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In Vivo Evaluation of the Protective Effects of *Lagenaria siceraria* (Molina) on Lead- and Cadmium-Induced Toxicity in Rats

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ABSTRACT

The present study investigated the protective effects of *Lagenaria siceraria* (Molina) fruit powder against lead- and cadmium-induced toxicity in male albino rats. Thirty Sprague Dawley rats (150 ± 10g) were divided into five groups (n = 6): a negative control, a positive control exposed to lead and cadmium, and three treatment groups receiving diets supplemented with 2.5%, 5%, and 10% dried *L. siceraria* powder for 28 days. Serum biomarkers of oxidative stress (MDA), liver and kidney function, lipid profile, and antioxidant enzymes were analyzed, and histopathological examination of liver tissue was performed. Exposure to heavy metals caused significant increases in serum glucose, total cholesterol, triglycerides, LDL-c, AST, ALT, ALP, and MDA levels, while HDL-c and antioxidant enzyme activities (SOD, CAT, GPx) decreased markedly. Dietary supplementation with *L. siceraria*, particularly at 10%, significantly ($p<0.05$) ameliorated these adverse effects, restoring biochemical parameters and improving liver tissue architecture. These findings demonstrate the hepatoprotective and nephroprotective potential of *L. siceraria* fruit, likely due to its rich content of bioactive antioxidants.

1. Introduction

Heavy metals are natural elements characterized by high atomic weight and densities exceeding 4 g/cm³, typically five times that of water. Due to their extensive use in industrial, agricultural, medical, and technological sectors, heavy metals and metalloids have become major environmental contaminants. Their widespread presence raises significant concerns regarding their adverse effects on ecosystems and human health. Generally, these elements are toxic even at trace levels, with their toxicity depending on factors such as dose, chemical form, exposure route, and host characteristics including age, sex, and nutritional status (Okpara et al., 2022; Omar et al., 2023). Among heavy metals, arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) are categorized as priority pollutants because of their systemic toxicity and capacity to damage multiple organs. They also contribute to oxidative stress by disturbing antioxidant enzymes activity, such as superoxide dismutase. (Gomaa, 2022). Cadmium is one of the most hazardous and persistent environmental contaminants. Anthropogenic sources include battery production, phosphate fertilizers, electronic waste burning, and tobacco smoke it is also used in pigments, the manufacturing of polyvinyl chloride plastics and electroplating. Soil contamination from industrial discharge or sewage sludge leads to cadmium bioaccumulation in crops. The recommended dietary intake is 0.007mg/kg body weight, and the permissible limit in drinking water is 0.005 mg/L. Acute ingestion causes gastrointestinal distress, while chronic exposure damages the kidneys, liver, bones, and nervous system and is linked to immunosuppression and reproductive toxicity. The World Health Organization classifies cadmium and its compounds as Group 1 carcinogens (Mehrdad et al., 2017, and Mohyudin et al., 2022).

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Lead has long been used in paints, pipes, and industrial materials. Although its environmental mobility is limited, it remains a common contaminant. Inorganic lead, once absorbed, accumulates primarily in the blood, soft tissues, and bones, with over 95% of total body burden stored in the skeleton. Children are particularly vulnerable due to their higher absorption rates and developing nervous systems. Lead exposure causes neurodevelopmental impairment, oxidative stress, DNA damage, and carcinogenic effects (Natasha et al., 2021; Tareq & Sheikh, 2025). Medicinal and edible plants have been used since ancient times plants, to treat various ailments, and their potential as natural protectors against chemical toxicity is increasingly recognized Many plant species possess antibacterial, antioxidant, organ protective qualities (Kalsait et al., 2017). Among these, *Lagenaria siceraria* (Molina) Standl., commonly known as bottle gourd, a member of the Cucurbitaceae family native to Asia and Africa, has shown promising pharmacological potential. The fruit of *L. siceraria* exhibits antihyperglycemic, antihyperlipidemic, antithrombotic, cardiotonic, hepatotonic, and anti-atherosclerotic properties. These effects are attributed to its rich phytochemical profile, including ascorbic acid, campesterol, spinasterol, kaempferol, palmitic acid, linoleic acid, quercetin, and isoquercetin (Zahoor et al., 2021, and Muhammad et al., 2022). These effects are attributed to its rich phytochemical profile, including ascorbic acid, campesterol, spinasterol, kaempferol, palmitic acid, linoleic acid, quercetin, and isoquercetin (Shirwakar & Sreenivasan, 2019; Zahoor et al., 2021; Muhammad et al., 2022). HPLC analyses have identified flavone-C glycosides and a novel polypeptide, lagenin, known for its antioxidant and antimicrobial activities (Chakraborty & Ghosh, 2020). Given the severe toxicological impact of cadmium and lead, and the reported antioxidant and protective effects of *Lagenaria siceraria*, this study aimed to evaluate the ameliorative and protective properties of different levels of *L. siceraria* fruit powder against cadmium–lead-induced toxicity in male albino rats. The evaluation was based on biochemical, antioxidant, and histopathological parameters to determine the plant's potential as a natural detoxifying agent. *Lagenaria siceraria* (Molina) fruit (Figure 1).

Figure 1. *Lagenaria siceraria* (Molina) fruit

2. Materials and Methods

Plant material for raw material

Fresh fruits of *Lagenaria siceraria* (Molina) were obtained from the greenhouses of the Vegetable Research Department of Humidity Pollination, Horticultural Research Institute, Agricultural Research Center, Giza, Egypt.

Experimental animals

The experiment was conducted on 30 healthy adult male albino rats (Sprague-Dawley strain) weighing 150±10g. The animals were obtained from the Animal House, Department of Medical Analysis, Faculty of Pharmacy, Mansoura University, Egypt. The approval letter was conducted from MU-ACUC (Mansoura University animal care and use committee) code number: MU-ACUC (OTH.R.25.10.5). The animals were obtained from the Animal House, Department of Medical Analysis, Faculty of Pharmacy, Mansoura University, Egypt.

Chemicals and diagnostic kits

Cadmium chloride and lead chloride were used as sources of cadmium and lead, respectively. All biochemical assays diagnostic kits were obtained from El-Gomhoria and Nasr Company for Chemical Industries, Egypt.

Dietary components

Ingredients used in the formulation of the basal diet, including casein, cellulose, starch, and premixed vitamins and minerals, were procured from Technogene Co., Dokki, Giza, Egypt. Components used for the preparation of ice cream formulations were obtained from local markets in Egypt.

Methods

Preparation of dried (*Lagenaria siceraria*) Molina powder

Ca 40kg of the whole fresh fruit of Bottle gourd (*Lagenaria siceraria*) Molina were harvested, washed, and cut to slices, followed by air-drying at ambient temperature. After complete drying, the obtained pieces of the dried fruits were grinded by mill, affording 2kg of dried powder (Aldewy et al., 2022).

Chemical analysis of dried (*Lagenaria siceraria*) Molina powder

Proximate Composition

Moisture, crude protein, crude fat, ash and crude fiber were determined according to AOAC (2012). Total carbohydrate and energy values contents were calculated by difference using the following Elizabete et al. (2004):

Total carbohydrates (%) =100−(Moisture% + Protein% + Fat%+Ash%+ Fiber%)

Total Energy Value (Kcal/100g) = 4 × (Protein % + Carbohydrates %) + 9 × (Fat %).

Estimation of mineral content

Mineral composition (Na, K, Ca) was analyzed using a flame photometer (Model Corning 410), while Cu, Zn, Se, Fe, P, and Mg were quantified using an atomic absorption spectrophotometer (Perkin Elmer Model 2380) as described by AOAC (2012) and Nzikou et al. (2009).

