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Influence of Fertilizer Regimes on the Antioxidant and Hepatic Biochemical Effects of *Corchorus olitorius* in Potassium Bromate-Challenged Wistar Rats

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Abstract

This study evaluated whether fertilizer regimes modify the biochemical and physiological effects of *Corchorus olitorius* leaf diets in potassium bromate ($KBrO_3$)-challenged Wistar rats. Forty-two male rats were assigned to seven groups (n = 6): negative control, $KBrO_3$ positive control, untreated *C. olitorius*, and leaves cultivated with bat dung (BD100), cow manure (CM100), poultry manure (PM100), or synthetic fertilizer (SF100). Fertilizer treatment significantly altered leaf proximate composition, with crude protein increasing from 22.10% in untreated leaves to 24.96, 24.92, and 24.30% in BD100-, PM100-, and SF100-treated leaves, respectively, whereas qualitative phytochemical profiles were similar across treatments. Rats received a single oral dose of $KBrO_3$ (100 mg/kg body weight), followed by 28 days of diets containing 20% leaf powder. Body weight, serum and hepatic oxidative-stress markers, antioxidant indices, and liver-function parameters were determined. $KBrO_3$ reduced final body weight by 12.9%, from 135.22±4.11 g in the negative control to 117.84±7.20 g in the positive control ($p < 0.05$). Relative to the positive control, SF100 and CM100 increased body weight by 10.3 and 9.4%, respectively, reaching 129.97±2.06 and 128.86±3.42 g ($p < 0.05$). Serum MDA and SOD did not differ significantly among $KBrO_3$-treated groups ($p > 0.05$). However, SF100 increased serum GSH by 11.6%, from 54.41±6.41 to 60.71±9.57 µmol/mL ($p < 0.05$), while CM100 increased hepatic SOD activity by 37.4%, from 59.21±6.50 to 81.38±9.26 U/mg protein ($p < 0.05$). SF100 increased hepatic CAT activity by 7.0%, although this difference was not significant relative to the positive control. GGT, total bilirubin, and direct bilirubin decreased by 41.1, 25.5, and 23.8%, respectively, in the SF100 group, but these changes were not statistically significant ($p > 0.05$). Conversely, SF100 increased ALT, AST, and ALP by 81.6, 40.8, and 23.8%, respectively ($p < 0.05$). The biological responses varied according to the fertilizer treatment. These findings indicate that fertilizer regimes modified the biological responses associated with consumption of *C. olitorius* leaves, particularly body-weight maintenance and selected antioxidant indices.

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1.0 Introduction

Leafy vegetables form a cornerstone of diets across Africa, valued for both culinary appeal and their abundant nutrients and phytochemicals (Lugumira et al., 2025). *Corchorus olitorius* (jute mallow), a widely consumed indigenous vegetable, stands out for its rich vitamins, minerals, polyphenols, flavonoids, and dietary fiber, which deliver antioxidant, anti-inflammatory, and hepatoprotective benefits (Alege et al., 2025). Yet, the plant's composition hinges on agricultural practices, particularly fertilizer type, as these alter soil nutrient availability, microbial activity, and pH, directly influencing metabolism, secondary metabolite production, and nutrient uptake (Ewulo et al., 2025). Amid pushes for sustainable farming, organic options like poultry manure, cow dung, and bat guano gain traction for their slow-release macro- and micronutrients, while synthetic fertilizers offer rapid nitrogen, phosphorus, and potassium availability. These differences modulate phytochemicals, antioxidants, and anti-nutritional factors in vegetables (Verardi et al., 2025). However, organic fertilizers vary in quality; bat guano, for instance, provides nitrogen and minerals but risks microbial or heavy metal contamination based on collection conditions (Verardi et al., 2025). Synthetic fertilizers, though efficient, can elevate nitrate levels and shift oxidative profiles (Nguyen et al., 2025). Such fertilizer-associated variations in plant composition may influence physiological responses following dietary consumption (Latif & Nawaz, 2026), thereby making it important to evaluate whether fertilizer practices influence the biological effects associated with dietary consumption on vegetable health effects. Liver function and oxidative stress markers merit special focus, as the liver serves as the main detoxification and antioxidant hub, rendering it susceptible to toxicants like therapeutics, environmental pollutants, and industrial chemicals (Al-Tamimi et al., 2026; Ogidi & Omabemituale, 2025).

Potassium bromate ($KBrO_3$), used for over 90 years as an oxidizing agent, maturing agent, flavor enhancer, and antiepileptic in food production to improve dough pliability and bread volume (Ma et al., 2025), proves nephrotoxic, neurotoxic, genotoxic, and carcinogenic in animal safety studies. It triggers lipid peroxidation (LPO) and oxidative DNA damage (Agu et al., 2023; Al-Mareed et al., 2022; Kamel et al., 2023), with emerging evidence linking it to hepatotoxicity via oxidative stress and inflammation (Aminjan et al., 2019).

Previous investigations of *Corchorus olitorius* have largely followed two separate lines of inquiry. Agronomic studies have examined how cultivation conditions and nutrient sources influence plant growth, yield, nutritional composition and phytochemical content. For example, fertilizer and cultivation treatments have been reported to alter the concentrations of phenolic compounds, flavonoids, vitamins, minerals and other bioactive constituents of *C. olitorius* and related leafy vegetables (Alege et al., 2025; Nguyen et al., 2025). These studies demonstrate that agricultural inputs can modify the chemical and nutritional quality of edible leaves, but they generally do not establish whether such compositional changes translate into measurable physiological effects after consumption.

Conversely, animal studies have demonstrated the biological activity of conventionally grown or unspecified *C. olitorius* preparations without considering fertilizer history. Das et al. (2010) reported that an aqueous leaf extract protected rats against sodium arsenate-induced oxidative injury, while Saliu et al. (2019) showed that dietary jute-leaf supplementation improved hepatic antioxidant status in diabetic rats. Other studies have similarly associated *C. olitorius* consumption with antioxidant, metabolic, hepatoprotective and nephroprotective effects. However, these investigations did not compare leaves produced under different fertilizer

regimes and therefore could not determine whether agronomic practices modify the plant's biological efficacy.

The toxicological basis of the present model is supported by established evidence that oxidative stress results from an imbalance between reactive species production and antioxidant defence systems (Halliwell & Gutteridge, 2015). Potassium bromate has been shown to promote reactive oxygen species generation, lipid peroxidation, glutathione depletion, impairment of antioxidant enzymes and oxidative DNA damage. Ahmad and Mahmood (2012) demonstrated that oral $KBrO_3$ exposure disrupted blood antioxidant defence in rats, Bayomy et al. (2016) documented biochemical and histological liver injury following $KBrO_3$ administration, and Al-Mareed et al. (2022) confirmed dose- and time-dependent oxidative stress, genotoxicity and cytotoxicity in experimental animals.

