

## Study the effect of electroacupuncture on liver– spleen and kidney function of experimental animals

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**ABSTRACT**

Studying the effects of electroacupuncture the acupoints LI4, PC6, Jiaji C7 in experimental inflamed rabbits show that, Electroacupuncture acupoints LI4, PC6, and Jiaji C7 have a protective effect, limiting the degree of degenerative and congestive damage in liver and kidney parenchyma in experimentally inflamed rabbits; stabilizing indicators evaluating the function of these organs (enzyme activity AST, ALT, urea, creatinine). Electroacupuncture acupoints LI4, PC6, and Jiaji C7 has prevented congestion in the parenchyma of the spleen, and in turn cause hyperplasia of lymphoid follicles of the spleen, increasing the number and percentage of lymphocytes in peripheral blood.

**Keywords:** electro acupuncture, liver, spleen, kidney

**1. BACKGROUND**

In many past decades, in Vietnam electro acupuncture has been widely applied to treat many diseases, including the treatment of various pain and surgical acupuncture with high efficiency. Acupuncture is used to treat some pain in clinical practice which has been proved to be an effective treatment, especially in surgical acupuncture (Sun et al., 2020; Zhang et al., 2014). Many subclinical studies have initially demonstrated the objective analgesic effect and safety of electro acupuncture through a number of research criteria of the authors in Vietnam and around the world (Li et al., 2020). Many different authors have also studied a number of aspects in the functional changes of the nervous system, the fluid system, various chemical intermediates to explain the mechanism of acupuncture in general and electro-acupuncture pain relief in particular have achieved certain results (Kawakita and Okada, 2014; Zhang et al., 2014).

With the purpose of continuing to research scientific bases to evaluate the impact of electro acupuncture on the function of some organs (liver, spleen, kidney) on experimental rabbits in order to contribute to assessing the safety and effectiveness of reduction pain of some electro acupuncture procedures to treat some pain without adjuvant medication. We conduct the research: Study on the effect of electro acupuncture on the change of function of liver, kidney

and spleen organs with the aim of studying the biochemical changes of blood and histopathology of organs liver, spleen and kidney of rabbit under the effect of electro acupuncture.

## 2. MATERIALS AND METHODS

### Study population

From January 2021 to December 2021, we conducted a study on 36 rabbits, regardless of male and female, weighing 1.9-2.2 kg, provided by Ba Vi experimental animal farm. Rabbits are divided into 03 groups (Figure 1): Group 1: 12 rabbits, does not cause inflammation and electroacupuncture. Group 2: 12 rabbits, cause inflammation but not treated with electro acupuncture. Group 3: 12 rabbits, cause inflammation, and are treated with electro acupuncture. All rabbits were take care under laboratory conditions, fed with industrial food and green vegetables, and had enough water to drink at the Department of Physiology – Military Medical Academy.



**Figure 1** Rabbits used in research

### Method of causing inflammation

Two groups of rabbits (group 2 and group 3) were inflamed by the method of Gerhard Vogel H (2002):

