



*Research Paper*

**ENUMERATION AND STRUCTURAL ASSESSMENT OF MURINE PERITONEAL MACROPHAGES EXPOSED TO CHROMIUM (VI): A PRELIMINARY STUDY**

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**Abstract**

Heavy metals have significant importance in altering the immune response. Chromium is found in the environment commonly in trivalent, Cr (III), and hexavalent, Cr (VI), forms. The reduction of Cr (VI) to Cr (III) results in the formation of reactive intermediates (ROS) that contribute to the cytotoxicity and genotoxicity. In this study mice were treated with chromium (VI) oxide (24 mg /kg body weight in 1 ml water / day for 21 days). Macrophage study was done after Iyengar et al. 1985. In treated group significant numbers of peritoneal macrophages were found to be pyknotic. The cellular death was confirmed by membrane blebbing and membrane rupture. Percentages of cells showing membrane blebbing was increased significantly after treatment. The result indicated that Cr (VI) induced toxicity may affect macrophage mediated immunity. So macrophage activation during heavy metal mediated reaction is an interesting area to be explored in future researches.

Key words: Macrophage, Blebbing, Apoptosis, Necrosis.

**INTRODUCTION**

A large number of biologically active substances, including heavy metals, may have direct or indirect effect on the immune system. Heavy metals have significant importance in altering the immune response by immunostimulatory or immunosuppressive mechanisms. Widespread pollution by heavy metals has important consequences for human health [1]. Among the toxic metals, chromium (Cr) is widely used in industries such as electroplating, alloy, steel manufacturing, leather tanning, metal finishing and pigment. Chromium commonly enters the environment in the effluents from these industries. Chromium is found in the environment commonly in trivalent, Cr (III), and hexavalent, Cr (VI), forms. The reduction of Cr (VI) to Cr (III) results in the formation of reactive intermediates that contribute to the cytotoxicity, genotoxicity and carcinogenicity of Cr (VI)-containing compounds [2]. It has been reported that inhalation of chromium does not affect lung morphology, but macrophages are enlarged, multinucleated or vacuolated and accumulate in intra-alveolar spaces as nodules [2]. Higher doses of Cr (VI) depress the phagocytic activity of alveolar macrophages and the humoral immune response, whereas lower doses of Cr (VI) stimulate phagocytic activity of the alveolar macrophages and increase the humoral immune response [3]. Chromium moderately suppresses inducible NO synthase, which suggests that it may directly modify enzyme or

cofactor activity [3]. Significant differences are found in the ability to phagocytose and level of respiratory burst elicited by macrophages of fish fed supplemented chromium [2]. The aim and objective of this study was to analyse the cytomorphology of peritoneal macrophage in mice, during chromium toxicity induced condition.

## MATERIALS AND METHODS

The study was carried out on healthy mice weighing between 80 to 100 g. The animals were housed in clean plastic cages under natural light and dark cycles at room temperature. Animals in all groups were fed normally *ad libitum* and allowed free access to water. All animals received human care.

After 5 days of acclimation, the animals were divided into two equal groups (n=5/group). Group I (Control group) were untreated animals. Group II animals were treated with chromium (VI) oxide (purified,  $\text{CrO}_3$ , M=99.99g/mol, MERCK, B.N. MH0M602462) 24 mg /kg body weight in 1 ml water / day for 21 days [4]. The i.p administration of Cr (VI) was selected for being the least aggressive [5].

The peritoneal cavity is a unique compartment within which a variety of immune cells reside, and from which macrophages are commonly drawn without altering their physiological properties. Sterile phosphate buffered saline (PBS), pH 7.2 was injected into mice peritoneum and the abdomen was gently massaged and the aspirate was taken for macrophage study [6]. Peritoneal fluid was placed and smeared directly on sterilized glass slides and incubated at 37°C in a humid chamber for 3 hours. After incubation the nonadherent cells were removed by washing three times with PBS. The adherent macrophages were fixed by methanol and stained by giemsa and methylene blue and observed under light microscope [6]. Cells were treated with 50  $\mu\text{l}$  of 0.25 % trypan blue solution for 5 minutes. Cells that have taken up the dye are dead, since the dye is normally excluded by the membranes which maintain their semi permeability intact and therefore, the percentage of blue-stained cells represents a mortality index. Cell counting was done by hemocytometer.