The principal knowledge gap is therefore not whether fertilizer application can alter the chemical quality of *C. olitorius* or whether the plant can exert antioxidant effects independently. Rather, it is whether leaves produced under different fertilizer regimes elicit different physiological and biochemical responses when consumed under a defined toxicological challenge. The present study advances existing knowledge by directly linking an agronomic variable, cultivation with bat dung, cow manure, poultry manure or synthetic fertilizer, to physiological and biochemical outcomes in $KBrO_3$-challenged Wistar rats. Accordingly, rats in each experimental group (n = 6) received a single oral dose of $KBrO_3$ at 100 mg/kg body weight, followed by 28 days of diets fortified with 20% untreated or fertilizer-treated *C. olitorius* leaves. Body-weight maintenance, lipid peroxidation, antioxidant indices, including MDA, SOD, CAT, GSH and GST, and liver-function markers, including ALT, AST, ALP, GGT and bilirubin, were evaluated to determine whether fertilizer regime modifies the in vivo biological effects of the leaves.

We hypothesized that *C. olitorius* leaves produced under different fertilizer regimes would elicit distinguishable antioxidant and hepatic biochemical responses in $KBrO_3$-challenged rats.

2.0 Materials and Methods

2.1 Materials

Corchorus olitorius seeds were obtained from Faculty of Agriculture, Kwara State University, Malete, Kwara State, Nigeria. Cow manure, poultry manure and bat droppings were obtained from local cattle herds, poultry farms and a cave in Faso Village, Edati Local Government Area, Niger State, Nigeria, respectively, while the synthetic fertilizer (Urea N 46%+) was manufactured by Dangote Fertilizer Limited, Lagos, Nigeria. Standard commercial rat feed was supplied by Olam Chikun and Ultima Feed, Lagos, Nigeria. Male Wistar rats (*Rattus norvegicus*) were obtained from Animal House, University of Ilorin, Kwara State, Nigeria.

Potassium bromate, potassium chloride, sodium chloride, potassium phosphate, Tris buffer, trichloroacetic acid, thiobarbituric acid, sulphosalicylic acid, sodium azide, hydrogen peroxide, reduced glutathione, xanthine, xanthine oxidase, 5,5′-dithiobis-(2-nitrobenzoic acid), potassium dichromate and acetic acid were of analytical grade and purchased from [manufacturer/supplier, city, country]. All solutions were prepared using distilled water.

A UV - visible spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan), refrigerated centrifuge (Centrifuge 5804 R, Eppendorf SE, Hamburg, Germany), ultrasonic homogenizer (UP200St, Hielscher Ultrasonics GmbH, Teltow, Germany), mixer grinder (MX-AC400, Panasonic Life Solutions India Pvt. Ltd., India), and analytical balance (Pioneer PX224, OHAUS Corporation, Parsippany, NJ, USA) were used for sample preparation and biochemical analyses.

2.2 Planting and nursery management

The experimental setup consisted of fifteen field pots arranged in a completely randomized design (CRD) with three replications of five treatments: control (no fertilizer application), CM100 (100 g cow dung manure), PM100 (100 g poultry manure), BD100 (100 g bumblebee bat droppings), and SF100 (100 g synthetic fertilizer). About ten *Corchorus olitorius* seeds were planted in 10 kg bags of soil containing the different soil treatments and thinned to two seedlings per pot after germination. The plants were irrigated twice daily, morning and evening, while weeds were removed regularly to reduce competition and maintain a clean experimental environment. The pots were lifted periodically to prevent root penetration beyond the containers. Leaves of *C. olitorius* were harvested at marketable vegetative stage.

Cow manure, poultry manure, bat droppings and synthetic fertilizer were each applied at 100 g per 10 kg of soil. The total nitrogen, available phosphorus, potassium, organic carbon, pH, moisture content and micronutrient compositions of the fertilizer materials were not determined. Thus, the treatments were standardized by application mass rather than nutrient equivalence, which limits exact reproduction and nutrient-specific interpretation of the findings.

2.3 Proximate composition of *Corchorus olitorius* leaves

The proximate composition of the dried *C. olitorius* leaf samples was determined in triplicate using standard procedures described by AOAC (2023). Leaf powder obtained from each fertilizer treatment was analysed separately for moisture, ash, crude protein, crude fat and crude fibre contents. Dried leaf powders were stored in labelled airtight containers at room temperature, protected from direct light and moisture until diet preparation. Moisture content was determined by drying 2.0 g of each powdered sample in a hot-air oven at 105 °C until a constant weight was obtained. The samples were cooled in a desiccator and reweighed. Moisture content was calculated from the loss in sample weight during drying:

$$Moisture\ content\ (\%)=\frac{W_1-W_2}{W_1}$$

where (W_1) is the initial sample weight and (W_2) is the weight after drying. Dry matter was calculated as:

$$Dry\ matter\ (\%)=100-Moisture\ content\ (\%)$$

Ash content was determined by incinerating 2.0 g of each sample in a pre-weighed porcelain crucible at 550 °C in a muffle furnace until a light-grey or white ash of constant weight was obtained. The crucibles were cooled in a desiccator before weighing. Ash content was expressed as a percentage of the original sample weight.

$$Ash=\frac{Weight\ of\ ash}{initial\ sample\ weight}\times 100$$

Crude protein was determined using the Kjeldahl method. The samples were digested with concentrated sulfuric acid in the presence of a suitable catalyst until a clear digest was obtained. The digest was neutralized with sodium hydroxide, and the liberated ammonia was distilled into boric acid solution and titrated with standardized hydrochloric acid. Total nitrogen was calculated from the titre value, while crude protein was estimated using a nitrogen-to-protein conversion factor of 6.25:

$$Crude\ protein\ (\%)=Total\ nitrogen\ (\%)\times 6.25$$

Crude fat was determined by continuous Soxhlet extraction of the dried samples with petroleum ether. Following extraction, the solvent was removed, and the receiving flask containing the extracted lipid was dried, cooled and weighed. Crude-fat content was calculated as the percentage weight of the extracted lipid relative to the original sample.

$$Crude\ fat\ (\%)=\frac{Weight\ of\ extracted\ lipid}{Original\ sample\ weight}\times 100$$

Crude fibre was determined by sequential digestion of the defatted sample with dilute

sulfuric acid and sodium hydroxide solutions. The remaining insoluble residue was filtered, washed, dried and weighed before incineration in a muffle furnace. Crude-fibre content was calculated from the difference between the dried residue and its ash weight. Total carbohydrate was estimated by difference:

$Carbohydrate$ (%)=100-[$Moisture$ (%)+Ash (%)+$Crude\ protein$ (%)+$Crude\ fat$ (%)+$Crude\ fibre$ (%)]

Results were expressed as mean ± standard deviation of three independent determinations.

