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Effects of black bone chicken on learning and memory in oxonic-induced hyperuricemia male rats

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ABSTRACT

Background: Black bone chicken is considered a nutritious food with a high protein content and low levels of lipids, cholesterol, and uric acid. It contains bioactive compounds such as melanin, carnosine, and anserine, which exhibit various pharmacological properties, including anti-inflammation, antioxidation, and potential enhancements in cognitive function. These bioactive compounds may also help lower serum uric acid levels while improving cognitive function. Hyperuricemia is correlated with a reduced quality of life due to its association with hypertension, kidney disease, cerebrovascular disease, and cognitive impairments in learning and memory. With its potential health advantages, black bone chicken is emerging as a functional food with promising applications for preventing and managing hyperuricemia-related cognitive decline.

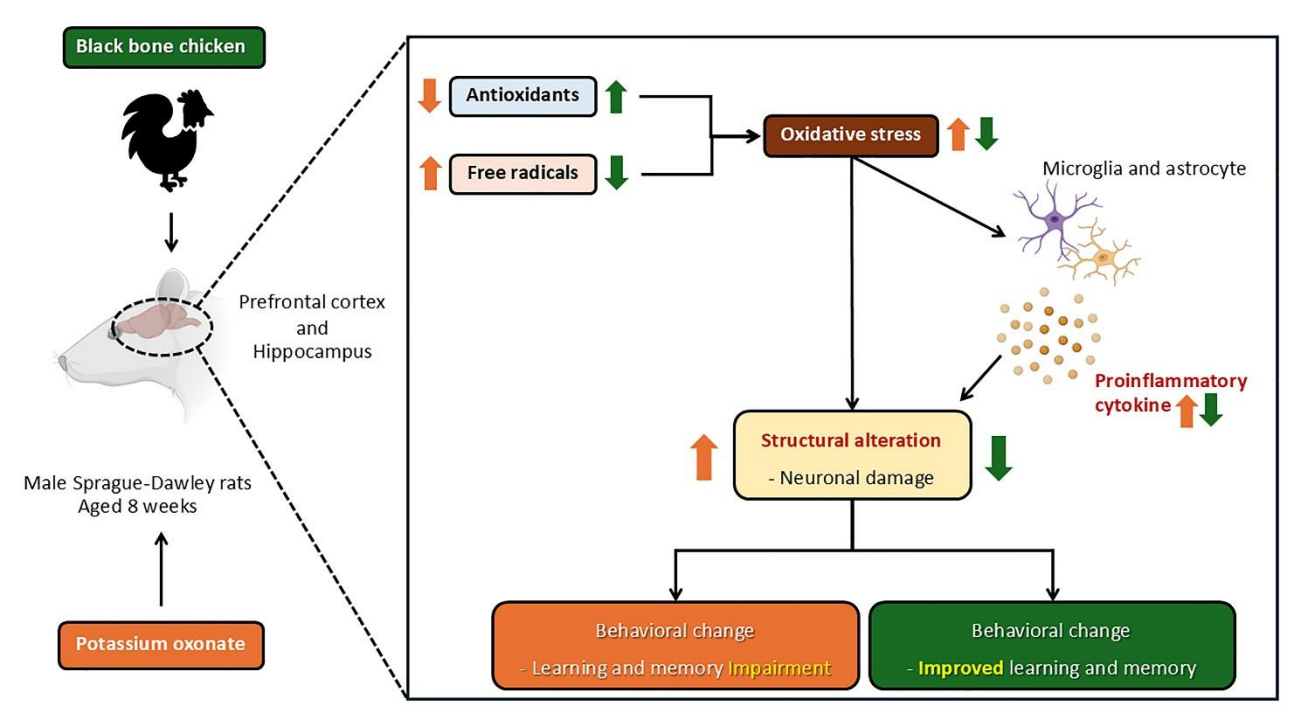
Objectives: This study aims to evaluate the effects of black bone chicken on learning memory and oxidative stress in both normal male rats and those induced with oxonic-induced hyperuricemia.

Methods: Male rats were categorized into two main groups: normal and hyperuricemia. They were fed eggs, white chicken, and black bone chicken via oral gavage for thirty consecutive days. The rats were sacrificed at the conclusion of the experiment. The prefrontal cortex and the hippocampus were extracted for biochemical and morphological study analysis.

Results: Black bone chicken exhibited the capacity to ameliorate learning and memory by diminishing MDA levels, inflammatory cytokine, and neuronal cell death, while concurrently elevating antioxidant activity.

Conclusion: The findings suggested that black bone chicken could improve cognitive function, specifically learning and memory performance in hyperuricemia rats. This study highlights its potential as a functional food with neuroprotective benefits, offering a dietary approach to improving cognitive health and alleviating the detrimental effects of hyperuricemia. Further studies could explore the underlying molecular mechanisms, while clinical trials are needed to assess its efficacy in humans.

Keywords: Black bone chicken, hyperuricemia, learning memory, oxidative stress, antioxidant, anti-inflammatory, neuronal damage, and functional food



Graphical abstract: Effects of black bone chicken on learning and memory in oxonic-induced hyperuricemia male rats

INTRODUCTION

Hyperuricemia, characterized by elevated serum uric acid levels, is linked to elevated interleukin-1β (IL-1β) secretion and reactive oxygen species (ROS) production, resulting in inflammation and oxidative stress, respectively. Hyperuricemia plays a role in the development of conditions such as gout, hypertension, metabolic syndrome, heart failure, chronic kidney disease, cerebrovascular disease, and cardiovascular disease [1]. Prolonged elevation of serum uric acid levels has been linked to an increased risk of cognitive impairment, potentially driven by oxidative stress, tumor necrosis factor-α, and β-amyloid peptide [2]. Furthermore, elevated uric acid levels trigger proinflammatory cytokines and activate the TLR4/NF-κB pathway in the hippocampus, which may contribute to

inflammation-related cognitive dysfunction, including deficits in learning and memory [3]. Current therapeutic strategies predominantly rely on pharmacological interventions, such as xanthine oxidase inhibitors and uricosuric agents. However, long-term use of these treatments has been associated with adverse effects, including gastrointestinal disturbances, interstitial nephritis, hepatitis, hypersensitivity reactions, and eosinophilia [4, 5]. In contrast, dietary approaches present a promising complementary strategy for managing hyperuricemia and its associated complications.

