COMPARATIVE HISTOPATHOLOGICAL CHANGES OF LIVER, KIDNEY AND APPENDIX OF RABBITS TREATED WITH INORGANIC NANO CHROMIUM TO AMELIORATE HEAT STRESS EFFECT

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Abstract: The objective of the current experiment was to describe the effects of nano-chromium chloride (CrCl₃) on chronic hyperthermia (32.8 ± 1.5 °C) on rabbit liver, kidney, and appendix in comparison with rabbits at room temperature (24.5 ± 1.3 °C) and treated with the same concentration of nano-chromium chloride. For this study, 108 rabbits of two different breeds (New Zealand White and Rex) were used and randomly allocated into 12 groups. The study was conducted as a completely randomized 2 × 2 × 3 factorial (n = 9) design. Treatments were temperature, breed, and concentration of nano-chromium chloride (0, 1, or 2 mg/L) the results showed that heat stress caused granular hepatic vacuolation, severe congestion of the central vein, and sinusoids in the liver. As well as degenerative changes within the epithelial lining of tubules in the kidney and lymphoid depletion in the appendix. The liver tissue of the New Zealand rabbits was affected more by heat stress than Rex Rabbits, but no difference was observed in the kidney or appendix tissues. The addition of 2 mg/L nano-chromium was more effective than 1 mg/L on the heat stressed rabbit tissues, but it caused hepatic vacuolation with glycogen infiltration in liver tissue and mild vacuolation in the renal tubular epithelium.

Key words: rabbit; nano-chromium; heat stress; histopathology

Introduction

The thermal comfort zone for rabbits is 21°C. Any elevation from this temperature is considered heat stress (1). Heat stress is divided into two types acute and chronic heat stress. This division depends on the period of exposure and the presence of tissue damage including: pyknosis, apoptosis, necrotic areas, and an increase in melanomacrophage centers in liver tissues through, DNA digestion and cell membrane destruction (2, 3). Previous studies (4-6) observed vacuolated hepatic degeneration with dilation and congestion of sinusoid, widespread necrosis, and infiltration of leukocyte in some parts of the liver and kidney in heat stressed rabbits. The authors also reported damage to the glomeruli of the kidneys. As a consequence, Ondruska et al. (7) reported high animal mortality rates, leading to economic losses for rabbit producers during summer season.
More recent studies have tried to reduce heat stress through different management and nutritional methods. Nano-chromium is one of the most important nutritional supplements that can be used to decrease and possibly eliminate heat stress damage. However, low doses must be used as higher doses may lead to adverse animal effects such as degenerative changes and necrosis in liver as well as hyaline casts in kidney and tubules and glomeruli (8).

Chromium is an essential trace mineral required for most vital metabolic processes. It is used in carbohydrate, protein, and lipid metabolism (9); therefore it has anabolic effects during periods of stress, including heat stress. Research has shown that nano-chromium has an anabolic effect when used in low concentrations and enhances the nucleic acid synthesis in the liver of the mouse (10). The objective of the current study was to determine what dosage of nano-chromium chloride is needed to ameliorate the degenerative changes in the vital tissues of heat stressed rabbits.

Materials and methods

This study was carried out at the Department of Animal Wealth Development in the Faculty of Veterinary Medicine at Kafrelsheikh University in Kafrelsheikh, Egypt. The experiment was approved by the guiding of committee on Animal Welfare and Ethics of the Faculty of Veterinary Science, Kafrelsheikh University, in accordance with Egyptian national laws regarding animal welfare. One hundred eight weaning aged rabbits of two different breeds (Rex and New Zealand White; 35 ± 3 and 33 ± 2 d weaning age, respectively) were used to evaluate the effect of nano-chromium chloride on liver, kidney, and appendix tissues.

Experimental design

Each breed had an initial body weight of 602 ± 9.5 and 531 ± 6.5 g for Rex and New Zealand Breed, respectively. Animals of each breed were divided equally into 6 groups with 9 rabbits in each group. Three groups of each breed were subjected to severe heat stress (32.8 ± 1.5 °C; HT) and the other three groups were reared under room temperature conditions (24.5 ± 1.3 °C; RT) (11). Each group contained 3 replicates with 3 rabbits in each replicate. Each replicated group was reared on wire cages 50 × 50 ×50 cm and fed commercial rabbit pellets (18% crude protein) ad libitum.

