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Effects of fermented *Crescentia cujete* L. on the profile of hematology, clinical chemistry, and circulatory CD4+/CD8+ in Sprague Dawley rats

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ABSTRACT

Background: The calabash (*Crescentia cujete* L.) is a tropical fruit that offers numerous health benefits. Its fermented calabash (FC) has been found to affect the neurological system positively. However, its impact on hematology, clinical chemistry, and CD4+ and CD8+ levels has yet to be documented.
Aim: Therefore, this study aims to examine the effects of FC on hematology, clinical chemistry, and the levels of CD4+ and CD8+ in the circulatory system using rat models.
Methods: This study used twenty male Sprague Dawley rats. The rats were divided into group 1 (0 mg/kg BW FC), group 2 (50 mg/kg BW FC), group 3 (500 mg/kg BW FC), and group 4 (2,000 mg/kg BW FC). The treatment was administered using a gastric probe once daily for 14 days. On day 15, the blood samples were collected and tested against hematology, clinical chemistry, quality of the erythrocytes, and CD4+/CD8+. The data were then analysed using SPSS with *p*-value at 0.05.
Results: The conducted study demonstrated that the utilization of FC at varying doses did not have a significant impact on the hematological profile changes ($p \geq 0.05$), except for total lymphocytes and a decrease in the neutrophils/lymphocytes (N/L) ratio ($p \leq 0.05$). Furthermore, FC did not influence the changes in clinical chemistry, circulatory protein, and electrolyte levels in rat models compared to the control ($p \geq 0.05$). The utilization of FC decreased the percentage of hemolysis and elevated the adenosine triphosphate (ATP) concentration ($p \leq 0.05$). Additionally, the use of FC led to a significant increase in CD4+ and the ratio of CD4+/CD8+ ($p \leq 0.05$), while no significant effect was observed regarding CD8+ ($p \geq 0.05$).
Conclusion: The study highlighted FC's beneficial effects on the haemorheology and immune system, specifically on the decrease in the percentage of hemolysis, elevated ATP concentration, number of lymphocytes, ratio N/L, CD4+, and the CD4+/CD8+ ratio, without causing significant changes to the hematological and clinical chemistry profiles in rat models.
Keywords: CD4+, CD8+, Clinical chemistry, Fermented *Crescentia cujete* (L), Haematology.

Introduction

The calabash (*Crescentia cujete* L.) is a tropical fruit found in various countries, including Indonesia. It is known for its rich content of antioxidants and various beneficial biochemical compounds (Das *et al*., 2014). Previous research has indicated that the calabash fruit contains compounds such as indomethacin, eriodyctiol, ferulic acid, ascorbic acid, apigenin, pinocembrin, and cinnamic acid, which have shown potential therapeutic properties against various diseases (Gonzales *et al*., 2023). Additionally, fermented calabash (FC) has been found to contain choline (Wilujeng *et al*., 2023); and has demonstrated potential in treating neurological defects and reducing brain infarct area in rat models of ischemic stroke (Hidayah *et al*., 2023). However, the mechanisms and effects of FC on the blood still need to be fully understood.

Blood, a vital component in humans and animals, consists of plasma and cells. Changes in the blood profile, such as erythrocytes, leukocytes, and erythrocyte indices, can serve as indicators of changes in organ function (Watson *et al*., 2022), pathological mechanisms (Samuel, 2023), and the success of therapy (Bodaghi *et al*., 2023). Additionally, plasma is a carrier for proteins, hormones, and waste products after metabolism, making it a valuable tool for clinical chemistry analysis (Haselbeck *et al*., 2022). Furthermore, increased levels of plasma markers, such as blood urea nitrogen (BUN) and creatinine, can indicate a decline in kidney function due to exposure

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to toxic agents (Griffin *et al*., 2019) and other plasma markers in the other organs.

