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Effects of T-Consciousness Charge Field on Skin Wound Healing in Mice Model with Evaluating of Kidney and Liver as Sensitive Organs

Mohammad Ali Taheri[1], Sara Torabi[2], Farid Semsarha[3]*

1- Sciencefact R&D Department, Cosmointel Inc. Research Center, Ontario, Canada.
2- Department of Plant Biology, School of Biology, College of Sciences, University of Tehran, Tehran, Iran.
3- Institute of Biochemistry and Biophysics (IBB), University of Tehran, Tehran, Iran.

** Corresponding author: Farid Semsarha Ph.D., Institute of Biochemistry and Biophysics (IBB), University of Tehran, P.O. Box: 13145-1384, Tehran, Iran*
Tel.: +98-9121786577
Email: Email: Semsarha@ut.ac.ir

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Abstract

T-Consciousness, with a non-physical entity, has been introduced by Taheri as the third element of the universe, apart from matter and energy. There are also various T-Consciousness Fields that can be examined through laboratory experiments. Although these fields cannot be measured with physical tools, their practical applications enable researchers to document their effects and gain insights into this unseen aspect of our universe. In this approach, the behavior of subjects under study changes as a result of receiving information from these fields. This study aimed to evaluate the possible effects of one type of these fields, named T-Consciousness Charge Field (TCCF) on the wound healing process in an animal model with a focus on two sensitive organs, including the kidney and liver. To achieve this, after creating a wound model, C57BL/6 mice were randomly divided into control and TCCF-treated groups. Over 14 days of the experiment, sterile distilled water was sprayed on the wound of the control group, while water exposed to the TCCF was sprayed on the wound of the treatment group. The results showed that the TCCF-treated samples, on average, exhibited more efficient wound healing in terms of wound size, with approximately 60% greater improvement compared to the control samples by the end of the study period. Additionally, histological analysis of the repaired skin revealed enhancements in the TCCF-treated samples in inflammation indices, angiogenesis, collagen deposition, and the formation of structured, normalized skin tissue. Furthermore, in terms of liver and kidney health, the samples influenced by TCCF-treated water demonstrated notable improvements in inflammation indices compared to the controls. Overall, the findings of this study indicate that water treated with TCCF, acquiring therapeutic properties, had a positive effect on the biological process of wound healing in an animal model. Further experiments are needed to expand these observations and explore the potential of this field in wound healing processes.

Keywords: Skin, Wound, Complementary and Alternative Medicine, T-Consciousness Charge Field, Kidney, Liver

Introduction

The structure and function of the skin have been shaped by thousands of years of evolution. Comprising more than 10% of our body mass, it is the largest organ in the human body (Walters and Roberts, 2002). In addition to serving as an environmental barrier, the skin performs various biological functions, such as sensation, thermoregulation, and immunological responses against foreign agents (Lima and Reis, 2018). Furthermore, its vital role in vitamin D synthesis makes it an independent endocrine organ (Monteiro-Riviere, 2010).

The skin is composed of three main layers: the hypodermis, the innermost layer, which conducts neural signals and performs other functions; the dermis, the largest layer, which contains fibroblasts, mast cells, and immune cells like macrophages; and the epidermis, the outermost layer, organized into different layers and interfacing with the external environment (Moniz et al., 2020).

A wound is an injury that damages the epidermis, disrupting its normal anatomy and function (Yazarlu et al., 2021). Various factors, such as cuts, burns, pressure, radiation, and pathological conditions like diabetes, can compromise skin integrity (Kolimi et al., 2022). The skin has evolved efficient mechanisms to respond to and close breaches. Wound healing is a complex physiological process that occurs in four phases: hemostasis, inflammation, proliferation, and tissue remodeling (Wilkinson and Hardman, 2020).

