

# Effect of seawater immersion on pathophysiological changes in rats combined burns with open abdominal injury

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## Research

**Keywords:** Seawater immersion, Burns, open abdominal injuries, Pathophysiological changes

**Posted Date:** August 11th, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-52957/v1>

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# Abstract

**Objective:** To observe the effects of seawater immersion on the pathophysiological changes in rats combined burns with open abdominal injury.

**Method:** Eighty-eight male Sprague-Dawley(SD) rats combined burns with open abdominal injuries were randomly divided into the seawater immersion group (SI) and the control group (Ctl).Rats in SI group were immersed in seawater at 15°C for 1 hour. The changes of respiration, blood pressure, rectal temperature, blood gases, blood electrolyte, liver and kidney function, coagulation function, heart damage and animal survival at different time points in the early stage (within 9 hours after injury) were observed.

**Result:** After immersed in seawater, the body temperature of rats in SI group were significantly decreased, the respiration were remarkably inhibited and the blood pressure were obviously declined as compared to those in Ctl group. The blood pH of rats in SI group were significantly lower than those in Ctl group, showing severe acidosis( $P<0.05$ ). The changes of  $\text{HCO}_3^-$ ,  $\text{PCO}_2$  and BE also presented the similar trends( $P<0.05$ ).  $\text{PO}_2$  and  $\text{PCO}_2$  in SI group were significantly higher than those in Ctl group ( $P<0.05$ ). However, the  $\text{SaO}_2$  in SI group were lower than those in Ctl group ( $P<0.05$ ). Blood sodium and chlorine in SI group were higher than those in Ctl group, showing severe hyponatremia and hyperchloremia( $P<0.05$ ). The concentration of potassium and calcium were not obviously changed. The liver and kidney function parameters(AST,ALT,Urea,Crea) in SI group were lower than those in Ctl group 4 hours after injury( $P<0.05$ ), while myocardial damage markers (TNT) in SI group were higher than those in Ctl group. There were no significant differences in coagulation function between the two groups. The survival time of the SI group rats were shorter than those in Ctl group and the mortality was higher than those in Ctl group (66.7% vs 41.7%).

**Conclusion:** Low-temperature seawater immersion might aggravate injury, accelerate animal death, induce hypothermia, respiratory depression, acidosis, hyponatremia and hyperchloremia. Immersing in low temperature seawater for 1 hour can cause myocardial damage, but it has protective effects on the liver and kidney. Therefore, more attentions should be paid to the secondary injury caused by seawater immersion in the emergency treatment of the wounded, such as rapidly rewarming, rectifying the acidosis and maintaining the stability of homeostasis of the wounded, so as to lay the foundation for the further definitive treatments.

## Background

In recent years, with the development of modern technology, human exploration and exploitation of Marine resources, Marine conflicts have become very common. When there is a Marine conflict, due to the dense personnel inside the ship, the wounded will appear in batches, and the injuries are mainly multiple injury, which include head, chest, abdominal trauma, what's more may combin with burns<sup>[1]</sup>.In addition, due to the rupture of the ship itself and the influx of seawater,trauma combined with seawater immersion is often seen<sup>[2]</sup>.

The medical treatment on the ship is very difficult due to the limited conditions, heavy waves and swaying. After soaked in sea water, the wounded are prone to trigger fatal complications such as hypothermia, acidosis and coagulation dysfunction<sup>[3]</sup>. In addition to the effect of hypertonic seawater, the water and electrolyte of the wounded are easily disordered, resulting in severe hypertonic dehydration, hypersodium and hyperchloremia<sup>[4]</sup>, and high wound infection rate, especially vibrio infection<sup>[5]</sup>. Therefore, the wounded combined with seawater immersion, suffered serious injuries and had a high mortality rate. In Marine conflicts, early and systematic assessment of the wounded facilitates early detection of life-threatening injuries and prioritization of treatment and intervention to save lives.

In the early stage, some scholars have carried out various models combined with seawater immersion experiments, such as hemorrhagic shock model, gunshot injury model, burn model, simple sea water drowning and so on. In gunshot injury combined with hemorrhagic shock model: low temperature seawater immersion will aggravate the body hypoxia, leading to severe hypothermia and acidosis<sup>[6]</sup>. In burn model: burn and sea water immersion can aggravate organ damage, the double whammy can aggravate the damage<sup>[7]</sup>. As for the simple sea water drowning models: hypertonic seawater inhaled into the lungs is likely to cause acute lung injury, leading to respiratory distress syndrome<sup>[8]</sup>. But for combined injury model such as burns combined with open abdominal injury, no study was conducted on the pathophysiological changes of burns combined with open abdominal injury plus low temperature seawater immersion. It is necessary to be studied, due to the incidence of burns and abdominal wounds is relatively high in Marine conflicts.

