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Effects of Electroacupuncture on Regulating Cholesterol and Superoxide Dismutase Levels in Cats After Ovariohysterectomy

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ABSTRACT

Electroacupuncture (EA) has gained attention as a non-pharmacological intervention that can influence metabolic and oxidative pathways through autonomic and neuroendocrine regulation. Cholesterol and superoxide dismutase (SOD) are important indicators of lipid metabolism and redox status. The present study aimed to investigate the modulation of cholesterol and SOD activity following EA in cats after ovariohysterectomy, a model of chronic estrogen depletion associated with dysregulated lipid control and increased oxidative susceptibility. Fifteen healthy, neutered adult female domestic cats weighing 2-3 kilograms, aged 1-2 years, were assigned to three groups in a completely randomized design. One group served as the control with no EA treatment (Group 1), the second group received EA at bladder 18 (BL-18), spleen 6 (SP-6), and small intestine 3 points (SI-3; Group 2), and the third group received EA at stomach 36 (ST-36), BL-19, and liver 3 points (LIV-3; Group 3). The EA was administered five times at three-day intervals, with each session lasting 10 minutes. Blood samples were collected before and after treatment and analyzed for cholesterol and SOD using standard biochemical assays and enzyme-linked immunosorbent assay. The present study was conducted over 15 days. Groups 2 and 3 demonstrated significant reductions in cholesterol levels compared to the control group. Group 2 achieved the lowest cholesterol level after EA, which was significantly lower than that of Group 3 and Group 1. Group 3 maintained significantly higher SOD levels than Group 2 and Group 1 before and after treatment, whereas no significant differences were observed between Group 1 and Group 2 at either time point. All groups demonstrated numerical decreases in SOD activity over the study period. The concurrent changes in cholesterol and SOD levels across treatment groups indicated interconnected physiological responses that improved lipid homeostasis and modulated oxidative stress. The present results demonstrated that EA supported coordinated regulation of metabolic and antioxidant pathways in neutered cats through distinct acupoint-specific mechanisms. These findings highlighted the potential of EA as a complementary approach for restoring metabolic-redox balance in animals with long-term endocrine alterations. The integrated changes in cholesterol and SOD underscored the relevance of acupuncture-based interventions for enhancing systemic homeostasis.

Keywords: Cat, Cholesterol, Electroacupuncture, Lipid metabolism, Ovariohysterectomy, Oxidative stress, Superoxide dismutase

INTRODUCTION

Domestic cats (*Felis catus*) have become increasingly popular companion animals as public awareness of animal welfare and the importance of long-term health management has increased (de Souza Machado et al., 2025). The increasing role of cats as indoor companion animals requires more comprehensive attention to their nutrition and diet, metabolic and reproductive health, as these three components directly contribute to the cat's physiological stability (Fascetti et al., 2024). Nutritional imbalances, particularly excessive fat consumption, are known to trigger hypercholesterolemia (Lara et al., 2025). Therefore, preventive practices related to reproductive health, including sterilization, have become an essential part of modern pet care (Root Kustritz, 2012).

Ovariohysterectomy (OHE) is a permanent sterilization procedure strongly recommended for population control and as a preventative method against reproductive disorders such as pyometra and mammary tumors (Kasa and Tesfaye,

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2023). While providing significant health benefits, the absence of ovarian hormones following OHE leads to long-term physiological changes that affect diet, activity, energy metabolism regulation, and lipid homeostasis (Lei et al., 2021). Post-neutering estrogen depletion has been associated with an increased risk of obesity, increased total cholesterol, and changes in lipoprotein composition in cats (Larsen, 2017; Chala et al., 2021; Saavedra et al., 2024). These changes reflect chronic endocrine-metabolic adaptations that are not only temporary but can persist throughout the animal's lifespan (Foreman-Worsley et al., 2025). In addition to impacting lipid metabolism, estrogen deficiency affects the endogenous antioxidant defense system (Xin et al., 2025). Estrogen is known to play an important role in maintaining the activity of antioxidant enzymes, including superoxide dismutase (SOD), which neutralizes superoxide radicals and maintains cellular oxidative stability (Payan-Carreira et al., 2025). Studies in mammals indicated that estrogen deficiency increases the production of reactive oxygen species (ROS), thereby compromising antioxidant defenses and reducing SOD activity (Cagnacci et al., 2023). In neutered cats, the interplay between oxidative stress and impaired lipid metabolism is even more relevant, as excessive ROS can accelerate lipid oxidation, impair hepatocyte function, and exacerbate dyslipidemia (Gözer et al., 2023).