Numerous biochemical pathways are activated to restore skin integrity. For example, during the inflammatory and hemostasis phases, the coagulation cascade and several cell types, including fibroblasts, keratinocytes, neutrophils, and macrophages, are involved in preventing excessive blood loss and infection (Tottoli et al., 2020). The migration and proliferation of keratinocytes, which comprise 90% of the cells in the epidermis (Than et al., 2019), are mediated by cytokines and interleukins such as TGF-β, FGF, IL-7, and IL-33 (Pakyari et al., 2013; Bartlett et al., 2016; Oshio et al., 2017), leading to the wound being covered by new epithelial cells (Wang et al., 2023).

To study the multifaceted nature of wound healing, various experimental models have been developed. In addition to *in vitro* models, such as the scratch assay (Abbas et al., 2019), *in vivo* models, particularly using rats and mice, are widely used (Sami et al., 2019). These animal models provide a realistic environment with diverse cell types and paracrine interactions. Moreover, compared to humans, small animals generally have a much shorter healing time, allowing the experimental duration to span several days rather than weeks or even months, as it would in humans (Masson-Meyers et al., 2020).

Many approaches have been developed to promote wound healing and protect wounds from infection. For example, hydrogels are used to maintain a moist environment, antimicrobial agents like combined silver with hydrogels to prevent infection, and collagen products are applied to chronic ulcers to create an environment that attracts essential cell types involved in healing while also reducing free radicals and proteases. Topical wound oxygen therapy has also been frequently evaluated (Han and Ceilley, 2017; Freedman et al., 2023). Additionally, traditional therapies using plant extracts, which are lower in cost and carry a reduced risk of bacterial resistance, have been explored as viable alternatives (Pereira and Bartolo, 2016).

In the field of consciousness studies, this is the first time that the concept of consciousness has been introduced as a practical method to promote wound healing. According to Taheri, there are various T-Consciousness Fields (TCFs) with distinct functions that can be examined through laboratory experiments (Taheri, 2013). This theory suggests that applying TCFs to samples induces changes in their functions and characteristics, even if the subjects under study are non-living. Previous experiments have explored the effects of TCFs on various subjects, from plants and animals to inanimate materials (Torabi et al., 2020; Taheri et al., 2022; Taheri et al., 2023). The data suggest that information transmitted through these non-physical fields alters the behavior of TCF-treated samples compared to controls.

The T-Consciousness Charge Field (TCCF) is one of these fields, specifically used to transmit information to a subject. Following exposure to TCCF, the treated subject acquires properties that may have alleviative effects. Thus, this study aimed to assess the potential wound-healing effects of water treated with TCCF.

Materials and Methods

Application of T-Consciousness Charge

This field was applied to the samples based on a protocol provided by the Cosmointel Research Center, established by the innovator of the theory, Mohammad Ali Taheri. More details have been explained in the general consideration of this issue.

Animals

Ten male and female C57BL/6 mice, weighing between 15-17 grams, were obtained from the Pasteur Institute of Iran and were kept at room temperature (25°C). The mice were randomly divided into two groups: the control and the group exposed to the TCCF. Ethical consideration: All procedures were conducted according to the guidelines of the Animal Care and Ethics Committee of Avicenna.Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran, and conducted in accordance with the NIH guidelines for the care and use of laboratory animals.

Experimental Design

The mice were anesthetized by intraperitoneal injection of 10% ketamine (80 mg/kg), 2% xylazine (8 mg/kg), and acepromazine (1 mg/kg). The area between the shoulders and lower back of the animal was shaved using a trimmer. During anesthesia, the animal's eyes were covered with an eye ointment to prevent corneal drying and body temperature was maintained within the normal range. The shaved area was sterilized with antiseptic agents, and a full-thickness wound with an 8-mm diameter was created between the shoulders using a disposable punch.

Sterile distilled water was sprayed on the wound of the control group, while water exposed to the TCCF was sprayed on the wound of the treatment group. The mice were kept in a calm and warm environment until full recovery from anesthesia, after which they were placed in separate cages with access to food and water. For 14 days, three times a day at four-hour intervals, water (each spray about 100 microliters) was sprayed on the wounds to fully cover them. Photographs of the wounds were taken every two days, and on day 14, tissue samples were collected for histological analysis. The effectiveness of the TCCF was assessed using hematoxylin-eosin staining.

