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# Hematological and Renoprotective Effects of Chia (*Salvia Hispanica*) and Flax (*Linum Usitatissimum*) Seeds in a Monosodium Glutamate-Induced Oxidative Stress in Wistar Rats

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## ABSTRACT

This study examined the effects of dietary chia and flaxseed supplementation on hematological parameters and kidney function in Wistar rats exposed to monosodium glutamate (MSG)-induced oxidative stress. Forty-eight male Wistar rats (120–150 g) were randomly divided into seven groups (n=5 each). Group A served as control (saline), Group B received MSG (15 mg/kg), and Groups C to F were given MSG along with either 20% or 40% chia or flax seeds. Group G received a combination of 20% chia and 20% flax seeds with MSG. After four weeks of oral treatment, blood samples were analyzed for hematological indices, including HGB, HCT, RBC, PLT, MCV, MCH, MCHC, MPV, and differential WBC counts, as well as kidney function markers such as serum urea, creatinine, and total protein. MSG significantly disrupted hematological and renal parameters. However, supplementation with chia and flax seeds significantly mitigated these effects ($p < 0.05$). The 40% flaxseed group showed the highest hemoglobin level (15.23±5.08 g/dL) and platelet count (725.67±153.02 $\times 10^9$/L), while the combination group exhibited synergistic improvements across most parameters. MSG increased creatinine (1.51±0.50 mg/dL) and urea (213.46±65.35 mg/dL) levels, but supplementation, especially the chia-flax combination, significantly lowered these markers toward control levels. Total protein also increased significantly in treated groups, with the most significant restoration observed in the combination group (4.24±1.43 mg/dL). These results suggest that chia and flax seeds, both individually and in combination, offer hematological and kidney-protective benefits against MSG-induced toxicity, supporting their use as functional foods in the dietary management of metabolic stress.

**Keywords:** Chia seed, Flax seed, Monosodium glutamate, Hematology, Kidney function, Wistar rats, Functional food.

## INTRODUCTION

The kidneys and hematopoietic system are important for maintaining physiological homeostasis, including fluid balance, waste removal, immune function, and blood cell production. However, exposure to synthetic food additives like monosodium glutamate (MSG) has raised toxicological concerns due to its possible effects on kidney and blood health (Eweka and Om' Iniabohs, 2007). MSG is commonly used as a flavor enhancer or additive but has been implicated in inducing oxidative stress through overproduction of reactive oxygen species (ROS), leading to lipid peroxidation, organ damage, and inflammatory responses (Farombi and Onyema, 2006; Ifemeje *et al*., 2020). Studies have shown that MSG exposure disrupts hematological parameters, including hemoglobin concentration and red blood cell counts, and contributes to nephrotoxicity as evidenced by elevated urea and creatinine levels (Al-Zuhairy and Taher, 2014).

On the other hand, dietary interventions incorporating natural antioxidants and omega-3-rich sources, such as chia (Salvia hispanica) and flax (Linum usitatissimum) seeds, have shown promising therapeutic potential. These seeds are rich in alpha-linolenic acid, lignans, flavonoids, and polyphenols, which are reported to combat oxidative stress and improve renal and hematological functions (Parikh *et al*., 2019; Marineli *et al*., 2015).

Flaxseed and chia seed supplementation have been previously shown to improve lipid profiles, modulate inflammatory markers, and protect against oxidative damage to renal and hematopoietic tissues (Sembratowicz *et al*., 2020). Chia's phenolic content and soluble fiber, and flax's lignans and omega-3 profile are particularly beneficial for erythrocyte membrane integrity and kidney function (Ullah *et al*., 2016).

This study builds on prior work such as that of Ifemeje *et al*. (2020), which demonstrated that food additives could induce oxidative stress and enzymatic alterations in laboratory animals. Our current investigation assesses the protective efficacy of chia and flaxseed supplementation, both individually and in combination, against MSG-induced hematological and renal dysfunctions in Wistar rats. The aim is to determine whether combined administration offers additive or complementary benefits over individual supplementation.

## MATERIALS AND METHODS

### Materials

Chia and flax seeds (1.5 kg each) were purchased from Roban Store in Awka and ground using a corona grinder. MSG was obtained from Sigma, while the grower's mesh rat feed was purchased from Top Feed Limited. Forty-eight (48) male Wistar albino rats (weighing 120–150 g) were acquired from Chris Animal Farm in Ifite-Awka and housed in stainless steel cages under normal conditions. The rats were kept in well-ventilated stainless steel cages and allowed to acclimate for 7 days before a 4-week experimental feeding period. The animals were maintained under ambient conditions with free access to standard rat chow and water.

