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Comprehensive impacts of black elderberry (*Sambucus nigra L.*) extract on mitigating acute heat stress, augmenting antioxidant defenses, and enhancing broiler chicken health and meat quality

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ABSTRACT

Poultry genotypes' low heat tolerance affects birds' health, which results in major economic losses and calls for an immediate, consumer-friendly dietary plan. This study assessed black elderberry extract (BE) on 120-Cobb chickens, 35-days old, to mitigate the adverse-effects of acute heat stress on clinical and pathological parameters, antioxidant status, and meat quality. Birds were divided into four-groups: NEG: maintained at thermoneutral temperature (24 ± 1°C); 2) BE: administered 0.15 g/L BE extract for 12-h while kept at 24 ± 1°C; HSBE: maintained at 38 ± 1°C for 6-h and treated with BE extract (0.15 g/L); HS: maintained at 38 ± 1°C for 6-h. BE supplementation under heat stress significantly mitigated these effects, reducing rectal temperature and droppings moisture ($p < 0.05$), lowering both macroscopic and histological lesion severity at 3 and 12 h ($p < 0.05$), and improving meat quality traits—lower cooking and thawing losses, enhanced water-holding capacity, and better tenderness – while also diminished muscle malondialdehyde concentration, and elevated levels of polyunsaturated fatty acids (PUFA) and omega-3 PUFA, particularly alpha-linolenic acid ($p < 0.05$). The results suggest that the use of BE extract in the water of broiler chickens during periods of heat stress is beneficial, and further research on heat stress-reducing efficacy of BE is needed.

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Heat stress; sambucol; antioxidants; broiler chicken; fatty acid profile;

1. Introduction

In tropical and subtropical nations, climate change, particularly elevated ambient temperatures, is the most significant environmental stressor threatening the poultry sector, which yields the most widely consumed animal-derived products globally, with no religious or cultural constraints (Abd El-Hack et al. 2020; Perini et al. 2021). Chickens housed in the thermoneutral zone (16-25°C) can maintain a steady internal temperature of 41–42°C (Ahmad et al. 2022). Birds experience heat stress when their body temperature increases above their ability to dissipate heat (Farag and Alagawany 2018). Commercial birds, especially broilers, are susceptible to heat stress damage when temperatures get above the thermoneutral zone because of their quick metabolism, feathers, and lack of sweat glands (Abd El-Hack et al. 2020). Most poultry breeds experience heat stress above 32°C (Hu et al. 2019). Acute heat stress can be described by a fast rise in temperature (27–38°C) and humidity for a short period (1–24 h) (Nawaz et al. 2021). High humidity and 32°C to 38°C can generate a lot of free radicals, which can damage cells (Pasri et al. 2024). Chickens stressed by heat have detrimental behavioural, physiological, immunological, metabolic, and oxidative alterations (Perini et al. 2021). Heat stress decreases movement, feed intake, and elevated wing position, increases both body temperature and water consumption, and causes severe pathological lesions in visceral organs (Perini et

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al. 2021; Kim et al. 2025). Stressed birds also paint, which helps evaporative cooling dissipate heat (Apalowo et al. 2024). Additionally, immune suppression, endocrine abnormalities, and electrolyte imbalances have been reported (Nawab et al. 2018). Moreover, it negatively affects the fatty acid composition and physicochemical quality of meat, including pH, tenderness, colour, and water-holding capacity (Gonzalez-Rivas et al. 2020; Li et al. 2023). It disrupts the cellular antioxidant-oxidant balance, leading to oxidative stress, lipid peroxidation, and protein oxidation in skeletal muscles, while also significantly reducing fatty acid composition (Zhao et al. 2021). The above measures reduced profitability, resulting in an estimated annual loss of $128 to $165 million (Hu et al. 2019), which is expected to rise owing to global warming.

Various interventions have been used to reduce heat stress in broiler chickens. Environmental changes, genetic selection, and dietary interventions like antioxidants to balance antioxidant capacity and free radical activity can reduce oxidative stress (Apalowo et al. 2024) These solutions yield variable responses depending on the bird's age, health, breed, sex, management, and location. There is a significant need to investigate various effective strategies to address the specific needs of stressed chickens (Nawab et al. 2018). Researchers are studying herbs and medicinal plants as natural antioxidants in the broiler industry to mitigate heat stress, enhance consumer health, and protect the environment (Bouassi et al. 2021). Secondary metabolites, such as polyphenols in the phytochemicals, have been shown to mitigate heat stress in poultry production (Jimoh et al. 2022).

The black elderberry (*Sambucus nigra L.*) is a plant supported by extensive research demonstrating various health benefits for both humans and animals (AL-Shammary 2023). In chickens, black elderberry has been shown to reduce H9N2 virus shedding when administered in drinking water for 7 days post-challenge at a dosage of 0.4 ml/kg body weight (Karimi et al. 2014). Moreover, it exhibited antibacterial efficacy, evidenced by enhancements in clinical and pathological indicators following *E. coli O78* challenge, as well as hepatic and renal protective effects in the challenged SPF chicks when administered at 0.15 g/L in drinking water for 5 days post-challenge (Elbasuni et al. 2024). Furthermore, the oral treatment of a 10% elderberry flower extract to heat-stressed laying hens improved their blood hematological, antioxidant enzyme levels, and overall production performance (AL-Shammary 2023). Elderberries modulated the quantity of reactive oxygen species (ROS) in intestinal contents and epithelial cells of human colon cells in vitro, due to their elevated quantities of bioactive chemicals with antioxidant characteristics (Olejnik et al. 2016). All parts of this plant (flower, bark, leaf, and fruit) are rich in dietary phytochemicals, encompassing carbohydrates, lipids, terpenoids, flavonoids (such as kaempferol, astragalin, quercetin, quercetin-3-O-glucoside, rutin, isoquercitrin, and hyperoside), phenolic acids, gallic acid, gentisic acid, lectins, essential oils, fatty acids, organic acids, vitamins, and minerals (Kolesarova et al. 2022; Nawirska-Olszańska et al. 2022; Vlaicu et al. 2025). The principal constituents of black elderberry fruits are polyphenols, particularly anthocyanins, which exhibit notable antioxidant properties, hence enabling this plant to impede viral and bacterial activity within the body. They are acknowledged as free radical scavengers, efficient in protecting the body from oxidative stress and peroxidative processes. Furthermore, anthocyanins can improve vascular health by promoting nitric oxide production, hence augmenting vascular permeability. Additionally, they can downregulate inflammatory promoters and markers, such as monocyte chemoattractant protein-1 (MCP-1), the transcription factor nuclear factor kappa B (NF-κB), and interleukin 8 (IL-8) (Kolesarova et al. 2022). The plant's significant antioxidant capacity is likely due to the presence of the physiologically active flavonols rutin and quercetin, together with gallic acids, which either directly neutralize free radical species or augment antioxidative enzymes within the cells. This subsequently mitigates oxidative stress, safeguards genomic DNA integrity, and exerts inhibitory effects on gut microbiota, inflammation, vascular health, carcinogenesis, and brain function (Kolesarova et al. 2022).

In the present study, we aimed to investigate the efficacy of sambucol (black elderberry extract) in mitigating the negative effects of acute heat stress on clinical and pathological characteristics, serum antioxidants, and meat quality in commercial broiler chickens.

2. Materials and methods

2.1. Chickens and diets

A total of 120 Cobb broilers aged 35 days and weighing 1740 ± 50 g were purchased from a commercial farm in Moshtohor, El-Quaubia, Egypt, and kept for 48 h under thermoneutral temperature for adaptation. The

basal diet used in this study followed (NRC 1994) recommendations and consisted of a standard corn–soybean meal base. The diet contained approximately 18% crude protein and 3200 kcal/kg ME. Egypt's Benha University Faculty of Veterinary Medicine lab animal research centre housed these chickens following the Animal Welfare Committee's standards (BUFVTM 04-06-24).

2.2. Acute heat stress induction

Heat stress was implemented using technique of Huang (2017) and (Goel et al. 2022) with slight modification. For 6 h, heat-stress groups were housed at 38 ± 1°C and 60 ± 10% relative humidity (measured by digital thermo-hygrograms). Control groups were kept in a separate room at 24 ± 1°C, while each group under heat stress was kept in two separate rooms.

