Efficacy of Zingiber Officinale (ZO) in the Treatment of Lead Acetate-Induced Hepatopathy in Rabbits
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Received 14/4/2023, Accepted 26/6/2023, Published 27/6/2024

Abstract
Lead acetate (Pb) is a hazardous heavy metal that is well-known to impair the functions of bodily systems and organs. This present study identifies the histopathological alterations that Pb toxicity causes in the liver of rabbits as well as the efficacy of ginger (Zingiber Officinale, ZO) in mitigating its negative effects. As ZO is a rich source of several antioxidants, this present study examines its ability to decrease Pb toxicity in exposed animals. A total of 30 rabbits were randomly divided into three groups (n = 10). The Control group was only fed distilled water, the Negative group was only fed a 2% Pb solution, while the Treated group was fed a 2% Pb solution as well as 100 mg of ZO extract per kilogram of body weight daily. Lead acetate (Pb) was found to significantly alter the histopathology of liver tissue by increasing inflammation, fibrosis, vacuolation, and degeneration; significantly decreasing plasma superoxide dismutase (SOD) and catalase (CAT) enzymatic activities; and increasing plasma malondialdehyde (MDA) concentrations. Furthermore, ZO extract not only significantly decreases the negative hepatic impacts of Pb but also prevents Pb-induced hepatopathy.

Keywords: Lead acetate, Hepatopathy, Ginger, SOD

Introduction
Lead (Pb) is considered a major environmental pollutant as it is used extensively in the manufacturing industry (1). However, industrialised and developing countries are both equally affected by environmental and occupational exposure to toxic pollutants (2). Toxic heavy metals, such as Pb; are hazardous to human and animal health even in low quantities (3). In humans, Pb poisoning causes a wide range of symptoms; such as anaemia, weight loss (4), infertility, nephropathy, hepatotoxicity, testicular problems, heart damage, and other complications (5,6). It increases the oxidative breakdown of hepatic cells by increasing the peroxidation of the lipid membrane (7); a destructive process that is caused by oxygen free radicals.8. Lead (Pb) toxicity generally causes Pb-induced oxidative stress to increase in the soft tissues or blood (9). If antioxidant and pro-oxidant levels are imbalanced the tissue damage is supposed to have occurred (10).

Lead (Pb) also increases lipid peroxide levels in the body and modifies the antioxidative defence system of the liver (11). Reactive oxygen species (ROS) plays a critical role in increasing Pb-induced toxicity in the body. Multiple recent studies have examined the significance of oxidants and antioxidants in medicine, biology, and nutrition (12). Studies revealed that the survival of aerobic life depends on the continuous production of pro-oxidants; such as free oxygen radicals. Multiple ROS signalling molecules, such as nitrogen oxide (NO), superoxide ion (O2.-), and hydroxyl radical (OH); are deemed highly reactive and are classified as free radicals as they possess an unpaired electron. Although they are produced naturally in the body via multiple different biological processes, they are confined to cell compartments. On the other hand, a few naturally occurring antioxidant molecules; such as superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx), vitamin C (L-ascorbic acid), and vitamin E (α-tocopherol); behave as scavengers of free radicals to offset excess ROS production (13). As free radicals are produced throughout the pathogenic processes post-Pb exposure, antioxidant supplements could be a viable alternative to chelation therapy (14). Ginger is the subterranean stalk or tuber portion of the Zingiber officinale roscoe (ZO) plant that contains...
many polyphenolic compounds; such as 6-gingerol and its derivatives; that exhibit exceptional antioxidative activity. Approximately more than 50 antioxidants can be successfully isolated from ZO rhizomes of the 50 compounds, 12 exhibit higher antioxidative activity than α-tocopherol. Ginger (ZO) and all its compounds display cholangue, antithrombotic, anti-inflammatory, anti-emetie, anti-hepatotoxic, androgenic, and antioxidative effects. Ginger (ZO) is a powerful antioxidant that can decrease and inhibit the formation of free radicals. As it is an innocuous herbal remedy with limited or negligible negative effects, multiple studies have extensively examined ZO extract for its biological properties, particularly its antioxidative activity. Apart from ZO, seashore mangosteen (Garcinia hombroniana) bark extracts as well as the essential oils of camphor bush (Tarchonanthus camphoratus) stems and leaves also exhibit significant antioxidative properties. In murine models, ZO extracts have been found to significantly decrease the peroxidation of lipids by retaining the activities of enzymatic antioxidants; such as catalase (CAT), SOD, and GPx. Meanwhile, other studies have examined the influence of Phoenicean juniper (Juniperus phoenicea) extracts on the activities of enzymatic antioxidants in the hepatic tissues of rats that have been treated with potassium oxonate. To the best of the researcher’s knowledge, no study has examined the ability of ZO root extract to decrease Pb-induced oxidative damage in homogenate hepatic tissue samples by using TEM and lesion scoring protocol. As such, this present study investigated if orally administered ZO root extract could prevent Pb-induced liver disorders/hepatopathy such as (hepatocyte degeneration, necrosis, steatosis and fibrosis) in rabbits.

