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SHORT COMMUNICATION

Alteration of Cytomorphology of Peritoneal Macrophages of Albino Rat Exposed to Mercuric Compound

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ABSTRACT

Mercury is among the heavy metals that have been reported to cause devastating health problems worldwide. The present work was aimed at investigating the effects of mercury chloride on the cytomorphology of the peritoneal macrophages of rat. The current study characterizes the mechanism by which mercury, a toxic metal, induces death in rat macrophages. In mercury 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 mercury 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.

***Keywords*:** Macrophage, Mercury, Rat, Blebbing, Apoptosis

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1. INTRODUCTION

Heavy metals have significant importance in altering the immune response by immune-stimulatory or immunosuppressive mechanisms. Widespread pollution by heavy metals has important consequences for human health (Abdel-Moneim, AM and Abdel-Mohsen, HA, 2010). Mercury (Hg) is a well-known toxic agent that produces various types of tissue damage including immune-mediated manifestations, autoantibody synthesis, apoptosis and necrosis of immune cells or macrophages (InSug, O *et al*., 1997; Jiang, Y and Moller, G, 1995). Mercury has toxic effects in mammalian organ systems and decreased host resistance or immunity to viral infections (Christensen, MM *et al*., 1996).

Macrophages play an important role in various aspects of inflammatory responses, that is, immunity, host defense, and tissue repair (Morrissette, N *et al*., 1999). Macrophages produce ROS (reactive oxygen species) that play an important role in the ability of macrophages to kill pathogens and infected cells (Gregory, SH and Wing, EJ, 2002). Excess production of ROS induces undesirable biological reactions, including cell death (Buttke, TM and Sandstrom, PA, 1994). It has been reported that mercury regulates redox status that results in the induction of apoptosis in human T cells and monocytes and macrophages (InSug, O *et al*., 1997). The aim and objective of this study was to analyse the cytomorphology of rat peritoneal macrophage, during mercuric compound toxicity induced condition.

2. MATERIALS AND METHODS

The study was carried out on albino rat 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) as follow:

- Group I (Control group): Untreated animals.

- Group II animals were treated with 52.5 mg/kg body weight of mercury chloride (Hg). The administration was done by oral route daily and lasted for 3 weeks (Lucky, TD, 1987; Ibegbu, AO *et al*., 2014).

Sterile phosphate buffered saline (PBS), pH 7.2 was injected into rat peritoneum and the abdomen was gently massaged and the aspirate was taken for macrophage study (after Iyengar, UR *et al*., 1985). 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 (after Iyengar, UR *et al*., 1985; Guria, S and Das, M, 2011; Guria, S *et al*., 2012a; Guria, S and Das, M, 2012; Guria, S *et al*., 2012b; Guria, S, 2016; Guria, S and Das, M, 2016). Cells were treated with 50 µl of 0.25 % trypan blue solution for 5 minutes. Mortality index was calculated. Cell counting was done by hemocytometer. Lysosomal enzymes of cells were detected by neutral red.

3. RESULTS

Effect of mercury on peritoneal macrophages

Photographs of control macrophages displayed an intact nuclei, membrane and pseudopodia (Fig. 1 A and B). In treated group significant numbers of peritoneal macrophages were found to be pyknotic (Fig. 2A). Fragmented nuclei in cells were found in treated group (as indicated by arrows) (Fig 2 B). The cellular death was confirmed by membrane blebbing and membrane rupture. Progressive stages of macrophage alteration were noticed in treated group when stained with Giemsa (Fig. 2 C).

