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Impact of Germinated Millet Grains on Hepatorenal Toxicity of Rats induced by Lead-Acetate

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ABSTRACT

This study evaluated the protective potential of germinated millet powder (GMP) against lead acetate-induced toxicity in male Sprague Dawley rats, focusing on growth performance and hepatorenal biomarkers for 4 weeks. Thirty-five rats were divided into five groups: a negative control, a positive control receiving lead acetate (20 mg/kg BW/week), and three groups fed diets containing 20%, 30%, or 40% GMP along with lead. Germination slightly improved millet's nutritional profile by increasing protein and carbohydrates while reducing fat, fiber, and ash. GMP also showed higher antioxidant activity than raw millet, demonstrated by greater DPPH scavenging. Lead acetate was exposure in male albino rats caused significant reduction in feed intake, body weight gain, and feed efficiency ratio (FER), along with elevated liver enzymes (AST, ALT, ALP), kidney markers (urea, creatinine), and malondialdehyde (MDA) levels, while catalase activity significantly decreased Relative liver and kidney weights also declined. GMP supplementation improved all parameters in a dose-dependent manner. The 40% GMP group showed near-normal growth performance, significantly reduced AST, ALT, ALP, urea, creatinine, MDA levels, and restored catalase activity. **In conclusion:** Germinated millet powder exhibited strong protective effects against lead-induced hepatic and renal dysfunction. The observed improvements in liver and kidney markers, antioxidant balance, and organ weights are attributed to the enhanced antioxidant content and nutritional quality acquired through germination. These results support the use of GMP as a functional food to combat oxidative stress and mitigate heavy metal-induced hepatorenal toxicity.

Key words: Germinated millet powder, Liver, Kidney, Toxicity, Oxidative Stress.

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INTRODUCTION

The concept of biological oxidative stress was developed in more detail later in" Oxidative stress: a concept in redox biology and medicine", and it was defined as "an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage". At that time, when free radicals as having a possible role in, and were considered primary agents of many diseases, oxidative stress was a much-welcomed concept linking the damaging (**Azzi, 2022**).

Heavy metal exposure stems from both natural environments and human-made sources. Everyday sources of heavy metal exposure include: dentistry materials used in crowns, bridges, fillings, and partial dentures, prescription medicines, vaccines, contrast agents, metal tungsten

used in orthopedics, vascular medicine, and prosthodontics, air pollution, polluted food, polluted groundwater/drinking water , jewelry, cookware, lead-based paint, improperly coated containers, aluminum foil, and aluminum-containing products (health and beauty products). The liver and kidneys are primary targets for lead (the second most hazardous substance) toxicity due to their central roles in metabolism and excretion. Lead exposure increases serum levels of hepatic enzymes (ALT, AST, and ALP) and renal markers (urea, creatinine), indicating hepatocellular damage and reduced glomerular filtration (**Akhter *et al*., 2023 and Lee *et al*., 2024**).

Millet is a whole grain known for its high nutritional value, being naturally gluten-free and rich in proteins, dietary fiber, and essential minerals such as magnesium, zinc, and selenium. It also contains significant amounts of bioactive compounds, particularly polyphenols and flavonoids, which contribute to its antioxidant potential (**Raval and Ranote, 2025**). Germination is a simple and cost-effective bioprocess that further enhances the nutritional and functional properties of millet by activating hydrolytic enzymes that induce structural modifications, biochemical transformations, and the synthesis of new compounds. This process improves the bioavailability of nutrients and increases the concentration of antioxidant phytochemicals, while simultaneously reducing anti-nutritional factors such as phytic acid (**Theodoro *et al.*, 2021**).

The present study was designed to investigate the protective role of germinated millet powder against lead-induced hepatic and renal toxicity in Experimental rats, by assessing biochemical parameters.

MATERIALS AND METHODS

Materials

- Millet purchased from Agricultural Research Center, Giza, Egypt.
- Rats, total of 35 adult male albino rats, Sprague Dawley strain, weight (150 ±5 gm).
- Basal diet (AIN-93), the ingredients purchased from El-Gomhouria Pharmaceutical Company, Cairo, Egypt.
- Soy oil, Sucrose and starch were obtained from Egyptian Local Market, Cairo, Egypt.
- Chemical Kits and lead acetate purchased from Gamma Trade, Giza, Egypt.

