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SHORT COMMUNICATION
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Electroacupuncture-Induced Hematological and Biochemical Changes in Ovariohysterectomized Cats: The Role of Alkaline Phosphatase as a Biomarker

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ABSTRACT

This study aims to explore the effect of electroacupuncture on the role of alkaline phosphatase (ALP) as a biomarker of physiological function in sterile female cats while analyzing hematological parameters such as mean corpuscular hemoglobin (MCH), corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), and differential leukocyte count. A total of nine sterile female cats were divided into three groups, namely P1 as a control without electroacupuncture stimulation, group 2 P2: sterile female cats were given electroacupuncture at points BL18, SP6, and SI3, and group three P3: cats were given electroacupuncture at points ST36, BL19, and LIV3 (=LR3). Electroacupuncture was administered five times at intervals of three days, with each session lasting 10minutes. Blood was taken after the last electroacupuncture session was completed. Data were analyzed using one-way ANOVA and followed by the Tukey Post Hoc test. The results of the analysis showed that ALP levels between the treatment groups were significantly different ($P<0.05$). There was no significant ($P>0.05$) difference in MCH, MCHC, or MCV levels. The differential leucocyte value was still within the normal range. The conclusion of this study showed that the administration of electroacupuncture, combined with specific acupuncture points, can potentially reduce ALP levels in sterile female cats.

Keywords: Electroacupuncture, Alkaline Phosphatase, Sterilized Cats, Reproduction, Alternative Treatment.

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INTRODUCTION

Cats are popular pets, and their population is increasing uncontrollably (Dutton-Regester & Rand, 2024). Many efforts have been made to control this through sterilization (Ferreira et al., 2024). Sterilization also helps suppress hormonally driven unwanted behaviours (Ferré-Dolcet & Romagnoli, 2023). In females, this procedure is called ovariohysterectomy, which is helpful in addition to controlling overpopulation and preventing reproductive tract diseases such as pyometra (Talukdar et al., 2022). However, this conditioning procedure can also reduce estrogen levels, which can contribute to osteoporosis in females (de Melo et al., 2021), increase the risk of neoplasia in several organ systems, lead to musculoskeletal issues, endocrinological disorders, urinary incontinence, and

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obesity (Jeong et al., 2024). In ovariectomized mice, this procedure has long-term effects on several organs, such as the liver, intestines, and myocardium, due to the sudden loss of estrogen (Klintip et al., 2022).

There was an increase in ALP levels in cats that had been ovariohysterectomized, although the results were not significantly different from cats that were not ovariohysterectomized (Teixeira et al., 2022). However, the increase in ALP indicates osteoblast activity associated with bone diseases such as fractures, bone tumours, rickets, osteoporosis, primary and secondary hyperparathyroidism, and periostitis (Abo-Soliman et al., 2020).

Alkaline Phosphatase ALP is an enzyme found in many body tissues, especially in the liver, bones, kidneys, and digestive tract (Sahraei et al., 2024). ALP is essential in phosphate metabolism and is associated with liver and bone function. Understanding the relationship between ALP and blood hematology can be very helpful in analyzing hematological disorders related to organ dysfunction or body metabolism. ALP levels can also be used to assess abnormalities in the hepatobiliary system (Oikonomidis & Milne, 2023).

In sterile female cats, leukocyte examination should accompany ALP testing to help confirm bone or liver disorders (Marahrens et al., 2023). Detection of an increase in leukocyte differential accompanied by changes in ALP levels can help determine whether the cause is infection, postoperative inflammation, or other disorders. This examination helps confirm post-surgical complications.

Certain meridians are commonly targeted in acupuncture for reproductive balance (Ye et al., 2022). Bai-hui is believed to influence and restore endocrine-metabolism-immune balance through neural pathways. Acupuncture for reproductive disorders is also considered relatively safe, with minimal side effects (Wang et al., 2024).

This study investigates whether electroacupuncture can stabilize ALP levels, a marker of disorders linked to estrogen decline (Hu et al., 2023). Therefore, the ALP levels obtained in this study are expected to be normal after electroacupuncture is administered to sterilized female cats. This study aims to provide scientific information about the effects of electroacupuncture on ALP as a biomarker of physiological function in sterile female cats while analyzing hematological parameters such as MCH, MCHC, MCV, and differential leukocyte count.