Hematoxylin-Eosin Staining

The collected samples were fixed in 10% neutral buffered formalin. The fixed tissues were dehydrated in a graded series of ethanol concentrations, cleared in xylene, and embedded in paraffin. Sections with a thickness of 5 micrometers were cut and, after staining with hematoxylin and eosin, were observed under a light microscope (Olympus BX51) connected to a digital camera (Olympus DP71). The slides were examined blindly by a veterinary anatomical pathologist. Histopathological parameters to evaluate wound healing included acute and chronic inflammatory cells, granulation tissue formation, collagen deposition, epithelial regeneration, and neovascularization.

Additionally, to assess potential toxicity, kidney and liver tissues were also examined. To semi-quantitative analysis, in kidney tissue, inflammation, necrosis, congestion/hemorrhage, and degeneration were scored on a scale of 0 to 4, with 0 indicating absence, 1 presence in up to 25% of the area, 2 presence in 26-50%, 3 in 51-75%, and 4 in more than 75% of the area.

In liver tissue, ballooning degeneration, hepatic cell necrosis, inflammation, and congestion were scored, with severity levels (except for necrosis) defined as 0 (absent), mild (1-20%), moderate (21-60%), and marked (61-100%). Hepatic cell necrosis was assessed based on the presence or absence of cellular degeneration or massive necrosis with associated inflammation.

Statistical Analysis

The wound dimensions were measured over the 14-day treatment period using ImageJ software v1.54f, and comparisons between the control and treatment groups were made using GraphPad software version 9. Semi-quantitative histological comparisons were also conducted as described in the previous section.

While medications play a critical role in treatment and enhancing patients' quality of life, they may sometimes negatively impact the wound healing process by affecting metabolism, immune cell function, angiogenesis, and coagulation. For example, in patients with acute or chronic wounds, the use of certain medications, such as antineoplastic drugs for cancer, non-steroidal anti-inflammatory drugs (NSAIDs), and immunosuppressants, can delay wound healing (Bennett et al., 2024). Therefore, TCCF treatment may be a helpful complementary option in these cases.

Results and Discussion

Evaluation of Wound Size Changes

The changes in wound size are presented in Figure 1. As shown, after 6 days of exposure to TCCF, a significant reduction in wound size (p-value < 0.0001) was observed compared to the control group. The alleviating effect of TCCF treatment persisted until the end of the experimental period. A comparison between control and TCCF-treated wounds at each time point revealed differences of 20%, 32%, and 33% on days 6, 12, and 14, respectively. By the end of the study period, skin integrity had been restored by approximately 56% in the TCCF-treated group, compared to about 35% in the control group, suggesting a promotive effect of TCCF treatment on wound healing. Figure 2 illustrates images from a representative animal in each experimental group, showing observable changes over the 14-day study.