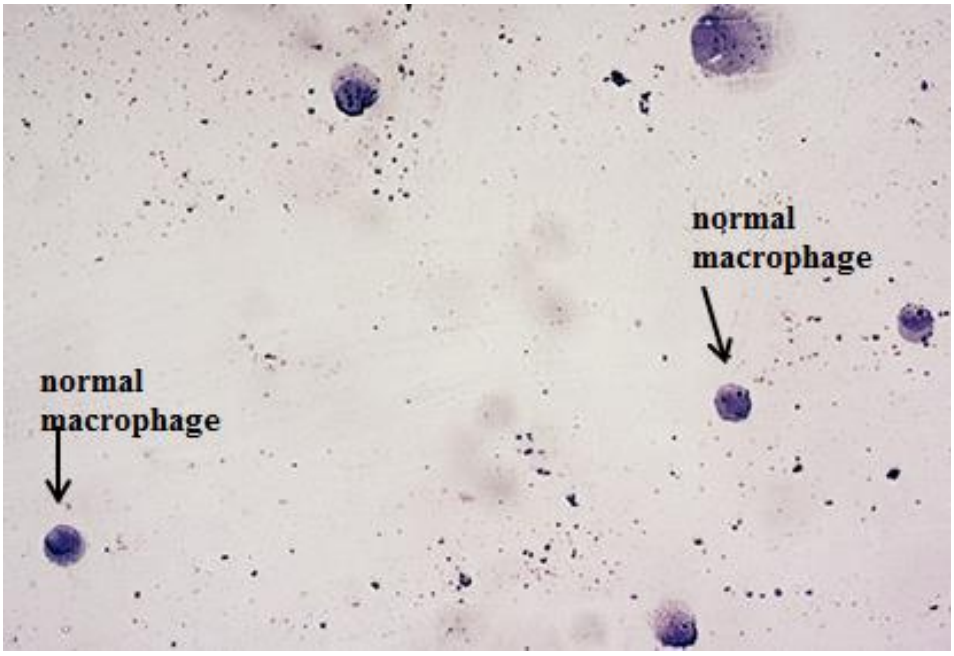

Figure 1 (A). Normal rat peritoneal macrophage population (x 100)

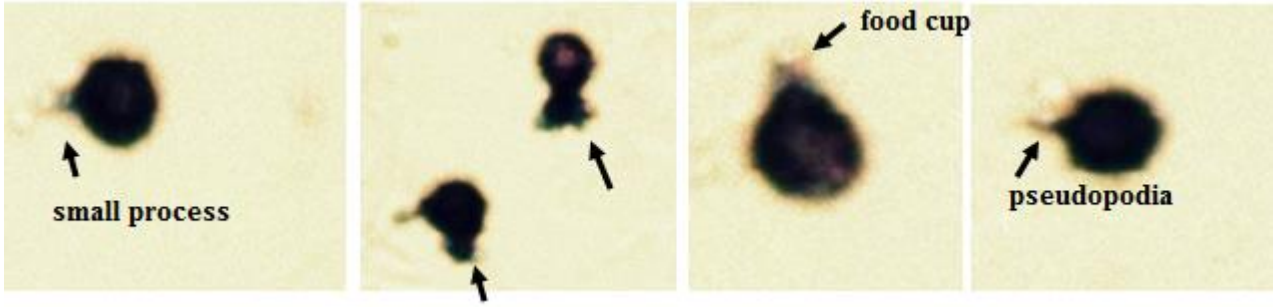

Figure 1 (B). Normal rat peritoneal macrophages with pseudopodia and short finger like process (x 400)