**Research Objectives**

1. To investigate the toxicological pathophysiology of Pb in a trial animal model using rabbits.
2. To better understand the detrimental effects of specific toxic concentration of Pb.
3. To facilitate future studies on the toxicology of Pb and other trace elements in human beings and animals.
4. To assess the antioxidant capabilities of whole ZO against Pb-induced liver damage.

**Materials and Methods**

**Trial strategy**

This present study used 30 male and female rabbits weighing 1200 to 1750 g and aged six months to a year. The animals were cared for in line with the standards outlined by the Laboratory Animal Facilities, the World Health Organization (WHO), Geneva. As such, the rabbits were fed a typical diet and water was easily accessible. The animals were housed in stainless steel coops that were placed in a temperature-controlled environment (25± 2°C) with a consistent humidity of 40 to 70% and provided with 12 hours of light and darkness.

**Animal handling and grouping**

The 30 rabbits were randomly sorted into three groups of 10. The rabbits in the Control group were fed distilled water while those in the Negative group were only fed a solution of 2% Pb. The rabbits in the Treated Group, however, were fed a solution of 2% Pb as well as 100 mg/kg of body weight of ZO extract. Standard alanine aminotransferase (ALT)/glutamic-pyruvic transaminase (GPT) and aspartate transaminase (AST)/serum glutamic-oxaloacetic transaminase (SGOT) test kits for rabbits were procured from the SAE Egyptian Company for Biotechnology while creatine kinase (CK) test kits were purchased from Biolabo S.A.S, France.

**Blood and tissue sampling**

All 30 rabbits were sacrificed three months upon completion of the last treatment. Blood samples were collected via heart puncture for biochemical analysis. Every blood sample was stored in two separate bottles; one was an empty bottle while the other contained ethylenediaminetetraacetic acid (EDTA). Hepatic tissue samples were collected from every animal and stored in 10% (v/v) solution of saline formalin. For the histopathological analysis, the stored samples were thinly sliced, affixed to a slide, and stained with haematoxylin and eosin (H&E). The blood samples and tissue extracts were also prepared according to the methods described by (Khaki et al.) to assess the enzymatic activities of CAT, plasma SOD and malondialdehyde (MDA).

**Tissue preparation and preservation**

Three months after the treatment ended, the animals in all three groups were sacrificed and liver tissue samples were collected immediately. The tissue samples were thinly sliced and preserved in a 10% (v/v) saline formalin solution before they were prepared for paraffin embedding. The tissue slices were 5 μm thick and H&E stained. A light microscope was used to examine all the samples.

**Microscopic analysis**

Tissue samples were collected from the sacrificed animals and fixed in 10% (v/v) neutral-buffered formalin. A rotary microtome was then used to produce 5 μm thick slices of paraffin blocks containing the fixed samples. They were H&E stained and examined using an Olympus® light microscope to determine the histopathologic alterations that occurred post-Pb exposure.

