The effect of laserpuncture at the LI4 Hegu point on the plasma levels of β-endorphin in healthy subjects

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Abstract. Laserpuncture is an acupuncture method for pain management. The goal of this study was to determine the effect of laserpuncture at the LI4 Hegu point on the plasma levels of β-endorphin in healthy subjects. A randomized, double-blind, controlled trial with placebo controls was conducted on 29 healthy subjects. Subjects were allocated into the laserpuncture group (n = 15) and the laserpuncture placebo group (n = 14). The plasma levels of β-endorphin were used to measure the output of the study assessed both before treatment and post-treatment. There are statistically significant differences in the mean plasma levels of β-endorphin before and after treatment in the laserpuncture group: changes in mean value from 0.22±0.06 ng/ml to 0.29±0.07 ng/ml with a p value = 0.005 (p < 0.05). There were no statistically significant differences in the mean plasma levels of β-endorphin before and after treatment in the laserpuncture placebo group: changes in mean value from 0.22±0.06 ng/ml to 0.26±0.09 ng/ml with p value = 0.195 (p > 0.05). Between groups, there was not a statistically significant difference in the baseline mean plasma levels of β-endorphin (p = 0.183, p > 0.05). The conclusion of this study is that laserpuncture can affect the plasma levels of β-endorphin in healthy subjects when there is no statistically significant difference in the mean plasma levels of β-endorphin between groups.

1. Introduction
Laserpuncture is a method of excitatory acupuncture that employs a laser beam. It is both a laser therapy and a phototherapy, combining the science of medical acupuncture with the modern physics principle [1]. Laserpuncture is used for a variety of conditions ranging from soft tissue injury to chronic ulceration. This treatment has also been recommended as an effective needle alternative to stimulate acupuncture points or musculoskeletal trigger points [2]. The use of acupuncture as an alternative medical treatment is quite popular in Indonesia, but there are no data on acupuncture utilization in the population. Laserpuncture is promoted as a safer alternative to needles, because it is non-invasive and can be used to stimulate difficult acupuncture points such as the ear or around the genitals, or perineum [2]. Laserpuncture energy is measured in volts, amperes, and joules. Like an integrative combination of drugs that blends conventional pharmaceuticals with traditional Chinese medicine, laserpuncture combines modern medical techniques with traditional Chinese methods [1].

Laserpuncture with non-thermal irradiation is considered to be equivalent to the low-intensity stimulation of acupuncture points in traditional acupuncture. Although the use of therapeutic laserpuncture is increasingly popular, it is difficult to objectively evaluate therapeutic effectiveness, because parameters such as wavelength, irradiation, and light profiles are rarely clearly described [3].
The depth of laser energy transmission is an important factor, but laserpuncture is also affected by variations such as a patient’s skin thickness, age, and pigmentation. Laserpuncture is one of the latest technological advances in the field of acupuncture [4,5].

The mechanism of acupuncture analgesia has been studied extensively in the last two decades in western countries. Many studies use biomedical instruments to explain the work of acupuncture in the brain. Stabbing acupuncture needles into the acupuncture point will stimulate the release of mediators such as histamine, bradykinin, substance P, and ATP in the tissue around the acupuncture point. Increased levels of peripheral nociceptor that occur in the acupoints will cause nerve depolarization that will pass the signal from the nerve to the brain. The transduction process produces a response from the central nervous system to the periphery via an efferent route, followed by the release of β-endorphins and other neurotransmitters. This study was conducted to evaluate laserpuncture potential, safety in therapy, and comfort in action to reduce the doubts associated with laserpuncture therapy. In this study, researchers wanted to know the effect of laserpuncture at the LI4 Hegu point on blood plasma levels of β-endorphin in healthy subjects. The hypothesis of this study was that laserpuncture could influence a change of blood plasma levels of β-endorphin in healthy subjects.

