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ORIGINAL RESEARCH

Effects of 10- and 30-minute Hepatic Ischemia on Total Protein, Albumin, Globulin Fractions, and LDH of Male Albino Rates; An Experimental Study

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Abstract: **Introduction:** Hepatic ischemia results in the dysrhythmia of the intrahepatic hemodynamics. This study aimed to evaluate the changes occurring in the protein metabolism and serum lactate dehydrogenase (LDH) level after hepatic ischemia and assess the effects of antioxidant therapy in this regard. **Methods:** In this experimental study (with manipulation of ischemia time and antioxidant therapy) 120 male rats were divided into groups of acute hepatic ischemia; acute hepatic ischemia +antioxidant; chronic ischemia using tetra chloromethane; and sham or intact control to evaluate the changes occurring in the protein metabolism (total protein, albumin, and globulin fractions) and LDH level 3, 7, 15, and 30 days after 10- or 30-minute hepatic ischemia induction and effects of antioxidant therapy in this regard. **Results:** In the 10-minute ischemia group, total protein decreased to 34.69 ± 2.49 g/L at 3 days, while albumin fell to 12.36 ± 0.85 g/L. The inflammatory response was evident through elevated α1-globulin (9.02 ± 1.50 g/L) and LDH (3476.37 ± 324.89 U/L) at day 3, which gradually normalized by day 30. In the 30-minute ischemia group, the effects were more pronounced, with total protein reaching 54.57 ± 1.93 g/L and albumin 34.33 ± 2.20 g/L at day 3, alongside marked increases in α1-globulin (10.35 ± 1.30 g/L), α2-globulin (3.19 ± 0.43 g/L), β-globulin (8.09 ± 2.27 g/L), γ-globulin (5.64 ± 1.08 g/L), and LDH (2301.44 ± 80.07 U/L). After 10- and 30-minute ischemia, α1, α2, β, and γ globulins as well as LDH level were significantly increased at the post-ischemic recovery. The group that received antioxidant showed significantly lower increases in the globulin fractions and LDH level at 3, 7, 15, and 30 days after the procedure. **Conclusion:** Based on the findings, 10- and 30-minute acute hepatic ischemia had a profound negative effect on protein metabolism, which was reflected in decreased total protein and albumin, and increased globulin fractions and LDH, indicating the presence of continuous hepatocellular injury and a significant inflammatory reaction. Riditox antioxidant therapy had a consistent, albeit incomplete, hepatoprotective effect, which attenuated these biochemical imbalances.

Keywords: Liver ischemia model; Protein metabolism; Oxidative stress; Antioxidant therapy; LDH activity

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1. Introduction

The liver is susceptible to ischemic injury owing to its huge involvement in detoxification and the metabolism (1, 2). Ischemic hepatic injuries occur at a cellular level and usually heal within 3-5 days on reperfusion (1, 3). In both hypoxia and reperfusion, reactive oxygen species (ROS) production by mitochondria triggers the onset of an inflammatory response that is dependent on Kupffer cell activation. These cells liberate superoxide anions, hydrogen peroxide and hydroxyl radical, which increase the hepatocellular damage even further (4, 5).

Both exogenous and endogenous intoxication cause ischemia that results in the dysrhythmia of the intrahepatic hemodynamics (6). A combination of the pestilential environmental body poisons, infectious diseases like viral hepatitis, and the surgical post-operative incidence are reported as the frequent causes of hepatic ischemia (7). There is a risk of a temporary shutdown of the blood flow during surgical operations, which, along with the duration of the shutdown, can result in ischemia in some parts of the liver (8) . Ischemic liver

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injuries are known to be a frequent issue in liver transplant and liver surgery as well as trauma (9). In the case of such procedures, special attention should be paid to reperfusion injury, since the metabolic and detoxification capabilities of the liver become impaired (10). In addition, there are hepatic disorders like non-alcoholic fatty liver disease (NAFLD) and hepatitis that predispose the liver to ischemic damage (11).

Several studies have confirmed that oxidative stress is a major factor in liver ischemia injuries (12, 13). Besides, antioxidant therapy has already demonstrated a mitigating effect on liver ischemia with increased mitochondrial activity and alleviated oxidative stress. The administration of antioxidants, including N-acetyl cysteine (NAC) facilitates the work of cell defense systems combatting the reduction of glutathione (GSH) level, ultimately minimizing the generation of ROS and renewing the synthesis of proteins (14, 15).

The connection between oxidative stress and liver ischemia is a subject of much research, but the molecular interaction between oxidative damage with protein metabolism is not well-researched. Most of the available studies have concentrated on oxidative stress or the protein metabolism individually. Moreover, though the antioxidants are shown to be crucial protective elements, the role they performed on the balance of protein metabolism in a process of ischemic injury is underrepresented.

Based on the mentioned points, this study aimed to evaluate the changes occurring in the protein metabolism and serum lactate dehydrogenase (LDH) level after hepatic ischemia and assess the effects of antioxidant therapy in this regard.

2. Methods

2.1. Study design and setting

This experimental study (with manipulation of ischemia time and antioxidant therapy) was conducted on male rats to evaluate the changes occurring in the protein metabolism (total protein, albumin, and globulin fractions) and LDH level 3, 7, 15, and 30 days after 10- or 30-minute hepatic ischemia induction and effects of antioxidant therapy in this regard.

The Ethical Review Board of Azerbaijan Medical University approved the study and all the procedures of the study met with the acceptable international ethical standards in conducting animal research. Rats were treated in a humane way throughout the course of the study and attempts were made to ensure the least level of discomposure and distress. Ethical recommendations of the World Health Organization were acceded to in the study. All the procedures were conducted diligently so that the welfare of the animals was guaranteed. Confidentiality was ensured by making the data anonymous; all results were made available in an aggregate manner to scientifically communicate the same. The rats were allowed free access to food and water and were maintained on a 12-h light/dark cycle at room temperature (24 ± 2 °C) with constant humidity (40 ± 15%).

2.2. Sampling strategy and study parameters

A group of 120 healthy male albino rats (Rattus norvegicus) aged between 8 and 12 weeks were randomly assigned to the following experimental groups:

Group (A): 10- or 30-minutes acute ischemia (n=40): The animals were subjected to 10 minutes (n = 20) or 30 minutes (n=20) of acute hepatic ischemia using hepatic artery ligation. In each duration of ischemia, there were subgroups of five animals that were euthanized after 3, 7, 15, and 30 days after the ischemia induction.

Group (B): Acute ischemia + antioxidant (n=40): The subjects underwent acute hepatic ischemia by hepatic artery ligation for 10 minutes (n=20) or 30 minutes (n=20) and then were given antioxidant Riditox (succinic acid–methionine complex; Pharm standard, Kursk, Russia) therapy. Like in the acute ischemia alone, five-animal subgroups were sacrificed at 3, 7, 15, and 30 days after ischemia induction.

