Iraqi Journal of Veterinary Sciences

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Alleviating hemorrhagic shock damage: Protective effects of non-invasive ultrasound therapy on the vagus nerve in the liver and kidneys of rabbits

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Article information

Article history:
Received 20 March 2025
Accepted 17 August 2025
Published 19 November 2025

Keywords:
Crash
Hemorrhagic shock
Organs
Ultrasound therapy
Vagus

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Abstract

This study aims to investigate the protective capability of noninvasive ultrasound therapy on the liver and kidneys after hemorrhagic shock. A total of 21 male New Zealand white rabbits weighing 2-3 kg were divided into 7 groups (sham, Ultrasound Therapy (UST), UST+Dexamethasone (Dx), Fluid Therapy (FT)+USR+Dx, FT+UST, FT+Dx, FT). Hemorrhage was induced to 30-35% of the total blood volume. A hypotensive resuscitation fluid therapy was administered 3 times the blood volume, lasting 60 min. Ultrasound therapy at 1 MHz was delivered every 30 min at 1 W/cm² for 10 min. Blood chemistry parameters Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Total Protein (TP), Blood Urea Nitrogen (BUN), and Creatinine (Cr) were tested at min 0 (before shock), min 60, and min 180. Histopathological examination of liver and kidney tissues was performed. The results showed that the FT+UST+Dx group had the highest protection in liver blood chemistry, while FT+Dx and FT provided the best protection in kidney blood chemistry. Histopathological evaluation showed that FT+UST and FT provided the best liver protection, whereas FT+UST+Dx and UST+Dx demonstrated the best-preserved kidney structures. In conclusion, the study is well coordinated. It shows that combining ultrasound therapy with fluid therapy and dexamethasone can provide adequate organ protection. Further research is needed to confirm its clinical applicability and review the effects of single treatment versus combination therapy.

DOI: 10.33899/ijvs.2025.158433.4188,

Introduction

Hemorrhagic shock (HS) can cause death due to hypoperfusion and progressive inflammation, which may lead to multiple organ dysfunction syndrome (MODS) (1). Several studies have shown the effects of liver and kidney dysfunction post-hemorrhagic shock (2,3). The mechanism of dysfunction in these two organs begins with tissue injury; however, the progression of injury differs between the two. Liver injury progression occurs due to circulating pro-inflammatory factors that concentrate at the injury site (4), while kidney injury progression results from the renal tubular vulnerability to hypoxia, exacerbated by intra-renal vasoconstriction induced by sympathetic activity and renin-angiotensin system activation (5). The key to protecting organs from damage post-hemorrhagic shock is suppressing the activation and circulation of inflammatory mediators. Hemorrhagic shock requires prompt and effective intervention. Fluid therapy is a primary approach to restore intravascular volume and maintain organ perfusion in critically injured patients (6). The limitations of fluid therapy alone in managing hemorrhagic shock have been demonstrated in previous studies. Although fluid resuscitation restores systemic hemodynamics, it fails to prevent reperfusion-related cellular damage and may even contribute to endothelial injury and organ dysfunction (5,7).

Inappropriate fluid volume or composition may trigger inflammatory cascades and worsen vascular leakage (8). To address these limitations, researchers have investigated pharmacological adjuncts such as dexamethasone. Administration of dexamethasone after hemorrhagic or traumatic injury has been shown to reduce leukocyte infiltration, support vascular integrity, and improve clinical outcomes (9,10). Dexamethasone also contributed to organ protection by modulating inflammation and preserving endothelial function (11). However, potential drawbacks include hyperglycemia and altered metabolism at higher doses (12). These findings suggest that while fluid therapy and dexamethasone offer benefits, their combined limitations warrant exploring alternative strategies, such as non-invasive neuromodulation. The vagus nerve, one of the cranial nerves, plays a critical role in regulating inflammation. Studies have shown that vagus nerve stimulation (VNS) activates the anti-inflammatory cholinergic pathway (13). VNS studies related to HS indicate that vagus nerve stimulation during HS can enhance hemostasis, improve blood flow, and reduce inflammatory responses (14-16). This study employs a different VNS approach than previous research, which used invasive electrical stimulation targeting the nerve (7-9). In this research, ultrasound therapy (UST) is used for stimulation. UST was chosen because several studies have demonstrated its ability to influence vagus nerve activity (neuromodulation) (17). UST operates through three mechanisms: thermal, cavitation, and mechanical. These mechanisms induce changes in neural action potentials (18).

