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RESEARCH ARTICLE

# Effects of Manual Acupuncture at GB34 on Carbon tetrachloride-induced Acute Liver Injury in Rats

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**Abstract**
Manual acupuncture at Yanglingquan (GB34) is reported to have a beneficial effect on chronic liver damage. We, therefore, studied the effect of manual acupuncture at GB34 on acute liver damage. Rats were administered carbon tetrachloride ($CCl_4$) or olive oil, and direct manual acupuncture was subsequently performed at GB34 or at a sham point (a nonacupoint). In rats administered with $CCl_4$, the serum levels of aspartate aminotransferase and alanine aminotransferase, the total cholesterol concentration, and the levels of hepatic thiobarbituric-acid-reactive substances were suppressed by acupuncture at GB34 when compared with acupuncture at the sham point. By contrast, there was little histological difference in the liver between acupuncture at GB34 and that at the sham point in rats administered with $CCl_4$. These results suggest that manual acupuncture at GB34 tends to improve acute liver damage but is not sufficient by itself to completely resolve the hepatic injury.

## 1. Introduction

Oriental medicine has a far longer history than modern medicine, and studies of the medical mechanisms of acupuncture are one important aspect providing information on this type of treatment. Acupuncture treatment is reported to be effective not only for pain of the locomotor system but also for various other symptoms, such as those relating to disorders of the digestive, respiratory, and circulatory systems [1]. The mechanisms of this effect are thought to include endogenous pain relief actions, such as a spinal segmental mechanism, a motion control mechanism, somatic reflexes, autonomic reflexes, and neurological and endocrine mechanisms. In particular, an endogenous opioid hormone is released in the central nervous system during acupuncture stimulation [2]. This controls pain by blocking the neural pathway through which the brain recognizes pain. Hence, the effectiveness of

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acupuncture as an analgesic method seems to be related to blockades of both pain recognition in the brain and transmission of nociception in the spinal cord. Moreover, stimulation of peripheral nerves by acupuncture facilitates blood flow, thereby hastening the removal of substances causing pain. Acupuncture has also been recently reported to be associated with temporary increases in cytokine levels, which stimulate the immune system [3].

The administration of carbon tetrachloride ($CCl_4$) or galactosamine is a proven method of creating an animal model of liver dysfunction. Within 24 hours of administration, $CCl_4$ causes acute liver damage characterized by hepatocellular necrosis in the vicinity of the central vein, infiltration of white blood cells, fat deposition, and remarkable increases in serum transaminase levels. These findings are almost completely restored to a normal state within 72 hours [4].

Acupuncture is effective against various symptoms, and acupuncture at Qimen, Ganshu and Yanglingquan (GB34) is said to be effective for liver dysfunction. Although the literature contains a report on the effect of acupuncture at GB34 for chronic liver damage [5], no studies seem to have been conducted on the effect of acupuncture at this point for acute liver damage. However, acupuncture at Zusanli and Taichong performed following the administration of $CCl_4$ has been reported to be effective against acute liver damage [6]. We, therefore, analyzed the effect of manual acupuncture at GB34 by using a rat model of acute liver damage.

## 2. Materials and methods

### 2.1. Laboratory animals

We purchased male Sprague Dawley rats at 8 weeks of age (200—250 g) from Clea Japan Inc. (Shizuoka, Japan). We maintained the animals at a room temperature of $22 \pm 3$ °C and a humidity of 60% in a 12-hour light/dark cycle for a 2-week adjustment period after purchase. Rats were given free access to animal feed (CE-2 from Clea Japan Inc.) and water. The approval of the Suzuka University of Medical Science Animal Experiment Ethical Review Board was obtained for the experiments.

### 2.2. Experimental method

Laboratory animals were randomly divided into six groups: Normal, $CCl_4$ (−) and no acupuncture; Control, $CCl_4$ (+) and no acupuncture; Sham/$CCl_4$ (−), $CCl_4$ (−) and acupuncture at a sham point selected on the gluteal region, Fig. 1; Sham/$CCl_4$ (+), $CCl_4$ (+) and acupuncture at a sham point; GB34/$CCl_4$ (−), $CCl_4$ (−) and acupuncture at left GB34; and GB34/$CCl_4$ (+), $CCl_4$ (+) and acupuncture at left GB34. Each group consisted of five rats.

Liver injury was induced by intraperitoneal injection of 500 mL /L solution of $CCl_4$ in olive oil (2 mL/kg) in the $CCl_4$ (+) groups or injection of olive oil in the $CCl_4$ (−) groups. After administration of $CCl_4$ or olive oil, we fixed each rat to a brace and applied manual acupuncture to GB34 or to the sham point three times every 4 hours for 12 hours. This method was followed because the effect of the acupuncture continues for at least 3 hours [7] and because acute liver damage induced by $CCl_4$ shows an invasion of white blood cells, fatty deposition, and an increase in serum transaminase 12 hours after $CCl_4$ administration [8].

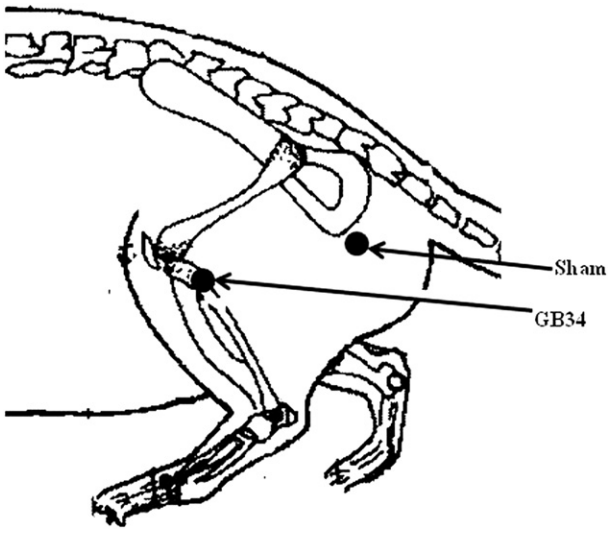


**Figure 1** GB34 and sham point for acupuncture. GB34 = Yanglingquan.

Sterilized disposable manual acupuncture needles (0.25 mm × 30 mm, Carbo deluxe, Global Medical Supplies Ltd, Hong Kong) were inserted perpendicularly to a depth of 2—3 mm at left GB34 or at a sham point. After the needles had been inserted, they were rotated 90° clockwise and then thrust downward nine times. Nine rotations and nine thrusts constituted one manipulation, and three manipulation units constituted one treatment session. The full manipulation took about 30 seconds. We performed an operation under 10% Nembutal anesthesia 24 hours after $CCl_4$ administration and surgically obtained blood and liver specimens.

### 2.3. Biochemical analysis

Blood was centrifuged at 3000 revolutions/minute for 20 minutes to separate the serum. The levels of serum transaminases, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), were measured as an index of liver damage by using 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride colorimetry (520 nm) after conjugation with glutamate dehydrogenase [9], and the serum total cholesterol was measured using a "Cholesterol E-test Wako" kit (Wako Pure Chemical Industries, Osaka, Japan).

Liver specimens were homogenized in 9 volumes of the homogenate buffer (50 mM Tris-HCl [pH 7.5], 1.14% KCl, 1 mM EDTA) in a Potter-Elvehjem homogenizer from As One Corp. (Osaka, Japan), and this homogenate was used as a crude extract. As an indicator of peroxidative disorder of the liver tissue, we measured the level of crude extract thiobarbituric-acid-reactive substance (TBARS) by using the thiobarbituric acid method [10] and expressed the level as the malondialdehyde quantity.