



Study the biochemical, hematological, and histopathological alteration in heat-stress fish treated with an immune stimulant

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Abstract

Levamisole effects on the biochemical, hematological, and Histopathological parameters on heat stress fish. 40 *Cyprinus carpio* fish were distributed into 4 groups, raised in a glass aquarium, and provided with a basic diet for ten days. Control groups as G1 and three treated groups were tested G2 with the elevated water temperature at 30°C, G3 was treated with a concentrated dose of 125 mg/L of levamisole, and the water temperature was 25°C. At the same time, G4 elevated water temperature to 30°C and treated with the same dose of levamisole. At the end of the experiment, all groups were euthanized. Blood samples were collected for packed cell volume, hemoglobin, mean corpuscular hemoglobin concentration, cortisol, glucose, immunoglobulin, albumin, and histopathological investing from all groups also done. The results showed that the G2 and G3 showed highly significant variation in the level of glucose and albumin in contrast to others, as well G4 showed highly significant variation in the level of the biochemical parameters, G4 showed a higher level of biological parameters changes in contrast to the other groups. At the same time, the hematological value was higher in the G3. The pathological alterations of G2, G3 and G4 represented vascular changes such as hemorrhagic congestion, disturbances of growth infiltration of inflammatory cell, necrosis, hyalin cast and dilution of bowman's capsule; all these changes were graded as mild, moderate, and severe changes and were more severe in G2. Our results refer that immune stimulant agents enhance the biochemical and hematological parameters, but it leads to changes in the liver and kidney due to the effects of heat stress.

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Introduction

The main vital ecological factors which affect organisms' persistence and growth in aquaculture farming is the physicochemical criteria of the water, mainly water temperature, which changes according to the climate and season, and this may affect the aquatic environment population and diversity of biota, fish are ectothermic. Which mean that any change in the culture water temperature (acute or chronic heat stress) could affect the fish homeostasis, survival, immune system and physiologically response (1-3).

Primary, secondary, and tertiary physiological responses to adverse environmental stressors have been identified in fish (4,5). Primary responses include the initial release of neurotransmitters such as dopamine and serotonin, which are examples of neuroendocrine responses, and releasing corticosteroid hormone to the blood circulation (6); cortisol is a secondary response that involves physiological biochemical and hematological response with alteration in the ionic acid-base balance and changes in the levels of the metabolites. In contrast, other responses include retired growth, abnormal behavior, fish performance, and

immunosuppressed, which lead to fish death during spawning and reduce fish resistance to diseases (7,8). Al-Hamdani and Al-Tae (8) reported that respiratory rate increases with elevated Hb concentration during exposure *Carrassius carrassius* to heat stress, also increased percentage of PCV and stress index in *Cyprinus carpio* exposed to an elevated temperature to 30, 32, and 34°C (9), with histopathological alteration in the gills of *Carrassius carrassius* (10). Immunostimulants such as antimicrobial agents (vaccination), probiotics, food additives, vitamins such as vitamin c, which improve the immune system against heat stress (11), and plant extract are important compounds that act to enhance specific and non-specific immunity and increase resistance to disease as hemorrhagic septicemia caused by *Aeromonas hydrophila* and other environmental stressor factors as heat stress and pollution (12,13). Levamisole is an anti-nematode used in *C. carpio* farming to stimulate non-specific immunity against bacterial diseases (14), and also stimulate grass carp (*Ctenopharyngodon idella*) (15).

We aimed in current study to determine the effects of levamisole on biochemical, hematological responses and histological in the internal organs of thermal stress fish.

Materials and methods

Ethical approve

The experiment were agreeable by the moral committee at Mosul University and conformed with ethics approve number UM.VET.2021.068.

Design experiment

Forty healthy *Cyprinus carpio* fish weighing 125±10 gm was obtained from the College of Agriculture and Forestry, University of Mosul from to the fish unit in the animal house care of Veterinary Medicine College -University of Mosul; fish has been put in the glass aquarium 30*30*40 cm for adaptation at last 10 day with contentious replacement de chlorinated and aeration water, temperature 25°C and feed with commercial pellet. Fish were distributed into four groups: G1 consider a control group with a 25°C water temperature; G2 was exposed to an elevated water temperature of 30°C while fish in G3 were treated with levamisole with a concentration dose of 125 mg/L (16). The water temperature was 25°C, and G4 was exposed to elevated water temperature 30°C and treated fish with the same dose of levamisole. Fish were allowed to adapt to the new tank condition for 2 weeks and then used for the experiment for 10 days.