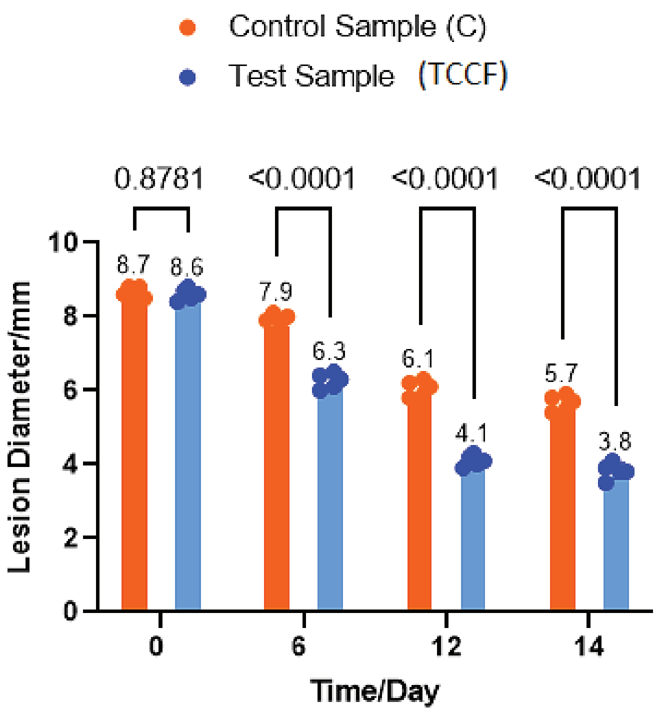


Figure 1. The diameter of skin wounds at four time points in the control and samples under the influence of T-Consciousness Charge Field (TCCF) treatment. In addition to the p-values, the measured dimensions are listed in millimeters at the top of each column for each experimental group.

Figure 2. Wound model in mice at three time points—initial, middle, and end of the study—comparing samples under the influence of T-Consciousness Charge Field treatment (right) and control samples (left).

Histology of the Healed Skin in the Control Group

In four cases, the skin matrix was relatively covered by stratified squamous epithelium (Figure 3). A magnified view in Figure 4 shows that the dermis contained relatively mature granulation tissue, characterized by a low to moderate number of new blood vessels, a moderate number of active fibroblasts, and mildly to moderately dense irregular collagenous connective tissue. Evidence of a mild inflammatory response was observed in all cases. The granulation tissue displayed a reduced number of vessels, particularly in the deeper layers of the wound (Figure 5). In one out of five control samples, normal skin morphology was noted.

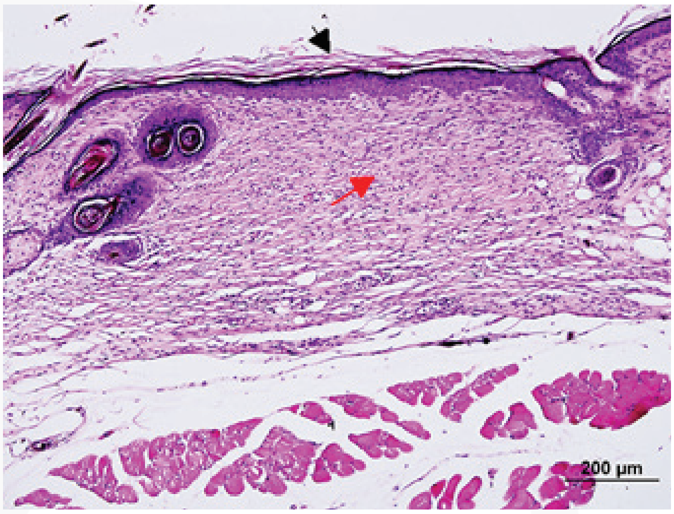


Figure 3. Stratified squamous epithelium (black arrow) and relatively mature granulation tissue in the newly formed dermis (red arrow). (H & E, Scale bar: 200 µm).

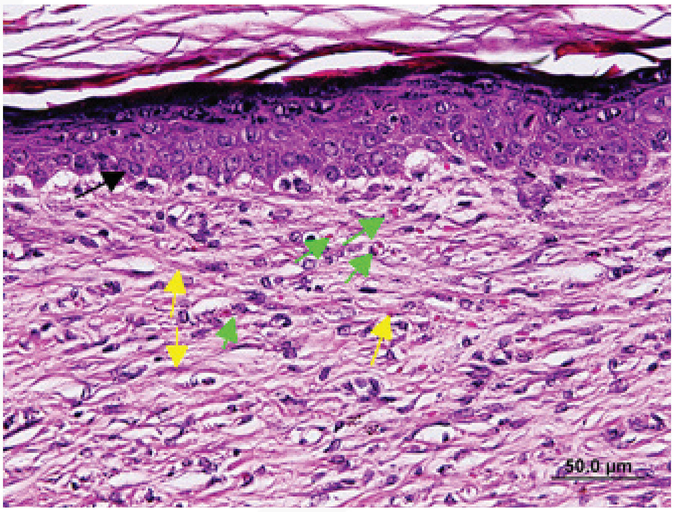


Figure 4. Magnified view of Figure 3, showing blood vessel density (green arrow) and collagen fibers (yellow arrow). (H & E, Scale bar: 50.0 µm).