Group (C): Chronic ischemia or chronic intoxication (n = 20): This group was added to provide a suitable pathological comparator of ischemic injury. They were given subcutaneous injections of 50 percent oil solution of tetra chloromethane (Carbon tetrachloride (CCl4)) at a dose of 0.5ml/100g body weight, twice per week and over four weeks, inducing chronic hepatic damage. The paradigm used is a proven method of inducing hepatic oxidative stress and fibrosis, which serves as a standard against which the specific proteomic changes induced by acute ischemia could be compared (16). The subjects in this group were sampled and analyzed using the same sampling and analytical procedures as the ischemic groups at the same time points (3, 7, 15, and 30 days).

Group (D): Sham or intact control (n = 20): The participants in this group were laparotomized, but no hepatic artery ligation was done, and the participants were not given any antioxidants. This population was further subdivided into four subpopulations (n = 5 each), which were euthanized on days 3, 7, 15, and 30, respectively, to have time matches of baseline controls.

2.3. Procedures

- Induction of acute hepatic ischemia

Selective ligation of the hepatic artery to cause acute hepatic ischemia was performed under sterile conditions, with 10 or 30 minutes of ligation. To exclude confounding factors related to reperfusion injury, the duration of ischemia was intentionally kept without the revival of ischemia.

Intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg) was used to attain anesthesia. Midline laparotomy was then carried out, allowing the isolation of the hepatic artery. The 5.0 silk suture was put in place, obstructing blood flow throughout the specified time (10 or 30 minutes). After the procedure, buprenorphine (0.05-1.0mg/kg) was administered to all animals as analgesics; they were kept hydrated and meticulously monitored after the operation to detect the indication of distress or pain.

The anesthesia and postoperative care in sham control group were the same as experimental groups. The acute ischemia + antioxidant groups were exposed to the same surgery as the ischemia-only groups, but these groups were subjected to Riditox therapy (0.2mL per rat and given weekly).

- Euthanasia and sampling schedule

Humane euthanasia of animals was done at 3, 7, 15, and 30 days after induction of ischemia. Initially, to euthanize animals, they were anesthetized with ketamine (80mg/kg) and xylazine (10 mg/kg) given intraperitoneally then euthanized by cardiac exsanguination under anesthesia, to ensure total insensibility and prevent the perception of pain. This was in accordance with institutional animal welfare regulations as well as the international ethics of animal care in the laboratory. The arguments behind euthanasia at the mentioned points were triple: Ethical and welfare justification: The volumes of serum necessary to perform biochemical and protein fraction analyses (around 1.5 to 2 mL each assay) were not consistently available using the collected serum of a single rat due to the risk of hypovolemia or death in such a situation. Experimental design: A time point was used as an independent cohort, which allowed assessing the temporal changes in the biochemical and histopathological parameters after an ischemic injury.

Histopathological validation: Liver tissue was taken as early as possible after the death, to be examined morphologically and cytologically, so that results of the biochemical findings could be supported by histopathological evidence of hepatic injury and its reparation.

Therefore, euthanasia done in the acute, subacute, and recovery periods was considered a particular observational endpoint, which made it possible to conveniently study time-varying metabolic changes caused by ischemia.

- Antioxidant therapy

The antioxidant therapy was applied to the group of acute ischemia plus antioxidant. Riditox (succinic acid–methionine complex; Pharm standard, Kursk, Russia) was used as an antioxidant (0.2 mL, intravenous injection, once per 7 days). Riditox is a detoxifying and antioxidant formula that has succinic acid, methionine, and vitamins, particularly E and C, and has been known to increase the antioxidant enzyme activity and reduce oxidative stress. Its primary mode of action is the free radical scavenging and stabilization of the hepatocellular membranes (17).

2.4. Data instrumentation and collection

Each group was sampled aseptically on days 3, 7, 15, and 30 on the retro-orbital plexus. Serum was centrifuged at 3,000 × g in 10 minutes in 4 °C and stored in -80 °C until examined. The amount of serum protein, including albumin and globulin subfractions (alpha 1, alpha 2, beta and gamma) was analyzed with enzyme-linked immunosorbent assay (ELISA) kits (Roche Diagnostics, Germany) as per the protocol. The ELISA assay entailed antigen-antibody interactions on the microplate wells, enzyme-linked detection, and development of the color at 450 nm on the microplate reader. The activity of serum lactate dehydrogenase (LDH) was determined using kinetic colorimetric assay (Roche Diagnostics, Germany) and expressed in U/L. These kits offer validated, repeatable and, hence, reliable measurement of biochemical variables.

2.5. Outcomes

The main outcome of study was exploring the changes in serum protein metabolism (total protein, albumin, and globulin fractions) and LDH of male rats after 10- and 30-minute induction of acute hepatic ischemia and to explore time-dependent effects of antioxidants on protein metabolism after acute hepatic ischemia.

2.6. Statistical analysis

Since the sizes of the subgroups were relatively small and the biochemical data were non-normally distributed, non-parametric statistical analyses were used. Biochemical markers in a number of groups were compared with the Kruskal–Wallis test with post-hoc pairwise testing to detect any particular difference. To determine the correlations between antioxidant treatment and protein metabolism markers, Spearman's rank correlation was used. $P < 0.05$ was considered as the level of significant differences.

3. Results

3.1. Protein metabolism changes after 10- and 30-minute hepatic ischemia

The changes of total protein, serum albumin, and globulin fractions were monitored 3, 7, 15, and 30 days after the induction of 10-minute (table 1, figure 1) and 30-minute (table 2, figure 2) hepatic ischemia. The findings were as follows:

- Total protein changes

After 10-minute hepatic ischemia

In group A, the total protein concentration dropped significantly at day 3 compared with group B (34.69 ± 2.49 g/L vs. 41.35 ± 3.87 g/L; p = 0.005), indicating early hepatic synthetic impairment. Although protein levels rose gradually by day 7 (45.36 ± 2.35 g/L) and day 15 (51.69 ± 2.85 g/L), these differences remained statistically non-significant compared with group B (p = 0.196 and p = 0.075, respectively). By day 30, total protein in group A (54.35 ± 2.39 g/L) was close to that of group B (56.61 ± 1.36 g/L; p = 0.293), suggesting partial recovery.

Group C showed intermediate levels throughout, with values of 46.95 ± 2.38 g/L (day 3) to 54.50 ± 4.16 g/L (day 30), not differing significantly from group B (p > 0.05). Overall, the data indicate that short (10-min) ischemia caused an initial decline in total protein, followed by a gradual normalization over 30 days.