Biochemical test

At the end of the experiment at 10 days of treatment, all fish per aquarium in G1, G3, and G4 were netted and then anesthetized according to (17), except G2, then blood was collected from the caudal vein by an insulin syringe, blood

was drawn and divided into part, the first part put right away into a test tube coated with ethylene diamine tetra acetic acid (EDTA) and mixed gently to prevent blood hemolysis. This part of the blood sample was utilized for determined packed cell volume (PCV), Hemoglobin (Hb) and Mean Corpuscular Hemoglobin Concentration (MCHC) (18). The second part of the blood was transferred to another test tube to obtain the serum to analyze glucose, cortisol, albumin and immunoglobulin (19).

Histopathological examination

At the end of the experiment, tissue samples from all the groups were obtained through anesthetized fish (20) except the G2 the tissue samples were obtained after 48 hours of the treatment. A part from the Liver and posterior kidney samples was excised, swill in physiological saline, and firm in aqueous Bouin's solution, and by ascending concentration of ethyl alcohol tissue samples were dried then embedded in paraffin wax, sectioned at 4-5mm, stained with H&E and finally all slides examined by a light microscopic (21-23).

Statistical analysis

one-way test of variation (ANOVA) was used to evaluate all data result with SPSS, and the significant value of differences between means was compared by multiple Duncan's tests (24).

Results

Hematological variables serum

PCV and Hb concentration have shown significant increase in the G3 (Table 1) when compared with the other groups. While PCV and Hb did not increase significantly between other treated groups and G1. At the same time, MCHC showed no significant variation in G2 in contrast to the other group, which showed a highly significant value.

The result presented in table 2 showed a significant difference and high-level increase in cortisol concentration in G4 in contrast to the control and other treated groups. There was no significant variation between G4 and G1, while glucose concentration, in general, showed high significant variation in all groups in contrast to G2, with a significant difference in the Ig concentration in G4 when compared with the other group, as well as the Albumin concentration showed high significant variation in all groups.

Histopathological result

Histopathological changes of the liver and kidney section in G2 showed vacuolar degeneration of hepatic cells, congestion of sinusoids, and hemorrhage in the hepatic tissue, while the kidney section showed deposition of proteins material in the renal tubules with a hyaline cast, hemorrhage in the renal tissue, infiltration of mononuclear inflammatory cells, coagulative necrosis of renal tubules with fibrosis of bile ducts (Figures 1-4).

Table 1: Effects of levamisole on PCV, Hb, and MCHC in *Cyprinus carpio* fish

Blood parameters	G1	G2	G3	G4
PCV (%)	33.67±4.04 b	0.00±0.00 c	40.50±1.73 a	35.33±3.21 b
Hb (g/dl)	11.40±0.35 b	0.00±0.00 c	13.10±0.58 a	11.60±1.00 b
MCHC (%)	32.30±0.22 a	0.00±0.00 b	32.35±0.06 a	32.26±0.12 a

The different letters mean a significant difference at P<0.05.

Table 2: Effects of levamisole on a biochemical test of *Cyprinus carpio* fish

Parameters	G1	G2	G3	G4
Cortisol	420.33±269.60 ab	0.00±0.00 c	231.00±132.82 bc	622.00±34.00 a
Glucose	7.10±5.50 a	0.0± 0.00 b	143.81±274.80 a	6.85±1.17 a
Ig	0.21±0.00 b	0.00±0.00 b	0.32±0.29 b	176.12±170.88 a
Albumin	3.65±0.05 a	0.00±0.00 b	5.45±4.09 a	5.15±0.85 a

The different letters mean a significant difference at P<0.05.