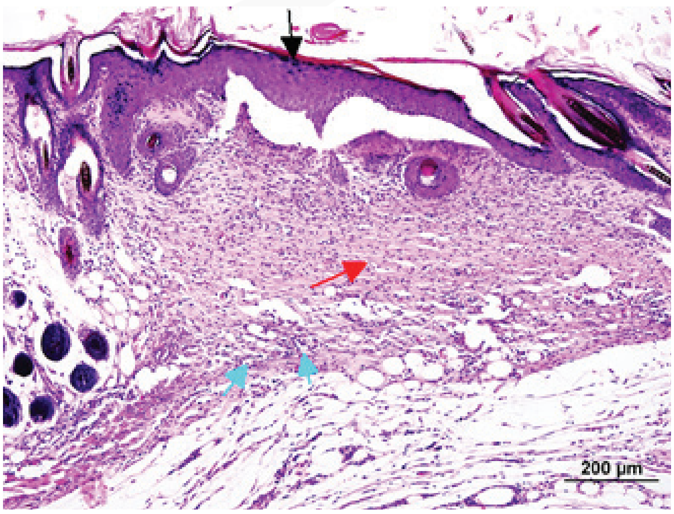
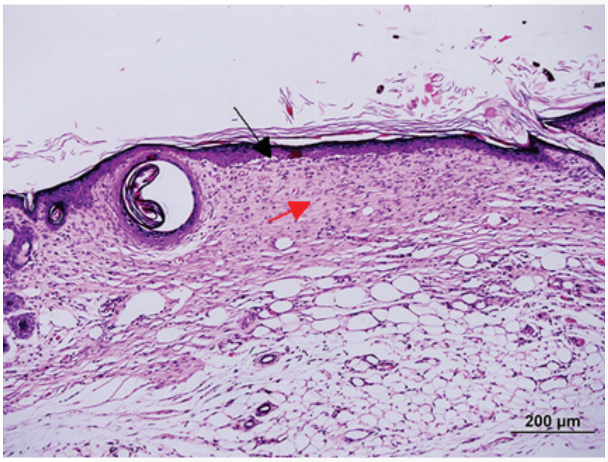


Figure 5. Stratified squamous epithelium (black arrow), relatively mature granulation tissue in the newly formed dermis (red arrow), and infiltration of inflammatory cells (blue arrow) in the dermis. (H & E, Scale bar: 200 µm).

Histology of the Healed Skin in the Treatment Group

In four cases, histopathological evaluation revealed well-developed stratified squamous epithelium and re-epithelialization (Figure 6). The dermis consisted of mature dense collagenous connective tissue and well-organized skin appendages. In most cases, the epidermal layer was nearly fully restored. However, in one case, the boundary between the epidermis and dermis was not completely established. The wound bed was primarily filled with fibroblast proliferation. Granulation tissue showed a reduced number of vessels, particularly in the deeper layers of the wound. Additionally, several newborn hair follicles were present at the wound site.



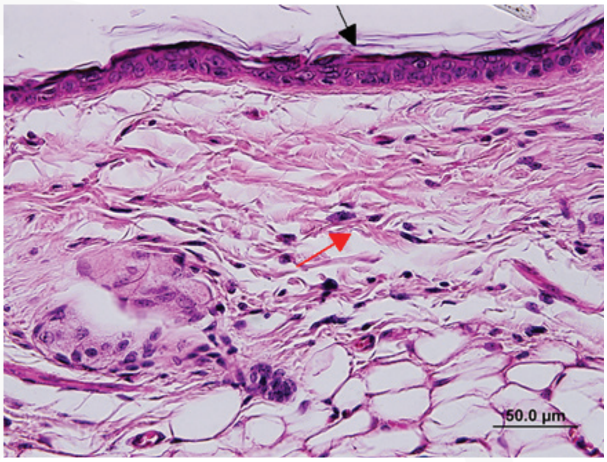


Figure 6. Fully restored epidermis (black arrow) and dermis (red arrow) in most samples exposed to T-Consciousness Charged Field. (H & E, Scale bar: Left: 200 µm, Right: 50.0 µm).

