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Potential hepatic-protective effect of *Physalis peruviana* against lead-induced toxicity in albino rats

Fatma I Gad[1], Eglal G Salem[2], Ossama A Abdullatef[3], Samar M Aborhyem[4]
[1]Nutrition Department, Faculty of Applied Health Sciences Technology, Pharos University in Alexandria, Alexandria Governorate, Egypt
[2]Environmental Health Department, High Institute of Public Health, Alexandria University, Alexandria Governorate, Egypt
[3]Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Pharos University in Alexandria, Alexandria Governorate, Egypt
[4]Nutrition Department, High Institute of Public Health, Alexandria University, Alexandria Governorate, Egypt

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ABSTRACT

Lead toxicity poses a serious threat facing humanity. Research aims to investigate the potential hepato-protective effect of *Physalis peruviana* (P.P) fruit against lead toxicity (10 ppm (1/45 LD50)) in male albino rats. Toxicological induction with lead acetate lasted for 28 days followed by treatment with 200, 300, and 500 mg/kg of lyophilized P.P. fruit extract for another 28 days. Superoxide dismutase, malonaldehyde, catalase, and glutathione peroxidase were measured using a spectrophotometer. Lead concentration in liver tissues was assessed using Atomic Absorption Spectroscopy after four and eight weeks, in addition to Aspartate aminotransferase, alanine aminotransferase, and complete blood count were measured. Lead acetate induced a substantial elevation in malonaldehyde levels, and a significant decrease in Superoxide dismutase, catalase, and glutathione peroxidase activities. Treatment with 200, 300, and 500 mg/kg P.P showed an improvement in the activity of the antioxidant enzymes in a dose-dependent manner. Co-administration of P.P. ameliorates the damage caused by lead acetate. *Physalis peruviana* solution showed a reduction in the mean lead concentration in a dose-dependent response of 0.26±0.02, 0.21±0.02, and 0.14±0.03 ppm for groups III, IV, and V, respectively. In conclusion, P.P. fruit extract can improve antioxidant activity and exert a beneficial effect in the treatment of Pb-induced hepatotoxicity.

Corresponding Author:

Samar M Aborhyem
Nutrition Department, High Institute of Public Health, Alexandria University
165 EL-Horreya Avenue, EL-Hadrah, 21561 Alexandria, Egypt
Email: samaraborhyem@yahoo.com

1. INTRODUCTION

Lead (Pb) is a common toxic heavy metal in the environment. It is a highly accumulative toxicant due to its non-biodegradable nature with a long biological half-life, therefore its prolonged persistence and tendency to accumulate in tissues with a steady supply, even in small amounts. Since the first issue of Environmental Health Perspectives was published twenty years ago, there has been significant progress in the understanding of the potential toxicity of heavy metals, especially lead (Pb). Lead is considered one of the most dangerous pollutants because of its widespread existence in the environment as shown in Figure 1.

Lead poses a serious health threat not only to individuals directly handling it, but also to the whole population. Chronic exposure to lead directly correlates with certain types of cancer. The International Agency for Research on Cancer (IARC) incorporated lead compounds into the list of possible carcinogens [1].

Exposure to lead over time has been associated with hepatotoxicity, nephrotoxicity, reproductive, and behavioral issues. Among the organs targeted by Pb toxicity, the liver is the most affected, followed by the kidneys [2]. The toxicity of lead in the live cells is caused by an ionic mechanism as well as oxidative stress. Studies have revealed that oxidative stress in live cells is generated by an imbalance between the creation of free radicals and the production of antioxidants for detoxification or repair of the reactive intermediates [3], [4]. Figures 2 and 3 show the attack of heavy metals on the cell and the effects of increased lead levels in the blood.

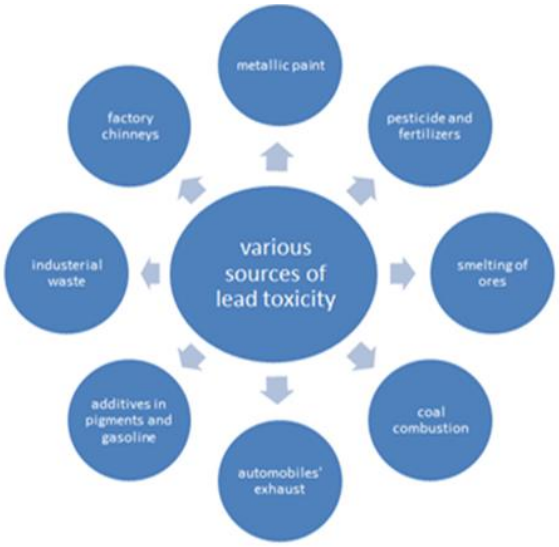

Figure 1. Various sources of lead toxicity

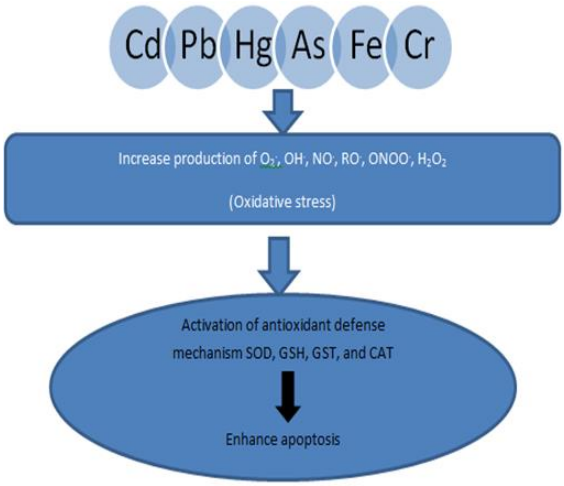

Figure 2. The balance between antioxidants and heavy metals attack

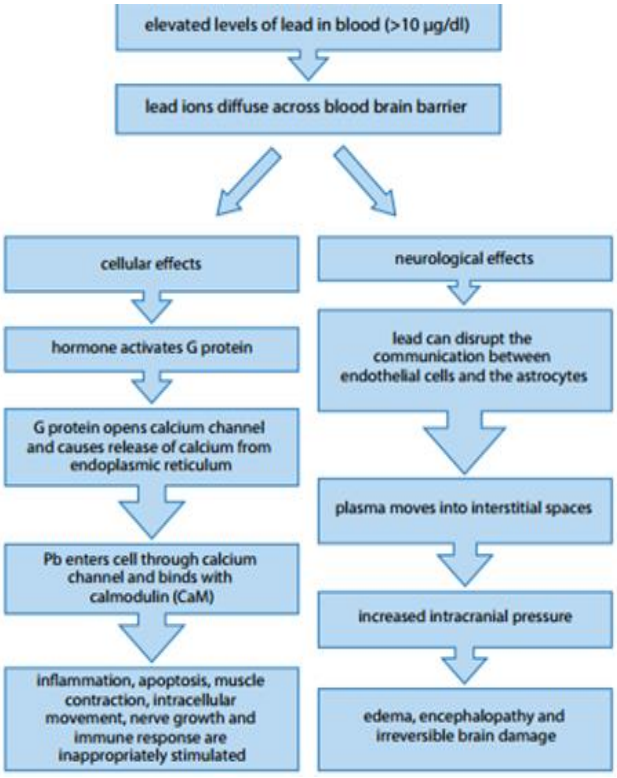

Figure 3. Effects of increased lead level in blood

Traditional herbal treatments have become increasingly popular in both developed and developing countries [5]. Various studies have investigated the protective effects of various plant extracts against Pb-induced oxidative stress and how they help reduce Pb accumulation in the liver by enhancing the liver and kidney profiles [6].