Methods

Preparation of Germinated Millet:

For germination: grains cleaned and sorted to remove stone, dust particles, and broken, undersized, and immature grains. The remaining cleaned and washed then millet grains soaked for 24 h at room temperature (20–25° C) in a seed-to-Water (1:3) ratio, with slight modifications. The steeping water and grains separated using a plastic sieve and the grains placed in muslin cloths. The soaked and washed grains germinated for 24h in plastic bags at room temperature (20–25°C) (**Yenasew and Urga, 2023).** Then, the germinated millet grains dried by solar energy at **Solar Energy Unit, National Research Center, Dokki, Giza.** then milled using Cyclo-miller and sieved at 1mm to get germinated millet flour or powder (GMP).

Chemical composition of millet powder and dried germinated millet determined according to, **Dubois *et al.*, (1956)** for carbohydrates and **AOAC, (2005)** for protein, ash and lipids as well as total phenolic content according to **Brand *et al.*, (1995)** and antioxidant activity according to **Blois, (1958).**

Animal Experiment Design:

The experiment was carried out at the **Post Graduated Lab of Home Economics Faculty, Helwan University.** The Basel diet (g/kg diet) consisted of casein (18%), sucrose (10%), soybean oil (7%), cellulose (5%), mineral mixture (3.5%), vitamin mixture (1%), choline (0.25%) and remainder will be corn starch. Basal diet formulated according to **Reeves *et al.*, (1993).** Thirty-five Sprague Dawley adult male rats which weighing (150±5g) were obtained from Helwan Experimental Animals Farm, Helwan, Egypt. Animals were be housed in well aerated cages under hygienic conditions and feed on basal diet for one week for adaptation. After this period, the rats divided into 5 groups (6 rats each), as follow:

- **Group1:** Served as (–ve) control and was fed on basal diet.
- **Group2:** served as (+ve) control that was inducted with 20 mg/kg BW lead acetate once / week/4 weeks by oral according to **Riaz *et al.*, (2019**), and fed basal diet.
- **Group3:** as the same as group 2 and it was fed on diet containing 20% GMP.
- **Group4:** as the same as group 2 and it was fed on diet containing 30% GMP.
- **Group5:** the same as group 2 and was fed it was fed on diet containing 40% GMP.

Biological Parameters: feed intake was recorded daily, body weight gain, Feed Efficiency ratio (FER) and relative weight for kidney, liver and heart calculated according to **Chapman *et al.*, (1959**).

Biochemical Analysis:

Assay of Liver Enzymes: Activity of aspartate aminotransferase (AST) & alanine aminotransferase (ALT) and alkaline phosphatase (ALP) enzymes were assessed according to the methods of **Canepari *et al*., (1994**), **Hsueh *et al*., (2011) and Roy, (1970)** respectively.

Assay of Kidney Functions: serum urea nitrogen according to **Lear, (1950**) and creatinine were determined using the methods of **Pundir *et al.*, (2019).**

Determination of Serum Malondialdehyde and Catalase Enzyme: serum malondialdehyde (MDA) and activity of catalase enzymes were measured according to **Shin, (2009) and Góth, (1991),** respectively.

Statistical analysis:

Results expressed as the mean ±SE. Data will statistically be analyzing for variation "ANOVA" test at $P \leq (0.05)$ using SPSS statistical software, version 20 will be used for the calculation **(Armitage and Berr, 1987).**

RESULTS AND DISCUSSION

Proximate chemical composition of millet grain powder (MP) and germinated millet grain powder (GMP): -

The comparative analysis presented in **Table 1** of the chemical composition of raw millet powder (MP) and germinated millet powder (GMP) reveals significant changes induced by germination, reflecting enhanced nutritional potential and functional value.

The proximate analysis revealed that germinated millet (GMP) showed a noticeable increase in protein content compared to millet powder (MP), with values rising from 6.80 to 12 g/100g. In contrast, fat content slightly decreased in GM (3.4 g/100g) relative to MP (4.7 g/100g). Crude fiber also showed a reduction, from 4.89 g/100g in MP to 3.10 g/100g in GM. Additionally, ash content declined significantly in GM (2.1 g/100g) compared to MP (5.29 g/100g), indicating a

loss of mineral contents. Meanwhile, carbohydrate content slightly increased in GM (79.4 g/100g) compared to MP (78.32 g/100g), reflecting changes in carbohydrate structure and availability during germination.