Black bone chicken (BC), a traditional food widely consumed in Asian countries, is known for its high nutritional value, including a rich protein content with low levels of lipids, cholesterol, and uric acid. [6]. BC has pharmacological properties that support heart health, improve blood circulation, protect the nervous system, reduce inflammation, and act as an antioxidant [7-9]. BC contains higher levels of melanin, carnosine, and anserine than white chicken (WC), which aid the inhibition of xanthine oxidase (XO), a crucial enzyme involved in the synthesis of uric acid. Ultimately, this inhibition could help reduce serum uric acid levels [10-12]. Furthermore, these bioactive compounds enhance the activity of antioxidant enzymes, including glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT), while simultaneously reducing free radical levels. This ultimately mitigates lipid peroxidation [13-18]. Additionally, melanin, carnosine, and anserine employ anti-inflammatory properties by suppressing the production and downregulating the expression of proinflammatory cytokines, such as interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-α), IL-1β, and interleukin-6 (IL-6) [19-21].

Apart from its basic nutritional composition, black bone chicken contains various bioactive compounds like melanin, carnosine, and anserine, which are known for their antioxidant, anti-inflammatory, and neuroprotective properties. A recent finding suggests that these bioactive compounds may contribute to reducing serum uric acid levels while simultaneously enhancing cognitive function. Given its potential health benefits, black bone chicken represents an emerging functional food with promising applications in preventing and managing hyperuricemia-related cognitive decline. This research examines the impact of black bone chicken on uric acid levels, cognitive function, and memory performance, as well as oxidative stress and inflammation parameters in both normal and hyperuricemia-induced male rats. By elucidating the neuroprotective and metabolic effects of black bone chicken, this research may provide valuable insights into its potential role as a functional food for improving cognitive health and mitigating the adverse effects of hyperuricemia.

MATERIALS AND METHODS

Preparation of BC and WC: The breasts of BC used in this study were obtained from Cherngwai Black Bone Chicken Herb Community Enterprises in the Taluktiem subdistrict, the Prompiram district, and the Phitsanulok Province. The BC and WC underwent a 5 min pressure-cooking process and were finely ground before being orally administered to the experimental animals.

Animals: Fifty-six male Sprague-Dawley rats (200–220 g, 8 weeks old) were provided by Nomura Siam International Co., Ltd. and acclimatized for one week before treatment. The rats were housed under a controlled 12:12-hour light/dark cycle with a humidity 55 ± 10% at a temperature of 22 ± 1°C. They had unrestricted access to food and water.

The experiment followed Naresuan University's ethical guidelines and was approved under reference number 630302. The rats were divided into 2 clusters: normal and hyperuricemia. The normal rats were randomly assigned to 3 groups (n=7), consisting of a control group, WC group (white chicken 0.49 g/kg

B.W./day), and BC group (black bone chicken 0.49 g/kg B.W./day), with the black bone chicken dose converse from a previous study of anserine of tuna extract (S 1) [22]. The hyperuricemia rats were randomly assigned to 5 groups (n=7), consisting of a control group (normal saline solution), PO group (potassium oxonium 250 mg/kg B.W./day), POWC group (potassium oxonate + white chicken), POBC group (potassium + black bone chicken), and POA group (potassium oxonate + allopurinol 27 mg/kg B.W./day). Rats received daily gavage for 30 days. During the final week of the experiment, behavioral assessments were conducted using the open-field test and the novel object recognition test. The end of the experiment, rats were euthanized with 100 mg/kg thiopental sodium. The right hippocampus and prefrontal cortex were collected for analysis of SOD, CAT, GPx, MDA, and IL-1β. The left hippocampus and prefrontal cortex were collected for the morphological study.

Determination of bioactive compounds in BC and WC:

The measurement of melanin: Melanin extraction adapted from Yoo et al. (2017) [23]. Minced white or BC and WC breast (20 g) was mixed with 40 mL of 1 M NaOH, boiled at 100°C for 30 minutes and centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected and adjusted to pH 2.5, followed by incubation at 25°C for 2 hours and at 5,500 xg for 30 minutes. The sediment was washed with distilled water, treated with 7 M HCl, and boiled at 100°C for 2 hours. The solution was cooled down prior to filtration with filter paper. The sediment was dissolved with 20 mL of 1 M KOH, and to 1 mL of this solution, 3% H_2O_2 was added. The mixture was heated at 100°C for 30 minutes, follow by centrifugation at 10,000 rpm for 1 minute. Absorbance was determined at 350 nm against a melanin standard.

The measurement of carnosine and anserine: The method of anserine and carnosine analysis by high-performance liquid chromatography (HPLC) was modified according to Tian et al. (2007) [24], using a 5 µm Alltima NH2 column at 25°C. The mobile phases were as follows: phase A was 0.1 M K_2HPO_4 and 0.1 M NaOH, and phase B was acetonitrile in a 30:70 ratio. The absorbance was assessed at 210 nm compared to the anserine and carnosine standards.

The measurement of total purine and uric acid: The measurement of total purine and uric acid was adapted from the methods of Inazawa et al. (2014) [25] and Yang et al. (2012) [26]. In this procedure, 2 g of each sample (minced WC and BC breast) was mixed with 20 mL of 70% perchloric acid and heated at 100°C for 1 hour. The mixture was subsequently subjected to centrifugation 5,500 xg for 10 minutes. The solution was adjusted to pH 4 using 1 M NaOH and passed through a 0.45 membrane µm filter. Total purines were analyzed using HPLC with an Agilent C18 column and a 7 mM KH2PO4-H3PO4 mobile phase (pH 4.0) at 25°C. Detection was at 254 nm, using adenine, guanine, hypoxanthine, and xanthine as standards. The levels of uric acid were subsequently determined with a uric acid assay kit (Sigma, cat. no. MAK077).

The measurement of protein, carbohydrate, and fat: The proximate analysis of protein, carbohydrate, and fat in minced WC and BC followed AOAC methods to determine macronutrient composition. This included moisture (oven-drying), ash (incineration), crude protein (nitrogen analysis), crude fat (solvent extraction), crude fiber (acid-alkali digestion), and carbohydrates, ensuring a standardized assessment of food and feed components [27].

Behavioral studies: Open field test: The open field test (OFT) was developed in 1932 by Hall and Ballachey [28]. The test includes two trials: habituation and testing. In habituation, rats explore an open field for 3 minutes to reduce novelty-induced stress. In testing, they are reintroduced for 10 minutes while behavior, distance traveled, and velocity are recorded and analyzed using video tracking software.