2.3. Experimental design

At 37 days of age, the broiler chickens were randomly assigned to four groups (30 birds, 5 replicates/group, $n = 6$ / replicate). The experimental groups included: (1) NEG: maintained at a thermoneutral temperature; (2) BE: maintained at a thermoneutral temperature and treated with black elderberry extract (Sambucol, sourced from Pharmacare, Inc., San Diego, USA) in water (0.15 g/L) for 12 h, (3) HSBE: exposed to acute heat stress for 6 h and treated with BE extract (0.15 g/L) for 12 h; (4) HS: exposed to acute heat stress for 6 h. The selected concentration of 0.15 g/L was based on prior efficacy studies by (Roschek et al. 2009; Elbasuni et al. 2024).

2.4. Assessed parameters

2.4.1. Ante-mortem parameters

Birds were observed for heat stress symptoms such as open mouth, laborious breathing, drooped wings, stretched neck, and watery droppings. The onset time (first panting after heat exposure) and frequency (detected by hand counting at the last 5 min of HS exposure) of panting were also recorded at 3 and 6 h post heat stress (PHS).

2.4.2. The rectal temperature

The rectal temperature (RT) was measured at 3, 6, 9, and 12 h from the onset of heat exposure (Goel et al. 2022) in all experimental groups by inserting a digital thermometer (±0.1°C, Huger Electronics GmbH, Germany) up to approximately 3 cm inside the cloaca for 1 min.

2.4.3. Relative dropping moisture

The droppings from each pen were collected at 6 and 12 h PHS to determine the initial moisture content of the dropping samples using the oven-drying method (Khodadadi et al. 2023). In this regard, samples were dried at 105°C for 24 h in an oven, then weighed before and after dryness for calculation of moisture ratio.

2.4.4. Cumulative lesion scoring (CLS)

At 3, 6, and 12 h PHS, 5 birds from each group were killed by neck dislocation and lesions (congestion, hemorrhages and enlargement) on subcutaneous tissues, blood vessels, muscles, the inner surface of the keel bone, liver, heart, spleen, and kidneys were scored, with 0 indicating normal conditions, 1 mild pathological alteration, 2 moderate, and 3 severe. The CLS for each group was calculated.

2.4.5. Relative weight of internal organs

The liver, heart, spleen, and kidneys ($n = 5$/group) were dissected and weighed at 3, 6, and 12 h PHS, and the relative weight of each organ was calculated by dividing organ weight at a certain time post heat stress by the live body weight at the same time. Relative organ weight (g/kg) was calculated [organ weight (g)/body weight (kg)] × 100 (Kim et al. 2025).

2.4.6. Serum antioxidant

Blood samples (5/group) were aseptically collected from the jugular vein at 3, 6, 9, and 12 h PHS. Serum was separated to quantify malondialdehyde (MDA), reduced glutathione (GSH), oxidized glutathione (GSSG), and nitric oxide (NO) (El-Bahr et al. 2021). After setting up the HPLC, a Bondapak column (30 cm, 3.9 mm, C18 l) was loaded with a mobile phase that included 0.005 M tetra butylammonium phosphate, 13% methanol, and 0.0025 M sodium phosphate buffer. The pH of the solution was 3.5.

2.4.7. Meat quality assay

The pH_{24}, water holding capacity (WHC), muscle drip loss (DL), thawing loss (ThL), cooking loss (CL), Warner–Bratzler shear force (WBSF), and Lightness (L*), redness (a*), and yellowness (b*) values were assessed to determine the physicochemical properties of meat (Fathi et al. 2023; Abdel Haleem et al. 2024), at 3, 6, and 12 h PHS (5/group). The malondialdehyde and GSH were assessed using commercial kits produced by Diamond Diagnostic Company, Giza, Egypt. The colorimetric method was employed to ascertain the fatty acid profile of meat (Abdel Haleem et al. 2024), with certain modifications. Briefly, tissue homogenates were generated by homogenizing and centrifuging the chicken breast meat samples at 4000 rpm for 15 min. Total lipids and total cholesterol were subsequently determined using a commercial reagent (Stanbio Laboratory Company; Boerne, TX 78202, USA) from the obtained supernatant. After vortexing for two minutes in a chloroform: methanol (2:1; v/v) solution, breast tissue samples were centrifuged at 1792 × g for 10 min to extract the total lipid content. The esterification reaction resulted in the production of fatty acid methyl esters (FAME) from the supernatant using a methanol/sulfuric acid mixture (95:5, v/v) and hexane. The FAME hexane extract was analyzed using a temperature gradient programme with hydrogen as the carrier gas, as well as a split model, using a gas chromatography system with an SP2330 column (30 mm × 0.32 mm × 0.2 µm film thickness; Supelco Analytical, USA) and a flame ionization detector. Hewlett-Packard Chem Station software (Agilent Technologies Inc., USA) was used to compare the retention times of the fatty acid standards (Cat. No. 24073, Sigma-Aldrich, USA) with FAME peak periods. To ensure reproducibility, all assays were conducted in duplicate, with quality control steps carried out in accordance with kit and GC-FID protocols.

2.4.8. Histopathological evaluation of organs

At 3 h PHS, sections from the liver, kidney, and heart ($n = 5$/group) were prepared for histopathological examination (Bancroft and Gamble 2008). The scoring assessment applied the 0–4 scoring using the following criteria: 0) normal histological structure to 4) severe pathological changes.

2.5. Statistical analysis

The data was analyzed as a completely randomized design by one-way ANOVA using the statistical application SPSS version 20.0 for Windows (SPSS, Inc., Chicago, IL, USA). Tukey's post hoc test ($p < 0.05$) was conducted to determine the significance level between the experimental groups. The data of physicochemical parameters of breast meat were analyzed by two-way ANOVA.

The normality of the data set was assessed using the Shapiro–Wilk test (Shapiro and Wilk 1965). The test did not reject the null hypothesis (p value > 0.05) that the data are from a regularly distributed population. Consequently, this population follows a normal distribution, allowing for the use of parametric tests.

The sample size was calculated using the following formula to test for equality between two proportions: $n = (Z_{\alpha/2} + Z_{\beta})^2 * (p_1(1\text{-}p_1) + p_2(1\text{-}p_2)) / (p_1 - p_2)^2$, where $Z_{\alpha/2}$ is the critical value of the Normal distribution at $\alpha/2$, Z_{β} is the critical value of the normal distribution at β and p_1 and p_2 are the expected sample proportions of the four groups.

3. Results

3.1. Ante-mortem parameters

At 3 and 6 h PHS, all birds in the HSBE and HS groups exhibited panting (65 breaths per minute), open mouths, watery droppings, and respiratory distress. At 3 h PHS, 6.67% of birds in both groups exhibited a stretched neck. Those in the thermoneutral zone did not show these symptoms (Table 1).

Table 1. Effect of black elderberry extract on clinical signs of heat-stressed broilers.

	NEG		BE		HSBE		HS	
Parameter/Time/ group*	3 h	6 h	3 h	6 h	3 h	6 h	3 h	6 h
Panting	–	–	–	–	min/65	min/65	min/65	min/65
Opened mouth	–	–	–	–	+ (6.67%)	+ (15%)	+ (6.67%)	+ (15%)
Extended neck	–	–	–	–	+ (6.67%)	–	+ (6.67%)	–
Watery dropping	–	–	–	–	+	+	+	+
Dropped wings	–	–	–	–	–	–	–	–
Labored breathing	–	–	–	–	20/20 (100%)	15/15 (100%)	20/20 (100%)	15/15 (00%)

* NEG: kept at thermoneutral temperature (24 ± 1°C); 2); BE: kept at thermoneutral temperature and treated by intracrop black elderberry extract (0.15 g/L), followed by water medication for 12 h. with the same dose; HSBE: birds subjected to acute heat stress for 6 h and treated with BE intracrop black elderberry extract (0.15 g/L), followed by water medication for 12 h. with the same dose; HS: subjected to heat stress for 6 h.

3.2. Rectal temperature

Compared to non-stressed groups, birds in HSBE and HS showed a significant ($p < 0.05$) increase in RT at 3 and 6 h PHS (Table 2). HSBE birds had a 0.28°C lower body temperature than HS birds at 6 h PHS ($p > 0.05$). The RT of the HSBE and HS birds fell to normal levels at 9 and 12 h PHS, comparable to the NEG and BE groups.