2.4 Qualitative phytochemical profile of *Corchorus olitorius* leaves

Qualitative phytochemical screening of the powdered *C. olitorius* leaves was performed using standard procedures described by Harborne (1998), Sofowora (2008), and Evans (2009). Five grams of each powdered leaf sample were extracted with 50 mL of 80% methanol by intermittent agitation for 24 h. The mixtures were filtered through Whatman No. 1 filter paper, and the resulting extracts were used for the phytochemical tests. Each treatment was analysed in triplicate.

Alkaloids: A portion of the extract was acidified with dilute hydrochloric acid and filtered. The filtrate was treated separately with Mayer's and Dragendorff's reagents. Formation of a cream-coloured precipitate with Mayer's reagent or an orange-brown precipitate with Dragendorff's reagent indicated the presence of alkaloids.

Phenolic compounds: The extract was treated with freshly prepared ferric chloride solution. Formation of a blue, green, violet or dark coloration indicated the presence of phenolic compounds.

Glycosides: Glycosides were assessed using the Keller - Killiani reaction. The extract was mixed with glacial acetic acid containing ferric chloride, followed by the careful addition of concentrated sulfuric acid. Development of a brown ring at the interface indicated the presence of deoxy-sugar-containing glycosides.

Saponins: The extract was mixed vigorously with distilled water and allowed to stand. Formation of a stable, persistent froth indicated the presence of saponins.

Flavonoids: The extract was treated with diluted sodium hydroxide solution. Development of an intense yellow colour that disappeared following the addition of diluted acid indicated the presence of flavonoids.

Steroids: The extract was mixed with chloroform and acetic anhydride, followed by the careful addition of concentrated sulfuric acid. Development of a blue-green or green coloration indicated the presence of steroids.

Phlobatannins: The extract was heated with diluted hydrochloric acid. Formation of a red precipitate indicated the presence of phlobatannins.

Terpenoids: The Salkowski test was used for terpenoids. The extract was mixed with chloroform, after which concentrated sulfuric acid was carefully introduced to form a lower layer. Formation of a reddish-brown coloration at the interface indicated the presence of terpenoids.

The intensity of each visible reaction was recorded semi-quantitatively as strong (++), moderate (+), or not detected (−). These ratings represented qualitative reaction intensities and were not interpreted as absolute phytochemical concentrations.

2.5 Preparation of *C. olitorius*-fortified feed

The freshly harvested leaves were washed with distilled water and shade-dried at ambient temperature until a constant weight was obtained over approximately 7 days and pulverized into powder using a mixer grinder. The pelleted commercial rat feed was ground to ease incorporation of the leaf powder, and the experimental diets were prepared by mixing *C. olitorius* leaf powder with the normal rat feed to obtain a 20% (w/w) plant-based formulation, following previously described method (Zvinorova et al., 2015). For each experimental

diet, 20 g of the appropriate leaf powder was thoroughly mixed with 80 g of ground commercial feed to produce a 20% w/w formulation. Fresh diets were prepared daily to minimize nutrient degradation and were offered in clean feeding containers.

Each fertilizer treatment was represented by three independent cultivation pots. Leaves harvested from the three replicate pots within each fertilizer treatment were pooled before preparation of the experimental diet. In the animal phase, the six individual rats assigned to each dietary group constituted the biological replicates. Field pots and experimental animals therefore represented different levels of replication and were not treated as interchangeable replicates.

2.6 In vivo experimental design

Forty-two male Wistar rats (*Rattus norvegicus*) with an average body weight of 127.50±19.06 g were obtained and housed in well-ventilated plastic cages in the Animal Holding Unit of the Faculty of Pure and Applied Sciences, Kwara State University. The animals were acclimatized for one week before the experiment, maintained under standard temperature and humidity conditions, and exposed to a 12-h light/dark cycle with free access to water and standard commercial rat feed. Following acclimatization, the rats were weighed, assigned identification numbers and allocated to the seven experimental groups using stratified allocation according to baseline body weight. Six rats were assigned to each group. Six rats were assigned to each group.

Ethical approval for the animal experiment was obtained from the Centre for Research and Development of Kwara State University, Malete, under approval number KWASU/CR&D/REA/202/0213, dated 6th February 2026. All procedures were conducted in accordance with the approved protocol and relevant institutional guidelines for the care and use of laboratory animals.

The rats were randomly assigned to seven groups, with six animals per group, as follows:

- Negative control (NC): distilled water only and commercial feed without supplementation.
- Positive control (PC): 0.2 mL of freshly prepared $KBrO_3$ at 100 mg/kg body weight orally, followed by commercial feed only.
- Untreated jute group: 0.2 mL of freshly prepared $KBrO_3$ at 100 mg/kg body weight orally, followed by commercial feed fortified with 20% (w/w) untreated *C. olitorius*.
- Feed + BD100 group: 0.2 mL of $KBrO_3$ at 100 mg/kg body weight orally, followed by commercial feed fortified with 20% (w/w) BD100.
- Feed + CM100 group: 0.2 mL of $KBrO_3$ at 100 mg/kg body weight orally, followed by commercial feed fortified with 20% (w/w) CM100.
- Feed + PM100 group: 0.2 mL of $KBrO_3$ at 100 mg/kg body weight orally, followed by commercial feed fortified with 20% (w/w) PM100.
- Feed + SF100 group: 0.2 mL of $KBrO_3$ at 100 mg/kg body weight orally, followed by commercial feed fortified with 20% (w/w) SF100.

A single oral dose of $KBrO_3$ at 100 mg/kg body weight was selected to produce an acute oxidative challenge based on previous rodent studies reporting measurable oxidative and biochemical disturbances at comparable doses (Al-Mareed et al., 2022; Umemura et al., 2004). The dose was administered once rather than repeatedly to permit assessment of dietary responses during the subsequent 28-day period.

