and two weeks of actinomycetes spores injection. Blood was taken in vials with anticoagulant (potassium- EDTA). Whole blood samples were used for the analysis of haematological parameters; red blood cells (RBCs), white blood cells (WBCs), hemoglobin concentration (Hb), haematocrit (HCT), platelets count (PLT), the mean corpuscular volume (MCV) level, the mean corpuscular hemoglobin (MCH) content, the mean corpuscular hemoglobin concentration (MCHC) content. Moreover differential leukocyte count (the percentage of lymphocyte cells and the percentage of neutrophils cells) were estimated. The complete blood capture (CBC) was done by cell counter system (Cell-DYN 3700).

D- **Biochemical Assay:**

The serum samples were used for the, analysis of liver enzymes, Aspartate amino transferase(AST) and Alanine amino transferase (ALT). The liver enzymes were measured by the automated system (Olympus AU 640).

E- Immunohistochemical Examinations:

All rats in the two groups were sacrificed and dissected. Their liver, was separated and fixed in 10% formalin and dehydrated in ascending series of ethanol, cleared and embedded in paraffin wax. Paraffin sections of 5 µ in thickness were prepared and stained with streptavidin-biotin-peroxidase staining method to detect apoptotic marker (caspase-3) according to Elias (1989) in the Egypt Cancer Institute.

Statistical analysis:

Analysis of Variance (ANOVA) and unpaired t- test were performed using data analysis tool of Microsoft Excel XP. The differences were considered significant at $P \leq 0.05$.

III. Results

Weight of rats:

All rats were weighed after one and two weeks of after i.p. injection of (1×10^7) *N. dassonvillei* spores. There was no significant change in weight after one week. On the other hand, a highly significant decrease in the weight of rats after two weeks ($p<0.05$). The results are demonstrated in Figure (1).

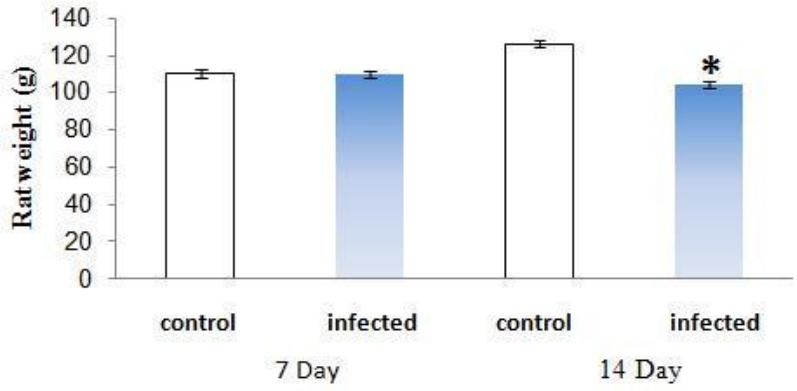

Figure (1) Rat body weights after i.p. injection of (1×10^7) *N. dassonvillei* spores in one and two weeks.
Values represent means ±S.E. (n=5-8). ***** $P \leq 0.05$ Significantly different from control (unpaired t- test).

Hematological Assay:

Hematological alterations resulting from the I.P. injection of (1×10^7) *N. dassonvillei* spores per rat are presented in Tables (1). It was observed that RBCs count and HCT% non significantly decreased after 7 and 14 days. In the same time, hemoglobin content was significant decrease after two weeks of infected ($P<0.05$). On the other hand, platelets count was significantly increased after two weeks of infection ($P<0.05$)**.**

Table (1) Change of hematological parameters of rats after i.p. injection of (1×10^7/rat) *N. dassonvillei* spores in one and two weeks.

Parameter	Time	Control	infected group
RBCs(10^6/UL)	7 days 14 days	7.24±0.18 7.24±0.19	6.67±0.28 6.78±0.14
HCT (%)	7 days 14 days	42±1.22 43±0.70	38.40±1.7 41.50±0.5
HB (g/dl)	7 days 14 days	13.34±0.34 13.60±0.13	12.40±0.56 12.90±0.21*
MCV (fl)	7 days 14 days	57.62±0.88 59.2±1.88	57.10±0.72 60.75±0.86
MCH (pg)	7 days 14 days	18.30±0.177 18.66±0.46	18.05±0.11 18.97±0.18
MCHC (g/dl)	7 days 14 days	31.78±0.40 31.56±0.33	31.47±0.33 31.23±0.24
PLT $\times10^3$ (UL)	7 days 14 days	490±58.81 655±70.07	447±35.49 860±60.84*

Values represent means ±S.E. (n=5-8). ***** $P \leq 0.05$ significantly different from control (unpaired t- test).