The nano-chromium particles were prepared by chemical precipitation method of chromium chloride salt (12), and then characterized by transmission electron microscope (TEM) at the Nanotechnology Institution of Kafrelsheikh University, Egypt. The resulting particles were 40 – 60 nm. These particles were then added to the drinking water at 3 different concentrations (0, 1 or 2 mg/L; C, 1, and 2, respectively) for the duration of the 8 week study. At the end of the experiment rabbits were slaughtered for tissue collection. The liver, kidney and appendix tissues were collected in 10% formalin, sectioned, and stained with H&E stain for microscopic examination (13).

Results

Liver

The liver of the control groups from both breeds and untreated with nano-chromium showed the normal arrangement of the hepatocytes in cords around the central vein and normal sinusoids (Figure 1- A and C). However, the liver of the groups that were exposed to heat stress but untreated with nano-chromium showed that the New Zealand breed was more susceptible to heat stress than the Rex breed. This is due to the appearance of severe congestion of the central vein and sinusoids, as well as granular hepatic vacuolation (Figure 1- B and D). Mild hepatic valuation in New Zealand rabbits treated with 1 mg/L nano-chromium at room temperature was observed (Figure 2). This is consistent with excessive glycogen storage in the liver. This was also seen when New Zealand rabbits received the same concentration of nano-chromium, but that were also exposed to heat stress (Figure 2- C). However, when Rex Rabbits were treated with 1 mg/L of nano-chromium under room temperature conditions, they appeared to have
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normal hepatocyte and liver tissue as seen in Figure 2-B. When exposed to heat stress with the same concentration of nano-chromium, the Rex rabbits responded the same as the New Zealand rabbits, and also appeared to have congestion of the sinusoids. The addition of 2 mg/L nano-chromium caused hepatic vacuolation with glycogen infiltration that was severe in New Zealand breeds (Figure 3-A) and mild in Rex Rabbits (Figure 3-B). While in animals exposed to heat stress, the same effect was observed in both breeds which appears as marked decrease in hepatic vacuolation.

Kidney

There was no observable difference in kidney tissues between breeds under the same temperature conditions. Under room temperature conditions, the two breeds showed normal renal glomeruli and tubules (Figure 4-A and C). However under heat stress, they showed degenerative changes within the lining epithelium of the renal tubules (Figure 4-B and D). In regards to renal tissues, there was no observable difference in the response between the breeds when treated with 1 mg/L nano-chromium and under the same temperature (Figure 5-A and C). The tissue appeared to be normal under room temperature conditions. Mild degenerative changes in the lining of the epithelium of the renal tubules appeared in heat stressed animals (Figure 5-B and D). In rabbits treated with 2 mg/L nano-chromium, there was no observable difference in response between two breeds under room temperature. Rex rabbits showed normal renal glomeruli and tubules (Figure 6-A). The New Zealand rabbits showed normal renal glomeruli, but showed mild vacuolation of the renal tubular epithelium (Figure 6-B). There was also no difference between the two breeds under heat stress compared with New Zealand under room temperature.

Appendix

Under room temperature conditions and treated with 0 mg/L nano-chromium, the two breeds showed normal lymphoid follicles (Figure 7-A and C), but heat stress caused a mild lymphoid depletion in the germinal center of the follicle of Rex rabbits and on the basal follicle of the New Zealand rabbits (Figure 7-B and D). When rabbits were treated with 1mg/L nano-chromium under room temperature conditions, they were not different from the untreated rabbits under the same temperature (Figure 8-A and C). However under heat stress, the addition of 1mg/L of nano-chromium ameliorated the lymphoid depletion that resulted from the heat stress in untreated rabbits (Figure 8-B and C). Figure 9 from A to D showed that there was no difference between breeds treated with 2 mg/L nano-chromium under different temperature treatment.