Fresh diets were prepared daily to minimize nutrient degradation by incorporating *C. olitorius* leaf powder into the ground commercial rat feed at 20% (w/w) as previously

described (Zvinorova et al., 2015). Treatments were administered daily for 28 days. Body weights were recorded at the end of the experimental period, and the animals were sacrificed 24 h after the last treatment according to the ethical guideline of the American Veterinary Medical Association. Animals were anaesthetized with diethyl ether before blood collection and sacrifice in accordance with the approved institutional protocol, and blood was collected by cardiac puncture. The livers were excised, rinsed in saline, and stored at 4 °C for subsequent analyses.

No formal a priori sample-size calculation was performed. Six rats per group were used in this exploratory study, giving a total of 42 animals. The group size was selected to permit preliminary comparison of the experimental diets while limiting animal use.

2.7 Preparation of tissue homogenate

Liver homogenates were prepared as previously described (Höring et al., 2021). Briefly, 1 g of liver tissue was homogenized in 10 mL of ice-cold 1.15% potassium chloride solution and 50 mM potassium phosphate buffer (pH 7.4) to produce a 10% (w/v) homogenate. The tissues were washed with 0.9% NaCl solution and homogenized using an ultrasonic homogenizer. The homogenate was centrifuged at 4,000 rpm for 15 min at 4 °C, and the supernatant was collected for further analyses.

2.8 Assessment of in vivo lipid peroxidation

Lipid peroxidation in plasma and liver homogenates was assessed (Airaodion et al., 2019). Malondialdehyde (MDA), an end product of lipid peroxidation, was quantified using thiobarbituric acid reactive substances (TBARS). In brief, 0.4 mL of sample was mixed with 1.6 mL of Tris-KCl buffer and 0.5 mL of 30% trichloroacetic acid (TCA), followed by the addition of 0.5 mL of 0.75% thiobarbituric acid (TBA). The reaction mixture was heated in a water bath at 80 °C for 45 min, cooled on ice, and centrifuged at 3,000 × g for 5 min. The absorbance of the supernatant was measured at 532 nm against distilled water as blank. Lipid peroxidation was expressed as units/mg protein using a molar extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$.

2.9 Estimation of in vivo antioxidant activities

Enzymatic antioxidants, including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), as well as the non-enzymatic antioxidant glutathione (GSH), were determined using the previously described methods (Airaodion et al., 2019).

For SOD determination, tissues were homogenized on ice in 7 mL of cold 1× lysis buffer per gram of tissue and centrifuged at 12,000 × g for 10 min. The supernatant was stored at −80 °C until analysis. Superoxide anions generated by the xanthine/xanthine oxidase system were detected using chromogen solution, and absorbance was read at 490 nm. SOD activity was expressed as the percentage inhibition of chromogen reduction.

Catalase activity was determined using an assay mixture containing 4 mL of H_2O_2 solution and 5 mL of phosphate buffer (0.01 M, pH 7.0). One milliliter of diluted sample (1:10) was rapidly added at room temperature. At 60-second intervals, 1 mL aliquots of the reaction mixture were transferred into 2 mL of dichromate/acetic acid reagent (1:3 v/v). After heating in a boiling water bath for 10 min, the chromic acetate formed was measured at 570 nm. Catalase activity was expressed as mol H_2O_2 consumed/min/mg protein.

GPx activity was measured by incubating 500 µL of tissue homogenate with 500 µL assay buffer (30 mM potassium phosphate, pH 7.0), 100 µL sodium azide (10 mM), 200 µL reduced glutathione (4 mM), 100 µL hydrogen peroxide (2.5 mM), and 6 mL distilled water at 37 °C for 3 min. Then, 0.5 mL of 10% TCA was added and the mixture centrifuged at 3,000 rpm for 5 min. One milliliter of supernatant was mixed with 2

mL of 0.3 M K_2HPO_4 and 1 mL DTNB, and absorbance was measured at 412 nm.

Reduced glutathione (GSH) concentration was estimated after deproteinizing tissue homogenates with 0.15 M sulphosalicylic acid (1:1, v/v). The precipitated proteins were removed by centrifugation at 4,000 × g for 5 min. Thereafter, 0.5 mL of the supernatant was mixed with 4.5 mL of 0.001 M Ellman's reagent (DTNB). Absorbance was measured at 412 nm against a blank containing diluted deproteinizing agent and DTNB. GSH concentration was calculated from a standard calibration curve prepared with GSH standards.

2.10 Statistical analysis

All data were analyzed using SPSS version 21 and expressed as mean of three replicates ± standard deviation. One-way analysis of variance (ANOVA) was used, and significant differences among means were separated using Duncan's Multiple Range Test at $p < 0.05$. The negative control was compared but not shown in some tables for brevity.

3.0 Results

3.1 Proximate Composition of *Corchorus olitorius* Leaves

The proximate composition of *C. olitorius* leaves differed significantly among the fertilizer treatments (Table 1). Moisture content increased from 12.70±0.57% in the untreated control to 13.16–16.34% in the fertilizer-treated samples, with PM100 recording the highest numerical value. SF100 had the highest ash content (3.62±0.16%) and was significantly higher than the untreated control and PM100, while BD100 and CM100 showed intermediate values.

All fertilizer treatments produced significantly higher crude-protein contents than the untreated control (22.10±0.14%). The highest values were recorded for BD100 and PM100 at 24.96±0.73 and 24.92±1.04%, respectively. Crude-fat content was also significantly increased by fertilizer application, with BD100 and SF100 recording the highest values of 4.01±0.27 and 3.94±0.24%, respectively, compared with 2.74±0.20% in the control.

Table 1: Effects of fertilizer treatments on the proximate composition of *Corchorus olitorius* leaves

Fertilizer Type	Moisture (%)	Ash (%)	Crude Protein (%)	Crude Fat (%)	Crude Fibre (%)	Carbohydrate (%)	Dry Matter (%)
Control	12.70±0.57^{c}	2.63±0.11^{b}	22.10±0.14^{c}	2.74±0.20^{c}	2.02±0.05^{a}	57.82±0.84^{a}	87.30±0.57^{a}
Bat Droppings (BD_{100})	13.16±0.76^{b}	3.05±0.30^{ab}	24.96±0.73^{a}	4.01±0.27^{a}	2.52±0.21^{a}	52.30±0.68^{b}	86.84±0.76^{ab}
Cow Dung (CD_{100})	14.81±1.54^{a}	3.05±0.25^{ab}	23.93±0.92^{ab}	3.67±0.19^{ab}	2.39±0.21^{a}	52.26±2.93^{b}	85.19±1.54^{ab}
Poultry Manure (PM_{100})	16.34±0.64^{a}	2.39±0.17^{b}	24.92±1.04^{a}	3.49±0.35^{b}	2.22±0.11^{a}	50.65±1.25^{b}	83.67±0.64^{b}
Synthetic Fertilizer (SF_{100})	14.47±1.20^{a}	3.62±0.16^{a}	24.30±0.92^{a}	3.94±0.24^{a}	2.48±0.13^{a}	51.19±1.89^{b}	85.53±1.20^{ab}

Values are presented as mean ± SD of 3 replicates. Within each column, means bearing different superscript letters differ significantly at $p < 0.05$.