2. Materials and Methods
This was a double-blind, randomized clinical trial with controls. The study was conducted from April 2016 to May 2016 at the RSUPN Dr. Cipto Mangunkusumo medical acupuncture clinic in Jakarta. The study population included healthy subjects from administrative personnel who met the study criteria. The inclusion criteria in this study were healthy adult subjects, 18–44 years of age, with normal vital signs, body mass index (BMI) <25 kg/m², and blood sugar levels <180 mg/dl, who were willing to sign informed consent. The exclusion criteria included subjects consuming drugs containing caffeine, codeine, and morphine (i.e., painkillers); subjects who consumed foods or beverages containing chocolate, caffeine, and alcohol or performed strenuous exercises at least 24 hours before the research; subjects with precancerous skin lesions, photoallergy, or epilepsy; and smokers. Subjects who discontinued the study were those who did not complete the research procedure, who were excluded during blood sampling, who were preshocked when treated with laserpuncture, and those who experienced pain during treatment.

The subjects were randomly divided into two groups: the intervention group with active laserpuncture and the control group with placebo laserpuncture. Active laserpuncture is an excitation performed on the subject using the RJ Handylaser Trion laser device. The wavelength was 785 nm with a power output of 50 mW. The device was affixed to the skin at the right and left LI4 acupuncture points. Radiation was performed for 2 x 40 seconds with a total dose of 4 J per point, with continuous wave. Therapy was administered three times with a frequency of once per day for three consecutive days. Placebo laserpuncture was performed using a non-compressing laser probe attachment made with the RJ Handylaser Trion device. The probe attachment was applied for 2 x 40 seconds per point with the same frequency as the active laserpuncture group.

The objective assessment in this study was performed by independent laboratories and used measurements of blood plasma β-endorphins at baseline and on the third day post-action. Research data was entered in the parent table and then processed statistically with IBM SPSS Statistics version 20. A paired t-test analysis was conducted to compare the blood plasma β-endorphins in each group after which a comparison was made between the two intervention groups using an unpaired t-test analysis. Results of such analyses are significant if they yield a value with p < 0.05. The data are presented below in tables and figures.
3. Results and Discussion

3.1 Results

Table 1 details the characteristics of the study subjects by gender, education, occupation, age, BMI, blood glucose, blood pressure, temperature, pulse frequency, and β-endorphin levels before testing to show that there was no significant difference between groups, making them acceptable for comparison.

| Variable                        | Intervention (n=15) | Control (n=14) | p-value |
|---------------------------------|--------------------|----------------|---------|
| Gender                          | Male               | 4 (26.7)       | 5 (35.7) | 0.700   |
|                                 | Female             | 11 (73.3)      | 9 (64.3) |         |
| Education                       | Bachelor degree    | 12 (80.0)      | 7 (50.0) | 0.128   |
|                                 | Junior-senior high school | 3 (20.0) | 7 (50.0) |         |
| Occupation                      | Employee           | 6 (40.0)       | 8 (57.1) | 0.302   |
|                                 | Entrepreneur       | 9 (60.0)       | 5 (35.7) |         |
|                                 | Housewife          | 0 (0.0)        | 1 (7.1)  |         |
| Age, years                      |                    | 34.07 (4.26)   | 31.29 (5.12) | 0.123* |
| BMI, median                     |                    | 22.66 (18.5–24.48) | 21.95 (18.67–23.83) | 0.252** |
| Blood sugar levels              |                    | 92.93 (13.57)  | 91.43 (11.22) | 0.748* |
| Blood pressure                  | Systolic           | 115.27 (9.09)  | 113.43 (10.37) | 0.615* |
|                                 | Diastolic          | 72.20 (7.82)   | 70.64 (8.27)  | 0.607* |
| Temperature                     |                    | 36.41 (0.32)   | 36.40 (0.46)  | 0.967* |
| Pulse frequency                 |                    | 77.26 (7.83)   | 78 (13.10)    | 0.855* |
| β-endorphin levels before       |                    | 0.22 (0.06)    | 0.22 (0.06)   | 0.881* |

