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Impact of Germinated Millet Grains on Lipid Profile of Rats induced by Lead-Acetate

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ABSTRACT

This study investigated the protective effects of germinated millet grain against lipid profile disturbances and oxidative stress induced by lead acetate in male rats. Thirty-five Sprague Dawley rats were assigned to five groups: a negative control, a lead-exposed positive control, and three groups received diets with 20%, 30% and 40% germinated millet powder respectively. Chemical analysis showed that germination improved millet's nutritional value by increasing protein and carbohydrates, and reducing fat, fiber, and ash. It also increased the levels of total phenols and flavonoids, and enhanced antioxidant activity. Lead exposure significantly impaired feed intake, weight gain, feed efficiency, and organ weights. These effects were alleviated in a dose-dependent manner with millet supplementation, with the 40% group showing the greatest improvement. Lead caused dyslipidemia—elevated cholesterol, triglycerides, LDL-c, VLDL-c, atherogenic index, and reduced HDL-c. Germinated millet significantly improved these parameters, indicated hypolipidemic and cardioprotective effects. Oxidative stress, marked by decreased catalase and increased malondialdehyde, was also observed in lead-exposed rats. Germinated millet-fed groups showed restored antioxidant status in a dose-dependent manner, likely due to its phenolic compounds. In conclusion, germinated millet grain demonstrated protective effects against lead-induced dyslipidemia and oxidative stress may be throughout improving lipid metabolism, boosting antioxidant defenses, and supporting liver and kidney function, suggesting its potential as a functional food against heavy metal toxicity.

Key words: Millet grain- Lead acetate - Lipid profile- Oxidative stress.

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INTRODUCTION

The concept of biological oxidative stress was developed in more details 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 were considered to play a possible role and act as primary agents in many diseases, oxidative stress was a much-welcomed concept linking the damaging." (**Azzi, 2022**).

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 & 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 disturbances and oxidative stress induced by lead acetate in rats, through the assessment of 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, weighed (150 ±5 GMP).
Basel diet (AIN-93): the ingredients purchased from El-Gomhouria Pharmaceutical Company, Cairo, Egypt.
Chemicals: chemicals and Kits 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, Research center, Dokki, Giza**. then milled using Cyclo-miller and sieved at 1mm to get germinated millet flour or powder (GMPP).
Chemical composition: chemical composition of germinated Millet determined according to **Dubois *et al.*, (1956),** as well as total phenolic content according to **Brand *et al*., (1995)** and antioxidant activity according to **Blois, (1958).**
Experimental 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 protein (18%), sucrose (10%), soybean oil (7%), cellulose (5%), mineral mixture (3.5%), vitamin mixture (1%), choline (0.25%) and remainder will be corn starch. Diet will be 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 housed in well aerated cages under hygienic conditions and fed on basal diet for one week for adaptation. After this period the rats were divided into 5 groups (6 rats each), as follow:

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

Biological analysis: feed intake was recorded daily, body weight gain, feed efficiency ratio and relative weight for kidney, liver and heart will be calculated according to **Chapman *et al.,* (1959**).

Biochemical tests:

Determination of lipid profile: Serum triglycerides (TG) was measured according to **Richmond, (1973)**, serum total cholesterol (TC) was measured according to **Wahlefeld, (1974)**, the concentration of high-density lipoprotein (HDL-c) measured according to **Albers *et al.,* (1983)**, LDL-c and VLDL-c concentrations measured according to **Friedewal *et al.,* (1972)** as follows**:**

LDL-c (mg/dl) =TC- (HDL-c + VLDL-c).

VLDL-c (mg/dl) = (Triglycerides /5)

Atherogenic index (AI) is calculated in keeping with **Dobiášová and Frohlich, (2001)** by the formula: log (TG/HDL-C).

Determination of catalase and malondialdehyde: Catalase was determined according to **Góth, (1991)** and malondialdehyde (MDA) according to **Shin, (2009).**

Statistical analysis:

Results will be 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.