Physalis peruviana (P.P.) is frequently used to treat cancer, malaria, asthma, hepatitis, dermatitis, and rheumatism. It was tested for its hepato-renal protective effects against a variety of hazardous chemicals. *Physalis peruviana* fruit extract was found to have a substantial protective effect in the hepatic and renal tissues of Cd-treated rats. This was accomplished by lowering lipid peroxidation and increasing enzymatic and non-enzymatic antioxidant activities, such as glutathione [7]. Lead toxicity is a significant public health problem because it's a multisystem toxicant that affects the quality of life and production. Searching for a solution to a serious toxin by using herbal medicine is considered a novel idea to minimize hepatic inflammation resulting from the usage of medicinal drugs. To the best of our knowledge, there aren't any reports on the protective effects of *Physalis peruviana* against hepatotoxicity induced by lead intoxication in rats. Therefore, the use of natural products in the treatment of hepatic toxicity induced by lead ingestion will be a great idea in the near future with low cost and minimal side effects.

2. RESEARCH METHOD

2.1. Chemicals

Lead acetate was purchased from Merk. Isoflurane and other chemicals and reagents used in the study were of analytical grade. Distilled water was used as a solvent.

2.2. Plant materials

Physalis peruviana fruits were collected from the markets of west Alexandria, Egypt in the months of February and March 2020. The plant materials were authenticated in the Botany Department, Faculty of Agriculture, Alexandria University, Egypt, by a plant taxonomist.

2.2.1. *Physalis peruviana* fruit extract preparation

A total of 10 kg of fresh fruits of *Physalis peruviana* were collected from the Alexandria markets, separated from their calyxes, sorted to obtain the full mature fruits, washed and homogenized with a grinder. Subsequently, the homogenized mixture was classified into three parts; three different extraction solutions were used (water, 70% ethanol, and 70% methanol). The solutions were kept for 48 hours with continuous shaking, and then filtered. A rotary evaporator was used for evaporation, followed by a lyophilization stage using a freeze-drier to obtain the powdered form of the whole fruit. The total antioxidant activity was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay for the three extraction solutions. The form with the highest antioxidant activity was freshly dissolved in water on a daily basis and administered to the animal via oral gavage.

2.3. Experimental animals

A total of 72 Rattus norvegicus male albino rats were selected because their growth potential is much higher than females' and thus the biological impact of the treatment with *Physalis peruviana* fruit as manifested by the difference in bodyweight can be determined. The animals were housed in plastic cages (4 rats per cage) at a well-ventilated animal facility. The authors followed the European Community Directive (86/609/EEC) and national rules of animal care implemented in accordance with the NIH guidelines for the Care and Use of Laboratory Animals, 8th edition. Animals were fed a standard commercial diet and supplied with water ad-libitum. Rats were acclimatized to laboratory conditions for two weeks before the commencement of the experiment and maintained in a friendly environment, which was enriched by shredded paper and Kleenex, with a 12h/12h light and dark cycle at room temperature (22-270 C) and relative humidity (45-46). Rats were monitored daily to check their normal physiological behavior and were weighted weekly at a fixed time. The experimental protocol and procedures were approved by the Institutional Animal Care and Use Committee (IACUC) AU0919062725 of the High Institute of Public Health, Alexandria University. Rats were classified into six groups; three groups included 8 rats each, and the other three included 16 rats each. The study lasted for eight weeks (56 days) and was divided into two phases (four weeks each) as revealed in Table 1.

Table 1. Summary of feeding protocol of studied rats during the study period

Groups	Total period of the experiment eight weeks Phase one (induction period for the intervention group) (day 1 to day 28)	Day 29th	Phase two (treatment period) (day 29 to day 56)	Day 57
I control (16 rats)	-Rats fed on basal diet and tap water (No lead or PP)	8 rats were euthanized using isoflurane inhalation > 5%	Rats fed on basal diet tap water	Rats euthanized by inhalation of isoflurane inhalation >5% then blood and tissue samples were collected
II control positive (16 rats)	-Rats fed on basal diet -10 ppm lead acetate in water (1/45 LD50)	8 rats were euthanized using isoflurane inhalation > 5%	Rats fed on basal diet tap water no treatment	
III (8 rats)	- Rats fed on basal diet - 10 ppm lead acetate in water (1/45 LD50)	-	Rats fed on basal diet and tap water + 200mg/kg bw of *Physalis peruviana* L daily by oral gavage	
IV (8 rats)	- Rats fed on basal diet - 10 ppm lead acetate in water (1/45 LD50)	-	Rats fed on basal diet and tap water + 300mg/kg bw of *Physalis peruviana* L daily by oral gavage	
V (8 rats)	- Rats fed on basal diet - 10 ppm lead acetate in water (1/45 LD50)	-	Rats fed on basal diet and tap water + 500mg/kg bw of *Physalis peruviana* L daily by oral gavage	
VI ((16 rats)	- Rats fed on basal diet - 10ppm lead acetate in water (1/45 LD50) - 300mg/kg body weight of *Physalis peruviana* L daily by oral gavage	8 rats were euthanized using isoflurane inhalation > 5%	Rats fed on basal diet and tap water + 300mg/kg bw of *Physalis peruviana* L daily by oral gavage	
Total	-	24	-	48

The parameters were measured after four and eight weeks for the different groups;

Part I: Anthropometry, rats were weighted weekly for eight weeks [8]. Part II: A biochemical examination was performed on the blood, including liver enzyme function tests (ALT and AST) [9] and a complete blood count (CBC). Blood samples were collected by cardiac puncture through the diaphragm after anesthetic inhalation (Isoflurane 5%). The blood was divided into two parts; anticoagulant (heparin) was used for the measurement of hematological parameters, and the remaining blood was collected in test tubes without anticoagulant for obtaining serum. Plasma and serum were separated and frozen at -20 ◦C, while antioxidant and lipid peroxidation markers [10], including glutathione (GSH), catalase (CAT), malonaldehyde (MDA), and super oxide dismutase (SOD), were measured in liver tissue by preparing a 10% tissue homogenate.