Amino acid profile

Amino acid composition was determined following Adeyeye and Afolabi (2004). Defatted samples were hydrolyzed with 6 MHCl at 110°C for 24h, and amino acids were quantified using a TMS amino acid analyzer.

Determination of antioxidant vitamins

Vitamin C content

The colorimetric method described by Klein and Perry (1982) was used to determine the ascorbic acid (vitamin C) content of dried *Lagenaria siceraria* (Molina) powder. In this method, ascorbic reacts with 2,6-dichlorophenolindophenol (DCPIP) and the extent of decolorization is measured spectrophotometrically, being directly proportional to the vitamin C concentration. Results were expressed as milligrams of ascorbic acid per 100 grams of sample.

Vitamin E content

The spectrophotometric method described by Rutkowski and Grzegorczyk (2007) was used to determine the vitamin E (α-tocopherol) content of dried *Lagenaria siceraria* (Molina) powder. In This method, vitamin E is extracted using an organic solvent and then reacts with ferric chloride and α, α′-dipyridyl to form a red-colored complex. The intensity of the color is measured using a UV–Vis spectrophotometer at 520 nm. The vitamin E concentration was calculated by comparing the sample's absorbance with a standard calibration curve prepared from known concentrations of α-tocopherol. Results were expressed as milligrams of vitamin E per 100 grams of sample.

β-carotene content

The β-carotene content of dried *Lagenaria siceraria* powder was determined using the colorimetric method described by Nagata and Yamashita (1992). Carotenoids were extracted with an acetone –hexane mixture, and absorbance was measured at 453 nm using a UV–Vis spectrophotometer. The β-carotene concentration (mg/100 mL) was calculated using the following equation:

$$\beta - \text{carotene} = 0216A_{663} - 1.220A_{645} - 0.304A_{505} + 0.452A_{453}$$

Where, A_{663}, A_{645}, A_{505} and A_{453} represent absorbance values at specific wavelengths. Results were expressed as milligrams of β-carotene per 100 grams of sample.

Phenolic composition as a whole

The total phenolic content of dried *Lagenaria siceraria* (Molina) powder was determined using the Folin–Ciocalteu colorimetric method described by Singleton and Rossi (1965). In this procedure, phenolic compounds react with the Folin–Ciocalteu reagent to form a blue-colored complex, which is measured spectrophotometrically at 765 nm. Gallic acid was used as a standard, and results were expressed as milligrams of gallic acid equivalents (mg GAE) per 100 grams of sample.

Flavonoid's content

The total flavonoid content of dried *Lagenaria siceraria* (Molina) powder was determined using the aluminum chloride colorimetric method, with detection at 415 nm. Quercetin was used as the reference standard, and the flavonoid concentration was expressed as milligrams of quercetin equivalents (mg QE) per 100 grams of sample.

Tannin content

The tannin content of dried *Lagenaria siceraria* (Molina) powder was determined using the method described by Bohm and Kocipai (1994). In this procedure, tannins were extracted and reacted with specific reagents, and the absorbance of the resulting solution was measured spectrophotometrically. Results were expressed as milligrams of tannic acid equivalents (mg TAE) per 100 grams of sample.

Biological experiment

Experimental Design

Rats were divided into five groups (n = 6 each):

- **Group 1 (Negative Control):** Fed the basal diet only.
- **Group 2 (Positive Control):** Exposed to cadmium and lead (5 mg/kg b.w.) without supplementation.
- **Group 3:** Exposed to cadmium and lead + diet containing 2.5% *L. siceraria* powder.
- **Group 4:** Exposed to cadmium and lead + diet containing 5% *L. siceraria* powder.
- **Group 5:** Exposed to cadmium and lead + diet containing 10% *L. siceraria* powder.

The experiment lasted for 28 days. Body weight gain, feed intake, and feed efficiency ratio were calculated weekly according to Chapman et al. (1959).

Sample Collection and Biochemical Analysis

At the end of the experimental period, blood samples were collected after 12 h fasting. Serum was separated by centrifugation and stored at −20 ° C for analysis of glucose, lipid profile, liver enzymes (AST, ALT, ALP), kidney function markers, total protein, and antioxidant enzymes (SOD, CAT, GPx, GST, TAC, and MDA) using standard methods (AOAC, 2012; Henry, 1974; Reitman & Frankel, 1957; Ohkawa et al., 1979).

Histopathological Examination

Liver tissues were fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic examination (Bancroft et al., 1996).

Basal diet Composition

The basal provided to all experimental rats was prepared in accordance with the guidelines of the American Institute of Nutrition's guidelines (AIN, 1993). The composition of the diet was as follows: 67.6% corn starch, 11.9% casein (protein source), 10% corn oil (fat source), 4% salt mixture, 1% vitamin mixture, 5% bran (fiber source), 0.3% methionine and 0.2% choline chloride. This diet was formulated to meet the nutritional requirements of growing laboratory rats while maintaining ensuring consistency across experimental groups.

Cadmium and lead toxicity induction

Cadmium and lead toxicity were induced by orally administering a mixture of cadmium chloride and lead chloride to the designated experimental groups at a dose of 5 mg/kg body weight per day, dissolved in distilled water, for three consecutive weeks. The protocol was adapted from El-Demerdash et al. (2004), which provides a validated model for simulating chronic environmental exposure to heavy metals in rodents.

Following exposure, serum analysis confirmed systemic absorption and bioaccumulation of the administered metals, with cadmium and lead concentrations of 2.5 µg/L and 40 µg/dL, respectively.

Biological evaluation

Feed intake (FI), Body Weight Gain (B WG), and Feed Efficiency Ratio (FER)

Throughout the 28-day experimental period, body weight was recorded weekly, and daily feed intake for each rat was monitored. At the end of the study, physiological performance parameters, including body weight gain (BWG), feed efficiency ratio (FER), and relative organ weights, were evaluated. These indices were calculated according to the method described by Chapman et al. (1959).

Feed Intake (FI) (g/day) =Total feed given(g) −Feed refused (g)

Body Weight Gain (BWG) (g)=Final body weight (g)−Initial body weight(g)

Feed Efficiency Ratio (FER)=Body weight gain(g) / Feed intake (g)

Blood parameters and organs collection

At the end of the 28-day experimental period, blood samples were collected from all groups after a 12-hour fasting period. Rats were anesthetized with ether and sacrificed by cardiac puncture through the abdominal aorta. Blood was drawn into clean, dry centrifuge tubes and allowed to clot for 30 minutes at room temperature. Samples were then centrifuged at 3000 rpm for 10 minutes to separate the serum. The obtained serum was carefully aspirated into labeled sterile plastic tubes and stored at −20°C until biochemical analysis (Malhotra, 2003).

Serum samples were analyzed for the following biochemical parameters:

- Glucose: Determined according to Trinder (1969).
- Total cholesterol and triglycerides: Determined according to Allain (1974) and Fassati and Prencipe (1982), respectively.
- Lipoproteins (LDL-C, VLDL-C, HDL-C): Determined according to Lopez (1977) and Lee and Nieman (1996).
- Total protein, albumin (Alb), globulin (Glb), and albumin/globulin ratio (A/G): Determined following Srivastava et al. (2002).
- Liver enzymes (AST, ALT, ALP): Measured according to Reitman and Frankel (1957), Henry (1974), and I.F.C.C (1983), respectively.
- Antioxidant enzymes and oxidative stress markers (GPx, SOD, CAT, GST, TAC, and MDA): Determined following Zhao (2001), Sun et al. (1988), Diego (2011), Koracevic et al. (2001), Hegsted et al. (1941), and Ohkawa et al. (1979).

Simultaneously, the liver, kidneys, lungs, heart, and spleen were excised, rinsed with saline, blotted dry with filter paper, and weighed according to the method of Drury and Wallington (1980).