The interaction between oxidative stress-induced antioxidant imbalance and lipid profile changes underscores the importance of simultaneously evaluating SOD activity and cholesterol, as redox status is closely linked to lipid metabolism in metabolic regulation (Grundler et al., 2020). Such an integrated approach is particularly relevant for characterizing metabolic status in cats after OHE (Nazari et al., 2025). However, studies specifically examining cholesterol levels and SOD activity in sterilized cats remain very limited, particularly those involving non-pharmacological interventions. Electroacupuncture (EA) is a modification of conventional acupuncture that uses controlled electrical stimulation at acupuncture points, resulting in more consistent physiological effects (Wu et al., 2025). In different animal models, EA has been reported to influence the autonomic nervous system, regulate neuroendocrine responses, reduce inflammation, and improve tissue perfusion (Harpin et al., 2020; Liu et al., 2025). Several studies have indicated that EA can increase the activity of antioxidant enzymes, including SOD, reduce oxidative stress, and improve lipid metabolism (Zhao et al., 2022; Luo et al., 2024). However, scientific evidence on the effects of EA on SOD activity and cholesterol profiles in cats that have experienced OHE remains very limited. Therefore, the present study aimed to assess the effects of EA on cholesterol levels and SOD activity to support metabolic-redox homeostasis in cats following OHE.

MATERIALS AND METHODS

Ethical approval

The present study has obtained ethical approval from the Animal Ethics Committee for Research, Faculty of Veterinary Medicine, Syiah Kuala University, Aceh, Indonesia, under No. 42/KEPH/IX/2019.

Study design

The present study was a laboratory experimental study using a completely randomized design with three treatment groups. The experimental animals were selected using purposive sampling from the area surrounding the Veterinary Teaching Hospital, in accordance with animal welfare guidelines and institutional ethics. All neutered female domestic cats, weighing 2-3 kg and 1-2 years old, underwent a 7-day acclimatization period at an average ambient temperature of 24-29°C and a humidity of 80-81%. Cats had free access to water and a commercially available dry extruded kibble diet (BOLT Tuna Cat Food Kibble Ikan®, PT Central Proteina Prima Tbk., Indonesia), formulated to meet the nutritional standards established by the Association of American Feed Control Officials (AAFCO, 2023). According to the manufacturer's specifications, the diet contained 28% crude protein, 9% crude fat, and 4% crude fiber, with a calculated metabolizable energy (ME) of 3850 kcal/kg. The animals were housed in clean and well-ventilated cages, and daily monitoring of their health and behavior. The sample size was determined in accordance with ethical approval guidelines and the reduction principle of the 3Rs framework. Additionally, the institutional Animal Ethics Committee reviewed and approved the use of 15 cats in a completely randomized design as sufficient to detect biologically relevant differences in cholesterol and SOD activity while minimizing animal use.

A total of 15 cats were randomly allocated into three groups, with five animals allocated to each group. The control group received no EA treatment (Group 1). The second group received EA at the first anatomical combination points, which included the bladder 18 (BL-18), spleen 6 (SP-6), and small intestine 3 (SI-3; Group 2), while the third group received EA at the second anatomical combination point, which was stomach 36 (ST-36), bladder 19 (BL-19), and liver 3 (LIV-3; Group 3; Figure 1). The selection of acupuncture combination points was guided by previous anatomical and physiological studies that investigated hepatobiliary function, lipid metabolism, and oxidative regulation in the liver, adrenal glands, kidneys, gastrointestinal tract, and related endocrine tissues (Xie and Preast, 2008; Pontes et al., 2015).