Novel object recognition test: The Novel Object Recognition Test (NOR), developed in 1988 by Ennaceur and Delacour, evaluates episodic memory by assessing the recognition of novel objects. [29]. The setup includes a video camera, an open field, and three distinct objects (square, triangle, and sphere). The experiment consists of three trials: habituation (3 min acclimatization), training (10 minute exploration of two objects), and testing (one object replaced, 10 minute recording). The %Recognition Index, a quantitative measure of recognition memory based on exploratory behavior towards objects A (T_A) and C (T_C), is calculated using the formula $[(T_C \times 100)/(T_A + T_C)]$.

Biochemical parameters:

Tissue preparation: The right hippocampus and prefrontal cortex were blended in 0.1M PBS (pH 7.4), and then centrifuged at 10,900xg for 15 minutes at 4°C. The supernatant was collected and preserved at -80°C for subsequent biochemical analyses.

Assessment of uric acid in serum: At the conclusion of the experiment, serum samples were obtained from the rats for uric acid level analysis. The measurement was conducted using appropriate kits with the parameter at the Biolab Medical Technology Clinic in Phitsanulok, Thailand.

Assessment of lipid peroxidation levels: The measurement of lipid peroxidation was conducted by assessing malondialdehyde (MDA) levels through the formation of thiobarbituric acid reactive substances (TBARS), following the method described by Liu et al. [30]. In this process, MDA molecules reacted with 2 molecules of thiobarbituric acid (TBA). The experimental solution comprised 0.8% sodium thiobarbituric, 8.1% sodium dodecyl sulfate (SDS), 20% acetic acid (pH 3.5), and standard (1,1',3,3' tetramethoxy propane) or sample. The resulting solutions were subjected to incubation at 95°C for 1 hour. The absorbance of the solutions was later determined at 532 nm using a spectrophotometer [31].

Assessment of catalase activity: Catalase (CAT) activity was evaluated by assessing the hydrogen peroxide decomposition reaction into water and oxygen [32]. The reaction mixture included 0.059 M hydrogen peroxide in the buffer, distilled water, 0.05 M sodium phosphate buffer (pH 7), and a sample. Kinetics were monitored at 240 nm every 30 seconds for 5 minutes at 25°C using a microplate reader. CAT activity was calculated using a molar extinction coefficient 43.6 and showed as U/mg protein.

Assessment of superoxide dismutase activity: The measurement of superoxide peroxidase (SOD) activity entailed assessing its capability to impede the autoxidation of pyrogallol [33]. The mixtures consisted of sample, 0.2 mM pyrogallol prepared in 50 mM Tris-HCl buffer at pH 7.4, 50 mM Tris-EDTA buffer at pH 8.2, and distilled water. The reaction kinetics were gauged by monitoring the alteration in optical density at 420 nm, 5 minutes at 25°C. A single unit of SOD activity was defined as the amount of SOD required to inhibit pyrogallol oxidation by 50%. The results were expressed as U/mg protein.

Assessment of glutathione peroxidase activity, IL-1β level, and protein content: The determination of glutathione peroxidase (GPx) activity in tissues was conducted utilizing a colorimetric assay kit (Abcam, cat no. Ab100785), while the determination of IL-1β levels was accomplished through the utilization of an Enzyme-Link Immunosorbent Assay kit (Abcam, cat no. Ab100768). The final protein content measurement was performed using a bicinchoninic acid (BCA) protein assay reagent kit (Thermo Fisher Scientific, Cat. No. 23225), following the manufacturer's instructions for the respective assay kit.

Morphological study: Brain tissues, including the left prefrontal cortex and hippocampus, were fixed in 10% neutral buffer formalin and coronally sectioned at 3 mm intervals. The hippocampal regions (CA1, CA3, DG) and medial prefrontal cortex (mPFC) were identified based on the brain atlas. Samples underwent dehydration with graded alcohol, clearing with xylene, and paraffin embedding. Hematoxylin and eosin (H&E) used to stain tissue sections (3 µm) and then analyzed under a light microscope. Cell death was assessed based on morphological criteria (shrinkage, nuclear condensation, vacuolation, pyknosis) and quantified using ImageJ software [34].

Statistical analysis: The data were examined by GraphPad Prism version 8.0.1 and existed as the mean ± standard error of the mean (SEM). Variation between the control and various treatment groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's Post hoc test. Statistical significance was determined as a P-value of less than 0.05 ($p<0.05$).

RESULTS

Bioactive compounds concentration in white chicken and black bone chicken: The results showed the concentration of protein, carbohydrate, fat, melanin, carnosine, anserine, uric acid, and total purine in the breast of WC and BC (Table 1).

Effects of black bone chicken on serum uric acid levels: The serum uric acid in normal rats showed significantly increased in the WC group compared to control groups ($P<0.05$) (Figure 1A). Additionally, the PO group revealed a significant increase compared to the control group ($P<0.01$). In contrast, the POA group decreased serum uric acid levels compared to the PO group in hyperuricemia rats ($P<0.05$) (Figure 1B).