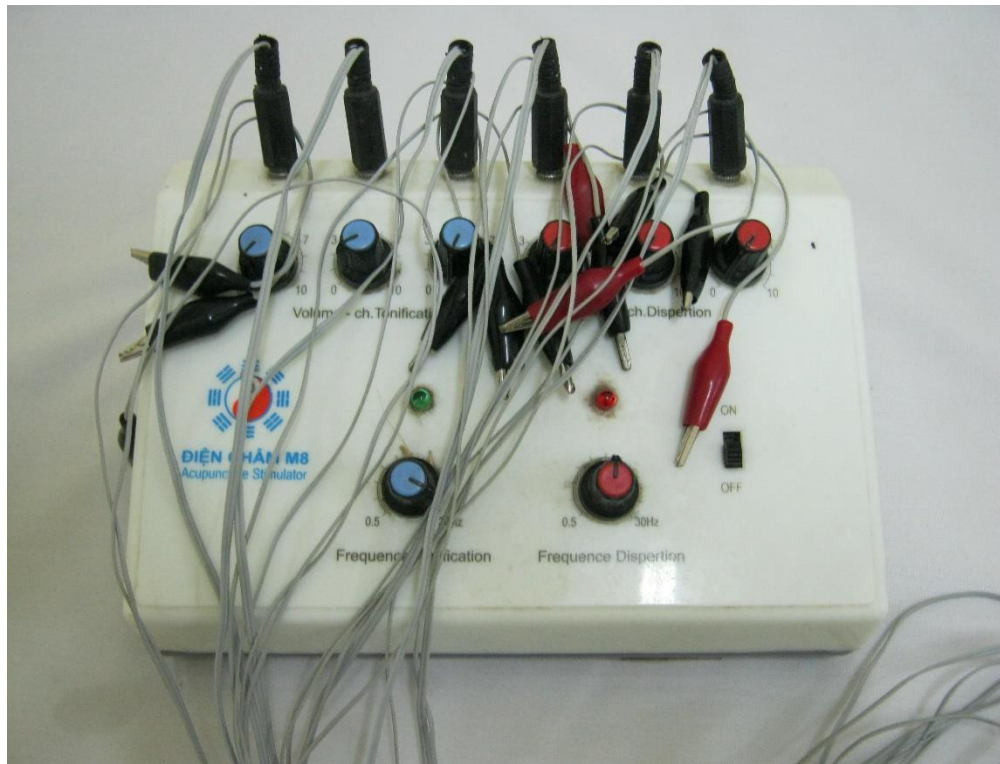
- Using chemicals: carrageenan (from Sigma Company - USA)
- Procedure: Inject to the soles of the rabbit's foot 5% carrageenan solution, try the dose of 0.25 mg / kg, 0.5 mg / kg and 0.75 mg / kg body weight. We determined the dose of 0.75 mg / kg achieved the best inflammatory effect and chose this dose to conduct inflammation for rabbits.

Inject 5% solution, 0.75mg / kg body weight into the front sole of the rabbit, and then release the rabbits freely in the cages.

### Electroacupuncture rabbit

Twelve rabbits of group 2 (with inflammation) and 12 rabbits of group 3 (non-inflammatory) were electro acupunctured to the corresponding locations of LI4, PC 6 and Jiaji C7 in humans determined by anatomical landmarks and described in acupuncture points atlas of animals by Plakhotin (Figure 2).

- Duration of electroacupuncturer: twice a day, lasting 20 minutes / time; at 8-9 a.m and 14-15 p.m, continuously for 7 days, by the Technical staff of National hospital of acupuncture implemented.
- Using M7 electroacupuncture stimulator with 2 frequency tonification - dispersion (produced by the National Hospital of Acupuncture). Acupuncture needles 5-7cm long in stainless steel.



**Figure 2** Electro-acupuncture stimulator M8

### Research targets

- Hematological indicators: the number of white blood cells (G / l) and the lymphocyte ratio (%).
- Blood biochemical indices: AST (U / l), ALT (U / l), urea (mmol / l), creatinine ( $\mu\text{mol} / \text{l}$ ), and glucose (mmol / l).
- Histopathology of liver, kidney, spleen.

All research indicators (except liver, kidney, spleen histopathology) were determined before conducting experiments (day No) and after 7 days (day N7) electro acupuncture or after 7 days causing inflammation (in rabbits without acupuncture).

How to identify research indicators

- Complete blood count was determined by automatic hematological analyzer K4500 (Japan). Reagents manufactured by Sysmex Company.
- Blood biochemical indices were determined by automatic biochemical analyzer Autohumalyzer 900s Plus of Humal Company (Germany).
- Research method of microscopic structure of liver, kidney, spleen.

After the end of the 07-day treatment session, on the 8th day, all rabbit research groups were operated. Samples of liver, kidney, and spleen were fixed by Borum solution, buried in paraffin, sliced 5-7 $\mu\text{m}$  thin, dyed hematoxylin-eosin (HE), read and analyzed on optical microscopy, at the Department Histopathology - Forensic, Military Medical Academy.

### Data processing

The research data was processed by the method of medical statistics, using SPSS 15.0 software and M.exell.

## 3. RESULTS

### *Functions of the liver, kidney and spleen organs of rabbit*

The liver, kidney, and spleen function of the rabbits were assessed by leukocyte formula index, blood biochemical indices of AST, ALT, urea, creatinine, glucose and histopathological images of these organs.