Ultrasound therapy offers advantages such as ease of use and non-invasiveness, making it a promising first-aid treatment for hemorrhage that does not require specialized expertise. To date, no studies have explored the benefits of ultrasound therapy as a vagus nerve stimulator for treating HS and preventing multiorgan dysfunction. In this study, UST is employed as a supportive therapy to prevent organ damage by suppressing inflammatory activity after hemorrhagic shock.

Materials and methods

Ethical approval

All procedures conducted in this study were approved by the School of Veterinary Medicine and Biomedical Sciences (SVMBS) IPB University Animal Ethics Committee with the number 033/KEH/SKE/III/2023.

Research methodology

This study used 21 male New Zealand White rabbits weighing 2–3 kg, divided into seven groups: sham, UST, UST+Dx, FT+UST+Dx, FT+UST, FT+Dx, and FT, respectively. All groups were subjected to inhalation anesthesia with isoflurane. All Groups were induced with hemorrhagic shock (HS) by withdrawing 30–35% of the total blood volume (60 ml x body weight) through the auricular artery over 15 minutes. The induction method used in this study was a fixed-volume hemorrhagic shock (19).

The ultrasound device used in this study was the Hanil HS-502 (Hanil-TM Co., Ltd., Korea), a physiotherapeutic unit operating at 1 MHz, equipped with a crystal vibrator probe (effective radiating area ~4 cm^2), and capable of continuous or pulsed output at adjustable intensities from 0.1 to 1.5 W/cm^2. Ultrasound therapy targeting the vagus nerve was applied to the ventral cervical region every 30 minutes for 10 minutes at a frequency of 1 MHz and an intensity of 1 W/cm^2. These parameters were selected based on ultrasound therapy for deep-tissue effects (20). They were supported by previous studies (21-23) demonstrating beneficial anti-inflammatory, cardioprotective, and immunomodulatory effects with ultrasound under comparable settings. The fluid therapy used in this study refers to hypotensive resuscitation with lactated Ringer's solution, combined with intramuscular administration of dexamethasone (5 mg/mL) at a dose of 0.6 mg/kg.

Blood chemistry was evaluated by comparing baseline values, shock at minute 60, and final values at minute 180. The tests included alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST), total protein (TP), blood urea nitrogen (BUN), and creatinine (Cr), measured using a blood chemical analyzer (Arkray® SPOTCHEM EZ SP-4430). After 180 minutes, euthanasia was performed using an overdose of anesthesia, followed by the collection of liver and kidney tissues for histopathological examination using hematoxylin and eosin (H&E) staining.

Data analysis

To determine changes in blood chemistry post-hemorrhagic shock, blood chemistry values at minute 0 were compared to those at minute 60. To evaluate differences in blood chemistry between treatment groups, values at minute 60 were compared with those at minute 180 across all groups (1–8). The data were analyzed using one-way ANOVA with Duncan's post hoc test. The blood chemistry variables that had been statistically tested were then examined for patterns (decrease, increase < 60%, or increase> 60% from the value during hemorrhagic shock). Variables that showed a reduction were given a score of 3, an increase < 60% was given a score of 2, and > 60% was given a score of 1. The scores for each group per variable were then summed and depicted in a graph.

Histopathological examinations were performed to evaluate tissue damage in both the liver and the kidney using a semiquantitative scoring method adapted from previous studies (24-26). In the liver, three variables were evaluated: hepatocyte degeneration, vascular and sinusoidal congestion, and hepatocyte apoptosis. Observations were conducted in zone 3 (central vein area), with degeneration evaluated at 20× magnification and congestion and apoptosis

at 40× magnification. The area of degeneration was measured, while hepatocyte counts for congestion and apoptosis were quantified using ImageJ® software. In the kidney, histopathological examination focused on the cortical area, where four pathological changes were evaluated: tubular epithelial edema, vascular congestion, Bowman's space dilatation, and hyaline leakage. All variables were observed at 20× magnification, and quantitative analysis was performed using ImageJ® software. Lesions and normal areas in the proximal tubules and glomeruli were calculated to determine percentages. Each variable was then scored based on the rate of affected cells: 0 for 0%, 1 for 1–25%, 2 for 26–50%, and 3 for >50%. Histopathological scoring data were statistically analyzed using the Kruskal-Wallis and Mann-Whitney tests. Final scores from liver and kidney examinations were ranked to evaluate protective effects.

Results

Blood chemistry in a shock condition

Induction of blood loss in Groups 1-7 resulted in significant changes ($p < 0.05$) in the blood chemistry parameters total protein (TP), blood urea nitrogen (BUN), and creatinine (Cr), while changes in alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were not statistically significant ($P > 0.05$) (Figure 1). Compared to baseline (minute 0), levels of ALP, AST, BUN, and Cr increased, whereas ALT and TP levels decreased. Furthermore, compared with the standard reference values for average rabbits, the levels of ALP, ALT, AST, and Cr in the shock condition were above the normal range, while TP and BUN were below the normal range.