### Determination of Acute Toxicity (LD50)

The LD50 of the additives was determined by Lorke's method (1983) using thirteen (13) rats. The animals received monosodium glutamate, chia, and flax seeds and were monitored for 24 hours for signs such as excitation, paw licking, increased respiratory rate, convulsions, and death. $LD_{50}$ estimation was conducted in two sequential phases to assess the acute toxicity effects of chia and flax seed supplementation in MSG-exposed rats.

**Phase**

In the initial phase, groups of rats were fed diets supplemented with low concentrations of chia or flax seeds, ranging from 10% to 30%. This phase served as a preliminary screening to observe any mortality or toxic signs and to identify dose ranges that cause minimal to moderate toxic effects.

**Phase2:**

Based on Phase 1 findings, a second phase involved feeding other groups of rats higher supplementation doses, ranging from 50% to 90% chia or flax seeds. This phase aimed to observe mortality rates at these elevated doses more precisely to identify doses causing significant toxicity, thereby narrowing down the dose-response relationship.

**The $LD_{50}$ is calculated using the formula:**

$$LD50 = \sqrt{D0} \times D100$$

### Experimental Design

After acclimatization, rats were randomized into seven groups (n=5):

- **Group A:** Control (saline, 3 ml/kg)
- **Group B:** MSG only (15 mg/kg)

- **Group C:** MSG + 20% chia seed
- **Group D:** MSG + 20% flax seed
- **Group E:** MSG + 40% chia seed
- **Group F:** MSG + 40% flax seed
- **Group G:** MSG + 20% chia + 20% flax seeds

Treatments were given orally for four weeks. After fasting overnight, rats were anesthetized, and blood was drawn through cardiac puncture. Serum was then isolated for hematological and renal tests.

### Hematological Analysis

Whole blood collected in EDTA tubes was analyzed for hematological parameters using an automated hematology analyzer. The parameters evaluated included hemoglobin concentration (HGB), hematocrit (HCT), red blood cell count (RBC), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean platelet volume (MPV), and white blood cell differentials: lymphocytes, granulocytes, and monocytes (WBC, LYMPH, MID, GRAN, EOS, BAS), following established protocols (Gulye *et al*., 2006).

### Kidney Function Test

### Serum Urea

The urea content of the serum samples was estimated using an automated analyzer, the Blood Urea Analyzer, from Beckman Coulter Inc., USA. The analysis procedure required setting up reagents, specifically a Hichem kit for the blood urea nitrogen analyzer. The kit is supplied by Elan Diagnostics, USA (Adekomi, 2010).

### Serum Creatinine

A Creatinine Analyzer-2 (Beckman Coulter Inc., USA) was used in combination with a specific kit of reagents (Hichem Creatine Pak, Elan Diagnostics, USA) to calculate the creatinine content of the serum samples (Adekomi, 2010).

### Statistical Analysis

The data obtained were expressed as mean ±SD of three replicates. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 23. One-way analyses of Variance were adopted for comparison, and the results were subjected to a post hoc test using the least significant difference (LSD) method. $p<0.05$ was considered significant for all the tests.

## RESULTS

### Determination of Acute toxicity ($LD_{50}$) of Flax seed and Chai Seed:

Results of the Acute toxicity of flax and Chai seed are shown in Tables 1 and 2 for phase 1 and phase II, respectively. The results of the study showed that feed supplemented with flax and chia seeds at percentages ranging from 10% to 30% for different groups of rats in phase 1 of the study did not cause any animal deaths or observable physiological changes.

Also, at a concentration of 50–90% supplementation, no deaths were recorded in rats fed pulverized flax and Chai seed-supplemented feed. However, at 90% supplementation with flaxseed, there was observable palpitation.

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The LD50 of pulverized flaxseed and Chai seed was obtained, showing that no deaths were recorded at a 90% supplementation level. Since no deaths were recorded at high doses, the pulverized seeds were not considered very toxic.