## RESULTS

### Cytomorphology of peritoneal macrophages

In treated group significant numbers of peritoneal macrophages were found to be pyknotic. The cellular death was confirmed by membrane blebbing and membrane rupture (Fig 1 B). Progressive stages of macrophage alteration were noticed in treated group when stained with giemsa and methylene blue (Fig 1 C). Percentage of cells showing membrane blebbing was increased significantly after treatment (Fig 1 D).

### Trypan blue (TB) response of macrophages

Significant number of treated mice peritoneal macrophages showed trypan blue positive response. Dead macrophages of mice were blue in colour whereas the viable cells of controls were white (Fig 2A and B). Mean mortality index was significantly increased in treated group (Fig3).

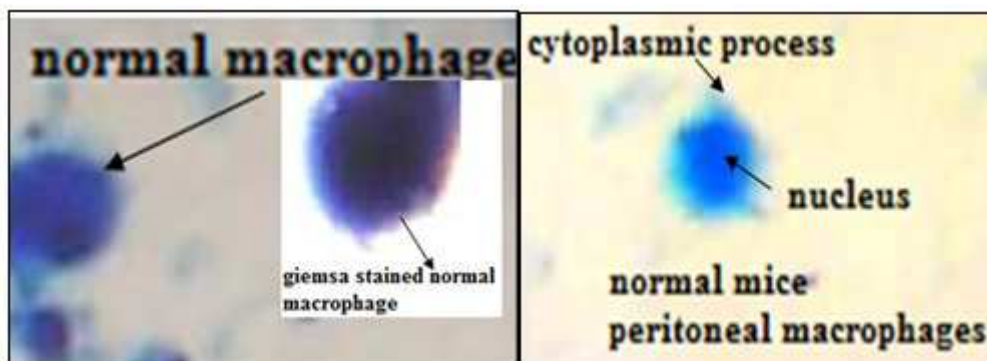


Figure 1 (A): Normal mice peritoneal macrophage (x 400).

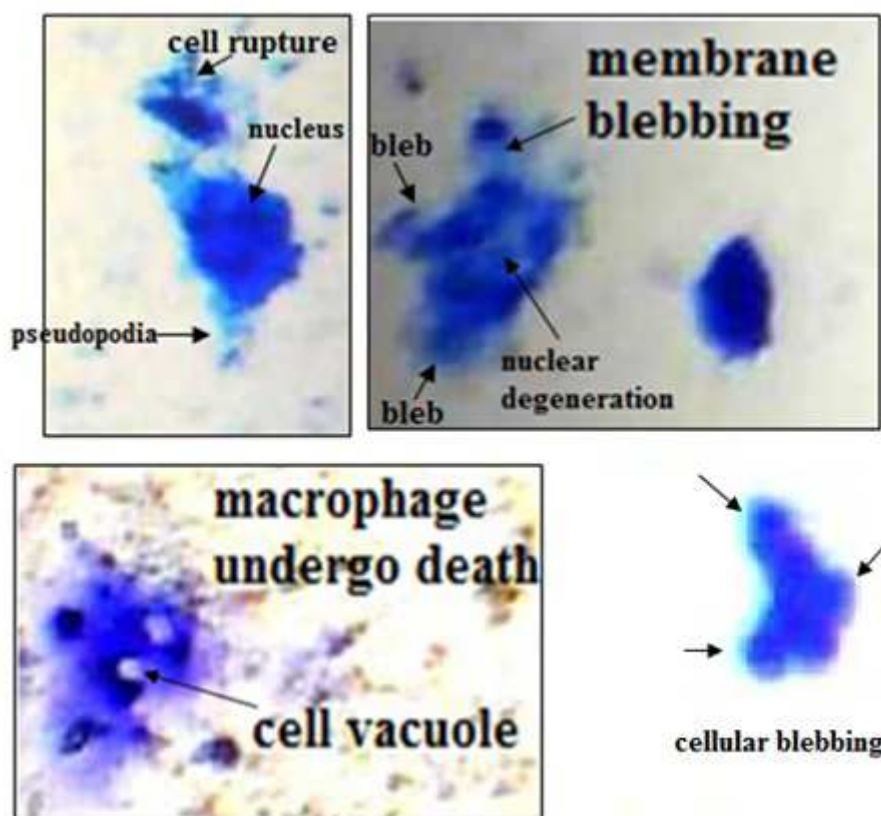


Figure 1 (B): Mice peritoneal macrophage with membrane blebbing and membrane rupture and vacuolation (x 400).