2.4. Ethical considerations

Approval of the ethics committee "Institutional Animal Care and Use Committee" (IACUC) of the High Institute of Public Health was obtained (AU0919062725). The study was carried out in compliance with the International Guidelines for Research Ethics. The authors declared that there was no conflict of interest.

2.5. Statistical analysis of data

The data was fed into the computer and analyzed using the IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Numbers and percentages were used to describe qualitative data. The Kolmogorov-Smirnov test was used to verify the normality of the distribution. Quantitative data was described using mean and standard deviation. The significance of the obtained results was determined at the 5% level. The F-test (ANOVA) for normally distributed quantitative variables allows comparisons between more than two groups, and the post-hoc test (Tukey) allows pairwise comparisons. A paired t-test for normally distributed quantitative variables was utilized to compare the two periods.

3. RESULTS AND DISCUSSION

Plants produce secondary metabolites or polyphenolic chemicals, such as flavonoids, anthocyanidins, alkaloids, glycosides, phenolic acid, and tannins; these are key sources of drugs such as antioxidants and anti-cancer agents. In terms of their ability to prevent oxidation, several phytonutrients play an antioxidant role. The DPPH radical scavenging method is commonly used to assess the ability of antioxidants to scavenge free radicals. This method has been widely used to predict antioxidant efficacy due to the short time required for the test and the fact that this method is more stable than others [11]. Different extracts were used for detection. The ethanol extract had the highest scavenging activity (67.60%), followed by the methanol extract (54.21%), and the water extract had the lowest (34.14%) as presented in Figure 4.

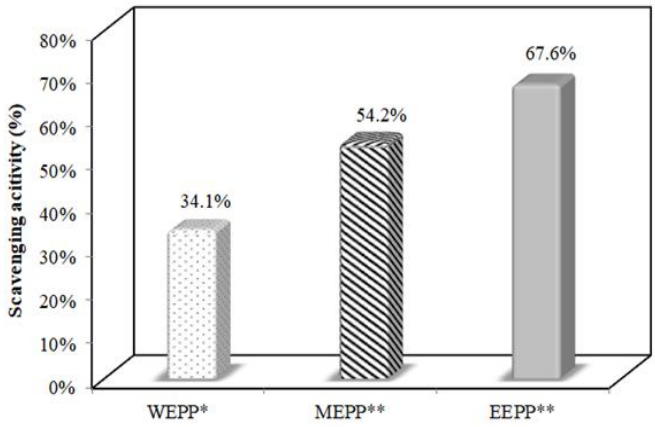

Figure 4. Free radical scavenging activity of *Physalis peruviana* fruit using DPPH assay

In a study conducted by Saratha *et al*. [12], the inhibition percentages of the ethanolic extract of *Physalis peruviana* fruit from 14.5 at 50 µg/Ml to 31.7 at 250 µg/mL were shown to be lower compared to the results of the current study. The DPPH assay results show that the extract of *Physalis peruviana* fruit possesses free radical scavenging activity. However, the antioxidant capacity is poor when compared to ascorbic acid, gallic acid, and butylated hydroxytoluene (BHT). Demİr *et al*. [13] got similar results when they used the DPPH radical scavenging procedure to assess the antioxidant activity of *Physalis peruviana* fruit collected by a local supplier from Antalya. The IC50 values for ascorbic acid and *Physalis peruviana* were found to be 1.06 g/ml and 430 g/ml, respectively. Chang *et al*. [14] suggest that the aqueous extract of *Physalis peruviana* has antioxidant activity in a concentration-dependent manner, when compared with vitamin C (5 µg/ml, scavenging 94.64%), their extract concentrations 50 µg/ml, 100 µg/ml, and 300 µg/ml showed the scavenging of 13.17%, 22.04%, and 52.72%, respectively. They argued that the fruit extract was as effective as vitamin C in terms of total antioxidant activity, but was less effective in scavenging DPPH radicals. However, some studies have used different methods to determine the antioxidant potency of *Physalis peruviana* fruit, implying that the fruit has good antioxidant activity. Differences in technique, ripeness level, or extraction method could account for the disparity in scavenging values across studies [12].

Body weight gain was progressively affected by exposure to lead acetate during the experimental period of all different groups. The mean body weight of rats in different groups is shown in Figure 5. The final body weight of intoxicated rats with lead was significantly lower than that of the control group. However, rats in group II (receiving no PP) showed significantly lower body weight ($p<0.001^{*}$) than those in the control group after eight weeks. There was a significant increase in the body weight of rats between four weeks and eight weeks.

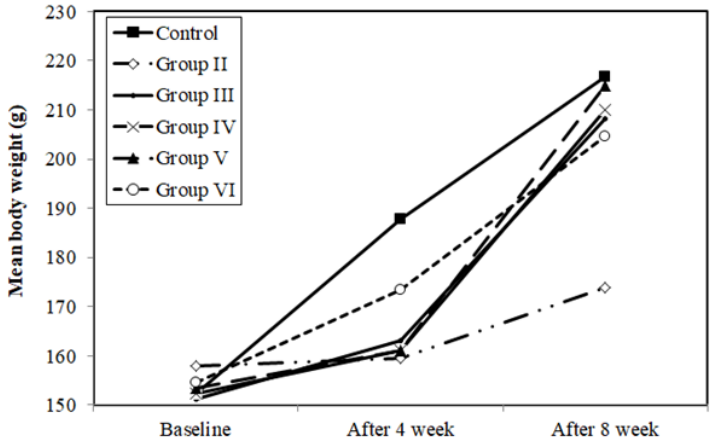

Figure 5. Comparison between mean body weight of rats at the baseline, after four and eight weeks of the study

Feeding on lead resulted in diminished body weight (groups II, III, IV, V, and VI), and the withdrawal of lead from the diet of rats induced an increase in weight, especially with the treatment with *Physalis peruviana*. In comparison to the control group, the feeding efficacy of lead-treated rats was lower. The findings are consistent with those of Ibrahim *et al*. [15], where the increase in body weight was reduced in rats given 1/20, 1/40, and 1/60 of the LD50 of lead acetate. The detrimental effects of lead were exacerbated as the amount of lead acetate was raised. The reduction in mean body weight caused by hazardous ions could arise from a variety of mechanisms, one of which is metabolic imbalance. This can be caused by low zinc levels in zinc-dependent enzymes, which are required for various metabolic activities [1].

Reckziegel *et al*. [6] perceived that decreased food intake at the satiety set-point due to the effect of Pb resulted in a non-significant increase in body weight. Animals given the Physalis solution had a significant readjustment to lower body weight after treatment, owing to changes in behavior, attitude, and physiological markers [6]. Since lead was administered simultaneously with therapy, the mean body weight of rats in group VI remained lower than the control. The findings revealed that the increase in body weight of rats treated with *Physalis peruviana* fruit solution was within normal limits and did not result in obesity. A prior study found that obese rats treated with *Physalis peruviana* fruit extract had a better lipid profile, with a substantial reduction in low-density lipoprotein cholesterol(LDL-c) and very low-density lipoprotein cholesterol (VLDL-c) values in rats treated with 500 mg/kg body weight fruit extract [16]. The latest findings support prior research showing that *Physalis peruviana* extracts from fruits, roots, leaves, and pomace can reduce heavy metal oxidative stress and reverse weight loss caused by CdCl2, CCl4, and acetaminophen [14], [17]–[19].