3.3. Relative dropping moisture

The results showed that the NEG group had the highest moisture ratio (91%) followed by the HS group (85%), and the HSBE group had the lowest moisture ratio (67%) at 6 h of heat exposure (Figure 1). On the other hand, at 12 h of the trial, the NEG group demonstrated the highest moisture ratio (83%), and the HSBE group demonstrated a moisture ratio of 67%.

3.4. Cumulative lesion scoring

The heat-stressed birds in the HS group displayed significant macroscopic abnormalities in visceral organs at 3, 6, and 12 h PHS, which correspondingly resulted in a markedly ($p < 0.05$) elevated CLS compared to the NEG and BE groups (Table 3). Group HSBE had a notable reduction in CLS values at 3 and 12 h PHS compared to the HS group ($p < 0.05$).

3.5. Relative weight of internal organs

A notable decline ($p < 0.05$) in the average kidney weight was recorded at 12 h PHS in the HS group in comparison to HSBE group (Table 4). No significant differences were observed in the average weights of the liver, heart, and spleen across different durations of heat stress in the treated group compared to the negative and positive control groups.

3.6. Serum antioxidants

Before heat stress, the data indicated no substantial variations in the activity of serum antioxidants and lipid peroxidation in the examined birds (Figure 2). At 3 and 6 h of exposure to heat, the HS group

Table 2. Effect of black elderberry extract on rectal temperature of heat stressed broilers (Mean ± SE).

Group*/Time	3 h	6 h	9 h	12 h
NEG	39.7 ± 0.00^{c}	40.0 ± 0.10^{b}	40.0 ± 0.03^{a}	40.0 ± 0.03^{a}
BE	39.9 ± 0.07^{c}	39.7 ± 0.15^{b}	39.9 ± 0.15^{a}	39.8 ± 0.15^{a}
HSBE	42.0 ± 0.33^{a}	40.9 ± 0.21^{a}	39.9 ± 0.12^{a}	39.9 ± 0.12^{a}
HS	41.1 ± 0.11^{b}	41.2 ± 0.28^{a}	39.9 ± 0.08^{a}	39.9 ± 0.08^{a}

Tukey's test represents the least profound differences between different groups at probability $p < 0.05$. $^{a–c}$ Means within a column not sharing a common superscript differ significantly when $p < 0.05$. SE: standard error. Values are given as the mean (n = 5). * NEG: kept at thermoneutral temperature (24 ± 1°C); 2); BE: kept at thermoneutral temperature and treated by intracrop black elderberry extract (0.15 g/L), followed by water medication for 12 h. with the same dose; HSBE: birds subjected to acute heat stress for 6 h and treated with BE intracrop black elderberry extract (0.15 g/L), followed by water medication for 12 h. with the same dose; HS: subjected to heat stress for 6 h.

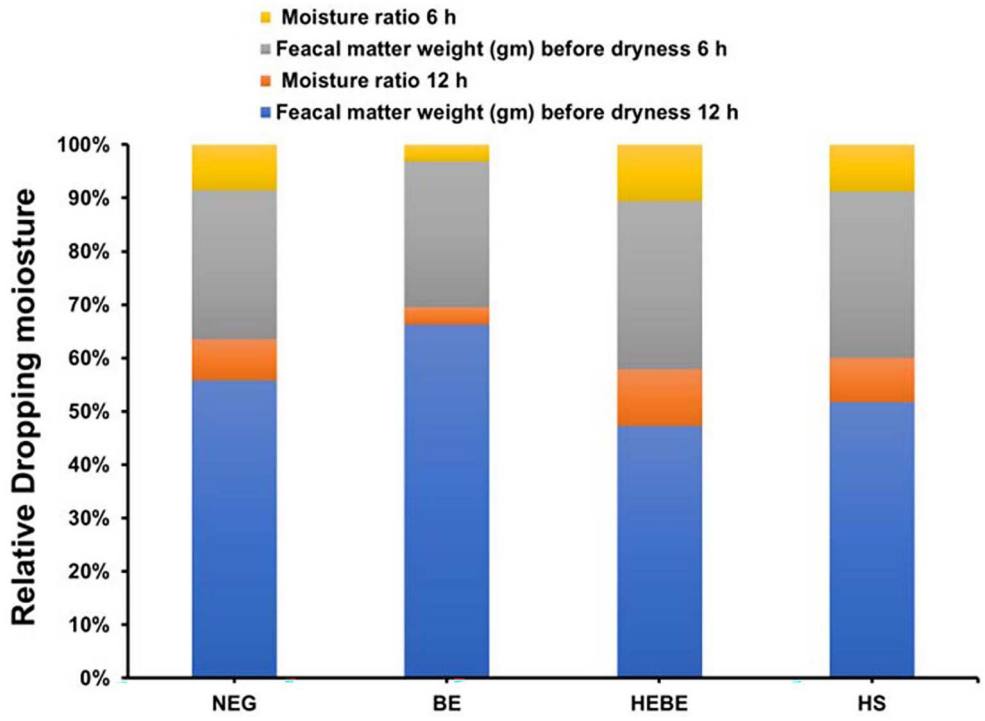

Figure 1. Effect of black elderberry extract on relative dropping moisture of heat-stressed broilers for 3, 6, and 12 h. NEG: kept at thermoneutral temperature (24 ± 1°C); 2); BE: kept at thermoneutral temperature and treated by intracrop black elderberry extract (0.15 g/L), followed by water medication for 12 h. with the same dose; HSBE: birds subjected to acute heat stress for 6 h and treated with BE intracrop black elderberry extract (0.15 g/L), followed by water medication for 12 h. with the same dose; HS: subjected to heat stress for 6 h.

Table 3. Impact of black elderberry extract on the cumulative macroscopic and histological lesion scores in heat-stressed broilers (Mean ± SE, $n = 5$).

Cumulative macroscopical scoring			
Group*/Time	3 h	6 h	12 h
NEG	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
BE	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
HSBE	5.40 ± 1.72^{b}	13.6 ± 3.43^{a}	3.40 ± 1.66^{b}
HS	14.4 ± 2.62^{a}	12.2 ± 2.29^{a}	12.2 ± 2.29^{a}
Histopathological scoring at 3 h.			
Group*/Organ	Liver	Kidney	Heart
NEG	0.80 ± 0.37^{c}	1.40 ± 0.25^{b}	1.00 ± 0.32^{b}
BE	0.80 ± 0.37^{c}	1.00 ± 0.32^{b}	1.00 ± 0.32^{b}
HSBE	3.00 ± 0.32^{b}	3.00 ± 0.32^{a}	2.80 ± 0.37^{a}
HS	4.00 ± 0.00^{a}	3.80 ± 0.20^{a}	3.60 ± 0.25^{a}

Tukey's test represents the least profound differences between different groups at probability $p < 0.05$. $^{a-c}$ Means within a column not sharing a common superscript differ significantly when $p < 0.05$. SE: standard error. Values are given as the mean (n = 5). * NEG: kept at thermoneutral temperature (24 ± 1°C); 2); BE: kept at thermoneutral temperature and treated by intracrop black elderberry extract (0.15 g/L), followed by water medication for 12 h. with the same dose; HSBE: birds subjected to acute heat stress for 6 h and treated with BE intracrop black elderberry extract (0.15 g/L), followed by water medication for 12 h. with the same dose; HS: subjected to heat stress for 6 h.

exhibited a considerable ($p < 0.05$) elevation in MDA levels relative to the other experimental groups, but this parameter dramatically diminished in the HSBE group compared to the HS group ($p < 0.05$). The activity of GSH was markedly reduced in the HS group when compared to the HSBE group at 6 h PHS. No substantial difference in the enzyme levels was noted between the HSBE and NEG groups ($p > 0.05$). The HS group exhibited elevation in GSSG at 6 h in comparison to the other experimental groups ($p < 0.05$). While it was markedly diminished in the HSBE group in comparison to the HS group ($p < 0.05$). The levels of nitric oxide significantly diminished in the HS group relative to the HSBE group at 6 h PHS ($p < 0.05$).