Fisher’s Exact Test, *Unpaired t-test, **Mann–Whitney U test

Figure 1 shows that there is a change in β-endorphin levels in both groups; an increase in 12 of the intervention group subjects and in seven of the control group subjects. Decreased levels of β-endorphins also occurred in three intervention group subjects and six control group subjects. The level of β-endorphins was unchanged in only one control group subject.
Table 2. Plasma β-endorphin levels before and after intervention

| Plasma β-endorphins | Before   | After   | p-value |
|---------------------|----------|---------|---------|
| Intervention        | 0.22 (0.06) | 0.29 (0.07) | **0.005** |
| Control             | 0.22 (0.06) | 0.26 (0.09) | 0.195   |

Paired t-test

Table 2 shows that there was a significant difference in the intervention group before and after the laserpuncture action, as indicated by the p value = 0.005, exceeding the p < 0.05 significance margin. The changes that occurred in the control group were not statistically significant (p = 0.195). Table 3 shows that differences in the β-endorphin levels between the intervention and control groups were not significant, with p = 0.183 with significance value of p < 0.05.

Table 3. Difference in β-endorphin levels before and after intervention

| Plasma β-endorphins | Intervention | Control | p-value |
|---------------------|--------------|---------|---------|
| Difference          | 0.06 (0.07)  | 0.03 (0.09) | 0.183   |

Mann–Whitney U test

3.2 Discussion

This study marked the first laserpuncture research conducted in Indonesia to evaluate the effects of β-endorphin biomarker levels on healthy subjects. The tool used for laserpuncture action was an infrared laser device with a wavelength of 785 nm, chosen because it had sufficient depth penetration ranging from 3–4 cm to provide stimulation at the acupuncture point. This study used the LI4 acupuncture points associated with β-endorphin expenditure [6]. The LI4 point stimulated by low-frequency electroacupuncture (2 Hz) can increase endogenous opioid production by 367% [7]. In a study of chronic low back pain by Yun XK, it was determined that the LI4 Hegu point provides better results than other standard acupuncture methods, especially for the long term [8]. It was hoped that the laserpuncture technique could affect the levels of β-endorphins, which are neurotransmitters with an important role in acupuncture treatment, particularly in the pain mechanism. This study was conducted on healthy subjects aged 18–44 years, because this age group is active and productive and has not yet undergone the changes in β-endorphin metabolism that occur in the elderly [9]. This study also limited the consumption of foods and medications that may affect β-endorphin levels [10,11]. Subjects with a BMI of 18.5–25 were part of the inclusion criteria to reduce the likelihood of metabolic differences that may affect β-endorphin levels [12,13].

The intervention procedure in this study was performed with laserpuncture in a similar manner to the research done by Ribas et al., where the subjects did not experience pain after the installation of dental rubber, application of analgesia, and laser treatment three times total over a period of three days [14]. Based on these studies, it can be assumed that there was an increase in β-endorphin levels, because the study subjects did not experience post-action pain. Laserpuncture has a primary and secondary stimulation effect: irradiation of 35–40 mW or a minimum power density of 20 W/cm² may cause mast cell degranulation effects and acupuncture needle effects [15]. The dose used in this study was 4 J, based on the Walt recommendation [16]. The tool used has a cross-sectional area of 0.03 cm² and a power of 50 mW. The instrument parameters used in laserpuncture had sufficient power density to stimulate mast cells.

In this study, there was no significant difference in β-endorphin level between the intervention group and the control group. Although the changes in the intervention group were statistically significant and showed a trend corresponding to the literature, when compared with the control group the results were not significant. This is because laserpuncture dose was not optimal, so there were no
significant changes in β-endorphin levels compared to the control group. In research conducted by Musawi et al. about the effect of laser irradiation on the number of red blood cells, irradiation with a dose of 72 J/cm² from a laser with a wavelength of 405 nm caused a significant decrease in red blood cell volume, so the study concluded that the optimum laser dose was 72 J/cm² [17]. In this study, the dose used was 2000 J/cm², exceeding the optimum dose in the study conducted by Musawi et al. However, there is a difference in the wavelength of the tool used in this study (785 nm) compared with research done by Musawi et al. (405 nm). The dose was determined by the duration of therapy, based on studies conducted by Zhang et al. against mice. The duration of different acupuncture treatments gave different treatment responses in mice with medial cerebral artery occlusion [18]. There are currently no studies comparing the effectiveness of laserpuncture with other acupuncture stimulation methods. Therefore, much remains to be examined in more detail in subsequent research to determine the dose, wavelength, excitatory technique with biomarker, and/or clinical effects on a case.