Table 2 shows that Pb was able to induce a significant increase ($p<0.05$) in Alanine aminotransferase (ALT) mean value when comparing group II (treated with lead) to the control. There was a significant increase in ALT levels to 86.20 ±8.81 ($p<0.001$*) and 88.20±7.40 ($p<0.001$*) after four and eight weeks, respectively. On the contrary, group VI (treated with 300 mg/kg body weight of PP solution with lead) showed a significant increase in mean ALT level after four weeks (59.0±10.93 $p<0.001$*) compared to the control group. ALT levels decreased to 46.80±3.35 for group VI due to the withdrawal of lead for four weeks. Meanwhile, the mean AST of group II (fed lead) was more than double (88.60±4.92) the value of the control group after four weeks and continued to increase to 91.80±7.96 by the end of week 8. In contrast, group VI (fed lead and treated with 300 mg/kg body weight of PP) showed a significant increase in mean AST level after four weeks (62.80±8.87 $p<0.002$*) compared to the control group. Nonetheless, it was significantly lower than group II. By the end of week eight, the AST level decreased to 56.40±4.62 for group VI due to the withdrawal of lead for four weeks. All groups treated with PP solution showed diminished AST levels compared to group II (91.80±7.96). The mean AST levels were 69.60±6.73, 55.20±4.92, 50.40±3.29, and 56.40±4.62 for groups III, IV, V, and VI, respectively. In this study, the administration of lead acetate exerts possible hepatic alterations as evidenced by the increase in serum ALT, AST activities. These enzymes are important markers of hepatocellular damage, as demonstrated by Jaishankar *et al*. [3].

Lead causes oxidative damage in the liver by increasing lipid membrane peroxidation, a harmful process carried out entirely by free radicals. The increase in serum ALT and AST functions confirms that Pb injection has the potential to cause hepatotoxicity. These enzymes have been implicated as important hepatocellular damage markers [20]. Bhattacharya [21], has reported that Pb causes hepatotoxicity and produces an elevation in liver enzymes. The abnormalities in liver function tests in lead-treated rats are known to be the key manifestations of hepatic damage. Results from the current study indicate the ability of PP solution to reduce the activity of liver enzymes ALT and AST and restore them to normal levels. Doses of *Physalis peruviana* fruit solution (300 and 500 mg/kg body weight) may protect the tissue against the toxic effects of Pb, which can reduce the systemic release of the enzyme. Previous research has shown that PP solution has a hepatoprotective effect. According to Chang *et al*. [14], PP water extract protects rats from acetaminophen (APAP)-induced hepatotoxicity. Pretreatment with PPWE at doses of 150, 300, and 600 mg/kg body weight considerably minimizes the rise in liver enzymes (ALT and AST), which are the most important indicators of liver hepatitis.

In the present study, the administration of lead acetate exerts hepatotoxicity as verified by the increase in serum ALT and AST activities. Treatment of rats with *Physalis peruviana* efficiently restored the liver enzymes of the control group. A previous study supported the current findings, where supplementation of Physalis juice exhibited a significant decrease in the levels of ALT and AST enzymes and restored them to control values in CCl4-treated animals with hepatotoxicity. The latter was indicated by the elevation of the activities of these markers [5]. Dkhil *et al*. [7] stated that pretreatment of Cd-treated rats with a methanolic extract of Physalis (MEPh) at a daily dose of 200 mg/kg bwt for five days reduced hepatorenal damage. Furthermore, a dose of 500 mg/kg body weight of PP leaf extract twice a week for six weeks effectively reduced the elevation of liver enzymes (alanine aminotransferase, alanine aminotransferase, and alkaline phosphatase) in rats with hepato-renal fibrosis caused by carbon tetrachloride (CCl4) [18].

Cellular antioxidant enzymes (CAT, SOD, GSH, and GPx) are critical components of the antioxidant defense system in the body, as they are involved in the reduction of reactive oxygen species (ROS) and peroxides produced in the living organism, as well as the detoxification of certain exogenous compounds; thus, they play a primary role in the maintenance of a balanced redox status [22]. Oxidative stress is an imbalance between the production of free radicals and the capacity of the cells to detoxify the highly reactive intermediates or repair the resulting damage. As a result of two separate and linked mechanisms, oxidative stress occurs: the production of reactive oxygen species (ROS) and the direct depletion of antioxidant reserves. In any biological system where ROS production increases, antioxidant reserves are depleted [23].

Table 2. Mean ALT and AST of rats in different studied groups after 4 and 8 weeks

ALT (U/L)	Control Mean ± SD.	Group II Mean ± SD.	Group III Mean ± SD.	Group IV Mean ± SD.	Group V Mean ± SD.	Group VI Mean ± SD.
After 4 weeks	28.0±0.53	86.20 ±8.81	–	–	–	59.0±10.93
p_1 vs. control		<0.001*	–	–	–	0.002*
p_2			–	–	–	<0.001*
After 8 weeks	28.40 ± 2.70	88.20±7.40	59.0±4.06	59.40±5.94	43.40±4.34	46.80±3.35
p_1 vs. control		<0.001*	<0.001*	<0.001*	0.001*	<0.001*
p_2			<0.001*	<0.001*	<0.001*	<0.001*
p_3			1.000		0.062	
p_4					<0.001*	0.067
AST (U/L)	Control	Group II	Group III	Group IV	Group V	Group VI
After 4 weeks	42.0± 0.90	88.60±4.92	–	–	–	62.80±8.87
p_1 vs. control		<0.001*	–	–	–	0.002*
p_2			–	–	–	<0.001*
After 8 weeks	43.20 ± 8.64	91.80±7.96	69.60±6.73	55.20±4.92	50.40±3.29	56.40±4.62
p_1 vs. control		<0.001*	<0.001*	<0.05*	<0.05*	<0.05*
p_2			<0.001*	<0.001*	<0.001*	<0.001*
p_3			0.051*		0.06	
p_4					<0.001*	0.067

Pairwise comparison between groups was done using Post Hoc Test (Tukey) for ANOVA test
p_1: p value for comparing between for each group and Control
p_2: p value for comparing between for each group and Group II
p_3: p value for comparing between Group III vs. Group IV and Group V vs. Group VI
p_4: p value for comparing between Group III vs. Group V and Group IV vs. Group VI
*: Statistically significant at $p \leq 0.05$

Lipid peroxidation is characterized by the attack of oxidants such as free radicals on lipids containing carbon-carbon double bonds, particularly polyunsaturated fatty acids. Lipid peroxidation triggers a chain reaction that results in the production of various breakdown chemicals like MDA and 4-HNE, which appear to be new indicators of oxidative organ damage. Proteins and DNA are two substrates that are particularly vulnerable to the effects of these aldehydes [20]. Malondialdehyde (MDA) was regarded as an indication of lipid peroxidation. The amount of MDA in the liver tissue of group II, which was exposed to lead acetate for the first four weeks of the trial, was significantly higher. Previous research has found that Pb-induced diseases can be linked to the generation of reactive oxygen species [20]. All cellular structures are attacked by ROS. The lipid peroxidation (LPO) process in the polyunsaturated fatty acid residues of phospholipids is accelerated, leading to the development of breakdown products such as malondialdehyde [24].