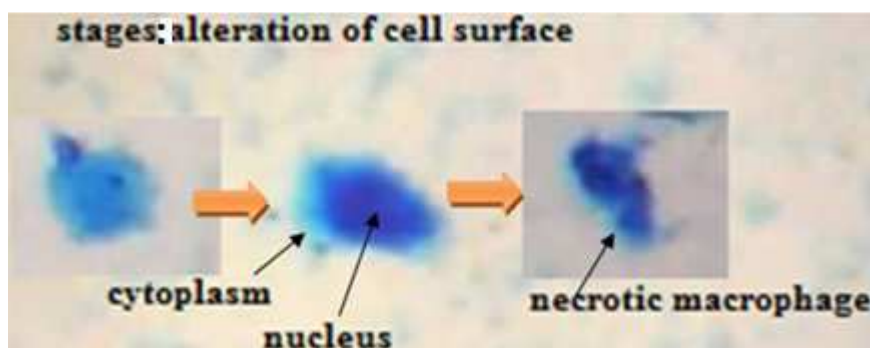


Figure 1 (C): Progressive stages of macrophage alteration (x 400).

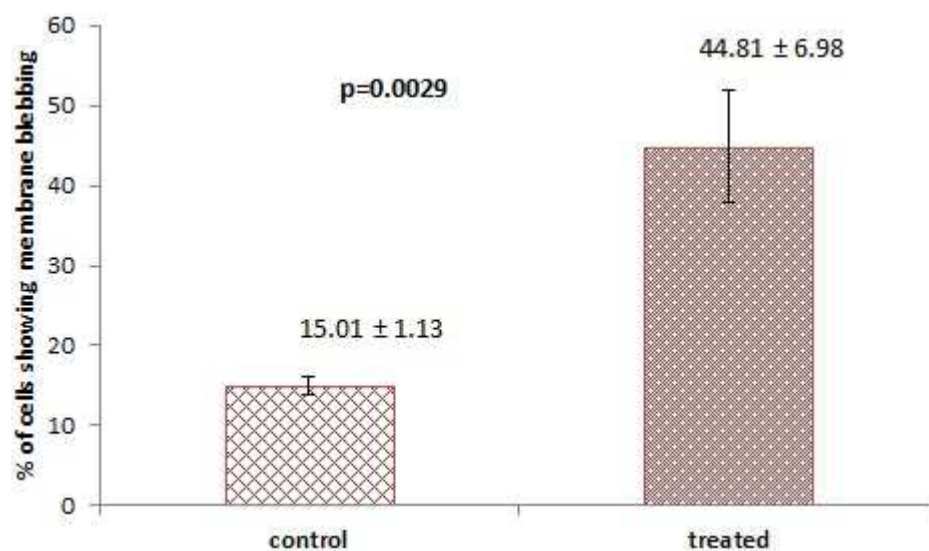


Figure 1 (D): Percentage of macrophages with membrane blebbing in normal and treated group. Values are expressed as Mean ± SEM