**Data collection and statistical analysis**

The liver samples of every rabbit were first homogenised before the supernatant was examined to determine their SOD, CAT, and MDA enzymatic
activities using the methods described by (Khaki et al.)\textsuperscript{26}.

The values were expressed as the mean value ± SE for the purposes of the statistical analysis. A one-way analysis of variance (ANOVA) was conducted in IBM® SPSS Statistics to compare differences in the mean values. A P of < 0.05 was deemed statistically significant.

**Findings and Discussion**

**Histopathological evaluations and lesion scoring of the Control and Negative groups.**

The multiple histopathological lesions observed in the hepatic samples were used to summarise the experimental findings of this present study. As the hepatocytes were organised normally around the central vein and sinusoidal capillaries, the liver slices of the Control group were within normal limits (Figures A1, A2). However, the liver slices of the Negative group presented with significant Pb-related histopathological changes; more specifically, hepatocyte vacuolation and swelling as well as the presence of mononuclear cells and tissue degeneration. Some of the liver slices also presented with changes due to advanced septal and capsular fibrosis. Furthermore, an increase in inflammatory cells and congestion was also noted, which indicated that the animals had developed hepatitis (Figures B2 to B7).

**Histopathological evaluations and lesion scoring of the Treated group.**

Structural changes were not noted in the hepatic samples of the Treated group (Figures C8, C9).

**Effects of ginger (ZO) and Pb-induced oxidative stress on the liver enzymes and biomarkers of rabbits.**

The results of the current study were provided in mean ± SE. The level of significance was set at P < 0.05*. The enzymatic analysis indicated that the plasma SOD and CAT enzyme activities of the Negative group (P < 0.05) were significantly lower than that of the Control group. However, the plasma SOD and CAT enzyme activities of the Treated group did not show a significant difference (P > 0.05) from that of the Control group (Table 1). Furthermore, the plasma MDA activity of the Negative group was significantly higher (P < 0.05) while that of the Treated group did not show a significant difference (P > 0.05) from that of the Control group (Table 1).

<table>
<thead>
<tr>
<th>Groups (n=10)</th>
<th>SOD</th>
<th>CAT</th>
<th>MDA</th>
</tr>
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<tbody>
<tr>
<td>(A) Control</td>
<td>1.958 ± 0.05</td>
<td>0.3874 ± 0.03</td>
<td>0.25 ± 0.04</td>
</tr>
<tr>
<td>(B) Negative [2% Pb]</td>
<td>1.124 ± 0.05*</td>
<td>0.2440 ± 0.02*</td>
<td>4.1 ± 0.06*</td>
</tr>
<tr>
<td>(C) Treated [2% Pb + 100 mg ZO/kg/rabbit/day]</td>
<td>1.783 ± 0.06*</td>
<td>0.3692 ± 0.01</td>
<td>2.2 ± 0.06*</td>
</tr>
</tbody>
</table>

Data is presented as mean ± SE. (*): Significant differences in the Negative, Control, and Treated group (P < 0.05).

**Control Group**

![Control Group](image)