Figures 6 and 7 demonstrate that lead acetate significantly increased malondialdehyde (MDA) ($p<0.001$*) levels of rats of different studied groups after four and eight weeks of group II (treated with lead). The MDA of group II represents 3 times the value of the control group after four weeks (63.51±2.91) and slightly decreased to (60.45±5.18) by the end of week 8. Furthermore, there was a significant increase in the MDA mean value of group VI (treated with 300 mg/kg PP in addition to lead) compared to the control group (47.13±1.56 P<0.001*). Nevertheless, the mean MDA of group VI was significantly lower than that of group II (63.51±2.91 P<0.001*) after 4 weeks and significantly decreased to 33.96±4.04 ($p<0.001$*) by the end of week 8. Mean MDA levels of groups III, IV, and V that were treated with a daily dose (200, 300, and 500 mg/kg BWT) of PP solution decreased in the dose- dependent response (25.43%, 33.66%, and 48.64% for III, IV, and V, respectively). On the contrary, a marked reduction occurred in MDA levels in the liver tissue of the studied animals following the oral feeding with P. Pervuviana, either in parallel or after lead toxicity induction. These results are supported by previous studies which reported that P. Pervuviana extracts led to a significant reduction in MDA levels in CCl4 intoxicated rats [25], Cadmium intoxicated rats [7], [19], and Acetaminophen-induced liver injury in rats [14], [26].

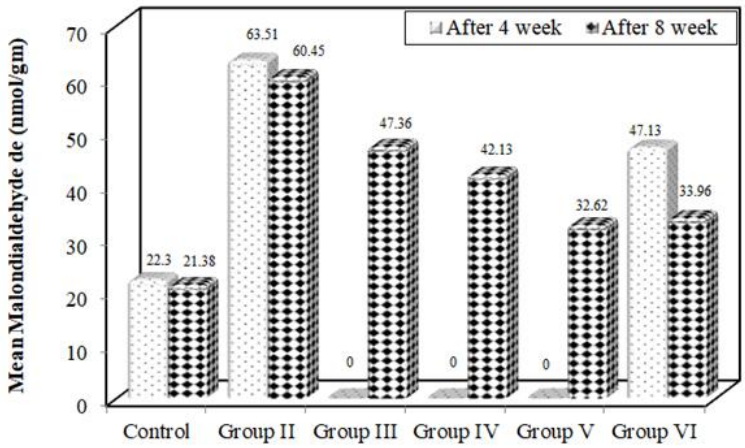

Figure 6. Mean Malondialdehyde (MDA) of rats in different studied groups after four and eight weeks

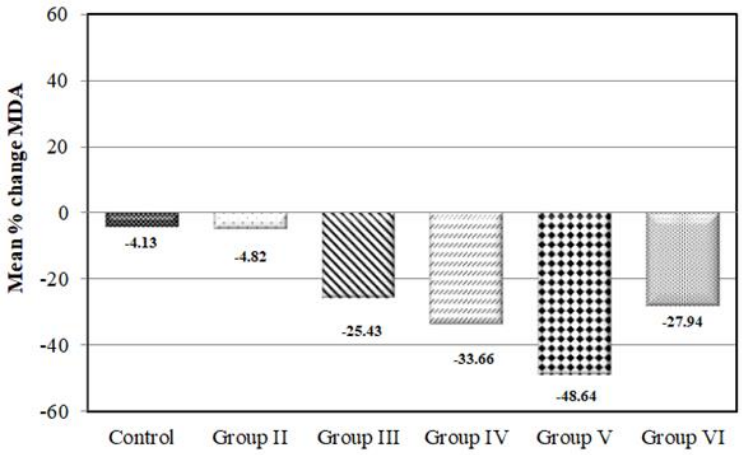

Figure 7. Comparison of the mean percent change of Malondialdehyde of rats in different studied groups during the 2nd and 4th weeks

The mean Superoxide dismutase (SOD) values are showen in Figure 8 to Figure 9. It decreased to 29.08±1.74 and 37.10±1.43 after four weeks for groups II and VI, respectively. However, the SOD value of group VI was significantly higher than that of group II due to simultaneous oral feeding with PP in the 1st four weeks. After eight weeks; group II levels continued to decrease, reaching a mean value of 25.34±2.50, while the control group recorded 46.23±4.16. Groups treated with P.P. solution showed relative improvement in a dose-dependent manner in SOD mean values (31.34±1.44, 30.67±0.51 and 39.39±0.46 for groups III, IV and V respectively). Furthermore, group VI showed a significant increase (40.92±0.22, $p<0.001$*) after eight weeks compared to group II, and the mean SOD was close enough to the normal level of the control group (46.23±4.16) with no significant difference ($p=0.062$). Superoxide dismutase (SOD) is considered as a front line of defense against the potentially cytotoxic O2 free radicals that cause oxidative stress. SOD transforms O_2 to the more stable hydrogen peroxide (H_2O_2), which is enzymatically converted to H_2O by catalase and glutathione peroxidase [11].

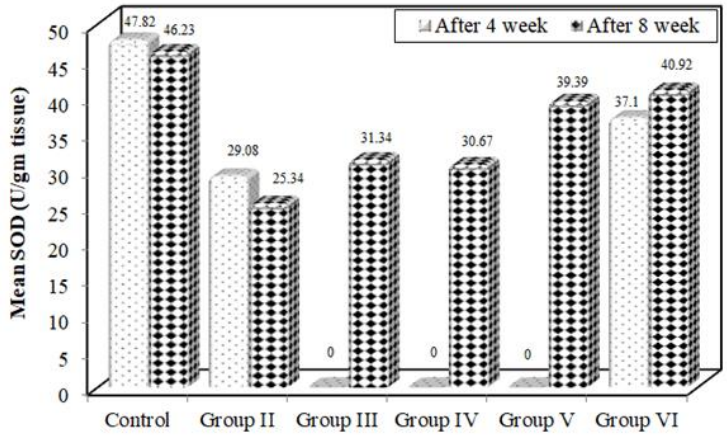

Figure 8. Mean superoxide dismutase (SOD) of rats in different studied groups after four and eight weeks of the study