The purpose of this study is to observe the effects of low-temperature seawater immersion on the pathophysiological changes in rats combined burns with open abdominal injury, so as to lay a foundation for the formulation of treatment principles in the next step.

## 1 Materials And Methods

### 1.1 Animal ethics

This study was approved by the Laboratory Animal Welfare and Ethics Committee of Third Military Medical University, and conformed to the Guide for the Care and Use of Laboratory Animals published by US National Institutes of Health (NIH Publication, 8th edition, 2011).

### 1.2 Experimental animals and groups

Eighty-eight male SD rats weighing 190-230g, provided by the Experimental Animal Center of Daping Hospital of Army Medical University, fasted for solids overnight and drank freely. Eighty-eight rats combined burns with open abdominal injury were randomly selected and randomly divided into the seawater immersion group (SI) and the control group (Ctl). 24 rats are used to record physiological indicators (respiration, blood pressure, anal temperature) and observation of survival time, the other 64 rats

are used for blood gases, electrolyte, liver (AST and ALT), kidney (Urea and Crea) function, heart damage (TNT) and coagulation function (PT, APTT, FIB, INR).

### 1.3 Animal models

SD rats were anesthetized by intraperitoneal injection of 3% pentobarbital sodium (30 ~ 50 mg/kg), and right carotid artery cannulation was done to monitor the blood pressure and 0.2ml of blood was drawn to test blood gas. Shave the fur of the rats with clipper at supine position with razor. A 6 cm x 6cm gauze was put into 99°C boiling water for ten seconds, and then was used to prepare 10% of rats' burn of rat<sup>[9-10]</sup>. Abdominal injury was made by opening the abdomen of the middle of the abdomen, the incision length is 3 cm located under the xiphoid<sup>[11]</sup>. After the process the rat is stable for about five minutes, rats in SI group were immersed in 15°C artificial seawater for one hour, the waterline is at the level of the xiphoid process, for maintaining its normal breathing. In the Ctl group, except no immersing in seawater, the other treatment methods were the same.

### 1.4 Seawater composition

The concentration of sea salt was 2.535% according to the formulation of the Third Institute of State Oceanic Administration. Main indicators: osmotic pressure 12501mmol/L, pH 8.2, sodium concentration 630mmol/L, potassium concentration 10.88mmol/L, chloride concentration 658.8mmol/L, sea water temperature 15°C<sup>[12]</sup>.

### 1.5 Drugs

3% pentobarbital sodium, heparin sodium injection (1:500) (systemic heparin, 30 ~ 50 mg/kg), heparin sodium injection (1:5) (flushing tube), 0.9% normal saline.

### 1.6 Observation index

Time points: The determination time points included before the injury, immediately out of seawater and 1h, 3h, 8h after out of seawater (corresponding to 1h, 2h, 4h and 9h after injury in the Ctl group respectively). For convenience, the following text was unified as before injury, 1h, 2h, 4h and 9h after injury.

Respiration was recorded by measuring the ups and downs of the chest, blood pressure was measured by mercury-type sphygmomanometer, anal the temperature was measured by (Medlinket AM-806-CS0531R-S) Vital sign monitor.

The animal survival was recorded at 9h after injury.

Blood gas and electrolyte were determined by automatic blood gas analyzer [potential of hydrogen (pH), partial pressure of oxygen (PO<sub>2</sub>) partial pressure of carbon dioxide (PCO<sub>2</sub>), base excess (BE), HCO<sub>3</sub><sup>-</sup>, sodium, potassium, chloride, calcium].

Liver and kidney function were measured by fully automatic biochemical analyzer.

Heart damage was measured by the cardiac injury marker detector.

Coagulation function(PT,APTT,FIB,INR) was measured by the automatic coagulometer.

## 1.7 Statistical Analysis

Statistical software Excel2010 and SPSS20.0 were used to analyze the data, and the measurement data were expressed as  $X \pm S$ . One-way ANOVA was used for the comparison of different time points of each group, and Least Significant Different(LSD) was used for the multiple comparison.  $P < 0.05$  was considered statistically significant.