Proximate analysis indicated that germinated millet (GMP) exhibited a marked increase in protein content, rising from 6.80 to 12 g/100 g compared to millet powder (MP). Conversely, fat content decreased slightly in GMP with value 3.4 g/100 g relative to MP with value 4.7 g/100 g. Crude fiber was also reduced, declining from 4.89 g/100 g in MP to 3.1 g/100 g in GMP. Ash showed a notable decrease from 2.1 g/100 g in GMP to 5.29 g/100 g in MP, suggesting a reduction in mineral content. Meanwhile, carbohydrate content increased marginally in GMP (79.4 g/100 g) compared to MP (78.32 g/100 g), likely due to structural and compositional changes occurring during germination.

The nutritional modifications induced by germination in millet reflect a series of complex biochemical processes that enhance its functional and dietary value. The notable elevation in protein content can be ascribed to increased enzymatic activity during germination, which facilitates the degradation of storage proteins and initiates de novo protein synthesis. This process increases the availability of free amino acids and subsequently improves the biological quality of protein (**Raval and Ranote, 2025**). A slight reduction in fat content was consistent

with the activation of endogenous lipase enzymes, which catalyze the hydrolysis of stored lipids. This mobilization serves to meet the energetic demands of the developing seedling during early sprouting stages (**Saleh *et al.*, 2013**).

The observed decrease in crude fiber content is likely associated with the partial conversion of insoluble fiber fractions into soluble forms. This transformation is a well-documented effect of germination and contributes to improved digestibility and gastrointestinal functionality (**Chethan *et al.*, 2022**). The marked reduction in ash content may be attributed to the leaching of mineral elements into the soaking medium and their redistribution during radicle and rootlet emergence. Such mineral loss is commonly reported in germinated cereals and pseudocereals (**Sharma *et al.*, 2023).**

Finally, the slight increase in carbohydrates is plausibly linked to the enzymatic hydrolysis of structural polysaccharides into simpler and more bioavailable sugars. This enhances both the energy density and metabolic accessibility of the germinated millet (**Gani *et al.*, 2012**). Collectively, these transformations underscore the role of germination in enhancing the nutritional profile of millet, positioning it as a promising ingredient for functional food development with potential health-promoting properties.

Table (1): Chemical Composition of MP and GMP

S.N	**Test**	**(MP) g/100g**	**(GMP) 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	79.4

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 (GMP) 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 (GMP).

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 (**SanGMPa *et al.,* 2021**).

The significant elevation in total phenolics in GMP was likely driven by the enzymatic activation of the phenylpropanoid pathway, which is stimulated under sprouting-induced stress, leading to elevated levels of antioxidant metabolites. Although the increase in flavonoid content was comparatively modest, it remains notable, contributing to the overall antioxidant potential of the grain through direct free radical scavenging and synergistic interactions with other phenolic compounds (**Raval & Ranote, 2025**).

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

S.N	Test Item Identifier	Total Phenols (mg gallic acid /100g)	Total Flavonoids (mg rutin /100g)
1	**MP**	110.58	39.72
2	**GMP**	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 GMP at 2% was more than double that of MP with mean values 31.74 and 13.99%, showed a significant early advantage. This difference narrows slightly at higher concentrations, yet GMP maintains its lead at 5% with values75.70% vs. 74.40% and 10% with values 86.35% vs. 83.96%, suggesting that germination enhances antioxidant potency, especially at lower doses.

These results confirmed that germination enriched the bioavailability and reactivity of phenolic compounds and flavonoids, which were primary contributors to antioxidant activity (**Raval and Ranote, 2025**). Additionally, enzyme activation during germination enhanced the synthesis of new antioxidant molecules. Studies show that germinated grains activated 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 GMP

Test Item Identifier	%DPPH Radical-Scavenging Activity		
	2%	**5%**	**10%**
MP	13.99	74.40	83.96
GMP	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 control group had the highest feed intake at 19.70 g/day/rat, reflecting normal appetite and metabolic function. In contrast, positive control 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 varied 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% GMP, which recorded 19.60 g/day. These findings indicated a dose-dependent recovery in appetite with increased levels of GMP supplementation.

