British Journal of Medicine & Medical Research
4(1): 451-467, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Alteration of Testicular Macrophage Morphology and Associated Innate Immune Functions in Cadmium Intoxicated Swiss Albino Mice

Sumana Chakraborty[1] and Mahuya Sengupta[1*]

[1]Department of Biotechnology, Assam University, Silchar- 788011, Assam, India.

Authors' contributions

This work was carried out in collaboration between both authors. Author MS Conceived and designed the experiments. Author SC performed the experiments, analyzed the data. Author MS contributed reagents/materials/analysis tools; Authors MS and SC wrote the manuscript. Both authors read and approved the final manuscript.

Research Article

Received 24th June 2013
Accepted 19th July 2013
Published 28th September 2013

ABSTRACT

Aims: The present study investigates in a mouse model the extent of immunomodulatory effects after exposure to cadmium chloride (*in vivo*) in the testes.
Study Design: Experimental study.
Place and Duration of Study: Department of Biotechnology, Assam University, Silchar, Assam, India; between may 2010 and march 2012.
Methodology: LD50 was determined and the percent mortality of mice was plotted against their respective decreasing levels of cadmium chloride. To elucidate the immunomodulatory effects of cadmium chloride, Swiss albino mice were divided into two groups: the 1st group was intraperitonially injected with cadmium chloride (0.35 mg/kg b.w.) and the 2nd group with isotonic saline solution for 15 days. The isolated testicular macrophages were used to determine the morphological alteration as well as cell function studies such as phagocytosis, intracellular killing capacity, myeloperoxidase, nitric oxide release and TNF-α release assay from cadmium chloride -treated and control group of adult male Swiss albino mice.
Results: The present work shows that cadmium chloride is responsible for a significant

**Corresponding author: Email: senguptamahuya35@gmail.com;*

alteration in morphology from 22.2 ± 0.05% to 60.1 ± 1.19% (P**), degenerative changes in scanning electron microscopy and reduced cell function such as phagocytosis (from 21000 ± 577.35 to 7100 ± 115.47; P**), myeloperoxidase release (from 46.8 ± 0.872 µM to 30.23 ± 1.041 µM; P*), nitric oxide release (from 11 ± 1.53 to 5 ± 1.2; P*) and the intracellular killing capacity was also reduced significantly (P**) in testicular macrophages probably by increasing oxidative damage. It also shows that TNF-α increases with cadmium chloride treatment (from 164 ± 4.62 to 235 ± 5.2; P*).

Conclusion: Thus it can be concluded that the toxic potential of cadmium chloride causes morphological changes as well as alterations in cell function in macrophages, rendering the animals more prone to infection, all of which may bear particular significance in heavy metal induced infertility.

Keywords: Cadmium chloride; phagocytosis; scanning electron microscopy; cytokines.

1. INTRODUCTION

Cadmium (Cd) exposure has been associated with a wide range of toxic effects including those on the hepato-biliary system, kidneys, reproductive and immune systems [1-2]. Cadmium is widely distributed in the environment at relatively low concentrations except where it has been concentrated anthropogenically. Since cadmium is a metal, it does not break down and can accumulate over time. The symptoms of cadmium toxicity are many, but common symptoms include hepatotoxicity, nephrotoxicity, loss of immune function, infertility, sub-fertility and depression. The present study aims to address immune-infertility due to cadmium exposure. Immune infertility is now estimated to be a considerable cause of sterility in couples seeking medical assistance [3-6].

There is now widespread agreement that the immune system and the intrinsic testicular functions, spermatogenesis and steroidogenesis, are intricately linked by a network of complex interactions. There is a delicate balance needed, between the suppression of the immune response to protect the germ cells from auto attack on the one hand and the ability to have an active immune response to prevent damage from infection, trauma, and cancer on the other [3,5].

There is general agreement that the existence of an immunoprivileged organ is an evolutionary adaptation to protect vulnerable tissues with limited capacity for regeneration, thereby avoiding loss of function [7-8].

For the testes this means safeguarding reproductive capability. The mechanisms responsible for the testes' immune privilege are still far from being understood, but it is apparent that the identified factors involved are multiple and probably redundant. Notwithstanding its immune privileged status, the testis is clearly capable of mounting normal immunogenic responses, as proven by its effective response to viral and bacterial infection. The present study addresses both of these immune privileged status as well as the capability to mount an immunogenic response against bacterial challenge after cadmium exposure.

The testis is known to be an immunopriviledged site largely due to the existence of the blood-testes-barrier (BTB). The main task of the BTB is to protect the developing germ cells from the immune system. It is now accepted that the BTB alone does not account for all the manifestations of the testicular immune privilege and some other mechanism, besides

physical separation, must exist to maintain testicular immune privilege, which requires more robust protection of the tolerogenic environment of the testis.

Testicular macrophages are the largest population of immune cells in the rodent testes among other cells like lymphocytes, mast cells and neutrophils. Macrophages are directly involved in the fight against invading microorganisms as sentinels of innate immunity as well as antigen-presenting cells which activate lymphocytes. Testicular macrophages originate from blood monocytes which move into the testes and then mature into macrophages. Testicular macrophages can respond to infectious stimuli and become activated macrophages [9] that are well differentiated. Given that macrophages are the pivotal cells in the initiation of inflammation and subsequent immune responses, determination of the functional properties of the testicular macrophages in consistency with the manifestations of testicular immunoprivilege after cadmium exposure was a potent question.