Blood, in addition to its other functions, also mirrors the immunological response through the transportation of biomarkers, including CD4+ and CD8+ (Waschbisch *et al*., 2014). Both of these biomarkers are essential for maintaining immune status, with CD4+ playing a critical role in wound healing (Prakoso and Kurniasih, 2018), infection, and internal organ repair after infection (Kervevan and Chakrabarti, 2021), and CD8+ being involved in protection against infection (Prakoso *et al*., 2021) and tumorigenesis (Xie *et al*., 2021). Therefore, analyzing the levels of CD4+/CD8+ after herbal treatment is crucial to demonstrate the potency of herbal remedies on immunity.

This study focuses on elucidating the effects of FC on hematology profiles, clinical chemistry, and circulatory CD4+/CD8+ using rat models.

Material and methods

Time and place of study

This study was performed from August 2023 until April 2024. The animal experimentation and laboratory tests were conducted in the Laboratory of Pharmacology, University of Wijaya Kusuma Surabaya. The calabash (*Crescentia cujete* L.) was gathered from the Botanical Garden of the University of Wijaya Kusuma Surabaya, and the fermentation was conducted in the Laboratory of Pharmacology, Faculty of Veterinary Medicine, Universitas Gadjah Mada.

Herbal preparation

The calabash was collected and washed using tap water. It was then peeled and weighed. The pulp was fermented following the procedure demonstrated in a previous study (Wilujeng *et al*., 2023). After the fermentation, the FC product was stored inside the fridge at 4°C until being used.

Animal model and research design

This study utilized Sprague Dawley rats from the Laboratory of Pharmacology, UWKS, as the animal model. The selection criteria included male rats weighing 259.00 ± 9.82 g, 6 months old, with a total of 20 rats collected. The rats were individually housed and provided with oven-husk bedding. The room temperature was maintained at 25°C using an air conditioner, with a humidity level of 70%, and light/dark intervals of 12/12 hours. The rats had ad libitum access to water and feed (Ratbio®, Indonesia). Prior to treatment, the rats were acclimated for 7 days and then divided into four groups: Group 1 (0 mg/kg BW FC), Group 2 (50 mg/kg BW FC), Group 3 (500 mg/kg BW FC), and Group 4 (2,000 mg/kg BW FC). The treatment was administered using a gastric probe once daily for 14 days.

Sample collection

The blood from all rats in this study was then collected on day 15 via plexus retro-orbital using microcapillary (Onemed, Indonesia). During the blood collection, all rats were under general anesthesia using 50 mg/kg BW ketamine (Ket-A-100 Agrovet Market, Peru) and xylazine (Xyla Interchemie, Holland). The anesthesia was carefully injected intraperitoneally using a 25G syringe (Onemed, Indonesia), ensuring the comfort and safety of the animals. The blood was then divided into two parts. The first was inserted into a 0.5 ml ethylenediamine tetraacetic acid tube (Onemed, Indonesia), and it was homogenized using a blood roller mixer (KJMR II, Indonesia). The other part of the blood was inserted into a plain tube. Blood without anticoagulant was then centrifuged, and serum was collected. The sample was stored inside a fridge at 4°C of temperature.

Hematology test

The blood was tested against routine hematological parameters, i.e., total erythrocytes, hemoglobin, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total platelets, total leucocytes, differential leucocytes count, and neutrophils/lymphocytes ratio (N/L). The hematology analysis was performed using an automated veterinary hematology analyzer (Vetscan® HM5, USA).

Clinical chemistry test

The serum was tested against several clinical chemistry parameters, including alanine transferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALKP), cholinesterase, gamma-glutamyl transferase (GGT), sorbitol dehydrogenase (SDH), BUN, albumin, globulin, triglyceride, cholesterol, high-density lipoprotein, low-density lipoprotein (LDL), inorganic phosphorus (IPHS), natrium (Na), kalium (K), calcium (Ca), chloride (Cl). The reagents for AST, ALT, ALKP, cholinesterase, GGT, and Ca were gathered from Diasys, Indonesia; BUN, creatinine, albumin, triglyceride, cholesterol, HDL, LDL, and IPHS from Elitech, Indonesia; however, Na, K, and Cl from Glory Diagnostics, Indonesia. All the procedures were followed the demonstrated procedure by the manufacturer.