Preparation of liver tissue

Following anesthesia, the liver of each rat was carefully excised and rinsed with 0.9% sodium chloride (NaCl) solution to remove residual blood. Tissue homogenates were prepared according to the method of Kumari et al. (2016) using a Teflon homogenizer in a 1:10 (w/v) ratio, 1g of wet tissue per 10mL of 0.05M ice-cold phosphate buffer (pH 7.5).

The homogenate was divided into two portions:

- **First portion:** Mixed with an equal volume of 10% trichloroacetic acid (TCA) and centrifuged at 5000 rpm for 10 minutes at 4°C.
- **Second portion:** Centrifuged at 15,000 rpm for 60 minutes at 4°C to obtain a clear supernatant for superoxide dismutase (SOD) determination (Ellman, 1959; Marklund and Marklund, 1974).

Antioxidant enzyme activities in liver tissue were determined using the same methods applied for serum samples.

Collection sample of cadmium and lead in serum for rats treated with different levels of Molina powder

Approximately collect 3–5mL of whole blood was collected from each rat using metal-free collection tubes (e.g., trace-metal grade EDTA tubes). Samples were Centrifuged at 3000rpm for 10 minutes to separate the serum. which was then

transferred into clean, acid-washed polyethylene tubes and stored at −20°C until analysis.

Sample digestion

For digestion, 0.5 mL of serum was placed into a digestion vessel, followed by the addition of 1.0 mL of concentrated nitric acid (HNO_3) and 0.5 mL of hydrogen peroxide (H_2O_2). The mixture was digested in a water bath at 90°C for 1–2 hours, or until the solution became clear. After cooling, the digested sample was diluted to 10mL with deionized water.

Preparation of standards

Calibration standards for lead (Pb) and cadmium (Cd) were prepared at concentrations of:0.5, 1.0, 2.0, 5.0µg/L (ppb). Standards were prepared using the same acid matrix as the samples to ensure matrix consistency.

Instrumental conditions (GFAAS)

Instrumental analysis (GFAAS)

The concentrations of Pb and Cd were determined using graphite furnace atomic absorption spectrophotometry (GFAAS) under the following conditions:

- **Wavelengths:** Pb, 283.3 nm; Cd, 228.8 nm
- **Atomizer:** Graphite furnace
- **Injection volume:** 20 µL

Matrix modifier (optional)

0.05% Pd $(NO_3)_2$ or Mg $(NO_3)_2$ to improve atomization accuracy. Results were expressed as micrograms of Pb or Cd per liter of serum.

B. Product application (Ice Cream Fortification).

Preparation of ice cream formulations

Ice cream formulations were prepared according to Mabrouk et al. (2021). The mix consisted of milk, cream, and sugar, with *L. siceraria* powder incorporated at 10% (based on the optimal biological activity observed in the animal experiment). The mixture was pasteurized at 80–85 °C for 15–20 min, homogenized, cooled to 4 °C, aged for 12–24 h, flavored, frozen, and stored at −18 °C until analysis.

Table 1. Formula of ice cream mixes (g/2 kg).

Ingredients	Control sample	*Lagenaria siceraria* product
Sugar (sucrose)	310	310
Skim milk powder	200	-----
Fresh milk	1270	1270
Fresh cream	200	200
Carboxymethyl cellulose.	10	10
Vanilla flavor	10	10
Dried fruit	----	200
Total	2000	2000

Physicochemical analysis of ice cream

Ice cream samples were analyzed for pH, titratable acidity, moisture, and protein content according to AOAC (2000). pH values were determined following Veena et al. (2020).

Sensory evaluation

Sensory evaluation was performed by 50 trained panelists from the Department of Nutrition and Food Science, Faculty of Specific Education, Mansoura University. Samples were coded and evaluated for flavor, texture, melting quality, and overall acceptability using the method of Kramer and Twigg (1962).

Statistical analysis

The mean ± standard deviation (SD) represents the results. Using the Statistical Analysis System, differences between treatment groups were deemed statistically significant at $P < 0.05$ (SAS, 1985).

3. Results and Discussion

The chemical makeup of powdered dry Molina *(Lagenaria siceraria)*

The proximate composition of dried (*Lagenaria siceraria*) Molina powder is presented in Table 2. The powder **c**ontained 9.23% moisture, 19.65% protein, 4.92% fat, 5.95% crude fiber, 4.26% ash, and 55.99% total carbohydrates with a total caloric value of 346.84 kcal/100g **(**dry weight basis).

The relatively low moisture content (9.23%) is advantageous for extended shelf life as it minimizes microbial growth and spoilage compared to fresh forms. The high Total carbohydrate content (55.99%) indicates that *L. siceraria* serves as an excellent energy source, while the substantial protein level was 19.61% highlights its nutritional potential as a dietary supplement. The ash content (4.26%) reflects a moderate mineral presence, which is comparable to that values reported for fluted pumpkin (4.66g/100g) and melon (4.14g/100g) by Olaofe et al. (2009) and Ogunbusola (2018). These findings confirm that *L. siceraria* is a nutrient-dense functional food capable of contributing macronutrients and minerals that support normal metabolic and physiological processes.

Table 2. Chemical composition of dried (*Lagenaria siceraria*) molina powder (%)

Chemical composition	Contents (g/100g)
Moisture	9.23±0.56 [d]
Protein	19.65±1.04 [c]
Fat	4.92±0.42 [e]
Fiber	5.95±0.09 [e]
Ash	4.26±0.23 [e]
Available carbohydrates	55.99±3.77 [b]
Total Calories	346.84±5.75 [a]

No statistically significant difference between the mean values of the three times values in the identical column that share the same superscript letters ($p \leq 0.05$)

The mineral composition of dry Molina powder *(Lagenaria siceraria)*

Table 3 presents the mineral composition (mg/100g) of the dried *Lagenaria siceraria* powder. Potassium (467.52mg/100g), was the most abundant mineral, followed by phosphorus, while selenium and zinc were present in the lowest concentration. Similar trends were observed by Ogunbusola et al. (2008). The high potassium content agrees with findings of Olaofe et al. (2018), who reported that potassium as the predominant mineral in several Nigerian agricultural products. The dried fruit powder also contained moderate amounts of selenium, and relatively low levels of copper. Owing to its high potassium potassium-to-sodium ratio, *L. siceraria powder* contribute to the regulation of blood pressure and cardiovascular health. The calcium-to-magnesium (Ca/Mg) ratio in the dried fruit was calculated as 0.824, which is considerably lower than the recommended dietary ratio of 2.2. This lower ratio is likely due to the fruit's limited calcium content. Therefore, if *L. siceraria* is to be utilized in dietary formulations particularly in weaning or complementary foods calcium fortification may be required to meet nutritional adequacy standards (Muhammad et al., 2022). Dried fruit of Molina (*L. siceraria*) was shown specifically to be rich in Copper (17.3mg/100g) and Chromium (0.132 mg/100 g) along with an abundance of Potassium and Iron according to Aldewy et al. (2022).