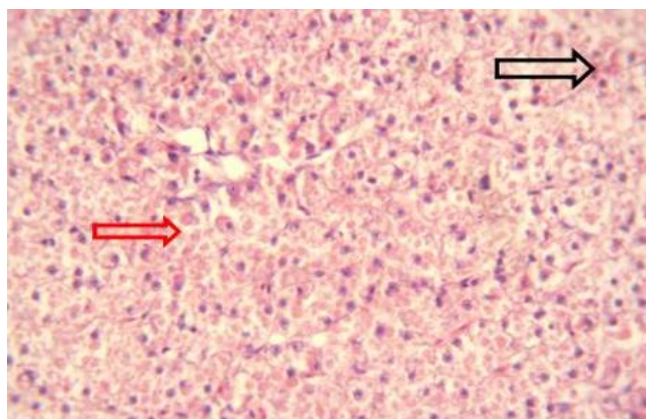


Figure 1: Histopathology section of the liver in G2 showed vacuolar degeneration of hepatic cells (red arrow) and congestion of sinusoids (black arrow). H&E, 40X.



Figure 3: Histopathology section of the kidney in G2 showed vacuolar degeneration (yellow arrow) and fibrosis of bile ducts (blue arrow). H&E, 40x.

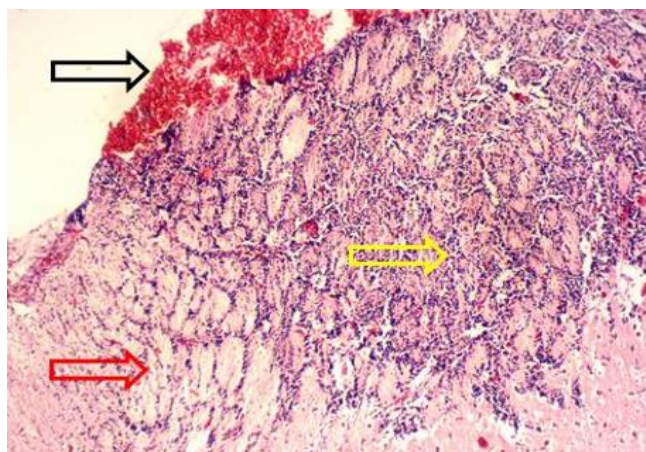


Figure 2: Histopathology section of the kidney in G2 showed diffused hemorrhage in renal tissue (black arrow), proteins material in the renal tubule (yellow arrow) and coagulative necrosis of renal tubules (red arrow). H&E, 10X.

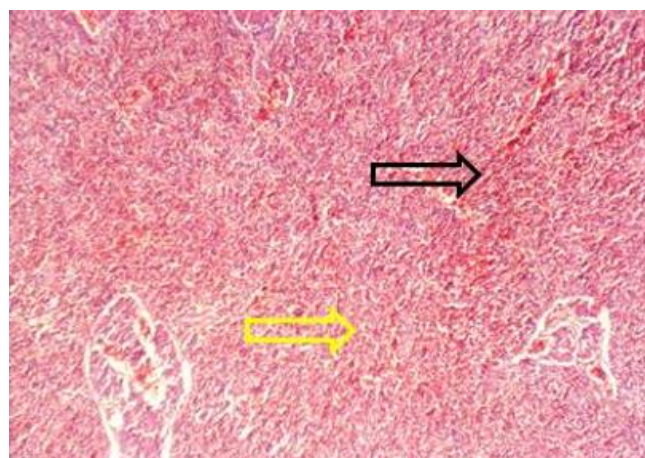


Figure 4: Histopathology section of the liver section in G2 showed vacuolar degeneration (yellow arrow) and hemorrhage (black arrow). H&E, 10X.

Also there was vacuolation and hydropic degeneration and dissemination of epithelial cells lining the renal tubules with congestion of blood vessel with desquamation of epithelial cells, necrosis of the renal tubules and infiltration of mononuclear inflammatory cells in the interstitial tissue (Figures 5 and 6), while G4 showed Congestion of the sinusoid, hydropic degeneration of hepatic and renal tubule, hemorrhage with dilatation of bowman's capsule (Figures 7-11).