# 2. Results

## 2.1 Animal Survival

The survival time of SI group was shorter than that of Ctl group, the mortality of SI group was higher than that of Ctl group, and the death peak of rats was 7 hours after injury, regardless of whether the rats were immersed in sea water in Fig 1. As a large number of rats died 7 hours after injury, statistical analysis was conducted on the changes of various indicators at time points 0, 1, 2 and 3(respectively correspond: before injury, 1h, 2h and 4h and after injury)

## 2.2 Changes of Respiration, blood pressure and body temperature

Respiration rate and anal temperature of rats in SI group were significantly decreased after injury and immersion ( $P < 0.05$  compared with that of Ctl group), and the trend was still observed at 2h and 4h after injury. Blood pressure in SI group was decreased more severe than that in Ctl group. The results showed that seawater immersion had great influence on respiration, body temperature and blood pressure in rats combined burns with open abdominal injury in Fig 2.

## 2.3 Changes of blood gases in SI and Ctl groups

Table 1 shows that the blood pH value in SI group at 1, 2h and 4h after injury was lower than that in Ctl group ( $P < 0.05$ ), and the lowest value was about 7.023. In addition, the base excess and  $\text{HCO}_3^-$  also presented this trend. Besides,  $\text{PO}_2$  and  $\text{PCO}_2$  in SI group was significantly higher than that in Ctl group at 1, 2, and 4h after injury ( $P < 0.05$ ), up to 205.0mmHg and 61.6mmHg, respectively. However, the  $\text{SO}_2$  in SI group was lower than that of Ctl group at 1, 2, and 4h after injury. The results showed that the rats combined burns with open abdominal injury presented more serious acidosis, respiratory inhibition and hypoxemia.

**Table 1 Changes of blood gas in rats combined burns with open abdominal injury after seawater immersion**

Index	Group	pre-injury	1h post-injury	2h post-injury	4h post-injury
pH	Ctl	7.3400.028	7.3130.052	7.3080.066	7.2400.079
	SI	7.3360.060	7.0230.091*	7.0610.098*	7.0590.114*
PO <sub>2</sub> [mmHg]	Ctl	119.610.96	139.511.97	143.521.16	152.530.56
	SI	123.910.37	205.047.77*	205.113.49*	181.759.66
SO <sub>2</sub> (%)	Ctl	96.62.60	96.91.91	97.31.63	96.61.23
	SI	97.11.98	91.419.05	96.631.56	96.52.80
PCO <sub>2</sub> [mmHg]	Ctl	39.74.59	31.95.65	25.97.16	25.38.06
	SI	40.24.40	61.613.50*	46.911.05*	29.09.55
BE [mmol/L]	Ctl	-4.082.25	-8.921.57	-11.642.31	-14.484.35
	SI	-4.222.26	-16.013.44*	-17.053.46*	-21.033.72*
HCO <sub>3</sub> <sup>-</sup> [mmol/L]	Ctl	21.11.73	17.51.12	15.61.58	14.12.79
	SI	21.01.76	12.82.30*	12.32.01*	10.21.73*

Note: \* P<0.05 compared with Ctl, seawater immersion group (SI) and control group (Ctl).

## 2.4 Changes of electrolyte in SI and Ctl groups

Table 2 shows that the blood electrolyte were significantly changed after seawater immersion, especially the sodium and chloride in SI group at 1, 2h and 4h after injury (P<0.05), and the highest value reached to 155.67 mmol/l and 129.83 mmol/l, respectively. But potassium and calcium do not show a clear change, only at 4h after injury, after seawater immersion blood potassium and calcium were increased (P<0.05). So burns combined with open abdominal wounds after immersion in seawater, the electrolytic changes were manifested as severe hypernatremia and hyperchloremia.

### Table 2 Changes of electrolyte in rats combined burns with open abdominal injury

Index	Group	pre-injury	1h post-injury	2h post-injury	4h post-injury
Na+	Ctl	143.92.47	140.22.66	140.02.80	139.52.2
mmol/L	SI	143.81.36	155.711.69*	151.88.34*	151.28.40*
K+	Ctl	3.530.49	3.400.37	3.320.61	3.980.31
mmol/L	SI	3.550.30	3.220.42	3.760.51	4.881.16*
Cl-	Ctl	113.35.79	114.23.51	116.93.60	116.54.20
mmol/L	SI	111.71.83	129.813.48*	129.09.57*	130.99.31*
Ca <sup>2+</sup>	Ctl	1.300.12	1.250.09	1.180.15	1.200.09
mmol/L	SI	1.250.12	1.290.07	1.270.10	1.320.15*

Note: \* P<0.05 compared with Ctl, seawater immersion group (SI) and control group (Ctl).