Table 3. Mineral content of dried (*Lagenaria siceraria*) Molina powder (mg/100g)

Minerals	Quantity
Iron	9.67
Phosphorous	250.93
Potassium	467.52
Zinc	0.673
Magnesium	5.59
Copper	9.07
Sodium	4.42
Calcium	12.86
Selenium	0.211

Amino acids content of dried (*Lagenaria siceraria*) Molina powder

Table 4 presents the amino acid profile of dried (*Lagenaria siceraria*) (Molina) powder. Amino acids are classified as either essential or non-essential based on the body's ability to synthesize them in sufficient quantities. Essential amino acids cannot be adequately produced by the human body and therefore must be obtained through dietary intake. In the analyzed sample, glutamic acid was identified as the most abundant non-essential amino acid. Among the essential amino acids, leucine, valine, and threonine were present in relatively high concentrations, whereas tryptophan, cystine, and methionine were detected in comparatively low amounts. The FAO/WHO/UNU (1991) reference amino acid pattern is commonly employed to assess the nutritional quality of food proteins by comparing their amino acid profiles. *Lagenaria siceraria* was found to contain several nutritionally important amino acids, including lysine and valine, which are

reported to be present in relatively high levels. According to Attar and Ghane (2019), valine is the only amino acid in *Lagenaria siceraria* that exceeds the FAO/WHO/UNU reference standard.

Table 4. Amino acids content of dried (*Lagenaria siceraria*) Molina powder (%)

Amino acids	Content
Essential amino acids	
Tryptophan	5.50
Theronin	15.27
Isolucine	5.51
Leucin	16.40
Methionin	3.48
Phenylalanine	12.59
Valine	17.86
Cystine	3.55
Non-Essential amino acids	
Arginine	13.84
Glutamic acid	23.38
Histidine	6.33

Antioxidant Vitamins, total phenols, total flavonoids, and tannin content of dried (*Lagenaria siceraria*) Molina powder

Table 5 presents the concentrations of antioxidant vitamins and phenolic compounds in dried *Lagenaria siceraria* (Molina) powder. The primary antioxidant vitamins identified were vitamin E, β-carotene, and vitamin C, with concentrations of 23.83mg/100g, 20.63mg/100g, and 10.96mg/100g, respectively. Vitamin E acts as a lipid-soluble antioxidant by interacting with lipid radicals generated during lipid peroxidation, thereby protecting cell membranes from oxidative damage (Azzi, 2007). Its antioxidant activity is attributed to the donation of a hydrogen atom or hydroxyl group from its chroman ring, which terminates radical chain reactions (Deshmukh and Sherkar, 2019). Reported vitamin E contents among different *Lagenaria siceraria* cultivars range from 5.5 to 25.0mg/100g, while Peter et al. (2013) recorded lower levels of 3.39mg/100g and 1.0mg/100g, respectively. Vitamin C plays a critical role in collagen synthesis, blood and hormone production, bone and tooth development, scurvy prevention, and antioxidant defense against free radicals. It donates hydrogen and electrons to convert ascorbic acid into dehydroascorbic acid, thereby halting the propagation of free radicals (Olaniyi, 2005). The measured vitamin C concentration (10.96 mg/100 g) aligns closely with previously reported values for *Lagenaria siceraria* 17.94 mg/100g (Shaneh, 2021) and 10.90mg/100g (Juee and Naqishbandi, 2020). In addition to these vitamins, the antioxidant components of dried *Lagenaria siceraria* powder include tannins, total flavonoids, and total phenols, with concentrations of 25.035±2.05mg/100g, 42.541±6.83mg QE/g, and 58.972±4.87µg GAE/g, respectively. Tannins exhibit antioxidant activity through metal ion chelation, free radical scavenging, and inhibition of lipid peroxidation. The tannin content observed in this study is comparable to the findings of Okonkwo (2009), who reported values ranging from 2.5 mg/100g (ethyl acetate extracts) to 12mg/100g (aqueous extracts). Attar and Ghane (2019) also reported a tannin content of 9.20mg/100g in *L. siceraria*. Tannins are known for their therapeutic potential in cancer prevention and for their astringent properties that promote wound healing and tissue recovery (Perron and Brumaghim, 2019). Phenolic compounds exert antioxidant effects primarily through free radical scavenging. Their concentrations vary among different fruit cultivars (Sharma et al., 2012). Tapkir et al. (2013) reported that short-hybrid bottle gourd (*L. siceraria*) contains high levels of total phenolics (10µg GAE/g) and flavonoids (17.9mg QE/g). Flavonoids contribute to antioxidant capacity by scavenging reactive oxygen species, chelating transition metals, and inhibiting lipid

peroxidation. The hydroxyl group at the C3 position of the flavonoid structure plays a crucial role in its metal-chelating and radical-scavenging functions (Litoto and Frey, 2020).

Table 5. Antioxidant Vitamins, total phenols, total flavonoids, and tannin content of dried (*Lagenaria siceraria*) Molina powder

Phytochemical	Content
Vit.C (mg/100g)	10.96± 1.56 [c]
Vit.E (mg/100g)	23.83± 1.85 [a]
β-carotene (mg/100g)	20.63± 0.94 [b]
Total phenols µg GA/g	58.972±4.87 [a]
Total flavonoid mg QE/g	42.541±6.83 [b]
Tannins mg/100g	12.035±2.05 [c]

No statistically significant difference between the mean values of the three times values in the identical column that share the same superscript letters ($p \leq 0.05$).

Biological evaluation

Effect of different levels of dried *Lagenaria siceraria* (Molina) powder on body weight gain, feed intake, and feed efficiency ratio in rats posed to cadmium and lead toxicity

The data presented in Table 6 illustrate the effects of different concentrations of dried *Lagenaria siceraria* (Molina) fruit powder on body weight gain (BWG), feed intake (FI), and feed efficiency ratio (FER) in rats subjected to lead and cadmium toxicity. As shown, the BWG, FI (g/28 days), and FER of the positive control group (+), which received cadmium and lead without supplementation with *Lagenaria siceraria* powder, significantly decreased ($P < 0.05$) compared with the negative control group (–). Conversely, rats in the toxic groups that were fed diets supplemented with 2.5%, 5%, and 10% dried *Lagenaria siceraria* powder exhibited gradual improvements in BWG, FI, and FER. The most pronounced enhancements were observed in the groups receiving 5% and 10% *Lagenaria siceraria* powder (Groups 4 and 5, respectively). These findings indicate that supplementation with dried *Lagenaria siceraria* powder can mitigate the adverse effects of heavy metal toxicity on growth performance and nutritional parameters. Similar observations have been reported in previous studies. Tianran et al. (2024) demonstrated that elevated lead and cadmium intake is associated with reduced energy consumption and weight gain, contributing to underweight conditions. Likewise, Stahr et al. (2021) reported a negative correlation between lead exposure and body weight in both children and adults. Flora (2019) also attributed weight loss to increased exposure to heavy metals such as lead and mercury from contaminated food sources and fertilizers. Statistical analysis in the present study revealed a strong positive correlation between supplementation with *Lagenaria siceraria* powder and improvements in BWG, FI, and FER when compared with the positive control group. These findings align with those of Shaneh (2021), who reported that *Lagenaria siceraria* extract contains bioactive compounds such as triterpenes, flavonoids, alkaloids, tannins, and coumarins that enhance growth performance. These bioactive constituents contribute to detoxification through mechanisms including ion exchange, hydrogen bonding, chemical binding, and electrostatic interactions, thereby reducing the bioavailability and toxic impact of heavy metals.