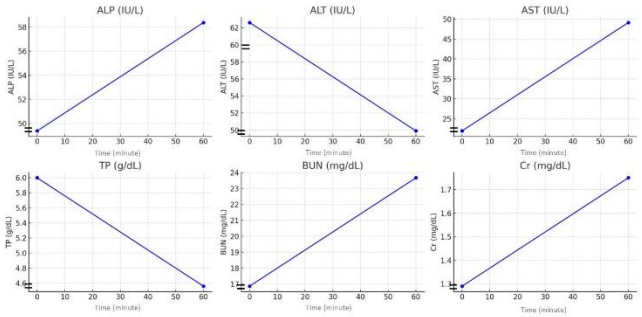

Figure 1: Blood chemistry in hemorrhagic shock.

Blood chemistry after treatment

Treatment affected blood chemistry values (Figure 2). Groups FT+UST+Dx, FT+Dx, and FT showed decreases in ALP and ALT levels. No treatment group showed a reduction in AST values (Figure 2). However, FT+UST+Dx, FT+Dx, and FT caused a slight increase (< 60%), while the others caused a significant increase (> 60%) in AST (Figure 3A). The protective effect on the liver in this study was evaluated based on blood chemistry values of ALP, ALT, and AST. Ranking the treatment groups from those with positive to adverse effects (decrease, slight increase, and significant increase), the order of liver protection based on blood chemistry values is FT+UST+Dx, FT, FT+Dx, FT+UST, UST+Dx, and UST (Figure 3B).

Treatment groups involving FT without UST showed decreases in TP and Cr. In contrast, FT combined with UST showed decreases in TP and increases in Cr. All treatment groups showed increased BUN values. Groups with FT combinations showed a slight increase in BUN values compared to groups without FT combinations. Groups without FT (UST+Dx and UST) showed increases in TP, BUN, and Cr (Figure 2). The protective effect on kidney damage in this study was evaluated based on blood chemistry values of TP, BUN, and Cr. Ranking the treatment groups from those with positive to adverse effects (decrease, slight increase, and significant increase), the order of kidney protection based on blood chemistry values is: FT+Dx and FT; FT+UST+Dx and FT+UST; UST+Dx; and UST (Figure 4).

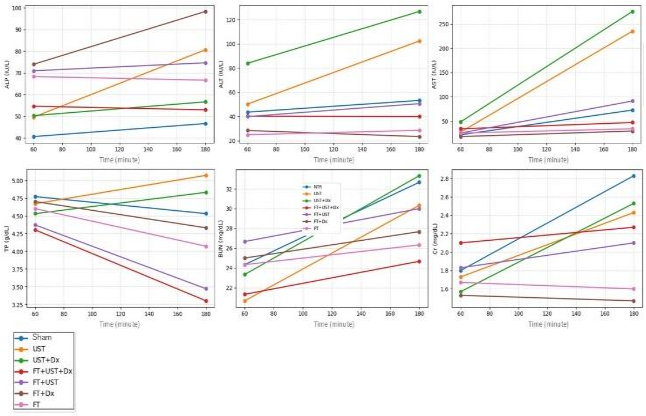

Figure 2: Blood chemistry after treatment.

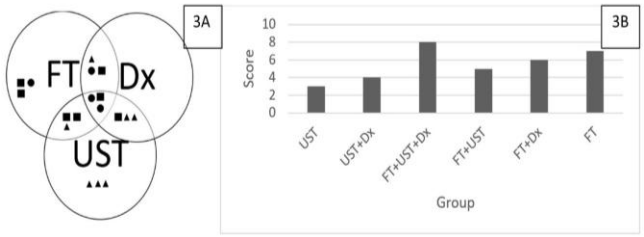

Figure 3: The effect of therapy on liver blood chemistry variables. 3A. Plot liver blood chemistry values as a function of therapy. Circle: decrease effect (score 3), square: slight increase (< 60%) (score 2), triangle: significant increase (> 60%) (score 1). 3B. Rank liver blood chemistry scores as an effect of therapy.