As seen in Figure (2A), infected rats with *N. dassonvillei* spores revealed a significant increase of white blood cells (WBCs) ($P<0.05$).Figure (2 B) show that segmented cells % (neutrophil) and lymphocyte cells % significantly increased ($P <0.05$) after one week although after two weeks non significant change was noticed in neutrophil cells % but Lymphocyte cells % showed significant increase ($P <0.05$) compared to control group. No significant changes occurs of monocytes, eosinophil or basophils percentage neither after one nor two weeks of infection ($P >0.05$).

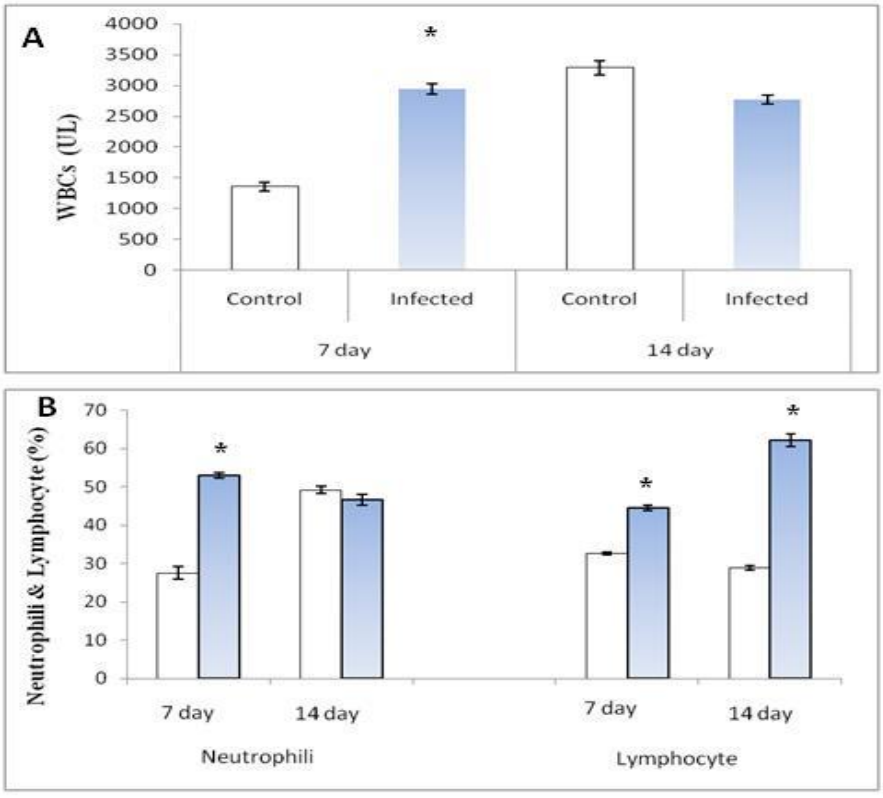

Figure (2 A & B) Changes in (A) total count WBCs, (B) Lymphocyte cells (%) and Neutrophil cell (%) after i.p. injection of *N. dassonvillei* spores per rat in one and two weeks.Values represent means ±S.E. (n=5-8).
***** $P \leq 0.05$ significantly different from control (unpaired t- test).

Biochemical Assay:

The indicators of the liver function were assessed by estimating of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) level in the serum. The present data demonstrated that I.P. injection of a dose (1×10^7) of *Nocardiopsis dassonvillei* spores did not exhibit significant increase of liver enzymes ($P > 0.05$) after one week while after two weeks there were highly significant decline in liver enzymes ($P < 0.05$). The results are demonstrated in Figure (3A, B).