Table 8: Phase 1 of Acute Toxicity ($LD_{50}$) of pulverized Chai seed and Flax seed

| GROUPS | DOSE (% OF PULVERIZED SEEDS | MORTALITY IN CHAI SEED | MORTALITY IN FLAX SEED | OBSERVATIONS |
|---|---|---|---|---|
| 1 | 10 | 0/3 | 0/3 | NORMAL |
| 2 | 20 | 0/3 | 0/3 | NORMAL |
| 3 | 30 | 0/3 | 0/3 | NORMAL |

Table 2: Phase II of Acute Toxicity ($LD_{50}$) of pulverized Chai seed and Flax seed

| GROUPS | DOSE (% OF PULVERIZED SEEDS | MORTALITY IN CHAI SEED | MORTALITY IN FLAX SEED | OBSERVATIONS |
|---|---|---|---|---|
| 1 | 50 | 0/1 | 0/1 | NORMAL |
| 2 | 70 | 0/1 | 0/1 | NORMAL |
| 3 | 90 | 0/1 | 0/1 | PALPITATION |

**Effect of Chia and Flaxseeds on haematological parameters in rats subjected to monosodium glutamate (MSG)-induced oxidative stress.**

The data presented in Figures 1 through 8 indicate that administering monosodium glutamate (MSG) results in significant hematological changes, suggesting metabolic stress, including decreases in hemoglobin (HGB), hematocrit (HCT), red blood cell counts (RBC), and platelet counts (PLT). These decreases suggest impaired red blood cell production, reduced oxygen transport, and weakened clotting ability, highlighting the harmful effects of MSG on blood composition. In contrast, incorporating chia and flax seeds into the diet at varying levels, particularly 40% flax seed and a combination of 20% chia and 20% flax seeds, notably helped bring these parameters closer to normal levels, highlighting their blood-protective properties. Hemoglobin levels in the flaxseed and combined seed groups matched those of the normal controls, showing a successful reversal of MSG-induced anemia. Likewise, hematocrit values, which were significantly lowered by MSG, were significantly increased by seed diets ($P < 0.05$), indicating improvements in blood volume and red cell mass.

Red blood cell counts followed a comparable trend, with treated groups showing values similar to those of the controls, highlighting the role of chia and flax seeds in enhancing erythropoiesis and red cell survival, likely mediated by their rich antioxidant and nutrient content. Platelet counts, markedly diminished by MSG exposure, were significantly increased in the flax seed 40% group and the combined seed group ($P < 0.05$), implying restoration of thrombopoiesis and improved hemostatic function.

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), which were reduced by MSG treatment, showed significant improvement ($P < 0.05$) in treated groups, indicating enhanced red blood cell size and hemoglobin content. Plateletcrit (PCT) and mean platelet volume (MPV), which MSG also suppresses, increased significantly ($P < 0.05$) following chia and flaxseed diets, with combined seed feeding yielding values similar to those of the control group. Overall, these results demonstrate that chia and flax seeds alleviate MSG-induced hematological disturbances, restoring red blood cell and platelet indices toward normal ranges.