Discussion

Liver

The granular hepatic vacuolations resulting from exposure to heat stress observed in Figure 1 may be due to the activation of mitochondrial reactive oxygen species (ROS; 14) which can lead to cytotoxicity, apoptotic cell death, and necrosis (15). This degenerative condition appears as vacuoles in hepatocytes. However, the severe congestion of the central vein was likely due to the fact that the liver has the densest concentration of mitochondria, which are overloaded during stress. This can lead to increased blood supply to this organ as a compensatory mechanism (16). Figure 2-A and B represent the liver tissue unexposed to heat stress and treated with 1mg/L nano-chromium. The New Zealand breed showed a better response to nano-chromium treatments than the Rex breed. This is evident in the mild hepatic vacuolation that is consistent with excessive glycogen storage. This is in agreement with Huskisson et al. (17) that illustrated that Cr III enhanced glucose uptake by the liver cells. On the other hand, Muthulingam et al. (18) observed that Cr lead to decreased glycogen in the gills, liver, and kidneys of fish. This was observed in Figure 3, where the degenerative changes and congestion of the liver caused by heat stress disappeared and were replaced by marked hepatic vacuolation due to the increased dose of nano-chromium.
Figure 1: A) Liver of room temperature (R) Rex rabbits showing normal hepatocyte arranged in cords around the central vein (arrow), H&E, bar = 40 µm. B) Liver of heat stressed (HS) Rex breed showing granular hepatic vacuolation (arrow), H&E, bar = 40 µm. C) Liver of New Zealand-R rabbits showing normal hepatocytes arranged in cords around the central vein (arrow), H&E, bar = 40 µm. D) Liver of HS-New Zealand rabbits showing severe congestion of the central vein and sinusoids (arrowheads) and granular hepatic vacuolation (arrow), H&E, bar = 40 µm

Figure 2: A) Liver of room temperature (R) Rex treated with 1 mg/L nano-chromium (T1) showing normal hepatocytes (arrow), H&E, bar = 40 µm. B) Liver of HS Rex rabbits T1 showing sinusoidal congestion (arrowhead) and mild hepatic vacuolation consistent with hydropic changes (arrow), H&E, bar = 40 µm. C) Liver of R-New Zealand rabbits T1 showing mild hepatic vacuolation consistent with over glycogen storage (arrow), H&E, bar = 40 µm. D) Liver of heat stressed (HS) New Zealand rabbits T1 showing hepatic vacuolation consistent with over glycogen infiltration (arrow), H&E, bar = 40 µm
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**Figure 3:**

A) Liver of room temperature (R) Rex rabbits treated with 2mg/L nano-chromium (T2) showing mild hepatic vacuolation consistent with glycogen storage (arrow), H&E, bar= 200 µm.  
B) Liver of Rex heat stressed (HS) rabbits T2 showing marked decrease of hepatic vacuolation (arrow), H&E, bar = 40 µm.  
C) Liver of R New Zealand rabbits T2 showing marked hepatic vacuolation consistent with glycogen infiltration (arrow), H&E, bar= 200 µm.  
D) Liver of HS New Zealand rabbits T2 showing marked decrease of hepatic vacuolation (arrow), H&E, bar= 40 µm.

**Figure 4:**

A) Kidney of room temperature (R) Rex rabbits showing normal renal glomeruli and tubules (arrowhead and arrow respectively), H&E, bar = 40 µm.  
B) Kidney of heat stressed (HS) Rex rabbits showing marked degenerative changes within the lining epithelium of the tubules (arrows), H&E, bar= 40 µm.  
C) Kidney of R New Zealand rabbits showing normal renal glomeruli and tubules (arrowhead and arrow respectively), H&E, bar= 40 µm.  
D) Kidney of HS New Zealand rabbits showing marked degenerative changes within the lining epithelium of the tubules (arrows), H&E, bar= 40 µm.
Figure 5: A) Kidney of room temperature (R) Rex rabbits with 1 mg/L nano-chromium (T1) showing normal renal glomeruli and tubules (arrowhead and arrow respectively), H&E, bar = 40 µm. B) Kidney of HS Rex rabbits T1 showing mild degenerative changes within the lining epithelium of the renal tubules (arrow), H&E, bar = 40 µm. C) Kidney of R New Zealand rabbits T1 showing normal renal glomeruli and tubules (arrowhead and arrow respectively), H&E, bar = 40 µm. D) Kidney of HS New Zealand rabbits T1 showing mild degenerative changes within the lining epithelium of the renal tubules (arrow), H&E, bar = 40 µm.