Although there are some studies reporting that cadmium exposure may inhibit the testicular macrophages the extent to which cadmium alters testicular macrophage function is not well-elucidated. As confusing reports were observed regarding the extent of tissue damage actually caused by cadmium, the present work thus aims to determine the functions of testicular macrophages in cadmium chloride exposed mice by studying their morphology. Since phagocytosis and intracellular killing are the primary functions of macrophages, these were also assayed in the testicular macrophages along with the enzyme release from them. Our findings reveal that cadmium chloride exposure causes immunomodulation of testicular macrophages. While there exists a general debility in the immune status of the macrophages leading to their immunogenic dysfunctions and loss of immune surveillance due to cadmium chloride exposure, one can observe an augmented TNFα level. The myriad and often conflicting effects mediated by TNFα indicate the existence of extensive signaling cross-talk between immune functions, the cytokine microenvironment and immunoprivilege.

2. MATERIALS AND METHODS

2.1 Reagents

The following reagents were used: collagenase Type IA, DNase I, Tosyl (Na-p-tosyl-L-lysine chloromethyl ketone), Histopaque-1077 (SIGMA St. Louis, MO); RPMI 1640 (Gibco Life Technologies, Grand Island, NY), fetal calf serum (FCS) (SIGMA-Aldrich); All other reagents were of analytical grade.

2.2 Animals

Ten adult (6 weeks) male Swiss albino mice (avg b.w 20g $\pm$ 2g) were taken and divided into two groups of five mice each: a) control, b) cadmium chloride treated. The treated group was injected (i.p.) with cadmium chloride solution (0.35 mg/kg b.w.) and the control group with 0.9% isotonic saline daily for 15 days. The animals were kept in plastic cages in the departmental animal house. Animal care and protocols were in accordance with and approved by the institutional animal ethics committee. These animals were kept in an environment with controlled temperature (25ºC), humidity (45-50%), and photoperiod (12:12-h light-dark cycle). All the animals were fed standard diet *ad libitum* and had free access to water.

2.3 Dose Response Study

The LD50 values of cadmium chloride (*in vivo*) in mice were found to be 7.00 mg/kg b.w. for 30 days. Sublethal dose of cadmium chloride at a concentration of 50% of LD50 was standardized for administration *in vivo* to study its toxic effect on murine immune system at 0.35 mg/kg b.w. for 15 days. All the experiments were performed in triplicate.

2.4 Determination of Cadmium Chloride Content in Testes by Atomic Absorption Spectroscopy

The sample was suitably digested to extract the metals, and the metals solubilized for eventual excitation when introduced into the flame as per the basic principle in atomic absorption as developed by Walsh in 1977 and cadmium chloride analysed with the model Perkin Elmer 3110 [10].

2.5 Isolation of Testicular Macrophages

Testicular macrophages were isolated following a slightly modified procedure of [11]. Macrophages from both control and cadmium chloride exposed mice were used for assays.

2.6 Preparation of Bacteria (*Staphylococcus aureus* MC524) for Intracellular Killing and Phagocytosis Assay

To obtain bacteria in the mid logarithmic phase 100 µl of an overnight culture made in nutrient broth was added to 10 ml of nutrient broth and incubated for 2-5h at 37ºC with orbital shaking. The bacteria was washed in 10 mM sodium phosphate buffer (pH 7.4) and their concentration was estimated by spectrophotometry at A_{620} on the basis of the relationship: A_{620} 0.2 = 5×10^7/ml [12].

2.7 Morphological Alteration of Macrophages

Cells were observed under oil immersion microscope. Any cell devoid of pseudopodia was scored as polarized and this was expressed as a percentage of the total number of cells counted [13].

2.8 Scanning Electron Microscopy

The tissues were observed using a JSM-6360 (Jeol) SEM at the Sophisticated Analytical Instrument Facility (SAIF), North-Eastern Hill University (NEHU), Shillong, Meghalalya, India [14-15].

2.9 Phagocytosis Assay

Testicular macrophages from both control and exposed groups were allowed to adhere separately on glass slides for one hour. Phagocytosis assay was performed with 10% SRBC and phagocytic index calculated [16].

2.10 Intracellular Killing Assay

Bacteria were incubated with testicular macrophages. After various time intervals (15, 30 and 45 min), sample was treated with Gentamycin to kill extracellular adherent bacteria and viability of intracellular bacteria was determined [17].

2.11 Myeloperoxidase Release Assay

Cell suspension was taken, stimulated with LPS and centrifuged. The supernatant was collected in separate microcentrifuge tubes. Supernetant and cell lysate were allowed to react with orthophenylenediamine (OPD) substrate and readings were taken at 492 nm in a spectrophotometer [18].

2.12 Nitric Oxide Release Assay

Testicular macrophages were suspended in DPBS-BSA and were stimulated with LPS. The cell-free supernatant was used for nitric oxide release assay using Griess reagent. Readings are taken in a UV spectrophotometer at 550 nm [19].

2.13 Cytokine Assay

Testicular cells were separated by density gradient centrifugation. Then testicular macrophages were obtained by adherence to plastic surface. A number of 1 X 10^5 viable cells in 0.2 ml RPMI 1640 medium supplemented with 5% FCS were distributed in microwells in flat 96 well microtitre plates and, after 24 h culture, supernatants were collected. Cytokine concentrations in culture supernatants were measured by sandwich ELISA estimating TNF-α using a mouse TNF-α ELISA kit (RayBiotech, USA). Biotinylated monoclonal secondary antibodies were used. The reaction was stopped with 3 M H_2SO_4 and the optical density of each well was measured in a 96- well plate reader at 492 nm. All determinations were done in triplicate. Standard curves were generated by recombinant mouse cytokines. Lower density limits was found to be 10 pg/ml (TNF-α).