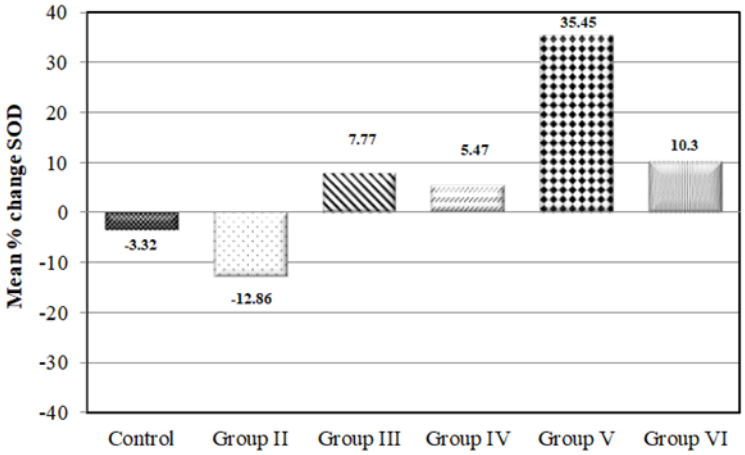

Figure 9. Comparison between mean percent changes of Superoxide dismutase of different studied groups during the second and fourth weeks

Endogenous antioxidant enzymes such as SOD, CAT, and GSH are the first lines of defense against free-radical attack. With respect to the findings, the activity of the antioxidant enzyme, SOD, was significantly reduced in the liver tissue of the lead-treated rats compared to the control group, which suggests the ability of lead to induce extreme oxidative stress [2]. The inhibitory effect of lead on SOD may be due to competition between lead and Zn or Cu, which is fundamental to SOD production and results in an inactive form of the enzyme. Simultaneous treatment of rats with 300 mg/kg body weight of Physalis Peruvian fruit contributed to preventing the rapid decline in SOD values and increased them to around the normal value of the control group by the end of week 8. The fruit of Physalis Peruvian does not only demonstrate the ability to improve the antioxidant capacity in the body; a previous study showed that SOD activity was increased and free radicals decreased by co-treatment with *Physalis peruviana* root extract in CCl4 treated rats [17].

Khalaf-Allah *et al*. [18] reported a significant increase in SOD value demonstrated in CCl4-treated rats after treatment with 500 mg/kg body weight of *Physalis peruviana* leaves extract twice a week for six consecutive weeks. The protective effect of *Physalis peruviana* was also evaluated against cadmium-induced toxicity and showed an improvement in the antioxidant status of the experimented animals [7], [19].

A remarkable decline in the mean CAT value of group II was detected at 35.05% with a statistically significant difference (p=0.001*) compared to the control group. The control group also experienced a slight decline without any indications. In addition, groups III, IV, and V showed varied increased percentages, ranging from 30.0% in group IV to 72.50% in group V. In the current study, administration of *Physalis peruviana* in parallel to lead exposure also showed a significant improvement and mitigating effect against lead-induced oxidative stress by means of increasing catalase activity. These results are consistent with the

previous study by Dkhil *et al*. [7] where pretreatment of rats with MEPh prevented Cd-induced alternated activities of the antioxidant enzymes.

Lead induced a significant decrease in the catalase mean value of groups treated with lead. However, group VI (treated with 300mg/kg of PP in parallel to lead toxicity) showed a significant increase (0.69±0.04 p 0.001*) compared to group II (untreated with PP), as displayed in Figure 10. In addition, by the end of week 8, there was a remarkable increase in CAT mean values of group V (treated with 500 mg/kg BWT of PP) with no significant difference compared to the control (0.69±0.03 p=0.062). However, the mean CAT value of group II (that left without treatment after lead toxicity induction) significantly decreased by the end of week 8, recording 0.26±0.04 (p<0.001*) compared to the control group. Meanwhile, group VI has increased from 0.69±0.04 at the end of week 4 to 0.72±0.04 by the end of week 8 with no significant difference (p=0.074) compared to the control group after 8 weeks. Catalase is a common hemeprotein enzyme found in nearly all living organisms exposed to oxygen. It catalyzes the decomposition of hydrogen peroxide into water and oxygen using either iron or manganese as a cofactor. It plays a significant role in protecting the cell from oxidative damage by reactive oxygen species such as H2O2 and OH [27].

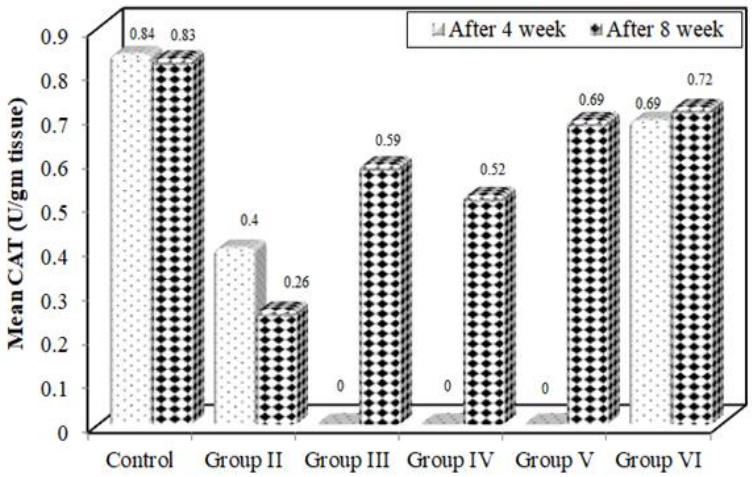

Figure 10. Illustrates the percent change of catalase (CAT) of different studied groups during the 2nd four weeks

Previous studies reported that in carbon tetrachloride intoxicated rats, the activities of CAT were decreased compared to the control groups. Rats supplemented with Physalis juice along with CCl4 for 12 weeks experienced a significant increase in CAT activity compared to the CCl4-treated group [5]. Not only the fruit juice, but also the extracts of the leaves and roots of *Physalis peruviana* showed a protective effect against the oxidative stress caused by CCl4, and administration of their extracts resulted in an increase in antioxidant system capacity, including CAT activity [17].

Figure 11 demonstrates that the mean value of glutathione (GSH) in the control group was 12.40±0.65 after 4 weeks and 12.62±0.51 after 8 weeks. However, the mean value for group II (not treated with PP) was 9.86±0.44 and 7.47±0.77 after 4 and 8 weeks, respectively, which was lower than that of the control with a statistically significant difference (p<0.001). Moreover, the mean GSH value of group VI was lower than the control group with no significant difference and remained higher than that of group II with a significant difference (11.14±0.41 p=0.004* and 12.12±0.15 p<0.001* after 4 and 8 weeks, respectively). Lead alters the antioxidant activities by inhibiting functional sulfhydryl groups (SH groups) in several enzymes, such as superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH).