After 30-minute hepatic ischemia

Prolonged (30-min) ischemia induced a more persistent decline in total protein levels across all time points in group A

compared with the control. The mean concentrations were 54.57 ± 1.93 g/L on day 3, 71.57 ± 4.97 g/L on day 7, 59.58 ± 4.06 g/L on day 15, and 60.25 ± 3.08 g/L on day 30, all showing significant differences relative to group B (p = 0.002, 0.002, 0.049, and 0.028, respectively).

Group B maintained relatively stable protein levels (61.57 ± 2.20 g/L to 61.36 ± 2.74 g/L), whereas group C consistently exhibited the lowest means (46.95 ± 2.38 g/L to 54.50 ± 4.16 g/L). By day 30, group A's values remained below control but indicated mild recovery.

These results demonstrate that extended ischemia significantly suppressed hepatic protein synthesis and that recovery remained incomplete even after 30 days.

- Serum albumin changes

After 10-minute ischemia

In group A, serum albumin levels showed a marked reduction at all observation intervals compared with group B. The mean albumin concentration was 12.36 ± 0.85 g/L on day 3 (p = 0.004), gradually rising to 16.86 ± 1.42 g/L on day 7 (p = 0.008), and reaching 25.46 ± 2.96 g/L and 22.38 ± 1.89 g/L on days 15 and 30, respectively (p = 0.264 and p = 1.000).

Although albumin levels recovered progressively after day 7, the early suppression indicates impaired hepatic protein synthesis following ischemic insult.

Group B exhibited relatively higher albumin values (13.49 ± 0.94 g/L at day 3 to 22.43 ± 1.27 g/L at day 30), while group C maintained intermediate levels without significant deviation from controls (p > 0.05). By day 30, no significant difference persisted between group A and group B, suggesting substantial restoration of liver synthetic function after 10 minutes of ischemia.

After 30-minute ischemia

Following prolonged ischemia, serum albumin concentrations in group A were significantly altered across all intervals. A sharp decrease was recorded at day 3 (34.33 ± 2.20 g/L; p = 0.002) and day 7 (27.12 ± 3.14 g/L; p = 0.007), followed by a pronounced decline on day 15 (17.25 ± 1.23 g/L; p = 0.002). By day 30, a modest recovery was noted (18.86 ± 1.24 g/L), but values remained significantly lower than those of group B (22.43 ± 2.69 g/L; p = 0.025). Group B maintained relatively stable albumin levels throughout the experiment (29.69 ± 1.07 g/L to 22.43 ± 2.69 g/L), whereas group C showed mild fluctuations but no significant differences compared with controls. These findings indicate that 30 minutes of hepatic ischemia induced a prolonged suppression of serum albumin synthesis, with only partial recovery evident after 30 days.

- Fraction 1 globulin (α1-Globulin) changes

After 10-minute ischemia

The α-globulin fraction in group A showed a notable elevation following ischemic insult. Concentrations increased from 9.02 ± 1.50 g/L on day 3 to 12.03 ± 1.55 g/L on day 7 (p = 0.024) and remained higher on day 15 (12.06 ± 0.70 g/L; p = 0.067). A significant surge was observed on day 30 (18.77 ± 4.00 g/L; p = 0.004) compared with the control group.

Group B exhibited lower α-globulin levels throughout (7.82 ± 0.89 g/L to 23.37 ± 3.27 g/L), while group C showed similar trends but with smaller magnitude. The rise in α-globulin in group A may reflect an acute-phase hepatic response to ischemic injury, particularly at the later recovery stage (day 30), where differences remained statistically significant.

After 30-minute ischemia

A progressive and sustained elevation in α-globulin was recorded in group A across all time points. The values increased from 10.35 ± 1.30 g/L on day 3 (p = 0.065) to 20.37 ± 2.53 g/L on day 7 (p = 0.002), peaking on day 15 (29.97 ± 3.86 g/L; p = 0.002) before slightly declining on day 30 (26.68 ± 2.72 g/L; p = 0.001).

Compared with group B, α-globulin levels in group A were consistently higher at all stages, indicating an intensified acute-phase response following prolonged ischemia. The magnitude of this increase, particularly on days 15 and 30, suggests persistent hepatocellular stress and compensatory protein synthesis.

- Fraction 2 globulin (α2-globulin) changes

After 10-minute ischemia

In the early phase post-ischemia, α-globulin levels in group A remained relatively stable (5.80 ± 0.71 g/L on day 3 and 5.97 ± 0.58 g/L on day 7; p = 0.046 and p = 0.174, respectively). By day 15, values showed mild variation (5.52 ± 0.75 g/L; p = 0.185), followed by a pronounced increase on day 30 (18.51 ± 2.02 g/L; p = 0.005).

This late surge was significant relative to both group B (19.89 ± 2.45 g/L) and group C (17.30 ± 1.88 g/L), suggesting delayed hepatic activation and increased synthesis of α-globulins as part of the repair phase. The marked rise at day 30 likely reflects post-ischemic inflammatory and regenerative processes.

After 30-minute ischemia

A sharp and statistically significant increase in α-globulin was observed in group A throughout all post-ischemic intervals. Levels rose from 3.19 ± 0.43 g/L at day 3 (p = 0.006) to 21.10 ± 1.12 g/L at day 7 (p = 0.002), peaking at 35.75 ± 1.70 g/L on day 15 (p = 0.002). Despite a slight decline on day 30 (29.94 ± 2.42 g/L), concentrations remained significantly higher compared with group B (p < 0.001).

The sustained elevation in α-globulin across the 30-day period indicates prolonged hepatic stimulation of acute-phase protein synthesis following extended ischemic damage, reflecting a stronger and more persistent inflammatory response than that observed after 10 minutes of ischemia.

- Fraction 3 globulin (β-globulin) changes

After 10-minute ischemia

β-globulin levels in group A displayed a significant increase throughout the observation period compared with the control group. The mean concentrations rose from 6.14 ± 0.34 g/L on day 3 (p = 0.018) to 5.34 ± 0.70 g/L on day 7 (p = 0.468), 5.11 ± 0.70 g/L on day 15 (p = 0.566), and markedly peaked at 21.61 ± 2.51 g/L on day 30 (p = 0.002).

While early changes (days 3–15) were minor and statistically

insignificant, a pronounced elevation was evident by day 30, indicating delayed β-globulin activation during hepatic recovery. Group B showed generally lower values (5.52 ± 0.51 to 26.58 ± 3.90 g/L), whereas group C remained stable (5.20 ± 0.25 to 20.91 ± 2.51 g/L). The late surge in β-globulin likely reflects enhanced hepatic synthesis of acute-phase transport proteins during tissue regeneration.