### Result of blood biochemical indices

The data from Table 1 show that the biochemical indices evaluating the rabbit's liver and renal function without causing inflammation with electro acupuncture were almost unchanged over the 7 days of the study ( $p > 0.05$ ). Results of blood biochemical

indices before and after 7 days of study of inflammatory rabbits not treated with electro acupuncture (group 2) in table 2 showed no statistically significant change ( $p > 0.05$ ). However, the activity of AST and ALT enzymes varies greatly between individuals. The biochemical indices of blood of rabbits in group causing inflammation with electro acupuncture (group 3) before and after 7 days of treatment presented in table 3 showed no significant change ( $p > 0.05$ ), there is no clear trend.

**Table 1** Change of some biochemical indicators of rabbit blood in group without causing inflammation with electro acupuncture

Indicators	Before inflammation ( $\bar{X} \pm SD$ )	After inflammation ( $\bar{X} \pm SD$ )	p
Glucose (mmol/l)	6.79 ± 3.19	8.62 ± 2.57	>0.05
Ure (mmol/l)	9.81 ± 4.66	9.04 ± 3.21	>0.05
Creatinin (µmol/l)	122.50 ± 25.27	108.70 ± 15.00	>0.05
AST (U/l)	26.90 ± 5.28	32.20 ± 8.44	>0.05
ALT (U/l)	78.50 ± 11.99	75.20 ± 19.02	>0.05

**Table 2** Change of some biochemical indicators of rabbit blood in group causing inflammation without electro acupuncture

Indicators	Before inflammation ( $\bar{X} \pm SD$ )	After inflammation ( $\bar{X} \pm SD$ )	p
Glucose (mmol/l)	7.24 ± 1.35	7.32 ± 1.07	>0.05
Ure (mmol/l)	8.78 ± 2.59	7.65 ± 1.84	>0.05
Creatinin (µmol/l)	118.00 ± 16.32	115.92 ± 20.11	>0.05
AST (U/l)	43.18 ± 37.34	28.00 ± 8.49	>0.05
ALT (U/l)	64.00 ± 22.55	69.50 ± 21.12	>0.05

**Table 3** Change of some biochemical indicators of rabbit blood in group causing inflammation with electro acupuncture

Indicators	Before inflammation ( $\bar{X} \pm SD$ )	After inflammation ( $\bar{X} \pm SD$ )	p
Glucose (mmol/l)	7.67 ± 1.56	8.22 ± 1.28	>0.05
Ure (mmol/l)	4.48 ± 1.60	6.35 ± 2.41	>0.05
Creatinin (µmol/l)	89.58 ± 17.38	101.17 ± 15.94	>0.05
AST (U/l)	25.58 ± 14.00	23.08 ± 5.22	>0.05
ALT (U/l)	62.58 ± 15.39	67.50 ± 13.37	>0.05

**Results of the change of white blood cell count in the research groups**

After electro acupuncture (Table 4), the number of white blood cells increased significantly in 10/12 rabbits (83.3%), with the average increase is 100% ( $p < 0.01$ ); Lymphocyte count increased significantly in 12/12 rabbits (100%) with an average increase of 250% ( $p < 0.01$ ). The percentage of lymphocytes also increased accordingly ( $p < 0.01$ ). For rabbits that were inflamed without electro acupuncture, the number of white blood cells increased in all rabbits with an average increase of 40.4% ( $p < 0.01$ ); however, the lymphocyte count decreased in 90% of the rabbits (41.7% on average) and the lymphocyte ratio in all rabbits (on average decreased 98%) ( $p < 0.05 - 0.01$ ) (Table 5). In rabbits that caused inflammation and were treated with an electro acupuncture after 7 days, there was no significant change in the number of white blood cells, but there was an increase in lymphocyte count in 11/12 rabbits (91.7%) with an increase in average of 105.1%, increase in lymphocyte ratio in 10/12 rabbits (83.3%) with an average increase of 83.3% (both with  $p < 0.01$ ) (Table 6).