The whole treatment protocol lasted 15 days, and treatments were administered in five sessions at three-day intervals, in accordance with previous EA protocols that used repeated treatments on separate days with several-day intervals (Chen et al., 2008). The duration of EA stimulation for each cat was 10 minutes (Wahyuni et al., 2025). All acupuncture procedures were performed in the morning at the Noerjanto Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Syiah Kuala University, Aceh, Indonesia. Anatomically, the acupuncture points were the BL-18 and BL-19, which are located in the thoracic dorsal region lateral to the spinous processes of the ninth and tenth thoracic vertebrae (T9 and T10; vertebrae thoracic IX and X, respectively). The SP-6 was on the medial hind limb, proximal to the medial malleolus, and SI-3 was on the lateral forepaw near the fifth metacarpophalangeal joint. The ST-36 was on the lateral proximal hind limb, distal to the patella, and LIV-3 was on the dorsal hind paw between the second and third metatarsal bones (Xie and Preast, 2008; Figure 2).

Figure 1. Electroacupuncture treatment in adult female domestic cats that had undergone ovariohysterectomy (aged 1-2 years)

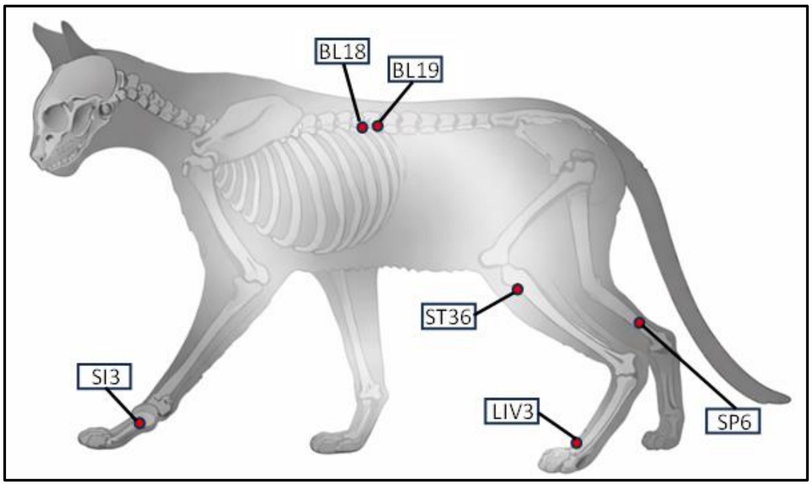

Figure 2. Anatomical locations of acupuncture points used in the electroacupuncture treatment groups. The first combination consisted of BL-18, SP-6, and SI-3, while the second combination included ST-36, BL-19, and LIV-3. The schematic image was generated using AI-assisted software (ChatGPT, OpenAI) and was conceptually developed based on the anatomical descriptions reported by Xie and Preast (2008).

Blood samples were taken from the cephalic vein before and after the EA treatment to evaluate cholesterol concentration (mg/dL) and SOD activity (U/mL) as indicators of metabolic and oxidative status. The enzyme-linked immunosorbent assay (ELISA) method was used to determine SOD levels, using a commercial SOD assay kit, water-soluble tetrazolium (WST), purchased from Dojindo Molecular Technologies, Inc., Japan. Cholesterol levels were determined using a hematology analyzer ABX Micros 60 (France). In general, 20 µL serum samples were added to microplate wells containing WST salt working solution and enzyme reaction mixture, and the reaction was incubated at 37°C for 20 minutes according to the manufacturer’s instructions. Absorbance was measured with a microplate reader (xMark™ microplate absorbance spectrophotometer, Bio-Rad Laboratories Inc., USA), and SOD activity was calculated from the degree of inhibition of WST-formazan formation, as described in the kit protocol and according to the method established by Peskin and Winterbourn (2017).

Statistical analysis

Data analysis was conducted using SPSS software to ensure the accuracy of the data processing. The normality and homogeneity of data distribution were evaluated using the Kolmogorov-Smirnov test to ensure suitability for parametric analysis. Further analysis was conducted to determine differences between treatment groups using one-way ANOVA. The significant differences were evaluated with a Tukey HSD Post Hoc test to identify treatment pairs that indicated statistically significant differences at a p-value less than 5% ($p < 0.05$).