## 2.5 Changes of liver and kidney function, heart damage marker and coagulation function in SI and Ctl groups

Table 3 shows that the liver (AST and ALT) and kidney (BUN and Cr) injury parameters were lower than the Ctl group, especially at 4h after injury (P<0.05). However, the heart damage (TNT) opposed result, especially at 4h after injury. It was higher in SI group than that in Ctl group (P<0.05). As for coagulation function, there was no significant difference between the two groups.

### Table 3 Changes of liver and kidney function, heart damage marker and coagulation function in rats combined burns with open abdominal injury

Index	Group	pre-injury	1h post-injury	2h post-injury	4h post-injury
AST U/L	Ctl	116.057.41	239.145.97	440.7171.15	574.0150.35
	SI	96.836.32	188.349.07	302.289.40	293.650.00*
ALT U/L	Ctl	58.528.03	59.29.78	88.134.95	124.241.97
	SI	41.311.11	51.613.93	79.014.68	69.130.10*
Urea mmol/L	Ctl	6.351.97	6.771.31	9.522.37	13.601.61
	SI	5.281.61	7.491.21	9.221.28	9.111.74*
Crea μmol/L	Ctl	22.24.03	31.79.29	40.65.78	49.810.54
	SI	19.94.91	29.35.80	37.43.54	26.17.86*
TNT μg/L	Ctl	0.0240.019	0.1150.107	0.1500.111	0.1860.092
	SI	0.0140.006	0.3280.371	0.5430.702	0.6410.400*
PT S	Ctl	12.52.60	12.60.75	13.52.52	12.71.40
	SI	12.11.27	12.12.14	11.81.13	15.87.34
APTT S	Ctl	28.413.52	36.719.44	45.049.88	28.210.38
	SI	23.54.72	25.76.17	41.030.50	50.934.71
FIB g/L	Ctl	1.180.40	0.940.22	1.080.15	1.080.09
	SI	1.260.22	1.160.06*	1.130.11	1.150.37
INR	Ctl	1.100.22	1.100.07	1.170.22	1.110.12
	SI	1.060.11	1.060.18	1.030.10	1.370.62

Note: \* P<0.05 compared with Ctl,seawater immersion group (SI) and control group (Ctl).

### 3. Discussion

Marine conflicts lead to a special type of trauma with the unique characteristics. Compared with the common trauma. Trauma combined with seawater immersion has the characteristics of complex injury and prognosis, and its treatment is more difficult<sup>[13-14]</sup>. Due to a large number of advanced high-tech weapons used in the sea operations, the proportion of blast injury is increasing gradually, especially in multiple trauma, compound injury, burns, open abdominal injuries and soon<sup>[7,15]</sup>. What's more, it is unavoidable for the wounds to be exposed to seawater after injury. What is the effect of seawater immersion on compound injuries? What is the difference in the treatment aspect of compound injuries combined with seawater immersion or not. Some scholars have studied hemorrhagic shock or gunshot injury combined with seawater immersion. For example, Lu songmin found that hemorrhagic shock

combined with seawater immersion 21°C resulted in increased blood viscosity, presenting severe decompensated acidosis and severe hypoxia<sup>[16]</sup>. Besides, Liu yanli found that hemorrhagic shock combined with seawater immersion caused high incidence of lethal triad—hypothermia, acidosis, coagulation disorders and aggravate organ function damage in seawater at 15°C for 4 hours<sup>[17]</sup>. For gunshot injury model, Lai xinan found that Bacteremia appeared earlier and more serious after seawater immersion. In the histopathology of internal organs, there were not only different degrees of circulation disorder observed, but also severe inflammatory response<sup>[18]</sup>. Therefore, in the study of different injuries combined with seawater immersion, the main observed focus is the impact of seawater immersion on the pathophysiological changes of the injury.

In the present study, the burn combined with open abdominal injury rat model was applied. It was found: Firstly, low temperature seawater immersion significantly inhibited respiration and blood pressure. Secondly, lethal triad were easy to occur after immersion in low temperature seawater, however, in this study coagulation function was not obviously changed. Thirdly, burns combined with open abdominal injuries resulted in severe electrolyte disturbance following low temperature seawater immersion, presenting severe hyponatremia and hyperchloremia. Fourthly, low temperature seawater immersion aggravated cardiomyocyte injury for burns combined with open abdominal injuries, but for less than an hour it's protective for liver and kidney function.