Body weight gain was significantly influenced by lead exposure and showed progressive improvement 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 (–ve), indicating impaired growth likely due to lead acetate induced toxicity. When compared to Group 2, GMP-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 suggested that millet may exert a protective effect against lead-related growth retardation, particularly at higher inclusion levels.

Regarding FER, Group2 positive control significantly decreases compared to negative 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 (40% millet) nearly restored feed intake to control levels and showed a 106% improvement in BWG% compared to Group 2. Germination 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.

Germinated millet is a valuable source of polyphenols, dietary fiber, and essential trace minerals such as magnesium, zinc, and selenium constituents that collectively contribute to its potential detoxifying and antioxidant properties. These bioactive components recognized for their role in promoting gastrointestinal health, improving nutrient bioavailability, and mitigating oxidative stress, which is a key pathway through which leads to exerts its cytotoxic and tissue-damaging effects (**Saleh *et al.*, 2013**). In support of findings, a study by **Ibrahim and Sayed (2023)** demonstrated that millet supplementation showed improvement in body weight and metabolic parameters in rats under oxidative stress. Also, **Li *et al.* (2021)** reported that millet positively.

Table (4): Effect of Millet Grain 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 negative group. This improvement suggested that millet may exert hepatoprotective and nephroprotective effects.

Lead exposure was 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.

In hepatic tissue, the antioxidant constituents of germinated millet were thought to facilitate hepatocyte regeneration and enhanced the activity of enzymatic detoxification systems. This occurs, in part, through modulation of the Nuclear factor erythroid 2–antioxidant response element (Nrf2–ARE) signaling pathway, which upregulated the expression of cytoprotective and antioxidant genes. Experimental evidence supported this mechanism; millet supplementation had been shown to significantly reduce serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lipid peroxidation products in toxin-challenged animal models, indicating improved liver function and oxidative balance (**Ibrahim and Sayed, 2023**).

In kidneys, germinated millet may reduce nephrotoxicity by preserving glomerular and tubular structure, maintaining electrolyte balance, and reducing inflammatory cytokine levels. **Li**

***et al*. (2021)** observed significant improvements in renal biomarkers in millet-treated diabetic rats, which share mechanistic parallels with oxidative damage from lead. The gradual normalization of liver and kidney weights with increasing millet inclusion supported the hypothesis that its protective benefits were dose dependent.

Table (5): Effect of Millet Grain in Organs (Liver, Kidneys) Relative 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 lipid profile:

Lipid profile is an indicator of overall metabolic health and cardiovascular risk, as it reflects the body's lipid metabolism, including levels of total cholesterol, triglycerides, HDL, LDL, and VLDL. Results presented in **Table 6** showed that there was a significant increase in TC levels in (+ve) group compared to the (–ve) group with mean values 319.99 and 102.17 mg/dL, indicating lead-induced hypercholesterolemia. Administration with germinated millet led to a significant and dose-dependent reduction in cholesterol levels across groups 3, 4 and 5 with mean values 248.42, 236.83 and 204.13 mg/dL respectively, indicating a clear hypo-cholesterolemic effect, especially at the 40% supplementation level.

Similarly, TG levels were significantly elevated in the lead-exposed group (+ve) compared to (–ve) group with mean values 99.33 and 63.66mg/dL, while Groups 3–5 demonstrated significant reductions with mean values 91.83, 84.00, and 73.66 mg/dL, respectively. The reduction in TG was particularly notable in group 5, which approached near-normal values, suggesting improved hepatic lipid regulation and reduced triglyceride synthesis under the influence of millet's antioxidant constituents.