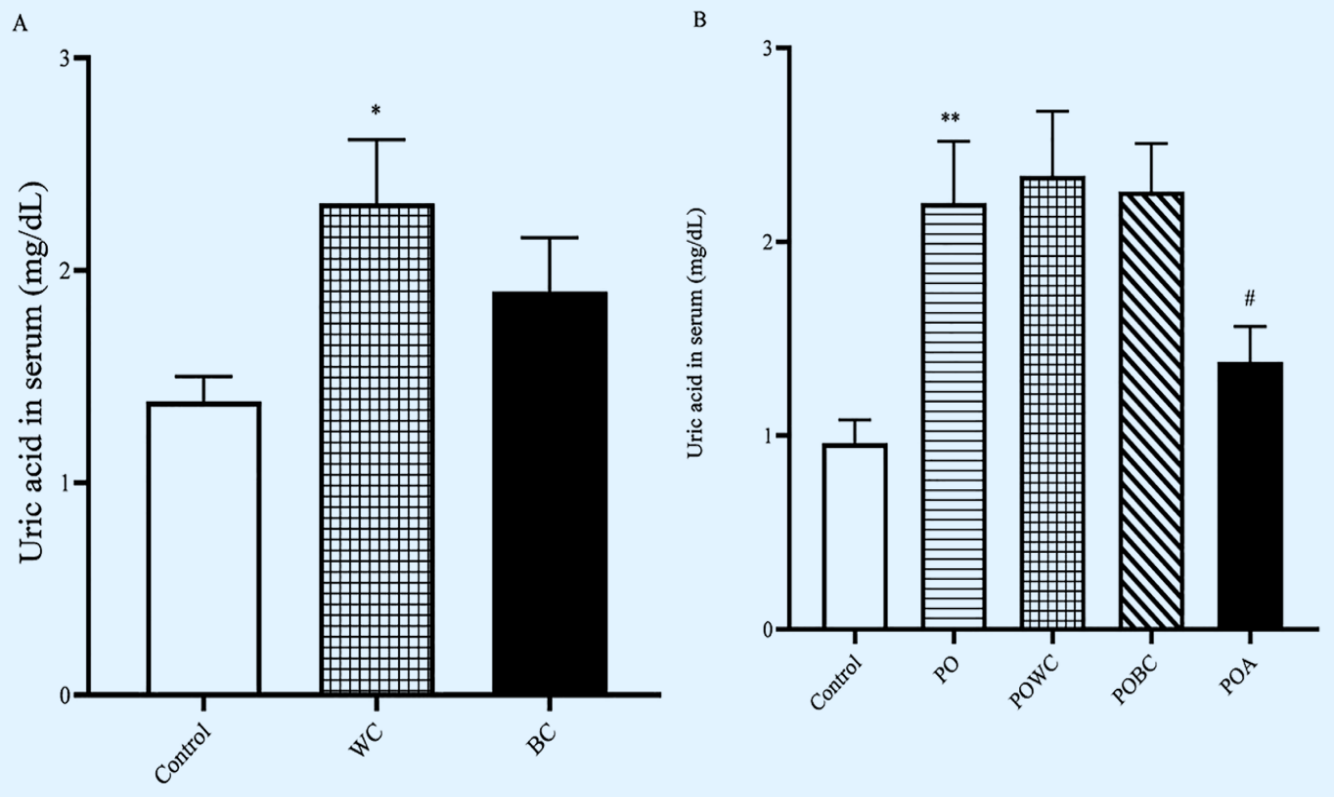


Figure 1. The effect of black bone chicken on serum uric acid level in the serum in normal rats (A) and hyperuricemia rats (B). WC, white chicken; BC, black bone chicken; PO, potassium oxonate; POWC, potassium oxonate + white chicken; POBC, potassium oxonate + black bone chicken; POA, potassium oxonate + allopurinol. Data are represented as the mean ± SEM. $^{*}P<0.05$ and $^{**}P<0.01$ indicate significance compared to the control group while $^{\#}P<0.05$ indicate significance compared to the PO group.

Table 1. Comparison of black bone chicken and white chicken.

Compounds	Black bone chicken breast	White chicken breast
Melanin (mg/g)	0.7	0.37
Carnosine (mg/g)	10.08	4.4
Anserine (mg/g)	11.77	2.15
Uric acid (pg/g)	28.5	64.9
Total purine (μg/g)	778.3	1,388.0
Protein (g/100 g)	21.4	20.8
Carbohydrate (g/100 g)	0.92	0
Fat (g/100 g)	3.99	2.6

Effect of black bone chicken on locomotor activity: The findings conclude that there are no significant differences in total distance and speed in normal rats (Table 2) and hyperuricemia rats (Table 3).

Effect of black bone chicken on recognition memory: The recognition index percentage did not exhibit a significant difference in the experimental group of normal rats (Figure 2A). In hyperuricemia rats, the PO group revealed a significant reduction in the percent recognition index compared to the control group ($P<0.01$), while the POBC group was significantly elevated compared to the PO group ($P<0.001$). The POA group significantly diminished the present recognition index compared to the POBC group ($P<0.001$) (Figure 2B).

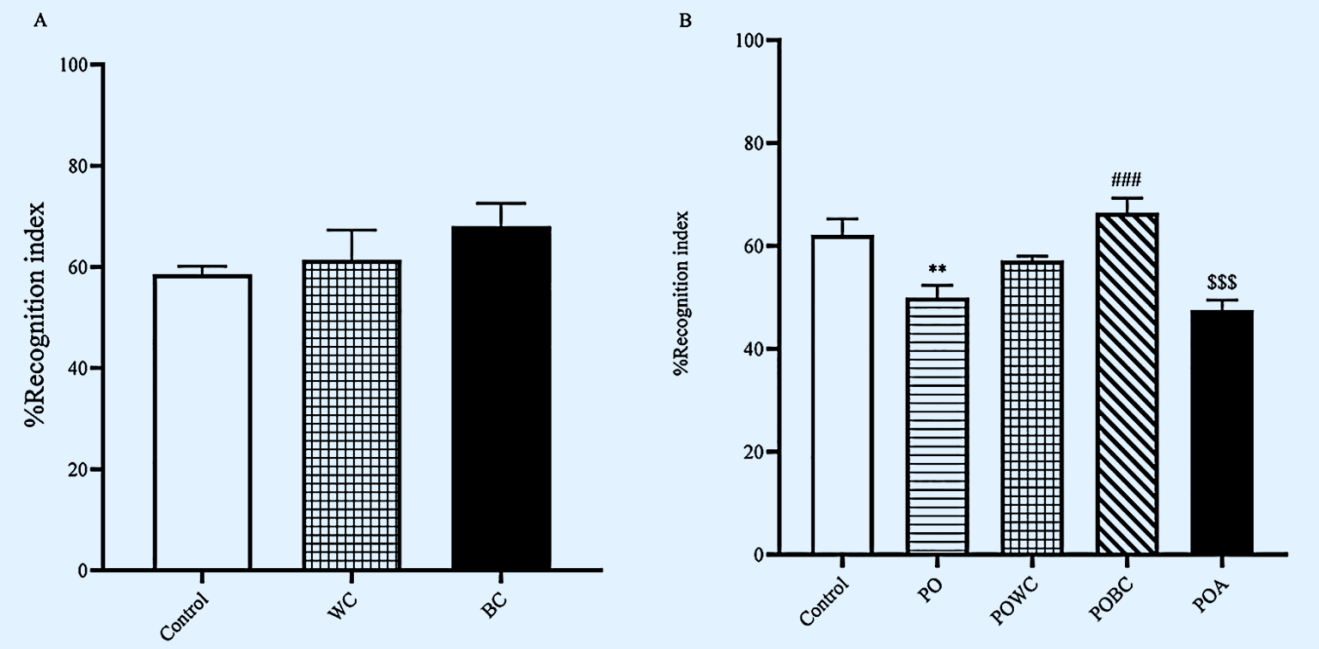


Figure 2. The effect of black bone chicken on recognition memory performance in normal rats (A) and hyperuricemia rats (B). WC, white chicken; BC, black bone chicken; PO, potassium oxonate; POWC, potassium oxonate + white chicken; POBC, potassium oxonate + black bone chicken; POA, potassium oxonate + allopurinol. Data are represented as the mean ± SEM. $^{**}P<0.01$ when compared to the control group, $^{###}P<0.001$ when compared to the PO group, and $^{\$\$\$}P<0.001$ when compared to the POBC group.