**Negative Group (2% Pb)**

![Negative Group (2% Pb)](image)
Figure 1. Photomicrographs of the rabbit livers examined in the sub-acute trial. (1) The histopathological changes of the Control group were within normal limits, with hepatocytes arranged normally around the central vein and with normal sinusoidal capillaries (2-7). The histopathological changes observed in the Negative group included severe congestion and dilatation of the sinusoidal space and central vein, extremely diffused centrilobular and periportal steatosis throughout the hepatocytes (black indicator), hepatocellular necrosis (red indicator), moderate Kupffer cell (KC) hypertrophy (green indicator), minor periductal fibrosis encompassing the bile ducts (blue indicator), clearly swollen hepatocytes with the presence of Mallory-Denk bodies (white indicator), the infiltration of inflammatory cells into the perportal region with periductal fibrosis (blue indicator), periportal KC hypertrophy and hyperplasia (green indicator), and hepatocellular hypertrophy (purple indicator) with a larger HHP area ≈ 365.89 µm² and HNHP area ≈ 63.74 µm²) than the hepatocytes area of the Control group (HHP area ≈ 83.51 µm² and HNHP area ≈ 14.31 µm²). (8) Histopathological observations of the Treated group indicated the restoration of the hepatocyte population to normal levels and significantly fewer hepatotoxic lesions. Therefore, ZO extract has a significantly hepatoprotective effect and can reverse the negative effects of Pb toxicity. These were proven by presence of significantly fewer KC hyperplasia, hepatocyte hypertrophy, necrosis, cystic and fatty cell degeneration, and inflammation. However, although the histopathological changes were minimal, some singular necrosis and congestion were noted under haematoxylin and eosin staining at 40X magnification.
Figure 2. (A) Transmission electron micrographs (TEM) of the hepatocytes of the Control group indicated that they appeared normal with euchromatic nuclei (N) and nuclear membranes (yellow indicator). The cytoplasm comprised typical mitochondria (m) as well as endoplasmic reticula that were both rough (rER) and smooth (sER), and ordinary peroxisome proliferation.
Hepatoprotective effects of Ginger in Pb-treated rabbits

Figure 3. (B) Transmission electron micrographs (TEM) of the hepatocytes of the Negative group indicated the presence of intracytoplasmic vacuolation (V), organelle clustering, significant liver steatosis (f), bloated mitochondria (m), and large-sized stellate or “Ito” cells in the Disse space. The blood sinusoids (S) were dilated with severe KC hypertrophy and deteriorated hepatic nuclei (N) encompassed in broken and uneven nuclear envelopes, extremely engorged sER, and increased peroxisome (P) proliferation as well as the presence of electro-dense bodies (mb), necrotic nuclei debris, multiple lysosomes (Ly), and a larger volume of cytosol directly next to the nucleus and collagen fibres near the KCs.

Figure 4. (C) Transmission electron micrographs (TEM) of the hepatocytes of the Treated group depict a marked decrease in hepatocellular deterioration and necrosis as well as KC hypertrophy with a significant reduction in previously elevated levels of sER and peroxisomes. The decrease in previously elevated cytoplasmic organelle levels, such as peroxisomes and sER; as well as the accrual of intracellular components, such as glycogen, lipid, and water; was significantly more pronounced. Furthermore, the hepatocytes appeared almost normal with euchromatic nuclei (N) and established nuclear membrane (yellow indicator). Furthermore, the cytoplasm comprised typical mitochondria (m) and endoplasmic reticula that was rough (rER) as well as a normal quantity (non-proliferative) of peroxisomes (P) and smooth endoplasmic reticula (sER). The blood sinusoids were normal with the endothelial lining and normal KC in the Disse. Only a handful of cytoplasmic vacuoles (V) were noted, and no fat droplets (f) were observed.

Therefore, the histopathological and enzymatic findings of this present study indicate that Pb-exposure damaged the livers of the rabbits. Increased plasma MDA activity was observed in the liver tissues of the animals that had been fed Pb. This is corroborated by the findings of (Attia et al)28 who reported higher plasma ALT, AST, and ALP activities as well as higher concentrations of hepatic MDA. The higher lipid peroxidation and decreased levels of GSH observed in the liver tissues also allude to the existence of oxidative stress due to Pb exposure. Therefore, animals that are exposed to Pb absorb it and retain it in their soft tissues, primarily the liver29. As the liver is the first bodily organ to encounter ingested toxins or nutrients via the portal vein, this present study histologically examined the liver tissues to ascertain the morphological alterations that occurred as it would clearly indicate the detrimental effects of Pb on hepatocytes.
A light microscope was used to examine the hepatocytes. Significant alterations were observed in the hepatocytes; more specifically, hepatocyte vacuolation and deterioration, severe fibrosis, and hepatitis due to congestion and inflammatory cell infiltration. Abdou and Newairy (2006) similarly reported that the ingestion of Pb-polluted water dilates the hepatic vein as well as congests, necrotises, and deteriorates blood vessels. The presence of hepatoxic lesions as well as multiple other histopathological changes; such as blood vessel and central vein dilatation and sinusoid haemorrhages have also been reported. Lead acetate (Pb) is also believed to be most destructive to liver tissues as it causes inflammation and hepatic fibrosis. Furthermore, the various histological alterations that occur in the liver post-Pb-exposure differ depending on the type of animal, exposure time, exposure length, and administration method.