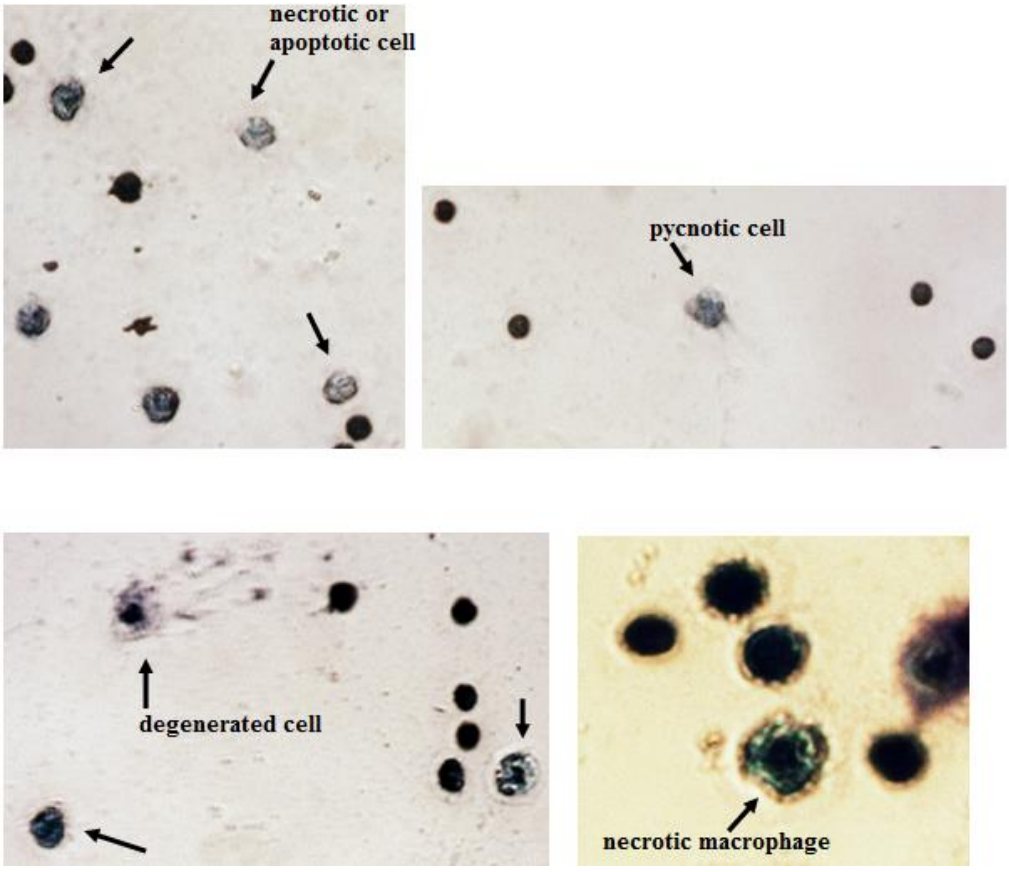

Figure 2 (A). Treated rat peritoneal pyknotic macrophages (as indicated by arrows) (x 400)

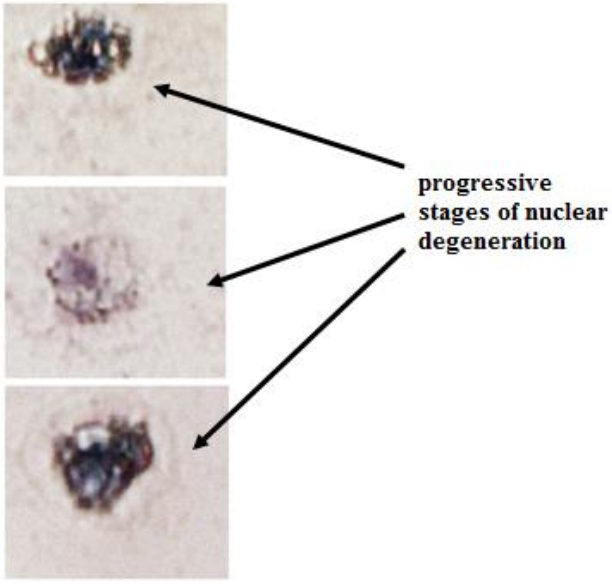

Figure 2 (B). Fragmented nuclei in cells were found in treated group (as indicated by arrows) (x 400)