Effect of black bone chicken on lipid peroxidation: The findings in normal rats revealed no significant difference in the hippocampus and prefrontal cortex (Table 2). In hyperuricemia rats, the PO group exhibited a significant elevation in MDA levels compared to the control group (P<0.05). Meanwhile, the POBC group exhibited a noteworthy increase in MDA levels compared to the PO group in both the prefrontal cortex and hippocampus (P<0.05). Additionally, the POA group diminished significantly in the MDA levels compared to the PO and POWC groups in the hippocampus (P<0.05) (Table 3).

Effect of black bone chicken on CAT activity: In normal rats' prefrontal cortex and hippocampus, CAT activity did not differ significantly from the control group (Table 2). In hyperuricemia rats, the CAT activity in the hippocampus and prefrontal cortex significantly reduced in the PO group compared to the control group (P<0.05). POBC group significantly increased CAT activity compared to the PO and POWC groups (P<0.05). In contrast, the POA group exhibited a significant decrease in CAT activity compared to the POBC group in both the prefrontal cortex and hippocampus (P<0.05) (Table 3).

Effect of black bone chicken on the SOD activity: The results revealed in the prefrontal cortex and hippocampus did not significantly alter the activity of SOD in normal rats (Table 2). However, in rats with hyperuricemia, the SOD activity in the PO group was diminished considerably compared to the control group (P<0.05). In contrast, the POBC group significantly elevated SOD activity compared to the PO group in the prefrontal cortex and hippocampus (P<0.05). The POBC group increased significantly compared to the POWC group (P<0.05). In contrast, the POA group significantly decreased SOD activity compared to the POBC group in the prefrontal cortex region (P<0.05) (Table 3).

Effect of black bone chicken on the GPx activity: The GPx activity concluded with no significant difference in normal rats' prefrontal cortex and hippocampus (Table 2). In rats with hyperuricemia, the PO group revealed a significant reduction in GPx activity compared to the control group (P<0.05). In contrast, the POBC group had significantly elevated levels compared to the PO group in both the prefrontal cortex and the hippocampus (P<0.05). In the hippocampus, GPx activity in the POBC and POA groups significantly increased compared to the PO and POWC groups (P<0.05). In contrast, the POA group significantly reduced compared to the POBC group (P<0.05) (Table 3).

Table 2. Effects of black bone chicken on the evaluated variable in normal rats.

Variable	Control group	WC group	BC group
Total distance (cm)	1684 ± 228.5	1867 ± 102.9	2128 ± 124.1
Mean speeds (cm/s)	2.806 ± 0.3802	3.110 ± 0.1721	3.544 ± 0.2060
Prefrontal cortex			
MDA levels (µmol/mg protein)	4.06 ± 0.58	3.97 ± 0.21	3.85 ± 0.31
CAT activity (U/mg protein)	2.03 ± 0.24	1.54 ± 0.16	1.70 ± 0.11
SOD activity (U/mg protein)	7.65 ± 0.54	9.23 ± 0.41	9.66 ± 0.0.73
GPx activity (nmol/min/mg)	718.27 ± 71.86	611.10 ± 114.50	847.8 ± 152.20
Dead cell in mPFC (cell/mm^2)	41.61 ± 3.99	55.77 ± 2.80	44.48 ± 6.41
Hippocampus			
MDA levels (µmol/mg protein)	1.10 ± 0.11	1.33 ± 0.12	1.36 ± 0.03

Variable	Control group	WC group	BC group
CAT activity (U/mg protein)	1.52 ± 0.15	1.37 ± 0.19	1.04 ± 0.13
SOD activity (U/mg protein)	7.63 ± 0.78	8.23 ± 1.19	6.91 ± 0.50
GPx activity (nmol/min/mg)	886.69 ± 105.62	887.30 ± 74.29	1156 ± 148.90
Dead cell in CA 1 (cell/mm^2)	40.59 ± 3.54	51.39 ± 2.39	43.05 ± 3.29
Dead cell in CA 3 (cell/mm^2)	31.49 ± 0.84	46.08 ± 5.81	38.87 ± 6.39
Dead cell in DG (cell/mm^2)	51.80 ± 2.33	59.31 ± 1.23	57.81 ± 2.93

Data are represented as the mean ± SEM. WC, white chicken; BC, black bone chicken; MDA, malondialdehyde; CAT, catalase; SOD, superoxide dismutase; GPx, glutathione peroxidase; mPFC, medial prefrontal cortex; CA 1, cornu ammonis 1; CA 3, cornu ammonis 3; DG, dentate gyrus.

Table 3. Effects of black bone chicken on the assessed variable in hyperuricemia rats.