The nutritional changes observed in germinated millet highlight the significant biochemical transformations that occur during the germination process. The marked increase in protein content can be attributed to enhanced enzymatic activity that breaks down storage proteins and stimulates de novo protein synthesis, making amino acids more available and improving overall protein quality (**Raval and Ranote, 2025**). The slight reduction in fat content is consistent with the activation of lipase enzymes, which mobilize stored lipids to support energy demands during sprouting (**Saleh *et al.*, 2013**).

The decrease in crude fiber is likely due to the conversion of insoluble fiber into soluble forms, which is commonly seen during germination and improves digestibility (**Chethan *et al.*, 2022**). The significant reduction in ash content suggests the leaching of minerals during soaking and rootlet formation, a common occurrence in germinated grains (**Sharma *et al.*, 2023).** Finally, the slight increase in carbohydrates may result from enzymatic hydrolysis of complex polysaccharides into simpler sugars, enhancing the energy density and bioavailability of the grain (**Gani *et al.*, 2012**). These combined changes reflect the ability of germination to enhance the nutritional profile of millet, making it a valuable functional food ingredient with potential health benefits.

Table (1): Chemical Composition of Millet Powder and Germinated Millet

S.N	Test	(MP) g/100g	(GM) g/100g
2	**Protein**	6.80	12
3	**Fats**	4.70	3.4
4	**Crude Fiber**	4. 89	3.1
5	**Ash**	5.29	2.1
6	**Total Carbohydrates**	78.32	79.40

Phenolic and flavonoid compounds concentration of MP and GMP: -

The presented in **Table 2** revealed the Phenolic and flavonoid compounds concentration of MP and GMP. Results showed that germination led to a notable increase in both total phenol and flavonoid contents in millet. Germinated millet (GM) showed a significantly higher concentration of total phenols compared to raw millet powder (MP) at 152.74 and 110.58 mg GAE/100g. Similarly, flavonoids increased slightly from 39.72 mg rutin/100g (MP) to 40.52 mg rutin/100g (GM).

These results align with numerous studies showing that germination enhances the biosynthesis and release of bioactive compounds, particularly phenolic acids and flavonoids, due to the activation of metabolic enzymes. During germination, hydrolytic enzymes break down complex

macromolecules, releasing bound phenolic compounds and converting them into more soluble and biologically active forms (**Sangma *et al.*, 2021**). The significant increase in total phenolics in GM can be attributed to enzymatic activation of the phenylpropanoid pathway, which is stimulated under sprouting-induced stress, leading to elevated levels of antioxidant metabolites (**Raval and Ranote, 2025**). Flavonoids, although less dramatically increased, still showed a modest enhancement, maintaining their role in free radical scavenging and synergizing with other phenolic compounds.

Table (2): Phenolic and Flavonoid Compounds Concentration of MP and GM

S.N	Test Item Identifier	Total Phenols (mg/100g gallic)	Total Flavonoids (mg/100g rutin)
1	**MP**	110.58	39.72
2	**GM**	152.74	40.52

The antioxidant activity of MP and GMP:

Results in **Table 3** showed the antioxidant activity of MP and MG at 2, 5 and 10%. The antioxidant activity of GM at 2% is more than double that of MP with mean values 31.74 and 13.99%, showing a significant early advantage. This difference narrows slightly at higher concentrations, yet GM maintains its lead at 5% with values 75.70% vs. 74.40% and 10% with values 86.35% vs. 83.96%, suggesting that germination enhances antioxidant potency, especially at lower doses.

These results confirm that germination enriches the bioavailability and reactivity of phenolic compounds and flavonoids, which are primary contributors to antioxidant activity (**Raval and Ranote, 2025**). Additionally, enzyme activation during germination enhances the synthesis of new antioxidant molecules. Studies show that germinated grains activate the nuclear factor erythroid 2–related factor 2 (Nrf2) pathway, improving the cellular antioxidant defense system and increasing free radical scavenging capacity (**Lin *et al.*, 2024**).