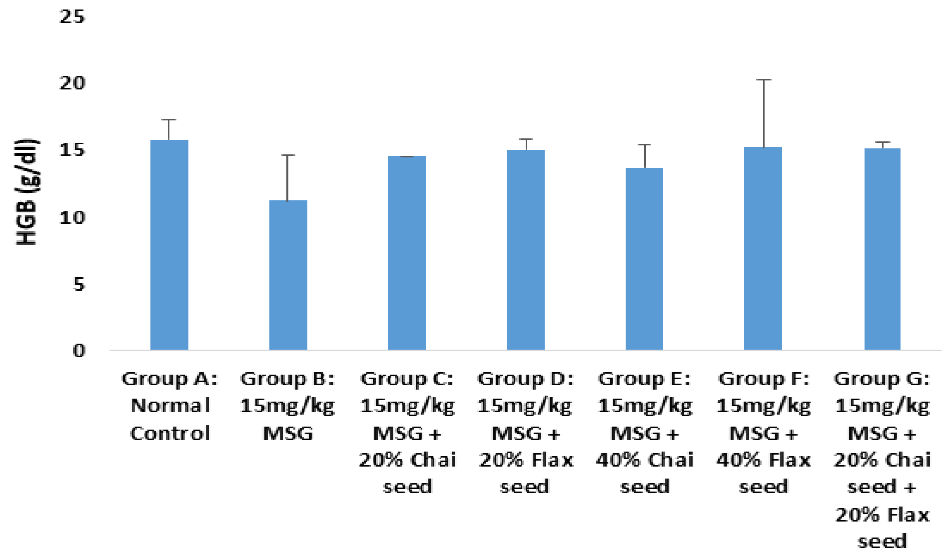


Figure 1: Haemoglobin

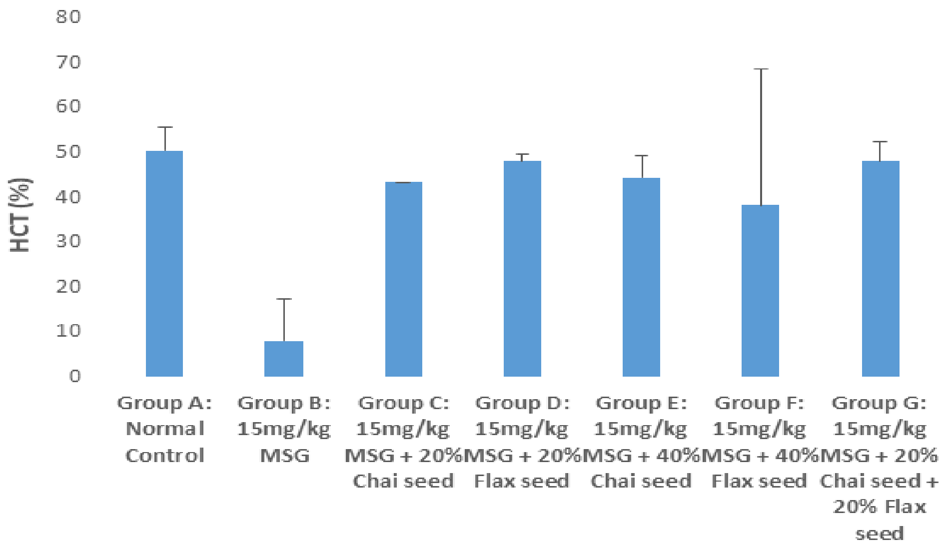


Figure 2: Hematocrit

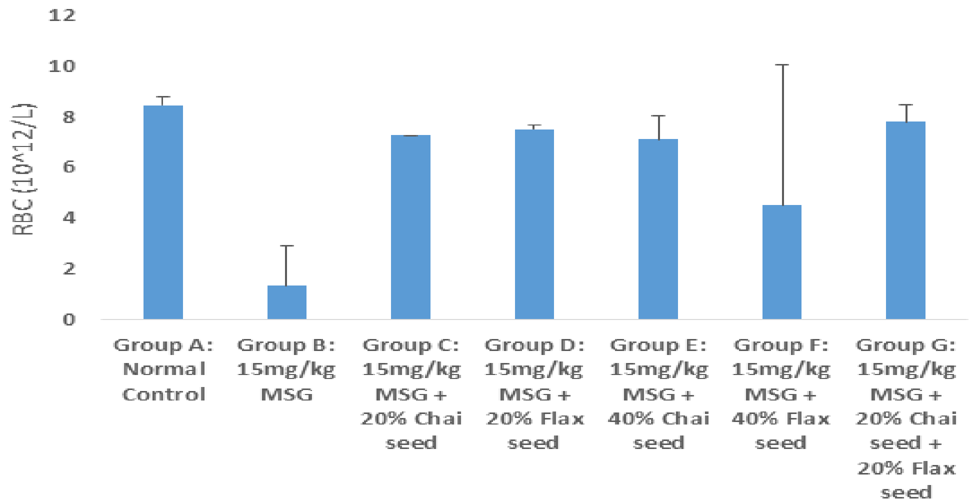


Figure 3: Red blood cell

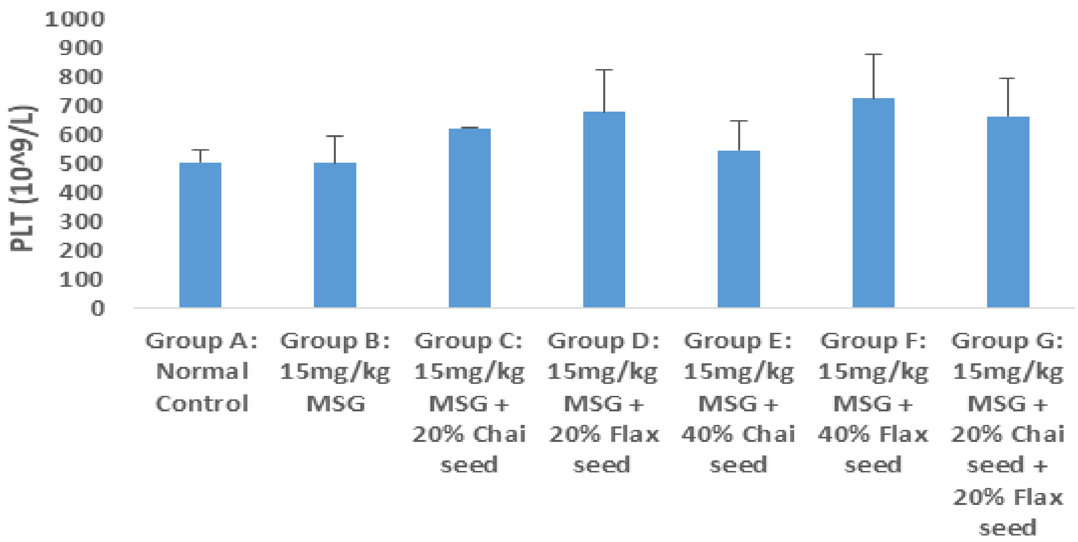


Figure 4: Platelet Count

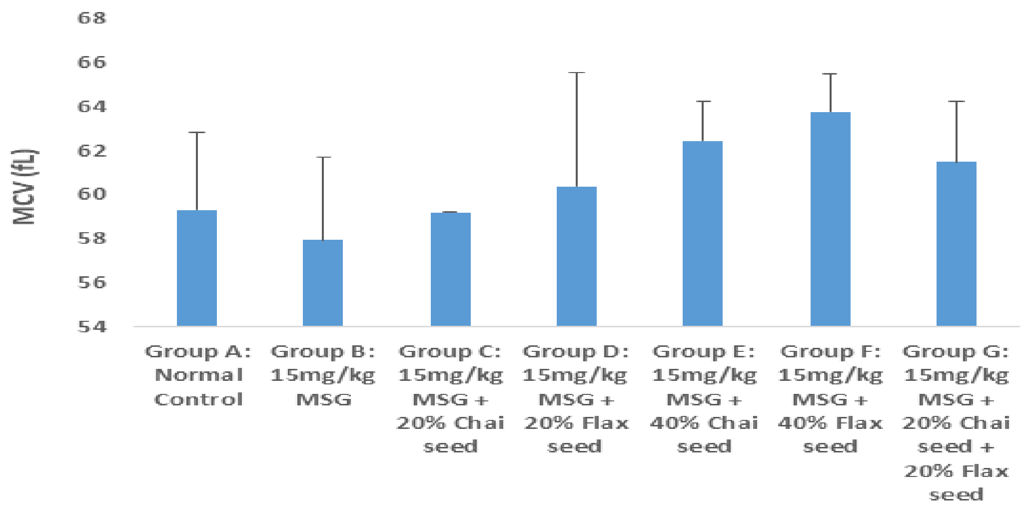


Figure 5: Mean Corpsular Volume

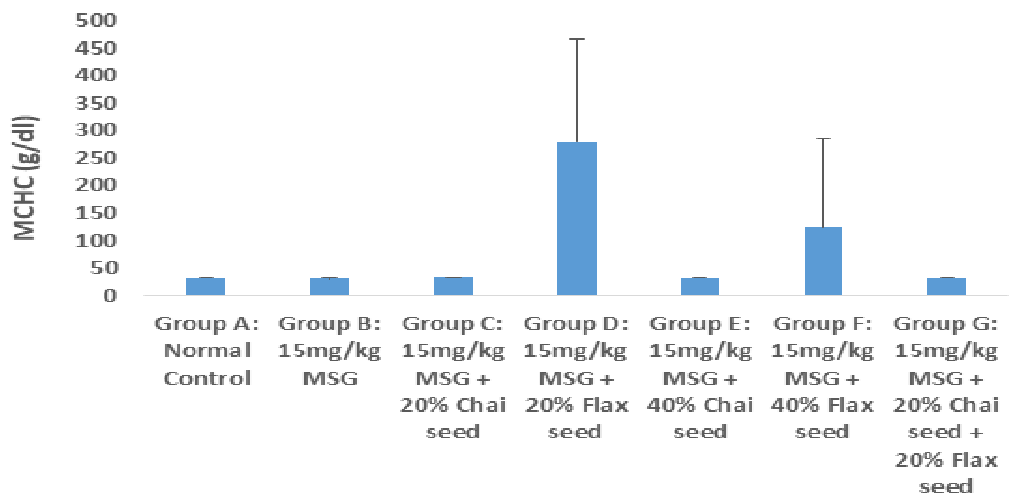


Figure 6: Mean Corpsular Haemoglobin Concentration

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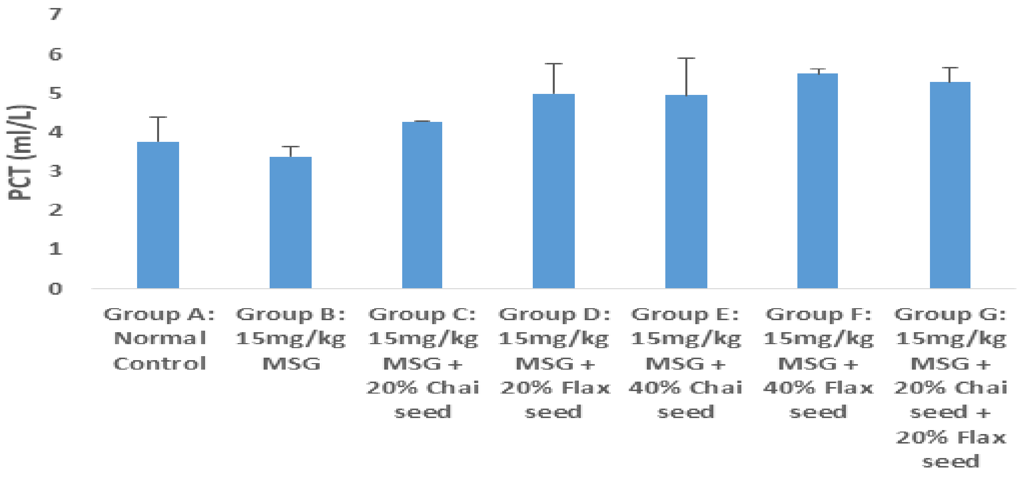


Figure 7: Procalcitonin

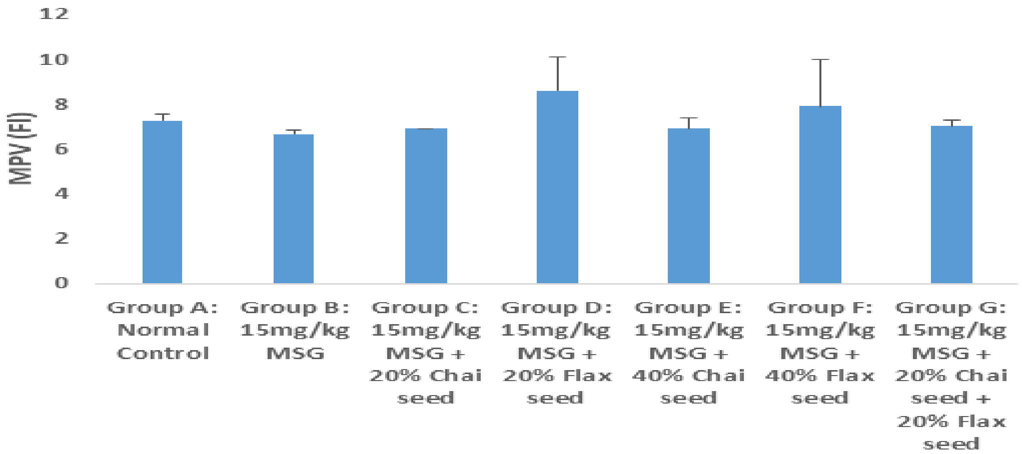


Figure 8: Mean Platelet Volume

**Effects on WBC Differential**

Differential leukocyte analysis showed that WBC and lymphocyte counts were significantly increased in all