2.14 Statistical Analysis

The data was expressed as mean ± SD. A two tailed student's t-test was performed to estimate the difference in means and the level of significance thereof. All the experiments were done in triplicate. P* = P<.05, P**= P<.001, comparing with control.

3. RESULTS

3.1 Dose Response Study for *In vivo* Exposure to Cadmium Chloride

To determine the LD50, mice were administered different concentration of cadmium chloride (1,3,5,7,10,20 and 40 mg/kg body weight) till 30 days period. The percent mortality of mice was plotted against their respective decreasing levels of cadmium chloride. The dose of cadmium chloride at which 50% mortality occurred (LD50) was extrapolated from the dose response curve of cadmium chloride. It was found that 50 % of the experimental population died at a concentration of about 7 mg/kg body weights and there is a significant decrease of survival according to the increment of dose (Fig. 1). Therefore 0.35 mg/kg body weight

(sublethal) dose was chosen for further experiments to elaborate the underlying mechanisms. The volume of drug administered (ip) was 50 µl (concentration of cadmium chloride was 0.14 mg/ml). The injections (ip) of both control groups and cadmium chloride treated group were given daily in the forenoon for a period of 15 days. No death was recorded among the control group.

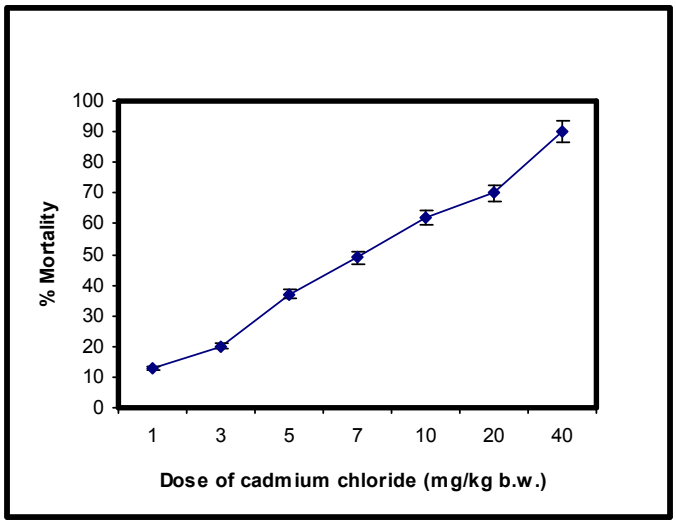

Fig. 1. The lethal dose of cadmium chloride 50 (LD_{50}) (Mean ± S.D.)

3.2 Concentration of Cadmium Chloride in Testes Tissue

Cadmium content in cadmium chloride exposed mice was found to be 0.021 ± 0.0015 µg/ml whereas no cadmium trace was detected in control group.

3.3 Effect of Cadmium Chloride on the Morphology of Testicular Macrophages

Morphology of testicular macrophage plays a very important role in their function. To demonstrate the effect of cadmium chloride on testicular macrophages study of morphologically altered macrophages in both *in vivo* study was performed. From the assay it was found that cadmium chloride treatment increases the number of undifferentiated macrophages from 22.2 ± 0.05% to 60.1 ± 1.19% (Fig: 2; P**).

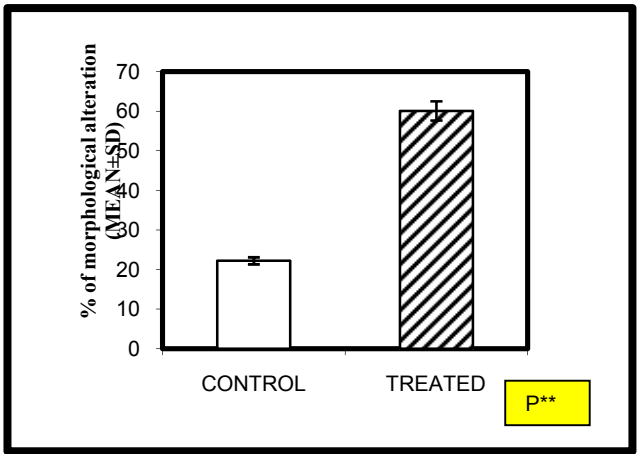

Fig. 2. *In-vivo* study of effect of cadmium chloride on the morphology of testicular macrophages in adult male Swiss albino mice. (Mean ± S.D., P).**

3.4 Effect of Cadmium Chloride on Morphology of Cadmium Chloride Intoxicated Testicular Macrophages by Scanning Electron Microscopy

Adherence of macrophages to a particular foreign body decides the ability to kill the pathogens. Macrophage adheres to the surfaces of the foreign body by means of extended dendritic morphology (pseudopods) and phagocytoses foreign body. The scanning electron micrograph of testicular macrophages isolated from the respective groups showed that cadmium chloride intoxication render somehow the differentiation of testicular macrophages and so, deficient of dendritic morphology (Plate 1B) as compared to control groups (Plate 1A). Cadmium chloride treatment shows macrophage having almost smooth and spherical outer surface when compared to control group of macrophages shows dendritic forms.

