

The Effect of Giving Celery Extract on Testicular Function of Wistar White Rats Exposed to Lead Acetate

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Abstract

The population is unaware of lead poisoning risks. Lead can cause tissue function and metabolism issues. Celery (*Apium graveolens L*), an antioxidant, is widely used in medicine and as a multivitamin. This study examines how celery extract affects SOD levels and testicular Caspase-3 expression in lead-exposed Wistar white rats (*Rattus norvegicus*). The accurate experiment-based quantitative research. 27 Wistar white rats (*Rattus norvegicus*) were sampled. This study examined celery extract as an independent variable and SOD and caspase-3 expression as dependent variables. Three treatment groups were presented: Control Group, Treatment Group 1 with 200 mg/kg BW celery extract, and Treatment Group 2 with 400 mg/kg BW. The Paired T-test and normality test were performed. SPSS 26.0 processed statistical data. The study indicated that celery extract increased SOD levels, but not as much as without lead introduction. Also, the caspase-3 decrease was not significant. In normality tests, SOD and caspase-3 were $p > 0.05$. Similarly, the Paired T-test showed $0.000 < 0.05$ for SOD caspase-3 levels. Celery leaf extract (*Apium graveolens Linn*) at 200 and 400 mg/kg BW provided active components like alkaloids, flavonoids, saponins, tannins, and steroids, which were beneficial. The body benefits from its antioxidant properties.

Introduction

Entry of live things, substances, or energy into the environment causes pollution (Ahmad Bhat et al., 2019). Heavy metal contamination is harmful. Active compounds from poisonous heavy metals can harm ecosystems (Zhang et al., 2023). The toxic power of heavy metal active substances inhibits physiological or metabolic enzymes. The metabolic process is disturbed. In addition, harmful elements can collect in the body, causing health issues (Onakpa et al., 2018). Heavy metals like lead can harm health. Lead is most commonly known as lead, but scientists use the sign (Pb). Not many know that Indonesia has a high lead poisoning rate. Lead contamination may have occurred in family members or their regular surroundings. Short-term lead pollution effects that do not yet create health symptoms worsen this problem (Rees & Fuller, 2020).

Fuel oil, which contains lead as a significant component to boost the octane rating, is a major contributor to lead pollution (Usman & Hayat, 2023). Many things we use daily, like cutlery, spoons, forks, and knives, contain lead. The cosmetics, glaze, and ceramics industries use lead oxide, a pigment or dye, in their products (Ranjbar et al., 2023). One common source of lead contamination in food is processed foods packaged in cans (Nerín et al., 2016). These impurities can originate from various sources, including the cans, due to the soldering process or the paint mixture used to coat the metal. Lead-lined pipes can significantly contaminate drinking water (Lu et al., 2022).

There are several metabolic and physiological problems lead poisoning can bring on. Various diseases and conditions impacting the body's ability to produce haemoglobin include kidney, reproductive, neurological, and lung health issues. We find it concerning that even a tiny child's intelligence can drop two points when exposed to 10-20 µg/dl pb in their blood (Jones et al., 2017). Multiple studies have also shown that lead can impair renal function, harm nerve tissue, impair learning ability, and cause hyperactivity in youngsters. Lead phosphate is absorbed by the brain, liver, spleen, kidneys, and spinal cord and then redistributed to other body parts sensitive to its effects (Balali-Mood et al., 2021).

The body's lead contamination can damage IQ (Heidari et al., 2022; Hou et al., 2013; Levin-schwartz et al., 2020; Schwartz, 1994). Children infected with up to 10 micrograms of Pb can become idiots. It can cause paralysis, impaired fertility, miscarriage, and fetal brain cell development in pregnant women. Even little doses of lead can disrupt early physical and mental maturation in children, affecting brain function and academic performance (Fraas et al., 2023).

Lead absorption uniformly distributes blood lead concentrations three months after exposure. Lead is primarily stored in the liver and kidneys and dispersed throughout the body (Charkiewicz & Backstrand, 2020; Grant, 2020). Adult bones contain lead, which increases tenfold with age, notably in the shins. After lead exposure, lead elimination has two phases: blood and soft tissue elimination takes 20–30 days, and bone excretion slows blood lead elimination. Lead has a biological half-life of one year in trabecular bone and 10-20 years in cortical bone. Most inorganic lead is eliminated in the urine (2/3), whereas 1/3 is passed through the bile into the intestines and faeces (Fraas et al., 2023).