The study of acupuncture’s effect on β-endorphins performed by Setiawardhani in 2011, using electroacupuncture, gave different results. Electroacupuncture is performed only once. Plasma β-endorphins increased by 50% (n = 9) in the intervention group and by only 5.6% (n=1) (p < 0.05) in the control group. The mean plasma β-endorphin levels in the intervention group increased from 34.6±3.5 pg/ml to 35.1±3.4 pg/ml. In the control group the mean plasma β-endorphin level decreased from 43.6±3.3 pg/ml to 10.3±1.8 pg/ml (p < 0.05) [19]. The use of laserpuncture in the current study showed subjects with increased levels of β-endorphin in the intervention group numbered 12 subjects (80%) and eight in the control group (57.14%), whereas subjects with decreased levels of β-endorphins numbered three in the intervention group (20%) and six in the control group (42.86%). The mean plasma β-endorphin level in the intervention group was 0.22±0.06 ng/ml to 0.29±0.07 ng/ml (p = 0.005) but 0.22±0.06 ng/ml to 0.26±0.09 ng/ml (p = 0.195) in the control group. These results showed that studies using laserpuncture are in line with research conducted by Setiawardhani et al. that both laserpuncture and electroacupuncture can increase the blood plasma β-endorphin levels of healthy people.

A study of the effects of laserpuncture on changes in blood plasma β-endorphin levels has never been done before this, although many studies have shown that the action of laserpuncture is effective. The role of laserpuncture in treating pain has been widely studied. β-endorphins are one type of the various neurotransmitters targeted in acupuncture work in cases of pain, and they play an important role. Biostimulation of laserpuncture stimulates degranulation of mast cells, which then provides stimulation to the central nervous system and provides feedback with elevated blood plasma β-endorphin levels [15]. The study conducted by Peres et al., showed that the analgesia effect in laser phototherapy is closely related to peripheral opioids [20]. Based on research by Zhang et al., mast cell degranulation by laser irradiation is possible through transient receptor potential channels (TRPV2) [21]. Laser irradiation provides stimulation of mitochondria that stimulate mast cell degranulation, which stimulates the migration of T-lymphocytes capable of producing β-endorphins [22].

Changes in β-endorphin levels are influenced by many things, including food, physical activity, smoking habits, psychological experiences, and the menstrual cycle. This study sought to limit the elements that can affect changes in β-endorphin levels, but psychological experiences and normal body cycles cannot be regulated under ideal conditions. This could affect the results of β-endorphins that occurred in the control group. The control group experienced a change in β-endorphin levels despite no laserpuncture treatment and, based on statistical changes that occurred before and after, the placebo of laserpuncture was not significant. These results showed that the placebo effect of the laserpuncture did not cause the changes to occur. One of the difficulties in research in humans is creating the ideal research conditions, due to various factors. The physical and psychological factors of each individual are different, so they can produce different results [23,24]. Lundeberg et al., recommended that the evaluation of the clinical effects of acupuncture needs to consider individual patient variations, as the results of a clinical trial of acupuncture give varying results. These varying results make the group comparison analysis less meaningful [24]. The relatively higher number of women in this study could have skewed the results Based on the characteristics of research subjects
other factors that may affect the menstrual cycle and ovulation in women that can affect β-endorphin levels [25,26].