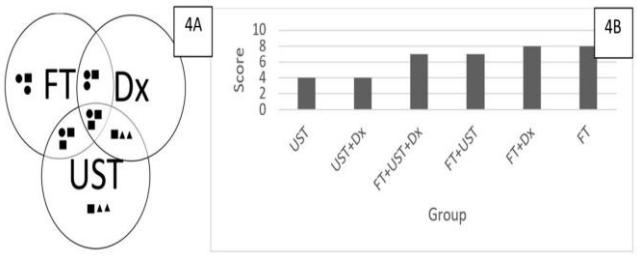

Figure 4: The effect of therapy on kidney blood chemistry variables. 4A. Plot kidney blood chemistry values as a result of therapy. Circle: decrease effect (score 3), square: slight increase (< 60%) (score 2), triangle: significant increase (> 60%) (score 1). 4B. Rank kidney blood chemistry scores as an effect of therapy

Histopathology

Based on liver histopathological examination (Figure 5), hepatocyte degeneration was observed, with scores ranging from 1.22 to 1.78 (1% < average < 25%), with the highest score in the FT group. Vascular and sinusoidal congestion was also observed in all treatment groups, with scores ranging from 1 to 2.22 (25% < average < 50%), with the highest score in the FT+Dx treatment. In contrast, apoptosis scores ranged from 0.14 to 1 (average < 25%), with the highest scores in the FT+UST+Dx and NTR groups. All treatment groups showed significant differences from the FT group (0.14 ± 0.24) (Table 1).

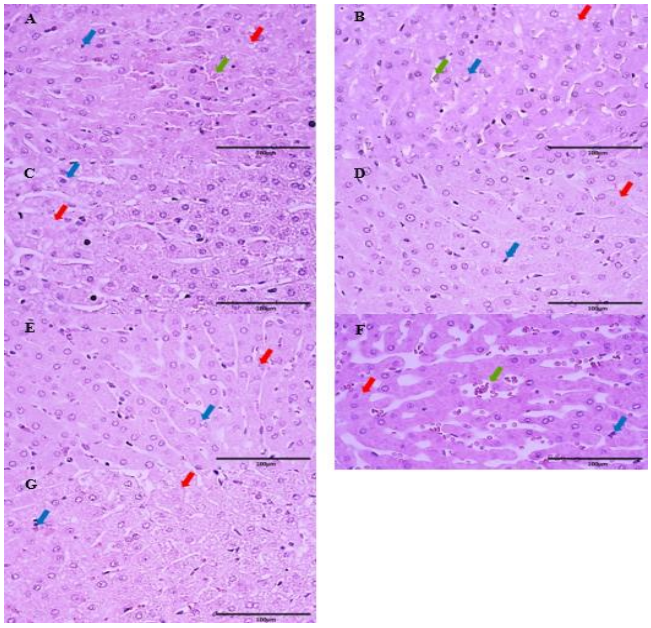

Figure 5: Histopathological features of liver tissue at the central vein margin by group. Apoptotic changes and sinusoidal congestion, scale bar 100 µm. 5A: Sham; 5B: UST group; 5C: UST+Dx group; 5D: FT+UST+Dx group; 5E: FT+UST group; 5F: FT+Dx group; 5G: FT group. Red arrows: degeneration; blue arrows: apoptosis; green arrows: congestion.

Table 1: Liver histopathological score

Group	Hepatocyte degeneration	Vascular and sinusoid congestion	Hepatocyte apoptosis
NTR	1.44 ± 0.24 [a]	1.56 ± 0.29 [ab]	1.00 ± 0.00 [a]
UST	1.63 ± 0.18 [a]	1.75 ± 0.31 [ab]	0.88 ± 0.13 [a]
UST+Dx	1.44 ± 0.18 [a]	1.67 ± 0.37 [ab]	0.78 ± 0.15 [a]
FT+UST+Dx	1.38 ± 0.18 [a]	1.38 ± 0.26 [ab]	1.00 ± 0.00 [a]
FT+UST	1.22 ± 0.15 [a]	1.00 ± 0.17 [a]	0.89 ± 0.11 [a]
FT+Dx	1.44 ± 0.18 [a]	2.22 ± 0.28 [b]	0.89 ± 0.11 [a]
FT	1.78 ± 0.15 [a]	1.11 ± 0.11 [a]	0.14 ± 0.24 [b]

Note: Data are presented as mean with standard deviation (x ± SD). The same superscript letters in the same column indicate no significant difference (P > 0.05).

In the liver, hepatocyte degeneration and apoptosis were observed in up to 25% of the tissue, with congestion reaching up to 50%. The characteristic histopathological change in the liver following hemorrhage is generally bleeding; however, since the method used in this study involved fixed-volume blood withdrawal, the resulting liver changes were predominantly degenerative. Degenerative changes are commonly observed in hepatocytes due to increased Reactive Oxygen Species (ROS) production. The protective effect on the liver in this study was evaluated by the number of pathological changes observed in the organ for each treatment group, as reflected in the scoring system. When ranked from the group with the least damage to the most, the therapeutic protective capacity for the liver was as follows: UST, FT+UST, FT+UST+Dx, FT+Dx, UST+Dx, and FT (Figure 6).