The public's use of multivitamins and natural substances in medicine has significantly increased in recent years. In addition to being less expensive, some individuals can process it without needing more advanced machinery. On top of that, this plant-based ingredient is abundant, natural, and easy to procure. Celery (*Apium graveolens L.*) is one plant that has health benefits (Dirjen Kefarmasian dan Alat Kesehatan Kemenkes RI, 2017; Duke et al., 2022). Flavonoid and phenolic chemicals, found in celery and other regularly eaten plants, are known to have antioxidant effects (Wang et al., 2018).

When lead binds to sulfhydryl and nucleophilic functional groups, it can disrupt several metabolic processes in the body. These groups contribute to the development of oxidative stress and excessive generation of reactive oxygen species (ROS). Damage to cell DNA caused by an overabundance of reactive oxygen species (ROS) activates nuclear factor kappa B (NF-κB), setting off a chain reaction of inflammatory signals. Caspase 8 and Caspase 3 are activated, leading to cell apoptosis, including cells in the testicles, and the production of pro-inflammatory cytokines like Tumor Necrotic Factor alpha (TNF-α) and Interleukin 6 (IL-6) rises due to NF-κB activation. This, in turn, induces the expression of Activator Protein 1 (AP-1) (Elgawish & Abdelrazek, 2014).

Celery is a medicinal and nutraceutical vegetable due to its health advantages and pharmacological qualities (Boonruamkaew et al., 2020; Hussain et al., 2023). In recent years, soil loading with Pollution-Potentially Toxic Elements (PTEs) has become a global concern, contaminating crops and reducing their quality and safety for human consumption. We tested celery and parsley grown on Cd-contaminated soil for quality and safety. We studied the prevalence of PTEs (As, Cu, Fe, Mn, Ni, Cu, and Cd) in soil and certain herbs and their physiological response to Cd exposure (control, 3 and 6 µg/g Cd from dry soil). After Cd increased in plants, both species increased As, Pb, and Cu, which exceeded acceptable levels except for Cu. In addition, celery demonstrated robust phytoextraction (99.9 µg/g Cd dry weight) and high Cd tolerance due to effective antioxidant machinery. Celery is an excellent herbal remedy (Arsenov et al., 2021).

Research shows that environmental pollutants can impair adult women's and men's reproductive function. Heavy metals (including lead), organic solvents, insecticides, and endocrine disruptors are pollutants (Ma et al., 2019). These findings suggest that environmental pollutants may affect human fertility, although the pattern of impacts is complex and contentious. They recommend further research on the genetic and cellular causes of infertility, as well as environmental factors and reproductive outcomes. Few studies have shown that celery can lower blood lead levels or, more specifically, testicular lead levels. This context inspired scientists to study the impact of celery extract on Superoxide Dismutase (SOD) levels and caspase-3 expression in the testes of lead acetate-exposed Wistar white rats (*Rattus norvegicus*).

Methods

This sort of study falls under the umbrella of quantitative research. Research in this area is characterized by utilizing controlled laboratory settings or actual experiments (Notoatmodjo, 2022) for as long as 27 Wistar rats (*Rattus norvegicus*) are involved (Weichbrod et al., 2018). Measurable and observable traits or features that differ among the groups under study are called variables (Suwarno & Nugroho, 2023). This study uses celery extract as its independent variable, SOD levels as its dependent variable, and caspase-3 as its expression variable.

This research utilized a variety of materials, including acetic acid and caspase three antibodies, immunohistochemistry staining tools, xylene, citrate buffer, ethanol, distilled water, rat diet, chloroform, and a SOD ELISA kit. The alcohol concentrations employed were 30%, 40%, 60%, 50%, 70%, 80%, 90%, and 96% (Bruce et al., 2018). The research began with a seven-day acclimatization period. The test animals were housed in the Medanese Herbarium at the University of North Sumatra's Faculty of Mathematics and Natural Sciences. Next, use the celery extract to create an ointment. Acclimating mice to the research site was Step I. Step II includes lead exposure and treatment. For 14 days, negative control mice received regular chow and 200 mg/kg BW lead acetate dissolved in 2 ml of distilled water orally. Treatment of rats was step III. The rats were given a simultaneous oral dose of 200 mg/kg BW lead acetate and celery extract. They continued their regular diet for 14 days. In the fourth stage, rats were given 200 mg/kg BW lead acetate orally in 2 ml distilled water. The rats received 400 mg/kg BW celery extract and were fed regularly for 14 days. Caspase-3 staining was the last step after blood samples, SOD level determination, and tissue sample collection. After doing a normalcy test using Shapiro-Wilk, the data was processed using SPSS 26.0. The paired T-test was then carried out.

