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Effects of Choline-Rich Fermented *Crescentia cujete* on Blood Parameters, Superoxide Dismutase, and Cerebral Interleukin-6 in Rats after Induced Ischemic Stroke

Puput Fiohana[1]*, Yos Adi Prakoso[2], Sitarina Widyarini[3], Jasir Hakim Hidayah[2,4], Puput Ade Wahyuningtyas[2], and Achmadi Susilo[5]

[1]*RSI Siti Hajar Sidoarjo, Sidoarjo, East Java, 61234, Indonesia*
[2]*Department of Pharmacology, University of Wijaya Kusuma Surabaya, Surabaya, East Java, 60225, Indonesia*
[3]*Department of Pathology, University of Gadjah Mada, Yogyakarta, East Java, 55281, Indonesia*
[4]*D'Luna Vet Care, Jakarta Pusat, DKI Jakarta, 10460, Indonesia*
[5]*Agrotechnology Study Program, Faculty of Agriculture, University of Wijaya Kusuma Surabaya, Surabaya, 60225, Indonesia*

*Corresponding author's Email: fiohanap@gmail.com

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ABSTRACT

Ischaemic stroke is a major vascular disorder that profoundly impacts human health. Choline-rich fermented *Crescentia cujete* (Ch-RFCC) has emerged as a promising adjunctive therapy for ischemic stroke; however, its effects on hematological parameters, superoxide dismutase (SOD) activity, and interleukin-6 (IL-6) levels remain unexplored. The present study aimed to assess the impacts of Ch-RFCC on hematological parameters, SOD activity, and brain IL-6 levels in the rats' ischemic stroke model (ISM). A total of 40 three-month-old male rats, weighing 247.31 ± 4.95 g, were randomly assigned into four groups, including healthy rats with a skin incision as the control group (sham-operated, T1), ISM without treatment (T2), ISM treated with 496 mg/kg body weight of piracetam (T3), and ISM treated with 11.84 mg/kg body weight of Ch-RFCC (T4). Treatments using piracetam (T3) and Ch-RFCC (T4) were administered orally via gavage twice daily for 14 consecutive days. The current results demonstrated that Group T4 maintained haemoglobin and haematocrit levels, normalised the platelet-to-leucocyte ratio and neutrophil counts, reduced fibrinogen levels, elevated SOD activity, and enhanced IL-6 immunoreactivity compared to the untreated ISM group (T2). Furthermore, rats in Group T4 exhibited the least body weight loss compared to those in groups T2 and T3. These findings indicated that Ch-RFCC may alleviate ischemic stroke in rats by enhancing antioxidant defenses, modulating IL-6 expression, and preserving hematological homeostasis.

Keywords: Choline-rich fermented *Crescentia cujete*, Hematology, Interleukin-6, Ischemic stroke, Superoxide dismutase

INTRODUCTION

Ischemic stroke is a major vascular disorder that significantly impacts human health, greatly impairs the quality of life, and exerts a long-term financial burden on individuals and healthcare systems (Dhanasekara et al., 2024). The occlusion of cerebral arteries deprives brain tissue of oxygen and essential nutrients, resulting in infarction and neurological deficiencies such as paralysis (Lowry and Jin, 2020). The infarct area expands as cerebral blood flow decreases and neuronal cell death progresses (Zhao et al., 2022). Neuronal mortality is caused by deprivation of oxygen and nutrients, a condition that has the potential to upregulate the intracellular expression of interleukin-6 (IL-6, Ciryam et al., 2023). Feng et al. (2015) have indicated that IL-6 is essential for maintaining neuronal integrity. Additionally, IL-6 acts as a neurotrophic-like factor, promoting neuroplasticity, development, and regeneration during the progression of neuronal diseases (Erta et al., 2012). Elevated IL-6 levels in the brain may further support the development of dopaminergic neurons and regulate voltage-gated ion channels (Grebenciucova and VanHaerents, 2023).

During ischemic stroke pathogenesis, cerebral injury triggers systemic changes in blood composition. Circulating inflammatory cells increase; key biomarkers of this response include the platelet-to-leukocyte ratio (PLR; Amalia and Dalimonthe, 2020) and the neutrophil-to-lymphocyte ratio (NLR; Graça et al., 2024). Furthermore, the acute-phase response may also involve an increase in total plasma protein (TPP), reflecting a broader systemic inflammatory shift. Concurrently, erythrocyte count, hemoglobin (HGB) concentration, and haematocrit (HCT) often decline when nutritional intake is compromised by dysphagia (Brito et al., 2020. Furthermore, neuronal damage results in a significant reduction in plasma antioxidant defenses, particularly in superoxide dismutase (SOD) activity (Zhang et al., 2022). In humans, these hematological and biochemical changes are linked to the severity and prognosis of ischemic stroke,

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highlighting the importance of comprehensive blood profiling during both the development and treatment stages (Liang et al., 2025).

