Comparison the Analgesic Effect of Electroacupuncture and Tramadol on Writhing Test in Rat

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Introduction
Acupuncture analgesia is a useful clinical technique for pain relief, and it has been using in treating diseases and pain and “Zusanli” point (St. 36) is one of the 365 classical acupuncture points. Electroacupuncture (EA) produces analgesia by endogenous substances including opioids. Also EA is one of the most desirable methods for the management of pain including chronic visceral pain and it is easy to apply, the cost is low and its adverse effects are minimum. Acupuncture can be an effective treatment in acute herpes zoster pain. EA was used in laboratory animals and the rat has 13 thoracic, six lumbar, four sacral, and 28 coccygeal vertebrae acupoints. Tramadol, a centrally acting analgesic structurally related to codeine and morphine, inhibits serotonin and norepinephrine reuptake, enhancing inhibitory effects on pain transmission in the spinal cord.

Materials and Methods
Fifty healthy Wistar female rats, 10-12 weeks of age, weighing 180-200g were purchased (Joundishapour laboratory animal center, Ahvaz, Iran) and housed polycarbonate cage for a 2-week acclimation period. Rats were fed ad libitum by standard laboratory pellet (Pars khurakdam, Shushtar, Iran.) and tap water. A 12-h light: 12-h dark cycle was maintained. Room temperature was at 23±2 °C. The study was carried in 5 groups of rats. One group was kept without restriction and drugs (group1) and another group was only restricted (group 2). Acetic acid was intraperitoneally injected in 3 to 5 groups of rats (10 rats each group) and the number of writhes was counted for 40 minutes after acetic acid injection. Group 3 received acetic acid and was kept as control. Tramadol was administered at dose 10 mg/kg intramuscularly in group 4 and rats of group 5 received EA and ST-36 (Zu San Li) and SP-6 (San Yin Jiao) points were stimulated at 1ms, 0.3 and 0.3 Hz, duration, intensity and frequency respectively. The amount of serum cortisol was measured by ELISA kit at end of study. Results: The results shown the mean of writhing was significantly decreased in group 4 and 5 with comparison to control group 3. In groups 3, 4 and 5 the mean writhing was 22.3, 3.4 and 3.6 per 10 minutes respectively. The mean of serum cortisol was greater in group 2 than other groups. This mean was significantly was lesser in group 4 and 5 in comparison to control group 3. In other hands, the mean cortisol was 0.7 and 1.16 ng/ml in groups 3, 4 and 5 respectively. Conclusion: Present study shows that tramadol and EA has similar analgesic effect on writhing test in rats.

Since the use of EA may be suitable for animals in veterinary medicine, effect of this technique on control of visceral pain was compared to tramadol in present study.
from Suzhou Acupuncture & moxibustion co. Ltd-China. Twenty minutes after acetic acid injection, tramadol (from Exir Co. Iran) was administered at dose 10mg/kg intramuscularly in group 4 and rats of group 5 received EA and ST-36 (Zu San Li) and SP-6 (San Yin Jiao) points were stimulated at 1ms, 0.3 and 0.3Hz, duration, intensity and frequency respectively. The amount of serum cortisol was measured by ELISA kit (Radim Co. Iran) at end of study. Statistical significance between groups was determined using SPSS program (version 16, USA). Significance of differences between groups were assessed using one-way analysis of variance (ANOVA) followed by least significant difference (LSD) post hoc comparison. The minimum level of significance was p<0.05.

**Results**

The results shown the mean of writhing number was significantly decreased in group 4 and 5 with comparison to group 3. In groups 3, 4 and 5 the mean (± SE) writhing was 22.3± 3.67, 3.4 ± 1.13 and 3.6 ± 0.8 per 10 minutes respectively in 2th, 3th and 4th count times (Table1). The EA and tramadol had similar analgesic and anti-inflammatory effect in writhing test in rat.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of writhes after acetic acid injection</th>
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<tbody>
<tr>
<td></td>
<td>0-10 (min)</td>
</tr>
<tr>
<td>Only acetic acid</td>
<td>9 ± 1.39\textsuperscript{a}</td>
</tr>
<tr>
<td>Acetic acid ± Tramadol</td>
<td>2.9 ± 0.77\textsuperscript{a}</td>
</tr>
<tr>
<td>Acetic acid ± electroacupuncture</td>
<td>7.1 ± 1.04\textsuperscript{a}</td>
</tr>
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The mean of serum cortisol was greater in group 1 (1.36 ±0.04 ng/ml) than other groups. This mean was significantly was lesser in group 4 and 5 in comparison to control group 3. In other hands, the mean (± SE) cortisol was 0.7 ± 0.04 and 1.16 ± 0.07 ng/ml in groups 3, 4 and 5 respectively (Figure 1).

**Discussion**

At present study, we used acetic acid model for anti-inflammatory effect of EA and tramadol. The acetic acid induces inflammation in peritoneal and causes writhing position. The endogenous opioid system and nonopioid systems may be involved in EA-induced analgesia because administration of opioid, serotonin and catecholamine antagonists including alpha-2 antagonists block EA-induced analgesia.\textsuperscript{2,5} We used electroacupuncture at “Zusanli” (ST.36) and "Sanyinjiao" (SP.6) points. These points are frequently used in animal research to study acupuncture effects on various physiological mechanisms and control systems changes including gastrointestinal disorders and visceral pain.\textsuperscript{10} There is evidence showing that EA stimulates the pain inhibitory system at the spinal cord, brain stem and other areas of the central nervous system, such as thalamus, 3rd ventricle, mesencephalon, diencephalon, hypothalamus, and hypophysis. Endogenous opioids, such as endorphins, enkephalins, and dynorphins have also been well documented to contribute to the analgesic effect of acupuncture. The dense-disperse mode of electrical stimulation, with the frequency ranging from 10 to 1000 Hz, used in this study probably produced the release of a combination of endogenous opioids. Fang and et al (2009) demonstrated electroacupuncture at ST.36 and SP.6 decreased production of cytokines in experimental arthritis in rats.\textsuperscript{11} Tramadol hydrochloride is a centrally acting opioid analgesic, the efficacies and potencies of which is only five to ten times lower than that of morphine. This drug has been used in rats\textsuperscript{12,13} and its effect was similar EA in our study on writhing test. The effect of tramadol could be reduced with yohimbine as well as naloxone. The efficacy of tramadol depends in part on the duration of nerve injury-evoked nociception, and that its antinociceptive mechanism changes over time.\textsuperscript{13} We demonstrated that EA significantly increased serum cortisol level even than tramadol (Figure 1). This finding is similar to Wang’s et al (2007) study in rats;\textsuperscript{14} but is different to some studies e.g. Radahmadi et al (2006) seen a significant increase in serum cortisol level in diabetes rats but stress did not cause significant increases in its level.\textsuperscript{15} In some studies, the plasma and serum level of cortisol was measured in rats for visceral dysfunction and inflammation.\textsuperscript{16,17} But it was reported a decrease in plasma cortisol concentration in human...
with EA. The mean of serum cortisol increased with tramadol administration with comparison to control group in our study. However, administration of tramadol in dog did not change plasma cortisol concentration.

Since corticosteron is major corticosteroid in rats and the precursor of corticosteron and cortisol is same, thus the amount of cortisol is decreased by elevation of corticosteron synthesis during stress and pain.

In conclusion, present study shows that tramadol and EA has similar analgesic effect on writhing test in rats.

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References
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