GSH, CAT, and SOD are potential targets for lead toxicity. The reduction in catalase activity by lead can be caused by a decrease in iron absorption, an important trace for catalase activity. Deficiency or malfunction of catalase is postulated to be related to the pathogenesis of many age-associated degenerative diseases [2]. Glutathione is the main cellular thiol involved in cellular redox reactions and thioether formation, and is capable of preventing damage to important cellular components caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides, and heavy metals [22].

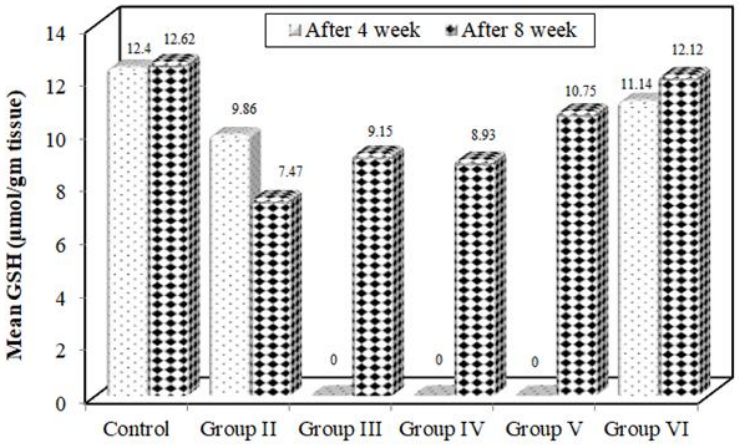

Figure 11. Mean glutathione of rats in different studied groups after four and eight weeks of the study

The most important antioxidant found in cells is glutathione (GSH). Glutathione exists in both reduced (GSH) and oxidized forms (GSSG). The reduced state of glutathione donates reducing equivalents ($H^+ + e^-$) from its thiol groups present in cysteine residues to ROS and makes them stable. After donating the electron, it readily combines with another molecule of glutathione and forms glutathione disulfide (GSSG) in the presence of the enzyme glutathione peroxidase (GP X). GSH can be regenerated from GSSG by the enzyme glutathione reductase (GR) [28].

Lead demonstrates the capacity for electron sharing that results in the creation of covalent attachments with the antioxidant enzymes present in the sulfhydryl groups, which are the most susceptible targets for lead and are ultimately inactivated. Using the previous mechanism, lead inactivates the glutathione enzyme. As a result, cysteine synthesis of GSH via the -glutamyl cycle occurs, which is generally ineffective in replenishing GSH supplies. Likewise, enzymes such as δ-amino levulinic acid dehydratase (ALAD), glutathione reductase (GR), glutathione peroxidase (GPX) and Glutathione-S-transferase are inactivated by lead, causing a further reduction in glutathione levels [4].

The role of *Physalis peruviana* in restoring and activating the production of GSH was also reported in previous studies. The protective effects of Physalis fruit methanolic extract in maintaining the GSH level towards control in cadmium-induced testes toxicity in rats were previously reported. It increased the capacity of endogenous antioxidant defense and increased the steady state of GSH and/or its rate of synthesis [19].

Dkhil *et al*. [7] reported that Cd injection reduced the content of GSH in hepatic and renal tissues. In addition, oral administration of MEPh at a daily dose of 200 mg/kg bwt for five days may keep the status of this molecule nearly normal. It was also found that *Physalis peruviana* extracts of roots, fruits, pomace and leaves have the capacity to restore GSH levels in CCl4 intoxicated rats [5], [17], [18] and Acetaminophen-Induced Liver Injury [14]. The total body burden of lead may be divided into four compartments. The residence times of lead in these four compartments are estimated to be about 35 days in the blood; 40 days in soft tissues; 3 to 4 years in trabecular bone; and 16 to 20 years in cortical bone. The disappearance time is largely dependent upon the degree of overall excess exposure. The greater the body's lead burden, the slower the rate of disappearance from the tissues, including blood. Nevertheless, blood lead measurements may not be effective in making a retrospective diagnosis. Lead injury (soft tissue and central nervous system (CNS) can last for a long time after blood lead levels have decreased due to distribution and elimination [4].

Table 3 illustrates that the exposure to lead acetate daily (10 ppm) in the drinking water of rats caused a significant increase ($p<0.001^*$) in lead concentration in the liver of rats after four and eight weeks in group II, which was (0.49±0.11 ppm.) about twice the mean value of group IV (0.26±0.13 ppm). The mean lead concentration in the control group was under the detectable level (limit of detection of atomic absorption spectroscopy schimadzu® 0.01 ppm). After eight weeks, the mean lead concentration remained undetected in the control group, while group VI showed a significant decrease from 0.26 ppm after four weeks to 0.07±0.01ppm after eight weeks of treatment with a daily dose of (300 mg/kg BW) *Physalis peruviana* fruit solution. Group II showed a mean lead concentration of 0.36±0.05 ppm after eight weeks, and this reduction may be due to the withdrawal of lead from the diet. The value of the mean lead concentration of group II remains high when compared with other groups. Groups III, IV, and V that were treated with 200, 300, 500 mg/kg BW of *Physalis peruviana* solution showed a reduction in the mean lead concentration in a dose-dependent response of 0.26±0.02, 0.21±0.02, and 0.14±0.03 ppm for groups III, IV, and V,

respectively. Since it was first described by Laennec in 1831, lead poisoning has been associated with anemia. Lead directly affects the hematopoietic system by limiting hemoglobin production through inhibiting different main enzymes involved in the pathway of heme synthesis. It also increases the fragility of cell membranes, which decreases the life span of circulating erythrocytes. Therefore, anemia results from the cumulative aftermath of these two mechanisms. Two types of lead poisoning anemia can occur; hemolytic anemia, which is associated with acute high-level lead exposure; and frank anemia, which is induced only when blood lead levels are significantly increased over long periods of time [4].

Table 3. Mean lead concentration of rats in different studied groups after four and eight weeks

Lead (PPM)	Control Mean ± SD.	Group II Mean ± SD.	Group III Mean ± SD.	Group IV Mean ± SD.	Group V Mean ± SD.	Group VI Mean ± SD.
After 4 weeks	0.00	0.49±0.11	–	–	–	0.26±0.13
p_1 vs. control		–	–	–	–	–
p_2			–	–	–	<0.001*
After 8 weeks	0.00	0.36±0.05	0.26±0.02	0.21±0.02	0.14±0.03	0.07±0.01
p_1 vs. control		–	–	–	–	–
p_2			0.06	0.05*	<0.001*	<0.001*
p_3				0.062	0.05*	
p_4					<0.001*	<0.001*

Pairwise comparison between groups was done using Post Hoc Test (Tukey) for ANOVA test
p_1: p value for comparing between for each group and Control
p_2: p value for comparing between for each group and Group II
p_3: p value for comparing between Group III vs. Group IV and Group V vs. Group VI
p_4: p value for comparing between Group III vs. Group V and Group IV vs. Group VI
*: Statistically significant at $p \leq 0.05$

A reduced red blood cell life span of less than 40 days leads to newly developed hypochromic cells and mild anemia, while there may be serious lead toxicity without anemia, or the anemia may be accidental. In cases of iron deficiency, some research has shown an increased susceptibility to lead poisoning, while high iron intake may decrease lead absorption. When iron deficiency and lead poisoning co-exist, especially in infants and children, the resulting anemia is more severe [29].