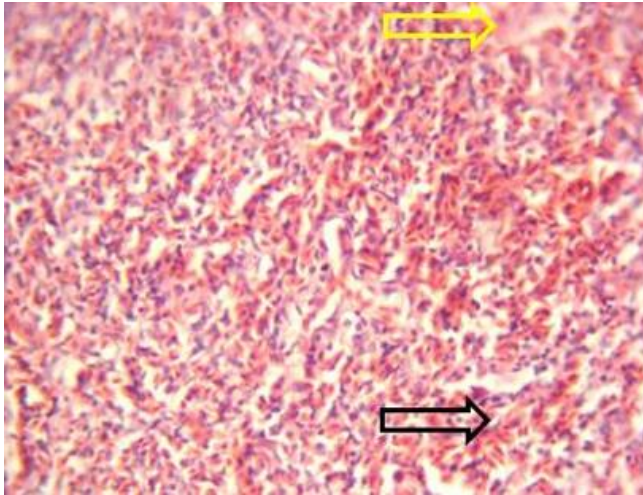


Figure 5: Histopathology section of the liver in G2 showed infiltration of inflammatory cells (black arrow) and fibrosis of hepatocytes (yellow arrow). H&E, 10X.

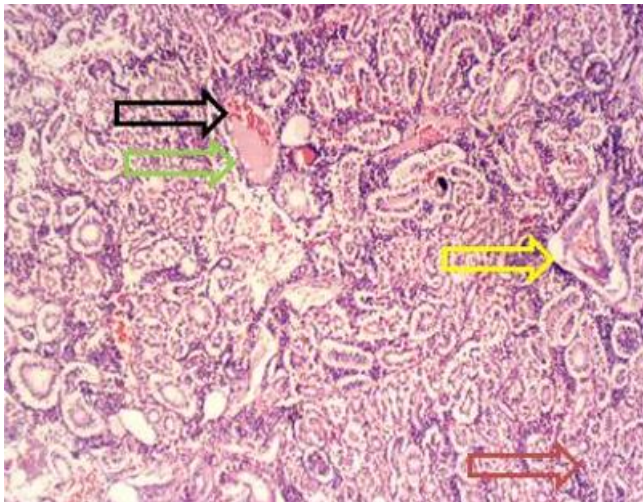


Figure 6: Histopathology section of the kidney in G3 showed recent thrombus (black arrow), proteinaceous material (green arrow), diffused vacuolation (yellow arrow), infiltration of the inflammatory cell (red arrow). H&E, 10X.

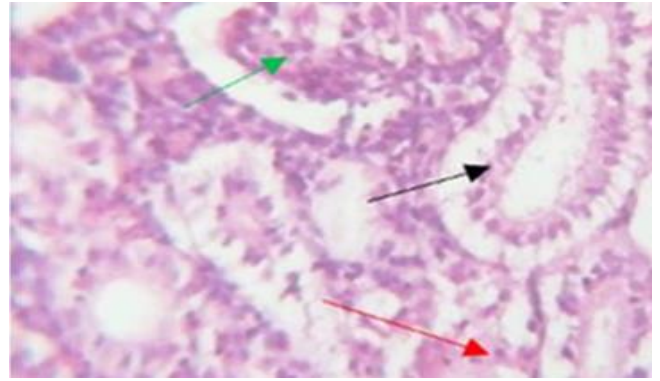


Figure 7: Histopathology section in G3 showed hydropic degeneration of the epithelial cell lining renal (black arrow), necrosis of the renal tubules (red arrow), hydropic degeneration of cells in the renal Taft of glomeruli with necrosis of some of them (green arrow). H&E, 40X.

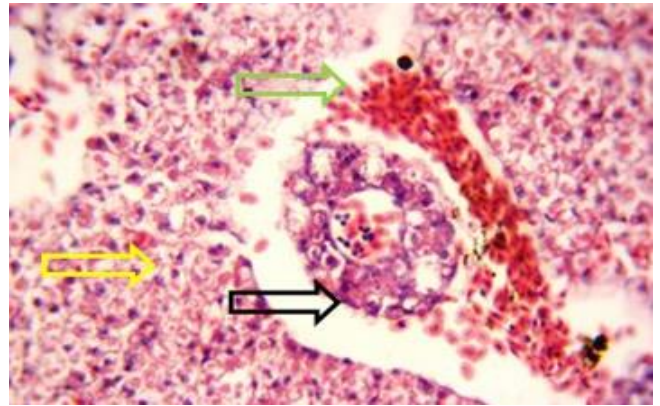


Figure 8: Histopathology section of the liver in G3 showed hemorrhage (green arrow), necrosis of pancreatic tissue (black arrow), infiltration of inflammatory cells (yellow arrow). H&E, 10X.