supplemented groups compared to MSG-treated rats ($p < 0.05$, Table 1). The combination group (G) exhibited WBC (6.20±2.42 × 10^9/L) and lymphocyte (88.37±5.05%) values similar to those of the control, indicating normalization of the immune status.

Table 3: White blood cell differentials of flax and chai seeds in Wistar Rats

| Groups | WBC ($10^9$/L) | LYMPH (%) | MID (%) | GRAN (%) | EOS (%) | BAS (%) |
|---|---|---|---|---|---|---|
| **Group A**: Normal Control | 5.20±2.76 | 83.57±1.53 | 8.80±2.61 | 7.63±1.88 | 0.00±0.00 | 0.00±0.00 |
| **Group B**: 15mg/kg MSG | 17.77±2.25 | 89.50±2.52 | 5.20±0.44 | 5.30±2.45 | 0.00±0.00 | 0.00±0.00 |
| **Group C**: 15mg/kg MSG + 20% Chai seed | 9.10±0.00 | 89.00±0.00 | 7.00±0.00 | 4.00±0.00$^{b}$ | 0.00±0.00 | 0.00±0.00 |
| **Group D**: 15mg/kg MSG + 20% Flax seed | 7.90±5.39 | 57.10±4.72 | 15.67±0.15 | 26.90±4.77$^{a}$ | 0.00±0.00 | 0.00±0.00 |

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue VII July 2025

| | | | | | | |
|---|---|---|---|---|---|---|
| **Group E**: 15mg/kg MSG + 40% Chai seed | 7.07±2.34 | 87.07±4.97 | 7.47±2.63 | 5.47±2.35[b] | 0.00±0.00 | 0.00±0.00 |
| **Group F**: 15mg/kg MSG + 40% Flax seed | 12.83±7.82 | 80.47±16.11 | 9.20±6.50 | 10.33±10.02[a] | 0.00±0.00 | 0.00±0.00 |
| **Group G**: 15mg/kg MSG + 20% Chai seed + 20% Flax seed | 6.20±2.42 | 88.37±5.05 | 7.23±2.87 | 5.53±3.07[b] | 0.00±0.00 | 0.00±0.00 |

Values are presented in mean ± SD (n=5) $p < 0.05$. [a,b,c] means values in a column with the same alphabetical superscripts are not significantly different.