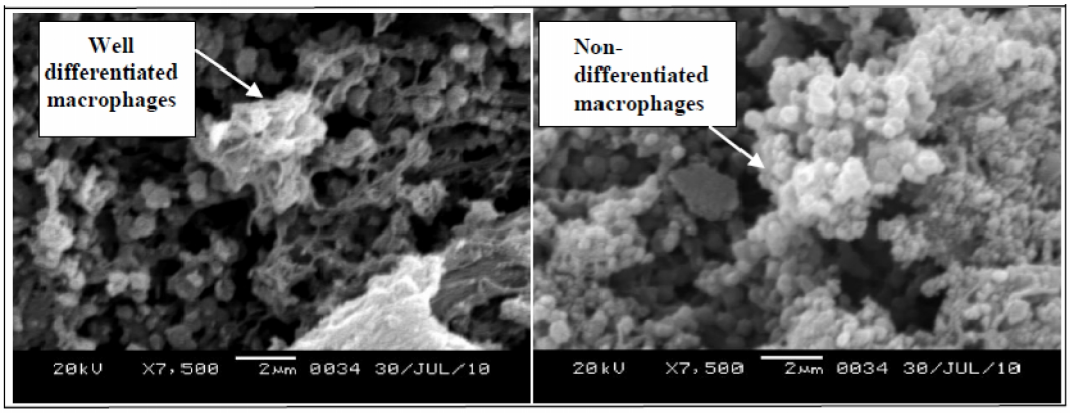

Plate 1. Effect of cadmium chloride on macrophage differentiation. (a) The micrograph of normal macrophages at X 7500 and the bar is 2µm. (b) Micrograph of cadmium chloride- treated macropahges at X 7500 and the bar is 2µm

3.5 Effect of Cadmium Chloride on Phagocytic Capacity of Cadmium Chloride Intoxicated Testicular Macrophages

In order to determine whether there was any alteration in phagocytic capacity of testicular macrophages due to cadmium chloride treatment, the phagocytosis of heat killed *S.aureus* by macrophages was assayed. Result shows that cadmium chloride causes a marked decrease in the phagocytic index from control 21000 ± 577.35 to 7100 ± 115.47 after cadmium chloride treatment (Fig. 3; P**).

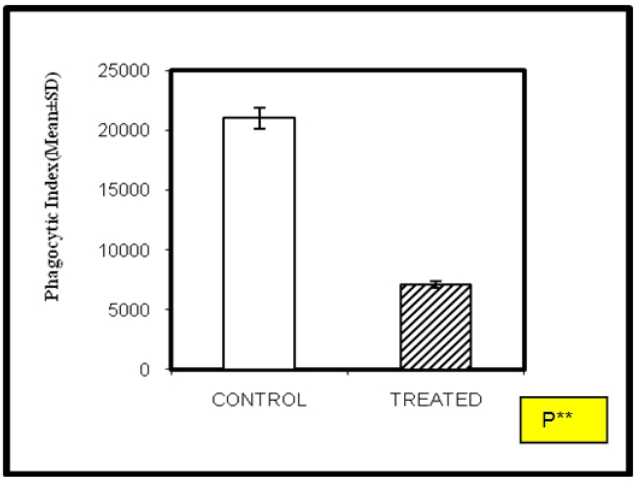

Fig. 3. *In-vivo* study of effect of cadmium chloride on phagocytic capacity of cadmium chloride intoxicated testicular macrophages in adult male Swiss albino mice (Mean ± S.D., P)**

3.6 Effect of Cadmium Chloride on Killing Capacity of Testicular Macrophages Isolated from Cadmium Chloride Intoxicated Male Swiss albino Mice

This assay was performed to determine the killing capacity of intracellular *S. aureus* in cadmium chloride treated and control group of mice. The result shows that cadmium chloride treated mice are prone to infection and less effective in clearing invading pathogens as it were evident that testicular macrophages from cadmium chloride exposed group could not able to kill the intracellular *Staphylococcus aureus* competently as show in (Fig. 4; P**).

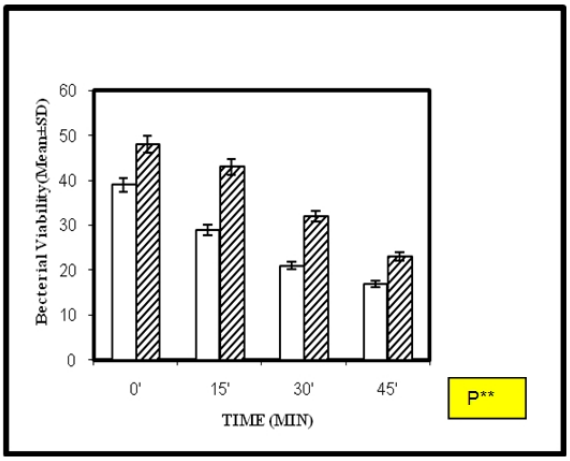

Fig. 4. *In-vivo* study of effect of cadmium chloride on killing capacity of testicular macrophages isolated from cadmium chloride intoxicated adult male Swiss albino mice (Mean ± S.D., P)**

3.7 Effect of Cadmium Chloride on Myeloperoxidase Release of Testicular Macrophages Isolated from Cadmium Chloride Intoxicated Male Swiss albino Mice

Activation of macrophages with bacterial cell wall lipopolysaccharide (LPS) begins to express high levels of myeloperoxidase (MPO) enzyme. MPO decreases the free radical level in our system. MPO release assay was performed to evaluate the effect of cadmium chloride exposure on the release of this enzyme after LPS stimulation. Significant decrease in MPO released (μM) was observed. MPO released from the control group with LPS stimulation, showed a value of 46.8 ± 0.872 μM and 30.23 ± 1.041 μM in the cadmium chloride treated group (Fig. 5; P*).