The clinical chemistry was tested using a photometer (Microlab 300, Vital Scientific, the Netherlands).

Erythrocytes quality test

The quality of erythrocytes was tested against the percentage of hemolysis and adenosine triphosphate (ATP) concentration. The percentage of hemolysis was performed using Drabkin's method (Sowemimo-Coker, 2002). While, the ATP concentration was tested using a spectrophotometry test following the demonstrated procedure in a previous study (Almizraq *et al*., 2013).

Microscopic observation of erythrocytes

During the blood collection, the blood was directly smeared on the object glass. The blood smear was dried and fixed using methanol. Further, the blood smear was stained using Giemsa and examined under a light microscope (Olympus CX33, Japan). The diameter of

erythrocytes was measured using Image J software (NIH, USA). The quality of erythrocytes in the blood smear was analyzed by an expert in veterinary clinical pathology under a blindfold condition.

Cell tube block (CTB)

The blood was also tested using CTB. The parameters of this test were CD4+, CD8+, and CD4+/CD8+ ratio. The antibodies used for this test were antibody anti-CD4+ (Novocastra RTU-CD4-1F6, USA) and antibody anti-CD8+ (Novocastra RTU-CD8-295, USA). The CTB was conducted following the previous study by Marcos *et al*., (2017). However, the staining procedure of the CTB was conducted following Prakoso *et al*., (2020). The result of CTB analysis was reported as a percentage (%) both of CD4+ and CD8+ expression.

Analysis data

The data were analyzed using SPSS version 23 (IBM Corp, USA). The data were tested regarding its homogeneity and normality. The normal and homogeny data were total erythrocytes, hemoglobin, PCV, MCV, MCH, MCHC, platelet, leucocytes, eosinophils, lymphocytes, monocytes, clinical chemistry, CD4+, ratio N/L, and ratio of CD4+/CD8+. Those data were then analyzed using ANOVA and it was confirmed using the Duncan test. However, the abnormal and non-homogeny data were basophils, LDL and CD8+. They were analysed using the Kruskal Wallis and Man Whitney-U test. The statistic was performed using p-value of 0.05.

Ethical approval

The ethical clearance committee from the Faculty of Veterinary Medicine, University of Wijaya Kusuma Surabaya, declared that this study is ethically appropriate. The approval number was 141-KKE-2023.

Results

The study findings revealed that the administration of FC at various doses did not produce a significant impact on the hematological profile of rat models compared to the control group ($p \geq 0.05$) (Table 1). Additionally, FC did not affect platelet count, leucocytes, N/L, basophils, and monocytes ($p \geq 0.05$). However, FC did lead to a significant increase in circulatory lymphocytes and a decrease in the ratio of N/L compared to the control group ($p \leq 0.05$) (Table 2). The observed increase in lymphocytes and decrease in N/L were dose-dependent in the rat models.

Furthermore, the study indicated that clinical chemistry parameters did not change following a 14-day administration of FC. This was evidenced by similar

Table 1. Profile of hematology in rats after utilization of FC.

Parameter	Unit	Group (dose in mg/kg BW)			
		0	50	500	2,000
Erythrocytes	$\times10^6$ cells/ mm^3	5.78 ± 0.20	5.78 ± 0.28	5.78 ± 0.31	5.77 ± 0.21
Haemoglobin	g/dl	14.37 ± 0.54	14.59 ± 0.93	15.16 ± 0.27	15.30 ± 0.24
PCV	%	42.18 ± 0.95	41.74 ± 1.04	41.09 ± 0.78	42.78 ± 1.55
MCV	fl	73.01 ± 3.16	72.36 ± 4.31	71.14 ± 3.44	74.20 ± 3.81
MCH	Pg	24.87 ± 1.44	25.25 ± 1.49	26.24 ± 1.16	26.55 ± 1.12
MCHC	%	34.09 ± 1.88	34.94 ± 1.99	36.90 ± 0.64	35.81 ± 1.63

Table 2. Profile of platelets, leucocytes, and differential leucocytes in rats after utilization of FC.