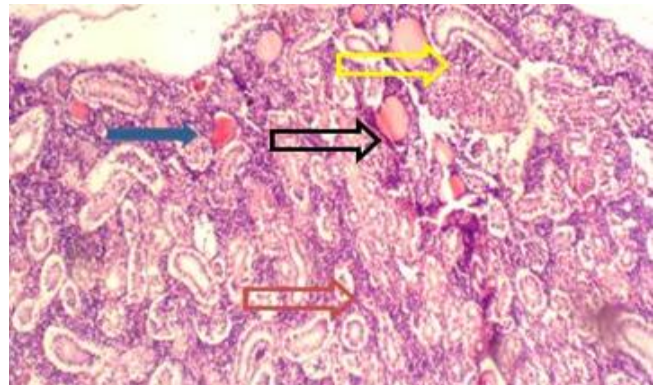


Figure 9: Histopathology section of the kidney in G3 showed proteinaceous material (black arrow), hemorrhage (blue arrow), inflammatory cell (red arrow), and necrosis of glomeruli (yellow arrow). H&E, 10X.

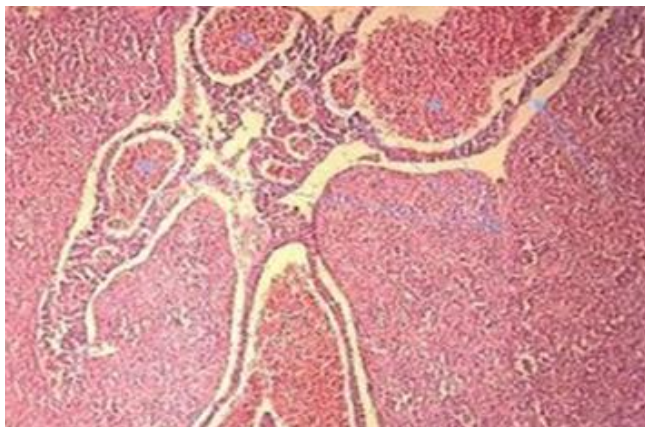


Figure 10: Histopathology section of liver in G4 showed server congestion with edema (blue arrow). H&E, 200X.

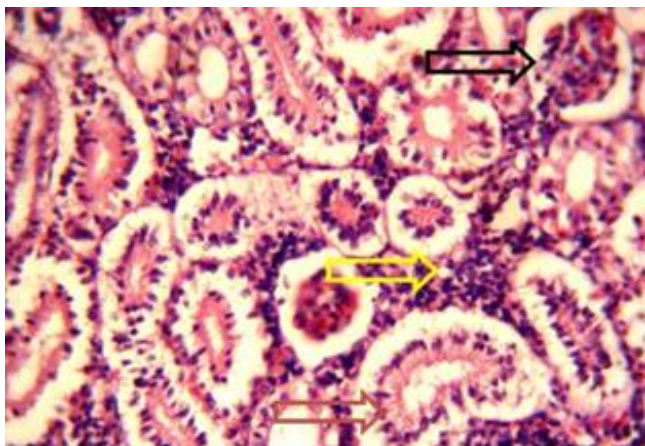


Figure 11: Histopathology section of the kidney showed tubular necrosis (black arrow) and severe infiltration of the inflammatory cell (yellow arrow) with the dilution of bowman's capsule (red arrow). H&E 300X.

Discussion

Aquatic creatures are exposed to local and international environmental stressors, like (chemical, biological, physical, and procedural stressors) (25,26). Stressors can cause various physiological and biochemical changes in living organisms. Following exposure to stressful events with cellular stress response (27). Numerous physiological changes in an organism's biological processes can be caused by variations in temperature. Particularly for cold-blooded fish species, increasing the temperature can seriously harm them and disrupt the body's metabolism (28).

According to established thermodynamic principles, increasing environmental temperatures raises oxygen consumption and accelerates several metabolic activities (29). Blood plasma comprises various proteins, mainly albumin, globulin, and fibrinogen. Among these, globulin

and albumin maintain the balance of fluid between blood and body tissues; our result showed a significant increase in albumin levels in all groups, and these related to the action of these sera in monitoring and diagnosis of inflammatory diseases; additionally, this serum is considered a crucial element in the transport of many substances, defense against pathogens, regulation of osmotic pressure and in other process (30). Our result showed a significant increase in glucose levels in all groups; body's response to stress is characterized by the secretion of cortisol, glucose, and catecholamine, which leads to hem dilution or hemp concentration due to alteration in the plasma concentration (31,32).