Sheps et al., conducted a study of psychophysical responses and their correlation with β-endorphin levels after psychological stress by public speaking. The role of β-endorphins in the body remains controversial. There are several contradictory studies relating β-endorphin levels to changes in pain perception. Some researchers have reported that plasma opioid peptide levels are associated with a pain perception index measured in skin tissue, while others did not find this to be true. This difference may be due to differences in sample populations, such as the possible differences in the management of psychological stress by different sexes, differences in examination techniques, differences in experimental protocols that may affect the status of baseline values, and differences in stress levels arising. These factors affect the levels of opioid peptide and differing pain perceptions [27]. Based on a study conducted by Sheps et al., subjects may have experienced a psychological response to the study that led to increased levels of β-endorphins in the control group.

One other factor that may have affected the results of β-endorphin levels is the effect of suppression at the time of the laserpuncture placebo action. Although efforts were made not to stress the subject, the placebo effect may have persisted. In a study of traditional Chinese acupuncture treatment groups, electrostimulation, TENS, and PTENS by Abenyakar et al., acupuncture and electrostimulation caused a significant increase in β-endorphin levels, whereas TENS and PTENS did not produce any significant results [28]. A study conducted by Fassoulaki et al. on the effect of acupressure on the bispectral index, serum melatonin, and β-endorphin plasma showed that acupressure has no effect on plasma levels of β-endorphins [29]. Based on the studies conducted by Abenyakar et al. and Fassoulaki et al., there is little possibility of changes in β-endorphin levels occurring as a result of the placebo laserpuncture effect, because, in those studies, TENS, PTENS, and acupressure did not give produce in β-endorphin levels.

In this study, there was a decrease in β-endorphin levels in both the intervention group and in the control group, in both male and female subjects. There are several conditions that may lead to a decrease in β-endorphin levels: excess physical exercise [30], depression [31], use of prednisone and steroids [32], irritable bowel syndrome [33], menstrual cycle [26], alcoholic addiction [34], and genetics [35]. Rhodin et al., in a study comparing the analysis of β-endorphins with genetic polymorphism, suggested there may be differences in men and women due to genetic variation. In addition, variations in the Na+, K+, and Ca2+ ion channels can provide different β-endorphin responses. The present study concluded that β-endorphin levels in the blood circulation are influenced by genetic and sex factors [35]. Rhodin et al.'s hypothesis supports the variations in results that occurred in this study. Genetic variation is a difficult factor to assess, as it requires more resources and more adequate facilities, so this became one of the limitations in this study.

This study found no side effects, such as infection or shock. During three interventions with laserpuncture and placebo laserpuncture, there were no complaints of pain or other adverse events. The kit used to examine the levels of β-endorphins in this study is the same as that used by Setiwardani et al. in a study of the effect of β-endorphins after electroacupuncture stimulation, but the results of the current examination gave a considerable difference. Normal β-endorphin levels are not known, and their role in the body is not fully explained. Based on research conducted by Harbach et al., the factors that affect variation in β-endorphin values are caused by the examination of radioimmunoassays, in which the detectable β-endorphin molecule is not pure but also includes derivatives of β-endorphins. There may be cross-reactions to the pure β-endorphin chain with the derivatives present in the blood [36].

4. Conclusion
The action of laserpuncture can cause changes in blood plasma β-endorphin levels in healthy subjects. The mean plasma β-endorphin level of the intervention group increased from 0.22±0.06 ng/ml to 0.29±0.07 ng/ml and was statistically significant (p = 0.005, p < 0.05). While the mean β-endorphin level of blood plasma in the control group also increased from 0.22±0.06 ng/ml to 0.26±0.09 ng/ml,
the variation was not statistically significant (p = 0.195, p > 0.05). Additionally, there was no statistically significant difference in the mean rate of blood plasma β-endorphin levels in the laserpuncture group compared with the placebo group (p = 0.183, p > 0.05). Further research involving gender and genetic factors would be warranted.