Variable	Control group	PO group	POWC group	POBC group	POA group
Total distance (cm)	15840 ± 133.3	1556 ± 55.94	1510 ± 198.8	1599 ± 165.2	1963 ± 42.12
Mean speeds (cm/s)	3.332 ± 0.2224	2.709 ± 0.0828	2.515 ± 0.3309	2.662 ± 0.2741	3.268 ± 0.0697
Prefrontal cortex					
MDA levels (µmol/mg protein)	3.54 ± 0.32	5.36 ± 0.24*	3.77 ± 0.54	3.19 ± 0.49$^{\#}$	4.02 ± 0.27
CAT activity (U/mg protein)	2.29 ± 0.11	1.46 ± 0.22*	1.68 ± 0.15	2.73 ± 0.12$^{\#, @}$	1.30 ± 0.12$^{\$}$
SOD activity (U/mg protein)	8.61 ± 1.06	5.09 ± 0.45*	8.27 ± 0.46	13.01 ± 1.33$^{\#, @}$	8.38 ± 0.40$^{\$}$
GPx activity (nmol/min/mg)	719.0 ± 76.86	264.26 ± 43.11*	493.96± 76.74	668.21 ± 46.74$^{\#}$	469.19 ± 21.70
Dead cell in mPFC (cell/mm^2)	39.17 ± 4.08	91.26 ± 7.92*	92.24 ± 5.65	60.44 ± 4.67$^{\#, @}$	59.81 ± 2.31$^{\#, @}$
Hippocampus					
MDA levels (µmol/mg protein)	1.45 ± 0.36	4.91 ± 0.51*	3.74 ± 0.20	2.67 ± 0.34$^{\#}$	2.48 ± 0.30$^{\#, @}$
CAT activity (U/mg protein)	2.65 ± 0.11	1.39 ± 0.15*	2.01 ± 0.26	2.90 ± 0.18$^{\#, @}$	1.44 ± 0.25$^{\$}$
SOD activity (U/mg protein)	7.63 ± 0.77	4.20 ± 0.53*	7.09 ± 0.48	8.00 ± 0.58$^{\#}$	6.44 ± 0.76
GPx activity (nmol/min/mg)	777.86 ± 12.61	386.31 ± 41.58	680.06 ± 130.62	1672.57 ± 160.59$^{\#, @}$	973.37 ± 104.16$^{\#, @, \$}$
Dead cell in CA 1 (cell/mm^2)	40.59 ± 3.54	55.41 ± 3.53*	50.57 ± 3.87	28.97 ± 2.74$^{\#, @}$	34.30 ± 2.68$^{\#, @}$
Dead cell in CA 3 (cell/mm^2)	31.49 ± 0.84	45.74 ± 2.03*	37.72 ± 4.39	28.04 ± 2.71$^{\#}$	34.28 ± 2.08$^{\#}$
Dead cell in DG (cell/mm^2)	51.80 ± 2.33	75.17 ± 1.55*	74.46 ± 3.09	53.63 ± 3.00$^{\#, @}$	50.18 ± 1.48$^{\#, @}$

Data are represented as the mean ± SEM. *P<0.05 when compared to the control group, $^{\#}$P<0.05 when compared to the PO group, $^{@}$P<0.05 when compared to the POWC group, and $^{\$}$P<0.05 when compared to the POBC group. PO, potassium oxonate; POWC, potassium oxonate + white chicken; POBC, potassium oxonate + black bone chicken; POA, potassium oxonate + allopurinol; MDA, malondialdehyde; CAT, catalase; SOD, superoxide dismutase; GPx, glutathione peroxidase; mPFC, medial prefrontal cortex; CA 1, cornu ammonis 1; CA 3, cornu ammonis 3; DG, dentate gyrus

Effect of black bone chicken on the inflammatory cytokine: In hyperuricemia rats, the IL-1β levels in the PO group exhibited a significant elevation when compared to the control group (P<0.05, P<0.01), while the POBC group revealed a significant decrease compared to the PO group in both brain regions (P<0.05, P<0.01). Additionally, the POA group showed significantly diminished values compared to both the PO (P<0.001) and POBC groups (P<0.05) in the prefrontal cortex (Figure 3).

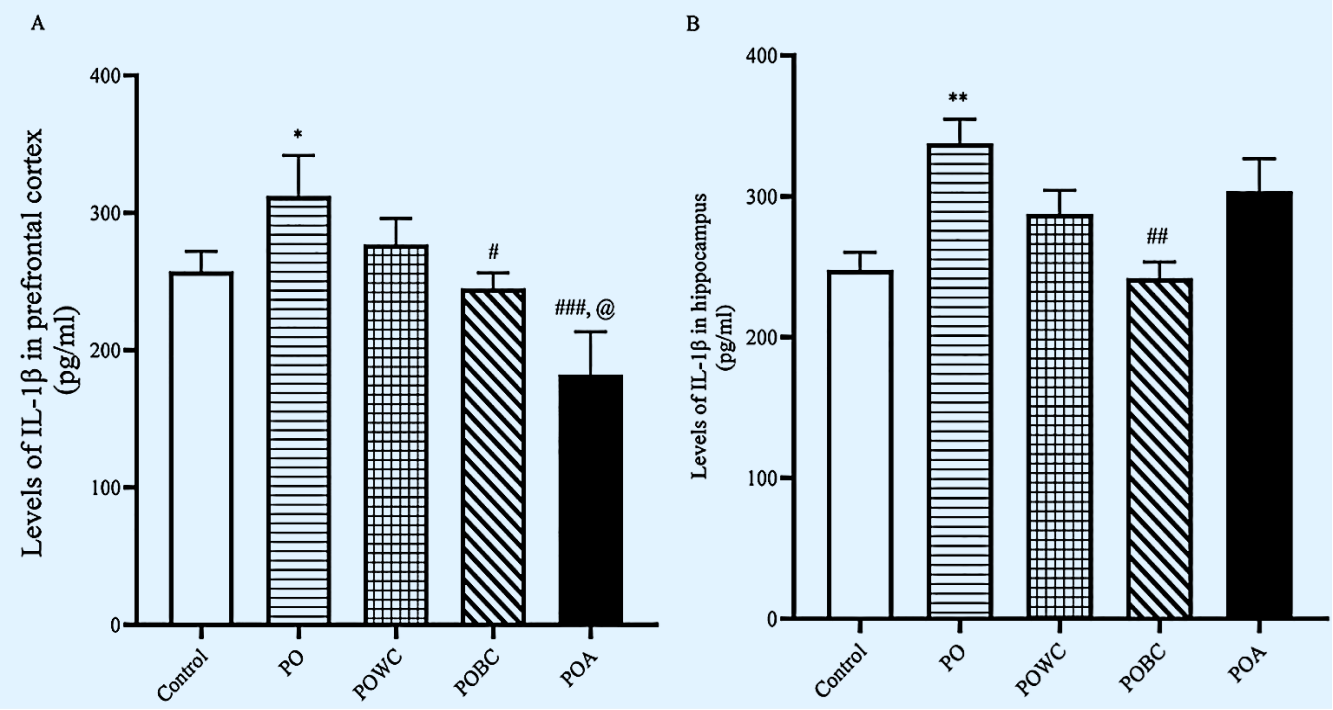


Figure 3. The effect of inflammatory cytokine levels in the prefrontal cortex (A) and the hippocampus (B). WC, white chicken; BC, black bone chicken; PO, potassium oxonate; POWC, potassium oxonate + white chicken; POBC, potassium oxonate + black bone chicken; POA, potassium oxonate + allopurinol; IL-1β, interleukin-1 beta. Data are represented as the mean ± SEM. *P<0.05 and **P<0.01 when compared to the control group, $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001when compared to the PO group, and $^{@}$P<0.05when compared to the POWC group.