Parameter	Unit	Group (dose in mg/kg BW)			
		0	50	500	2,000
Platelets	$\times10^5$ cells/ mm^3	5.12 ± 0.24	5.11 ± 0.13	5.11 ± 0.32	5.16 ± 0.25
Leucocytes	$\times10^3$ cells/ mm^3	6.10 ± 0.39	6.09 ± 0.41	6.30 ± 0.63	6.59 ± 0.35
Neutrophils	$\times10^3$ cells/ mm^3	1.67 ± 0.15	1.533 ± 0.15	1.61 ± 0.14	1.66 ± 0.07
Eosinophils	$\times10^3$ cells/ mm^3	0.13 ± 0.08	0.19 ± 0.05	0.15 ± 0.07	0.07 ± 0.05
Basophils	$\times10^3$ cells/ mm^3	0.09 ± 0.03	0.12 ± 0.01	0.10 ± 0.03	0.00 ± 0.00
Lymphocytes	$\times10^3$ cells/ mm^3	3.67 ± 0.29	3.62 ± 0.22	3.95 ± 0.43*	4.33 ± 0.25**
Monocytes	$\times10^3$ cells/ mm^3	0.52 ± 0.02	0.60 ± 0.06	0.48 ± 0.10	0.51 ± 0.10
N/L	-	0.45 ± 0.04	0.42 ± 0.03*	0.40 ± 0.01**	0.38 ± 0.01***

N/L = neutrophils/lymphocytes ratio, */**/*** indicated significant differences ($p \leq 0.05$).

Table 3. Clinical chemistry parameters of liver and kidney function in rats after utilization of FC.

Parameter	Unit	Group (dose in mg/kg BW)			
		0	50	500	2,000
ALT	U/l	24.03 ± 0.71	23.40 ± 1.03	23.38 ± 1.56	23.13 ± 1.08
AST	U/l	72.74 ± 3.07	71.50 ± 1.97	72.50 ± 4.69	71.07 ± 2.01
ALKP	U/l	71.74 ± 2.49	72.39 ± 1.95	71.08 ± 2.35	71.26 ± 3.15
Cholinesterase	$\times 10^3$ U/l	6.88 ± 1.87	6.71 ± 0.72	6.44 ± 0.51	6.69 ± 0.59
GGT	U/l	3.24 ± 0.43	3.32 ± 0.48	3.08 ± 0.23	3.14 ± 0.26
SDH	U/l	17.79 ± 0.81	17.56 ± 1.31	17.84 ± 1.33	18.42 ± 1.05
BUN	mg/dl	10.89 ± 0.80	10.41 ± 1.62	10.48 ± 0.58	10.89 ± 1.03
Creatinine	mg/dl	0.32 ± 0.06	0.31 ± 0.08	0.28 ± 0.06	0.28 ± 0.05

Table 4. Circulatory protein in rats after utilization of FC.

Parameter	Unit	Group (dose in mg/kg BW)			
		0	50	500	2,000
Albumin	g/dl	3.56 ± 0.45	3.63 ± 0.49	3.49 ± 0.43	3.40 ± 0.32
Globulin	g/dl	1.81 ± 0.14	1.84 ± 0.10	1.88 ±0.07	1.83 ± 0.11
Triglyceride	mg/dl	68.98 ± 1.29	69.70 ± 3.39	71.80 ± 1.24	70.90 ± 1.31
Cholesterol	mg/dl	45.39 ± 2.02	45.16 ± 3.26	45.33 ± 4.05	45.18 ± 3.30
HDL	mmol/l	0.79 ± 0.03	0.80 ± 0.03	0.73 ± 0.04	0.74 ± 0.06
LDL	mmol/l	0.23 ± 0.01	0.21 ± 0.01	0.22 ± 0.03	0.23 ± 0.05

Table 5. Circulatory electrolytes in rats after utilization of FC.