References

[1] Weber M and Kreisel V 2012 Laser Acupuncture A Practical Handbook Successful Treatment Concepts. 1st ed. A F, ed. (Germany: Fuchtenbusch Verlag).
[2] Baxter G D, Bleakley C and McDonough S 2008 Clinical effectiveness of laser acupuncture: a systematic review. J. Acupunct. Meridian. Stud. 1 65-82.
[3] The Royal Society of Medicine Press Limited 2001 Effective health care acupuncture. Effective Health Care. 2001 1-12.
[4] Whittaker P 2004 Laser Acupuncture: Past, Present, and Future. Lasers Med. Sci. 19 69-80.
[5] Round R, Litscher G and Bahr F 2013 Auricular acupuncture with laser. Hindawi Evid. Based. Complement. Alternat. Med. 2013 1-22.
[6] Leung W, Jones AY, Wong CY 2011 Electroacupuncture in reduction of discomfort associated with barostat-induced rectal distension--A randomized controlled study Journal of Gastrointestinal Surgery 15 660-6.
[7] Han JS 2001 Opioid and Antiopioid Peptides: A Model of Yin-Yang Balance in Acupuncture Mechanisms of Pain Modulation. In: Stux G, Pomeranz B, eds. Clin. Acupunct. Sci. Basis. (Berlin Heidelberg: Springer-Verlag) p. 230.
[8] Yun M, Shao Y, Zhang Y, He S, Xiong N, Zhang J, et al. 2012 Hegu acupuncture for chronic low-back pain: a randomized controlled trial. J. Alternat. Compliment. Med. 18 130-6.
[9] Stewart C, Schofield P, Gooberman-Hill R, Mehta S and Reid M C 2014 Management of Pain in Older Adults. Practical Management of Pain. 5th ed. (Philadelphia: Elsevier) p. 9.
[10] Jamurtas A Z, Tofas T, Fatouros I, Nikolaidis M G, Paschalis V, Yfanti C, et al. 2011 The effects of low and high glycemic index foods on exercise performance and beta-endorphin responses. J. Int. Soc. Sports. Nutrition. 8 15.
[11] Nogueiras R, Romero-Pico A, Vazquez M J, Novelle M G, Lopez M and Dieguez C 2012 The opioid system and food intake: homeostatic and hedonic mechanisms. Europ. J. Obesity. 5 196-207.
[12] Cabyoglu M T, Ergene N and Tan U 2006 The mechanism of acupuncture and clinical applications. Int. J. Neurosci. 116 115-25.
[13] Harahap H, Widodo Y and Mulyati S 2005 Penggunaan berbagai cut-off indeks massa tubuh sebagai indikator obesitas terkait penyakit degeneratif di Indonesia. Gizi. Indonesia. 28 76-87.
[14] Ribas M A, Dominguez J A and Puigdollers A 2013 Analgesic effect of a low-level laser therapy (830 nm) in early orthodontic treatment. Lasers. Med. Sci. 28 335-41.
[15] Sehikara D 2008 Laserneedle acupuncture: a critical review and recent results. Med. Acupunct. 20 37-42.
[16] Therapy WaoL 2010 Recommended treatment doses for Low Level Laser Therapy.
[17] Musawi M S, Jafar M S, Al-Gailani, Ahmed N M, Suhaimi F H and Suardi N 2016 In vitro mean red blood cell volume change induced by diode pump solid state low-level laser of 405 nm. Photomed. Laser. Surg. 34 211-4.
[18] Zhang C, Wen Y, Fan X N, Tian G, Zhou X Y, Deng S Z, et al. 2015 Therapeutic effects of different durations of acupuncture on rats with middle cerebral artery occlusion. Neural. Regeneration. Res. 10 159-64.
[19] Setiawardhani L 2011 Efek Elektroakupunktur Titik Li 4 Hegu Terhadap Kadar Beta-Endorfin Plasma Pada Subjek Sehat. (Jakarta: Universitas Indonesia).
[20] Peres e Serra A and Ashmawi H A 2010 Influence of naloxone and methysergide on the analgesic effects of low-level laser in an experimental pain model. Revista. Brasileira. De. Anestesiologia. 60 302-10.
[21] Zhang D, Spielmann A, Wang L, Ding G, Huang F, Gu Q, et al. 2012 Mast-Cell degranulation induced by physical stimuli involves the activation of transient-receptor-potential channel TRPV2. *Physiol. Res.* **61** 12.