Table (3): The Antioxidant Activity of MP and MG

Test Item Identifier	%DPPH Radical-Scavenging Activity		
	2%	**5%**	**10%**
MP	13.99	74.40	83.96
GM	31.74	75.70	86.35

Effect of germinated millet grain on feed intake (FI), body weight gain (BWG) and feed efficiency ratio (FER):

The results in **Table 4** recorded the effect of millet grain on FI, BWG and FER. Results showed that negative group had the highest feed intake at 19.70 g/day/rat, reflecting normal appetite and metabolic function. In contrast, positive group exhibited a significantly reduced intake of 16.54 g/day, indicating appetite suppression likely caused by lead toxicity. Compared to Group 2, all germinated millet-supplemented groups (3, 4 and 5) demonstrated varying degrees of improvement with mean values 17.4, 18.9 and 19.6 g/day, respectively. The highest improvement was observed in Group feed with 40% germinated millet, which recorded 19.60 g/day. These findings indicate a dose-dependent recovery in appetite with increasing levels of millet supplementation.

Lead exposure significantly affected body weight gain, which progressively improved with germinated millet supplementation. Group 1 (-ve) achieved the highest gain with a mean value of 39.50%, reflecting normal growth conditions. In contrast, group 2 (-ve) exhibited a marked reduction to 15.66% compared to negative group, indicating impaired growth likely due to lead acetate induced toxicity. When compared to Group 2, millet-supplemented groups (3, 4 and 5) demonstrated a dose-dependent recovery with mean values 21.66, 27,16 and 32.33%, respectively. Group 5, which fed 40% germinated millet, exhibited the greatest recovery among the treated groups. These results suggest that millet may exert a protective effect against lead-related growth retardation, particularly at higher inclusion levels.

In FER, Group2 (+ve) significantly decreases compared to (-ve) group with mean values 0.02 vs. 0.07. indicating poor feed utilization. While Germinated millet supplementation groups (3, 4 and 5) improved FER progressively with mean values 0.03, 0.04 and 0.05 respectively.

Results of the experiment demonstrate the detrimental impact of lead toxicity on growth performance, as evidenced by the significantly reduced feed intake, body weight gain, and feed efficiency in the (+ve) group compared to the (–ve) group. These findings are consistent with previous reports showing that lead disrupts hypothalamic appetite regulation, damages intestinal absorption, and interferes with metabolic pathways essential for growth and energy balance (**Patrick, 2006; Flora *et al*., 2012**).

Germinated millet supplementation showed a dose-dependent recovery across all parameters. Group 5 (containing 40% millet) nearly restored feed intake to control levels and showed a 106% improvement in BWG% compared to Group 2. Germination is known to enhance the digestibility and bioavailability of millet by increasing enzyme activity and reducing anti-nutritional factors (**Chethan *et al.,* 2022**), which likely contributed to better nutrient absorption and metabolic efficiency in the treated groups.

Millet is rich in polyphenols, dietary fiber, and essential minerals such as magnesium, zinc, and selenium all of which may contribute to its detoxifying and antioxidant effects. These bioactive compounds are known to support gastrointestinal health, enhance nutrient absorption, and reduce oxidative stress a major mechanism by which lead causes cellular and tissue damage (**Saleh *et al.,* 2013**). In support of findings, a study by **Ibrahim and Sayed, (2023)** demonstrated that millet supplementation improved body weight and metabolic parameters in rats under oxidative stress. Also, **Li *et al.* (2021)** reported that millet positively.

Table (4): Effect of Germinated Millet Powder on Feed Intake (FI), Body Weight Gain (BWG) and Feed Efficiency Ratio (FER):

Parameters / Groups	FI (g\d\rat)	BWG%	FER
G1 (-ve)	19.70	39.50±00.76[a]	00.07±00.001[a]
G2 (+ve)	16.54	15.66±01.02[e]	00.02±00.001[e]
G3 (20%)	17.40	21.66±00.66[d]	00.03±00.002[d]
G4 (30%)	18.90	27.16±00.79[c]	00.04±00.001[c]
G5 (40%)	19.60	32.33±00.88[b]	00.05±00.003[b]

*Mean values are expressed as means ± SE.
*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.

Effect of germinated millet grain on relative organs weight (liver and kidneys):

Organ weights are sensitive indicators of systemic toxicity. Results presented in **Table 5** showed that rats exposed to lead acetate (+ve) showed a marked reduction in the relative weights of both liver and kidneys compared with (–ve) group with values 3.31 and 4.66%, indicating organ atrophy or dysfunction, likely resulting from oxidative stress, inflammation, and cellular degeneration caused by lead toxicity.