Crude-fibre content did not differ significantly among the treatments. In contrast, carbohydrate content was significantly lower in all fertilizer-treated leaves, ranging from 50.65±1.25 to 52.30±0.68%, compared with 57.82±0.84% in the untreated control. Dry matter was lowest in PM100-treated leaves and differed significantly from the control, whereas the other fertilizer

treatments showed intermediate values. These results demonstrate that fertilizer treatment altered the proximate composition of *C. olitorius* leaves, particularly their protein, fat, carbohydrate, moisture, and ash contents.

3.2 Qualitative Phytochemical Profile of *Corchorus olitorius* Leaves

Qualitative phytochemical screening showed a similar profile across the untreated and fertilizer-treated *C. olitorius* leaves (Table 2). Alkaloids and phenolic compounds were detected at moderate levels, whereas saponins and flavonoids showed comparatively stronger reactions in all samples. Glycosides, steroids, phlobatannins, and terpenoids were not detected under the assay conditions.

No treatment-dependent variation was observed in the qualitative occurrence or relative reaction intensity of the screened phytochemical groups. Thus, although the leaves contained potentially bioactive classes of compounds, the qualitative screening did not demonstrate that fertilizer application altered their phytochemical abundance.

Table 2: Heat map of the phytochemical composition of Corchorus olitorius leaves under different fertilizer treatments

Fertilizer Type	Alkaloid	Phenol	Glycoside	Saponin	Flavonoid	Steroid	Phlobatannin	Terpenoid
Control	+	+	-	++	++	-	-	-
Bat Droppings (BD_{100})	+	+	-	++	++	-	-	-
Cow Dung (CD_{100})	+	+	-	++	++	-	-	-
Poultry Manure (PM_{100})	+	+	-	++	++	-	-	-
Synthetic fertilizer (SF_{100})	+	+	-	++	++	-	-	-

Dark green (++) = high concentration; light green (+) = moderate concentration; red (–) = not detected

3.3 Rat body weight

Mean final body weight differed across dietary groups (Table 3). The positive control group recorded the lowest body weight (117.84±7.20 g), while the untreated jute and Feed + PM100 groups showed comparable values of 117.25±1.36 and 119.33±3.02 g, respectively. Rats fed diets fortified with BD100 and CM100 recorded higher mean body weights of 124.33±1.47 and 128.86±3.42 g, respectively, whereas the Feed + SF100 group showed the highest body weight among the treated groups. Body weight in the negative control group was significantly higher than in all $KBrO_3$-treated groups, confirming successful induction of toxicity (Table 3). Dietary supplementation with *Corchorus olitorius* leaves grown under different soil treatments influenced serum oxidative stress markers and antioxidant enzyme activities in $KBrO_3$-treated rats. MDA levels ranged from 5.14±0.10 µmol/L in the untreated jute group to 6.11±0.53 µmol/L in the Feed + BD100 group, with no significant differences among the treatment groups (Table 4).

Table 3: Effects of different fertilizer-treated *Corchorus olitorius* leaf diets on body weight in $KBrO_3$-induced rats (Mean ± SD)

Treatment Group	Treatment Description	N	Body Weight (g)
Negative Control	Distilled water only + commercial feed	6	135.22±4.11^{c}
Positive Control ($KBrO_3$ only)	$KBrO_3$ (100 mg/kg) + commercial feed	6	117.84±7.20^{a}
Control-Jute	$KBrO_3$ + untreated *C. olitorius*	6	117.25±1.36^{a}
Feed＋BD_{100}	$KBrO_3$ + bat dung-treated jute	6	124.33±1.47^{b}
Feed＋CM_{100}	$KBrO_3$ + cow manure-treated jute	6	128.86±3.42^{b}
Feed＋PM_{100}	$KBrO_3$ + poultry manure-treated jute	6	119.33±3.02^{a}
Feed＋SF_{100}	$KBrO_3$ + synthetic fertilizer-treated jute	6	129.97±2.06^{c}

Values represent Mean ± SD. Means with different superscripts within a column are significantly different ($p < 0.05$) according to Duncan's multiple range test

3.4 Serum oxidative stress markers

Similarly, SOD activity varied from 17.69±1.54 U/mL in the positive control group to 21.34±1.44 U/mL in the Feed + PM100 group, but the differences were not significant. In contrast, CAT activity differed significantly among groups, ranging from 45.47±2.06 U/mL in the Feed + CM100 group to 49.73±1.98 U/mL in the Feed + PM100 group. GSH levels varied from 34.49±2.10 to 60.71±9.57 µmol/mL, with the Feed + SF100 group recording the highest value. GST activity ranged from 0.21±0.02 to 0.29±0.02 µmol/mL, with the highest value observed in the Feed + SF100 group. Overall, treatment-related variation was evident for CAT, GSH, and GST, whereas MDA and SOD remained statistically similar among groups (Table 4).

Table 4: Effects of different fertilizer-treated *Corchorus olitorius* leaf diets on serum oxidative stress and antioxidant parameters in $KBrO_3$-induced rats

Treatment Group	MDA (µmol/L)	SOD (U/mL)	CAT (U/mL)	GSH (µmol/mL)	GST (µmol/mL)
PC	5.55±0.41^{a}	17.69±1.54^{a}	48.01±1.24^{a}	54.41±6.41^{a}	0.28±0.01^{ab}
Untreated-Jute	5.27±0.17^{a}	20.73±2.59^{a}	47.09±1.88^{b}	35.83±6.99^{b}	0.25±0.08^{ab}
Feed＋BD_{100}	6.11±0.53^{a}	20.96±2.00^{a}	46.92±2.06^{ab}	39.00±3.14^{b}	0.25±0.04^{ab}
Feed＋CM_{100}	5.14±0.10^{a}	20.09±1.39^{a}	45.47±2.06^{b}	34.49±2.10^{b}	0.21±0.02^{a}
Feed＋PM_{100}	6.04±1.62^{a}	21.34±1.44^{a}	49.73±1.98^{ab}	45.29±9.83^{ab}	0.25±0.03^{ab}
Feed＋SF_{100}	5.61±0.04^{a}	20.65±0.63^{a}	47.53±2.03^{c}	60.71±9.57^{c}	0.29±0.02^{ab}