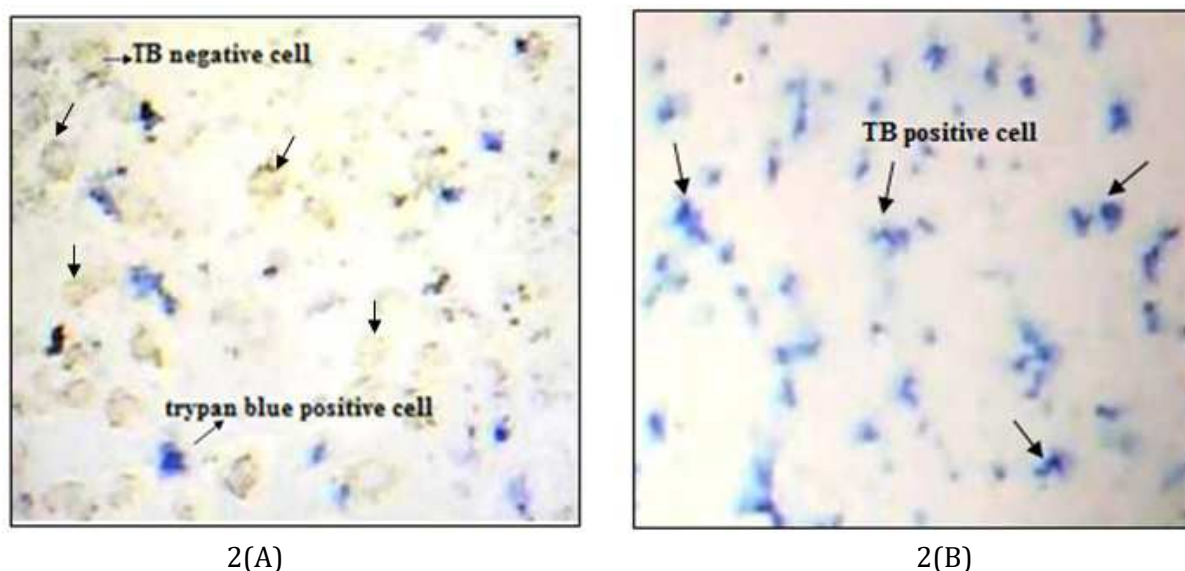


Figure 2 (A, B): (A) Normal mice viable (white) macrophages, (B) chromium treated dead (blue) macrophages

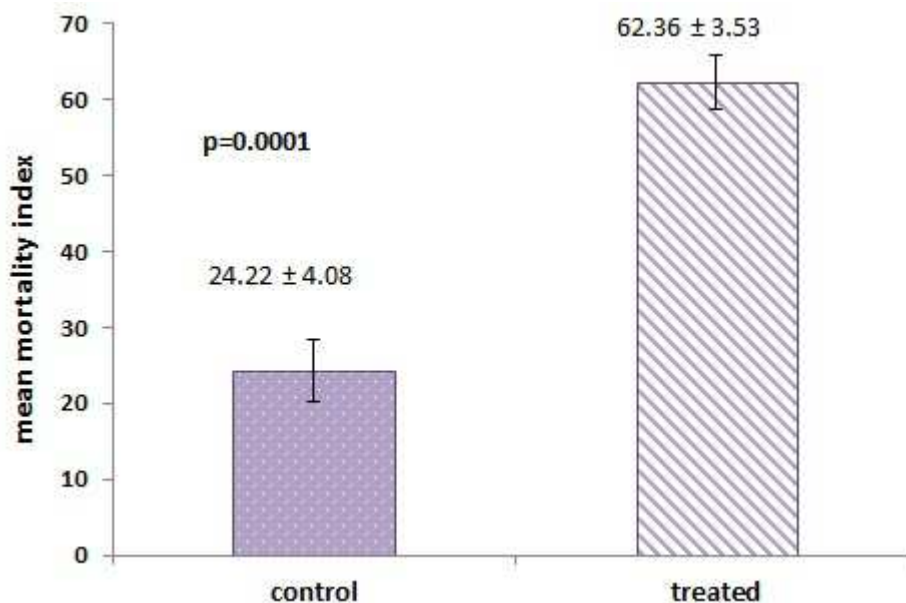


Figure 3: Mean mortality index in control and treated mice. Values are expressed as Mean  $\pm$  SEM

## DISCUSSION

In the present study significant changes were observed in the cytomorphology of peritoneal macrophages in the experimental group. Normal morphology was predominately found in the control cells, showing a well-defined plasma membrane and intact nucleus (Fig1A). Treated macrophages showed membrane blebbing, rupture of plasma membrane (Fig1B) and loss of viability (Fig2B and Fig3). The oxygen free radicals generated by chromium-mediated reactions may activate apoptosis which is a possible indicator of an immunosuppressive action. Previous researches reported that due to the inhalation of chromium macrophages are enlarged, multinucleated or vacuolated [2]. Higher doses of Cr (VI) depress the phagocytic activity of alveolar macrophages and the humoral immune response, whereas lower doses of Cr (VI) encourage phagocytic activity of the alveolar macrophages and increase the humoral immune response [3]. In this study result showed cell membrane rupture, nuclear degeneration, pyknosis which suggesting that cell death was mainly due to necrosis induction whereas some cells revealed membrane blebbing which is the indicator of apoptosis. After chromium treatment, some cells displayed vacuoles which may resemble another type of programmed cell death (PCD), called paraptosis (Fig 1B). Chromium may modify the defence via inhibition or enhancement of macrophage function. Our result indicates that Cr (VI) induced toxicity may affect macrophage mediated immunity. So macrophage activation during heavy metal mediated reaction is an interesting area to be explored in future researches.

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