RESULTS AND DISCUSSION

The present results indicated that EA significantly reduced cholesterol levels in both treatment groups compared to the control group ($p < 0.05$; Table 1). In Group 3, cholesterol levels decreased from 206 ± 14.43 to 124.8 ± 10.59 mg/dL, while in Group 2, levels decreased from 190.6 ± 82.49 to 110.4 ± 2.88 mg/dL. The current results demonstrated that after treatment, both Group 2 and Group 3 had significantly lower cholesterol levels than the control group ($p < 0.05$). Furthermore, a significant difference was observed between Group 2 and Group 3 ($p < 0.05$), with Group 2 achieving a lower mean cholesterol level (110.4 ± 2.88 mg/dL) than Group 3 (124.8 ± 10.59 mg/dL). Although Group 3 indicated a numerically greater percentage reduction (46.6%) compared to Group 2 (42.1%), the final cholesterol levels differed significantly between the two treatment groups.

Table 1. Cholesterol levels before and after electroacupuncture in 1–2-year-old neutered female domestic cats over 15 days

Treatments	Cholesterol level before electroacupuncture (mg/dL)	Cholesterol level after electroacupuncture (mg/dL)
Group 1	$217.6^{a} \pm 30.41$	$212.6^{a} \pm 74.06$
Group 2	$190.6^{c} \pm 82.49$	$110.4^{c} \pm 2.88$
Group 3	$206^{b} \pm 14.43$	$124.8^{b} \pm 10.59$

Group 1: Control (No electroacupuncture), Group 2: Electroacupuncture at BL-18, SP-6, and SI-3 points, and Group 3: Electroacupuncture at BL-19, ST-36, and LIV-3 points. [a,b,c] Mean with different superscript letters in the same column indicate a significant difference ($p < 0.05$). Data are presented as Mean ± SD