Result and Discussion

Making Celery Plant Extraction

Approximately 600 grams of celery (*Apium graveolens linn*) are finely sliced, dried at 50-60°C, and mixed into a dry powder. The dry powder is macerated with 95% ethanol, filtered using filter paper, and the filtrate is collected. The residue on the filter paper is macerated again. A rotating evaporator evaporates ethanol to a thick extract. The thick extract is refrigerated at 2-8°C till treatment.

Results of Phytochemical Screening Research

The secondary metabolite test was run to determine how many organic components were in the celery leaf extract. According to the findings, the active components in celery leaf extract (*Apium graveolens linn*) include steroids, alkaloids, flavonoids, saponins, and tannins. Therefore, the antioxidant properties of celery leaf extract are beneficial to health.

Results of Giving Celery Extract to SOD Levels in Rats Exposed to Lead Acetate

Table 1. Data on SOD levels

| Variable | Group | | | P-Value |
|--|--|--------------------------------------|--------------------------------------|---------|
| | Negative Control n = 5 Mean ± SD | Treatment (P1) n = 5 Mean ± SD | Treatment (P2) n = 5 Mean ± SD | |
| SOD levels | 2,786 ± 0,209 | 3,2314 ± 0,604 | 3,044 ± 0,353 | |
| <i>Shapiro Wilk Paired sample test</i> | 0,060 | 0,826 | 0,237 | 0,000 |

Table 1 shows that Celery Extract significantly raises SOD levels relative to the negative control and Treatment 1 (P1) groups. The data also suggests that Celery Extract increases SOD. Continuous celery extract treatment elevated SOD levels in rats compared to the negative control group was given 200 mg/kg BW lead acetate orally and standard feed, with an average result and standard deviation of 2.786 ± 0.209 and an increase in the average in the treatment group 1 (P1), mice received 200 mg/kg BW lead acetate and 200 mg/kg BW celery extract, with an average result and standard deviation of 3.2314 ± 0.604 . In treatment group 2 (P2), rats were given 200 mg/kg BW lead acetate, 400 mg/kg BW celery extract, and a standard diet. The average result was 3.044 ± 0.353 standard deviation. Despite not being similar to normal settings without Lead induction, celery extract increases SOD levels.

The normality of SOD levels in each group was assessed using the Shapiro-Wilk test. The results of the normality test indicated that the Control group (P0) had a normality test result of 0.060, treatment group 1 (P1) had a result of 0.826, and treatment group 2 (P2) had a result of 0.237. In all groups, the p-value was more significant than 0.05, suggesting that the data is usually distributed. Therefore, a parametric paired t-test can be conducted to determine the significance in all groups. The paired t-test yielded a result of 0.00, indicating significant differences in all treatment groups, as indicated by the value ($p < 0.05$).

Results of Giving Celery Extract on Caspase 3 in Rats Exposed to Lead Acetate

Table 2. Data Caspase 3 levels

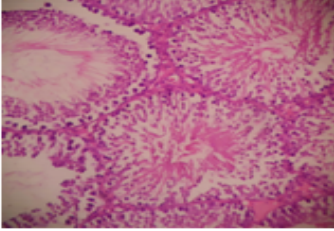
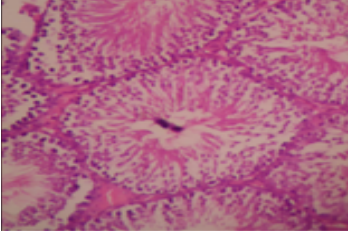
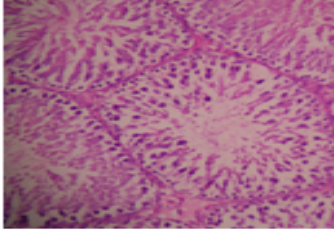
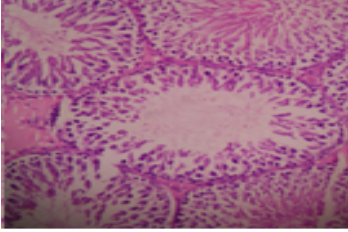
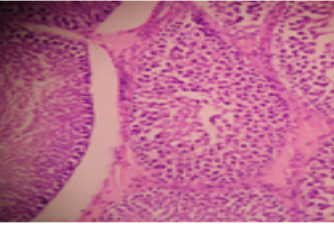
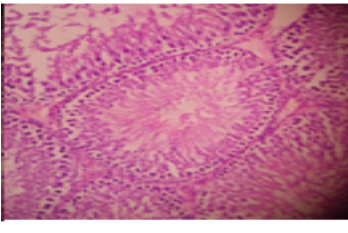
| Variable | Group | | | P-Value |
|--|--------------------------------------|------------------------------------|------------------------------------|---------|
| | Negative Control n = 5 Mean±SD | Treatment (P1) n = 5 Mean±SD | Treatment (P2) n = 5 Mean±SD | |
| Caspase-3 levels | 12,273 ± 0,101 | 8,363 ± 0,253 | 9,436 ± 0,294 | |
| <i>Shapiro Wilk Paired sample test</i> | 0,433 | 0,067 | 0,220 | 0,000 |