**Table 4** Changes in the number of lymphocytes in rabbits without causing inflammation, with electro acupuncture.

Indicators	Before acupuncture (n = 12)	After 7 days of acupuncture(n=12)	p
WBC (G/l)	2.44 ± 1.00	4.88 ± 1.79	< 0.01
L (G/l)	0.75 ± 0.39	2.63 ± 0.95	< 0.01
L% (%)	34.23 ± 16.17	54.62 ± 13.24	< 0.01

**Table 5** Changes in the number of lymphocytes in rabbits causing inflammation, without electroacupuncture.

Indicators	Before inflammation	7 days after inflammation	p
WBC (G/l)	6.06 ± 1.62	8.51 ± 1.72	< 0.01
L (G/l)	2.92 ± 1.01	2.06 ± 0.55	< 0.05
L% (%)	48.96 ± 11.09	24.71 ± 6.82	< 0.01

**Table 6** Changes in the number of lymphocytes in rabbits causing inflammation, with electro acupuncture

Indicators	Before inflammation	After 7 days of acupuncture	p
WBC (G/l)	6.99 ± 3.26	7.24 ± 3.05	>0.05
L (G/l)	1.18 ± 0.64	2.42 ± 1.36	< 0.01
L% (%)	17.57 ± 7.51	32.21 ± 8.68	< 0.01

**Histopathological results of liver, kidney, and spleen**

In non-inflammatory rabbits with electro acupuncture (group 1), no damage in the renal parenchyma were found, glomerular, renal tubular and renal slit clear and normal. Spleen histology revealed a slight hyperplasia of lymphoid follicles and slight congestion in the medullary region.

In rabbits cause inflammation non-electro acupuncture (group 2): congestion of the lobes of the liver, cases of severe congestion and necrosis, disruption of the structure of the lobules and the raft of the liver cells. In the kidney, there was an image of congestion in the glomerular and the renal slit. Spleen has clearly congestion of lymphoid follicles and medullary region. In rabbits causing inflammation, with acupuncture (group 3): Normal liver parenchymas, some cases of liver parenchyma have slight degeneration, but structure of hepatic lobules and the hepatocellular rafts are normal. The image of glomeruli, renal tubules and renal slit was normal, no signs of injury. Pictures of spleen histology showed that lymphoma follicles had hyperplasia phenomena, no manifestation of congestion.

**4. DISCUSSION**

Histopathological results of liver, kidney, and spleen show: In non-inflammatory group with electro acupuncture (group 1): liver parenchyma has some cases of mild congestion in the center of the lobes, images of the lobules, the hepatocellular rafts and the veins of the center lobe were clear. No damage of renal parenchyma was observed, the glomerular picture, renal tubules and renal slits were clear and normal. Histological images of the spleen showed mildly hyperplastic lymphoma and mild congestion in the medullary region.

In rabbits causing inflammation, non-electro acupuncture (group 2): In the liver: the hepatic lobules are congested, there are cases of severe congestion and necrosis phenomenon, disrupting the structure of the liver lobes and the hepatocellular rafts. In the kidneys: there is an image of congestion in the glomeruli and the cleft. In the spleen: there is a clear congestion of lymphoid follicles and medullary region. In rabbits causing inflammation, with electro acupuncture (group 3): Normal liver parenchymas, some cases of liver parenchyma have slight degeneration, but the structure of liver lobes and liver cells are normal. The image of glomerular, renal tubules and renal clefts was normal, no damages appeared.

Histologic image of spleen showed lymph follicles with hyperplasia, no manifestation of congestion. Thus, the histopathological results showed that in rabbits belonging to the group not treated with electro acupuncture, after 7 days of inflammation, organs liver, kidney, and spleen all showed congestion at different levels. There is congestion in the spleen in lymphoid follicles and medullary region; clear congestion in the glomeruli and cleft; severe congestion in the liver and associated necrosis of cells that change the structure of liver lobes. These results are consistent with the results of the study of changes in lymphocyte number and blood biochemical indices evaluating liver and kidney function of the rabbits. Due to congestion in the lymphoid follicles and medullary region, the absolute number and percentage of lymphocytes in the peripheral blood were significantly reduced. Due to congestion and necrosis of hepatocytes, ALT activity, especially AST enzyme in rabbit blood, has been increased after 7 days of inflammation.