Decreases in cholesterol levels were observed in both treatment groups. The current results indicated that after treatment, Group 2 and Group 3 had significantly lower cholesterol levels than the control group ($p < 0.05$). Group 3 (BL-19, ST-36, and LIV-3 points) demonstrated a statistically significant reduction in serum cholesterol levels before and after treatment ($p < 0.05$). Additionally, Group 2 (BL-18, SP-6, and SI-3 points) demonstrated a significant reduction compared to the control group, with the final cholesterol level being significantly lower than that of Group 3. These differences in therapeutic response can be explained by the meridian function and the target organ of each point. The LIV-3 and BL-18 have anatomical and functional relationships with the liver, the primary organ involved in cholesterol biosynthesis (Xie and Preast, 2008). Han et al. (2020) reported that acupuncture improved lipid metabolism in rats by regulating intestinal lipid absorption and hepatobiliary function, and increased cholesterol elimination by enhancing bile acid turnover. At the molecular level, liver cholesterol synthesis occurs via the mevalonate pathway, in which 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) is converted to mevalonate by HMG-CoA reductase. This rate-limiting enzyme regulates cholesterol production (Teng, 2025), and modulation of this pathway may explain the significant decrease in serum cholesterol observed in Group 3 after EA stimulation. The current findings are consistent with experimental evidence reported by Jin et al. (2023), who indicated that EA reduced serum cholesterol in rats by influencing hepatic HMG-CoA reductase activity through regulation of HMGCR deubiquitinating, thereby directly impacting the mevalonate pathway and reducing hepatic cholesterol synthesis. This mechanism was consistent with the current findings, particularly in both treatment groups, where a notable decrease in cholesterol levels was observed after stimulation of ST-36, BL-19, and LIV-3 points. In a previous study, the role of ST-36 has been linked to improved gastrointestinal metabolism, immune modulation, and stabilization of the neuroendocrine system, which indirectly supports lipid metabolism (Pontes et al., 2015). Stimulating the ST-36 point might increase the metabolic clearance rate of dietary lipids, providing an additional mechanism for regulating blood cholesterol levels (Landgraaf et al., 2023). Pontes et al. (2015) reported that stimulating ST-36 and ST-25 in obese rats primarily affected triglyceride rather than total cholesterol, which was partially consistent with the present results. This difference between the studies regarding triglyceride modulation and overall cholesterol reduction suggested that metabolic outcomes were strongly influenced by the selection of acupuncture points and their corresponding target organ associations. The stimulation of the LIV-3 point has traditionally been associated with regulating liver function and has been reported to lower total cholesterol and triglyceride levels (Taha et al., 2021). Stimulation of the BL-19 point increased gallbladder contractions and accelerated bile flow, allowing more cholesterol to be excreted into the intestinal lumen (Xie and Preast, 2013). This enhanced biliary excretion of cholesterol, combined with the activity of ST-36, which optimized digestion and absorption, allowed for greater cholesterol reduction. Such physiological adjustments contributed to a more sustained lipid-lowering effect, which was consistent with the marked decrease in total cholesterol in Group 3. Coordinated stimulation of acupuncture points related to metabolism can increase intracellular signals involved in cholesterol regulation, potentially contributing to higher cholesterol reduction (Jin et al., 2023), as observed in Group 3. The simultaneous activation of LIV-3, BL-19, and ST-36 points indicated interactions at different levels of physiological regulation (Nguyen et al., 2023). The greater reduction observed in Group 2, despite utilizing different acupoints, suggests that multiple acupoint combinations can effectively modulate lipid metabolism. The observed response pattern in the present study indicated that EA modulated lipid metabolism through systemic neurohumoral regulation rather than through local point stimulation, which was consistent with the findings of Ding et al. (2023), who confirmed that EA modulated systemic metabolic pathways rather than producing isolated local effects. Additionally, Petrescu et al. (2018) reported that the roles of the hypothalamic-pituitary-adrenal and autonomic pathways in regulating hepatic metabolism aligned with the present findings. The variability in metabolic responses across studies supported the present outcomes that the specific combination of ST-36, BL-19, and LIV-3 points provided a more potent lipid-modulating effect, while the combination of BL-18, SP-6, and SI-3 points yielded an even lower final cholesterol level, highlighting the importance of acupoint selection. These findings indicate the potential of EA as a practical adjunct to help restore lipid balance in cats after OHE with long-term hormone deficiency. In feline clinical settings, EA has been reported to influence anesthetic requirements and cardiorespiratory stability, indicating broader systemic physiological effects that may relate to the metabolic responses observed in the present study (Wahyuni et al., 2025).

The SOD activity pattern of changes demonstrated a distinct trend. Group 2 indicated a decrease in SOD activity from 12.423 ± 6.23 to 11.613 ± 2.63 U/mL, while Group 3 decreased from 17.289 ± 2.74 to 16.768 ± 3.63 U/mL. In contrast, the control group demonstrated a slight reduction from 11.029 ± 2.67 to 10.445 ± 1.19 U/mL. The current results indicated that SOD activity in Group 2 was not significantly different from that of Group 1 ($p > 0.05$); however, both Group 1 and Group 2 exhibited significantly lower SOD activity compared to Group 3, both before and after EA ($p < 0.05$). No significant differences were observed between Group 1 and Group 2 before and after EA treatment ($p > 0.05$; Table 2).

Table 2. Serum superoxide dismutase levels before and after electroacupuncture in 1–2-year-old neutered female domestic cats over 15 days

Treatments	SOD level before electroacupuncture (U/mL)	SOD level after electroacupuncture (U/mL)
Group 1	$11.029^{bc} \pm 2.67$	$10.445^{bc} \pm 1.19$
Group 2	$12.423^{b} \pm 6.23$	$11.613^{b} \pm 2.63$
Group 3	$17.289^{a} \pm 2.74$	$16.768^{a} \pm 3.63$

Group 1: Control (No electroacupuncture), Group 2: Electroacupuncture at BL-18, SP-6, and SI-3 points, Group 3: Electroacupuncture at BL-19, ST-36, and LIV-3 points. SOD: Serum superoxide dismutase. ^{a,b,c} Mean with different superscript letters in the same column indicated a significant difference ($p < 0.05$). Data are presented as Mean ± SD