Parameter	Unit	Group (dose in mg/kg BW)			
		0	50	500	2,000
IPHS	mg/dl	8.63 ± 0.41	8.64 ± 0.70	8.04 ± 1.85	8.98 ± 0.28
Na	mmol/l	106.58 ± 6.48	103.08 ± 5.16	103.00 ± 8.96	103.86 ± 7.60
K	mmol/l	8.00 ± 0.76	8.67 ± 0.93	8.01 ± 0.94	7.98 ± 0.66
Ca	mg/dl	9.81 ± 0.82	9.69 ± 1.37	9.18 ± 0.87	9.41 ± 0.67
Cl	mmol/l	91.36 ± 6.05	89.87 ± 7.47	95.06 ± 5.15	89.28 ± 1.10

results in various clinical chemistry parameters in the treated group using various doses of FC compared to the control group ($p \geq 0.05$) (Table 3). Moreover, FC did not influence circulatory protein and electrolyte levels in rat models ($p \geq 0.05$) (Tables 4 and 5).

The analysis of the erythrocytes revealed a significant decrease in the percentage of hemolysis after treatment with FC ($p \leq 0.05$). The lowest percentage of hemolysis occurred in the group treated with 2,000 mg/kg BW, followed by the group treated with 500 mg/kg BW. However, the group treated with 50 mg/kg BW FC did not show a significant difference compared to the control ($p \geq 0.05$) (Fig. 1A). Conversely, the ATP concentration in the treated groups increased with the dosage used in the study ($p \leq 0.05$), reaching the highest level in the group treated with 2,000 mg/kg BW ($p \leq 0.05$) (Fig. 1B). However, the statistical analysis indicated that the different doses of FC did not have a significant effect on the diameter of erythrocytes ($p \geq 0.05$) (Fig. 1C). The qualitative assessment of erythrocytes in the