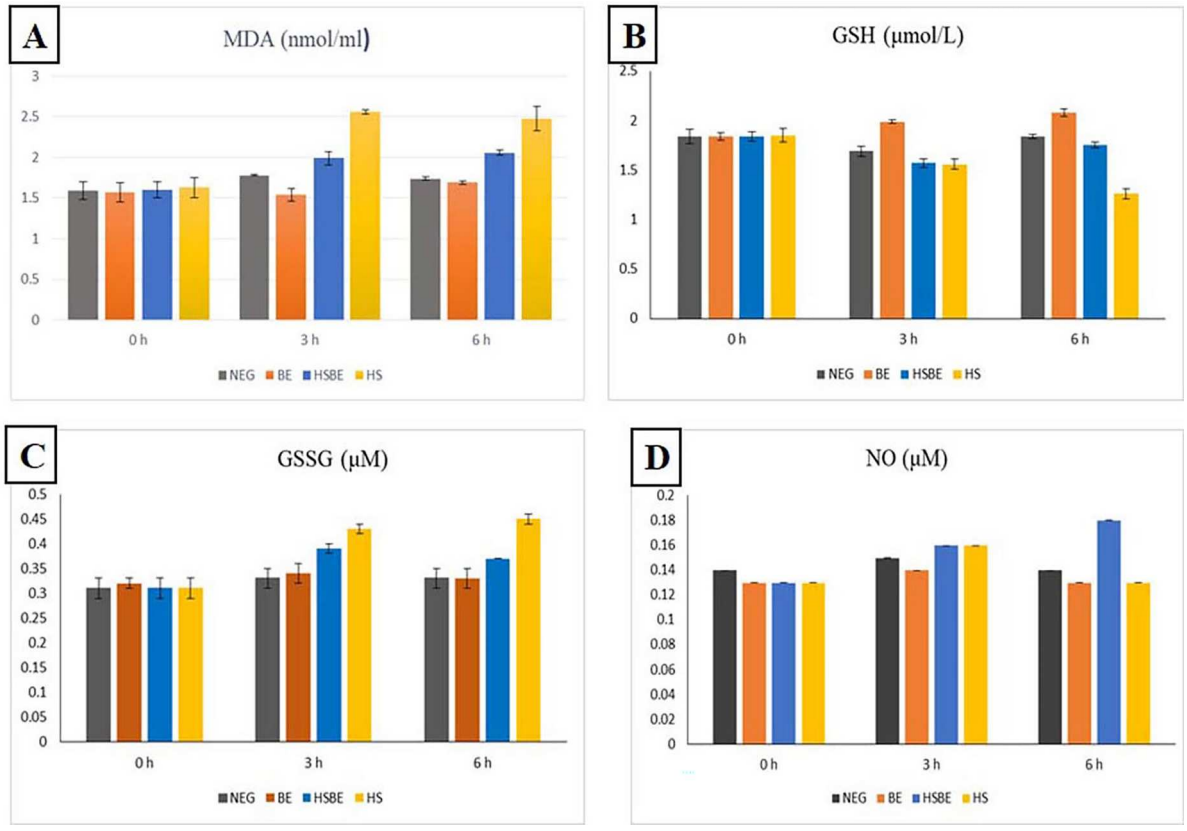

Figure 2. Effect of black elderberry extract on serum antioxidant capacity of the heat-stressed broilers for 3, 6, and 12 h. Tukey's test represents the least profound differences between different groups at a probability $P < 0.05$. MDA: malondialdehyde; GSH: reduced glutathione; GSSG: oxidized glutathione; NO: nitric oxide. NEG: kept at thermoneutral temperature (24 ± 1°C); 2); BE: kept at thermoneutral temperature and treated by intracrop black elderberry extract (0.15 g/L), followed by water medication for 12 h. with the same dose; HSBE: birds subjected to acute heat stress for 6 h and treated with BE intracrop black elderberry extract (0.15 g/L), followed by water medication for 12 h. with the same dose; HS: subjected to heat stress for 6 h

3.7. Meat quality assay

Regarding the physicochemical properties of breast meat, there were significant effects of HS time, BE treatment, or both on WHC, ThL, CL, SF, and meat colour (L*, a*, and b*), except for ultimate pH (pH_{24}) and DL (Table 5). At 3 h PHS, the cooking loss was elevated substantially in the HS group compared to other groups ($p < 0.05$). Nevertheless, the BE group exhibited a substantially higher WHC than the HS group at 6 h PHS ($p < 0.05$). Additionally, the BE supplementation improved the thawing loss in both BE and HSBE groups compared to the HS group ($p < 0.05$). In contrast to other groups, L* tended to increase in the HS group ($p < 0.05$). Likewise, at 12 h PHSWBSF, CL, and WHC were the most relevant meat quality parameters altered by the heat stress condition. At 12 h PHS, there was a notable enhancement in CL of both BE and HSBE groups compared to the HS group ($p < 0.05$).

Regarding the antioxidant capacity of broiler meat (Figure 3), the breast meat of broiler birds in the BE group had a significant decline in MDA concentrations ($p < 0.05$) as well as a significant increase in GSH activity than the NEG group ($p < 0.05$). Similarly, the HSBE group exhibited a substantial decrease in breast meat MDA levels and a marked rise in reduced glutathione activity compared to the HS group ($p < 0.05$).

There was a tendency for increased levels of alpha-linolenic acid (C18:3-n3, ALA) and the sum of n3-PUSFA in the BE group (Figure 4), at 3 h PHS compared to the NEG group ($p < 0.05$). The HS group exhibited a higher ratio of n6/n3-PUFAs, but the level of ALA (C18:3-n3), the sum of n3-PUFAs, and the ratio of n3/n6-PUFAs decreased compared to the HSBE group ($p < 0.05$) at 3 h PHS. Furthermore, at 6 h PHS, the ALA (C18:3-n3), the sum of n3-PUFAs, and the sum of PUFA increased in the BE group relative to the NEG group ($p <$

Table 4. Effect of black elderberry extract on internal organs relative weight of heat-stressed broilers (Mean ± SE, $n = 5$).

	Liver			Heart			Spleen			Kidney		
Group*/Time	3 h	6 h	12 h	3 h	6 h	12 h	3 h	6 h	12 h	3 h	6 h	12 h
NEG	0.03 ± 0.0^{a}	0.01 ± 0.00^{a}	0.03 ± 0.00^{a}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
BE	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.01 ± 0.00^{b}	0.00 ± 0.00^{b}	0.01 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
HSBE	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{b}	0.01 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
HS	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{b}	0.01 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.00 ± 0.00^{b}

Tukey's test represents the least profound differences between different groups at probability $p < 0.05$. $^{a–c}$ Means within a column not sharing a common superscript differ significantly when $p < 0.05$. SE: standard error. Values are given as the mean (n = 5). * NEG: kept at thermoneutral temperature (24 ± 1°C); 2); BE: kept at thermoneutral temperature and treated by intracrop black elderberry extract (0.15 g/L), followed by water medication for 12 h. with the same dose; HSBE: birds subjected to acute heat stress for 6 h and treated with BE intracrop black elderberry extract (0.15 g/L), followed by water medication for 12 h. with the same dose; HS: subjected to heat stress for 6 h.

Table 5. Effect of groups and heat stress on physicochemical parameters in the meat of heat-stressed broilers.