After 30-minute ischemia

In the prolonged ischemic group, β-globulin concentrations increased significantly at all time points in group A relative to the control. The mean levels were 8.09 ± 2.27 g/L at day 3 (p = 0.009), 20.39 ± 4.83 g/L at day 7 (p = 0.009), 34.57 ± 8.47 g/L at day 15 (p = 0.005), and 27.15 ± 9.21 g/L on day 30 (p = 0.009). Compared with group B, β-globulin remained consistently higher, indicating a robust and persistent hepatic synthetic response following prolonged ischemic stress. In contrast, group C exhibited stable but lower values across all intervals. The magnitude and duration of β-globulin elevation in group A suggest a strong association with prolonged inflammatory and repair mechanisms during hepatic recovery.

- Gamma globulin (γ-Globulin) changes

After 10-minute ischemia

Serum γ-globulin levels in group A exhibited moderate fluctuations at early intervals, followed by a significant rise during the later phase of observation. Mean concentrations were 10.22 ± 0.89 g/L on day 3 (p = 0.065) and 9.67 ± 1.29 g/L on day 7 (p = 0.145), with a decline to 8.65 ± 1.65 g/L at day 15 (p = 0.024). A substantial increase occurred by day 30, reaching 14.18 ± 2.15 g/L (p = 0.031).

Group B maintained lower γ-globulin levels (8.51 ± 0.48 to 12.47 ± 4.60 g/L), while group C showed minor fluctuations with no significant intergroup difference (p > 0.05). The late-phase elevation in group A indicates a delayed immunoglobulin response, potentially reflecting recovery-associated immune activation following hepatic ischemia.

After 30-minute ischemia

Prolonged ischemia induced a strong and sustained rise in γ-globulin concentrations in group A across all time points. Levels increased from 5.64 ± 1.08 g/L on day 3 (p = 0.035) to 17.96 ± 2.32 g/L on day 7 (p = 0.002), followed by 16.35 ± 2.27 g/L at day 15 (p = 0.006) and 19.29 ± 1.23 g/L at day 30 (p = 0.001).

In all cases, γ-globulin levels were significantly higher compared to both groups B and C, demonstrating enhanced immune globulin synthesis in response to extended hepatic injury. The persistence of elevated γ-globulin levels up to day 30 highlights the prolonged immune activation phase that accompanies delayed hepatic regeneration following extended ischemic stress.

Effect of antioxidant therapy on globulin fractions

In group A, α1, α2, β, and γ globulins were significantly increased during post-ischemic recovery, after 10- and 30-minute ischemia. Conversely, group B showed lower increases at these globulin fractions.

- LDH changes

After 10-minute ischemia

A pronounced rise in LDH activity was observed in the acute ischemia group (A), reaching 3476.37 ± 324.89 U/L on day 3 and 2935.73 ± 94.37 U/L on day 7 (Table 1, Figure 3). Although the enzyme activity remained above the control values throughout the observation period, the differences were not statistically significant at these early time points (p > 0.05). By day 30, LDH levels had decreased to 1753.51 ± 106.08 U/L, which was significantly lower than both the control (p = 0.002) and comparison group C values. The enzyme activity pattern therefore suggested an early transient increase followed by gradual normalization by day 30 post-ischemia.

After 30-minute ischemia

In the prolonged ischemia model, LDH activity in group A increased significantly on days 3 and 7 compared with the control (p = 0.014 and p = 0.003, respectively), reaching 2301.44 ± 80.07 U/L and 2425.01 ± 148.27 U/L, respectively (Table 2, Figure 3). Thereafter, LDH activity declined markedly, measuring 1611.79 ± 116.26 U/L on day 15 and 1395.54 ± 76.88 U/L on day 30 (p = 0.006 and p = 0.001, respectively). In comparison with group C, LDH levels in the ischemic group remained consistently lower at later stages, suggesting a compensatory recovery in hepatocellular enzyme release.

Effect of antioxidant therapy on LDH

In group A, the LDH level was significantly high in the early post-injury stages, after 10- and 30-minute ischemia. The values were relatively high with consistent changes over time. Conversely, group B registered a lower LDH response at the respective time points.

4. Discussion

The antioxidant therapy showed a buffering effect on the changes that occurred in the globulin fractions after hepatic ischemia. In the ischemia-only group (A), α 1, α 2, β, and γ globulins were significantly increased at the post-ischemic recovery, indicating an extreme reaction of the acute phase and inflammation. This increase was especially apparent at later periods, during which the chronic oxidative stress and hepatocellular damage probably favored the increment of synthesis of acute-phase cytokines and mobilization of immunoglobulins. Conversely, the ischemia plus antioxidant (B) group showed significantly lower increases in these globulin fractions, the values adjusted towards physiological levels over time.

The weaker reaction in this group indicates that the antioxidant supplementation was useful in stabilizing the hepatocellular integrity, decreasing oxidative-mediated inflammatory signaling, and restricting the excessive synthesis of acute-phase proteins. On the whole, these results suggest that antioxidants helped perform the regulation of the hepatic inflammatory cascade; therefore, maintaining proteomic profile balance during ischemic injury recovery.

The LDH profile also helps determine the protective effect of the antioxidant therapy during hepatic ischemia. In the is-

chemia only group (A), the LDH level was significantly high in the early post-injury stages, which demonstrates that there is a significant displacement of hepatocellular membranes and release of intracellular enzymes into the circulatory system. The values were relatively high with consistent changes over time, though they were lower than those of the treated group, indicating persistent cellular activity and delayed structural restoration. Conversely, the ischemia plus antioxidant group (B) registered a significantly lower LDH response at the respective time points. This inhibited increase indicates that antioxidant supplementation served to alleviate oxidative injury to hepatocytes, stabilize cellular membranes, and constrain enzyme release. The relatively higher rate of LDH normalization in group B thus represents the increased speed of recovery and less impact of ischemic damage in the presence of antioxidant support.

In the current experimental study, we gave an in-depth temporal breakdown of the metabolic effects that follow a short-term hepatic ischemia episode. The article puts the emphasis directly on the finer points of protein synthesis and cellular recovery, and as early as ten minutes, the results already show a deep, biphasic perturbation of protein turnover, a transient inhibition of the synthetic process, and a long, acute-phase inflammatory reaction (18). The incomplete but impressive alleviation that was obtained with the antioxidant substance Riditox highlights the importance of oxidative insult in the pathophysiology of ischemic hepatic injury (14).

The 30-minute ischemia caused more severe liver damage, which triggered a powerful compensatory immune response. This is proven by the massive increase in γ-globulins (antibodies) in Table 2. While the liver produced fewer proteins (like albumin), the immune system produced far more γ-globulins. This surge in one protein fraction offset the loss in others, resulting in a smaller net decrease in the total protein value compared to the 10-minute model, which did not show this strong immune reaction. In short, after 30 minutes of ischemia, the total protein decreased less because a severe immune response masked the severity of the liver's failure.