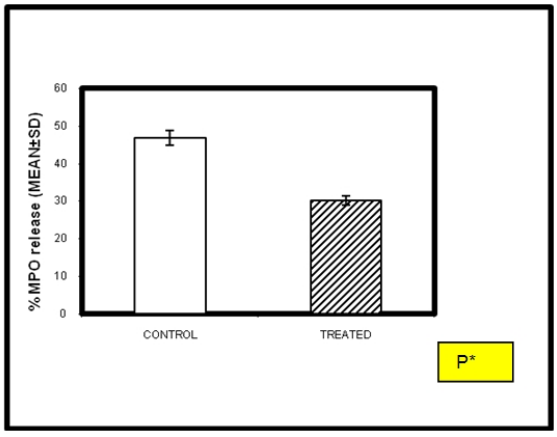

Fig. 5. *In-vivo* study of effect of cadmium chloride on myeloperoxidase enzyme release in testicular macrophages isolated from cadmium chloride intoxicated adult male Swiss albino mice. (Mean ± S.D., P*)

3.8 Effect of Cadmium Chloride on Nitric Oxide (NO) Release of Testicular Macrophages Isolated from Cadmium Chloride Intoxicated Male Swiss albino Mice

Macrophages when activated with bacterial LPS, they begin to express high level of nitric oxide synthase which oxidizes L-argininine to yield citrulline and nitric oxide (NO). NO itself has potent antimicrobial acitivity and it can also combine with superoxide anion to yield even more potent antimicrobial substances. The effect of cadmium chloride on NO release seeks to demonstrate the immuno-modulatory effect of cadmium. Significant decrease in NO released (μM) was observed in cadmium chloride intoxicated group of mice. Result shows that cadmium chloride causes a marked decrease in nitric oxide release from control 11 ± 1.53 to 5 ± 1.2 after cadmium chloride treatment (Fig. 6; P*).

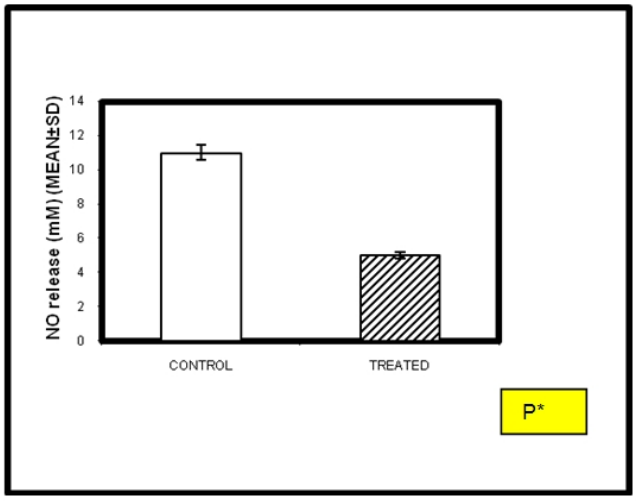

Fig. 6. *In-vivo* study of effect of cadmium chloride on nitric acid release in testicular macrophages isolated from cadmium chloride intoxicated adult male Swiss albino mice. (Mean ± S.D., P*)

3.9 Effect of Cadmium Chloride on Cytokines Pro-inflammatory Release (TNF-α) of Testicular Macrophages Isolated from Cadmium Chloride Intoxicated Male Swiss albino Mice

Intraperitoneal administration of cadmium chloride in male Swiss albino mice led to a increase in the levels of pro-inflammatory cytokine; TNF-α from 164 ± 4.62 to 235 ± 5.2 (Fig. 7; P*)

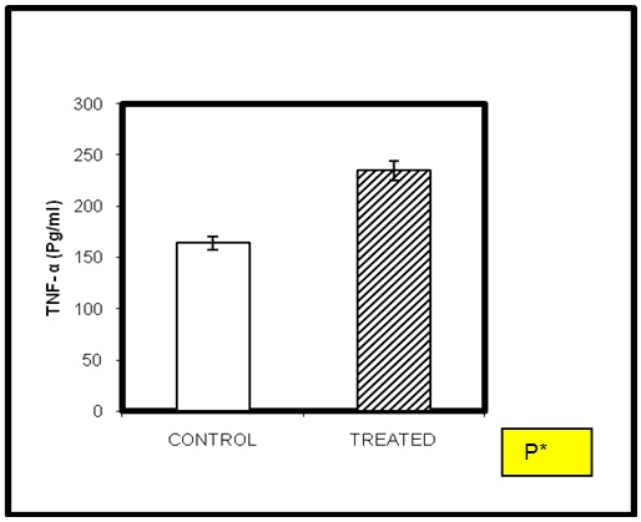

Fig. 7. *In-vivo* study of effect of cadmium chloride on pro-inflammatory (TNF-α) cytokine release in testicular macrophages isolated from cadmium chloride intoxicated adult male Swiss albino mice. (Mean ± S.D., P*)

4. DISCUSSION

Cadmium is extremely hazardous to life and is a serious lethal occupational and environmental toxin, known for its high toxicity, which may affect living systems in various ways [20].

The form of cadmium and the route of exposure can greatly affect the absorption and distribution of cadmium to various target sites, and therefore, the concentration at the target site and the severity of the observed effects were both considered. An intraperitoneal (i.p.) route was chosen to administer cadmium (Cd) as it has a very long biological half life and once deposited, is persistent for a longtime irrespective of the route of administration. Thus the precise route of exposure becomes less important compared with other chemical toxicants [21]. Repository injection of cadmium, such as the i.p. injection we used, increases systemic values of cadmium accumulation in tissues [22].

The effective dose values (ED_{50}) of cadmium as documented in environmental health criteria for a single intraperitoneal dose of cadmium in the form of CdCl2 for the control mice was ranging between 5, 6.75 and 7mg/kg b.w. [23-25]. But still an LD_{50} test was necessary as the continuous changes in different factors could affect Cd toxicity. Results of the current study (Fig. 1) show that the LD_{50} values for cadmium chloride via intraperitoneal injection was 7 mg/kg b.w. for 15 days exposure in male mice. A sublethal dose of 50% of the LD_{50} i.e., 0.35 mg/kg b.w. daily for 15 days was selected as the administrable dose.