Semi-quantitative Analysis of Wound Healing

Table 1 presents a semi-quantitative analysis of several parameters related to wound healing in both experimental groups at the end of the study period. A histological comparison of skin wounds in the experimental groups revealed greater epidermal regeneration in the treated samples, whereas granulation tissue formation was more pronounced in the control group. Angiogenesis, which supports wound healing by delivering nutrients and oxygen, especially during granulation tissue formation (Johnson and Wilgus, 2014), was also higher in the control group compared to the treatment group. Moreover, the control samples exhibited a moderate number of active fibroblasts. In response to tissue injury, these spindle-shaped cells become activated and transform into myofibroblasts, which contribute to wound healing by contracting the wound and producing extracellular matrix proteins (Li and Wang, 2011). Once the wound is closed, these activities are terminated, and the myofibroblasts disappear through apoptosis. However, myofibroblasts persist only in pathological conditions (Gabbiani, 2003). The lower presence of spindle-shaped cells with higher collagen deposition in the treated samples suggests an accelerated healing process. Additionally, signs of inflammation were less evident in the TCF-treated samples compared to the control group.

Table 1. Semi-quantitative evaluation of tissue parameters in wound healing assessment (C: control sample, TCCF: T-Consciousness Charge Field).

Parameters/ Cases	Epidermal regeneration	Granulation tissue	Inflammatory cell infiltration	Angiogenesis	Spindle-shaped cell proliferation (fibroblasts/ myofibroblasts and endothelial cells)	Collagen deposed
C-1	2+	3+	1+	2+	3+	1+
C-2	3+	0	0	3+	1+	3+
C-3	2+	3+	1+	2+	3+	1+
C-4	2+	3+	2+	3+	3+	1+
C-5	2+	2+	2+	3+	3+	1+
TCCF-1	3+	0	0	0	1+	3+
TCCF-2	3+	2+	0	2+	3+	3+
TCCF-3	3+	3+	0	1+	3+	1+
TCCF-4	3+	0	1+	1+	1+	3+
TCCF-5	2+	0	1+	0	1+	2+

Histological Investigations of Sensitive Organs to Drugs

Kidney

Here, the changes in histological parameters of the kidney were compared between control and TCCF-treated samples. Semi-quantitative analysis showed that inflammation and congestion or hemorrhage in TCCF-exposed samples were lower than in controls (Table 2).

Table 2. Semi-quantitative analysis of parameters related to kidney tissue in sampled areas (C: control, TCCF: T-Consciousness Charge Field).

Parameters/Cases	Inflammation	Necrosis	Congestion/ Hemorrhage	Degeneration
C-1	1+	0	3+	0
C-2	1+	0	3+	0
C-3	1+	0	3+	0
C-4	1+	0	2+	0
C-5	1+	0	3+	0
TCCF-1	0	0	0	0
TCCF-2	0	0	0	0
TCCF-3	0	0	0	0
TCCF-4	1+	0	1+	0
TCCF-5	1+	0	2+	0

Figures 7-9 show normal tissues as well as tissues with inflammation and multifocal interstitial nephritis. These disorders were observed in all samples from the control group and in two samples from the TCCF treatment group.

Figure 7. Normal kidney morphology was observed in three out of five samples under the influence of T-Consciousness Charge Field treatment (H & E, Scale bar: 200µm).