Our ultimate objective was to trace the development of the protein metabolism and lactate dehydrogenase (LDH) activity after acute hepatic ischemia. The statistics show that it has a distinct biphasic trend (19). A significant decrease in total protein and albumin is observed during the first week after ischemia, which is an immediate inhibition of the biosynthetic apparatus of the liver, which is a natural result of an energy crisis (20). Hepatocytes, which have a high metabolic turnover, are highly dependent on sustained oxygen supply to sustain their aerobic metabolic activity, and ischemia causes rapid depletion of ATP. Such depletion impairs the function of the endoplasmic reticulum, which arrests the translation and folding of complex proteins like albumin (21). Hypoalbuminemia that follows is more severe and persistent after the 30-minute ischemic insult than the 10-minute and demonstrates the dose-dependent nature of the energetic insult (22).

Conversely, the second phase of the response, a vigorous production of inflammatory and immune activation, was manifested by progressive and significant increase of all globulin fractions (α1, α2, β, and γ) (23). The acute-phase and hepatic response to injury was a sharp increase in α1 and α2-globulins that contain acute-phase proteins like 1-antitrypsin and haptoglobin. The activation process is caused by the cytokines such as IL-6 produced by hepatocytes, and the process is initiated by activated Kupffer cells released during ischemia-reperfusion (24). The simultaneous rise in γ-globulins, which represents the production of immunoglobulins, was evidence that the adaptive immune system was activated, and this stimulation could have been due to the neoantigens released on necrotic hepatocytes (25). The serum LDH profile is a strong support of cellular injury. A huge release of LDH right after an ischemic episode signals the break of the plasma membrane and necrotic cell death (10). A gradual decrease with time after 30 days indicates reparative and regenerative mechanisms of the hepatic tissue. However, the partial normalization of LDH, particularly following the 30-minute ischemic insult, is evidence of the partial cellular damage or continued inflammation beyond the initial insult (26, 27).

Our observations on inhibited protein synthesis support classical studies of Nemeth et al. (28) who established quick hepatic protein secretion inhibition during ischemia. The fall of albumin matches equally with clinical investigations on patients undergoing liver resection, where postoperative hypoalbuminemia is associated with the time period of vascular inflow discontinuation.

The literature is highly favorable to the pronounced acute-phase response, demonstrated by the elevated levels of globulins (28). The pivotal finding of Yu et al. (29) has outlined the necessity of Kupffer-cell activation and tumor necrosis factor (TNF)-release in early liver ischemia. Our data further support these results by indicating the continued increase of α2 globulin up to 30 days, indicating that ischemia triggers a protracted reorganization of the hepatic proteome, as opposed to a short-lived burst (30). The temporal variations of the globulin fractions, especially the subsequent peaks of 205 (beta) and 70 (gamma) components, are presumably due to a shift in the innate to adaptive immune interaction, which is becoming increasingly popular in modern ischemia-reperfusion research (11).

The protective effect of Riditox is consistent with an extensive body of antioxidant studies. Sun et al. demonstrated that N-acetylcysteine prevents mitochondrial oxidative stress in the aftermath of ischemia (31). Riditox is a succinic acid, methionine derivative and works presumably through a similar mechanism, restoring the Krebs cycle and increasing the production of glutathione and strengthening cellular response to reactive oxygen species. Our results offer the biochemical support of this cytoprotective ability in a regulated ischemic model (32). Our findings are based on the cascade of ischemic injury, which has biological foundations. Disruption

of hepatic perfusion leads to a hypoxic microenvironment, forcing cells to shift to anaerobic glycolysis. This metabolic change is depleting ATP and causes lactic acidosis that interferes with ion gradients and ends in cellular swelling (33).

The critical point is when reperfusion takes place. Recovery of oxygen in the damaged cells results in the abrupt activation of the reactive oxygen species (ROS) of mitochondria through xanthine oxidase enzyme activity (34-36). ROS directly damage macromolecules such as enzymes and ribosomal apparatus used to make proteins, which is the explanation of the first decline in total protein and albumin. Meanwhile, ROS serve as signaling molecules that trigger the expression of proinflammatory transcription factors in Kupffer cells (NF- -KB) and the production of proinflammatory cytokines (TNF, IL-1, IL-6) (37, 38). These cytokines cause the remaining hepatocytes to divert their synthesis of housekeeping proteins in favor of emergency acute-phase proteins' production, explaining the sudden increase in globulin fractions (39, 40). The action of Riditox is likely to be direct scavenging of ROS and supplying substrates to succinate dehydrogenase and glutathione synthesis, which improves the mitochondrial energy and redox homeostasis of the body (41). This dual response alleviates both the priming oxidative attack and secondary inflammatory signaling, which was observed as less pronounced changes in protein fractions and LDH observed in cured cohorts (22, 42, 43).

The implication of this study is twofold. Clinically, our information brings out the fact that even brief periods of ischemia, which could be inevitable during liver surgery, have non-immediate metabolic effects (44, 45). The albumin-to-globulin ratio and LDH level approaches were followed, which might be used as subtle measures of the extent of ischemic injury and recovery effectiveness. Moreover, this is reinforced by the proven effectiveness of Riditox, which justifies the prophylaxis of using antioxidants in the operating room when hepatic ischemia is expected, like transplantation and major resections (11). It gives a scientific foundation for future clinical trials to be conducted to test antioxidant adjuvants in order to enhance patient outcomes.

This study presents a number of avenues to be used in future research. The sustained inflammatory process implies the possible association of the transient ischemic events and the progression to the chronic liver pathology, including fibrosis. These biochemical changes have long-term histopathological correlates that require investigation. Also, further proteomic analysis may help point to particular acute-phase proteins that are the best biomarkers of ischemic injury (46).

To sum up, our findings outline a complicated temporal sequence of hepatic metabolic dysfunction after acute ischemia, that is, decreased synthesis and increased inflammation. The fact that antioxidant therapy partially reduces this injury confirms the pathogenic effect of oxidative stress and suggests an exciting approach to the treatment of liver damage, which is feasible in a clinical setting in the case of a vascular compromise.

5. Limitations

This research can be viewed as providing useful information, some weaknesses should be noted. Though necessary to have a controlled experimental design, the use of an animal model restricts the direct transferability of the results to human physiology. Each group (n=5) is rather small; however, such a sample size is typical of animal research, which decreases the statistical power and exposes the research to Type II errors. Lastly, the role of oxidative stress was deduced indirectly based on the effect of an antioxidant by the study, as/but direct measurements of ROS, lipid peroxidation, or antioxidant enzyme activities would have been more mechanistic evidence to support the proposed pathway.