Increases or decreases in SOD activity are typically associated with oxidative stress, hormonal balance, and ongoing tissue metabolic activity (Rosa et al., 2021). Different combinations of acupuncture points activated different physiological mechanisms, producing antioxidant responses of varying magnitude, indicating location-specific modulation of oxidative stress markers. The observed differences in SOD activity between Group 3 and the other two groups might be associated with variations in tissue blood flow, local bioelectrical conductivity, and nerve activation patterns at each acupuncture point. The finding that SOD activity in Group 3 was significantly higher than in Group 1 and Group 2, both before and after EA treatment, indicated that the mechanism of action of EA was complicated, involving a hierarchical relationship among metabolic factors, the nervous system, and endocrine regulation rather than a single, stand-alone pathway (Ding et al., 2023). The numerical decreases in SOD activity observed after EA across all groups may reflect an increase in redox homeostasis rather than a reduction in antioxidant capacity. Previous studies have indicated that EA modulated autonomic nervous system activity and improved microcirculation, thereby increasing tissue oxygenation (Li et al., 2022). Improved oxygen delivery and stabilization of mitochondrial membrane potential may reduce excessive ROS production, thereby contributing to the numerically lower SOD activity observed after EA. Additionally, Lee et al. (2024) reported that stabilization of mitochondrial membrane potential and reduction in electron leakage in acupuncture-based interventions provided a mechanistic basis for the decrease in superoxide production. This mechanism aligns with current findings, particularly in Group 3, where the highest baseline SOD activity was observed, followed by a numerical reduction after EA. Luo et al. (2024) demonstrated that EA significantly increased SOD activity in a rat model of intracerebral hemorrhage by activating the PPAR-γ/Nrf2 signaling pathway. Meanwhile, Zhao et al. (2022) reported an increase in the antioxidant response following EA in an inflammatory rat model, indicating enhanced endogenous antioxidant defense. These findings were in contrast to the present results, in which numerical decreases in SOD activity were observed across all groups. However, this discrepancy among the results was likely attributable to differences in physiological conditions, particularly the chronically stable post-OHE status, rather than contradictory biological effects. Increased SOD activity in acute injury or inflammatory models demonstrated a compensatory response to elevated ROS. In contrast, the present study involved cats that had undergone OHE and were in a stable hormonal condition, without acute inflammation. In cats after OHE, hormonal status is one factor influencing endogenous antioxidant activity (Pech et al., 2019). Studies in rats that had undergone OHE reported decreased estrogen levels that led to reduced SOD expression, accompanied by increased free radical production in blood vessel walls (Anwar et al., 2025). Although previous studies in rats that had undergone OHE highlighted the role of estrogen in regulating the antioxidant system, the numerical decreases in SOD activity observed in the present study after EA likely reflected an improvement in redox balance rather than oxidative stress caused by estrogen deficiency. Conversely, estradiol administration can increase the expression of antioxidant enzyme isoforms (Elliot et al., 2022). These findings confirmed that estrogen modulated antioxidant enzyme activity through direct effects on gene expression and other physiological processes, such as vasodilation, blood flow regulation, and metabolic control (Jaimes et al., 2019). Cats in the present study were post-permanent OHE (without ovaries); changes in SOD activity were associated with a chronically reduced and hormonally stable estrogen state rather than with acute perioperative effects. These findings were consistent with previous studies of Zhou et al. (2013) and Jaimes et al. (2019), who indicated that decreased ovarian hormone variability reduced damaging effects on antioxidant activity. Sustained low estrogen levels created a stable hormonal environment, allowing the effects of EA to be evaluated without the cyclic changes in estrogen concentration, antioxidant enzyme expression, and lipid metabolism that normally occur during the estrous cycle. Furthermore, chronically low estrogen levels can make tissues more vulnerable to persistent, mild oxidative stress. Therefore, hormonal status is a key element in understanding biomarker dynamics in animals undergoing permanent OHE.