MATERIALS & METHODS

Study Design and Ethics Approval

This study was approved by the Veterinary Ethics Committee, Faculty of Veterinary Medicine, Syiah Kuala University (No. 42/KEPH/IX/2019). It was conducted at the Dr. Noerjanto Veterinary Teaching Hospital, Banda Aceh, Aceh, Indonesia.

Experimental Animals and Study Design

This study used eighteen sterilized female cats (aged 1–3 years, weighing 2–3kg) divided into three groups: P1 (control, without electroacupuncture) (Kaptchuk, 2020), P2 (electroacupuncture in combination 1) and P3 (electroacupuncture in combination 2). The first combination of acupuncture points used was Bladder 18 (BL18, Gan-Shu), Spleen 6 (SP6, San-Yin-Jiao), and Small Intestine 3 (SI3, Hou-Xi), which have physiological roles in the liver, reproductive health, and spinal pain relief. The second combination was P3 Stomach 36 (ST36, Hou-San-Li), Bladder 19 (BL19, Dan-Shu), and Liver 3 (LIV3 = LR3, Tai-Chong), which have roles in regulating digestion, liver function, and the gallbladder, including immune modulation. The location of the acupuncture points can be seen in Fig. 1.

Acclimatization and Animal Management

All experimental animals were adapted to the cage conditions and research environment for one week (Al-Sagheer et al., 2023). Each cage was equipped with scented cat litter; cats were fed commercial dry food three times a day, with wet food as additional feed after each electroacupuncture treatment. Drinking water was provided as much as possible.

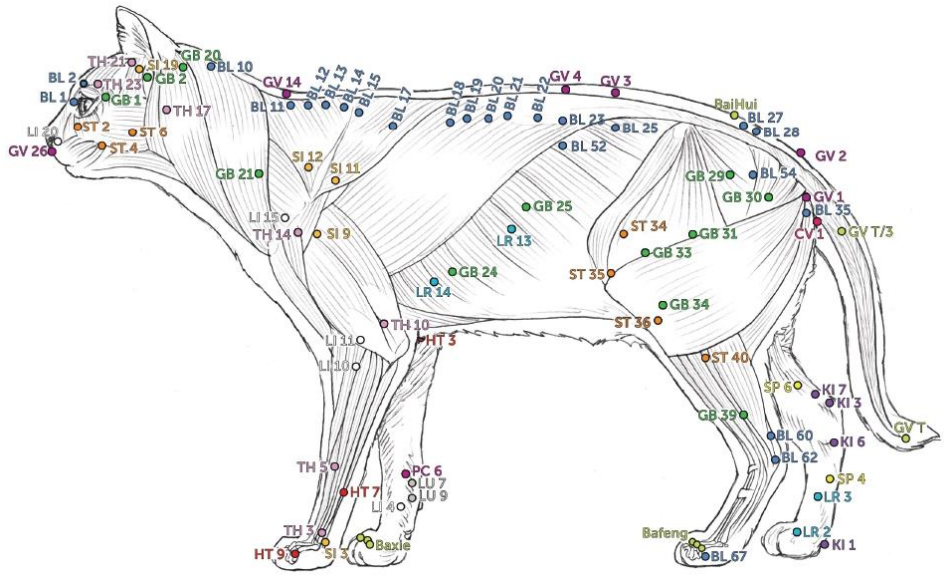

Fig. 1: Location of acupuncture points to be applied to research cats, BL18, SP6, SI3, ST36, BL19, and LR3. (Source: https://curacore.org/vet/2023/11/23/free-download-cat-acupuncture-point-chart/).

Electro Acupuncture Procedure

Electroacupuncture was given five times at a three-day interval, each lasting 10 minutes at a frequency of 1–2 Hz (Ingerson et al., 2023). After the last treatment, blood samples were taken aseptically from the cephalic or saphenous vein.