		Groups#				SEM	*P* value		
Items	Time (h)	NEG	BE	HSBE	HS		G	T	G*T
pH_{24}	3	6.01	5.96	5.88	5.84	0.019	0.842	0.393	0.111
	6	5.92	6.07	5.92	5.99				
	12	5.90	5.93	6.06	6.05				
WHC	3	84.2	84.3	84.3	83.8	0.502	0.010	0.561	0.098
	6	85.8^{ab}	88.1^{a}	86.8^{ab}	81.1^{c}				
	12	79.6^{b}	89.8^{a}	85.0^{ab}	84.0^{ab}				
$DL_{48h.}$	3	1.82	1.63	2.37	3.09	0.164	0.562	0.179	0.328
	6	3.02	1.97	2.03	3.52				
	12	1.80	1.76	1.87	2.00				
ThL	3	3.03	2.76^{A}	5.96	7.44	0.439	0.227	0.102	0.544
	6	2.54^{ab}	1.12^{bB}	2.50^{ab}	3.44^{a}				
	12	4.89	2.24^{A}	2.64	3.68				
CL	3	14.6^{b}	14.4^{bB}	16.0^{bB}	23.3^{a}	0.848	0.119	<0.001	
	0.004								
	6	21.4	18.9^{A}	22.0^{A}	24.13				
	12	13.6^{abB}	4.66^{bC}	8.13^{bC}	21.06^{a}				
WBSF	3	3.04	2.65	3.03	3.15^{A}	0.052	0.166	0.017	0.349
	6	2.77	2.50	2.70	2.86^{B}				
	12	2.44^{b}	2.40^{b}	2.69^{ab}	2.90^{aB}				
Instrumental colour									
L*	3	55.4	55.5	52.4	53.6^{A}	0.337	0.078	0.049	0.056
	6	53.0^{b}	54.3^{ab}	54.9^{ab}	55.7^{aA}				
	12	51.5	56.2	50.71	48.9^{B}				
a*	3	10.4	11.4	11.2	10.8	0.309	0.166	0.042	0.940
	6	8.84	11.0	10.5	10.1				
	12	11.4	14.0	12.1	11.2				
b*	3	14.0	14.5^{A}	12.9	12.2	0.224	0.736	0.094	0.011
	6	12.7	13.2^{AB}	13.9	15.6				
	12	13.4	10.7^{B}	12.6	13.8				

$^{a-c}$Values with different superscript letters in the same raw differ significantly ($p < 0.05$).
$^{A-C}$Values with different superscript letters in the same column differ significantly ($p < 0.05$).
#NEG: kept at thermoneutral temperature (24 ± 1°C); 2); BE: kept at thermoneutral temperature and treated by intracrop black elderberry extract (0.15 g/L), followed by water medication for 12 h. with the same dose; HSBE: birds subjected to acute heat stress for 6 h and treated with BE intracrop black elderberry extract (0.15 g/L), followed by water medication for 12 h. with the same dose; HS: subjected to heat stress for 6 h.$^{\$}$ pH_{24}: Ultimate pH, WHC: water-holding capacity, $DL_{48h.}$: drip loss after 48 h, ThL: thawing loss, CL: cooking loss, WBSF: Warner- Bratzler shear force *L: Lightness of colour, a*: redness, b*: yellowness, SEM: standard error of mean, G: group, T: time.

0.05). Compared to the HSBE group, the HS group had a higher level of palmitoleic acid (C16:1), and the ratio of n6/n3-PUFA increased, but ALA (C18:3-n3) and the sum of n3-PUFAs decreased ($p < 0.05$). There was a tendency for increased levels of arachidonic acid (C20:4, AA), sum of n3-PUFA, and the sum of PUFAs in breast muscle at 12 h PHS in the BE group compared to the NEG group ($p < 0.05$).

3.8. Microscopical evaluation of organs

The results in Table 3 and Figures 5–7 indicate that the HS group exhibited the highest lesion scores ($p < 0.05$) in multiple organs at 3 h PHS, with significant histological alterations noted in liver, kidney, and heart relative to the negative control groups. A significant enhancement in liver lesion score was seen in the HSBE group ($p < 0.05$) relative to the HS group. The lesions in the kidneys and hearts of the HSBE group decreased numerically ($p > 0.05$) compared to the HS group.

4. Discussion

Heat stress, a significant climatic concern resulting from global warming, is an area of extensive research. Heat intolerance adversely affects the output, reproduction, and growth of broilers. Tropical and subtropical countries, such as Egypt, encounter significant monetary costs. Exogenous phytogenic antioxidants can mitigate heat stress in broiler chickens (Chen et al. 2021). The current study investigates the effects of BE extract on mitigating the adverse consequences of acute heat stress in broiler chickens.

High ambient temperatures and elevated humidity levels are significant contributors to heat stress in poultry (Ahmad et al. 2022). Most poultry species will undergo heat stress when temperatures exceed 32° C, resulting in physiological and metabolic problems (Hu et al. 2019). Consequently, in alignment with

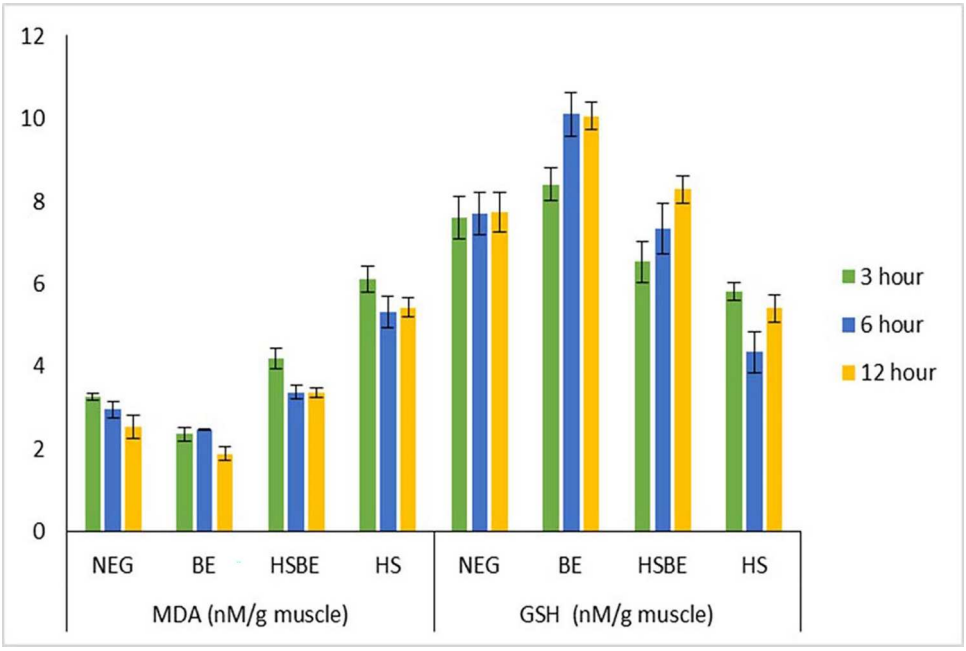

Figure 3. Effect of black elderberry extract on the meat antioxidant capacity of the heat-stressed broilers for 3, 6, and 12 h. Tukey's test represents the least profound differences between different groups at probability $P < 0.05$. MDA: malondialdehyde; GSH: reduced glutathione. NEG: kept at thermoneutral temperature (24 ± 1°C); 2); BE: kept at thermoneutral temperature and treated by intracrop black elderberry extract (0.15 g/L), followed by water medication for 12 h. with the same dose; HSBE: birds subjected to acute heat stress for 6 h and treated with BE intracrop black elderberry extract (0.15 g/L), followed by water medication for 12 h. with the same dose; HS: subjected to heat stress for 6 h

the aforementioned study, the present experiment maintained broiler chickens at a temperature of 38 ± 1°C and a relative humidity of 60 ± 10% for 6 h to effectively cause acute heat stress. It is well known that broiler exposure to approximately 38°C for a few hours causes acute heat stress, which can result in increased body temperature, oxidative imbalance, decreased feed efficiency, and changes to immunological and metabolic processes (Fathi et al. 2025).

The birds in groups HS and HSBE displayed the characteristic clinical signs of heat stress at 3 and 6 h PHS. These findings confirm prior studies, suggesting that changes in bird behavioural and physiological traits are the most reliable indicators of heat stress across all bird species (Goel 2021; Kim et al. 2025). The documented behavioural and physiological changes in this study help the birds to dissipate the heat through evaporative cooling of water in the respiratory tract and superficial blood circulation, owing to the absence of sweat glands in their skin (Hu et al. 2019; Kim et al. 2025)

The rectal temperature is utilized as an indicator of the response to heat stress in broilers (Kim et al. 2025). The heat-stressed groups showed a significant increase in rectal temperature ($p < 0.05$) at 3 and 6 h PHS, in comparison to the control group birds, which aligned with other previous reports (Del Vesco et al. 2020; Kim et al. 2025). This finding is attributed to the reduced rate of heat exchange with the surrounding environment (Goel et al. 2022). The body temperature of birds receiving the BE plant extract showed a slight decrease at 6 h post-heat stress, suggesting that the birds have developed enhanced tolerance to heat stress, attributed to the significant increase in the overall antioxidant capacity in serum and meat of the stressed birds.