6. Conclusions

Based on the finding, hepatic ischemia of 10 and 30 minutes had a profound negative effect on protein metabolism, which was reflected in decreased total protein, albumin, and increased globulin fractions and LDH, indicating the presence of continuous hepatocellular injury and a significant inflammatory reaction. Riditox antioxidant therapy had a consistent, albeit incomplete, hepatoprotective effect, which attenuated these biochemical imbalances.

7. Declarations

7.1. Acknowledgments

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7.2. Author contributions

Shalala Garib İsmayilova: Conceptualization, experimental design, data acquisition, drafting of the manuscript.

Zumrud Amirgulu Abaszade (Corresponding Author): Supervision, methodology, statistical analysis, manuscript revision, correspondence with the journal.

Aygun Vugar Kazimli: Laboratory experiments, biochemical analyses, interpretation of protein metabolism results.

Nigar Taryel Guliyeva: Histological and cytological examinations, validation of experimental findings.

Hijran Faramaz Khidirova: Animal model handling, Ischemia Model procedures, and data collection.

Maryam Rauf Abbasova: Literature review, data curation, and drafting of background section.

Kamil Sahib Alkishiev: Clinical interpretation, discussion of therapeutic implications, and manuscript editing.

All authors read and approved the final manuscript.

7.3. Ethics approval and consent to participate

All experimental procedures involving animals were conducted in accordance with international guidelines for the care and use of laboratory animals and were approved by the Ethics Committee of Azerbaijan Medical University. Ethical recommendations of the World Health Organization were acceded to in the study. Every effort was made to minimize animal suffering and reduce the number of animals used.

7.4. Consent for publication

Not applicable. The manuscript does not contain data from individual persons that require consent for publication.

7.5. Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

7.6. Competing interests

The authors declare that they have no competing interests.

7.7. Funding

This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

7.8. Using artificial intelligence chatbots

There was no conception, design, data analysis, or writing of this manuscript with the help of any artificial intelligence (AI) tools or chatbots. The authors were the sole contributors to all the content, interpretations, and conclusions based on their original research.

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Table 1: Comparing the blood proteins and lactate dehydrogenase changes after 10-minute hepatic ischemia between studied groups

Parameter	Time after procedure							
	3 days	P	7 days	P	15 days	P	30 days	P
Total Protein (g/L)								
A	34.69 ± 2.49	0.001	45.36 ± 2.35	0.006	51.69 ± 2.85	0.033	54.35 ± 2.39	0.309
B	41.35 ± 3.87		48.42 ± 1.67		58.25 ± 4.90		56.61 ± 1.36	
C	46.95 ± 2.38		47.17 ± 3.17		53.90 ± 2.60		54.50 ± 4.16	
D	56.02 ± 0.87		56.34 ± 1.05		56.56 ± 0.69		55.64 ± 2.68	
Albumin (g/L)								
A	12.36 ± 0.85	0.001	16.86 ± 1.42	0.001	25.46 ± 2.96	0.028	22.38 ± 1.89	1.000
B	13.49 ± 0.94		20.42 ± 0.57		27.24 ± 1.81		22.43 ± 1.27	
C	16.42 ± 1.09		18.39 ± 1.22		25.23 ± 1.15		22.76 ± 2.58	
D	22.90 ± 0.27		22.80 ± 0.27		22.78 ± 0.28		22.52 ± 0.33	
α1 globulin (g/L)								
A	9.02 ± 1.50	0.043	12.03 ± 1.55	0.002	12.06 ± 0.70	0.003	18.77 ± 4.00	0.008
B	7.82 ± 0.89		11.48 ± 0.84		10.82 ± 0.63		23.37 ± 3.27	
C	7.99 ± 0.55		9.63 ± 1.01		10.81 ± 1.12		21.41 ± 2.84	
D	7.12 ± 0.29		7.18 ± 0.35		7.12 ± 0.29		6.62 ± 0.58	
α2 globulin (g/L)								
A	5.80 ± 0.71	0.010	5.97 ± 0.58	0.010	5.52 ± 0.75	0.119	18.51 ± 2.02	0.009
B	4.71 ± 0.55		5.30 ± 0.58		4.31 ± 1.76		19.89 ± 2.45	
C	5.32 ± 0.70		5.28 ± 0.72		4.53 ± 1.01		17.30 ± 1.88	
D	4.44 ± 0.29		4.46 ± 0.35		4.50 ± 0.22		4.40 ± 0.58	
β globulin (g/L)								
A	6.14 ± 0.34	0.013	5.34 ± 0.70	0.317	5.11 ± 0.70	0.782	21.61 ± 2.51	0.004
B	5.52 ± 0.51		5.08 ± 0.50		5.65 ± 1.14		26.58 ± 3.90	
C	5.20 ± 0.25		5.53 ± 0.37		5.40 ± 0.90		20.91 ± 2.51	
D	5.12 ± 0.13		5.14 ± 0.11		5.08 ± 0.08		5.10 ± 0.17	
γ globulin (g/L)								
A	10.22 ± 0.89	0.013	9.67 ± 1.29	0.045	8.65 ± 1.65	0.025	14.18 ± 2.15	0.053
B	8.51 ± 0.48		8.15 ± 1.48		7.27 ± 0.74		12.47 ± 4.60	
C	9.22 ± 1.93		8.35 ± 1.12		10.46 ± 1.44		12.06 ± 2.69	
D	7.70 ± 0.35		7.60 ± 0.41		7.70 ± 0.29		7.11 ± 0.78	
Lactate dehydrogenase (U/L)								
A	3476.37 ± 324.89	0.004	2935.73 ± 94.37	0.011	2575.54 ± 183.41	0.008	1753.51 ± 106.08	0.004
B	3120.41 ± 739.35		2976.93 ± 187.62		2686.28 ± 117.29		1815.10 ± 72.20	
C	2676.43 ± 473.69		3126.31 ± 385.66		2704.48 ± 170.62		1915.84 ± 81.94	
D	780.00 ± 44.72		771.00 ± 38.79		798.00 ± 20.80		775.84 ± 121.01	

Data are presented as mean ± standard deviation. A: 10-minute ischemia; B: 10-minute ischemia +antioxidant; C: Chronic ischemia; and D: Sham or control.