Changes in the kidneys were assessed using four parameters, but only two showed differences among groups (Figure 7). In the kidney tissue, tubular swelling, Bowman's space expansion, and hyaline leakage were observed in up to 25% of cases, and vascular congestion in up to 50%. The Bowman's space expansion parameter, with scores ranging from 0.22 to 1.33, and hyaline leakage, with scores ranging from 0.00 to 0.33, showed no significant differences among

groups (Table 2). Bowman's space expansion had the highest score in the NTR group. At the same time, the lowest was observed in the Dx combination group. The UST group exhibited Bowman's space expansion in the moderate-to-severe category. This finding is consistent with the effects of analgesic drugs that act by inhibiting neurotransmitter release, including glutamate, which have also been reported to induce similar renal changes. This suggests that UST may potentially cause nephrotoxic effects resembling the pattern of damage associated with certain analgesics.

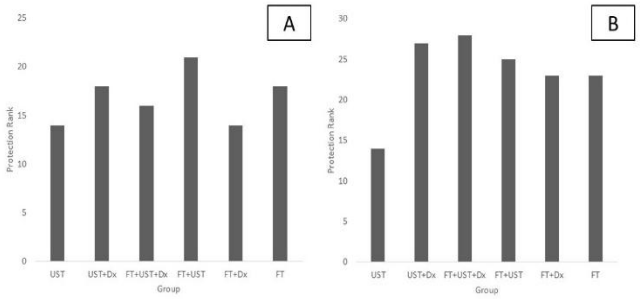

Figure 6: Rank of protection score. 6A: Ranking of liver protection scores. 6B: Ranking of kidney protection scores.

Hyaline leakage had the highest score in the UST group. For tubular swelling, the NTR group had the highest score of 1.67, with a range of 1.00-1.67 (1% < average < 25%), and showed a significant difference from the FT+UST group. Based on the vascular congestion parameter, the score range was 0.00 to 2.22 (0 < average < 50%). The FT group had a score of zero, indicating no congestion observed. In contrast, the UST treatment group had the highest congestion score of 2.22 (Table 2). Significant differences were found between the FT group and the UST and NTR groups. When ranked from the group with the least damage to the most, the therapeutic protective abilities on the kidney organ are ranked as follows: FT+UST+Dx, UST+Dx, FT+UST, FT+Dx, FT, and UST (Figure 6).

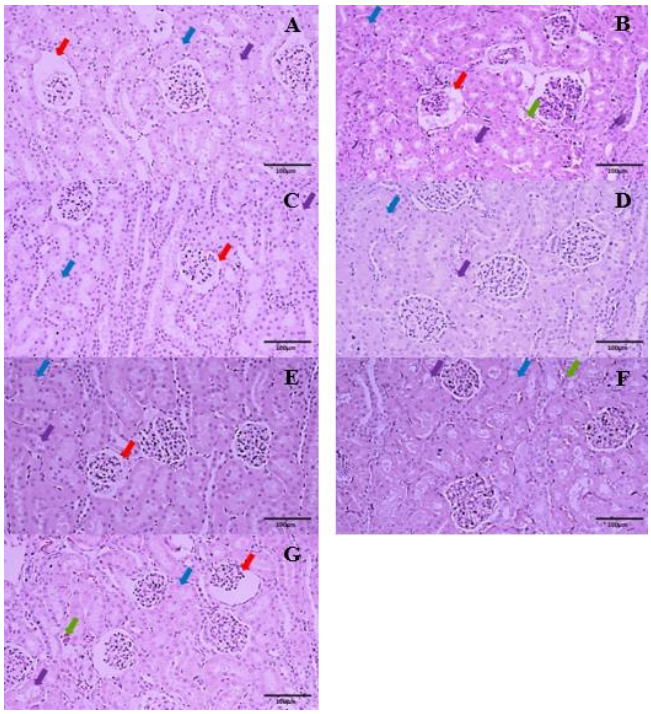

Figure 7: Kidney histopathological appearance in the cortex by group. Tubular swelling and Bowman's space dilation observed, bar 100 µm. 7ANTR group; 7B UST group; 7C UST+Dx group; 7D FT+UST+Dx group; 7E FT+UST group; 7F FT+Dx group; 7G FT group. Red arrow: Bowman's space dilation, blue arrow: tubular swelling, green arrow: congestion, purple arrow: hyaline deposition.