Table 6. Effect of different levels of dried *Lagenaria siceraria* (Molina) powder on body weight gain, feed intake, and feed efficiency ratio in rats exposed to cadmium and lead toxicity

Parameters / Groups	BWG (g/28 d)	FI (g/day)	FER (g/rat/day)
Negative control (G1)	35.52[a]±0.02	12.15[a]±0.002	0.104 [a]±0.009
Positive control(G2)	4.14[e]±0.01	6.36[d]±0.005	0.023[e]±0.002
2.5% dried *Lagenaria siceraria* (Molina) powder (G3)	7.43[d]±0.02	6.25[d]±0.005	0.042[d]±0.001
5% dried *Lagenaria siceraria* (Molina) powder (G4)	13.36[c]±0.03	8.83[c]±0.005	0.054[c]±0.005
10 % dried *Lagenaria siceraria* (Molina) powder (G5)	19.38[b]±0.05	10.47[b]±0.001	0.07[b]±0.005

Effect of different levels of dried *Lagenaria siceraria* (Molina) powder on organ weight in rats exposed to cadmium and lead toxicity

The results presented in Table 7 demonstrate the effects of cadmium and lead toxicity, as well as dietary supplementation with dried *Lagenaria siceraria* (Molina) fruit powder, on the relative weights of selected organs in rats. As shown, the greatest improvement in organ weights was observed in Group 5, which received 10% dried *Lagenaria siceraria* powder along with cadmium and lead exposure, followed by Group 4, which was supplemented with 5% dried fruit powder. Notably, lung weight did not differ significantly among the experimental groups.

The fruit *Lagenaria siceraria* is rich in carbohydrates, dietary fiber, vitamins, lipids, proteins, amino acids, and vital minerals, all of which contribute to its high nutritional value. Beyond its nutrient composition, dried *Lagenaria siceraria* powder has been reported to exhibit potent antioxidant and antimutagenic properties. Numerous studies have demonstrated that extracts derived from *Lagenaria siceraria* provide protective effects in conditions associated with oxidative stress and free radical damage. These include immunomodulatory, hepatoprotective, antioxidant, antihyperglycemic, antihyperlipidemic, and cardiotonic activities, which are often attributed to elevated levels of endogenous antioxidant enzymes such as glutathione, catalase, and superoxide dismutase (Deshpande et al., 2017). Furthermore, Diab and Aboul (2012) attributed the free radical scavenging capacity of dried fruits powder to the presence of ellagitannins in the epicarp, along with anti-angiogenic elements such as selenium. These bioactive constituents likely play a key role in the protective effects observed contributing to the preservation of organ weights and enhancing overall physiological resilience under heavy metal toxicity.

Table 7. Effect of different levels of dried *Lagenaria siceraria* (Molina) powder on organs weight (g) in rats exposed to cadmium and lead toxicity

Groups / Organ Weight (g)	Negative control (G1)	Positive control (G2)	2.5% dried *Lagenaria siceraria* (Molina) powder G3)	5% dried *Lagenaria siceraria* (Molina) powder (G4)	10% dried *Lagenaria siceraria* (Molina) powder (G5)
Liver	5.700 ^{d}±0.16	7.409 ^{a}±0.571	7.110 ^{a} ±0.55	6.910 ^{b} ±0.35	6.554^{c}±0.244
Heart	0.775 ^{c}±0.125	0.925 ^{a}±0.050	0.900 ^{a}±0.016	0.850 ^{b}±0.141	0.800 ^{c}±0.0577
Kidneys	1.350 ^{b}±0.122	1.62 ^{a}±0.302	1.605 ^{a}±0.095	1.580 ^{a}±0.276	1.53^{a} ±0.144
Lungs	1.250^{a}±0.216	1.300^{a}±0.129	1.290^{a}±0.129	1.270^{a}±0.090	1.265^{a}±0.057
Spleen	0.720^{d}±0.048	1.300^{a}±0.016	1.290 ^{a}±0.011	1.120 ^{b}±0.083	0.990^{c}±0.020

No statistically significant difference between the mean values of the three times values in the identical column that share the same superscript letters ($p \leq 0.05$).

Effect of different levels of dried *Lagenaria siceraria* (Molina) powder on serum glucose and lipid profile in rats exposed to cadmium and lead toxicity

The data presented in Table 8 highlight the effects of cadmium and lead mixture-induced toxicity and dietary supplementation with dried *Lagenaria siceraria* fruit powder on serum glucose levels and lipid profiles in rats. As shown the most favorable serum glucose level, closely resembling that of the normal control group, was observed in Group 5, which received 10% *Lagenaria siceraria* fruit in the diet alongside cadmium and lead exposure. Previous studies have demonstrated that *Lagenaria siceraria* (dried Molina fruits) exerts by reducing blood glucose levels and enhancing serum insulin concentrations. For instance, in alloxan-treated animals, blood glucose levels increased to approximately 210mg/dL compared to normal rats (~70 mg/dL); however, administration of *L. siceraria* extract reduced glucose levels to 89–106.5mg/dL. Dried *Lagenaria* fruit powder is edible and serves as an excellent source of essential nutrients,

including vitamin C, β-carotene, B-complex vitamins, pectin, and choline a key lipotropic factor. The hypoglycemic effect of dried fruit powder is primarily attributed to its high content of dietary fiber, antioxidants, and phenolic compounds. According to Randive et al. (2019), dietary fiber can lower blood glucose by inhibiting amylase activity, reducing glucose absorption, and slowing glucose diffusion in the digestive tract. The observed glucose reduction in the 10% *Lagenaria* group may therefore be-linked to its elevated high fiber content. Both in vitro and clinical studies have demonstrated that hydrated fibers delay glucose absorption and mitigate postprandial blood glucose spikes. Furthermore, the presence of fructose, a low glycemic index sugar, in dried fruit powder may further contribute to its hypoglycemic action (Barh and Mazumdar, 2019). Regarding lipid metabolism, the results presented in Table 8 indicate the cadmium–lead toxicity in the positive control group (+) significantly decreased serum levels of LDL-c, VLDL-c, total cholesterol TC, and triglycerides (TG), while significantly increasing HDL-c (P≤0.05), compared to the negative control group (–). Conversely, rats fed diets supplemented with 2.5%, 5%, and 10% dried *Lagenaria siceraria* powder exhibited showed marked improvements in lipid parameters, characterized by significant reductions in LDL-C, VLDL-C, TC, and TG alongside significant increases in HDL-C concentrations. The most pronounced ameliorative effects were recorded in Group 5 (10% fruit powder), followed by Group 4 (5% dried fruit powder). These improvements in lipid profile may be attributed to the presence of dietary fiber and polyphenolic compounds in *Lagenaria siceraria* powder, which are known to enhance lipid metabolism and reduce cardiovascular risk. As reported by Tacker and Thomas (2019), dietary fiber can lower LDL cholesterol by binding bile acids in the intestine and promoting their excretion. Thereby stimulating the conversion of endogenous cholesterol into bile acids. This mechanism contributes to the hypocholesterolemic effect of dietary fiber and helps reduce the risk of cardiovascular disease.

Table 8. Effect of different levels of dried *Lagenaria siceraria* (Molina) powder on serum glucose and lipid profile (mg/dl) in rats exposed to cadmium and lead toxicity

Groups Parameter (mg/dl)	Negative control G1	Positive control G2	2.5% dried *Lagenaria siceraria* (Molina) powder G3)	5% dried *Lagenaria siceraria* (Molina) powder (G4)	10 % dried *Lagenaria siceraria* (Molina) powder (G5)
Glucose	115.42 ^{d} ± 4.00	203.55 ^{a} ± 11.0	167.32 ^{b} ± 8.88	153.56 ^{c} ± 7.28	149.35 ^{c} ±3.52
Total cholesterol (TC)	80.15 ^{f} ± 4.25	135.01 ^{a} ± 1.37	126.9 ^{b} ±1.62	120.62 ^{c} ±2.24	114.13 ^{d} ±2.38
Triglycerides (TG)	79.1 5 ^{e} ± 0.946	124.83 ^{a} ± 2.7	120.80 ^{b} ±2.40	110.23 ^{c} ±4.20	97.41 ^{d} ±2.56
LDL-c	23.23 ^{d} ± 1.8	53.42 ^{a} ± 1.5	47.12 ^{b} ± 2.7	43.22 ^{b} ± 2.7	40.5 ^{c} ±3.5
HDL-c	41.09 ^{c} ± 3.7	56.62 ^{ab} ± 1.4	55.62 ^{ab} ± 1.4	55.35 ^{b} ±2.3	54.15 ^{b} ±2.6
VLDL-c	15.83 ^{c} ± 0.187	24.97 ^{a} ± 0.585	24.16 ^{a} ± 1.29	22.05 ^{a} ± 1.29	19.48 ^{b} ±0.01

No statistically significant difference between the mean values of the three times values in the identical column that share the same superscript letters (p≤0.05).