Table 2: Comparing the blood proteins and lactate dehydrogenase changes after 30-minute hepatic ischemia between studied groups

Parameter	Time after procedure							
	3 days	P	7 days	P	15 days	P	30 days	P
Total Protein (g/L)								
A	54.57 ± 1.93	0.001	71.57 ± 4.97	0.000	59.58 ± 4.06	0.067	60.25 ± 3.08	0.026
B	61.57 ± 2.20		64.39 ± 1.11		59.47 ± 2.98		61.36 ± 2.74	
C	46.95 ± 2.38		47.17 ± 3.17		53.90 ± 2.60		54.50 ± 4.16	
D	56.02 ± 0.87		56.34 ± 1.05		56.56 ± 0.69		55.64 ± 2.68	
Albumin (g/L)								
A	34.33 ± 2.20	0.000	27.12 ± 3.14	0.001	17.25 ± 1.23	0.001	18.86 ± 1.24	0.029
B	29.69 ± 1.07		29.13 ± 2.51		21.83 ± 1.86		22.43 ± 2.69	
C	16.42 ± 1.09		18.39 ± 1.22		25.23 ± 1.15		22.76 ± 2.58	
D	22.90 ± 0.27		22.80 ± 0.27		22.78 ± 0.28		22.52 ± 0.33	
α1 globulin (g/L)								
A	10.35 ± 1.30	0.018	20.37 ± 2.53	0.000	29.97 ± 3.86	0.000	26.68 ± 2.72	0.002
B	8.73 ± 3.32		12.95 ± 1.64		19.59 ± 1.59		19.46 ± 2.25	
C	7.99 ± 0.55		9.63 ± 1.01		10.81 ± 1.12		21.41 ± 2.84	
D	7.12 ± 0.29		7.18 ± 0.35		7.12 ± 0.29		6.62 ± 0.58	
α2 globulin (g/L)								
A	3.19 ± 0.43	0.003	21.10 ± 1.12	0.001	35.75 ± 1.70	0.001	29.94 ± 2.42	0.001
B	3.95 ± 0.67		13.80 ± 1.52		22.28 ± 1.11		21.17 ± 0.98	
C	5.32 ± 0.70		5.28 ± 0.72		4.53 ± 1.01		17.30 ± 1.88	
D	4.44 ± 0.29		4.46 ± 0.35		4.50 ± 0.22		4.40 ± 0.58	
β globulin (g/L)								
A	8.09 ± 2.27	0.002	20.39 ± 4.83	0.001	34.57 ± 8.47	0.002	27.15 ± 9.21	0.019
B	8.93 ± 2.20		19.64 ± 3.15		24.88 ± 4.45		23.20 ± 4.83	
C	5.20 ± 0.25		5.53 ± 0.37		5.40 ± 0.90		20.91 ± 2.51	
D	5.12 ± 0.13		5.14 ± 0.11		5.08 ± 0.08		5.10 ± 0.17	
γ globulin (g/L)								
A	5.64 ± 1.08	0.023	17.96 ± 2.32	0.001	16.35 ± 2.27	0.001	19.29 ± 1.23	0.002
B	7.93 ± 2.03		13.69 ± 2.32		12.35 ± 2.34		14.25 ± 1.22	
C	9.22 ± 1.93		8.35 ± 1.12		10.46 ± 1.44		12.06 ± 2.69	
D	7.70 ± 0.35		7.60 ± 0.41		7.70 ± 0.29		7.11 ± 0.78	
Lactate dehydrogenase (U/L)								
A	2301.44 ± 80.07	0.001	2425.01 ± 148.27	0.001	1611.79 ± 116.26	0.001	1395.54 ± 76.88	0.001
B	1994.56 ± 172.18		2091.82 ± 210.05		1759.03 ± 182.36		1562.47 ± 130.00	
C	2676.43 ± 473.69		3126.31 ± 385.66		2704.48 ± 170.62		1915.84 ± 81.94	
D	780.00 ± 44.72		771.00 ± 38.79		798.00 ± 20.80		775.84 ± 121.01	

Data are presented as mean ± standard deviation. A: 10-minute ischemia; B: 10-minute ischemia +antioxidant; C: Chronic ischemia; and D: Sham or control.