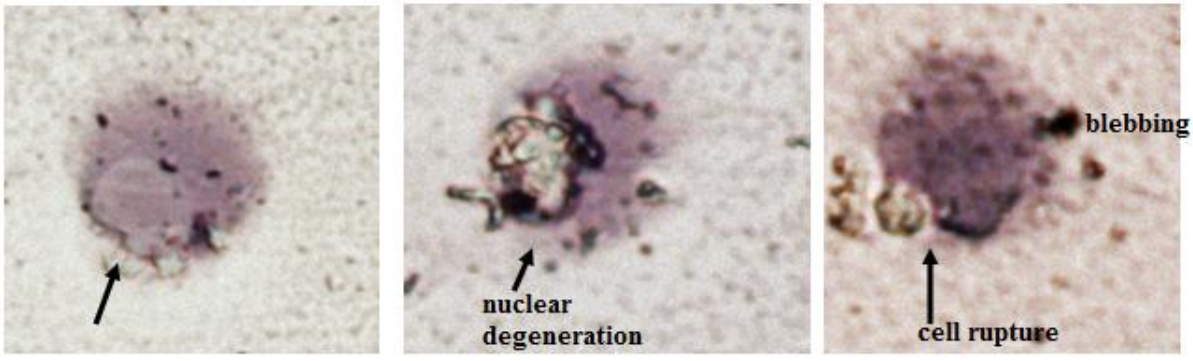

Figure 2 (C). Rat peritoneal macrophages with membrane blebbing and membrane rupture indicating progressive stages of macrophage alteration(x 400)

Trypan blue (TB) response of macrophages

Significant number of treated peritoneal macrophages showed trypan blue (TB) positive response. Dead macrophages were blue in colour whereas the viable cells of controls were white (Fig 3A and B). Mean mortality index was significantly increased in treated group.

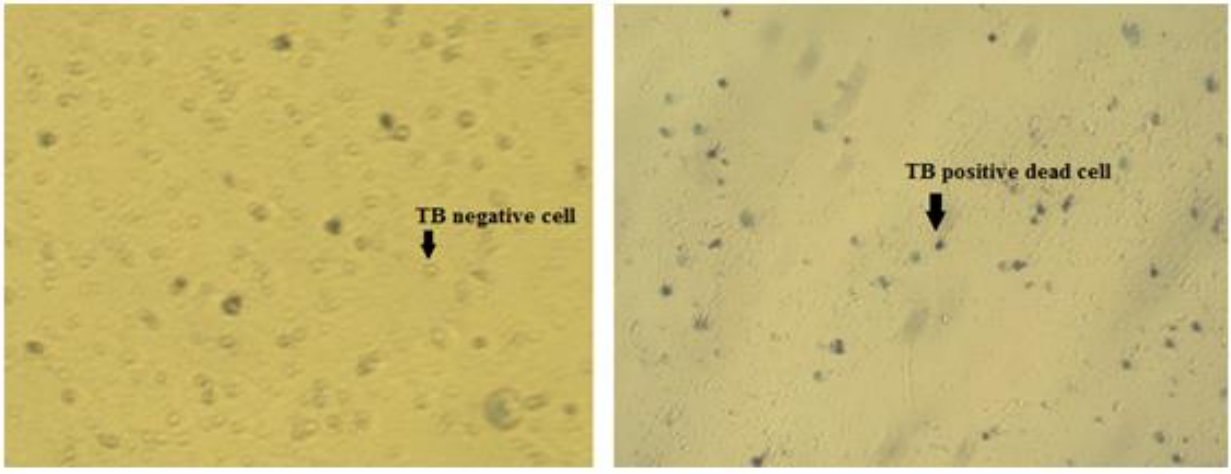

Figure 3 (A, B): (A) Normal rat viable (white) macrophages, (B) treated dead (blue) macrophages

Neutral red indicator in rat macrophages

Control rat macrophages showed neutral red stained lysosomal compartments. Treated macrophages showed neutral red positive response in their cytoplasm.