Data Analysis

Laboratory results (ALP, MCH, MCV, MCHC, RDW-SD: , RDW, and differential leukocytes) were presented as mean ± SD per subject. ALP, MCH, MCV and MCHC obtainers were tested for normality and homogeneity of variance. The significance level was tested using one-way ANOVA in a completely randomized block design, followed by Duncan's multiple range test to determine differences between treatment means (SAS Institute Inc 2004).

RESULTS

Results of Electroacupuncture Treatment on ALP Level in Sterile Female Cats

Table 1 presents ALP levels in U/L cats undergoing ovariohysterectomy and receiving electroacupuncture treatment. Different superscripts in the ALP column indicate significant differences (P<0.05) among the groups, suggesting that electroacupuncture significantly reduced ALP levels compared to the control group. These findings support the potential use of electroacupuncture as an adjunct therapy in postoperative management for cats undergoing ovariohysterectomy**.** The highest ALP levels were in the control group (P1), while the lowest was in P3, who received a second combination of electroacupuncture.

Table 1: Serum ALP levels (U/L) units in Ovariohysterectomized Cats Treated Electroacupuncture

Treatments	ALP (U/L)
P1(Control)	111.67±6.68a
P2	78.17±10.38b
P3	41.67±41.67c

Values (Mean+SD) bearing different alphabets in the same column indicate a significant (P<0.05) difference

Results of Electroacupuncture Treatment on Hematological Testing of Cats

Table 2 summarises the hematological profile. No significant differences (P>0.05) were observed in MCH, MCHC, or RDW-CV across groups. However, MCV and RDW-SD showed considerable variation (P<0.05). P3 exhibits the lowest MCV and P2 the lowest RDW-SD.

Table 2: Hematological Profile of Ovariohysterectomized Cats Treated with Electroacupuncture with Standart Hematology of Cats

Treatments	MCH (g/dL)	MCHC (g/dL)	MCV (fL)	RDW-SD (fL)	RDW-CV (%)
P1(Control)	15.72±1.28	29.86±1.04	52.93±3.22a	29.98±3.08a	17.43±1.39
P2	15.32±1.24	32.18±1.82	47.27±4.51ab	25.32±2.62ab	16.98±0.52
P3	14.90±0.67	29.93±3.20	49.03±1.56b	29.95±1.02b	16.38±0.80

Values (Mean+SD) bearing different alphabets in the same column indicate a significant (P<0.05) difference

Results of Electroacupuncture Treatment on the Percentage of Leucocyte Differentiation in Sterile Female Cats

Table 3 presents leukocyte differentiation in sterile cats treated with electroacupuncture. Significant differences (P<0.05) in lymphocyte and monocyte counts were observed among groups. Lymphocyte levels in the P3 group were higher than in P1 and P2, while monocyte counts in P2 and P3 were significantly higher than in the control group (P1). No significant differences were observed in granulocyte counts among the treatment groups. These results indicate that electroacupuncture may modulate immune responses, particularly in monocyte and lymphocyte activity.

Table 3: The number of each type of leukocytes of cats treated with laser acupuncture comparison with standard leucocyte number of cat's

Leucocytes (%)	Treatments		
	P1 (control)	P2	P3
Lymphocytes	3.77±0.75a	3.05±1.01ab	4.12±0.66b
Monocytes	26.30±4.72a	36.07±8.82b	37.15±7.12b
Granulocytes	46.88±8.62	48.13±12.42	44.67±9.28
Standard	0-5%	20-55%	35-75%

Values (Mean+SD) bearing different alphabets in a row indicate a significant (P<0.05) difference

DISCUSSION

Highest ALP levels occurred in P1, followed by P2 and. Elevated ALP signifies heightened osteoblastic activity (), driven physiologically by disruptions in bone metabolism or hepatobiliary function or pathologically by accelerated osteoclast-mediated resorption (Oikonomidis & Milne, 2023). P3 (ST36, BL19, LIV3 electroacupuncture) exhibited the lowest values, suggesting point-specific efficacy in reducing ALP potentially mitigating post-ovariohysterectomy bone turnover disorders (Ma et al., 2025). Control group elevation reflects compensatory osteoblast activation due to estrogen depletion, wherein osteoblasts promote osteoclast maturation via RANKL-RANK signaling a pathway normally suppressed by estrogen-regulated bone remodeling (Takegahara et al., 2022).