The specific acupuncture points applied in the present study might explain the changes in SOD activity. The SP-6 point was associated with the reproductive and endocrine systems and was known to influence hormonal regulation and energy flow in the Yin meridians (Xie and Preast, 2008). The ST-36 and SP-6 together have been reported to modulate immune function, increase cellular metabolic activity, and influence reproductive endocrine regulation. Clinical studies in cats undergoing OHE indicated that EA at ST-36 and SP-6 points reduced the need for postoperative analgesics and improved physiological recovery, indicating systemic regulatory effects of these acupuncture points (Nascimento et al., 2019). The BL-18, BL-19, and LIV-3 points were all associated with liver and gallbladder function, which are centers of oxidative metabolism and free radical detoxification. In the present study, Group 3 (BL-19, ST-36, and LIV-3) demonstrated significantly higher SOD activity compared to Group 2 (BL-18, SP-6, and SI-3) and the control group, suggesting that this acupoint combination was associated with a distinct antioxidant profile. Combined stimulation of these points produced a systemic effect that can improve metabolic balance and reduce free-radical accumulation. Consistent with the present findings, decreased SOD activity was not a sign of dysfunction, but rather a key factor that indicated the body was in a more stable physiological state (Zhao et al., 2022). Stimulation of liver-related acupuncture points can increase liver antioxidant capacity and bile secretion, thereby contributing to more efficient processing of metabolic byproducts (Yang et al., 2024).

Combined stimulation of LIV-3, BL-19, and ST-36 in the present study was associated with a distinct pattern of metabolic and antioxidant responses, supporting the concept of intermeridian interconnectivity, whereby stimulation at one point influences functionally related organs (Lu et al., 2022; Jin et al., 2023). The concurrent reduction in cholesterol and SOD activity in the present study reflected an improved metabolic-redox balance rather than a disruption of antioxidant defense, which aligned with the findings of Csonka et al. (2016), who demonstrated that hypercholesterolemia increased oxidative stress and the need for antioxidant enzymes. The coordinated adjustments in cholesterol and SOD activity in the present study demonstrated a coordinated systemic adaptation for lipid metabolism and oxidative balance rather than independent biochemical changes (Cho et al., 2022). Similarly, Li et al. (2022) and Luo et al. (2024) reported that EA modulated key regulators of metabolic homeostasis, including neuroendocrine signaling and autonomic pathways, which are important to redox regulation. Additionally, the metabolic response to EA can be influenced by individual biological characteristics such as breed and age. Previous studies have reported that oxidative balance can differ among cat breeds (Bilgiç et al., 2024). The differences in SOD responses observed in the present study following EA might be attributed to different oxidative status, suggesting that individual biological characteristics can influence antioxidant responses. The reduction in cholesterol levels across both EA groups might be associated with the numerical decreases in SOD activity, as hypercholesterolemia is known to promote free radical production and lipid peroxidation (Csonka et al., 2016). This mechanism is consistent with current findings, indicating that a decrease in cholesterol levels was accompanied by a trend toward reduced SOD activity following EA. Furthermore, lipid regulation in cats has been shown to vary under different physiological conditions (Mo et al., 2023). Changes in lipid regulation under different physiological conditions may explain the cholesterol responses observed in the present study following EA, suggesting that physiological conditions can influence lipid-related metabolic responses. This variability in cholesterol and SOD responses observed in the present study is consistent with the findings of Fang et al. (2017), who demonstrated that the physiological effects of EA depend on the selection of acupuncture points, stimulation parameters, and the biological condition of the animals. The present findings indicated that EA applied to both sets of acupuncture points induced measurable changes in serum cholesterol levels and SOD activity, reflecting modulation of hepatobiliary function of lipid metabolism and antioxidant status. These mechanisms played crucial roles in maintaining internal body stability, or homeostasis, especially after animals underwent physiological changes following OHE. Ingerson (2023) indicated that EA can influence physiological recovery after OHE in cats.