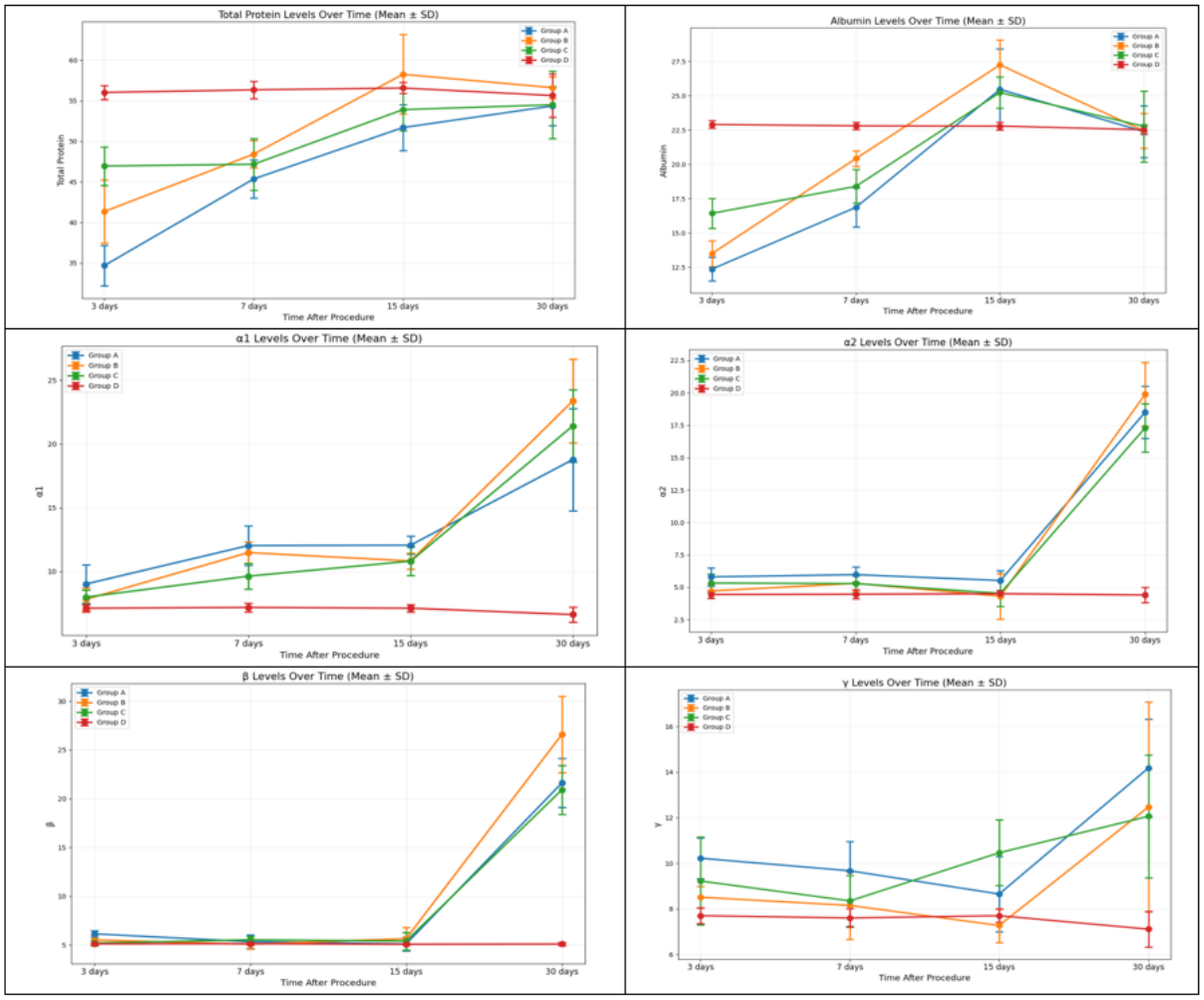

Figure 1: Time-dependent changes of total protein, albumin, and globulins' ($\alpha 1$, $\alpha 2$, β, γ), level in the 4 studied groups following 10 minutes of liver ischemia. Group A = ischemia (10 min), group B = ischemia + Riditox, group, C = chronic ischemia with CCl4 intoxication, and group D = sham control. SD: standard deviation.

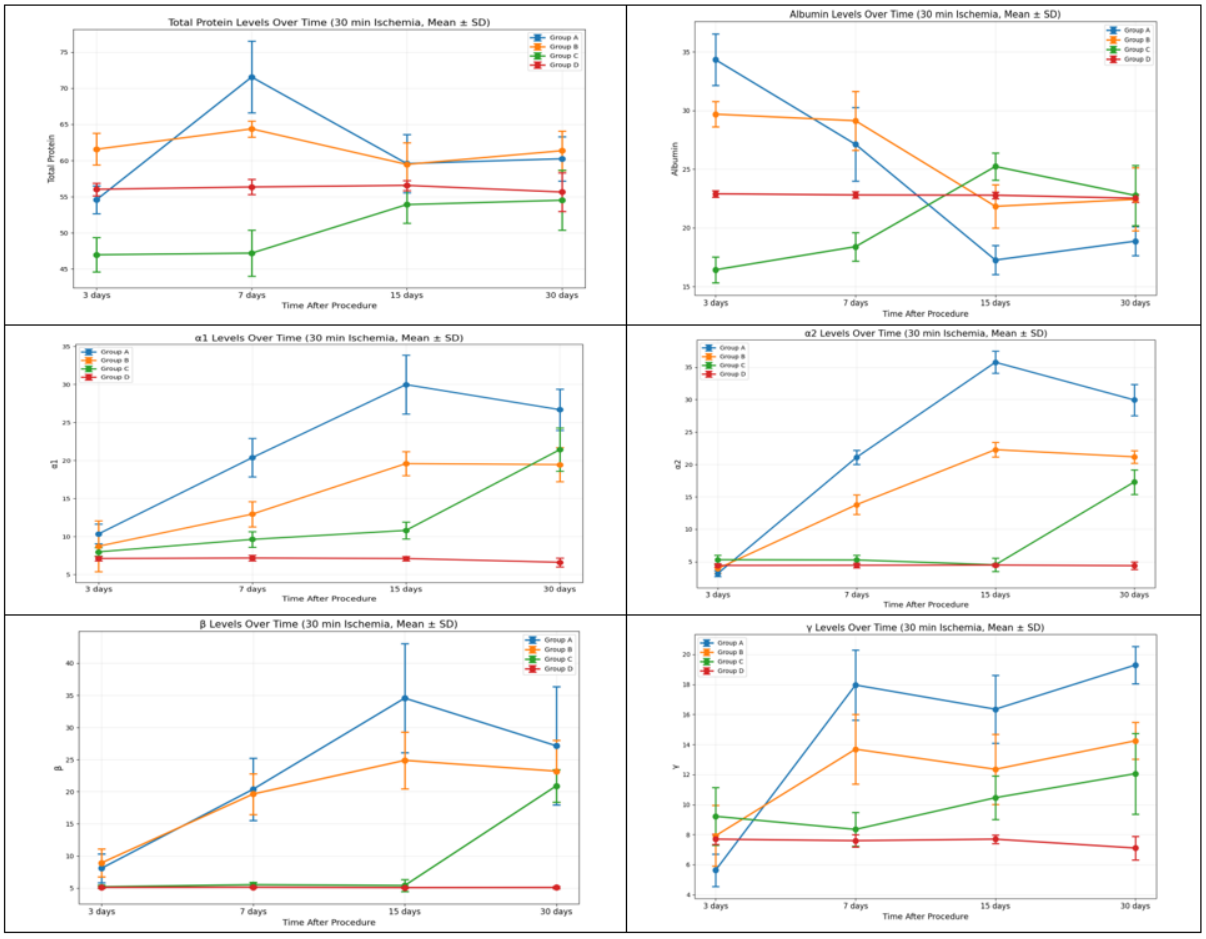

Figure 2: Time-dependent changes of total protein, albumin, and globulins' ($\alpha 1$, $\alpha 2$, β, γ) level in the four studied groups following 30 minutes of liver ischemia. Group A = ischemia (30 min), group B = ischemia + Riditox, group C = chronic ischemia with CCl4 intoxication, and group D = sham control. SD: standard deviation.

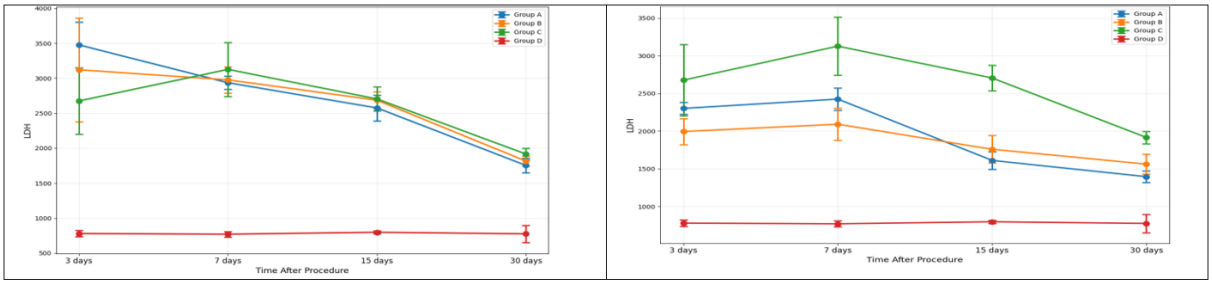

Figure 3: Time-dependent changes of lactate dehydrogenase (LDH) level in the four studied groups following 10 (left) and 30 (right) minutes of liver ischemia. Group A = ischemia (30 min), group B = ischemia + Riditox, group C = chronic ischemia with CCl4 intoxication, and group D = sham control.