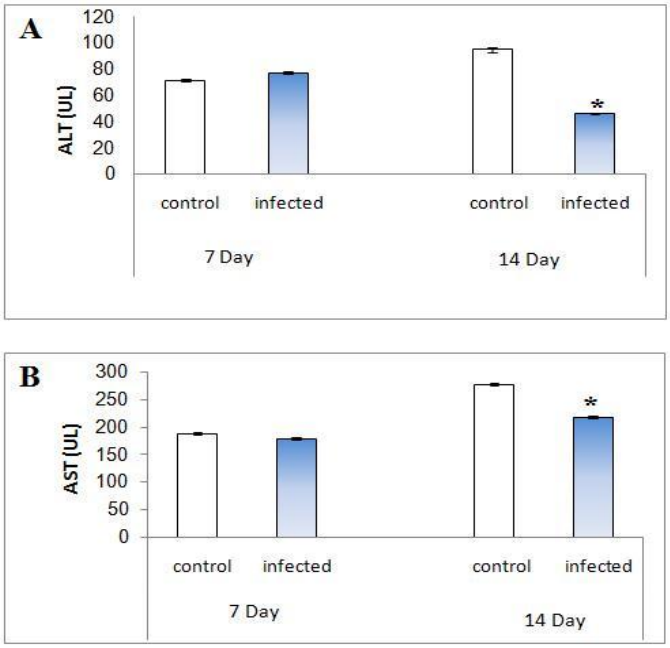

Figure (3 A & B) liver enzymes (ALT) and (AST) level after I.P. injection of *N. dassonvillei* spores in one and two weeks. Values represent means ±S.E. (n=5-8). ***** $P \leq 0.05$ significantly different from control (unpaired t-test).

Immunhistochemical Examination

Immunohistochemistry examination of the liver sections were investigated with caspase-3 antibodies obtained from rats infected with *N. dassonvillei* spores showed apoptotic lineage cells in the liver tissue. The caspase-3 activity is illustrated in Figure (4). Caspase-3 immune-reactive cells were high levels observed around of the central vein and lobular areas in the infected with *N. dassonvillei* spores group. It was reported that the numbers of apoptotic cell were increased in the infected group when compared with control group.

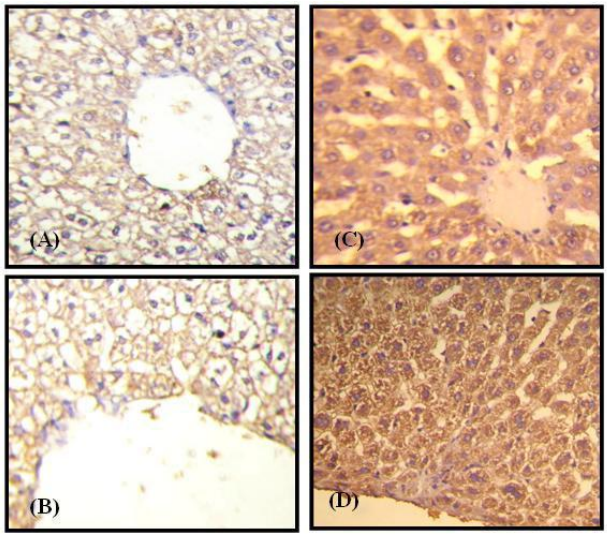

Figure (4) Caspase-3 positive reactions stained with streptavidin-biotin-peroxidase in rat liver tissue of control and *N. dassonvillei* infected rats-A & B show control liver with no apoptotic tissue - C &D show infected group with moderate and sever apoptotic tissue (40 X).

IV. Discussion

Evaluation of the toxicity responses in rats after administration of the spores of *N. dassonvillei* strain indicated that the rats lose their weight after two weeks comparing with control group this may be because actinomycetes cell wall contains endotoxin polysaccharide that can cause loss of body weight. Ekizlerian et *al.*(1986) indicated that heat-killed *Actinomadura madurae, 'Streptomyces pelletieri'and Nocardia brasiliensis* contain components with toxic and inflammatory properties; producing loss of body weight, formation of inflammatory exudates, granulomas and death when inoculated into mice. The initial chemical analysis of these toxic component showed that they were composed mainly of polysaccharide and the mycolic acid whichwas only detected in the lipid extract of *N. brasiliensis*.

El-Shatoury *et al*. (2012) reported that *Nocardia* like group were represented in ICU, with high frequency of resistance to antibiotics and also had an ability to cause blood haemolysis *in vitro* which is consistent with the observed

decline of RBCs and hemoglobin % in rats infected with *N. dassonvillei* strain.