Atomic Absorption spectroscopy (AAS) has been routinely used to detect and quantify cadmium contents in human seminal plasma [26-28]. Consequently, AAS was selected as the analytical technique to determine cadmium content in testes from each sacrificed mice (both control and cadmium chloride treated group). The results of this study show that cadmium accumulates within the testes of the mice exposed to cadmium chloride where as

no corresponding changes were observed in the tissue of control mice. Thus cadmium chloride exposure may directly be implicated in the alteration of testicular macrophage functions and associated reproductive functions (data not presented here).

A major characteristic feature of the testes relevant to immunity is the large population of resident macrophages within the interstitial tissue [29]. In the present study, the testicular macrophages in the cadmium chloride exposed group have been shown to be alternatively activated, with greatly augmented production of the proinflammatory cytokine TNF-α. Proinflammatory cytokines and other immune modulators must be tightly regulated in order to maintain immune privilege in the testis. TNFα is a multifunctional cytokine with effects not only in the proinflammatory response but in immunoregulatory and apoptosis.

Given that macrophages are the pivotal cells in the initiation of inflammation and subsequent immune responses, the functional properties of the testicular macrophages are entirely consistent with the manifestations of testicular immunoprivilege. Immune responses within the testicular environment tend toward suppression of antigen-specific cell-mediated responses, favoring tolerogenic immune responses instead. This is countered in cases of cadmium chloride exposure.

The results show that in cadmium chloride treated mice the alteration in testicular macrophage morphology is significantly higher than that of control (Fig. 2). Cadmium induced deviation in the normal shape of macrophages may be the cause of the reduced functional status of testicular macrophages. The groups exposed to cadmium chloride presented evident morphological alterations relative to control animals. But the extent of morphological damage was not well determined. The results demonstrate that noncytotoxic concentrations of cadmium chloride markedly impair differentiation of murine testicular macrophages. Inhibition of macrophagic differentiation by a clinically relevant concentration of cadmium chloride may therefore lead to deleterious adverse effects in cadmium chloride - exposed patients. From the present study it is confirmed that morphological alterations occur in the testicular macrophages due to cadmium chloride exposure. Killing mechanism by macrophages is very closely related with the adherence capacity of macrophage to the foreign body. Macrophages adhere to the foreign particles by means dendritic extensions. Testes from mice intoxicated with cadmium chloride were examined by means of scanning electron microscopy and comes out with the result that cadmium chloride caused a rapid decrease in the number of villous cells, with a constant increase in the number of cells with smoother surfaces. Scanning electron micrograph of randomly selected area of cadmium chloride intoxicated testes showed undifferentiated macrophages (Plate 1b) as compared to control group (Plate 1a).

Exposure of organisms to bacterial infection results in the activation of a variety of host defense mechanisms such as phagocytosis. It is evident that cadmium chloride causes a marked decrease in the phagocytic index after cadmium chloride intoxication in male mice as compared to control (Fig. 3). It can also be suggested that they cannot phagocytose efficiently, and as a result, cannot clear out the invading microorganism, which may lead to a diseased state upon bacterial invasion. These results help us to come to the conclusion that as testicular macrophage loses their phagocytic capacity due to the exposure of cadmium chloride, they are prone to infection.

Staphylococcus aureus has the ability to cause a variety of potentially life threatening infection varying from superficial soft tissue abscesses to septic shock. Despite the availability of effective antimicrobial agents, *S.aureus* continues to cause life threatening

infection [30]. It is evident from the study that cadmium chloride intoxication help the *S.aureus* to survive within testicular macrophages, since increased number of *S.aureus* colonies were observed in testicular macrophages from cadmium chloride exposed group as compared to control. *In vivo* cadmium chloride exposure may reduce the ingestion capacity of testicular macrophages, suggested that the cells were not active enough or somehow less potent to kill the ingested bacteria efficiently (Fig. 4). So the results demonstrated that murine macrophages constitute sensitive targets of inorganic cadmium chloride, which may lead to immunotoxicity and immunosuppressive properties of this environmental contaminant.

Activated phagocytes produce a number of reactive oxygen intermediates (ROIs) and reactive nitrogen intermediates and during phagocytosis, a metabolic process known as the respiratory burst occurs in activated macrophage. As the lysosomes fuses with the phagosome, the activity of myeloperoxidase produces hypochlorite from hydrogen peroxide and chloride ions. It is observed that in the cadmium chloride treated group the myeloperoxidase release significantly decrease in cadmium chloride intoxicated group when compared to control group (Fig. 5).

When macrophages are activated with bacterial cell wall lipopolysaccharide, they begin to express high level of nitric oxide synthase, which oxidizes L-arginine to yield citrulline and nitric oxide (NO). Nitric oxide plays a significant role in killing of phagocytisized pathogens within macrophages. Nitric oxide has potent antimicrobial activity; it also can combine with the superoxide anion to yield even more potent antimicrobial substances. In this study, it was found that, NO release significantly decreases in the cadmium chloride challenged as compared to that of control group (Fig. 6).