The emission of ammonia gas, which is influenced by the moisture content of chicken manure, is one of the most significant environmental concerns that are associated with the production of poultry. Heat stress has been demonstrated to impair digestive functions in chickens by reducing feed intake, elevating water consumption, modifying the intestinal tract's architecture, and affecting patterns of secretory activity, motility, and digesta viscosity. Furthermore, it diminishes feed digestion, enhances the permeability of the digestive system, and causes electrolyte imbalances (Lara and Rostagno 2013; Karl et al. 2018; Xing et al. 2019). This can result in diarrhea, which may cause the chicken to become dehydrated from moisture loss in the

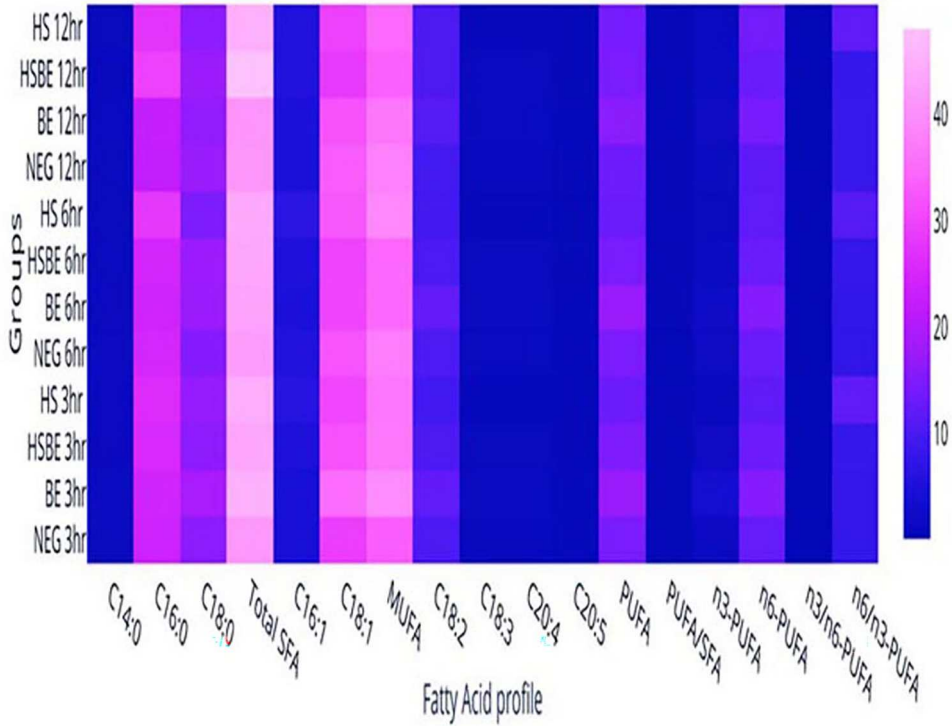

Figure 4. Heat map analysis of the effect of black elderberry extract on fatty acid profile in the meat of heat-stressed broilers for 3, 6, and 12 h. The pink colour indicates an increased level while the blue indicates decreased levels. C14:0 myristic acid, C16:0 palmitic acid, C18:0 Stearic acid, SAF: Saturated fatty acids, C16:1 palmitoleic acid, C18:1 Oleic acid, MUFA: monounsaturated fatty acids, C18: 2 linoleic acid, C18:3 linolenic acid, C20:4 Arachidonic acid, C20:5 eicosapentaenoic acid (EPA), PUFA: polyunsaturated fatty acids,n3-PUFA: Omega 3 polyunsaturated fatty acids, n6-PUFA: Omega 6 polyunsaturated fatty acids. NEG: kept at thermoneutral temperature (24 ± 1°C); 2); BE: kept at thermoneutral temperature and treated by intracrop black elderberry extract (0.15 g/L), followed by water medication for 12 h. with the same dose; HSBE: birds subjected to acute heat stress for 6 h and treated with BE intracrop black elderberry extract (0.15 g/L), followed by water medication for 12 h. with the same dose; HS: subjected to heat stress for 6 h

body (Lambert 2009). The HSBE group had a lower moisture ratio at 6 and 12 h PHS than the HS group. In accordance with these findings, the BE supplementation was found to protect the morphology of the chicken intestine without causing an increase in intestinal permeability or the tendency to cause diarrhea.

The present study indicated that acute heat stress in broiler chickens has resulted in severe pathological lesions in visceral organs, leading to a significant increase in CLS compared to the negative control groups ($P < 0.05$). These results align with previous research that documented cellular and organ damage in heat-stressed animals, attributed to a sequence of physiological responses: increased body temperature, rapid respiration, enhanced blood flow to the body surface, and activation of the antioxidant system (Kim et al. 2025). The administration of BE extract in the drinking water of heat-stressed birds demonstrated a therapeutic effect, as indicated by the decreased cumulative scoring of internal organs. The results may be ascribed to the substantial increase in the overall antioxidant capacity of experimental stressed birds in both serum and meat due to components in BE extract as flavonoids, phenolic acids, and anthocyanins, which directly neutralize free radical species and augment antioxidative enzymes within the cells hence safeguard cells and organs from oxidative damage induced by heat stress (Ahmad et al. 2022; Kolesarova et al. 2022)

The present experiment indicated no significant difference in the relative weights of the liver, heart, and spleen between the heat-stressed groups and the control groups ($p > 0.05$). The results correspond with a previous study that shown no significant effect on relative liver and spleen weights on the 37th day of life when birds experienced 6 h of heat stress (Goel et al. 2022). Although they disputed earlier findings (Shim et al. 2006; Lu et al. 2019). The discrepancies in results regarding the relative body weight of the liver under heat stress may be attributed to variations in the age of the birds, the intensity of heat stress, and the duration of exposure (Goel et al. 2022). The limited variation in the relative weight of the spleen observed in this study