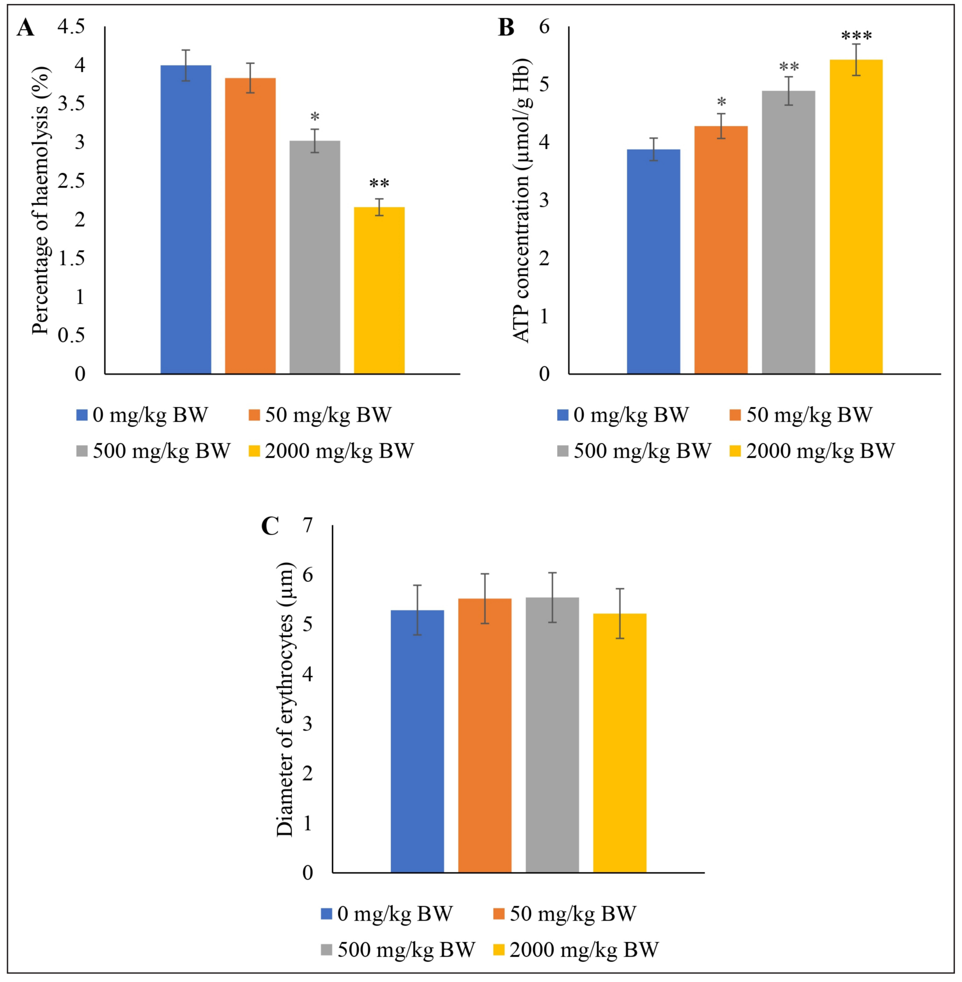

Fig. 1. Percentage of hemolysis, ATP concentration, and diameter of erythrocytes in rats after utilization of FC.

blood smear indicated the presence of anisocytosis in the control group and the groups treated with 50 and 500 mg/kg BW FC. However, anisocytosis was not observed in the group treated with 2,000 mg/kg BW FC. Acanthocytes, marked by spiculated erythrocytes, were observed in all groups, with minimal presentation of this change occurring in the group treated with 2,000 mg/kg BW (Fig. 2).

Finally, the assessment of circulatory lymphocytes using CTB revealed that FC potentially increased the expression of circulatory CD4+ and the ratio of CD4+/CD8+ ($p \leq 0.05$), with no significant impact on CD8+ ($p \geq 0.05$) (Table 6).

Discussion

The FC is a product resulting from the fermentation of calabash fruit. This study demonstrates that FC has notable effects on the number of circulatory lymphocytes and a reduction in the N/L ratio rather than impacting the hematological profile. These positive effects are attributed to the choline content in FC. A prior study indicated that FC contains choline at 110.33 mg/kg of fermented product (Wilujeng *et al.*, 2023). Another study supported this hypothesis, which found that choline supplementation promotes the proliferation of leucocytes and lymphocytes rather than affecting the hematological profile in broiler chickens (Khose *et al.*, 2018). Additionally, Ramalho de Lima *et al.* (2024) suggested that choline supplementation enhances broiler performance without altering the birds' clinical parameters.

Choline deficiency promotes the apoptosis of leucocytes and negatively impacts the immune system (da Costa *et al.*, 2006). Choline is essential for enhancing immunity due to its ability to induce the proliferation of lymphocytes (Garcia *et al.*, 2018). Choline's capacity to promote leucocyte proliferation stems from its metabolism into several essential products (e.g., phosphatidylcholine and betaine), which are essential as transporters and receptors at the cellular level