It appears that immune privilege in the testis is maintained by a unique testicular environment that controls immune cell activity, inducing and maintaining peripheral tolerance and suppressing adaptive immunity in a tissue-localized manner [31,32]. Interstitial testicular macrophages have been described a possible a source of TNFα. Normally, a rise in the TNFα causes heightened immune activity. However, contrary to this, we found that cadmium chloride caused a rise in TNFα levels and inflammation, leading to immunosuppression, thus indicating multiple targets and sites of action of cadmium chloride, probably at the receptor level. That cadmium chloride affects the cellular microenvironment and the signaling cross-talk within is evident from the functional status of the testicular macrophages. The inflammatory as well as the functional loss of immune surveillance may well be attributed to oxidative stress induced changes from an increased TNFα titer. Moreover, a heightened TNF response also leads to a feedback inhibition on testosterone release causing a vicious cycle of inflammatory damage, subfertility and loss of immunoprivilege. At least one study suggests that the levels of TNFα produced in the testis may be an important factor. Low levels of TNFα may preferentially regulate normal testicular homeostasis; whereas, elevated levels influence or initiate pathological conditions in the testis.

At low concentrations, TNFα in combination with other cytokines and lipopolysaccharides has been shown to stimulate inducible nitric oxide synthase (iNOS) and nitrite in Sertoli cells as well as seminiferous pertitubular cells by triggering the NF-kβ and MAPK pathways.

However, at higher concentrations, it causes potentiation of the apoptotic patways the death domain of p55 TNFR [33]. The present study shows a decrease in NO and MPO in cadmium chloride exposed testicular macrophages despite a rise in TNF-α at levels leading to the conjecture that NO and MPO escape the phagocytic milieu of the testicular macrophages to

increase oxidative stress and associated inflammatory damage in the testicular environment, including the developing sperm cells [34]. Cadmium chloride probably, alters the composition of macrophage population drastically and shifts the cytokine balance in favor of an inflammatory response with potential to overcome immune privilege.

The existence of immune privilege has important implications for both normal testicular function and male reproductive disease. Although in a unique immunological environment [35, 36], the testis does not display an increased susceptibility to tumors or infections compared with other tissues. In fact, infections of the testis are relatively rare in comparison with more distal tissues of the male reproductive tract [37]. The intensity of inflammatory responses in the testis may be reduced, due to the unique regulatory properties of the testicular macrophages, and antigen-specific immunity may be compromised, but it appears that the ability of the testes to resist and clear infections is intact nonetheless. This may be due to an increased reliance on innate immunity, and there has been a steady increase in interest in the role of innate immunity in testicular function recently [38-40]. Cadmium chloride exposure reverses this condition leading to immunocompliance of the macrophage mediated innate or nonspecific host response, augment inflammatory damage and subsequently, a loss of immune privilege.

5. CONCLUSION

Thus, the current *in vivo* study demonstrates that exposure of male mice to cadmium chloride resulted in alteration in morphology of testicular macrophages; reduced phagocytosis index of testicular macrophages indicate that cadmium chloride treated groups are more prone to infection, as they cannot phagocytose efficiently and so cannot clear out the invading microorganism. Cadmium chloride intoxicated testicular macrophages were not able to kill the intracellular *Staphylococcus aureus* competently as compared to control.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Institutional Ethics Committee, Assam University.

COMPETING INTERESTS

We declare that there is no competing interest among the authors.

REFERENCES

1. Henson MC, Chedrese PJ. Endocrine disruption by cadmium, a common environmental toxicant with paradoxical effects on reproduction. Exp. Biol. Med. 2004;229:383-92.
2. Thompson J, Bannigan J. Cadmium: Toxic effects on the reproductive system and the embryo. Reprod. Toxicol. 2008;25(3):304-15. doi:10.1016/j.reprotox.2008.02.001.

3. Mahmoud A, Comhaire FH. Immunological causes. In: Schill W-B, Comhaire FH, Hargreave TB (eds) Andrology for the clinician. Springer, Berlin. 2006;47–52.
4. McLachlan RI. Basis, diagnosis and treatment of immunological infertility in men. J Reprod Immunol. 2002;57:35–45.
5. Naz RK. Modalities for treatment of antisperm antibody mediated infertility: novel perspectives. Am J Reprod Immunol. 2004;51:390–97
6. WHO. Towards more objectivity in diagnosis and management of male infertility. Int J Androl. 1987;(Suppl 7):1–53
7. Filippini A, Riccioli A, Padula F, et al. Control and impairment of immune privilege in the testis and in semen. Hum Reprod Update. 2001;7:444–49.
8. Setchell BP, Uksila J, Maddocks S, Pollanen P. Testis physiology relevant to immunoregulation. J Reprod Immunol. 1990;18:19–32.
9. Meinhardt A, Bacher M, Metz C, Bucala R, Wreford N, Lan H, et al. Local regulation of macrophage subsets in the adult rat testis: examination of the roles of the seminiferous tubules, testosterone, and macrophage-migration inhibitory factor. Biol Reprod. 1998;59(2):371-78.
10. Walsh A. Atomic Absorption Spectroscopy and Its Application Old And New. Pure & Appi. Chern. 1977;49:1621-1628.
11. Hutson JC. Testicular macrophages. J Int. Rev. Cytol. 1994;149:99–143.
12. Yao L, Berman JW, Factor SM, Lowy FD. Correlation of histopathologic and bacteriologic changes with cytokine expression in experimental murine model of bacteremic Staphylococcus aureus infection. Infect. Immun. 1997;65:3889-95.
13. Qu J, Condliffe MA, Lawson M, Plevin RJ, Chlivers E. Lack of effect of recombinant platelet derived growth factor on human neutrophil function. J. Immunol. 1997;159:2952-59.
14. Dey S. A new rapid air drying technique for scanning electron microscopy using tetramethylsilane. Application to mammalian tissue. Cytobiology. 1993;73:17-23.
15. Dey S, Basu Baul TS, Roy B, Dey D. A new rapid method of air-drying for scanning electron microscopy using tetramethylsilane. J. Microsc. 1989;156:2259–61.
16. Czuprynski CJ, Henson PM, Campbell PA. Killing of Listeria monocytogenes by inflammatory neutrophils and mononuclear phagocytes from immune and non immune mice. J. Leukocyte Biol. 1984;35:193-208.
17. Leigh PCJ, Van Furth R, Zwet TL. In vitro determination of phagocytosis and intracellular killing by polymorphonuclear neutrophils and mononuclear phagocytes. In: Handbook of Experimental Immunology. Weir D.M. (ed.), 4th ed. Blackwell Scientific Publications. 1986;46:1-46.19.
18. Bos AR, Weaver R, Roos D. Characterization and qualification of the peroxidase in human neutrophils. Biochem Biophys. Acta.1990;525:4133-41.
19. Sasaki S, Miura T, Nishikawa S, Yamada K, Hirasue M, Nakane A. Protective role of nitric oxide in S. aureus infection in mice. Infect. Immun. 1998;66(3):1017-18.
20. Ostrowski SR, Wibur S, Chou CH, Pohl HR, Stevens YW, Allred PM, et al. Latent effects –carcinogenesis, neurotoxicology and development deficits in humans and animals. Toxicol. Ind. Heath. 1999;15:602-44.
21. Waalkes MP, Anver MR, Diwan BA. Chronic toxic and carcinogenic effects of oral cadmium in the Noble (NBL/Cr) rat: Induction of neoplastic and proliferative lesions of the adrenal, kidney, prostate, and testes. J. Toxicol. Environ. Health. 1999;58:199–214.