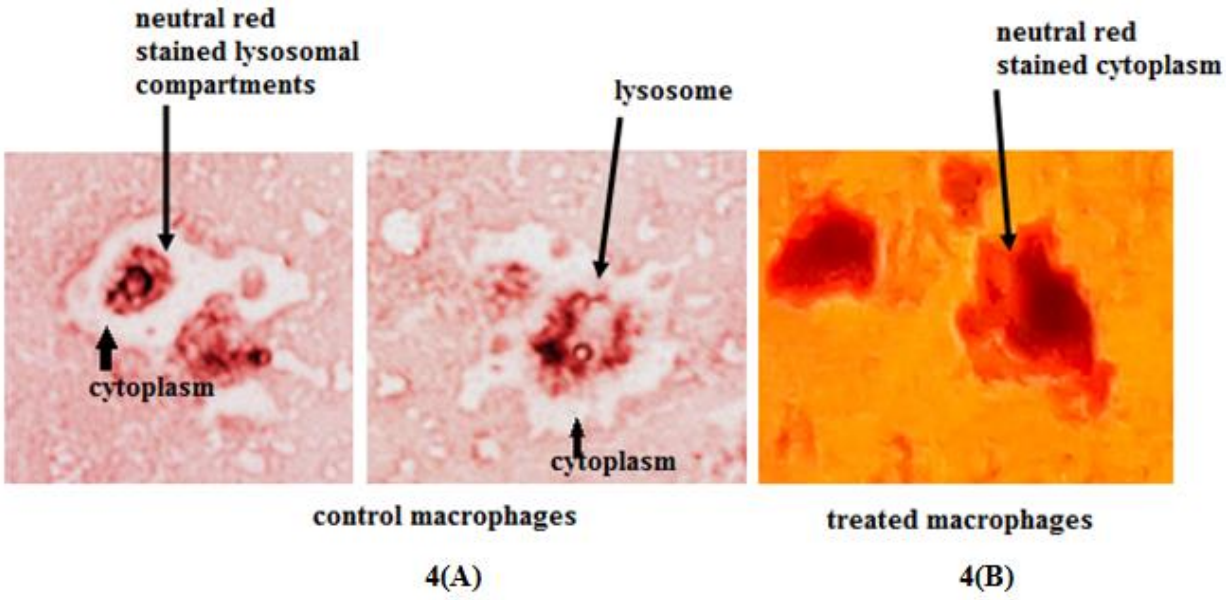

Figure 4 (A, B). (A) Lysosomal membrane stability of normal cells, (B) Arrows indicates the diffused neutral red in cytoplasm of treated rat peritoneal macrophages

4. DISCUSSION AND CONCLUSIONS

It has been known for many years that mercury impairs immune system function most likely via its deleterious effects on the poly morphonuclear leukocytes (PMNs) (Shenker, BJ *et al*., 1992). Mercury also affects PMN function by inhibiting their ability to destroy foreign substances (Shenker, BJ *et al*., 1992). Mercury-sensitive individuals are more likely to have allergies, asthma, and autoimmune-like symptoms. Mercury can induce alterations in immune cell production and function (Shenker, BJ *et al*., 1992). 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 (Fig1). Treated macrophages showed membrane blebbing, rupture of plasma membrane (Fig 2) and loss of viability (Fig 3). The time course of mercury-induced cytotoxic events in the experiments may be due to ROS generation. NF-kuppa B, a pleiotropic transcriptional factor, promotes cell survival and protects cells from apoptosis. Mercury, impairs NF-kuppa B activation and transcriptional activity (Woods, JS *et al*., 2002). Toxin exposure often leads to damage of lysosomal membrane leading to the release of hydrolases in the cytoplasm. This may result in impairment in the structural profile of cells. The degree of lysosomal membrane fragility can be quantitated by neutral red retention assay (Ray M *et al*., 2013). The lysosomal membrane stability of cells has been claimed as biomarkers of environmental toxicity (Ray M *et al*., 2013). Lysosomal membrane stability of cells is an established parameter of screening toxicity. In the present experiment, neutral red stained the cytoplasm of treated macrophages indicating the damage of lysosomal membrane leading to the release of hydrolases in the cytoplasm. The oxygen free radicals generated by mercury-mediated reactions may activate apoptosis which is a possible indicator

of an immunosuppressive action. The result indicates that mercury 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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