Choline-rich fermented *Crescentia cujete* (Ch-RFCC) has emerged as a promising adjunctive therapy for ischemic stroke in animal models (Hidayah et al., 2023). This liquid fermentation product of *Crescentia cujete* is enriched in choline and has demonstrated immunostimulatory (Wijayanti et al., 2024), complementary effects on pneumonia (Prakoso et al., 2024a), and neuroprotective effects in preclinical models of stroke (Hidayah et al., 2023). A previous study reported that Ch-RFCC elevated brain levels of granulocyte-macrophage colony-stimulating factor (GM-CSF) and vascular endothelial growth factor (VEGF), thereby supporting cerebral angiogenesis and oxygenation (Prakoso et al., 2024b). However, its downstream effects on brain IL-6 expression and systemic hematological parameters have not yet been evaluated. The present study aimed to assess the impact of Ch-RFCC on hematological parameters, SOD activity, and brain IL-6 levels in the rats' ischemic stroke model (ISM).

MATERIALS AND METHODS

Ethical statement

The Animal Research Ethics Committee of the Faculty of Veterinary Medicine, University of Wijaya Kusuma Surabaya, Indonesia, approved the present study (Registration No. KKE-243/I/2025). The committee registered and observed all procedures, and the study was conducted in the Pharmacology Laboratory at the Faculty of Veterinary Medicine, University of Wijaya Kusuma Surabaya, from January to July 2025.

Preparation of fermented *Crescentia cujete*

The fruit of *Crescentia cujete* was collected from the garden of the University of Wijaya Kusuma Surabaya, Indonesia. A total of 400 g of fruit pulp was weighed and combined with 1000 mL of distilled water. The mixture was supplemented with 40 mL of pectinase (Pectinex Ultra AFP, United Kingdom) and 40 g of sugar, then fermented at 25°C for 30 days with stirring for 15 minutes once daily. The 1000 mL final fermentation product was referred to as Ch-RFCC. This procedure was adapted from the study conducted by Wilujeng et al. (2023). According to Prakoso et al. (2024a), each milliliter of the final product contained 114.12 mg of choline.

Animals

The present study comprised 40 male Sprague-Dawley rats, three months old, weighing 247.31 ± 4.95 g, obtained from the Laboratory of Pharmacology at the Faculty of Veterinary Medicine, University of Wijaya Kusuma Surabaya, Indonesia. Before the experiment, the animals were acclimated for seven days in cages (90 × 50 × 60 cm) maintained at 25°C. The cages were bedded with oven husk, and a commercial food (RatBio®, Indonesia) and water (Cleo®, Indonesia) were provided *ad libitum*. Relative humidity was kept at 60% using a Leka DH6628 dehumidifier (Indonesia). After the adaptation period, the rats were randomly divided into four groups. Each group consisted of 10 rats, including healthy rats with a skin incision without common carotid artery (CCA) ligation as the control group (sham-operated, T1), untreated ISM (T2), the ISM rats that received 496 mg/kg body weight of piracetam (T3; Bernofarm, Indonesia, Paliwal et al., 2018), and ISM rats that received 11.84 mg/kg body weight of Ch-RFCC (T4; Hidayah et al., 2023). Treatments using piracetam (T3) and Ch-RFCC (T4) were administered orally twice daily via gavage at 6:00 a.m. and 6:00 p.m. for 14 consecutive days.

Induction of ischemic stroke in rats

The rats in groups T1, T2, T3, and T4 were anesthetized with a combination of xylazine (Xylazil, Ilium, Australia) and Zoletil® (Virbac, India). Xylazine was administered intramuscularly at 4 mg/kg body weight (Bhatia et al., 2022). Fifteen minutes later, each rat received Zoletil® at 25 mg/kg body weight via intramuscular injection (Kosenko et al., 2020). Rats in Group T1 had their anterior necks shaved with surgical clippers. The skin was disinfected with isopropyl alcohol (Onemed, Indonesia), and a 2 cm midline incision was made. A skin incision was made in all groups. In Group T1, the incision was closed with simple interrupted sutures using 2-0 silk. In Groups T2, T3, and T4, the same incision was followed by ligation of the right common carotid artery (CCA) and its external and internal branches using the same suture material before closure. The ligations were removed to permit reperfusion after four hours of occlusion, following the method described by Prakoso et al. (2023).