The HDL-$_C$, known for its protective cardiovascular role, was severely reduced in (+ve) group indicating impaired reverse cholesterol transport due to oxidative stress compared with (–ve) group with mean values 14.83 and 48.33 mg/dL. However, germinated millet supplementation increased HDL-C significantly, mean values 21.16, 27.33, and 37.00 mg/dL in

Groups 3, 4, and 5 respectively. Results indicated that millet reversed the adverse effects of lead on protective cholesterol fractions.

LDL-$_C$, the most atherogenic lipid parameter, was drastically elevated in the (+ve) compared to (–ve) group with mean values 285.3 and 41.17 mg/dL A gradual and statistically significant decline in LDL-C was noted in germinated millet groups (3-5) with mean values 208.9, 109.7 and 152. mg/dL respectively. Group 5 represented an almost 50% reduction from the untreated toxic group.

VLDL-$_C$ was also significantly higher in (+ve) group compared to (–ve) group with mean values 19.86 and 12.66 mg/dL. Germinated millet supplementation produced significant reductions in VLDL-$_C$ across treated groups (3-5) with mean values 18.36, 16.80 and 14.73 mg/dL respectively, group 5, showed best improvement supporting the lipid-modulating effect of millet under oxidative insult.

Lastly, the atherogenic index (AI), an important predictor of cardiovascular risk, was significantly elevated in the (+ve) group compared to the (–ve) group with mean values 0.83 and 0.12. germinated millet-fed groups (3-5) displayed significant improvement, with the index dropping to 0.64, 0.49, and 0.23 respectively. The results confirmed the beneficial role of millet in reducing cardiovascular risk markers associated with lead-induced dyslipidemia.

Lead acetate induced dyslipidemia resulted from its disruption of lipid metabolism through enhanced lipid peroxidation, inhibition of lipoprotein lipase activity, and increased oxidative degradation of polyunsaturated fatty acids (**Ibrahim *et al.*, 2023**). Lead exposure also upregulated hepatic HMG-CoA reductase, a key enzyme in cholesterol biosynthesis, contributing to elevated serum LDL-C and total cholesterol (**Omugha *et al.*, 2024**). These pathological mechanisms explained the pronounced lipid abnormalities observed in +ve group and highlighted the importance of antioxidant-rich dietary interventions like germinated millet to restore lipid balance.

The hypolipidemic and hepatoprotective effects of germinated millet observed in lead-exposed rats were mediated by multiple, interconnected biological pathways. Millet naturally rich in polyphenols, flavonoids, and dietary fiber, which together exerted powerful antioxidant effects. These compounds were capable of scavenging ROS generated as a result of lead-induced oxidative stress, thereby preventing lipid peroxidation, protecting hepatic cells, and improving lipid metabolism (**Lin *et al.*, 2024; Zhang *et al.*, 2025**).

Germination enhanced millet's phytosterol profile and soluble dietary fiber content, which played a crucial role in lowering serum cholesterol levels. Phytosterols compete with dietary cholesterol for absorption in the intestine, effectively reducing LDL-$_C$ concentrations, while fibers bind bile acids and promote their excretion, leading to increased hepatic cholesterol turnover (**Liu *et al.*, 2023**). Furthermore, studies on germinated millet had demonstrated upregulation of genes involved in lipid metabolism, such as ABCA1 and PPAR-α, contributing to improved HDL-C synthesis and lipid clearance from plasma (**Zhang *et al.*, 2025**).

Another important mechanism is the modulation of gut microbiota. Based on study millet polyphenols selectively enhanced the growth of beneficial gut bacteria, which in turn regulated lipid metabolism, reduced endotoxemia, and suppressed hepatic inflammation. This gut–liver axis plays a key role in protecting against lipid disorders induced by heavy metals such as lead (**Ghani *et al.*, 2024**). Oxidative stress markers such as catalase and malondialdehyde (MDA) are indicators of the balance between pro-oxidant and antioxidant activity in the body. Catalase is an antioxidant enzyme that protects cells by decomposing hydrogen peroxide into water and oxygen, thereby reducing oxidative damage. MDA, on the other hand, is a byproduct of lipid

peroxidation and serves as a reliable marker of oxidative damage to cell membranes. Together, these biomarkers are widely used to assess oxidative stress status and the impact of toxic agents, disease conditions, or protective dietary interventions (**Sies and Jones, 2021**).