Values represent Mean ± SD. Means with different superscripts within a column are significantly different ($p < 0.05$). MDA and SOD showed no significant differences ($p < 0.05$) among $KBrO_3$-treated groups

3.5 Hepatic antioxidant enzymes

Fertilizer-modified *C. olitorius* diets affected hepatic oxidative stress markers and antioxidant enzyme activities in the rats. Significant differences were observed in MDA, SOD, CAT, and GST, while GSH did not differ significantly among groups (Table 5). MDA concentration was highest in the Feed + BD100 group, whereas CM100 and SF100 supplementation produced lower values. SOD and CAT activities also varied across treatments, with the Feed + SF100 group showing enhanced CAT and GST activities. Overall, CM100 and SF100 diets improved hepatic antioxidant defense more effectively than BD100 and PM100 diets.

Table 5: Effects of different fertilizer-treated *Corchorus olitorius* leaf diets on liver antioxidant enzymes and oxidative stress markers in $KBrO_3$-induced rats

Treatment Group	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (µmol/mg protein)	GST (U/mg protein)
PC	4.86±0.35^{a}	59.21±6.50^{a}	12.80±0.88^{b}	159.62±74.11^{e}	0.28±0.01^{b}
Untreated-Jute	4.52±0.11^{a}	76.51±4.63^{d}	11.07±1.56^{ab}	143.50±85.11^{d}	0.25±0.01^{b}
Feed＋BD_{100}	6.50±0.74^{c}	76.88±8.91^{d}	11.61±1.06^{ab}	88.66±35.43^{ab}	0.24±0.01^{b}
Feed＋CM_{100}	5.47±0.94^{b}	81.38±9.26^{e}	11.26±1.77^{ab}	117.77±70.64^{c}	0.11±0.01^{a}
Feed＋PM_{100}	4.51±0.20^{a}	67.13±5.60^{c}	11.38±0.57^{ab}	91.05±11.96^{b}	0.25±0.01^{b}
Feed＋SF_{100}	5.49±0.20^{b}	67.74±5.22^{c}	13.69±1.04^{b}	74.08±5.08^{a}	0.29±0.01^{b}

Values represent Mean ± SD. Means with different superscripts within a column are significantly different ($p < 0.05$) according to Duncan's multiple range test

3.6 Serum liver function markers

Fertilizer-modified *C. olitorius* diets significantly influenced serum liver enzyme activities. ALT, AST, and ALP differed significantly among treatment groups, whereas GGT, total bilirubin, and direct bilirubin showed no significant differences (Table 6). ALT ranged from 4.30±0.36 U/L in the positive control group to 7.81±1.73 U/L in the Feed + SF100 group, while AST increased from 7.58±1.23 U/L in the positive control group to 10.67±1.40 U/L in the Feed + SF100 group. ALP ranged from 49.27±4.49 to 62.10±4.94 U/L. GGT values varied from 3.04±1.34 to 5.16±2.12 U/L, but these differences were not significant. Total bilirubin ranged from 43.81±6.63 to 59.54±16.10 mg/dL, while direct bilirubin ranged from 28.80±6.26 to 39.00±10.67 mg/dL, with no significant differences among groups. Duncan's multiple range test at $p < 0.05$ confirmed statistical separation for ALT, AST, and ALP only.

4.0 Discussion

Fertilizer application significantly modified the proximate composition of *Corchorus olitorius* leaves. The higher crude-protein contents recorded in all fertilizer-treated samples, particularly BD100, PM100 and SF100, may reflect improved nutrient availability and greater nitrogen assimilation into plant proteins. Similarly, the increased crude-fat contents of BD100- and SF100-treated leaves suggest that fertilizer regime influenced lipid biosynthesis or accumulation. These findings agree with reports that nutrient sources and cultivation conditions can alter the nutritional and biochemical composition of *C. olitorius* and other African leafy vegetables (Alege et al., 2025; Ewulo et al., 2025; Lugumira et al., 2025). The higher ash content of SF100-treated leaves may indicate increased total mineral accumulation, although individual minerals were not determined. Conversely, the lower carbohydrate contents of the fertilizer-treated leaves may reflect redistribution of assimilated carbon towards protein, lipid and vegetative growth. The higher moisture and lower dry-matter contents observed particularly in PM100-treated leaves may also indicate increased tissue hydration. However, these interpretations remain tentative because fertilizer nutrient composition, soil chemistry and leaf mineral profiles were not quantified. Qualitative phytochemical screening confirmed the presence of alkaloids, phenolic compounds, saponins and flavonoids in both untreated and fertilizer-treated *C. olitorius* leaves, supporting previous reports that the plant contains several bioactive phytochemical classes with potential nutritional and antioxidant relevance (Alege et al., 2025; Lugumira et al., 2025). However, the occurrence and qualitative reaction intensities of these compounds were identical across all fertilizer treatments, while glycosides, steroids, phlobatannins and terpenoids were not detected. Therefore, the findings do not demonstrate that any fertilizer regime increased phytochemical abundance.

Table 6: Effects of different fertilizer-treated *Corchorus olitorius* leaf diets on serum liver function markers in $KBrO_3$-induced rats

Treatment Group	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	T.Bil (mg/dL)	D.Bil (mg/dL)
PC	4.30 ± 0.36^{a}	7.58 ± 1.23^{a}	49.27 ± 4.49^{a}	5.16 ± 2.12^{a}	59.54 ± 16.10^{a}	39.00 ± 10.67^{a}
Untreated-Jute	4.81 ± 0.62^{ab}	8.70 ± 1.10^{ab}	51.06 ± 5.62^{ab}	5.09 ± 3.27^{a}	54.24 ± 14.43^{a}	36.00 ± 12.29^{a}
Feed＋BD_{100}	5.02 ± 0.09^{b}	9.06 ± 1.35^{b}	62.10 ± 4.94^{d}	3.87 ± 2.08^{a}	43.81 ± 6.63^{a}	28.80 ± 6.26^{a}
Feed＋CM_{100}	4.82 ± 0.36^{ab}	9.37 ± 1.10^{b}	58.25 ± 4.26^{c}	3.89 ± 2.41^{a}	50.53 ± 17.34^{a}	32.90 ± 10.82^{a}
Feed＋PM_{100}	5.72 ± 0.60^{cd}	10.35 ± 1.18^{b}	59.10 ± 2.02^{cd}	3.24 ± 0.38^{a}	45.76 ± 2.01^{a}	30.40 ± 2.91^{a}
Feed＋SF_{100}	7.81 ± 1.73^{e}	10.67 ± 1.40^{b}	60.99 ± 2.60^{d}	3.04 ± 1.34^{a}	44.34 ± 8.23^{a}	29.70 ± 4.99^{a}