However, supplementation with germinated millet grain in Groups 3 to 5 showed a significant improvement in the relative weights of these organs with values 3.99, 4.01 and 4.31% respectively, approaching the values observed in the (-ve) group. This improvement suggests that millet may exert hepatoprotective and nephroprotective effects.

Lead exposure is well known to induce histopathological and functional damage in various organs, particularly the liver and kidneys, which are major sites for detoxification and metal accumulation (**Flora *et al*., 2012**). Germinated millet's higher polyphenol and antioxidant content enhances tissue repair and protects against structural degradation caused by oxidative damage (**Zhang *et al.*, 2025**). The improvement in organ weight reflects restoration of cellular function and reduction in inflammation and necrosis.

Table (5): Effect of Germinated Millet Powder in Organs (Liver, Kidneys) Relative Body Weight

Parameters / Groups	Liver	Kidneys
	%	
Group1 (-ve)	**04.66±00.40[a]**	**00.90±00.08[a]**
Group (+ve)	**03.31±00.30[b]**	**00.75±00.04[b]**
Group3 (20%)	**03.99±00.19[a]**	**00.84±00.07[a]**
Group4 (30%)	**04.01±00.12[a]**	**00.86±00.01[a]**
Group5 (40%)	**04.31±00.14[a]**	**00.86±00.02[a]**

*Mean values are expressed as means ± SE.
*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.

Effect of germinated millet grain on liver enzymes (AST, ALT and ALP) in serum:

Liver serum (AST, ALT and ALP) biomarkers are indicators of hepatic function and integrity, as they reflect the liver's ability to synthesize proteins, metabolize substances, and maintain biochemical balance. Results in **Table 6** showed that AST levels were significantly elevated in the lead acetate-intoxicated group positive, reaching 127.30 IU/L compared to 31.83 IU/L in the negative group. This sharp increase reflects substantial hepatocellular injury caused by oxidative stress from lead exposure. In contrast, Groups 3, 4, and 5, which received germinated millet at 20%, 30%, and 40%, respectively, exhibited progressively lower AST levels with mean values 93.60, 75.50, and 60.60 IU/L, respectively. The results suggest that germinated millet supplementation helped mitigate hepatocellular damage in a dose-dependent manner.

ALT, a cytoplasmic enzyme more liver-specific than AST, followed a similar pattern. Group 2 (+ve) displayed markedly significantly elevated ALT levels compared to (–ve) group with mean values 143.60 and 39 IU/L due to lead-induced hepatotoxicity. Germinated Millet-treated groups (3, 4 and 5) showed significant improvement with mean values 96.00, 80.00 and 71.00 IU/L, respectively, these results highlight millet's ability to restore liver cell membrane integrity and limit enzyme leakage. The reduction of ALT by over 50% in group 5 compared to group 2 further confirms the effectiveness of the highest millet dose in reversing liver damage.

ALP levels, an indicator of biliary function and hepatobiliary injury, were also significantly elevated in (+ve) group compared to (–ve) group with mean values 200.7 and 100.0 IU/L. Elevated ALP may result from cholestasis or biliary inflammation caused by lead acetate accumulation. Germinated millet grain led to a significant gradual reduction in ALP values across groups 3, 4 and 5 with mean values 186.5, 177.0, and 149.8 IU/L, respectively, with the lowest value in the group receiving 40% millet. While ALP remained above control levels even in Group 5, the downward trend strongly supports a protective effect of millet on the biliary system.

The current results demonstrate that germinated millet grain supplementation significantly reduced serum levels of liver enzymes in lead acetate-exposed rats, suggesting a hepatoprotective effect. These findings are strongly supported by previous research investigating

the impact of millet on liver function. **Nishizawa *et al.* (2002)** reported that rats fed a diet containing 20% millet protein for 14 days exhibited significantly lower levels of AST and ALT, following chemically induced liver injury using D-galactosamine. In a study, **Zhang *et al.* (2023)** found that protein hydrolysates derived from millet bran exhibited a therapeutic effect against non-alcoholic fatty liver disease (NAFLD) in rats.