Table 2: Kidney histopathological score

Group	Tubular epithelia oedema	Vascular congestion	Bowman space dilatation	Hyalin leakage
NTR	1.67 ± 0.33 [b]	1.56 ± 0.50 [bc]	1.33 ± 0.44 [a]	0.11 ± 0.11 [a]
UST	1.44 ± 0.24 [ab]	2.22 ± 0.43 [c]	0.89 ± 0.39 [a]	0.33 ± 0.24 [a]
UST+Dx	1.00 ± 0.00 [a]	1.00 ± 0.50 [abc]	0.67 ± 0.44 [a]	0.00 ± 0.00 [a]
FT+UST+Dx	1.00 ± 0.00 [a]	0.33 ± 0.33 [a]	0.56 ± 0.38 [a]	0.11 ± 0.11 [a]
FT+UST	1.00 ± 0.00 [a]	0.25 ± 0.25 [a]	1.00 ± 0.50 [a]	0.25 ± 0.25 [a]
FT+Dx	1.22 ± 0.15 [ab]	1.22 ± 0.49 [abc]	0.22 ± 0.22 [a]	0.11 ± 0.11 [a]
FT	1.00 ± 0.00 [a]	0.00 ± 0.00 [a]	1.25 ± 0.45 [a]	0.13 ± 0.13 [a]

Note: Data are presented as mean with standard deviation (x ± SD). The same superscript letters in the same column indicate no significant difference (P > 0.05).

Discussion

Liver injury parameters in hemorrhagic shock

One of the effects of hemorrhagic shock (HS) on the liver is ischemia (hepatic ischemia). This occurs due to reduced vascular resistance (27-29). Ischemic hepatocytes subsequently lead to hepatocyte injury, characterized by mitochondrial dysfunction, excessive reactive oxygen species, and increased apoptosis (29). Hepatocyte injury, whether reversible or irreversible, induces the release of enzymes such as ALP, AST, ALT, and others like GLDH, SDH, LDH, and alpha-glutathione S-transferase (α-GST).

The presence of these enzymes in the bloodstream does not always indicate cell death (30). It may also occur in conditions of increased oxidative stress and imbalance with the body's antioxidant defenses (31). In this study, different results were observed. During shock conditions, ALP and AST levels increased, whereas ALT levels decreased. The increase in ALP and AST levels is attributed to HS-induced hypoxia, which leads to cell damage. Elevated ALP and AST levels indicate cellular injury, primarily in the liver. These values are typically observed in the acute phase of hepatocellular injury (30). ALP levels increased earlier because this enzyme is released after hypoxia triggers anaerobic metabolic shifts, ATP depletion, membrane integrity disruption, and the release of pro-inflammatory agents, particularly IL-1, IL-6, and TNF-α (32). AST levels were observed to rise earlier than ALT levels, likely because AST is more widely distributed in organs such as skeletal and cardiac muscles, the kidneys, and red blood cells (33). The decrease in ALT levels is likely due to its cytosolic localization, with mitochondrial ALT also found in mitochondria, particularly in the periportal zone of the liver (34). This zone is closest to the oxygen supply, resulting in a slower response to hypoxia (34).

Kidney parameters in shock conditions

Elevated BUN and creatinine (Cr) levels are key indicators of acute kidney injury (AKI) (35). The kidneys are among the most sensitive organs to hypoxic conditions, making them highly susceptible to tubular and endothelial hypoxia injuries that can lead to AKI (36). During hemorrhagic shock (HS), excessive blood loss disrupts both macro and microcirculation. These disruptions occur due to slowed red blood cell (RBC) flow in the vasculature and a decrease in oncotic pressure, characterized by reduced vascular turgor (5).

During shock, systemic and renal autoregulatory mechanisms are activated to compensate for blood flow, perfusion, and oxygen delivery through sympathetic activity stimulation and activation of the renin-angiotensin system. This compensatory response leads to intrarenal vasoconstriction. Although hemodynamic normalization through resuscitation can restore blood pressure and alleviate vasoconstriction, it cannot completely halt the injury process, particularly ischemic reperfusion injury (5).

A decrease in total protein (TP) levels following hemorrhagic shock is attributed to mechanical effects, such as the direct loss of protein molecules with the shed blood and the potential leakage of proteins into the interstitial space. This leakage may result from increased capillary membrane permeability and endothelial glycocalyx damage secondary to shock-induced stress (37).