Table 2 shows that the negative control group, given lead acetate at 200 mg/kg BW and standard diet, had the highest average caspase-3 findings (12.273 ± 0.101) compared to the Treatment 1 (P1) group. In rats, oral lead acetate (200 mg/kg BW) and celery extract (200 mg/kg BW) led to a decrease in caspase three yield, with a standard deviation of 8.363 ± 0.253 at treatment 2 (P2). Orally induced rats administered 200 mg/kg BW lead acetate and 400 mg/kg BW celery extract while fed regular chow had lower caspase-3 findings. The average and standard deviation were 9.436 ± 0.294 ; however, Treatment Group 1 (P1) results had the lowest caspase three reductions compared to P2. Even though the caspase-3 decrease was insignificant, the adverse control treatment and treatments 1 and 2 had different average results. According to the statistical analysis of the Shapiro-Williams test in Table 2, the results of the Caspase-3 expression in lead acetate-exposed mice on day 14 demonstrated a normal

distribution of data. The results for the negative control group were 0.433, the P1 group was 0.067, and the P2 group was 0.220, with a consequence of $p > 0.05$, indicating that the data is usually distributed. The paired t-test yielded a p-value < 0.05 , showing statistically significant differences across all treatment groups, with a result of 0.000.

His pathology of Rat Testicular Tissue

Table 3. Histopathologic Features of Testicular Tissue

| Group | Histopathological Image of Fibroblasts | |
|--|--|---|
| <p>The control group (K) was exposed to lead acetate (Pb) but not given celery extract.</p> |  |  |
| <p>Treatment group I (P1) was exposed to lead acetate (Pb) and given celery extract at 200 mg/kg BW.</p> |  |  |
| <p>In treatment group II (P2), the group was exposed to lead acetate (Pb) and given celery extract at 400 mg/kg BW.</p> |  |  |

Observing the histopathological image of the control group reveals the absence of sperm within the tubule lumen. Many germ cells may be experiencing apoptosis, which may explain why the seminiferous tubules have shrunk in diameter. This represents a barrier to spermatogenesis. The histological image of the first treatment group (P1) reveals that spermatogenesis is beginning to appear normal. This is evident in all germ cells within the seminiferous epithelium, including spermatogonia, primary spermatocytes (non-pachytene and pachytene), and spermatids (round and elongated). The tubules also include spermatogenic cells that are packed closely together. The histology image from the second treatment group (P2) revealed that the seminiferous tubules had a smaller diameter. Despite the apparent density and compactness of the tubules formed of spermatogenic cells, the seminiferous epithelium contains spermatogonia, primary spermatocytes (both non-pachytene and pachytene), and spermatids (both round and elongated).

For this research, few studies have linked celery to lowering lead in the blood or testicles. This laboratory study explored how celery extract affected SOD levels and Caspase-3 expression in lead-acetate-exposed Wistar white rats (*Rattus norvegicus*) testes. Celery leaves were examined for phytochemicals in celery extract before further research. Celery leaf extract was tested for metabolite chemicals using phytochemistry. Tests showed celery leaf extract (*Apium graveolens Linn*) included alkaloids, flavonoids, saponins, tannins, and steroids. Celery leaf extract contains antioxidant properties.

The poisonous element lead (Pb) seems to play a significant role in male infertility by gradually lowering the quality of semen (sperm). Multiple pathways mediate this Pb element's harmful effects on reproduction. Celery (*Apium graveolens L.*) is one plant that has health benefits. Flavonoid and phenolic chemicals, found in celery and other regularly eaten plants, are known to have antioxidant effects (Wang et al., 2018). Results were found after mice were exposed to lead acetate and treated with celery leaf extract. Next, the mice's blood was drawn. Analysis of blood samples begins immediately. SOD levels are limited to 12 hours following sampling. Lead-induced oxidative stress processes decrease ROS generation and release in cells and tissues, damaging membranes, DNA, and proteins. Lead acetate reduces catalase and SOD, increasing lipid peroxidation and nitric oxide generation. Lead dramatically raised the Caspase-3 threshold, indicating apoptosis.