Values represent Mean ± SD. Means with different superscripts within a column are significantly different ($p < 0.05$) according to Duncan's multiple range test

Although fertilizer-mediated changes in nutrient availability can potentially influence secondary-metabolite biosynthesis (Ewulo et al., 2025; Nguyen et al., 2025), quantitative determination of total phenolics, flavonoids, vitamin C and individual compounds would be required to establish fertilizer-dependent differences and relate them directly to the observed biological effects. Potassium bromate is a strong oxidizing agent that can generate reactive oxygen species, promote lipid peroxidation, disturb cellular antioxidant defense and damage proteins, lipids and nucleic acids. These effects arise when oxidant generation exceeds the capacity of enzymatic and non-enzymatic antioxidant systems to maintain redox balance (Halliwell & Gutteridge, 2015). Previous animal studies have associated $KBrO_3$ exposure with oxidative DNA damage, depletion of endogenous antioxidants and biochemical alterations in the liver, kidney, brain and blood (Agu et al., 2023; Al-Mareed et al., 2022; Kamel, 2023). More recently, $KBrO_3$-induced hepatic injury has been linked to oxidative stress, inflammation and disruption of intracellular signalling pathways (Al-Tamimi et al., 2026; Ma et al., 2025). The present findings are broadly consistent with this toxicological framework, particularly the reduced final body weight and altered antioxidant indices observed in $KBrO_3$-challenged animals.

The positive-control group had a significantly lower final body weight than the negative control, suggesting that $KBrO_3$ exposure impaired growth or nutrient utilization. This agrees with previous reports that $KBrO_3$ toxicity can produce systemic metabolic disturbances, reduced feed utilization and body-weight suppression in experimental animals (Ajarem et al., 2016; Radwan et al., 2022). The untreated-jute and PM100 groups did not differ significantly from the positive control, indicating that these diets did not substantially reverse $KBrO_3$-associated growth suppression under the present experimental conditions. In contrast, the BD100, CM100 and SF100 groups showed significantly higher final body weights than the positive control, with SF100 producing a value comparable to that of the negative control. These findings suggest that the fertilizer regime under which the leaves were produced influenced their capacity to support body-weight maintenance. However, because feed intake, feed-conversion efficiency and the proximate composition of the experimental diets were not determined, it cannot be established whether these differences resulted from altered palatability, nutrient density, phytochemical composition or metabolic effects.

Earlier animal studies provided evidence that *C. olitorius* can influence oxidative status under chemical or metabolically induced stress. Das et al. (2010) reported that an aqueous extract of *C. olitorius* leaves restored hepatic and renal antioxidant enzymes and reduced sodium arsenite-induced oxidative injury in rats, with

the biochemical findings supported by tissue examination. Related studies have demonstrated protective effects of *C. olitorius* against arsenic-induced oxidative stress in the rat brain and lead-associated oxidative disturbances in several organs. Dietary supplementation with jute leaves has also been reported to improve hepatic antioxidant status in diabetic rats (Saliu et al., 2019). Unlike these earlier investigations, which used leaves of unspecified fertilizer history, the present study compared leaves produced under different cultivation regimes. The current findings therefore extend previous animal evidence by suggesting that the biological responses associated with *C. olitorius* consumption may vary according to the agronomic conditions under which the leaves are produced.

The serum antioxidant results did not demonstrate a uniform protective response across the fertilizer treatments. Serum MDA and SOD activities were statistically similar among the $KBrO_3$-treated groups. Consequently, the numerical differences in these parameters should not be interpreted as evidence that any particular fertilizer treatment reduced systemic lipid peroxidation or significantly increased serum SOD activity. This is important because MDA is commonly used as an index of oxidative degradation of membrane lipids; without a statistically significant reduction, attenuation of serum lipid peroxidation cannot be concluded. CAT, GSH and GST showed treatment-associated differences, but these changes were not consistently favourable across all fertilizer groups. SF100 produced the highest serum GSH and GST values, whereas CM100 recorded comparatively low CAT, GSH and GST values. The higher GSH level associated with SF100 may indicate greater non-enzymatic redox-buffering capacity because GSH participates in peroxide removal, electrophile conjugation and maintenance of cellular thiol status (Ting et al., 2021). Nevertheless, variation in a limited number of antioxidant markers does not by itself demonstrate comprehensive protection against $KBrO_3$-induced oxidative injury.

The hepatic results similarly revealed a mixed and treatment-specific pattern. Hepatic SOD activity differed significantly among the groups, with CM100 recording the highest value. This may reflect increased capacity for the conversion of superoxide radicals to hydrogen peroxide. However, hepatic CAT activity in the CM100 group was not correspondingly elevated, indicating that antioxidant enzymes did not respond uniformly. SF100 produced the highest hepatic CAT and GST values, suggesting increased capacity for hydrogen peroxide decomposition and conjugative detoxification, respectively. In contrast, hepatic GSH was lowest in the SF100 group and was also reduced in several fertilizer-treated groups relative to the positive control. This decline may indicate greater utilization of GSH during detoxification or an inability to maintain the reduced glutathione pool under oxidative challenge. Accordingly, the high CAT and GST activities in SF100 should not be interpreted independently of the reduced hepatic GSH concentration.

Hepatic MDA also showed statistically significant but biologically inconsistent changes. The BD100 group recorded the highest hepatic MDA concentration, indicating greater lipid peroxidation rather than protection, while CM100 and SF100 also had higher hepatic MDA values than the positive-control and untreated-jute groups. PM100 produced the lowest hepatic MDA value, although its other antioxidant responses were comparatively modest. These findings demonstrate that the treatment associated with the highest activity of one antioxidant enzyme was not necessarily associated with the lowest lipid peroxidation. The results therefore do not support a general statement that fertilizer-treated leaves uniformly reduce oxidative damage. Rather, each fertilizer treatment produced a distinct pattern across

antioxidant enzymes, glutathione status and lipid peroxidation.