The respiratory system responds strongly to hypothermia, hyperventilation may occur initially, but as the body temperature drops further, pulmonary edema may develop, pulmonary ventilation may decrease, and even respiratory arrest<sup>[19]</sup>. After respiratory depression, a large amount of CO<sub>2</sub> accumulates, presenting respiratory acidosis. Hypoxia, lead to a large number of acid metabolites, and eventually manifested severe decompensated acidosis.

Study showed that severe hypothermia could further inhibit clotting, Liu yanli in hemorrhagic shock model combined with seawater immersion for 4 hours found that in the seawater soaking group, APTT and PT were significantly prolonged<sup>[20]</sup>. Perhaps this is due to the high consumption of clotting factors after hemorrhagic shock, coupled with hypothermia and acidosis that inhibit clotting factor activity, so clotting dysfunction is significant. In this study, due to the small amount of blood loss and the short immersion time, no serious clotting dysfunction was observed.

Because of the high permeability and high salinity of seawater, open abdominal wound through a serious membrane dialysis effect, would result in tissue and high blood osmolar such as dehydration. In addition, the chest and peritoneum were in direct contact with low-temperature seawater, resulting in a large amount of heat loss and rapid decrease of body temperature. The longer the immersion time is, the more obvious the electrolyte changes in blood are. Animal experiments by Yu JY et al. confirmed that the time of seawater immersion at any time is prolonged, and the concentration of plasma sodium ion and chloride ion is further increased. The present study indicated that the pathophysiological changes of burns combined with open abdominal injury combined with seawater immersion were significantly different from those of burns combined with open abdominal injury alone, and seawater immersion was

one of the main causes of hyperosmotic injury in rats, which provided theoretical basis for the treatment of burns combined with open abdominal injury combined with seawater immersion. Firstly, in the emergent management of seawater immersion injury, the casualty should be rapidly rewarmed and homeostasis protection. Secondly, the seawater in the abdominal cavity should be discharged as soon as possible, and the low-tension fluid should be infused in the early stage, so as to correct the electrolyte disturbance as soon as possible.

In the present study, after seawater immersion, the animals' PaO<sub>2</sub> showed a significant increase while the SaO<sub>2</sub> was significantly decreased. Analysis of the reasons may be related to the following: Firstly, hypothermia lead to a decrease in aerobic metabolic rate and tissue oxygen consumption. Secondly, hypothermia causes the oxygen dissociation curve to shift to the left, and the release of blood and tissue oxygen is reduced, leading to the increase of PaO<sub>2</sub><sup>[21]</sup>. Thirdly, due to the low temperature, hypertonic seawater immersion and combined with burns, the animals showed severe dehydration and hypovolemic shock, so the SaO<sub>2</sub> was decreased.

As for the organ function damage, the myocardial damage was more serious after 1 hour immersion in low temperature seawater, but liver and kidney damage is less. These means that myocardial has poor tolerance to low temperature. When the core temperature drops, ventricular fibrillation is likely to occur, or even cardiac arrest<sup>[22]</sup>. What's more, due to the heart is an active blood supplier, low-temperature seawater immersion caused cardiac acceleration to ensure blood supply to vital organs throughout the body, thus increasing myocardial metabolism. But liver and kidney are a passive organ that receives blood, low-temperature seawater would reduce the metabolic rate of liver and kidney function<sup>[23]</sup>. Therefore, in the treatment of seawater immersion injury, "heart muscle is life", and the treatment must race against time. However, as low temperature leads to a decrease in various metabolism of the body, a short period of low temperature seawater immersion has a protective effect on the liver and kidney organs, which also indicates that salvage should be carried out as soon as possible after a patient is found drowning to reduce the risk of further aggravation of organ functions.

This study has the following limitations: Firstly, the immersion time was only 1 hour without multiple time controls. The observed time was only 8 hours after out of water, which could only represent the early stage of the injury. Secondly, this study only observed the changes of pathophysiology after seawater immersion, but did not further study the molecular mechanism and treatment approaches.