Therapeutic effects on the liver and kidney

Based on blood chemistry and histopathological comparisons, it can be concluded that UST therapy provides protective effects only in groups combined with FT or Dx. UST therapy without FT does not yield good protective results. The influence of FT on resuscitation is evident in decreased blood chemistry values of ALP and ALT, although AST values remain elevated to a small degree. Similarly, the values of TP, BUN, and Cr indicate that all treatments incorporating FT have higher protective scores compared to UST+Dx and UST alone. Based on histopathological observations, UST combined with FT or Dx consistently shows higher protective rankings than standalone UST.

The use of intravenous fluid therapy to manage hemorrhagic shock (HS) has long been practiced to restore physiological parameters (38). Fluid resuscitation during the "golden hour" is critical to maintaining organ function and preventing mortality (39,40). Post-HS fluid resuscitation is an essential therapeutic approach to enhance intravascular volume and organ perfusion in critically injured patients (6). However, depending on its composition and volume, fluid therapy is also reported to have adverse effects on organ function (5).

Fluid therapy helps improve organ perfusion but does not fully protect the liver from damage. Degeneration due to hypoperfusion may be mitigated, but hepatocyte apoptosis can worsen during reperfusion due to increased ROS production, inducible Nitric Oxide Synthase (iNOS) induction, and disruption of calcium homeostasis (32,41). The use of lactated Ringer's may reduce bcl-2 expression and hepatic ATP reserves while increasing apoptosis, possibly due to the D-lactate content, which influences immune response and macrophage polarization (42,43). In the kidneys, crystalloids readily shift into interstitial spaces, leading to hemodilution and glycocalyx damage, elevating creatinine and BUN levels (44,45). Although it does not entirely prevent injury, FT aids metabolic recovery and reduces acidosis (46), which contributes to inflammation and vascular dysfunction (47).

The expectation for UST use in hemorrhagic shock management is to prevent the development of post-HS multi-organ dysfunction. Its proposed mechanism of action involves suppressing inflammation through stimulated vagus nerve activity. It is anticipated that by reducing inflammation, circulating oxidative stress will also decrease, thereby allowing liver enzyme levels and kidney filtration capacity to return to normal (31,48). A study has demonstrated its inhibitory effects on TNF-α release and splenic macrophages. This indicates that afferent (sensory) signals from the vagus nerve modulate efferent (motor) signals via inflammatory reflexes, which contribute to the regulation of innate immune responses and cytokine homeostasis (49). The inflammatory reflex is defined as a physiological signaling mechanism in which afferent vagus nerve signals, activated by cytokines or pathogen-derived products, trigger efferent vagus nerve responses to regulate pro-inflammatory cytokine production (50). Vagus nerve stimulation can attenuate inflammatory responses by

activating neuroimmune circuits known as the cholinergic anti-inflammatory pathway (50).

Based on this study's findings, it can be concluded that the UST used likely suppresses inflammatory mediators. However, its application as a standalone therapy without FT needs reevaluation. The mechanism by which UST exerts protective effects on the liver and kidneys post-hemorrhagic shock likely involves neuromodulation via three mechanisms: thermal, cavitation, and acoustic radiation (19). The thermal impact occurs when acoustic energy from ultrasound penetrates tissue, inducing vibrations. These vibrations are then converted into heat (51). Neurons are susceptible to temperature changes; even small increases (<1°C) can alter action potential kinetics and ion channel activity (52). Various temperature-sensitive transient receptor potential (TRP) channels, such as TRPV1 and TRPV4, exist in neuronal membranes. These receptors can even be activated at 37°C (53). Specifically, slight, moderate, or high-temperature increases open calcium (Ca) channels, leading to intracellular Ca2+ influx through TRP channels (53).

The second mechanism is cavitation. Cavitation is a non-thermal ultrasound therapy mechanism that uses sound-wave-induced vibrations to create spaces and bubbles within tissues. These cavities oscillate in response to acoustic waves, producing acoustic emissions, radiation, and flow, which can cause biological effects (53). This phenomenon deforms the lipid bilayer membrane, ultimately triggering action potentials and enhancing synaptic transmission (54).

The final mechanism involves acoustic radiation pressure, also known as mechanical effects. The mechanical force exerted by stable acoustic pressure on target neurons stretches the cell membrane, inducing conformational and structural changes in mechanosensitive ion channels (55). This process enables these ion channels to respond to mechanical stimuli, thereby modulating cellular activity and nerve function (53). Exposure to ultrasound at 1 W/cm², continuous-wave, can increase the amplitude of compound action potentials (CAPs) (56). CAP is a signal recorded from nerve trunks consisting of many axons. The increased CAP amplitude by ultrasound is due to non-thermal effects (mechanical stimulation) for two reasons. The first is that the stretch-activated ion channels are much more sensitive to mechanical than thermal stimulation. The more ion channels open, the more Na+ and K+ ions move in and out of the cell membrane, thereby increasing CAP amplitude. The second reason is that increased temperature does not enhance CAP amplitude. This suggests that ultrasound-induced thermal effects can partially deactivate ion channels, allowing fewer ions to pass through the membrane and thereby reducing CAP amplitude. This is evidenced by increasing intensity (2 and 3 W/cm²), which decreases CAP amplitude (56).