Meanwhile, the histopathological images of rabbit liver and kidney organs in the inflammatory group were treated with electro acupuncture almost normal, similar to that in the non-inflammatory rabbit (control group). Except for the liver, there is a slight degeneration image but it has not changed the structure of cell rafts and liver lobes. But the results of the study of biochemical blood indices evaluate the function of these two organs, there is no significant change. The above research results show that electro acupuncture has effect to prevent damage to a certain extent the liver parenchyma and kidney of rabbit due to the impact of the

inflammatory process; mobilizing mechanisms of clearing activities of organs and the whole body so that it cannot cause big changes in their function. To fully and accurately answer the question about this effect of electro acupuncture is due to what mechanism? Further studies are needed.

Previous research by several authors has also noted changes in the activity of enzymes plays a role of increasing resistance and repair damage to cells, such as Na<sup>+</sup> -K<sup>+</sup> -ATPase and 5'-nucleotidase enzymes of hepatocytes, brain, small intestine in rabbits have been increased under the influence of electro acupuncture LI4, PC6, St36 (Fujiwara et al., 1991; Nguyen and Nghiem, 1994; Pan et al., 1990; Still and Konrad, 1985). The study results also noted that when the electro acupuncture points LI4, PC6, St36 (alone or in combination) increased the secretory activity of adrenocortical cells (Nguyen and Nghiem, 1994), increasing the content of cortisol (Fujiwara et al., 1991; Still and Konrad, 1985; Le, 1993), the hormone that has the strongest anti-stress and anti-inflammatory effects.

The results of the study of spleen histology are completely consistent with the test results for lymphocytes in the peripheral blood. In inflammatory rabbits treated with electro acupuncture, the spleen showed no congestion, whereas hyperplasia of lymph follicles resulted in an increase in the number and percentage of lymphocytes in peripheral blood. This result is also consistent with the judgment of many domestic and foreign researchers (Fujiwara et al., 1991; Nguyen and Nghiem, 1994; Still and Konrad, 1985; Le, 1993; Wu, 1995). Nguyen Van Tu (1998), when electro acupuncture St36 of rabbit was found that there is an increase in spleen mass and an increase in the number of activated Lympho T leukocytes. Wu et al., (1995) found that acupuncture points in LI4, PC6, and St36 for patients with malignancies may increase TCD3, TCD4, ratio TCD4 / TCD8, concentration  $\beta$ -endorphin and reduces receptors with interleukin-2. The weight of lymph nodes, spleen, thymus and sensitive rose-forming cells increased when electro acupuncture points of Sp5, Liv8, and K2. Most authors believe that acupuncture plays a role in regulating the immune system by altering the number of immune cells or / and activating them (producing antibodies, secreting cytokines), thereby having effect with the whole body.

## 5. CONCLUSION

The inflammatory process has caused glomerular congestion, renal clefts; causing severe congestion and degeneration, hepatocellular necrosis in rabbits not treated with electro acupuncture; increases the activity of serum ALT enzymes. Electroacupuncture points of LI4, PC6, and Jiaji C7 have a protective effect, limiting the degree of degenerative and congestive damage in the liver and kidney parenchyma in experimentally infected rabbits; stabilizing indicators evaluating the function of these organs (enzyme activity AST, ALT, urea, creatinine). Inflammatory rabbits not treated with an electro acupuncture cause congestion of lymphoid follicles and medullary spleen, reducing the number and percentage of lymphocytes in peripheral blood. In inflammatory rabbits treated by electro acupuncture of acupun points LI4, PC6, Jiaji C7 has prevented spleen congestion in the parenchyma, in contrast, hyperplasia of lymph nodes of the spleen, increased number and percentage of lymphocytes in peripheral blood.

### Author Contributions

Pham Hong Van and Nguyen Duc Minh contributed equally to this article

### Ethical approval

The study was approved by the Medical Ethics Committee of Military Medical Academy (approval number: 1629/QD).

### Funding

This study has not received any external funding.

### Conflicts of interest

The authors declare that there are no conflicts of interests.

### Data and materials availability

All data associated with this study are present in the paper.

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