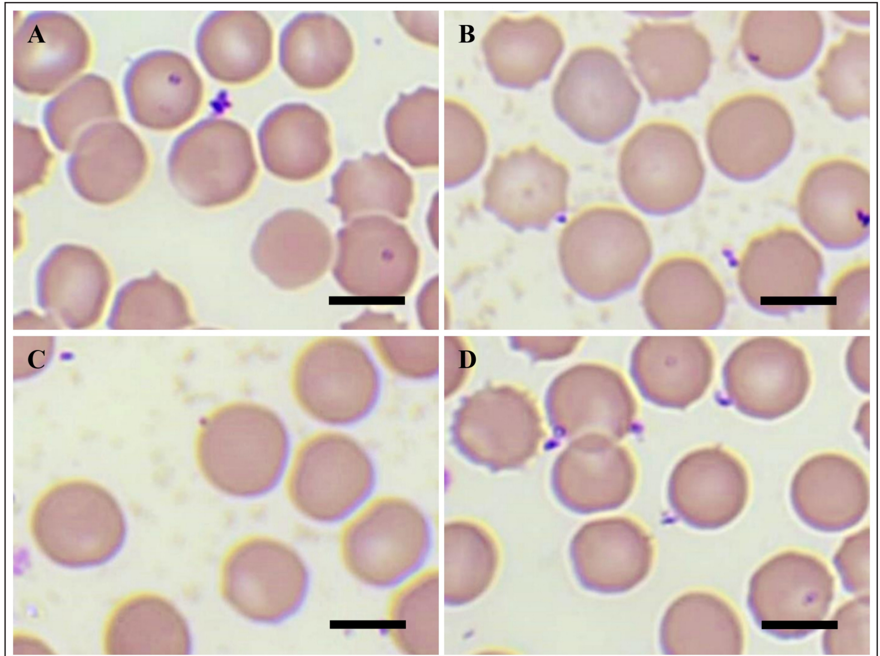

Fig. 2. Qualitative assessment of erythrocytes in rats after utilization of FC. Anisocytosis in blood smear of the control group (A); 50 mg/kg BW FC (B); and 500 mg/kg BW FC (C); the size of erythrocytes appears uniform in group 2,000 mg/kg BW FC (D). Giemsa staining, 1,000× (A-D), scale bar: 5 µm.

Table 6. Percentage of expression of CD4+, CD8+, and CD4+/CD8+ in rats after utilization of FC.

Parameter	Unit	Group (dose in mg/kg BW)			
		0	50	500	2,000
CD4+	%	4.51 ± 0.94	5.48 ± 0.81*	6.79 ± 1.80**	7.38 ± 1.09***
CD8+	%	2.45 ± 0.63	2.31 ± 0.53	2.00 ± 0.23	2.03 ± 0.10
CD4+/CD8+	−	1.94 ± 0.69	2.41 ± 0.21	3.42 ± 1.01*	3.66 ± 0.70**

CD4+/CD8+ = ratio of CD4+/CD8+, */**/*** indicated significant differences ($p \leq 0.05$).

(Mori *et al*., 2003). Choline also activates the mRNA of lymphocytes, which is crucial in regulating the immune system's function (Fujita *et al*., 2021). Moreover, choline's anti-inflammatory effect prevents excessive activity of neutrophils in secreting cyclooxygenase-2, which is essential in preventing oxidative stress (Zhang *et al*., 2023).

The FC does not impact rat models' clinical chemistry parameters, circulatory protein, and electrolytes. This is attributed to choline in FC, which plays a vital role in maintaining the metabolism of nephrons and hepatocytes. Previous studies have demonstrated that choline improves renal function in acute kidney injury in mouse models (Hasson *et al*., 2022) and has beneficial effects in ameliorating oxidative stress in kidney injury (Baris *et al*., 2023). Additionally, choline intake in hepatocytes influences epigenetics on histones and DNA and modulates gene expression to uphold liver function (Mehedint and Zeisel, 2013). These findings suggest that rich in choline, FC enhances liver and kidney function. The optimal functioning of the liver and kidneys may contribute to the regulation of circulatory serum protein (Ko *et al*., 2020) and electrolyte levels (Chen *et al*., 2021), aligning with the outcomes of this study.