Body weight and sample collection

The body weight of each rat was recorded on the day before induction (day 0), and the body weight was measured again on day 14 post-treatment. Percentage weight gain was calculated using the formula conducted by Beale et al. (2011).

W (%) = (WDx – WD0) / WD0 × 100 (Formula 1)

W (%) is the growth percentage, WDx is body weight on day x (g), and WD0 is body weight on day 0 (g).

After 14 days of treatment, rats were anesthetized with 25 mg/kg body weight Zoletil®. Blood was collected via the retro-orbital plexus using microhematocrit tubes. One aliquot was transferred into EDTA tubes (Onemed, Indonesia) for hematology analysis, and the residue into plain tubes for SOD analysis. Whole blood, plasma, and serum were stored at 4°C. Following blood collection, rats were euthanized by cervical dislocation. Brains were harvested and fixed in neutral-buffered formalin 10% (Leica, USA) for 24 hours.

Hematology test

Blood samples were analyzed against total red blood cells (RBC, 10^6 cells/mm^3), HGB (g/dL), HCT (%), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, Pg), mean corpuscular hemoglobin concentration (MCHC, %), PLT, (10^5 cells/mm^3), PWR, white blood cells (WBC, 10^3 cells/mm^3), lymphocytes (LYM, 10^3 cells/mm^3), monocytes (MONO, 10^3 cells/mm^3), neutrophils (NEU, 10^3 cells/mm^3), eosinophils (EOS, 10^3 cells/mm^3), basophils (BAS, 10^3 cells/mm^3), and neutrophil per lymphocyte ratio (NLR) using a hematology analyzer (VetScan HM5, Zoetis, USA). Plasma was then assayed for TPP (Hunsaker et al., 2016) and fibrinogen (Schlimp et al., 2015). Superoxide dismutase activity was measured following the protocol described by Afrazeh et al. (2015).

Immunohistochemistry

The brains of rats from all groups, with ten brains per group, were subjected to routine histopathological examination as outlined by Ma et al. (2024). For the routine histopathology processes, the organ was dehydrated and cleared using graded alcohol (70%, 80%, 90%) and graded xylene (1 to 3). The brains were then embedded using liquid paraffin and blocked. The brain blocks were then sectioned using a microtome (Leica RM2125 RTS, USA). After sectioning, the slides were immunostained for the detection of IL-6 using an anti-IL-6 antibody (Santa Cruz, USA) at a 1:100 dilution. The immunohistochemistry protocol followed the manufacturer's instructions provided with the secondary antibody kit (Novocastra, Leica Biosystem, USA). Interleukin-6 immunoreactivity was visualized using diaminobenzidine (DAB; Leica, USA) at a 1:50 dilution. The IL-6 was then analyzed using ImageJ software (NIH Image, United States) and quantified as the percentage of positive staining area and the number of IL-6-positive cells.

Statistical analysis

The current data were analyzed using SPSS Statistics version 26 (IBM, United States). Body weight, hematology, TPP, fibrinogen, and SOD measurements were calculated by one-way ANOVA followed by Duncan's multiple range test. Immunohistochemistry data were analyzed using the Kruskal-Wallis test and the Mann-Whitney U test. Statistical significance was defined at the 95% significance level ($p \leq 0.05$).

RESULTS AND DISCUSSION

On the first day of induction, no significant differences in body weight were observed among the rats ($p \geq 0.05$), indicating that all groups had a similar weight, which suggested that the body weights of the rats were homogeneous on this day (Table 1). However, after 14 days of treatment using 11.89 mg/kg body weight of Ch-RFCC, significant divergences emerged between groups ($p \leq 0.05$). The Group T2 exhibited the lowest body weight (229.00 ± 10.73 g) compared to groups T1 (250.50 ± 5.32 g) and T4 (247.25 ± 3.40 g; $p \leq 0.05$), although it did not differ significantly from Group T3 (235.25 ± 6.84 g; $p \geq 0.05$). Group T4 was more effective at maintaining weight (247.25 ± 3.40 g) than groups T2 and T3 ($p \leq 0.05$), and its weights remained comparable to those of Group T1 (250.50 ± 5.32 g; $p \geq 0.05$). Furthermore, Group T1 experienced the most significant weight gain (over 1.83%) among all groups ($p \leq 0.05$), while Group T4 had the lowest percentage of weight loss compared to Groups T2 and T3 ($p \leq 0.05$; Table 1).