Celery Extract significantly increased SOD levels compared to the negative control group and Treatment 1 (P1). The data also suggests that Celery Extract increases SOD. The data indicates that successive administration of celery extract increases SOD levels compared to the negative control group (200 mg/kg BW lead acetate, standard feed) with an average increase of 2.786 ± 0.209 , with the highest increase in treatment group 1 (P1), which received both lead acetate and celery extract. In treatment group 2 (P2), rats were given 200 mg/kg BW lead acetate and 400 mg/kg BW celery extract, still fed standard feed, with an average deviation of 3.044 ± 0.353 . Despite not being similar to traditional settings without Lead induction, celery extract increases SOD levels. Next, the caspase three expression was measured. A protein breakage cascade activated by caspases causes apoptosis. Apoptotic caspases are divided into apical (caspase-2, -8, -9, and -10) and effector (caspase-3, -7, and -6). Death receptors like TNF or FAS receptors activate caspase-8, while intrinsic stimuli like BCL2 family BH3-only protein BIM or puma depolarize mitochondria and activate caspase-9 (Slee et al., 1999).

Negative control mice treated with lead acetate at 200 mg/kg BW and given standard feed had the highest average caspase-3 results and standard deviation (12.273 ± 0.101). In comparison, Treatment 1 rats (P1) were given celery extract at 200 mg/kg BW and traditional food, resulting in decreased results. The average and standard deviation were 9.436 ± 0.294 ; however, Treatment Group 1 (P1) results had the lowest caspase three reductions compared to P2. Thus, celery extract at 200 mg/kg BW and 400 mg/kg BW reduces caspase-3 levels in testicular tissue in male Wistar rats, but treatment group 1's 200 mg/kg BW dose reduces them most. The suboptimal testing dose may also cause this. Subsequently, the research data was examined for normality using the Shapiro-Williams test. Later, the paired t-test ($p < 0.05$) determined significance in all groups. The statistical analysis verified that the SOD and caspase three observation data followed a normal distribution, as the p-value was more significant than 0.05, and the paired t-test yielded a substantial result of $p < 0.05$. This suggests that there were notable differences across all treatment groups.

Then, the researchers examined testicular histopathology to determine treble function in mice exposed to Pb lead and treated with celery leaf extract. The histopathological image of the negative control group shows no sperm in the tubule lumen. The shortened diameter of seminiferous tubules may hinder spermatogenesis and be attributed to the significant number of germ cells undergoing apoptosis. Spermatogenesis seemed normal in all germ cells, including spermatogonia, primary spermatocytes (non-pachytene and pachytene), and spermatids (round and elongated) in the seminiferous epithelium, in treatment group 1 (P1). Additionally, the tubules contain densely packed spermatogenic cells. Seminiferous tubule diameter decreased in treatment group 2 (P2). Even though the tubules are made of spermatogenic cells, the seminiferous epithelium's spermatogonia, primary spermatocytes (non-pachytene and pachytene), and spermatids (round and elongated) appear compact and dense.

Conclusion

This study found that after exposure to lead acetate (Pb), the administration of celery extract affected testicular function in Wistar white rats (*Rattus norvegicus*). Even though it was not yet comparable to standard settings without Lead induction, it was possible to raise SOD levels with a dosage composition of 200 mg/kg BW and 400 mg/kg BW of celery extract. Next, the authors discovered that male Wistar rats' testicular caspase-3 levels were reduced when given celery extract at 200 and 400 mg/kg BW doses. On the other hand, treatment group 1 saw the best reduction at 200 mg/kg BW. According to the histopathological picture, this study concludes that celery extracts at 200 mg/kg BW and 400 mg/kg BW can alter the histological structure of lead acetate-exposed testes. In the first treatment group (P1), which received 200 mg/kg BW of celery extract, spermatogenesis appeared normal. This was seen in all germ cells in the seminiferous epithelium, including spermatogonia, primary spermatocytes (non-pachytene and pachytene), and spermatids (round and elongated). The tubules also include spermatogenic cells that are packed closely together. Treatment group 2 (P2) demonstrated a decrease in seminiferous tubule width when 400 mg/kg BW of celery extract was administered. Despite the presence of spermatogenic cells in the tubules, the seminiferous epithelium's spermatogonia, primary spermatocytes (both non-pachytene and pachytene), and spermatids (both round and elongated) give the impression of being thick and compact.

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