In the current study there was a significant increase in white blood cells and platelets count, which increased after two weeks in the infected rat group. Both of them are important and necessary response to bacterial infection and contain antimicrobial peptides that act against a broad range of pathogens (Elzey *et al.,* 2005 and Fitzgerald *et al.,* 2006) demonstrated that platelets are an integral part of inflammation and can also be potent effector cells of the innate immune response. The platelet (CD154 molecule) has a vital importance to adaptive immune responses, is expressed by activated platelets and has been implicated in platelet-mediated modulation of innate immunity and inflammatory disease states (Elzey *et al.,* 2005, Sprague *et al*., 2007)

Antimicrobial peptides from white blood cells and platelets exert a rapid, potent and direct antimicrobial effect that contributes to limiting the infection. In addition, both of neutrophil and lymphocyte cells increased after one week, although after two weeks only lymphocyte cells increased. This is because neutrophils are normally found in the blood stream during the beginning (acute) phase of inflammation, particularly as a result of bacterialinfection, environmental exposure and some cancers where neutrophils are one of the first-responders of inflammatory cells which migrate towards the site of inflammation (De Larco *et al.* 2004, Waugh and Wilson, 2008 , Jacobs *et al.,* 2010). On the other hand, lymphocytes are the second-responders of inflammatory cells, Beaman and Beaman (1994) reported that mixed lymphocytes and macrophages were present in chronic phase of nocardial infection.

ALT and AST levels as indicators of the liver function decreased in infected group after 2weeks compared with control group. This might be due to a sudden death of cells which was approved by presence of moderate and sever apoptotic tissues using immunohistochemical examination of the liver tissues with caspase-3 antibodies. In addition, pervious histopathological results (El-Shatoury *et al*., 2012) confirmed the hepatotoxicity of *N. dassonvillei* where different pathological changes in liver cells were noticed as hydropic degeneration, hepatic necrosis and apoptosis which characterized by Karyolysis, Karyorrhexis and Pyknosis; according to Knight *et al*. (2005) liver cell necrosis may be due to inhibition of synthesis of DNA needed for the growth and maturation of the liver.

In general, exposure to indoor air microbes or their products has been suspected to cause immunosuppression and the increased frequency of infections among occupants in moisture-damaged buildings. So future studies should be focus on the factors affecting indoor air distribution and control in hospital critical areas. Health care facilities, such as hospitals, have to pay particular care to prevent the spread of airborne infectious diseases as well.

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References

[1]. Anonymous. 1997. Indoor air guideline. Edita, Helsinki. Applied Microbiology, 10:567–71. (In Finnish.)
[2]. Beaman, L. and Beaman, B.L . 1990. Monoclonal antibodies demonstrate that superoxide dismutase contributes to protection of *Nocardia asteroids* within the intact host. Infect immune, 58(9):3122-3128
[3]. Beaman, B.L. and Beaman, L. 1994. Nocardia species: host-parasite relationship. Clin. Microbiol. Rev., 3:213-264.
[4]. Beaman, B.L. and Moring, S.E. 1988. Relationship among cell wall composition, stage of growth, and virulence of *Nocardia asteroids* GUH-2. Infect Immune, 56(3):557-563.
[5]. Beaman, B.L., Black, C.M., Doughty, F. and Beaman, L. 1985. Role of superoxide dismutase: importance in resistance of microbicidal activities of human polymorphonuclear neutrophils. Infect immune, 47:135-141.
[6]. Black, C.M., Paliescheskey, M., Beaman, B.L., Donovan, R.M. and Goldstein, E. 1986. Acidification of phagosomes in murine macrophages: blockage by *Nocardia asteroids*. J Infect Dis., 154(6):952-958.