[22] Hsieh Y, Hong C, Chou L, Yang S, Yang C 2015 Fluence-dependent effects of low-level laser therapy in myofascial trigger spots on modulation of biochemicals associated with pain in a rabbit model. *Lasers in Medical Science* **30** 209-216.

[23] Arbol J L, Munoz J R, Ojeda L, Cascales A L, Irles J R, Miranda M T, et al. 2000 Plasma concentrations of beta-endorphin in smokers who consume different numbers of cigarettes per day. *Pharmacol. Biochem. Behavior* **67** 25-8.

[24] Lundberg T and Lund I 2009 Treatment recommendations should take account of individual patient variation not just group responses. *Acupunct. Med.* **27** 31-2.

[25] Straneva P A, Maixner W, Light K C, Pedersen C A, Costello N L and Girdler S S 2002 Menstrual cycle, beta-endorphins, and pain sensitivity in premenstrual dysphoric disorder. *Health. Psychol.* **21** 358-67.

[26] Giulio GD, Reissing ED 2006 Premenstrual dysphoric disorder: Prevalence, diagnostic considerations, and controversies *Journal of Psychosomatic Obstetrics and Gynecology* **27** 201-10.

[27] Sheps D S, Ballenger M N, Gent G D, Krittayaphong R, Dittman E, Maixner W, et al. 1995 Psychophysical responses to a speech stressor : correlation of plasma beta-endorphin level at rest and after psychological stress with thermally measured pain threshold in patient with coronary artery disease. *J. Amer. College. Cardiol.* **25** 1499-503.

[28] Abenyakar S and Boneval F 1994 Increase plasma beta-endorphin concentration after acupuncture: comparison of electroacupuncture, traditional chinese acupuncture, TENS and placebo TENS. *Acupunct. Med.* **12** 21-3.

[29] Fassoulaki A, Paraskeva A, Kostopanagiotou G, Tsakalozou E and Markantonis S 2007 Acupressure on the extra 1 acupoint: The effect on bispectral index, serum melatonin, plasma -endorphin, and stress. *Anesth. Analg.* **104** 312-7.

[30] Cunha G S, Ribeiro J L and Oliveira A R 2008 Levels of beta-endorphin in reponse to exercise and overtraining. *Arq. Bras. Endocrin. Metab.* **52** 589-98.

[31] Kubryak O V, Umriukhin A E, Emeljanova I N, Antipova O S, Guseva A L, Pertsov S S, et al. 2012 Increased beta-endorphin level in blood plasma as an indicator of positive response to depression treatment. *Bull. Exp. Biol. Med.* **153** 721-3.

[32] Ciriaco M, Ventrice P, Russo G 2013 Corticosteroid-related central nervous system side effects *Journal of Pharmacology & Pharmacotherapeutics* **4** (Suppl1) S94-S98.

[33] Hughes P A, Moretta M, Lim A, Grasby D J, Bird D, Brierley S M, et al. 2014 Immune derived opioidergic inhibition of visceral sensory afferents is decreased in Irritable Bowel Syndrome patients. *Brain. Behav. Immun.* **42** 191-203.

[34] Dai X, Thavundayil J, Gianoulakis C 2015 Differences in the Peripheral Levels of $\beta$-endorphin in Response to Alcohol and Stress as a Function of Alcohol Dependence and Family History of Alcoholism *Alcoholism: Clinical and Experimental Research* **29** 1965–1975.

[35] Rhodin A, Gronbladh A, Griya H, Nilsson K W, Rosenblad A, Zhou Q, et al. 2013 Combined analysis of circulating $\beta$-endorphin with gene polymorphisms in OPRM1, CACNAD2 and ABCB1 reveals correlation with pain, opioid sensitivity and opioid-related side effects. *Mol. Brain.* **6** 8.

[36] Harbach H, Hell K, Gramsch C, Katz N, Hempelmann G and Teschemacher H 2000 Beta-endorphin (1-31) in the plasma of male volunteers undergoing physical exercise. *Psychoneuroendocrinol.* **25** 551-62.