Mean values, 6 rats' results, in the same row sharing the same superscript letters are not statistically significantly different at (p≤0.05)

Effect of different levels of dried *Lagenaria siceraria* (Molina) powder on liver functions in rats exposed to cadmium and lead toxicity

Data in Table 9 confirm that rats exposed to cadmium and lead toxicity and subsequently treated with 2.5%, 5%, or 10% dried *Lagenaria siceraria* (Molina) powder exhibited a significant (P ≤ 0.05) reduction in liver enzyme levels compared to the positive control group (+), which received the toxic mixture without any supplementation. Among the treated groups, Group 5 (10% dried fruit powder) showed the most pronounced improvement, indicating the effectiveness of this dosage in ameliorating liver enzyme disorders.

Patrick (2016) emphasized that lead exposure plays a critical role in damaging various organs, including the liver. Elevated levels of liver enzymes such as AST, ALT, and ALP are widely recognized biomarkers of hepatotoxicity resulting from heavy metal exposure. Cadmium intake through contaminated food and beverages interferes with hepatic metabolism, promotes lipogenesis, and causes ATP depletion. Both cadmium and lead act as free radicals, inducing oxidative stress and damaging lipids, proteins, and nucleic acids. This oxidative stress has been associated with several pathological conditions, including carcinogenesis, mutagenesis, neurodegenerative diseases, aging, atherosclerosis, and depression (Sapolsky, 2019). The hepatoprotective effect of *L. siceraria* is primarily attributed to its strong antioxidant properties, which help neutralize free radicals and prevent lipid peroxidation, one of the main mechanisms underlying hepatotoxicity. The dried fruit powder is also rich in vitamin C, phenolic compounds, and flavonoids, which contribute to its antioxidant activity and support hepatic detoxification processes, including those required for metabolizing xenobiotics such as carbamazepine (Owais, 2019). In addition to liver enzyme levels, total protein and albumin concentrations serve as important biomarkers for hepatic function, since they are synthesized predominantly in the liver. Hypoalbuminemia is often observed in advanced liver disease and reflects impaired hepatic protein synthesis. In the present study, treatment with *L. siceraria* resulted in measurable reduction in total protein (by 9.9%) and albumin (by 10.3%), which may indicate a partial impairment of protein synthesis due to residual hepatocellular damage (Mayakrishnan et al., 2013, and Mehjabeen et al., 2016). Nevertheless, these findings suggest that *L. siceraria* fruit powder mitigates and protects, though may not completely reverse, the toxic effects of cadmium and lead on liver function.

Table 9. Effect of different levels of dried *Lagenaria siceraria* (Molina) powder on liver functions in rats exposed to cadmium and lead toxicity

Groups / Parameter	Negative control (G1)	Positive control (G2)	2.5% dried *Lagenaria siceraria* (Molina) powder G3)	5% dried *Lagenaria siceraria* (Molina) powder (G4)	10 % dried *Lagenaria siceraria* (Molina) powder (G5)
AST(IU/L)	39.6 ^{e}± 9.52	80.73 ^{a} ± 4.0	71.36 ^{b}±4.4	65.13 c± 6.81	56.09 ^{d}± 3.38
ALT(IU/L)	38.72 ^{e}± 4.70	72.57 ^{a}±2.29	68.68 ^{a}± 6.32	53.38 ^{c}± 6.01	47.92 ^{d}± 1.41
AST/ ALT	1.02 ^{c}± 0.36	1.11 ^{b}± 0.65	1.04 ^{c}± 0.58	1.22 ^{a}± 0.92	1.17 ^{a}± 0.47
ALP(IU/L)	44.21 ^{d}±0.210	73.21 ^{a} ±0.210	67.21 ^{b} ±0.210	61.87 ^{b}± 0.210	54.98^{c}± 0.210
Globulin mg/dl	2.74 ^{a}± 0.227	2.22 ^{b}± 0.147	2.24 ^{b}± 0.252	2.31 ^{b}± 0.355	2.42 ^{a}± 0.192
ALP/ globulin	16.14 ^{d}±0.210	32.98 ^{a}± 0.300	30.004 ^{a} 0.197 $^{\pm}$	26.78 ^{b}± 0.228	22.72 ^{c}± 0.146
Albumin (mg/dl)	4.28 ^{a}± 0.395	3.48 ^{b}± 0.359	3.47 ^{b} ± 0.396	3.53 ^{b}± 0.510	3.86 ^{a}± 0.447
Total protein (mg/dl)	7.03 ^{a}± 0.378	6.240 ^{a}±0.120	6.100 ^{a} ±0.252	6.540 ^{a}± 0.569	6.570 ^{a}±0.4911

No statistically significant difference between the mean values of the three times values in the identical column that share the same superscript letters ($p \leq 0.05$).

Effect of different levels of dried *Lagenaria siceraria* (Molina) powder on serum and liver oxidative enzymes in rats exposed to cadmium and lead toxicity

Results presented in Table 10 show that the activities of antioxidant enzymes (GPX, SOD, and CAT) in both serum and liver tissue were significantly decreased ($P \leq 0.05$) in the positive control group (+) compared to the negative control group (–), indicating severe oxidative stress induced by cadmium–lead toxicity. In contrast, all rats fed diets supplemented with 2.5%, 5%, or 10% dried *Lagenaria siceraria* (Molina) powder exhibited a significant and dose-dependent increase ($P \leq 0.05$) in GPX, SOD, and CAT levels relative to the positive

control group (+) compared to the negative control group (–), indicating severe oxidative stress induced by cadmium–lead toxicity. In contrast, all rats fed diets supplemented with 2.5%, 5%, or 10% dried *Lagenaria siceraria* (Molina) powder exhibited a significant and dose-dependent increase ($P \leq 0.05$) in GPX, SOD, and CAT levels relative to the positive control group. The most pronounced effect was observed in Group 5, which received 10% dried fruit powder, suggesting a strong antioxidative response that effectively countered heavy metal-induced oxidative damage. Studies by Ahmed and Ashiq (2018) support these findings, emphasizing that the alkaloids, flavonoids, and phenolic compounds in *L. siceraria* play a critical role in protecting against oxidative stress. These compounds have been shown to reduce DNA fragmentation, inhibit malondialdehyde formation, and alleviate liver damage associated with cadmium and lead exposure. Their antioxidant activity is linked to reduced structural damage, decreased inflammatory infiltration in hepatic tissue, and prevention of apoptosis, ultimately leading to improved total antioxidant capacity and liver function (El-Said et al., 2025).