A histological analysis of the liver tissues indicated that the Treated group experienced significantly fewer of the histopathological alterations observed in the Negative group. Furthermore, the Treated group did not present with any changes, unlike the Negative group. Therefore, the findings of this present study indicate that ZO can protect against the histological alterations that Pb-induced hepatotoxicity causes in animals. They also corroborate the findings of extant studies that ZO can effectively prevent Pb-induced hepatotoxicity as well as those of who reported that the administration of 160 mg/kg/rat prevented the lipid peroxidation levels of Pb-exposed rats from increasing. Therefore, this ZO dose successfully prevents Pb-induced oxidative stress and damage in exposed animals.

Multiple modern studies indicate that ROS may affect Pb-induced toxicity. As such, several animal and human studies have been conducted to examine how various antioxidative enzymes and molecules affect Pb-induced oxidative damage. Lower concentrations of GSH and glutathione disulphide (GSSG) as well as altered SOD activity are the most typical signs of Pb-induced toxicity in hepatic tissues or blood. It has been hypothesised that antioxidant supplementation could successfully replace chelation therapy as free radicals are produced during the pathogenic processes that occur post-Pb exposure. Apart from that, ascorbic acid; a popular chelating substance that possesses antioxidant characteristics; is renowned for shielding cells from oxidative stress. Tomato and tomato-based products are also effective antioxidative substances as they comprise many beneficial antioxidants; such as lycopene, ascorbic acid, and retinol.

Significant differences were not observed (P > 0.05) between the plasma SOD activities of the Control and Treated groups. The plasma SOD activity of the Negative group was significantly lower (P < 0.05) than that of the Control group. These findings corroborate that of an extant or previous study. The Negative group also had significantly lower plasma CAT activity (P < 0.05) than the Control group. The Plasma CAT activity of the Treated group did not differ significantly from that of the Control group (P > 0.05). Therefore, the ZO dosage that this present study used helped decrease Pb-induced oxidative stress and damage in exposed animals. No significant differences were observed between the concentrations of tissue and plasma MDA in the Control and Treated groups (P > 0.05). However, the plasma MDA levels of the Negative group increased significantly (P < 0.05). Therefore, the antioxidant-rich ZO extract can successfully reduce Pb-induced oxidative stress in the ZO-fed rats. In conclusion, as it is a rich source of antioxidants, ZO can considerably lessen the toxic effects of Pb-induced oxidative stress and damage on hepatic tissues.

Finally, the possible mechanism by which ZO produced a hepatoprotective effect against pathological events and the oxidative deteriorations induced by Pb, was possibly through the prevention of the rapid exhaustion and/or preservation of intracellular GSH levels. It is known that the rapid depletion of Glutathione levels highly increases the susceptibility of cells to irreversible changes and lesions. Furthermore, ZO possess hydroxyl radical scavenging properties, and pronounced inhibitory action towards the generation of thromboxane B2. These mechanisms have been analysed in recent reports for current year, with respect to lowered oxidative stress biomarkers, cytoprotective effects, and hepatoprotective effects of ZO.

**Conclusion**

The findings of this present study indicate that ZO roots have powerful cytoprotective, anti-proliferation, and antioxidative properties. The oral ingestion of 100 mg of ZO/kg daily can prevent the development and onset of hepatotoxicity in Pb-exposed rabbits. As such, ZO roots are extremely effective at preventing hepatotoxicity which, in turn, averts or decreases the intensity of lesions in a timely fashion.

**Ethical Statement**

This study was approved by the Animal Care and Use Committee (ACUC) of the Faculty of Biomedical Science, Universiti Putra Malaysia (UPM) (UPM/IACUC/AUP-R082/2015).

**Conflict of Interest**

The authors declare no conflicts of interest.

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