Furthermore, the distinct response patterns between the two treatment groups suggested that the appropriate selection of acupuncture points was crucial for the therapeutic effects. Group 2, which included BL-18, SP-6, and SI-3 points, produced the most pronounced cholesterol-lowering effect, while Group 3, combining ST-36, BL-19, and LIV-3 points, was associated with significantly higher SOD activity, indicating a different antioxidant profile. Combinations involving the liver and gallbladder points simultaneously had a greater impact on SOD activity and appeared more effective at maintaining higher antioxidant enzyme levels, whereas the combination that included SP-6 and SI-3 was particularly effective in reducing cholesterol. The observed reductions in cholesterol and SOD activity in the present study were consistent with the basic principle of traditional acupuncture that a broader balance across multiple organ systems leads to improved physiological function (Xie and Preast, 2008). Following the stimulation of liver- and gallbladder-related meridian points, bile secretion increased and led to improved hepatic microcirculation and lipid metabolism, which was associated with the reduction in cholesterol levels observed in the present study. The functional relationship between the liver and gallbladder is crucial to a wide range of metabolic processes; therefore, optimizing both organs simultaneously can enhance therapeutic effects (Ma et al., 2025). These findings are increasingly important given the increasing practice of sterilization in pets, while non-pharmacological intervention options specifically supporting long-term metabolic health remain limited.

CONCLUSION

The present study demonstrated that EA targeting a combination of specific anatomical points could modulate lipid metabolism and redox balance in neutered cats. The EA treatments resulted in significant decreases in cholesterol levels in both treatment groups compared to the control group, with Group 2 (BL-18, SP-6, and SI-3) achieving the lowest post-treatment cholesterol levels. Although SOD activity decreased numerically across all groups during the study period, Group 3 (ST-36, BL-19, and LIV-3) consistently exhibited significantly higher SOD activity than both Group 2 and the control group before and after treatment. The BL-18, SP-6, and SI-3 combination was particularly effective for cholesterol reduction, whereas the ST-36, BL-19, and LIV-3 combination was associated with a higher antioxidant enzyme profile. The changes in cholesterol and SOD reflected an integrated physiological adjustment in which improved hepatobiliary and metabolic function reduces oxidative load, thereby reducing the need for endogenous antioxidant enzyme activity. The current findings confirmed that EA did not act through a single pathway but rather through a multi-organ interaction involving the liver, gallbladder, gastrointestinal tract, and tissues involved in endocrine regulation. Overall, EA served as a complementary therapeutic pathway to restore redox metabolic homeostasis in animals experiencing long-term hormonal changes following OHE. Future studies can assess the response patterns across different electrical stimulation doses and frequencies, as well as long-term biomarker monitoring. Additionally, larger sample sizes and assessment of additional oxidative metabolic parameters are needed to strengthen the mechanistic understanding and clinical application of EA in companion animals.

DECLARATIONS

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Authors' contributions

Mudhita Zikkrullah Ritonga supervised field sampling, data collection, laboratory investigation, and data entry. Wahyu Eka Sari and Muhammad Hanafiah conceived, designed, and coordinated the study. Triva Murtina Lubis, Lailia Dwi Kusuma Warndami, Wisnu Rahmadan Almanda and Lian Varis Riandi conducted field sampling and data collection. Gholib Gholib, Alfiana Laili Dwi Agustin, and Mustafa Sabri performed statistical analysis and interpretation of results. All authors have read and approved the final edition of the manuscript.

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Competing interests

The authors declared no conflict of interest.

Ethical considerations

All authors hereby confirm that the manuscript has been thoroughly reviewed for ethical compliance, including verification of originality, approval for publication, integrity of the research process, accuracy of the data, and assurance that no duplicate or redundant submissions have been made. The authors further declared that AI tools (ChatGPT and Grammarly) were used solely for language refinement and grammar editing, as well as for assistance in drafting the graphical abstract. Additionally, ChatGPT (OpenAI) was used to illustrate Figure 2 in the present study. All authors checked and revised the entire article after refinement, and take full responsibility for using AI tools for language and graphical abstract preparation. The authors confirm that No AI tools were used in preparing this study. The authors take full responsibility for the scientific integrity and content of the manuscript.

Availability of data and materials

The data set generated and analyzed during this study is available upon reasonable request from the corresponding author.

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