Hematological parameters serve as critical indicators of systemic metabolic status, reflecting organ function through the blood-tissue interface (Zelmer et al., 2023). Analysis of erythrocyte indices (MCH, MCHC, MCV, RDW-SD, RDW-CV) provides insight into oxygen transport dynamics, which may indirectly influence or reflect biomarkers like ALP. The absence of significant alterations in MCH, MCHC, and RDW-CV post-electroacupuncture confirms the therapy's hematological safety. However, significant variations in MCV and RDW-SD across treatment groups suggest electroacupuncture modulates erythrocyte morphology and anisocytosis. Notably, reduced MCV in group P3 implies accelerated erythrocyte turnover, potentially attributable to enhanced microcirculatory efficiency and regulated erythropoiesis, warranting further investigation into long-term clinical implications.

Elevated ALP in contexts like hepatobiliary disease (e.g., cholestasis, hepatitis) stems from localized tissue production rather than systemic hematological disruption (Gonzalez & Gordon, 2023). Concomitant normal leukocyte differentials and erythrocyte profiles particularly absent markers of inflammation indicate that such ALP elevations occur independently of immune activation or hematopoietic stress (Oh & Kim, 2022). This dissociation

underscores that ALP dynamics are primarily governed by focal organ pathology (e.g., bone metabolism, hepatobiliary function) rather than hematopoiesis. Consequently, persistent ALP elevation without hematological correlates necessitates targeted diagnostic modalities (e.g., hepatic imaging, radiology) to identify underlying localized etiologies.

Differential leucocyte profiles remained within standard ranges across all groups, confirming the absence of infection or pathology in electroacupuncture-treated sterile female cats (Rahman et al., 2020). Specifically, P3 (ST36/BL19/LIV3) exhibited significant monocyte and lymphocyte elevation indicating enhanced immunomodulation of inflammatory pathways and tissue repair concurrently with the lowest ALP levels (40.3 ± 15.88 U/L). This dual efficacy demonstrates meridian-specific neuromodulation: ST36 optimizes gastrointestinal-immune-metabolic crosstalk, while BL19 and LIV3 synergistically regulate hepatic function and systemic energy flow, establishing targeted point selection as critical for biochemical and immunological homeostasis (Tian et al., 2022).

The ALP reduction in P3 reflects electroacupuncture's disruption of post-ovariohysterectomy bone dysregulation. Estrogen depletion elevates osteoblastic ALP/osteocalcin by impairing osteoprotegerin (OPG) inhibition of RANKL (OPGL), thereby accelerating osteoclastogenesis and bone resorption (Takegahara et al., 2022). Electroacupuncture counters this cascade by modulating neurohumoral-meridian pathways: 1) Restoring estrogen-mediated OPG/RANKL equilibrium to suppress osteoclast maturation (Gao et al., 2021) and 2) Enhancing bone density through systemic endocrine regulation (Ritonga et al., 2021). Molecular validation of this RANKL suppression mechanism remains essential for clinical translation.

Conclusion

The conclusion of this study is that electroacupuncture at the ST36/BL19/LIV3 point emerges as the optimal non-pharmacological intervention for reducing ALP levels and enhancing immunomodulation in cats that have undergone ovariohysterectomy, effectively reducing post-sterilisation bone turnover disorders and metabolic dysregulation without haematological side effects. Future research should prioritise molecular validation of neuroendocrine-mediated suppression of the osteoclastogenic RANKL/OPG pathway, accompanied by longitudinal studies to assess sustained effects on bone mineral density, protocol optimisation based on stimulation parameters, and translational efficacy in clinical cat populations with comorbidities to confirm therapeutic durability and clinical applicability.

DECLARATIONS

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Data Availability: All the data is available in the article.

Ethics Statement: The Animal Care and Use Committee of the Veterinary Medicine Faculty approved the study, Universitas Airlangga (No. 42/KEPH/IX/2019).

Author's Contribution: MZR, SW, and HY designed the study. WPL and TRF developed instruments. SH, MJ, and MM were responsible for data collection. MFA, SR, and MA analyzed data and drafted the manuscript. All authors approved the final version.

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