Effect of black bone chicken on morphological study: The study assessed the number of dead cells in normal and hyperuricemia rats. The number of dead cells showed no significant difference in the mPFC of the prefrontal cortex area and CA 1, CA 3, and DG of the hippocampus area in the normal rats (Table 2, Figure 4). In hyperuricemia rats, the PO group exhibited a significant elevation in the number of dead cells in the mPFC, CA 1, CA 3, and DG compared to the control group. The number of dead cells of the POBC and POA groups in the mPFC, CA 1, CA 3, and DG were significantly decreased compared to the PO group. In contrast, the POWC group exhibited a substantially increased number of dead cells in the mPFC, CA1, and DG regions compared to the POBC and POA groups (Table 3, Figure 5).

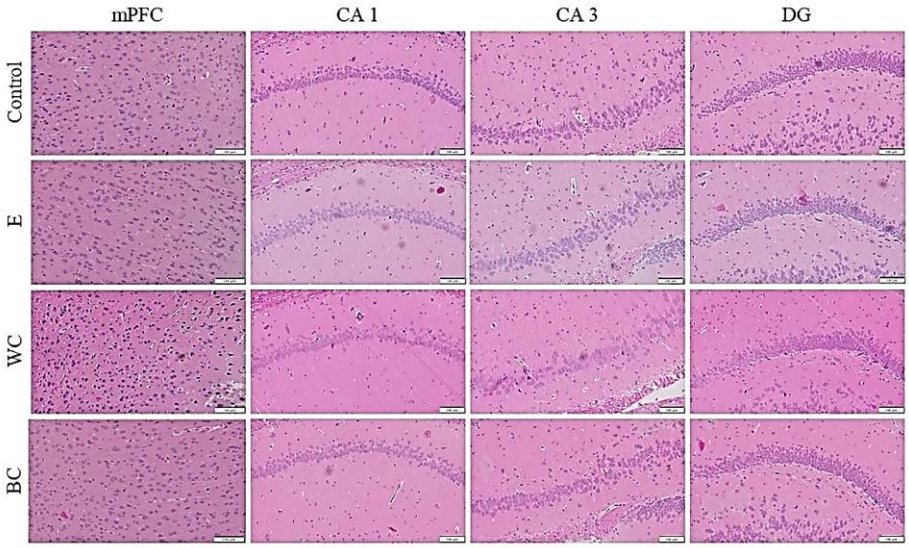


Figure 4. Expression of dead cells was evaluated by H&E staining of mPFC, CA 1, CA 3, and DG area in the normal rats. Hematoxylin stains nuclei a violet color, while eosin stains the cytoplasm pink. Magnification, x20; scale bar 100 μm. WC, white chicken; BC, black bone chicken; mPFC, medial prefrontal cortex; CA 1, cornu ammonis 1; CA 3, cornu ammonis 3; DG, dentate gyrus.

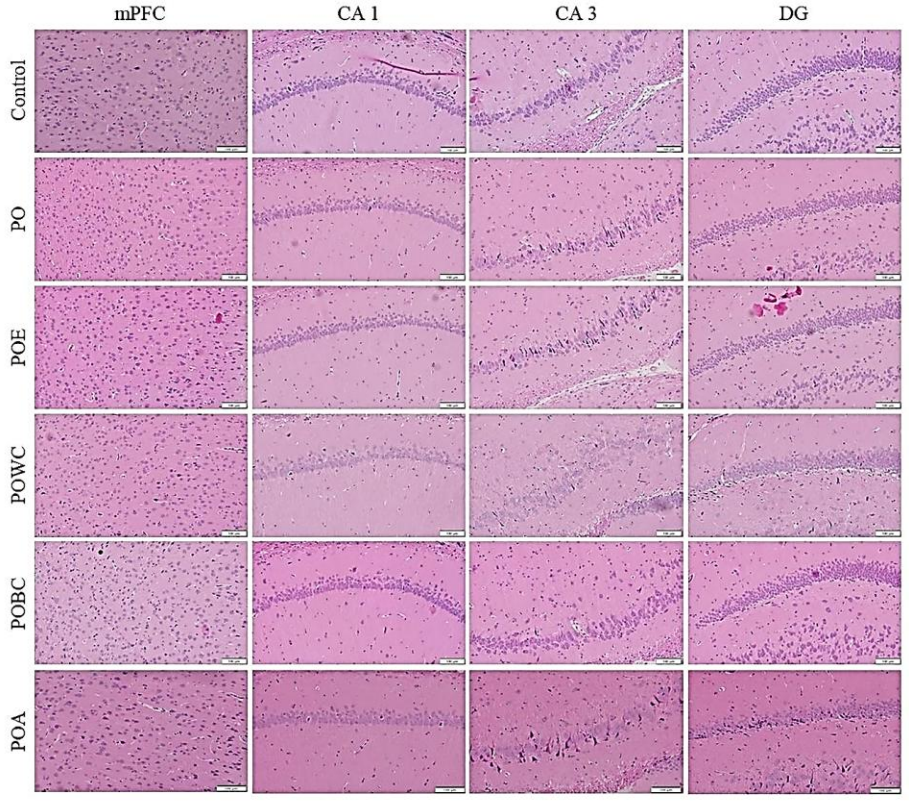


Figure 5. Expression of dead cells was evaluated by H&E staining of mPFC, CA 1, CA 3, and DG area in the hyperuricemia rats. Hematoxylin stains nuclei a violet color, while eosin stains the cytoplasm pink. Magnification, x20; scale bar 100 μm. PO, potassium oxonate; POWC, potassium oxonate + white chicken; POBC, potassium oxonate + black bone chicken; POA, potassium oxonate + allopurinol; mPFC, medial prefrontal cortex; CA 1, cornu ammonis 1; CA 3, cornu ammonis 3; DG, dentate gyrus.

DISCUSSION

Chicken consumption has been widely associated with increased uric acid levels due to its purine content, which contributes to hyperuricemia. However, the findings from this study suggest that while white chicken consumption led to elevated serum uric acid levels, black bone chicken did not significantly affect uric acid levels, behavioral performance, oxidative stress markers, antioxidant activity, or brain morphology in normal rats. The lack of increased uric acid levels following black bone chicken consumption may be attributed to its unique nutritional and biochemical profile. Black bone chicken contains bioactive compounds such as carnosine, anserine, and melanin, which have been reported to exhibit antioxidant and anti-inflammatory properties [13-17]. These compounds may play a role in modulating purine metabolism or enhancing uric acid excretion, preventing its accumulation in serum. Additionally, the balance of nutrients and lower lipid content in black bone chicken compared to white chicken could contribute to its neutral effect on uric acid regulation. Although white chicken increased serum uric acid levels, it did not induce significant changes in cognitive function or oxidative stress markers in the brain. This suggests that a short-term increase in uric acid levels alone may not be sufficient to cause immediate neurochemical or behavioral alterations in normal rats. However, long-term exposure to elevated uric acid levels has been implicated in oxidative stress, neuroinflammation, and cognitive decline, particularly in hyperuricemic conditions. These results indicated that consuming black bone chicken at a rate of 0.49 mg/kg B.W./day continuously for one month did not increase uric acid levels, which was a concern associated with chicken consumption.