The hepatoprotective effect of millet grain observed in this study can be attributed to its rich composition of bioactive compounds that counteract the oxidative damage and inflammation induced by lead exposure. Lead toxicity disrupts cellular redox balance, resulting in excessive generation of reactive oxygen species (ROS) that damage hepatocyte membranes, mitochondria, and Deoxyribonucleic acid (DNA). Millet polyphenols, flavonoids, and antioxidant peptides directly scavenge ROS and reduce lipid peroxidation, thus preserving hepatocyte integrity and preventing enzyme leakage into the bloodstream (**Zhang *et al.*, 2025**).

Studies have shown that germinated millet activates the Nrf2 antioxidant pathway, which enhances the expression of endogenous antioxidant enzymes such as catalase and glutathione peroxidase, while simultaneously inhibiting the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)-mediated inflammatory response. This dual modulation not only reduces hepatic oxidative damage but also limits the release of pro-inflammatory cytokines, thereby preserving hepatocyte membrane integrity and maintaining the proper function of liver enzymes (**Lin *et al.*, 2024).**

Table (6): Effect of Germinated Millet Powder on Liver Enzyme Activity

Parameter / Group	sAST	sALT	sALP
	lU/L		
Group1 (-ve)	**31.83±00.79^{e}**	**39.00±00.36e**	**100.0±00.36e**
Group2 (+ve)	**127.30±00.84^{a}**	**143.60±00.76a**	**200.7±00.61a**
Group3 (20%)	**93.60±00.71^{b}**	**96.00±01.09b**	**186.5±00.56b**
Group4(30%)	**75.50±00.92^{c}**	**80.00±00.36c**	**177.0±00.57c**
Grou5 (40%)	**60.60±010.88^{d}**	**71.00±00.36d**	**149.8±00.48d**

*Mean values are expressed as means ± SE.

*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.

Effect of germinated millet powder on kidney functions in serum:

Rustles presented in **Table 7** showed that urea nitrogen level in the lead-exposed group (+ve) was significantly elevated compared to the (–ve) group with mean values 102.5 and 40.66 mg/dL, indicating severe impairment in renal excretory function due to lead-induced nephrotoxicity. Administrated with germinated millet significantly reduced urea nitrogen levels in a dose-dependent manner: groups 3, 4 and 5 with mean values 90.16, 83.83 and 86.86 mg/ dL respectively, demonstrating significant gradual recovery of kidney function, with Group 5 showing the most notable improvement.

Creatinine levels were also significantly increased in positive control group relative to negative control group with mean values 2.37 and 0.33 mg/dL, confirming reduced glomerular filtration and renal clearance capacity due to oxidative damage from lead. Germinated millet groups (3, 4 and 5) displayed significantly reduced creatinine levels with mean values 1.02, 1.87 and 1.07 respectively, indicate that germinated millet supplementation improved renal filtration and minimized nephron damage in a dose-dependent manner.

Several recent studies have confirmed the nephrotoxic effects of lead exposure. Lead toxicity has been shown to significantly impair kidney function by reducing the estimated glomerular filtration rate (eGFR) and elevating renal injury markers such as cystatin C and β-trace protein (**Akhter *et al.*, 2023**). Even low blood lead levels below 5 μg/dL were associated with increased mortality risk in patients with chronic kidney disease (CKD). Mechanistically, lead is known to disrupt mitochondrial function in proximal tubule cells, leading to overproduction of ROS, cellular injury, and chronic inflammatory responses in renal tissues (**Lee *et al.*, 2024**). The reno-protective effects can be attributed to several interrelated mechanisms. Millet grains are rich in polyphenols, flavonoids, and antioxidant peptides, which scavenge Reactive Oxygen Species (ROS) generated by lead toxicity. By reducing lipid peroxidation and oxidative cellular injury, these compounds help preserve renal tubular and glomerular structure (**Zhang *et al.*, 2025**).

Germinated millet is reported to activate the Nrf2 signaling pathway, leading to increased expression of renal antioxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutase, while simultaneously suppressing the pro-inflammatory NF-κB pathway (**Ghani *et al.*, 2024**). These mechanisms collectively reduce tubular necrosis, preserve glomerular filtration function, and support renal tissue regeneration. Supporting studies have shown similar protective effects of millet-based interventions in heavy metal nephrotoxicity models, reinforcing the relevance of dietary strategies in mitigating chronic kidney damage (**Ibrahim *and Saied* 2023**).