## 4. Conclusion

Low-temperature seawater immersion would aggravate burns combined with abdominal open injury, which leads to hypothermia, respiratory depression, acidosis, hypernatremia, hyperchloremia and higher animal mortality. Soaking in low temperature seawater for 1 hour can cause myocardial damage, but for the liver and kidneys it's protective. Therefore, more attention should be paid to the secondary injury caused by seawater immersion and in the treatment of those wounded.

# Abbreviations

**SD**

Sprague-Dawley

**TNT**

troponin t

**ALT**

alanine aminotransferase

**AST**

aspartate aminotransferase

**BE**

base excess

**PT**

prothrombin time

**APTT**

activated partial thromboplastin time

**FIB**

fibrinogen

**INR**

international normalized ratio

**LSD**

Least Significant Different

# Declarations

## Availability of data and materials

All data were presented in this manuscript or Appendix.

## Acknowledgements

Not applicable.

## Funding

This subject was supported by Scientific Research plan(AWS16J032).

## Contributions

All corresponding and first authors contributed to the study concept and design.LY finished the experiment,analyzed the data and drafted this letter.All authors reviewed and approved the final manuscript.

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## Ethics approval and consent to participate

This study was approved by the Laboratory Animal Welfare and Ethics Committee of Third Military Medical University(number:AMUWEC20191329;approved June 30,2019),and conformed to the Guide for the Care and Use of Laboratory Animals published by US National Institutes of Health (NIH Publication, 8th edition, 2011).

## Consent for publication

Not applicable.

## Competing interests

The authors declare no potential conflict of interest.

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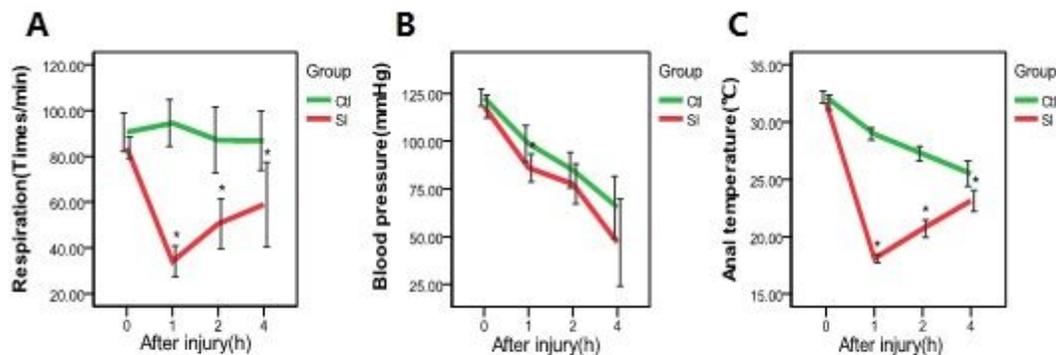
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## Figures

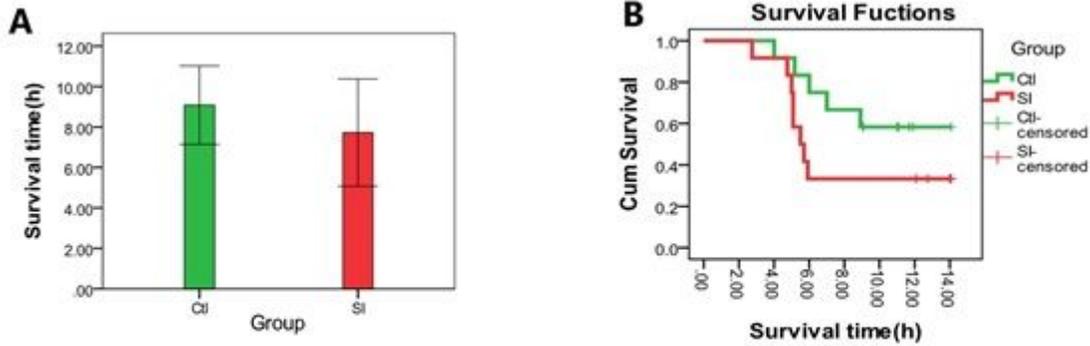


Note: \* P<0.05 compared with Ct, seawater immersion group (SI) and control group (Ct).

**Fig 2 Changes of Respiration, blood pressure and body temperature**

## Figure 1

Animal Survival



Note: \*  $P < 0.05$  compared with Ctl, seawater immersion group (SI) and control group (Ctl).

**Fig 1 Animal Survival**

## Figure 2

Changes of Respiration, blood pressure and body temperature

## Supplementary Files

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