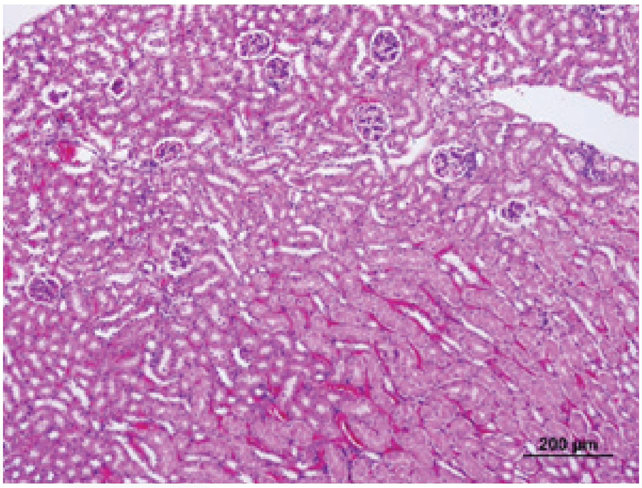


Figure 8. Inflammatory cell infiltration in the pelvis and surrounding tissue (black arrow) was observed in all control samples and in two of the samples exposed to T-Consciousness Charge Field (H & E, Scale bar: 100 µm).

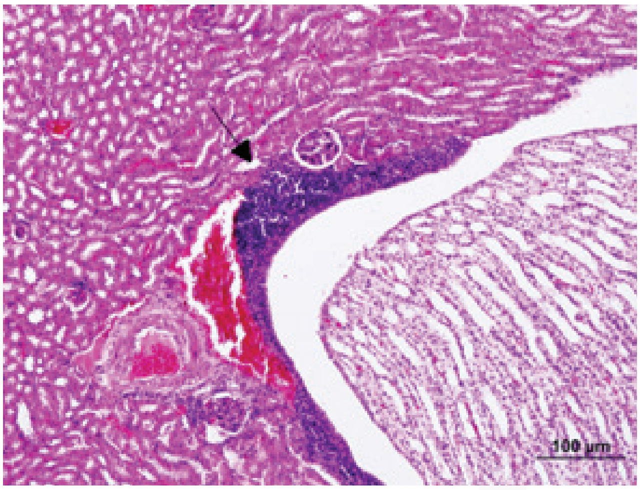
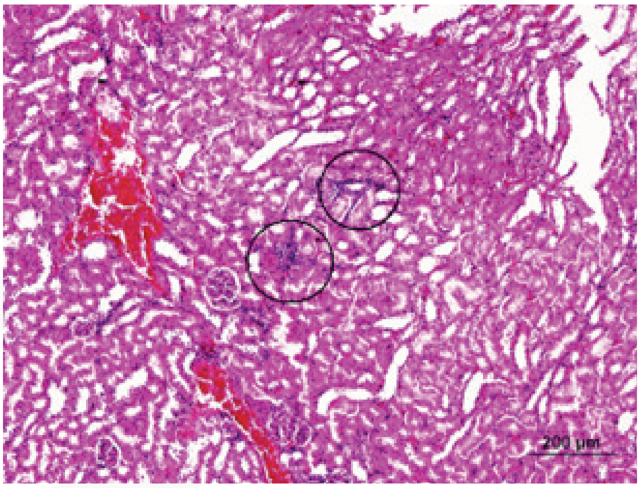


Figure 9. Multifocal interstitial nephritis (indicated by the circle) was observed in all control samples and in two samples exposed to the T-Consciousness Charge Field (H & E, Scale bar: 200 µm).



Liver

As described in the methods section, pathological changes in liver tissue were assessed according to severity levels. This approach enables a comparison of alterations between control and TCCF-treated samples throughout the experiment. A semi-quantitative analysis of several parameters is presented in Table 3, along with a histological image in Figure 10.

Table 3. Semi-quantitative analysis of parameters related to liver tissue in sampled areas (C: control, TCCF: T-Consciousness Charge Field).