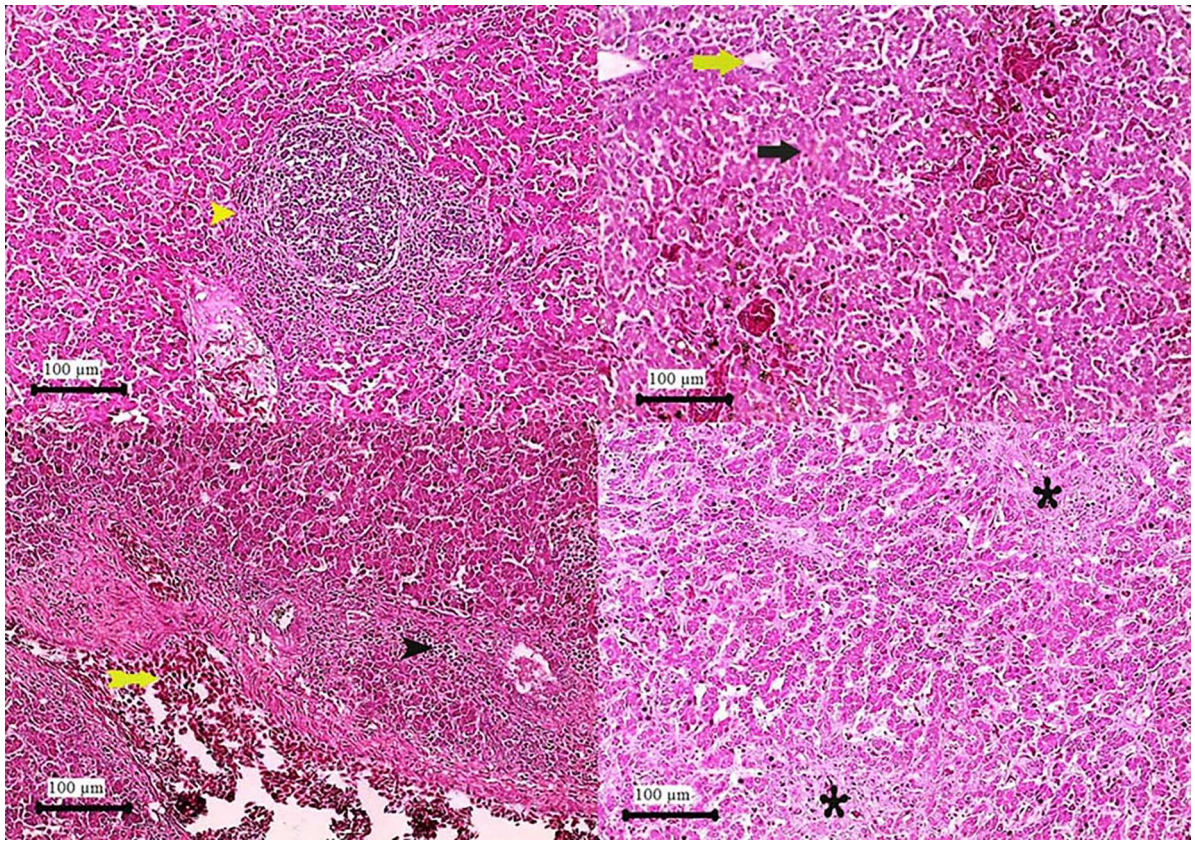

Figure 5. Effect of black elderberry extract on histopathological alteration in the liver of heat-stressed broilers for 3 h. (A) Mild hepatitis characterized by focal lymphocytic cell aggregation (yellow arrowhead) and congestion of hepatic sinusoids; (B) Moderate hepatitis with degeneration (black arrow), multifocal inflammatory cell infiltration, and dilated sinusoids (yellow arrow); (C) Moderate hepatic degeneration featuring focal necrotic areas infiltrated by abundant lymphocytic cells (black arrowhead), congested sinusoids, and hepatic blood vessels exhibiting evident thrombus formation(tailed yellow arrow); (D) Diffuse hepatic necrosis (black stars) accompanied by thrombotic blood vessels, focal lymphocytic cell infiltration, and hemorrhages (H&E, 100x).

may be attributed to the brief duration of acute hemorrhagic shock (Goel et al. 2022). At 12 h PHS in the HSBE group, the kidneys' relative weight matched that of the negative control group. The results can be attributed to the increased serum antioxidant activity observed in the stressed treated birds likely owing to the polyphenols and flavonols found in the BE extract, which could enhance the antioxidative enzymes and downregulate the inflammatory promoters and indicators within the kidney cells (Kolesarova et al. 2022).

Heat stress elevates cellular energy demands, resulting in increased mitochondrial ROS production, which in turn causes oxidative stress. To avoid oxidative damage, the body employs its antioxidant system to neutralize the ROS, including a sophisticated array of both nonenzymatic and enzymatic antioxidants that function synergistically to diminish the harmful effects of ROS. Therefore, both antioxidant enzymes and lipid peroxidation products may be utilized selectively as indicators for heat stress (Mahasneh et al. 2024). In the current study, exposing broiler chickens to acute heat stress for 6 h significantly impaired the activity of serum antioxidants, increased serum MDA, and reduced NO ($p < 0.05$), which aligns with several prior studies (Mujahid et al. 2007; Pirincciogluet al. 2010; Akbarian et al. 2016; Habashy et al. 2019; Yang et al. 2021; Sun 2023; Mahasneh et al. 2024). The administration of BE extract enhanced antioxidant status in the experimental birds subjected to thermal stress at 3 and 6 h compared to the HS group. These findings align with prior research that documented the in vitro antiradical activity of *Sambucus nigra L.* fruits and confirmed its antioxidant capacity (Sidor and Gramza-Michałowska 2015). The BE extract's preventive capacity is likely due to the presence of active chemicals such as polyphenols (anthocyanins) and active flavonols (rutin and quercetin) in the plant, which downregulate inflammatory promoters and indicators, such as MCP-1, NF-κB, and IL-8 (Kolesarova et al. 2022).

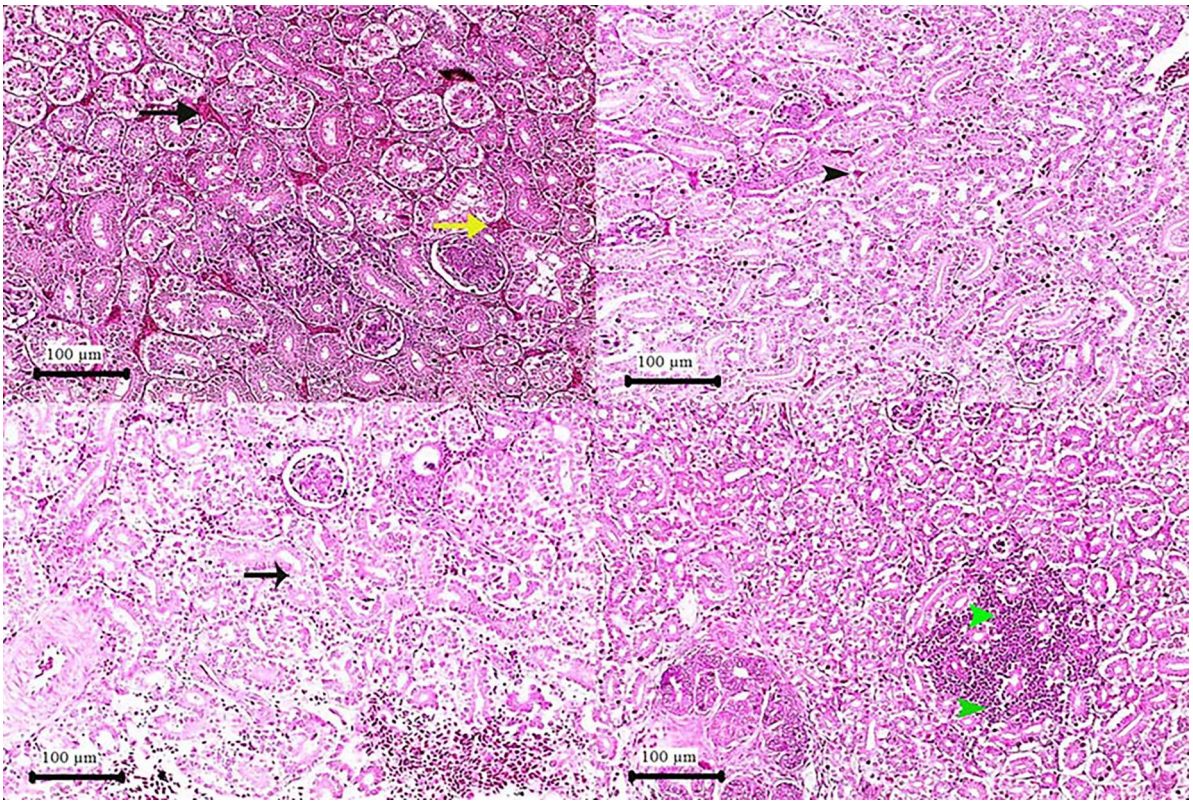

Figure 6. Effect of black elderberry extract on histopathological alteration in the kidney of heat-stressed broilers for 3 h. (A) Mild glomerulonephrosis and tubular degeneration, intertubular hemorrhages (yellow arrow) with focal infiltration of mononuclear inflammatory cells; (B) Mildly degenerated tubules and glomerulonephrosis with focal hemorrhages (black arrowhead); (C) Severe nephropathy characterized by diffuse tubular degeneration (black arrow) and glomerulonephrosis, extensive intertubular hemorrhages with pronounced ureter wall thickening; (D) Severe glomeruloadenopathy with significant intertubular infiltration of lymphocytic and heterophilic cells (green arrowheads) (H&E, 100x).

Heat stress adversely impacts the pH, tenderness, colour, and water-holding capacity of broiler meat, resulting in economic losses and poor customer acceptance (Zhao et al. 2021). The current study indicates that HS decreased the physicochemical quality of meat, aligning with earlier research on pigs (Pardo et al. 2021) and broiler chickens (Zhang et al. 2020). The detrimental effect of HS on meat quality is associated with accelerated postmortem glycolytic metabolism, resulting in increased cooking loss and reduced pH (Zhao et al. 2019). The HS initiates anaerobic glycolysis prior to and following slaughter, resulting in ATP hydrolysis and lactic acid buildup, which decreases pH, reduces water-holding capacity, and leads to pale, soft, exudative meat (Strasburg and Chiang 2009). Our findings demonstrated that the application of BE extract, in both thermoneutral and thermal stress environments, enhanced the overall physicochemical qualities of breast meat. This discovery corresponds with Kamboh and Zhu (2013), who indicated that dietary hesperidin supplementation decreased drip loss in broiler breast muscles. The current findings may be attributed to the BE extract's abundance of flavonoids (anthocyanins), which improve vascular health and act as free radical scavengers, thereby protecting the body from oxidative stress, peroxidative processes, and inflammation, and thus improving meat quality under heat stress (Nakajima et al. 2004; Vlachojannis et al. 2010; Kolesarova et al. 2022).