**Effects of Chia and Flax Seed Supplementation on Kidney Function**

Data illustrated in Figures 9 and 10 show that dietary supplementation with chia and flax seeds at both 20% and 40% concentrations, as well as their combination, resulted in a significant improvement in kidney function parameters compared to the MSG-only group ($p < 0.05$). Specifically, MSG administration led to a marked elevation in serum urea and creatinine levels (213.46±65.35 mg/dl and 1.51±0.50 mg/dl, respectively), indicating renal impairment. However, all supplemented groups showed significantly reduced urea and creatinine values, with the most pronounced effect observed in the combination group (G), which recorded the lowest urea levels (34.30 ± 17.83 mg/dL) and creatinine levels (0.49 ± 0.18 mg/dL). These results suggest that both chia and flax seeds, especially when used in combination, exhibit significant renoprotective effects in the context of MSG-induced metabolic stress.

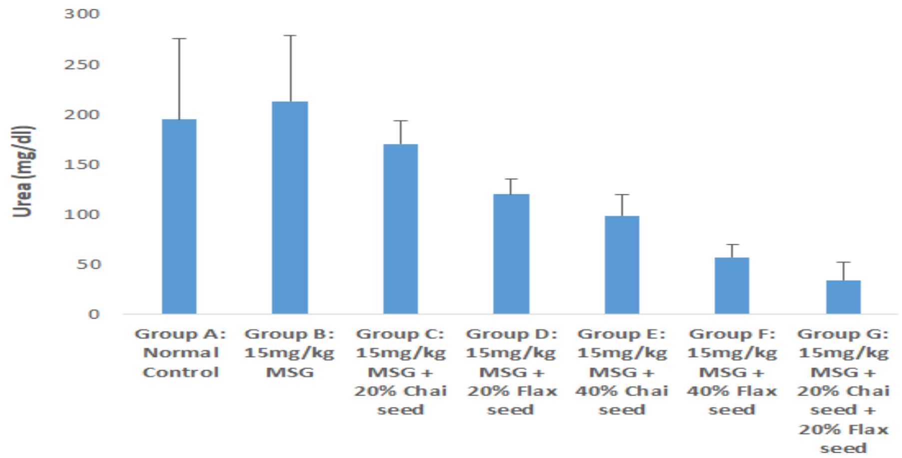


Figure 9: Urea

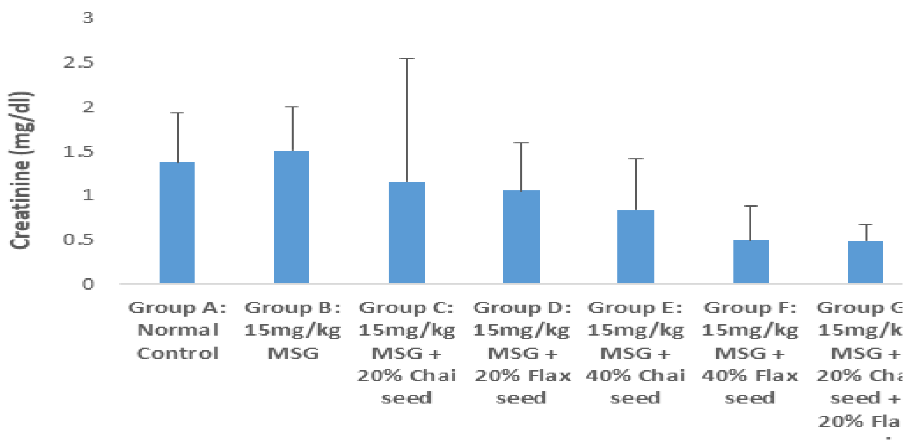


Figure 10: Creatinine

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue VII July 2025

## DISCUSSION

### Hematological Parameters

The present study on Wistar Rats exposed to MSG (Group B) showed significant reductions in hematological parameters, including hemoglobin (HGB), packed cell volume (PCV), red blood cell (RBC) count, and platelet (PLT) count, compared to the normal control (Group A). This decrease may indicate anemia and hematopoietic suppression caused by oxidative damage induced by MSG, consistent with the findings of Egbuonu *et al*. (2009) and Kolawole (2013), who reported similar anemia-like symptoms in MSG-treated rodents. This finding is also in agreement with reports by Ashaolu *et al*. (2011), who observed reductions in RBC, HGB, and HCT levels in MSG-exposed Wistar rats, attributing these changes to MSG-induced oxidative damage that disrupts erythropoiesis and red cell survival.