Table 10. Effect of different levels of dried *Lagenaria siceraria* (Molina) powder on serum and liver oxidative enzymes in rats exposed to cadmium and lead toxicity

Parameter / Groups	Negative control (G1)	Positive control (G2)	2.5% dried *agenaria siceraria* (Molina) powder G3)	5% dried *Lagenaria siceraria* (Molina) powder (G4)	10 % dried *Lagenaria siceraria* (Molina) powder (G5)
Serum oxidative enzymes					
GPX (ng/dl)	82.53^{a}±2.23	59.30^{d}±1.13	60.14^{d}±1.67	64.85^{c}±1.34	70.96^{b}±1.68
SOD (U/L)	54.75^{a}±1.98	35.91^{e}±0.79	39.98^{d}±1.23	43.95^{c}±1.85	49.94^{b}±1.92
CAT (mmoL/L)	75.74^{a}±2.88	41.23^{e}±1.71	50.99^{d} ±1.78	59.28^{c}±2.67	67.55^{b}±255
GST (mmoL/L)	35.97^{a}±1.79	21.66^{d}±1.08	22.91^{c}±1.56	27.66^{b}±1.33	31.99^{a}±1.220
TAC (nmoL/L)	1.93^{a}±0.461	0.91^{c}±0.045	1.26^{b}±0.064	1.52^{a}±0.075	1.84^{a}±0.092
MDA (nmoL/L)	16.17^{d}±0.80	31.41^{a}±1.77	26.88^{b}±1.44	21.88^{c}±1.22	18.97^{c}±0.16
Liver oxidative enzymes					
GPX (U/mg protein))	42.04^{a} ±2.23	22.41^{d}±2.73	25.71^{d}±1.67	29.31^{c}±1.34	32.89±1.68^{b}
SOD U/mg protein	87.93^{a} ±1.98	52.06^{e}±0.79	56.66^{d}±1.23	62.55^{c}±1.85	67.54±1.92^{b}
CAT U/mg protein	81.12^{a} ±2.88	36.88^{e}±1.71	40.27^{d} ±1.78	46.23^{c}±2.67	52.49±255^{b}
GST U/mg protein	50.32^{a} ±1.79	23.61^{e}±1.08	28.18^{d}±1.56	33.21^{c}±1.33	38.63±1.220^{b}
TAC (µmol Trolox equivalents/mg protein)	8.56±0^{a}.461	2.73^{d}±0.045	2.99^{d}±0.064	3.27^{c}±0.075	3.79±0.092^{b}
MDA (nmol/mg protein)	4.11^{e}±0.80	45.61^{a} ±1.77	41.56^{b}±1.44	36.02^{c}±1.22	30.14^{d}±0.16

No statistically significant difference between the mean values of the three times values in the identical column that share the same superscript letters ($p \leq 0.05$). glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT), glutathione S-transferases (GST), total antioxidant capacity (TAC), and malondialdehyde (MDA)

Effect of dried *Lagenaria siceraria* (Molina) powder on Levels of cadmium and lead in blood for rats

Data presented in Table 11 illustrate the effect of *Lagenaria siceraria* (Molina) dried fruit on a rat model of cadmium chloride–induced toxicity. The positive control group (G2) exhibited markedly elevated blood cadmium (5.212µg/L) and lead (18.534µg/dL) compared to the negative control (G1), which recorded of 0.341µg/L and 3.521µg/dL respectively, confirming the heavy-metal burden resulting from cadmium exposure. Dietary supplementation with *L. siceraria* powder produced a clear dose-dependent decline in both metal

concentrations. at 2.5% supplementation, cadmium and lead levels decreased to 4.911µg/L and 16.416µg/dL respectively. Further reductions were observed at 5%, (cadmium: 4.557µg/L; lead: 13.782µg/dL); and the greatest decrease occurred at 10%, with cadmium and lead levels dropping to 3.628µg/L for cadmium and 8.531µg/dL respectively. These findings correspond with the well-documented phytochemical richness of bottle gourd, which contains abundant flavonoids, phenolics, saponins, and other bioactive compounds. A recent phytochemical investigation by Mondal et al. (2023) reported that *L. siceraria* cultivated in Egyptian habitats possesses a diverse array of bioactive constituents, specifically seventeen phenolic analogs, ten flavonoids, and four isoflavonoids supporting its antioxidant and metal-chelating potential. Collectively, these results suggest that dietary inclusion of *L. siceraria* powder may serve as a natural and effective strategy for mitigating cadmium and lead toxicity through its combined chelating and antioxidative actions.

Table 11. Effect of dried *Lagenaria siceraria* (Molina) powder on Levels of cadmium and lead in blood for rats

Parameters / Groups	Cadmium µg/L	Lead µg/dL
Negative control (G1)	0.341	3.521
Positive control(G2)	5.212	18.534
2.5% dried *Lagenaria siceraria* (Molina) powder (G3)	4.911	16.416
5% dried *Lagenaria siceraria* (Molina) powder (G4)	4.557	13.782
10 % dried *Lagenaria siceraria* (Molina) powder (G5)	3.628	8.531

Sensory evaluation scores of the resultant ice cream samples

The sensory evaluation scores for the ice cream samples are presented in Table 12**.** The results revealed notable differences in overall acceptability, particularly in terms of flavor and body, among the various treatments. The incorporation of 10% dried *Lagenaria siceraria* (LS) powder enhanced the flavor compared to the control sample. This improvement is likely attributed to the natural sweetness imparted by the fruit powder, which contributed to a more pleasant and balanced taste profile in the final product. Furthermore, higher levels of LS powder positively influenced the texture and body of the ice cream. Samples containing 10% LS powder exhibited a firmer consistency better structural integrity. This enhancement in body and texture was accompanied by improved melting quality of the ice cream, indicating greater stability and creaminess. The total sensory scores indicated that all treatments were generally acceptable; however, samples with 10% LS powder consistently achieved the highest overall scores across all sensory attributes. These findings demonstrate that both the type and concentration of the LS fruit powder influence the sensory characteristics of the final ice cream product, with nonsignificant differences compared to the control.–According to Tidake et al. (2023), *L. siceraria* powder can serve as an effective alternative to milk solids–nonfat in ice cream production, acting as both a protein supplement and a natural stabilizer, thereby improving texture and shelf life.

Table 12. Sensory evaluation scores of ice-creams made using the best level of dried (*Lagenaria siceraria*) Molina powder

Samples	Flavor (50)	Body Texture (40)	Melting Quality (10)	Total (100)	Comments
Control ice cream	46.21 ^{a}±1.24	39.02 ^{a}±1.52	8.03 ^{a}±0.22	94.23^{a}±2.77	Excellent
Ice cream with 10%dried fruit powder	48.55^{a}±0.05	38.33^{a}±0.71	7.35^{a}±0.41	93.26 ^{a}±1.83	Excellent

No statistically significant difference between the mean values of the three times values in the identical column that share the same superscript letters ($p \leq 0.05$).

The physicochemical properties of the resultant ice cream samples

The physicochemical properties of the resultant ice cream samples, including pH, titratable acidity (TA%), moisture content, total protein, and protein-to-dry matter ratio, are presented in Table 13. The results showed that titratable acidity (TA%) increased proportionally with the addition of (*Lagenaria siceraria*) Molina fruit powder although the changes were not statistically significant. This slight increase in acidity can be attributed to differences in the inherent acidity and protein composition of the dried fruits powder compared to skim milk powder. Notably, samples containing 10% *L. siceraria* fruit powder exhibited the highest TA%, consistent with findings reported by Muhammad et al. (2022). In contrast, pH values exhibited an inverse trend, decreasing as TA increased, which aligns with the natural relationship between pH and acidity. In contrast, pH values exhibited an inverse trend, decreasing as TA increased, which aligns with the natural relationship between pH and acidity. A slight increase in moisture content was also observed across all treatments with higher levels of fruit powder, ranging from 62.42% to 63.52%. This may be due to the water-holding capacity of the dried fruit powder and its influence on the overall composition of the ice cream mixture. Regarding protein content, supplementation with *L. siceraria* fruit powder led to a proportional and significant increase in total protein across treatments. This enhancement is likely attributed to the higher protein concentration in *L. siceraria* fruit compared with skim milk powder. The protein content of the ice cream treatments followed the order: 10% *L. siceraria* fruit powder > control. The sample fortified with 10% fruit powder recorded the highest total protein value, highlighting its potential as a functional protein-enhancing ingredient in dairy-based frozen desserts (Gupta et al., 2022).