Table 1. Average body weight of ischemic stroke rats before and after 14 days of treatment

Parameter	Unit	T1	T2	T3	T4
Body weight before treatment	Gram	246.00 ± 5.35^a	247.00 ± 6.68^a	245.75 ± 5.73^a	250.50 ± 2.38^a
Body weight after treatment	Gram	250.50 ± 5.32^a	229.00 ± 10.73^b	235.25 ± 6.84^b	247.25 ± 3.40^a
Percentage of body weight loss	%	(+) 1.83 ± 0.87^a	(-) 7.31 ± 2.59^b	(-) 4.42 ± 3.15^c	(-) 1.29 ± 0.60^d

T1: Control group, T2: ISM without treatment, T3: ISM treated with 496 mg/kg body weight of piracetam, T4: ISM treated with 11.84 mg/kg body weight of Ch-RFCC, (+): Increase of body weight, (-): Decrease of body weight. $^{a,b,c, \text{ and } d}$ Different superscript letters indicate significant differences ($p \leq 0.05$).

After 14 days of treatment, there were no significant differences among groups T1, T2, T3, and T4 in the erythrocyte profile, MCV, MCHC, MONO, and EOS counts ($p \geq 0.05$). Group T2, however, indicated significant increases in HGB (12.59 ± 1.13 g/dL), PLT counts (1347.00 ± 97.59 × 10^5 cells/mm^3), PWR (134.63 ± 5.11), total leukocytes (10.20 ± 0.48 × 10^3 cells/mm^3), LYM (6.49 ± 0.50), and NLR (0.45 ± 0.09) compared to the other groups ($p \leq 0.05$). Group T2 exhibited a significant decrease in HCT (33.17 ± 3.10 %) and MCH (18.86 ± 2.06 Pg) compared to the other groups ($p \leq 0.05$; Table 2).

Furthermore, groups T3 and T4 restored several hematological parameters (RBC, HGB, HCT, MCV, MCH, MCHC, PWR, MONO, NEU, EOS, and NLR) to levels similar to those in Group T1 ($p \geq 0.05$), except for total PLT and LYM, which continued to exhibit significant differences ($p \leq 0.05$). After 14 days of treatment, groups T3 and T4 still exhibited significantly higher PLT (939.00 ± 13.54 and 864.50 ± 73.67 × 10^5 cells/mm^3, respectively) and LYM counts (5.46 ± 0.90 and 5.55 ± 0.71 × 10^3 cells/mm^3, respectively) than Group T1 (PLT: 647.75 ± 46.59 and LYM: 4.31 ± 0.39; $p \leq 0.05$), although their levels remained lower than those in Group T2 (PLT: 1374.00 ± 13.54 × 10^3 cells/mm^3 and LYM: 6.49 ± 0.50 × 10^3 cells/mm^3; $p \leq 0.05$). There were no significant differences between groups T3 and T4 for haematological parameters ($p \geq 0.05$; Table 2).

In Group T2, the TPP (8.40 ± 0.40 g/dL) and fibrinogen (440.25 ± 39.22 mg/dL) levels increased significantly compared to T1 (TPP: 6.77 ± 0.30 and fibrinogen: 225.00 ± 15.81 mg/dL; $p \leq 0.05$). In groups T3 and T4, both TPP (7.22 ± 0.33 and 7.55 ± 0.55 g/dL, respectively) and fibrinogen (323.25 ± 9.91 and 273.00 ± 23.07 mg/dL, respectively) decreased significantly compared to Group T2 ($p \leq 0.05$), yet remained higher than in Group T1 ($p \leq 0.05$). In contrast, the SOD activity dropped markedly in groups T2 (47.46 ± 6.68 U/mL) and T3 (49.08 ± 5.16 U/mL), whereas in Group T4 (60.21 ± 3.50 U/mL), SOD levels were not different from those of Group T1 (63.99 ± 6.68 U/mL; $p \geq 0.05$; Table 3). The percentage level of IL-6 immunoexpression did not differ significantly among groups T1 (17.49 ± 5.06 %), T2 (13.49 ± 2.43 %), and T3 (16.74 ± 2.32 %, $p \geq 0.05$), while Group T4 (22.96 ± 2.39 %) exhibited significantly higher expression than the other groups ($p \leq 0.05$). The quantity of IL-6-immunoreactive cells exhibited a pattern consistent with the percentage area measurements (Table 4). A representative qualitative evaluation of IL-6 is illustrated in Figure 1.