Figure 6: A) Kidney of room temperature (R) Rex rabbits with 2 mg/L nano-chromium (T2) showing normal renal glomeruli and tubules (arrowhead and arrow respectively), H&E, bar = 40 µm. B) Kidney of heat stressed (HS) Rex rabbits T2 showing normal renal glomeruli and mild vacuolation of the renal tubular epithelium (arrow), H&E, bar = 40 µm. C) Kidney of R-T2 New Zealand rabbits showing normal renal glomeruli (arrowhead) and mild vacuolation of the renal tubular epithelium (arrow), H&E, bar = 40 µm. D) Kidney of HS-T2 New Zealand rabbits showing normal renal glomeruli (arrowhead) and mild vacuolation of the renal tubular epithelium (arrow), H&E, bar = 40 µm.
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Figure 7: A) Appendix of room temperature (R) Rex rabbits showing normal lymphoid follicles covered with epithelial covering (arrow), H&E, bar = 200 µm. B) Appendix of heat stressed (HS) Rex rabbits showing mild degree of lymphoid depletion of the germinal center of the follicles (arrow), H&E, bar = 200 µm. C) Appendix of R New Zealand rabbits showing normal lymphoid follicles covered with epithelial covering (arrow), H&E, bar = 200 µm. D) Appendix of HS New Zealand rabbits showing lymphoid depletion of the germinal center of the basal follicles (arrow), H&E, bar = 200 µm.

Figure 8: A) Appendix of room temperature (R) rabbits treated with 1 mg/L nano-chromium (T1) showing normal lymphoid follicles (arrow), H&E, bar = 200 µm. B) Appendix of heat stressed (HS)-T1 Rex rabbits showing normal lymphoid follicles (arrow), H&E, bar = 200 µm. C) Appendix of R-T1 New Zealand rabbits showing normal lymphoid follicles (arrow), H&E, bar = 200 µm. D) Appendix of HS-T1 New Zealand rabbits showing normal lymphoid follicles (arrow), H&E, bar = 200 µm.
Kidney

When both breeds were exposed to room temperature conditions and untreated with nano-chromium they showed normal glomeruli and tubules (Figure 4-A and C). Kidneys of animals exposed to heat stress, but also untreated with nano-chromium particles showed degenerative changes within the lining of the epithelium of the renal tubules. This is likely due to the cytolytic response of the tissues by the heat followed by endotoxemia that enhanced the release of inflammatory cytokines and consequently caused vascular endothelium injury (19). This appeared as degenerative changes in tubular lining epithelium in this study.

Figure 5-A and C showed normal renal and glomerular structures when 1mg/L of nano-chromium was administered. This is interrupted to mean that this concentration of nano-chromium is safe and does not alter the renal structure in rabbits housed in room temperature conditions. This level of nano-chromium also decreased, but did not remove, the degenerative changes resulting from heat stress in both breeds. While the addition of 2mg/L nano-chromium did ameliorate the degenerative changes in the heat stressed rabbits, this was replaced by mild vacuolation in lining epithelium. This may be due to glucose uptake by the cell increased by addition of this concentration (17) rather than toxicity.

Appendix

Animals of both breeds that were not treated with nano-chromium and kept under room temperature conditions appeared to have normal lymphoid follicles, as they were covered with epithelium. This indicates that this temperature was comfortable for the immune system of the rabbits. On the other hand, the groups that were exposed to heat stress and untreated with nano-chromium appeared to have a lymphoid depletion of the germinal center of the follicle (Figure 7-B and D). This may be due to the heat stress enhanced the production of ROS (20) that lead to death of...
most of cells (21), including immune cells and resulted in lymphoid depletion.

The addition of both 1 and 2mg/L of nano-chromium had the same effect on appendix tissues during heat stress. The addition of nano-chromium prevented the lymphoid depletion resulting from this stress (Figure 8-B and D; Figure 9-B and D). These results may be due to the catalytic effect of Cr III on the H2O2 inactivating enzyme, consequently decreasing the cell apoptosis (22). The structure of the appendix was not altered in groups treated with 2mg/L of nano-chromium, but not exposed to heat stress. This in the agreement with Yucesoy et al. (23), that illustrated that Cr salts do not have an effect on the immune system cells.

Conclusion

In conclusion, addition of 2 mg/L nano-chromium particles to the drinking water of the heat stressed rabbits could be used to alleviate the granular degenerative changes and the sinusoidal congestion of the rabbit liver. As well as to ameliorate the effects of the previous concentration on the degenerative changes in the epithelium lining renal tubules. It is also able to reduce the lymphoid depletion in the lymphoid follicles of the rabbit appendix.

References