Table 13. The physicochemical properties of the resultant ice cream samples

Treatment	pH	Treatable acidity (%)	Moisture (%)	Protein (%)	Protein/Dry matter
Control ice cream	6.53 ^{a}±0.07	0.19 ^{a}±0.04	62.78 ^{a}±2.55	5.47 ^{b}±0.72	14.69 ^{b}±1.84
Ice cream with 10% dried fruits powder	6.43 ^{a}±0.45	0.21 ^{a}±0.01	62.69 ^{a}±1.91	6.43 ^{a}±0.08	17.11 ^{a}±0.87

No statistically significant difference between the mean values of the three times values in the identical column that share the same superscript letters ($p \leq 0.05$).

Histopathological examination of liver

Microscopic examination of liver sections from rats in Group 1 (negative control) revealed a normal histological architecture of the hepatic lobules, with well-organized hepatocytes and intact central veins and sinusoids (Photo 1). In contrast, liver sections from Group 2 (positive control, cadmium–lead toxicity) exhibited marked pathological alterations, including fatty degeneration of hepatocytes (Photo 2), as well as along with dilation and congestion of the central vein and hepatic sinusoids (Photo 3). Liver tissue from Group 3 (treated with 2.5% *Lagenaria siceraria* fruit) showed evidence of portal infiltration with inflammatory cells (Photo 4) and activation of Kupffer cells (Photo 5**),** indicating a mild inflammatory response and partial hepatic stress. In Group 4 (treated with 5% *L. siceraria* powder (Molina fruit), liver sections demonstrated Kupffer cell activation (Photo 6) but no other significant histopathological changes (Photo 7**),** suggesting a clear improvement in hepatic condition. Remarkably, liver sections from Group 5 (treated with 10% *L. siceraria* fruit powder displayed no observable histopathological alterations **(**Photo 8**),** indicating that this treatment provided effective protection against cadmium–lead-induced hepatic damage. These Results confirm the hepatoprotective effects of *L. siceraria*, which may be attributed to its rich phytochemical composition, including phenolics, carotenoids, and ascorbic acid (Owais, 2019; Muhammad et al., 2022).

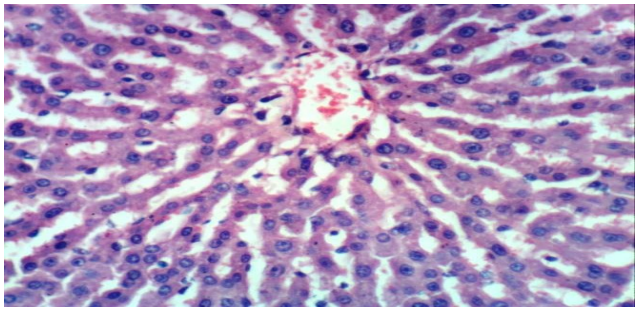


Photo (1): Liver of rat from group (1) (control"-") showing the normal histological structure of hepatic lobule (H&E X 400).

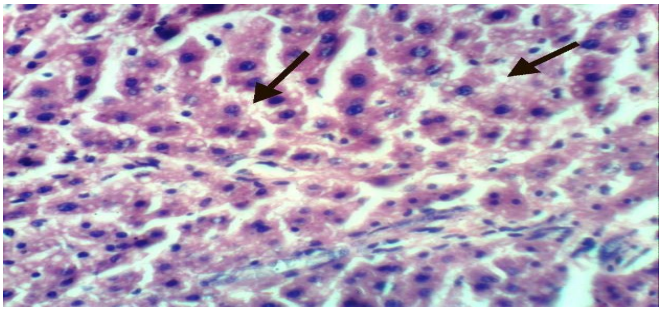


Photo (2): Liver of rat from group (2) (control"+") showing fatty change of hepatocytes (H & E X 400).

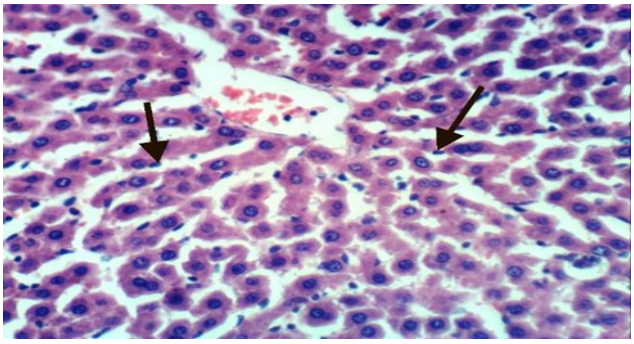
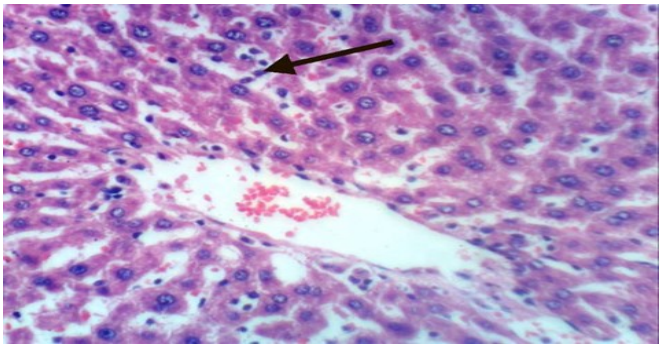


Photo (3): Liver of rat from group (3) (2.5% molina powder) showing portal infiltration with inflammatory cells and slight Kupffer cells activation (H& E X400)



Photo(4): Liver of rat from group (4) (5% molina powder) showing Kupffer cells activation (H & E X 400)

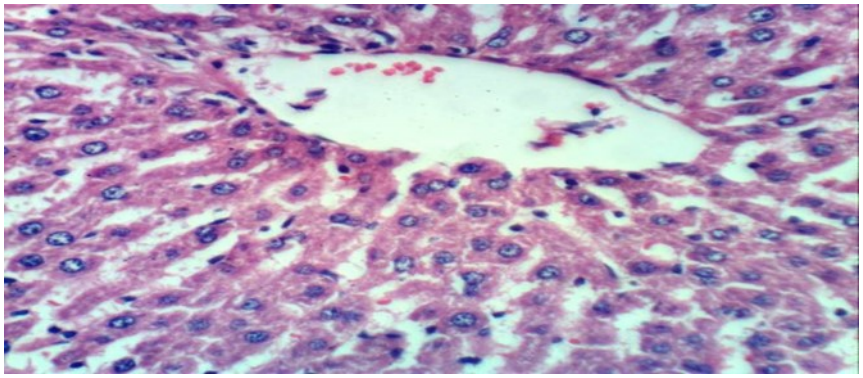


Photo (5): Liver of rat from group(5) (10% molina powder) showing no histopathological changes (H&EX400)

4. Conclusion

In the present study, the experimental design aimed to evaluate the dose-dependent protective effects of *Lagenaria siceraria* against cadmium- and lead-induced toxicity. The results demonstrated that supplementation with dried *L. siceraria* (Molina) fruit powder effectively mitigated the toxic effects of these heavy metals in rats. Dietary inclusion of the fruit powder led to significant improvements in biological performance, biochemical parameters, and the histological structure of liver tissues. These findings support the hypothesis that *L. siceraria* fruit contains a diverse range of free and conjugated bioactive compounds with antioxidant, anti-inflammatory, and detoxifying properties capable of counteracting heavy metal–induced oxidative damage. Based on these results, it is recommended that moderate amounts of *L. siceraria* fruit be considered for inclusion in daily diets or functional beverages as a natural dietary supplement to help protect against environmental heavy metal exposure.

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