The HS alters cellular antioxidant-oxidant equilibrium, resulting in oxidative stress, lipid peroxidation, and protein oxidation in skeletal muscles (Gonzalez-Rivas et al. 2020). This study revealed that BE supplementation mitigated the adverse effects of HS on antioxidant enzyme activity in meat. Comparable effects were noted with dietary grape pomace and quercetin supplementation, which augmented antioxidant enzyme activity and decreased lipid oxidation (Hosseini-Vashan et al. 2020). The present results correspond with our findings concerning the improvement in antioxidant activity in the serum of the experimental birds. Furthermore, the active flavonols rutin and quercetin, together with gallic acids in BE can mitigate oxidative

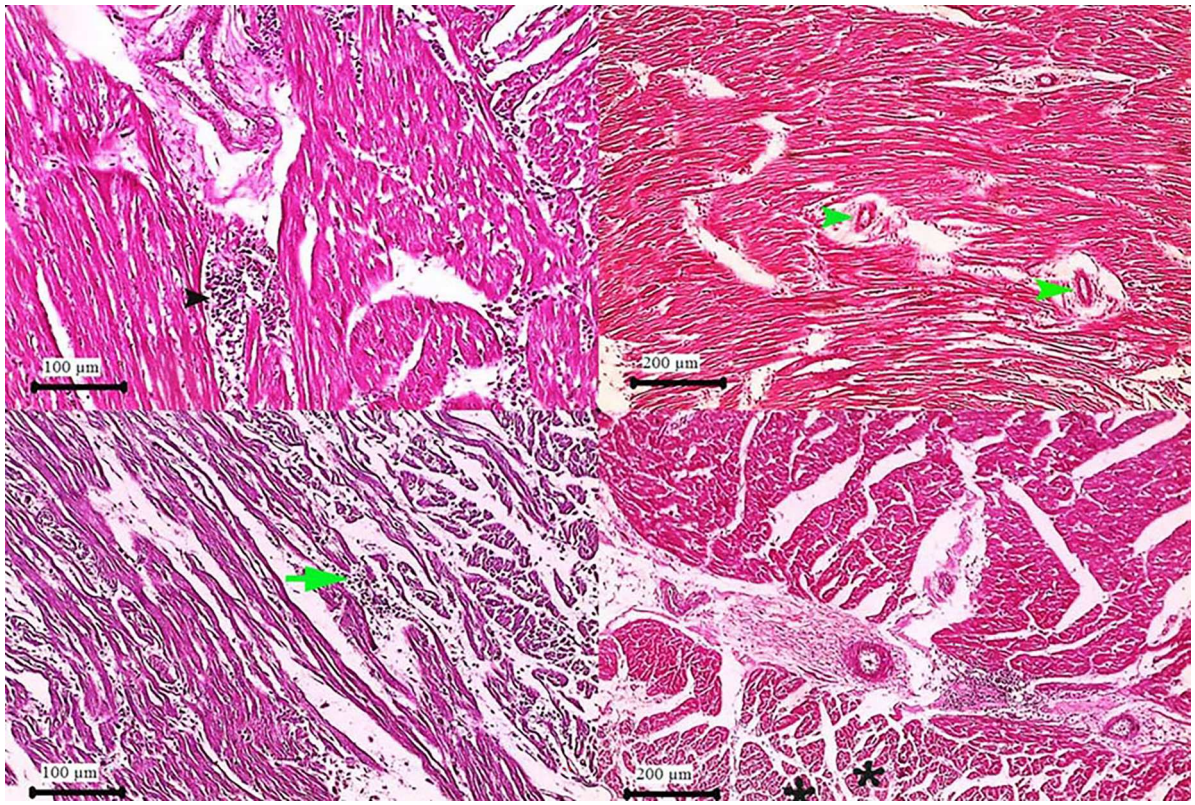

Figure 7. Effect of black elderberry extract on histopathological alteration in the heart of heat-stressed broilers for 3 h. (A) Normal cardiac muscle exhibiting mild edema and congested blood vessels, with focal infiltration of inflammatory cells (mononuclear and heterophilic) (black arrowhead) (100x); (B) Mild myocardial edema accompanied by congested blood vessels (green arrowhead) (200x); (C) Mild myocarditis characterized by lymphocytic cell infiltration (green arrow) and myocardial edema (100x); (D) Severe pericarditis and myocarditis with extensive lymphocytic cell infiltration and focal myocardial necrosis (black stars) (200x).

stress and safeguards genomic DNA integrity, hence maintaining muscle integrity and meat quality (Goliomytis et al. 2015; Kolesarova et al. 2022)

The composition of fatty acids is essential for meat quality. Polyunsaturated fatty acids, such as linoleic and linolenic acids, affect lipid metabolism, blood cholesterol levels, and cardiovascular health (Xu et al. 2022). This study demonstrated that HS markedly decreased ALA, C18:3-n3, and EPA, C20:5, attributable to its capacity to activate stress-related metabolic pathways (Kuo et al. 2013). The BE extract supplementation enhanced the fatty acid composition of broiler meat during heat stress, consequently improving flavour and nutritional quality. These findings are consistent with prior research indicating that rutin supplementation enhances n3- and n6-PUFAs in broiler breast muscle (Li et al. 2023). The improvement in the fatty acid profile in our study may have resulted from upregulation of Δ5 and Δ6 desaturase enzymes (Kühn et al. 2018).

This study showed that acute heat stress caused notable histological changes in visceral organs compared to those maintained in the thermoneutral zone. The findings align with earlier studies that indicate diverse pathological alterations in the liver, kidney, and heart of heat-stressed broiler chickens (Rebez et al. 2023). The observed pathological alterations may be attributed to the documented increase in body temperature and the decrease in serum and meat antioxidant activity in the experimental birds. Furthermore, an increase in mitochondrial ROS can lead to oxidative damage, lipid peroxidation, and protein oxidative modification in tissues (Gonzalez-Rivas et al. 2020). Treatment with the BE extract had shown a significant therapeutic effect on liver tissues compared to the HS group. The degenerative changes in kidney and cardiac tissues were numerically reduced in this group relative to the HS group. The improvement in histological evaluation of the visceral organs aligns with our recent findings of decreased CLS and elevated antioxidant activity in the blood and meat of treated stressed chickens. Furthermore, the BE extract has a considerable

concentration of active flavonols rutin and quercetin, along with gallic acids, which are able to directly neutralize free radical species, thereby mitigating oxidative stress and inflammation and improving vascular health (Sidor and Gramza-Michałowska 2015; Kolesarova et al. 2022).

This study was designed to assess the preventive role of black elderberry extract in acute heat stress situations; thus, the findings cannot be immediately applied to chronic heat stress scenarios, which may have distinct physiological effects. Furthermore, just one dose of BE extract was evaluated, and dose-response connections need to be investigated. Finally, no molecular or microbial investigations were conducted, limiting the mechanistic interpretation of the reported effects. Future research on these topics will provide a more complete picture of the possible applications of BE in poultry agriculture.

5. Conclusion

Understanding and managing high ambient temperatures is crucial for tropical and subtropical chicken productivity and welfare. The BE extract reduced clinical and behavioural deficits caused by heat stress, reduced macroscopic and microscopic visceral organ damage, increased serum and meat antioxidant activity, and improved broiler meat fatty acid profile and quality. These findings lay the groundwork for further study on BE extract's antioxidant properties in alleviating heat stress in conjunction with traditional management methods to improve their efficacy.

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Consent to publish

The Author allowed the publisher to publish the work.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

All data are represented in the manuscript and supplementary materials are available upon request.

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