Table (7): Effect of Germinated Millet Powder on Kidney Enzyme Activity

Parameter / Group	Urea Nitrogen	Creatinine
	mg/Dl	
Group1 (-ve)	**40.66±00.80^{e}**	**00.33±00.02^{e}**
Group2 (+ve)	**102.5±00.92^{a}**	**02.37±00.33^{a}**
Group3 (20%)	**90.16±00.31^{b}**	**02.02±00.31^{b}**
Group4 (30%)	**83.83±00.54^{c}**	**01.87±00.21^{c}**
Group5 (40%)	**68.50±00.56^{d}**	**01.07±00.21^{d}**

*Mean values are expressed as means ± SE.

*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.

Effect of germinated millet grain on oxidations enzymes:

Results presented in **Table 8** showed that Catalase activity, a key indicator of antioxidant defense, was significantly reduction in positive group compared to negative group with mean values 79.50 and 31.50 lU/mL, indicating severe oxidative stress and depletion of antioxidant enzymes due to lead acetate toxicity. Germinated millet supplementation in groups 3 to 5

significantly improved catalase levels in a dose-dependent manner with mean values 37.00, 45,17 and 54.50 lU/mL respectively.

The malondialdehyde (MDA) results revealed significant differences among the experimental groups, highlighting the impact of lead toxicity and the potential protective role of millet. group 2 (+ve) exhibited the significant highest MDA level compared to (–ve) group with mean values 3.11 and 1.72μM, indicating severe oxidative stress due to lead acetate exposure. Compared to group 2, all germinated millet-supplemented groups (3, 4 and 5) demonstrated significant reductions in MDA levels in a dose-dependent manner with mean values 2.85, 2.51 and 1.99 μM respectively, group 5 that fed 40% GMP showed the best result. These findings suggest that GMP supplementation mitigates oxidative damage and enhances the antioxidant status in a concentration-dependent manner.

Lead-induced oxidative stress is primarily mediated by the excessive generation of ROS, which disrupts cellular homeostasis and damage lipids, proteins, and DNA. One of the earliest and most sensitive indicators of oxidative stress is the reduction in antioxidant enzyme activity, such as catalase, and an increase in lipid peroxidation by-products like MDA (**Flora *et al.*, 2012**). Germination enhances the concentration of phenolic antioxidants, flavonoids, and peptides that activate the Nrf2 signaling pathway, leading to increased transcription of genes encoding antioxidant enzymes such as catalase, superoxide dismutase (SOD), and glutathione peroxidase. These changes reinforce the body's ability to neutralize ROS, thereby reducing oxidative damage. Furthermore, millet polyphenols have been shown to suppress ROS generation by down regulating Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase and modulating mitochondrial redox status (**Lin *et al.*, 2024 and Zhang *et al.*, 2025**).

Anther studies have also demonstrated that germinated millet enhances antioxidant enzyme activity more effectively than ingeminated forms due to its enriched phytochemical profile post-germination (**Kumar *et al.*, 2024**). This enhanced antioxidant capacity is crucial in mitigating lipid peroxidation, as evidenced by the substantial reduction in MDA, a key biomarker of oxidative membrane damage in lead-exposed models.

Table (8): Effect of Germinated Millet Powder on Oxidations Enzymes Activity

Parameter / Group	Catalase	MDA
	lU/mL	μM
Group 1 (-ve)	**79.50±00.62^{a}**	**01.72±00.10^{e}**
Group 2 (+ve)	**31.50±00.56^{e}**	**03.11±00.05^{a}**
Group 3 (20%)	**37.00±00.52^{d}**	**02.85±00.02^{b}**
Group 4 (30%)	**45.17±00.40^{c}**	**02.51±00.08^{c}**
Group 5 (40%)	**54.50±00.23^{b}**	**01.99±00.02^{d}**

*Mean values are expressed as means ± SE.

*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.

Conclusion

Present study highlights the protective role of germinated millet powder against lead acetate-induced hepatorenal toxicity in rats. Supplementation with GMP resulted in significant improvements in growth performance, liver and kidney function, and oxidative stress markers in a dose-dependent manner. These effects are likely attributed to the enhanced nutritional profile and antioxidant content developed through germination. Therefore, germinated millet may serve as a promising functional food for mitigating heavy metal-induced organ damage.

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