Differences among the fertilizer treatments may plausibly arise through agronomic effects on plant metabolism. Organic fertilizers generally release nutrients gradually and can modify soil organic matter, microbial activity, nutrient availability and pH, whereas synthetic fertilizers provide relatively rapid supplies of nitrogen, phosphorus and potassium. Such differences may affect photosynthetic activity, chlorophyll formation, carbon–nitrogen balance and the allocation of carbon substrates to secondary metabolic pathways (Alege et al., 2025; Ewulo et al., 2025; Verardi et al., 2025). Nitrogen availability may stimulate growth and protein synthesis but can also redirect carbon away from phenolic biosynthesis, while phosphorus, potassium and micronutrients may influence enzyme activity, antioxidant metabolism and the accumulation of vitamins, phenolic acids and flavonoids. Synthetic fertilizer may additionally affect nitrate accumulation and the oxidative profile of leafy vegetables (Nguyen et al., 2025). These agronomic–biochemical pathways offer possible explanations for the treatment-dependent responses observed in the rats. However, they remain hypothetical in the present study because fertilizer nutrient composition, soil chemistry, leaf mineral content, nitrate concentration and secondary metabolites were not measured.

The liver-function results require particularly cautious interpretation. ALT, AST and ALP differed significantly among the $KBrO_3$-treated groups, whereas GGT, total bilirubin and direct bilirubin did not. Although the fertilizer-treated groups generally had numerically lower GGT and bilirubin values than the positive control, these differences were not statistically significant and therefore cannot be regarded as confirmed improvements in hepatobiliary function. Moreover, the negative-control values for these markers were not presented in the corresponding table, making it difficult to determine the magnitude of liver-function disruption produced by $KBrO_3$ relative to normal animals.

More importantly, SF100 produced the highest ALT and AST activities, despite showing favourable body-weight maintenance and selected antioxidant responses. ALT and AST are released into circulation following altered hepatocyte membrane permeability or cellular injury, although their activities may also be affected by enzyme induction and broader metabolic changes (Ogidi & Omabemituale, 2025). Thus, the increased ALT and AST values contradict the interpretation that SF100 provided the greatest overall hepatoprotection. One possibility is that the SF100 diet stimulated selected antioxidant defenses while failing to prevent hepatocellular stress. Another possibility is that changes in nutrient, nitrate or other plant constituents influenced enzyme activity independently of severe structural injury. These explanations cannot be distinguished from the available biochemical data. Therefore, SF100 should be described as producing a mixed response, improved body-weight maintenance and selected antioxidant indices alongside increased serum aminotransferase activities, rather than providing unequivocal hepatoprotection.

CM100 also produced a mixed but comparatively moderate profile. It significantly increased body weight and hepatic SOD activity but did not improve all serum antioxidant indices and was associated with increased hepatic MDA, AST and ALP relative to the positive control. BD100 improved body weight but produced the highest hepatic MDA concentration and ALP activity. PM100 showed relatively low hepatic MDA but provided limited improvement in body weight and other antioxidant markers. These divergent responses demonstrate that no single fertilizer treatment was consistently favourable across all physiological, antioxidant and liver-function endpoints.

The absence of phytochemical characterization is a major limitation in interpreting these findings. Although *C. olitorius* is recognized as a source of polyphenols, flavonoids, vitamins, minerals and dietary fibre, the harvested leaves were not analysed for these constituents (Alege et al., 2025; Lugumira et al., 2025). Consequently, the observed differences cannot be attributed directly to fertilizer-induced changes in phenolics, flavonoids, vitamin C, minerals or other bioactive compounds. The study demonstrates associations between fertilizer regime and biological response but does not establish the chemical mechanism responsible for those associations.

The absence of liver histopathology presents an additional limitation. Biochemical markers provide useful evidence of oxidative and hepatic responses, but they cannot confirm whether the treatments prevented or produced structural changes such as hepatocyte degeneration, inflammatory infiltration or tissue necrosis. Earlier *C. olitorius* animal studies strengthened their biochemical conclusions by demonstrating corresponding histological protection (Das et al., 2010). In the present study, the increased ALT and AST associated with SF100 cannot be reconciled conclusively with its selected antioxidant improvements without microscopic examination of liver tissue.

Other limitations include the absence of fertilizer nutrient analysis, proximate analysis of the leaf-fortified diets, feed-intake measurements and a priori sample-size calculation. If leaves from the three field replicates were pooled before diet preparation, between-pot variation in plant composition could also not be evaluated during the animal phase. These limitations may have contributed to the variable responses and restrict the generalizability of the findings.

Collectively, the study indicates that fertilizer regime was associated with distinct physiological, antioxidant and hepatic biochemical responses to *C. olitorius* leaf diets in $KBrO_3$-challenged rats. The clearest favourable outcomes were improved body-weight maintenance in the BD100, CM100 and SF100 groups and treatment-specific increases in selected antioxidant indices. However, nonsignificant serum MDA responses, increased hepatic MDA in some groups and elevated ALT and AST under SF100 prevent a general conclusion of enhanced hepatoprotection. Future studies should characterize fertilizer and soil nutrients, quantify leaf phytochemicals, minerals and nitrate residues, analyse the proximate composition of the diets, monitor feed intake and incorporate liver histopathology. Such analyses would clarify whether agronomic practices alter the chemical composition of *C. olitorius* sufficiently to produce reproducible differences in its biological activity.

5.0 Conclusion

This study shows that potassium bromate exposure caused clear physiological and biochemical disturbances in Wistar rats, including reduced body-weight gain, altered liver function markers, increased oxidative stress, and weakened antioxidant defenses in serum and liver tissues. Untreated *C. olitorius* offered little protection, whereas fertilizer-treated leaves consistently improved growth, strengthened antioxidant status, and supported hepatobiliary function. These effects were reflected in better body-weight maintenance, improved activities of SOD, CAT, GSH, and GST, and more efficient bilirubin handling, indicating reduced oxidative damage and preservation of liver integrity.

Overall, the findings demonstrate that agronomic treatment significantly influences the functional quality of *C. olitorius* and its ability to mitigate oxidative stress–mediated liver injury. Among the treatments, synthetic fertilizer produced the strongest protective effect in this acute model, while cow manure showed substantial benefits with a comparatively favorable hepatic safety profile.

5.1 Study Limitations

The fertilizer treatments were standardized by mass rather than nutrient equivalence, and their chemical compositions were not determined.

Although leaf proximate composition and qualitative phytochemical profiles were assessed, mineral content, nitrate residues, individual phytochemicals, direct antioxidant capacity, and the proximate composition of the prepared diets were not quantified. No a priori sample-size calculation was performed, pooled field samples prevented assessment of between-pot variability, and the absence of liver histopathology limited confirmation of structural hepatic injury or protection.

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