[7]. Bowden, G.H. and Goodfellow, M. 1990. The actinomycetes: Actinomycetes, Nocardia and related genera. In: Topley and Wilson's Principles of Bacteriology, Virology and Immunity, 8thEdn., Vol. 2 (Parker M.T. and Duerden, B.I., Eds.), p. 51. Edward Arnold Advision of Hodeler and Stoughton, London.
[8]. Burge, H. A. 1990. Bioaerosols: Prevalence and Health Effects in the Indoor Environment. Allergy Clin Immunol., 86:687-701.
[9]. Davis, S.C. and Beaman, B.L. 1980. Interaction of *Nocardia asteroids* with rabbit alveolar macrophages: association of virulence, viability, ultrastructural damage, and phagosome lysosome fusion. Infect Immun., 28(2):610-619.
[10]. De Larco, J.E.,Wuertz, B.R.andFurcht, L.T. 2004. The potential role of neutrophils in Promoting the metastatic phenotype of tumors releasing interleukin-8. *Clin Cancer Res*.,10 (15): 4895–900.
[11]. Ekizlerian,S. M., Brandaofilho, S.L. andSillva,C. L.1986. Mouse Toxicity Induced by Lipids and Cell Walls Isolated from Actinomycetes.J Gen Microbiol., 132(9): 2647-2651.
[12]. Elias, J.M., Margiotta, M. and Gaborc, D. 1989. Sensitivity and detection efficiency of the peroxidase antiperoxidase (PAP), avidin-biotin peroxidase complex (ABC), peroxidase-labeled abidin-biotin (LAB) methods. Am J Clin Pathol., 92:62–67.
[13]. Elias, J.M., Margiotta, M. and Gaborc, D. 1989. Sensitivity and detection efficiency of the peroxidase antiperoxidase (PAP), avidin-biotin peroxidase complex (ABC), peroxidase-labeled abidin-biotin (LAB) methods. Am J Clin Pathol., 92:62–67.
[14]. El-Shatoury S. A., Saad M. M., Ahmed S. H. and Nabil Z. I. (2012). Toxicity of Actinomycetes isolated from the indoor air of Hospitals. Egyptian Journal of Natural Toxins, Vol. 9(1,2) – under publication
[15]. Elzey, B.D., Sprague, D.L. and Ratliff, T.L. 2005. The emerging role of platelets in adaptive immunity. Cell Immunol., 238 (1): 1-9.
[16]. Emeruwa,A.C. 1986. Isolation and some properties of beta-hemolysin produced by *Nocardia asteroids* Mycopathologia, 95(1):29-35.
[17]. Fitzgerald, J.R., Foster, T.J.andCox, D. 2006. The interaction of bacterial pathogens with platelets. Nat Rev Microbiol., 4 (6): 445 - 457.
[18]. Rottenberg, H., Waring, A. and Rubin, E. 1984. Alcohol- induced tolerance in mitochondrial membranes. Science, 223: 193 – 194.
[19]. Jacobs, L., Nawrot, T. S., De Geus, B., Meeusen, R., Degraeuwe, B., Bernard, A., Sughis, M., Nemery, B. and Panis, L.I. 2010. Subclinical responses in healthy cyclists briefly exposed to traffic-related air pollution. Environ. Health, 9 (64): 64.
[20]. Knight, B., Yeap, B.B., Yeoh, G.C. andOlynyk, J.K.2005. Inhibition of adult liver progenitor (oval) cell growth and viability by an agonist of the peroxisome proliferator activated receptor (PPAR) family member γ, but not α or δ.Carcinogenesis, 26 (10):1782–1792.
[21]. McCarthy, A.J. and Williams, S.T. 1992. Actinomycetes as agents of biodegradation in the environment - a review. Gene, 115 (1-2): 189-192.
[22]. Mikami,Y.,Yu, S.F., Yazawa, K.,Fukushima, K., Maeda, A,Uno, J., Terao, K., Saito, N., Kubo, A. and Suzuki, K. 1990. A toxic substance produced by *Nocardia otitidiscaviarum* isolated from cutaneous nocardiosis. Mycopathologia, 112 (2):113-118.
[23]. Oudea, M.C.; Collette, M. and Oudea, P. 1973 a. Morphometric study of the ultrastructure changes induced in rat liver by chronic alcohol intake. Dig. Dis., 18 (5):398 – 402.
[24]. Oudea, M.C., Collette, M. Dedien, P.H. and Oudea, P. 1973 b. Morphometric study of the ultrastructure of human alcoholic fatty liver. Biomedicine, 19 (10):455 – 459.
[25]. Popov, S.G., Popova, T.G., Grene, E., Klotz, F., Cardwell, J., Bradburne C, Jama, Y., Maland, M., Wells, J., Nalca, A., Voss, T., Bailey, C. and Alibek, K. 2004. Systemic cytokine response in murine anthrax. Cell Microbiol., 6:225-233.
[26]. Salinas- Carmonas, M.C. 2000. *Nocardia brasiliensis*: From microbe to human and experimental infections. Microbes infect., 2 (11):1373-1381.
[27]. Sprague, D.L., Sowa, J.M., Elzey, B.D., Ratliff, T.L. 2007. The role of platelet CD154 in the modulation in adaptive immunityImmunol Res.,39(1-3):185-93.
[28]. Waugh, D.J.; Wilson, C. 2008. The interleukin-8 pathway in cancer. Clin Cancer Res.14 (21): 6735–41.
[29].