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Effect of chemical industry effluent on macrophage functions of mice

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ABSTRACT

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To study the effect of chemical industry effluent on macrophage functions in mice, 128 mice of 2 weeks age were procured and divided in four equal groups. These mice were vaccinated with Ranikhet disease vaccine (R_2B) strain intraperitonealy @ 0.1ml and were treated with chemical industry effluent as drinking water for 4 months. The functional activity of macrophages was assessed by nitroblue tetrazolium reduction assay. The test was conducted at 90 days and 120 days of experiment. The results indicated a significant depression of macrophage functions as evidenced by a reduction in NBT positive cells in chemical industry effluent treated mice at 90 days and 120 days of experiment in comparison to controls. This depression of macrophage activity may result in increased susceptibility of host to various infections.

Keywords: Chemical, effluent, macrophage function, mice, NBT.

Environment is referred to as the interaction of all social, biological, physical or chemical factors, which make up the surrounding of all creatures. Since old times, pollution of the environment has been an undesirable part of man's activity. Industrialization and technologies have added another dimensions to environmental pollution.

It is central to modern world economy, converting raw materials (oil, natural gas, air, water, metals, minerals) into more than 70,000 different products. Polymers and plastics, especially polyethylene, polypropyl, polyvinyl chloride, polyethylene terephthalate, polystyrene and polycarbonate, comprise about 80% of the industry's output worldwide. Effluents from chemical industries are released abundantly into the environment many times without proper treatment. Environmental pollution as a result of industrial waste and use of pesticides, fertilizers has lead to increase level of heavy metals in ecosystem and food chain. Heavy metals such as cadmium, lead, mercury etc present in soil, water, air, food and feed additives may exert deleterious effect on immune system of animals and man[1]. The immunosuppressive activity of the heavy metals have been correlated with their toxic potential and inhibition of lymphocyte proliferation[2] and, or by stimulating synthesis and secretion of corticosteroids[3]. Keeping in view the nature of chemical industry effluent, a survey was conducted around chemical industries and effect of chemical industry effluent was studied on macrophage functions in mice.

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For the proposed study, samples of effluent were collected from and nearby effluent passing area through the naala near Jubilant organosys (Gajraula). The samples of effluent were collected using standard sampling protocol and brought to the laboratory. The toxicity of the effluent was studied in laboratory animals (mice) by giving effluent water *ad libitum* for four months.

To study the effects of chemical industry effluent in mice, 128 mice of 2 weeks age were procured from Laboratory Animal Resource Section, IVRI, Izatnagar, Bareilly. Before keeping the mice, the experimental house was thoroughly cleaned with water and then with 1% phenyl solution. Cages, waters and feeders after washing with water and phenyl solution were cleaned with potassium permanganate solution.

Total 128 mice were randomly divided into four groups of 32 mice each viz. control (group-1), R2B vaccine+effluent treated (group-2), effluent treated (group-3) and R2B vaccinated (group-4). The vaccine used was R2B strains given to group-2 and 4 @ 0.1 ml by intraperitoneal route. The experiment was conducted with permission from IAEC and the mice were housed under ideal conditions of hygiene and management as per the guidelines of CPCSEA. The mice of effluent treated and vaccine + effluent treated groups were given *ad libitum* effluent water for drinking. Mice of all groups were maintained on the feed supplied by the Animal Experimental House of College of Veterinary & Animal Sciences, Pantnagar. Mice from each group were sacrificed at 90 and 120 days of experimentation for estimation of functional activity of macrophages.

Table. Macrophage function of experimental mice by NBT reduction assay (Mean % NBT positive cells ± SE).

Groups	Days of Observation	
	90	120
Control (Gp. 1)	24.0±0.80	24.5±0.20
Effluent + vaccine treated(Gp. 2)	20.0±0.40*	18.4±0.10*
Effluent treated (Gp. 3)	20.2±0.09*	18.0±0.90*
Vaccine treated (Gp. 4)	24.5±0.60	25.1±0.04

* P<0.05

The metabolic activity of phagocytic cells was measured by the Nitro-Blue-Tetrazolium (NBT) reduction assay employed at 90 and 120 day by the modified procedure described by Talwar[4] and Chauhan[5]. For this a total 100 ml of NBT solution (0.2 %) was taken in a clean eppendorf, a 50 ml of blood with EDTA was added to it, followed by the addition of 20 ml stimulant (LPS). The tubes were then rolled gently within palm for mixing. After mixing, the vials were kept at 37°C for 10 minutes in incubator, followed by incubation at room temperature for 30 minutes. The vials were again rolled gently for mixing. A smear was prepared by taking a drop on the slide and dried gently in air. The smear was then stained with Wright's stain for 30 seconds and followed by addition of distilled water (1ml) for 30 second. The smear was rinsed with water and dried in air and NBT positive cells were counted under oil immersion objective.

The NBT exposed cells which were able to convert yellow coloured dye into distinctive bluish dark granules were designated as NBT positive cells and expressed as per cent positive cells. The NBT reduction test was performed at 90th and 120th day in different groups and the results are summarized in Table.

The percentage of NBT positive cells decreased significantly (p<0.05) at 90th and 120th day in groups 2 and 3 as compared to groups 1 and 4. The values in groups 2 and 3 on 120th day were 18.4±0.10 and 18.0±0.90, respectively. The mean % of NBT positive cells on day 120 in groups 1 and 4 were 24.5±0.20 and 25.1±0.04, respectively.

Significantly reduced natural killer cell activity in animals exposed to mercury was recorded[6]. However, no alteration in macrophage function of mouse peritoneal macrophages on exposure to environmental pollutants lead and cadmium has been recorded[7]. In the present study, the percentage of NBT positive cells decreased significantly (P≤0.05) at 90th and 120th day in group 2 and 3 as compared to groups 1 and 4 values. Similar finding were also observed in mice given paper and pulp industry effluent[8].

Macrophages which are involved in the generation of an immune response are adversely affected by cadmium toxicity and reason behind this could be the reduction of antigen recognition by macrophages and an impairment of optimal cell-cell contact that is considered as a potential mechanism of action[9,10]. A similar observation was also recorded in cadmium exposed animals[1]. The reduction in the number of active phagocytic cells in effluent and effluent + vaccine treated mice may also lead to decreased resistance to infections and occurrence of epidemics and vaccination failure.

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