Interestingly, the findings indicate that FC potentially benefits erythrocyte haemorheology by decreasing erythrocyte hemolysis and maintaining ATP concentration. The mechanism is attributed to the choline-rich composition of FC, which promotes erythrocyte membrane integrity, thereby preventing disruption due to changes in plasma osmolarity. The integrity of the erythrocyte membrane is potentially related to erythrocyte deformability and aggregation (Santos *et al*., 2003). Membrane integrity also contributes to maintaining erythrocyte

fluidity (Li and Lykotrafitis, 2014). A higher number of intact erythrocytes may be correlated with ATP concentration. This study's findings are similar to a previous study that demonstrated significant ATP release from erythrocytes due to blood hemolysis (Sikora *et al*., 2014). Intact erythrocytes also provide high storage capacity, including ATP storage (Zhong *et al*., 2023). ATP is an important energy source for cells and acts as an extracellular signaling molecule (McMahon *et al*., 2021). Within erythrocytes, ATP is released due to hypoxia or hemolysis (Ferguson *et al*., 2021). Furthermore, the study indicates that FC increases erythrocyte capability to maintain ATP concentration, thereby reducing oxidative stress within the circulatory system via its choline-derived herbal component. These findings are supported by qualitative assessments of erythrocytes post-FC use, as evidenced by the uniformity of erythrocyte size aligning with the increase in doses.

The utilization of FC has been demonstrated to elevate the percentage of CD4+ cells and the CD4+/CD8+ ratio in rat models. The choline constituent in FC upregulates lymphocytes expressing CD4+ rather than CD8+ in the circulatory system. Previous research has indicated that acetylcholine, a choline metabolite released from T-cells, may help mitigate inflammation and regulate vasculature (Cox, 2023). Hence, the use of FC is presumed to augment choline levels in cells to counterbalance choline release during disease pathogenesis.

The increase in CD4+ cells influences the CD4+/CD8+ ratio and plays a significant role in immune regulation. CD4+ lymphocytes act as regulators, promoting differentiating and stimulating macrophages, B lymphocytes, CD8+ cells, and other cells within the immune system (Shete *et al*., 2010). These cells are also crucial during the resolution of chronic pulmonary disease (Qin *et al*., 2022), carcinogenesis (Han *et al*., 2022; Xie *et al*., 2023), prevention of viral replication (Kervevan and Chakrabarti, 2021), and facilitation of T-cell adhesion to cells expressing MHC. Furthermore, CD4+ cells in the circulatory system also contribute to initiating kinase signals to T-cell receptors (Glatzová and Cebecauer, 2019).

The activation of CD4+ cells and the increased CD4+/CD8+ ratio signify a robust immune system. This activation accelerates the immune system's response to exposure to pathogenic agents (Vidya Vijayan *et al*., 2017). Additionally, activating circulatory CD4+ cells is imperative in carcinogenesis and can reflect the body's response during immunotherapy (Poncette *et al*., 2022). It is essential to acknowledge the various benefits of FC on the circulatory system, and further research is critical to validate its effects, including its efficacy during disease pathogenesis and its impacts on the host body.

In light of the study's outcomes, the following recommendations have been proposed: the utilization of FC for treating ailments associated with erythrocyte hemolysis in rats or other animal species, the administration of FC to enhance the immune system of animals, and further exploration of the effects of FC on a variety of animal species.

Conclusion

The study's findings indicate that the administration of FC did not have a statistically significant impact on the hematological profile and clinical chemistry parameters in rat models compared to the control group. However, noteworthy observations include a significant decrease in erythrocyte hemolysis, promoting the maintenance of ATP concentration.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Authors' contributions

ADW supervised this study. ADW and YAP contributed to the design and performed the study. KJVI performed statistical analysis. ADW, YAP, and KJVI contributed to the drafting, revising, and approved the final version of the manuscript.

Data availability

All the data supporting the findings are available within the manuscript.

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