The difference between UST combined therapy and UST without FT is not significant. This is likely due to the suboptimal ultrasound intensity. The UST therapy applied was 10 minutes per session, with a 30-minute interval between applications, at 1 MHz, 1 W/cm², and 5 applications in total. The frequency and intensity applied were lower than those used in previous studies (18). This aimed to determine the potential of non-invasive UST for vagus nerve stimulation. Low-intensity continuous ultrasound therapy (LICUS) is sufficient to induce thermal effects and produce nerve-regulation and thrombolysis effects. In this study, we did not examine the changes in the vagus nerve, either in terms of anatomy, microanatomy, or neurotransmitter levels. Further research is needed to investigate changes in inflammatory mediator levels in serum and organs through immunohistochemistry, which may help clarify the cellular mechanisms underlying the protective effects of UST via vagus nerve stimulation pathways.

Conclusion

Ultrasound therapy in combination tends to show better protective effects on the liver and kidneys than ultrasound therapy alone. It is necessary to test both single and combined ultrasound therapies at higher intensities and with a larger sample size to determine the optimal protective effects against organ damage.

Acknowledgment

The author is grateful to the Veterinary Teaching Hospital, the Division of Surgery and Radiology, and the Division of Pathology, School of Veterinary Medicine and Biomedical Science, IPB University, for providing the location for this research. The authors also express their gratitude for the financial assistance received from the Center for Higher Education Funding (BPPT) and the Indonesia Endowment Fund for Education (LPDP), as acknowledged in decree 01802/J5.2.3/BPI.06/9/2022.

Conflict of interest

There is no conflict of interest.

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تخفيف أضرار الصدمة النزفية: التأثيرات الوقائية للعلاج بالموجات فوق الصوتية غير الجراحية على العصب التائه في الكبد والكلى لدى الأرانب

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الخلاصة

تهدف هذه الدراسة إلى التحقق من القدرة الوقائية للعلاج بالموجات فوق الصوتية غير الجراحية على الكبد والكلى بعد الصدمة النزفية. تم تقسيم ٢١ من ذكور الأرانب البيضاء التي تزن ٢-٣ كجم إلى ٧ مجموعات (سيطرة، علاج بالموجات فوق الصوتية (UST)، علاج بالموجات فوق الصوتية (UST) + ديكساميثازون (Dx)، علاج السوائل (FT) + USR + Dx، (FT + UST، (FT + Dx، FT). تم إحداث نزيف بنسبة ٣٠-٣٥% من إجمالي حجم الدم. تم إعطاء علاج سائل الإنعاش الخافض لضغط الدم بمقدار ٣ أضعاف حجم الدم، واستمر لمدة ٦٠ دقيقة. تم تقديم العلاج بالموجات فوق الصوتية بتردد ١ ميجاهرتز كل ٣٠ دقيقة عند ١ وات/سم٢ لمدة ١٠ دقائق. تم اختبار معايير كيمياء الدم الفوسفاتيز القلوية (ALP)، ألانين أمينوترانسفيراز (ALT)، أسبارتات أمينوترانسفيراز (AST)، البروتين الكلي (TP)، نيتروجين اليوريا في الدم (BUN)، والكرياتينين (Cr) عند الدقيقة صفر (قبل الصدمة)، والدقيقة ٦٠، والدقيقة ١٨٠. تم إجراء الفحص النسيجي لأنسجة الكبد والكلى. أظهرت النتائج أن مجموعة FT+UST+Dx تتمتع بأعلى حماية في كيمياء دم الكبد، بينما توفر FT+Dx وFT أفضل حماية في كيمياء دم الكلى. أظهر التقييم النسيجي المرضي أن FT+UST وFT يوفران أفضل حماية للكبد، في حين أظهر FT+UST+Dx وUST+Dx أفضل هياكل الكلى المحفوظة. وفي الختام، فإن الدراسة منسقة بشكل جيد. ويبين أن الجمع بين العلاج بالموجات فوق الصوتية والعلاج بالسوائل والديكساميثازون يمكن أن يوفر حماية كافية للأعضاء. هناك حاجة إلى مزيد من البحث لتأكيد قابليته للتطبيق السريري ومراجعة آثار العلاج الفردي مقابل العلاج المركب.