Hyperuricemia induced by potassium oxonate in rats elevated serum uric acid, MDA, and pro-inflammatory cytokines, indicating increased oxidative stress and inflammation. Reduced CAT, SOD, and GPx activity further highlighted an impaired antioxidant defense. These changes were linked to cognitive decline, behavioral impairments, and neuronal damage in the prefrontal cortex and hippocampus. The findings align with studies associating oxidative stress and neuroinflammation with cognitive deficits in middle-aged individuals. Potassium oxonate (250 mg/kg B.W.) raised uric acid and IL-1β levels and reduced hippocampal cell layers, suggesting its negative impact on brain function [2, 35, 36]. Moreover, high levels of uric acid in the serum were associated with impaired spatial memory and working memory, alongside a notable decrease of 19% in SOD activity [3].

BC administration improved cognitive function in hyperuricemic rats without directly affecting serum uric acid levels, suggesting that its neuroprotective effects are mediated through alternative mechanisms. The study findings indicate that BC enhances antioxidant defense by increasing the activity of key antioxidant enzymes, including CAT, SOD, and GPx. This antioxidant response effectively reduces oxidative stress, as evidenced by decreased MDA levels, a marker of lipid peroxidation. Additionally, BC supplementation suppressed neuroinflammation by lowering IL-1β levels. Histopathological analysis further confirmed reduced neuronal damage in critical brain regions associated with learning and memory, including the prefrontal cortex and hippocampus. The observed neuroprotective effects of BC may be attributed to its bioactive compounds, such as melanin, carnosine, and anserine. Previous studies have reported that melanin helps reduce superoxide levels during the hypoxanthine-xanthine oxidase reaction, potentially mitigating oxidative stress [15]. Meanwhile, carnosine and anserine function as natural antioxidants that enhance CAT, SOD, and GPx activity, thereby preventing lipid peroxidation and preserving neuronal integrity [13, 14, 16]. Beyond their antioxidative properties, melanin, carnosine, and anserine have been shown to modulate inflammatory pathways. These

compounds suppress and downregulate IL-1β expression, a pro-inflammatory cytokine linked to neurodegeneration. A prior study demonstrated that these bioactive compounds significantly reduced IL-1β levels in the hippocampus of aged mice modeling Alzheimer's disease, further supporting their potential role in neuroprotection [19-21]. Carnosine has been reported to enhance recognition memory performance, a crucial component of episodic memory, by counteracting oxidative stress and neuroinflammation. Similarly, anserine has been shown to improve episodic memory by mitigating chronic glial neuroinflammatory responses in mice, enhancing spatial working memory in the Y-maze, and improving memory retention in models of diabetic-induced cognitive dysfunction [18, 34, 35]. These findings suggest that the cognitive benefits of BC may be attributed to the combined action of these bioactive compounds.

Allopurinol (27 mg/kg B.W. for 30 days) reduced uric acid by 79.57%, lowered MDA levels, and increased GPx activity in the hippocampus. It also decreased IL-1β in the prefrontal cortex but did not affect cognitive behavior. While allopurinol reduces inflammation and oxidative stress, studies show it does not significantly impact cognitive decline or Alzheimer's disease risk. It increases glutathione and GPx activity but does not affect CAT or SOD, suggesting its antioxidant effects may be independent of these enzymes. These results are consistent with previous studies that found uric acid-lowering medications, such as allopurinol, may not be significantly associated with an increased or decreased risk of cognitive decline, dementia, or Alzheimer's disease [37]. Additionally, previous studies found that it does not significantly influence CAT or SOD activity, indicating that its antioxidant effects in certain brain regions are independent of these enzymes [38]. Further research is needed to fully understand its impact on the nervous system.

According to the results, the utilization of BC is associated with an increased recognition index, driven by its potent antioxidant and anti-inflammatory properties and a reduction in MDA levels. These effects contributed to a decrease in cell death and the mitigation of structural alterations in the prefrontal cortex and hippocampus, which were the regions associated with learning and memory, culminating in enhanced cognitive function. The observed improvement in learning and memory performance in hyperuricemia rats suggests that black bone chicken could be a valuable natural resource for cognitive enhancement.

CONCLUSION

The findings suggest that black bone chicken may serve as a functional food capable of improving cognitive function, specifically learning and memory, in hyperuricemia conditions. Given its bioactive properties, black bone chicken could be further explored for its potential applications in dietary interventions for neurodegenerative disorders and metabolic diseases linked to hyperuricemia. Future research should investigate its long-term benefits, optimal consumption levels, and underlying mechanisms contributing to its neuroprotective effects. Additionally, clinical studies could provide valuable insights into its efficacy in humans, reinforcing its role as a functional food with therapeutic potential.

List of Abbreviations: BC, black bone chicken; CA, Cornu ammonis; CAT, catalase; DG, dentate gyrus; GPx, glutathione peroxidase; HPLC, high-performance liquid chromatography; IL, interleukin; MDA, malondialdehyde; mPFC, medial prefrontal cortex; NF-κB, nuclear factor kappa B; NOR, novel object recognition test; OFT, open field test; ROS, reactive oxygen species; SDS, sodium dodecyl sulfate; SOD, superoxide dismutase; TLR 4, toll-like receptor 4; TNF-α, tumor necrosis factor-alpha; WC, white chicken; XO, xanthine oxidase.

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Authors' contributions: Kotchakorn Klinprathap: Methodology; formal analysis; data curation; investigation; writing original draft; writing-reviewing and editing; validation; visualization. Waraporn Kasekarn: Conceptualization; writing-reviewing and editing; resource; validation. Sarawut Sattayakawee: Conceptualization; writing-reviewing and editing; resource; validation. Thanyaphon Phothi: Methodology; investigation; writing original draft; writing-reviewing and editing. Onrawee Khongsombat: Conceptualization; formal analysis; data curation; investigation; writing original draft; writing-reviewing and editing; validation; visualization; software; supervision; project administration; funding acquisition.

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