Parameters/ Cases	Ballooning degeneration			Hepatocellular necrosis		Inflammation portal/ lobular			Congestion	
Severity	Mild	Mod	Marked	Individual cell degeneration	Necrosis with inflammation	Mild	Mod	Marked	Mild	Mod/ Marked
C-1	-	-	-	-	-	+	-	-	-	-
C-2	-	-	-	-	-	+	-	-	-	+
C-3	-	-	-	-	-	+	-	-	-	-
C-4	-	-	-	-	-	+	-	-	-	+
C-5	-	-	-	-	-	+	-	-	-	+
TCCF-1	-	-	-	-	-	-	-	-	-	-
TCCF-2	-	-	-	-	-	+	-	-	-	-
TCCF-3	-	-	-	-	-	-	-	-	-	-
TCCF-4	-	-	-	-	-	-	-	-	-	-
TCCF-5	-	-	-	-	-	+	-	-	-	+

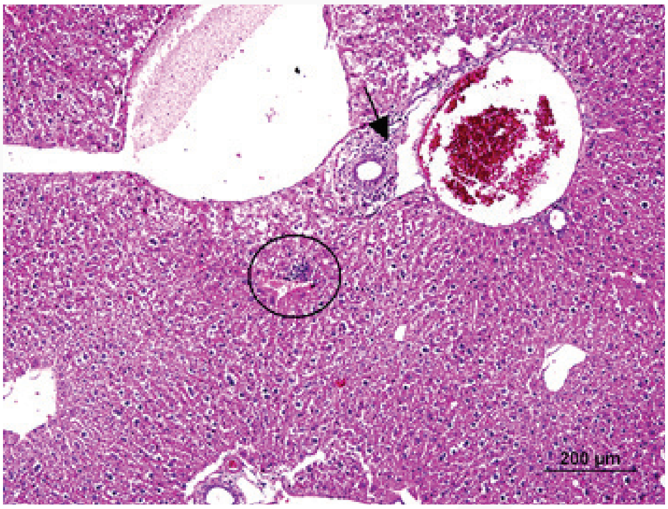


Figure 10. Multifocal infiltration of mononuclear cells in the parenchyma (circle) and the portal area (black arrow) was observed in all control samples and in two samples exposed to T-Consciousness Charge Field (H & E, Scale bar: 200 µm).

The results indicated that while all control samples exhibited mild inflammation in the liver tissue, three of the samples treated with TCCF showed no signs of inflammation. Additionally, most liver tissues in the control group displayed moderate to marked congestion; however, this condition was reduced to just one mouse in the treatment group.

It has been found that there is a link between skin injury and the development of organ dysfunction. In fact, inflammation, which plays a crucial role in the wound healing process, can adversely affect multiple organs, including the kidneys and liver (Huebener and Schwabe, 2013; Skopelja-Gardner et al., 2021; Anders, 2012). To mitigate these negative influences, several strategies are implemented. For instance, infection control is targeted through the use of antibiotics, anti-inflammatory treatments are applied to manage excessive inflammation (Khalil et al., 2017), and techniques such as negative pressure wound therapy are used in acute cases (Orgill and Bayer, 2013). In this experiment, we have evaluated the effectiveness of a non-energetic and non-material treatment on wound healing for the first time. This treatment influences the healing process by transmitting information through a type of TCFs, named T-Consciousness Charge Field.

In this study, water was used as a medium to be charged under the influence of the TCCF. This method requires no physical intervention but only a brief attention to TCCF. To illustrate, a trained individual can apply this treatment by establishing a connection between the medium—here, water—and the TCCF. According to the results of this study, water, acting as a recipient of information from the TCCF, has effectively enhanced key biological processes essential to wound healing, including cell migration, cell proliferation, inflammation prevention, and the production of vital macromolecules like collagen. The TCCF not only imparts therapeutic properties to water molecules for the repair and regeneration of the primary target tissue (skin) but also supports the structure and function of sensitive organs, such as the liver and kidneys, which are commonly assessed for toxicity in preclinical studies.

In conclusion, the findings of this study offer preliminary evidence of TCCF's potential to enhance wound healing in an animal model. We recommend further experiments to expand these observations and explore the practical application of this non-physical field in promoting wound healing.

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