Table 2. Hematology profile of ischemic stroke rats after 14 days of treatment

Parameter	Unit	T1	T2	T3	T4
RBC	10^6 cells/mm^3	6.70 ± 0.38[a]	6.69 ± 0.27[a]	6.52 ± 0.25[a]	6.68 ± 0.41[a]
HGB	g/dL	14.31 ± 0.37[a]	12.59 ± 1.13[b]	14.13 ± 0.58[a]	14.20 ± 0.36[a]
HCT	%	40.07 ± 0.72[a]	33.17 ± 3.10[b]	39.07 ± 2.28[a]	39.57 ± 3.40[a]
MCV	fL	28.01 ± 1.24[a]	26.48 ± 3.27[a]	27.64 ± 0.66[a]	27.86 ± 2.14[a]
MCH	Pg	21.40 ± 1.52[a]	18.86 ± 2.06[b]	21.70 ± 1.57[a]	21.31 ± 1.90[a]
MCHC	%	35.74 ± 1.54[a]	38.18 ± 4.68[a]	36.19 ± 0.84[a]	36.04 ± 2.62[a]
PLT	10^5 cells/mm^3	647.75 ± 46.59[a]	1374.00 ± 97.59[b]	939.00 ± 13.54[c]	864.50 ± 73.67[c]
PWR	-	101.14 ± 9.87[a]	134.63 ± 5.11[b]	115.89 ± 13.30[a]	103.50 ± 8.78[a]
WBC	10^3 cells/mm^3	6.42 ± 0.41[a]	10.20 ± 0.48[b]	8.17 ± 0.84[c]	8.39 ± 0.95[c]
LYM	10^3 cells/mm^3	4.31 ± 0.39[a]	6.49 ± 0.50[b]	5.46 ± 0.90[b]	5.55 ± 0.71[b]
MONO	10^3 cells/mm^3	0.51 ± 0.24[a]	0.53 ± 0.38[a]	0.54 ± 0.30[a]	0.55 ± 0.39[a]
NEU	10^3 cells/mm^3	1.46 ± 0.20[a]	2.91 ± 0.46[b]	2.07 ± 0.51[a]	2.15 ± 0.57[a]
EOS	10^3 cells/mm^3	0.12 ± 0.04[a]	0.25 ± 0.05[b]	0.09 ± 0.07[a]	0.12 ± 0.04[a]
BAS	10^3 cells/mm^3	ND	ND	ND	ND
NLR	-	0.33 ± 0.04[a]	0.45 ± 0.09[a]	0.38 ± 0.12[a]	0.39 ± 0.11[a]

T1: Control group, T2: ISM without treatment, T3: ISM treated with 496 mg/kg body weight of piracetam, T4: ISM treated with 11.84 mg/kg body weight of Ch-RFCC, RBC: Total erythrocytes, HGB: Hemoglobin, HCT: Haematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PLT: Platelets, PWR: Platelet-to-leucocytes ratio, WBC: Leucocytes total, LYM: Lymphocytes, MONO: Monocytes, NEU: Neutrophils, EOS: Eosinophils, BAS: Basophils, NLR: Neutrophil per lymphocyte ratio, ND: Not detected. [a,b, and c] Different superscript letters indicate significant differences ($p \leq 0.05$).

Table 3. Level of total protein, fibrinogen, and superoxide dismutase in ischemic stroke rats after 14 days of treatment

Parameter	Unit	T1	T2	T3	T4
TPP	g/dL	6.77 ± 0.30[a]	8.40 ± 0.40[b]	7.22 ± 0.33[c]	7.55 ± 0.55[c]
FIB	mg/dL	225.00 ± 15.81[a]	440.25 ± 39.22[b]	323.25 ± 9.91[c]	273.00 ± 23.07[d]
SOD	U/mL	63.99 ± 4.31[a]	47.46 ± 6.68[b]	49.08 ± 5.16[b]	60.21 ± 3.50[a]

T1: Control group, T2: ISM without treatment, T3: ISM treated with 496 mg/kg body weight of piracetam, T4: ISM treated with 11.84 mg/kg body weight of Ch-RFCC, TPP: Total plasma protein, FIB: Fibrinogen, SOD: Superoxide dismutase. [a,b,c, and d] Different superscript letters indicate significant differences ($p \leq 0.05$).