Hematological factors are reliable markers that help explain a fish's health condition or metabolic problems (33,34). Our result showed a significant increase in PCV and Hb concentration in G3 compared to the other group. These contributed to the physiological status of fish and the stimulant agent's action in enhancing innate and adaptive immunity, resulting in agreement with (35). The Histopathological examination of the internal organs in all treated groups showed mild to severe pathological changes (36,37). Liver and kidney sections showed disturbance of growth with dilution of Bowne's capsule and infiltration of inflammatory cells; these changes may be attributed to continuous tissue and cell irritation by different type of inflammatory factor such as heat stress, toxic material, injury and even infection (38,39). Degenerative changes which was more pronounced in G2 (increase temperature group) represented by necrosis which occurs in organism tissue in which the cell completely damaged and die. Pyknosis, hyperchromatosis, and complete paralysis occur inside the cytoplasm, stained with haematoxylin and eosin (40). In this condition, extracellular fluid is produced from the cell, irritating neighbouring cells and producing inflammation (41). Vascular changes, including edema, congestion, and recent thrombus with diffused vasculitis, are also noted inside hepatocytes and glomeruli. These may be associated with physiological causes, environmental conditions, time of the experiment, type of fish, and other adjuvant material leading to mild, moderate, and severe pathological changes (42).

Conclusions

Our results refer that immune stimulant agents enhance the biochemical and hematological parameters, but it leads to Histopathological changes in the liver and kidney due to the effects of heat stress.

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Conflict of interest

The researcher declares no competing interests.

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دراسة التغيرات البايوكيميائية والدموية والنسجية في الأسماك المجهددة حرارياً والمعالجة بالمنشطات المناعية

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الخلاصة

تم تقييم تأثير الليفاميزول على المعايير الكيموحيوية، الدموية والنسجية للإجهاد الحراري في ٤٠ سمكة من أسماك الكارب الاعتيادي. قسمت الأسماك بصورة عشوائية إلى أربع مجاميع وضعت في أحواض زجاجية وأعطيت العلف، مثلت المجموعة الأولى مجموعة السيطرة وبدرجة حرارة ٢٥ درجة مئوية، المجموعة الثانية عرضت إلى درجة الحرارة ٣٠ درجة مئوية، المجموعة الثالثة تم معاملتها بمادة الليفاميزول وعرضت إلى درجة حرارة ٢٥ درجة مئوية والمجموعة الرابعة عوملت بمادة الليفاميزول بالإضافة إلى رفع درجة الحرارة إلى ٣٠ درجة مئوية. في نهاية التجربة تم التضحية بالأسماك وتم جمع الدم لغرض قياس حجم الخلايا المرصوصة، صباغ الدم، ومتوسط تركيز الهيموكلوبين في الخلية، وقياس مستوى الكورتيزول وسكر الدم والكلوبيولين المناعي والألبومين بالإضافة إلى اخذ نماذج من الأعضاء الداخلية متمثلة بالكبد والكلى لغرض إجراء الفحص النسجي المرضي. أظهرت نتائج المجموعة الثانية والثالثة ارتفاعاً معنوياً ملحوظاً في مستوى تركيز الكلوكلوز والألبومين مقارنة ببقية المجاميع، وأظهرت نتائج المجموعة الرابعة ارتفاعاً معنوياً في مستوى المعايير الكيموحيوية، تضمنت التغيرات المرضية اضطرابات وعائية تمثلت بالنزف والاحتقان، ارتشاح الخلايا الالتهابية والنخر وتوسع محفظة بومان أيضاً وكانت شدة هذه الآفات بين الخفيفة والمتوسطة والشديدة وأكثرها شدة بالمجموعة الثانية، نستنتج من نتائج الدراسة الحالية إلى أن المواد المحفزة للمناعة مثل الليفاميزول قد حسنت من المعايير الكيموحيوية والدموية لكنها لم تحمي من التغيرات النسجية المرضية نتيجة تأثير عامل الإجهاد الحراري.