The significant improvement of HGB, HCT, and RBC in groups receiving chia and flax seeds aligns with evidence from recent studies highlighting the hematoprotective effects of these seeds. The antioxidant compounds, including polyphenols and omega-3 fatty acids in chia and flax seeds, are thought to reduce oxidative damage to hematopoietic cells, improving blood cell production and function, returning these parameters to normal levels. These findings are consistent with Mihafu (2024), who also reported hematological improvements in rats fed with chia seed-based diets. The observed increase in platelet count (PLT) in the flax and combination groups suggests an additional hematopoietic benefit, possibly mediated by the seeds' bioactive components that enhance megakaryocyte proliferation and platelet production. This aligns with findings by Al-Daraji *et al*., (2010), who reported that flaxseed supplementation led to significant increases in erythrocyte count, PCV, hemoglobin concentration, and leukocyte count in poultry.

Additionally, mean corpuscular hemoglobin concentration (MCHC) increased significantly in flax and chia-supplemented groups, indicating improved hemoglobinization of red blood cells. The low MCV and MCH values observed in the MSG-only group (B) are consistent with microcytic anemia, which may result from issues with iron bioavailability and erythrocyte shrinkage caused by oxidative stress. The hematoprotective effects observed in seed-fed groups likely originate from the omega-3 fatty acids, polyphenols, and lignans in chia and flax, which are known to stabilize red blood cell membranes and promote erythropoiesis (Marineli *et al*., 2015; Sembratowicz *et al*., 2020). White blood cell (WBC) and lymphocyte counts were significantly elevated in the MSG group, reflecting immune activation and systemic inflammation. Feeding with chia and flax seeds, especially in combination, normalized WBC and lymphocyte levels, indicating immunomodulatory effects. The immunomodulatory properties of omega-3 fatty acids, which modulate phagocytosis, natural killer cell activity, and cytokine production, have been well-documented (Oarada, 2008).

### Kidney Function Indices

Monosodium glutamate administration also resulted in significant increases in serum urea and creatinine, as well as a decrease in total protein, indicating renal dysfunction ($P < 0.05$, Table 2). These findings align with the nephrotoxic effects of MSG, which are mediated by oxidative damage to renal tubular and glomerular cells, resulting in impaired filtration and protein synthesis (Abdou, 2025). Supplementation with flax and chia seeds, particularly in combination, significantly reduced serum urea and creatinine levels and restored total protein levels. The combination group (Group G) showed the most pronounced improvement, with urea and creatinine values even lower than those of the control group. This suggests a combined renoprotective effect, likely due to the complementary antioxidant, anti-inflammatory, and membrane-stabilizing properties of the bioactive compounds in both seeds. These results corroborate the findings of Mostafa (2021), who demonstrated that omega-3-rich seeds significantly improved kidney function markers in rats, and Marineli *et al*., (2015), who found that chia seed supplementation reduced oxidative renal injury.

The mechanism underlying these renoprotective effects likely involves the scavenging of ROS, reduction of lipid peroxidation, and modulation of inflammatory pathways, as supported by Mihafu (2024). The restoration of total protein in supplemented groups further indicates preservation of renal synthetic function, likely through reduced oxidative damage and improved cellular integrity.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue VII July 2025

This present study provides new evidence for the enhanced efficacy of combined chia and flax supplementation, which produced greater improvements than either seed alone. These combined efforts may be attributed to the complementary action of chia's soluble fiber and phenolic content with flax's lignans and omega-3 fatty acids.

# CONCLUSION

Chia and flaxseed supplementation, especially when combined, significantly reduces MSG-induced hematological and renal dysfunction in Wistar rats. These findings support the use of these supplements as functional dietary aids for managing metabolic stress and highlight the additional benefits of combined supplementation. However, further research is needed to assess their potential clinical application in humans.

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### Competing Interests

The authors declare no competing interests.

### Authors' Contributions

Iloh Patience N. designed the study, performed statistical analysis, and drafted the manuscript. Ifemeje J.C. supervised the experiments and contributed to the manuscript revision. Both authors approved the final version.

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ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue VII July 2025


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