Table 4. Immunohistochemistry of the brain's IL-6 in ischemic stroke rat models after 14 days of treatment

Parameter	Unit	T1	T2	T3	T4
Percentage area	%	17.49 ± 5.06[a]	13.49 ± 2.43[b]	16.74 ± 2.32[c]	22.96 ± 2.39[d]
Average number of immunoreactive cells	Cells	155.25 ± 30.34[a]	139.25 ± 27.41[b]	149.00 ± 19.54[c]	184.00 ± 20.14[d]

T1: Sham-operated, T2: ISM without treatment, T3: ISM treated with 496 mg/kg body weight of piracetam group, T4: ISM treated with 11.84 mg/kg body weight of Ch-RFCC group. [a,b,c, and d] Different superscript letters indicate significant differences ($p \leq 0.05$).

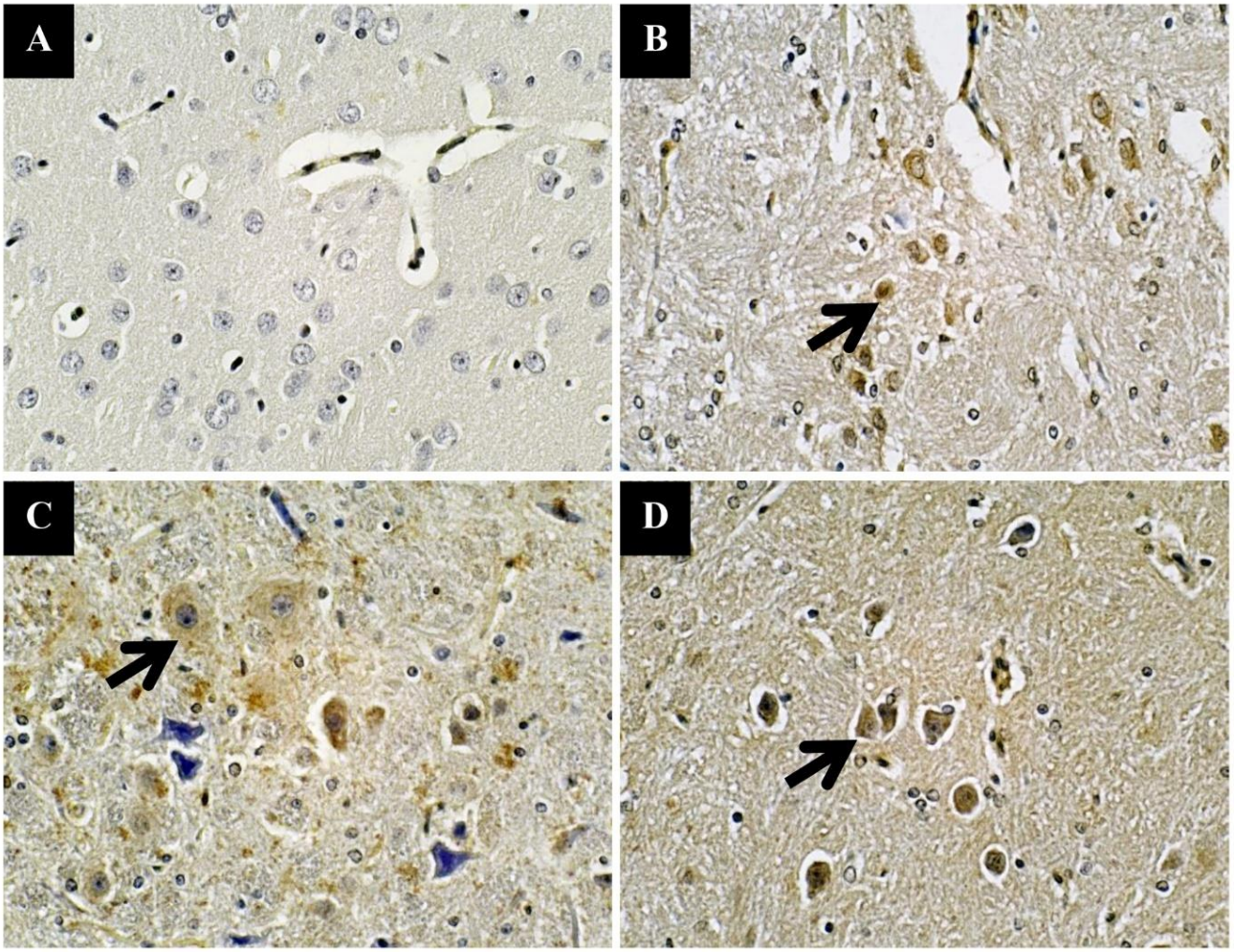

Figure 1. Immunohistochemical detection of IL-6 in brain sections from ischemic stroke rats after 14 days of treatment. **A**: The control group showing no IL-6 signal (400×), **B**: Group T2 treatment, IL-6 immunoreactivity evident (arrow; 100×), **C**: Group T3 treatment, IL-6 immunoreactivity evident (arrow; 400×), **D**: Group T4 treatment, IL-6 immunoreactivity evident (Arrow; 400×). Sections were incubated with anti-IL-6 antibody (1:100) and visualized using diaminobenzidine.

DISCUSSION

The present study demonstrated that cerebral infarction disrupted systemic circulation, evidenced by reductions in MCH and HCT levels, alongside increases in PLT count, PWR, total leukocytes, LYM, and NEU. The drop in MCH and HCT during ischemic stroke was associated with poor clinical outcomes (Kellert et al., 2012), mainly due to reduced delivery of nutrients, oxygen, and essential amino acids to the ischemic penumbra (Abuhulayqah et al., 2025).

Furthermore, damage to brain tissue increases the leukocytes and NEU levels as components of the inflammatory response (Jickling et al., 2015). Although NEU infiltration is essential for the removal of damaged tissue (Chen et al., 2021), it also increases local oxidative stress, consequently exacerbating neuronal injury (Mu et al., 2023). Oxidative stress within the ischemic region further promotes the release of prothrombotic factors that activate PLT (Amalia and Dalimonthe, 2020) and elevate the PWR (Prakoso et al., 2023). Concurrent inflammation increases TPP (Kalani et al., 2023) and fibrinogen levels (Prasad et al., 2023). Consequently, monitoring these hematological parameters provided a reliable way to evaluate the efficacy of interventions for ischemic stroke, as reported by Khandait and Barai (2019), who found that ischemic stroke leads to increased fibrinogen levels, which aligns with the present results. Similarly, Yang et