Table (6): Effect of Millet Grain on Lipid Profile

Parameters / Groups	CH	TG	HDL-C	LDL-C	VLDL-C	AI
	mg\dl					
Group1 (-ve)	102.17±01.79 e	63.33±01.20 e	48.33±01.05 a	41.17±02.69 e	12.66±00.24 e	00.12±00.01 e
Group2 (+ve)	319.99±01.46 a	99.33±01.64 a	14.83±00.94 e	285.3±01.53 a	19.86±00.32 a	00.83±00.02 a
Group3 (20%)	248.42±00.99 b	91.83±00.90 b	21.16±00.60 d	208.9±01.08 b	18.36±00.18 b	00.64±00.01 b
Group4 (30%)	236.83±01.85 c	84.00±00.96 c	27.33±00.71 c	192.7±01.64 c	16.80±00.19 c	00.49±00.01 c
Group5 (40%)	204.13±01.25 d	73.66±00.95 d	37.00±00.85 b	152.4±01.55 d	14.73±00.19 d	00.23±00.01 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 7** showed that Catalase activity, a key indicator of antioxidant defense, was significantly reduction in (+ve) group compared to (–ve) group with mean values 79.50 and 31.50 U/mg, indicated 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 U/mg respectively.

The malondialdehyde (MDA) results revealed significant differences among the experimental groups, highlighting the impact of lead toxicity and the potential protective role of

germinated millet. group 2 (+ve) exhibited the significant highest MDA level compared to (–ve) group with mean values 3.11 and 1.72μM, indicated 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 suggested that GMP supplementation mitigated oxidative damage and enhanced the antioxidant status in a concentration-dependent manner.

Lead-induced oxidative stress is primarily mediated by the excessive generation of ROS, which disrupted cellular homeostasis and damage lipids, proteins, and DNA. One of the earliest and most sensitive indicators of oxidative stress was the reduction in antioxidant enzyme activity, such as catalase, and an increase in lipid peroxidation by-products like MDA (**Flora *et al.*, 2012**). Germination significantly elevated the levels of phenolic antioxidants, flavonoids, and bioactive peptides capable of activating the Nrf2 signaling pathway. This activation enhances the transcription of genes encoding key antioxidant enzymes, including catalase, superoxide dismutase (SOD), and glutathione peroxidase, thereby strengthening endogenous defense mechanisms against oxidative stress. By bolstering the cellular capacity to neutralize reactive oxygen species (ROS), these changes helped attenuate oxidative damage. Additionally, millet-derived polyphenols had been reported to inhibit ROS production by downregulating NADPH oxidase activity and modulating mitochondrial redox balance, contributing further to their cytoprotective effects (**Lin *et al.*, 2024; Zhang *et al.*, 2025**).

Recent studies had also demonstrated that germinated millet enhanced antioxidant enzyme activity more effectively than ungerminated forms due to its enriched phytochemical profile post-germination (**Kumar *et al.*, 2024**). This enhanced antioxidant capacity was 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 (7): Effect of Millet Grain on Oxidations Enzymes

Parameter / Group	Catalase	MDA
	U/mg	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

Lead exposure caused marked dyslipidemia characterized by hypercholesterolemia, hypertriglyceridemia, increased LDL and VLDL, and reduced HDL, contributing to an elevated atherogenic index. Dietary supplementation with germinated millet grain significantly ameliorated these effects in a dose-dependent manner. The 40% millet group showed the most favorable lipid profile, suggesting that the bioactive compounds in germinated millet such as flavonoids, phenolic acids, and fibers exerted lipid-lowering, antioxidative, and protective actions against oxidative stress–induced lipid disturbances.

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