22. Kasprazak KS, Poirier LA. Effects of calcium and magnesium actetates on tissue distribution of carcinogenic doses of cadmium chloride in Wister rats. Toxicology.1985;34:221-30.
23. Leber PA, Miya TS. Amechanism for cadmium and zinc –induced tolerance to cadmium toxicity: involvement of metallothionein. Toxicology and Applied Pharmacology. 1976;37(3):403-14.
24. WHO. Cadmium (Environmental Health Criteria 134), Geneva; 1992.
25. Bench G, Corzeh MM, Martinelli. Cadmium concentration in the testes, sperm, and spermatids of mice subjected to long-term cadmium chloride exposure. Cytometery. 1999;35:30–36.
26. Noack-Fuller G, De Beer C, Seibert H. Cadmium, lead, selenium and zinc in semen of occupational unexposed men. Andrologia. 1993;25:7-12.
27. Omu AE, Dashti H, Mohamed AT, Mettappallil AB. Significance of trace elements in seminal plasma of infertile men. Nutrition. 1995;(suppl-5):502-5.
28. Saaranen M, Kantola M, Saaroski S, Vanha-Perttula T. Human seminal plasma cadmium: comparison with fertility and smoking habits. Andrologia. 1989;21:140-45.
29. Hedger MP. Macrophages and the immune responsiveness of the testis. J Reprod Immunol. 2002;57(1-2):19-34.
30. Peleg AY, Munckhof WJ, Kleinschmidt SL, Stephens AJ, Huygens F. Life-threatening community-acquired methicillin-resistant Staphylococcus aureus infection in Australia. Eur J Clin Microbiol Infect Dis. 2005;24(6):384-87.
31. Sheagren JN. Staphylococcus aureus: the persistent pathogen. New Eng.J.Med. 1984;310:1437-42.
32. Raburn DJ, Coquelin A, Reinhart AJ, Hutson JC. Regulation of the macrophage population in postnatal rat testis. J Reprod Immunol. 1993;24:139–51.
33. Duckett RJ, Wreford NG, Meachem SJ, McLachlan RI, Hedger MP. Effect of chorionic gonadotropin and flutamide on Leydig cell and macrophage populations in the testosteroneestradiol-implanted adult rat. J Androl. 1997b;18:656–62.
34. Jeffrey JL. The role of tumor necrosis factor-alpha and interleukin-1 in the mammalian testis and their involvement in testicular torsion and autoimmune orchitis. Reproductive Biology and Endocrinology. 2004;2:1-10.
35. Gerdprasert O, O'Bryan MK, Muir JA, et al. The response of testicular leukocytes to lipopolysaccharide-induced infl ammation: further evidence for heterogeneity of the testicular macrophage population. Cell Tissue Res. 2002;308:277–85.
36. Hudson MM, Frankel LS, Mullins J, Swanson DA. Diagnostic value of surgical testicular biopsy after therapy for acute lymphocytic leukemia. J Pediatr. 1985;107:50–53.
37. Kim TH, Hargreaves HK, Chan WC, Brynes RK, Alvarado C, Woodard J, et al. Sequential testicular biopsies in childhood acute lymphocytic leukemia. Cancer. 1986;57:1038–41.
38. Krieger JN. Epididymitis, orchitis, and related conditions. Sex Transm Dis 1984;11:173–81.
39. Com E, Bourgeon F, Evrard B, Ganz T, Colleu D, Je´gou B, et al. Expression of antimicrobial defensins in the male reproductive tract of rats, mice, and humans. Biol Reprod. 2003;68:95–104.

40. Bhushan S, Tchatalbachev S, Klug J, Fijak M, Pineau C, Chakraborty T, et al. Uropathogenic Escherichia coli block MyD88-dependent and activate MyD88-independent signaling pathways in rat testicular cells. J Immunol. 2008;180:5537–47.
41. Starace D, Galli R, Paone A, Cesaris PD, Filippini A, Ziparo E, et al. Toll-like receptor 3 activation induces antiviral immune responses in mouse Sertoli cells. Biol Reprod. 2008;79:766–75.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=215&id=12&aid=2081