al. (2019) observed that elevated PLT counts are associated with poorer clinical outcomes in patients with ischemic stroke, which aligns with the present findings.

Piracetam is commonly used to treat ischemic stroke. As a nootropic agent, it facilitates the preservation of neuronal function and integrity (Chen et al., 2019) and protects the brain against further ischemic damage. In the current study, piracetam restored several blood parameters disrupted by stroke, including the normalization of HGB, HCT, PWR, and NEU counts, while reducing PLT and leukocyte counts as well as TPP and fibrinogen levels, compared to the untreated ISM group. However, these measures remained higher than in the control group and the Ch-RFCC treatment. Early administration of piracetam has been shown to improve blood flow to the infarcted area in the brain tissue (Winblad, 2005); however, Ricci et al. (2012) found no clinical benefit in over 1,000 patients. Ricci et al. (2012) concluded that piracetam should not be recommended as routine therapy for ischemic stroke. These conflicting findings highlighted the complexity of piracetam's efficacy and underscored the need for further evaluation of its study. Hence, an alternative therapy should be found, such as using Ch-RFCC. The choline inside the Ch-RFCC is a compound that contains phospholipid to promote brain recovery and neuroprotection. A study by Hidayah et al. (2023) indicated that Ch-RFCC promoted the clinical outcome of ischemic stroke via the histopathology and neurodeficits score analysis. Prakoso et al. (2024b) stated that Ch-RFCC improves brain oxygenation by activating VEGF and enhances the resolution of brain inflammation through microglia activation, thereby activating GM-CSF. The role of choline from Ch-RFCC has beneficial direct effects on neuronal cell survival through its capacity to act as a precursor for phosphatidylcholine (Zhong et al., 2021). The benefits of Ch-RFCC in the current study were demonstrated by the normalization of HGB, haematocrit, PWR, and NEU compared to the untreated ISM group, and indicated a reduction in PLT, leucocytes, and TPP, similar to the piracetam group. Additionally, the Ch-RFCC demonstrated a promising outcome in decreasing fibrinogen. As a previous study stated that fibrinogen is a key component of the inflammatory biomarker (Prasad et al., 2023).

Apart from hematological metrics, assessing antioxidant activity, particularly SOD, is essential for evaluating the success of ischemic stroke therapy (Anwar et al., 2025). An increase in SOD activity reflects enhanced detoxification of oxidative stress in the brain (Chidambaram et al., 2024) and supports functional neuronal recovery (Briones-Valdivieso et al., 2024). In the current study, the Ch-RFCC group exhibited a similar level of SOD activity comparable to that of the control group, indicating a promotion of brain tissue repair. The present findings align with those of Pawluk et al. (2024), who reported that reduced SOD activity is associated with worse neurological outcomes, larger infarct volumes, and a higher risk of poststroke cognitive impairment. Moreover, the meta-analysis conducted by Golenia and Olejnik (2025) concluded that heightened oxidative stress in cases of ischemic stroke, coupled with the depletion of catalase and SOD, undermines the integrity of the blood-brain barrier and results in neuronal damage and death. Adequate SOD activity is neuroprotective, protecting neurons from free radicals that cause cell death, inflammation, and blood-brain barrier breakdown (Haorah et al., 2011). Superoxide dismutase catalyzes the dismutation of superoxide radicals into hydrogen peroxide, which is then neutralized by other antioxidants such as glutathione peroxidase and catalase (Chidambaram et al., 2024). By this mechanism, SOD prevents the oxidative cascade that damages brain tissue. The brain's high metabolic rate, combined with limited antioxidant defenses, makes it especially susceptible to oxidative injury during ischemia (Zhang et al., 2022). Therefore, increasing SOD activity is important for supporting brain tissue remodeling after ischemic stroke.

The administration of Ch-RFCC resulted in a more effective increase in SOD levels compared to piracetam, and this increase suggests superior neuroprotective effects. This finding aligns with those of Jin et al. (2015), who described choline treatment as improving neurological outcome and reducing damage in the brain. The IL-6 expression induces a neuroprotective increase in SOD (Jung et al., 2011). Although IL-6 is generally considered an inflammatory marker, its elevation during ischemic stroke is associated with improved clinical outcomes (Tirandi et al., 2023). A study conducted by Feng et al. (2015) demonstrated that IL-6 mitigates inflammatory cell infiltration, prevents blood-brain barrier disruption by reducing Evans blue leakage, and downregulates matrix metalloproteinase-9 (MMP9) levels in ischemic brain tissue. The reduction of inflammatory cells, prevention of blood-brain barrier disruption, and decrease in MMP9 levels contribute to an improved prognosis following a stroke. These findings aligned with the current results, which indicated that the Ch-RFCC group exhibited higher increases in brain IL-6 and SOD levels compared to the piracetam-treated group.

Ultimately, improved prognosis after ischemic stroke in rat models can be evaluated by monitoring changes in body weight. Rats treated with piracetam or Ch-RFCC lost less weight compared to untreated controls. Notably, the Ch-RFCC group exhibited the least weight loss, even surpassing the weight loss of the piracetam group. The upward trend in body weight suggested that Ch-RFCC promoted neuronal recovery, which helped maintain feeding and drinking behaviors. Ciobanu et al. (2017) demonstrated that reduced weight loss is associated with promising outcomes in rat models of ischemic stroke.

CONCLUSION

The present study established that Ch-RFCC delivered superior recovery in rat models of ischemic stroke compared to piracetam. The advantages of Ch-RFCC were evidenced by its ability to sustain hemoglobin and hematocrit levels, normalize PWR and NEU counts, decrease fibrinogen levels, increase SOD activity, and improve IL-6 levels immunoreactivity. It is suggested that comprehensive toxicity and safety assessments should be conducted before considering Ch-RFCC for clinical evaluation in humans.

DECLARATIONS

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Authors' contributions

Puput Fiohana, Yos Adi Prakoso, Jasir Hakim Hidayah, Sitarina Widyarini, Puput Ade Wahyuningtyas, and Achmadi Susilo performed the experiments. Yos Adi Prakoso supervised the study. Puput Fiohana formulated the Ch-RFCC compound. Yos Adi Prakoso, Jasir Hakim Hidayah, and Sitarina Widyarini collected specimens and carried out immunohistochemistry. Puput Fiohana and Puput Ade Wahyuningtyas conducted hematology and antioxidant analyses. Achmadi Susilo performed the statistical analysis. All authors contributed to the drafting, revising, and approval of the final manuscript.

Ethical considerations

This manuscript represents an original manuscript by the authors. Ethical issues, including plagiarism, consent to publish, misconduct, duplicate publication, data fabrication, and redundancy, have been reviewed and addressed. The authors did not use any AI application for preparing data, writing and revising the draft of this study.

Availability of data and materials

Data supporting the findings of the present study are available from the corresponding author upon reasonable request.

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Competing interests

The authors declared no competing interests.

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