Lead inhibits the body's ability to make hemoglobin. Primarily, by inhibiting d-aminolevulinic acid dehydratase (ALAD) and ferrochelatase (FECH) activity, lead decreases heme biosynthesis. Ferrochelatase is very susceptible to lead, which catalyses the incorporation of iron into protoporphyrin IX [30]. The mean complete blood count (CBC) of rats after four and eight weeks is presented in Table 4. It shows that the mean hemoglobin concentration (Hb) of the control group was 14.70±0.52 gm/dl after four weeks and increased to 15.50±0.68 gm/dl at the end of week 8. This increase occurred along with a decrease in the mean hemoglobin concentration of group II (untreated with PP solution), where it recorded 11.82±0.75 and 10.33±0.81 gm/dl at the end of weeks 4 and 8, respectively. The mean hemoglobin concentration of group VI was statistically significant higher than that of group II (12.63±0.18 p= 0.019* and 12.93±0.33 p<0.001* at the end of weeks 4 and 8 respectively) but remained lower than that of the control group. The mean value of red blood cell count (RBCs) showed a remarkable decrease in all groups treated with lead compared to the control group. However, group II recorded the lowest RBCs after four and eight weeks (6.03±0.41 and 5.74±0.49). The mean RBCs of group VI that was treated with 300 mg/kg b.w during the 1st 4weeks (7.18±0.33 & 7.32±0.32 after four and eight weeks, respectively) was significantly higher than that of group II (6.03±0.41 p=0.011*, 5.74±0.49 p= 0.044*). Nevertheless, it remained lower than that of the control group.

The mean hematocrit value (HCT) of all groups was lower than that of the control with a statistically significant difference (p<0.001*). The drop was observed in group II (35.29% ±4.41) while group VI recorded the highest value (40.83±2.23). On the contrary, the mean HCT of group V (treated with 500 m/kg bwt of PP solution,) was close to that of group VI after a 4.27% increase by the end of week 8 (HCT were 39.83±3.13 & 40.80±1.30 for group V and VI, respectively). This decline was also observed in the white blood cells count of all groups, which was also significantly reduced when compared to the control group (p> 0.001*). However, the WBC count of rats fed 500 mg /kg bwt *Physalis peruviana* fruit (group V) and rats in group VI, (fed with 300 mg/kg bwt *Physalis peruviana* fruit over 8 weeks) was increased by the end of week 8 (7.98±0.51&7.61±0.50) with no statistical difference when compared to the control group (p=1.000 and 0.697 for group V and VI, respectively).

Table 4. Mean CBC of rats after in different studied groups after 4 and 8 weeks

		Control	Group II	Group III	Group IV	Group V	Group VI
Hb (gm/dl)	After 4 weeks	14.70±0.52	11.82±0.75	–	–	–	12.63±0.18
	p_1 vs. control		<0.001*	–	–	–	0.041*
	p_2			–	–	–	0.019*
	After 8 weeks	15.50±0.68	10.33±0.81	11.77±0.60	11.57±0.34	12.62±0.17	12.93±0.33
	p_1 vs. control		<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
	p_2			<0.001*	0.002*	<0.001*	<0.001*
	p_3			0.983		1.000	
	p_4					0.092	0.017*
RBCs (M)	After 4 weeks	8.26±0.83	6.03±0.41	–	–	–	7.18±0.33
	p_1 vs. control		<0.001*	–	–	–	0.035*
	p_2			–	–	–	0.011*
	After 8 weeks	7.95±0.41	5.74±0.49	6.16±0.11	6.21±0.08	6.40±0.47	7.32±0.32
	p_1 vs. control		<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
	p_2			0.208	0.126	0.016*	0.044*
	p_3			1.000		0.999	
	p_4					0.830	0.992
HCT (%)	After 4 weeks	48.0±0.02	38.20±2.17	–	–	–	40.80±1.30
	p_1 vs. control		<0.001*	–	–	–	<0.001*
	p_2			–	–	–	0.066
	After 8 weeks	50.50±4.09	35.50±4.41	36.29±2.81	35.29±2.69	39.83±3.13	40.83±2.23
	p_1 vs. control		<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
	p_2			0.997	1.000	0.189	0.060
	p_3			0.993		0.995	
	p_4					0.421	0.056
WBCs (thousands)	After 4 weeks	8.43±0.69	7.10±0.23	–	–	–	7.47±0.10
	p_1 vs. control		0.001*	–	–	–	0.009*
	p_2			–	–	–	0.262
	After 8 weeks	8.04±0.58	5.81±0.54	7.04±0.35	7.14±0.59	7.98±0.51	7.61±0.50
	p_1 vs. control		<0.001*	0.016*	0.036*	1.000	0.697
	p_2			0.001*	<0.001*	<0.001*	<0.001*
	p_3			0.999		0.813	
	p_4					0.028*	0.571

Pairwise comparison between groups was done using Post Hoc Test (Tukey) for ANOVA test
p_1: p value for comparing between for each group and Control
p_2: p value for comparing between for each group and Group II
p_3: p value for comparing between Group III vs. Group IV and Group V vs. Group VI
p_4: p value for comparing between Group III vs. Group V and Group IV vs. Group VI
*: Statistically significant at $p \leq 0.05$

4. CONCLUSION

Treatment with *Physalis peruviana* contributed to reversing the damage that occurred in the antioxidant system as a result of lead toxicity. Moreover, co-administration of *Physalis peruviana* with lead, as a prophylaxis measure, reduces the deterioration by inhibiting the increase in MDA levels and enhancing the antioxidant activity. Long-term research on *Physalis peruviana* administration and the Maximum Tolerated Dose determination are highly recommended to avoid misuse. Affordable and innovative techniques for the extraction and isolation of natural products are needed to facilitate large-scale production and maximize the value of medicinal plants. Because lead toxicity is a devasting problem facing humanity, finding a natural remedy will have a great impact on improving quality of health and life with minimal side effects.

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BIOGRAPHIES OF AUTHOR

Fatma I Gad is a lecturer of public health /nutrition and food safety. Working on projects to improve the health and nutritional status in Egypt. PhD in public health science /food hygiene and control. Her research interest in global health, food security, WASH nutrition in emergency, and food control. She can be contacted in fatmaisamil.gad@gmail.com.

Eglal G Salem is a professor of public health science food hygiene and control at the High institute of public health - Alexandria University- Egypt. Consultant in food inspection to fast food chains in Egypt. She has taught courses of post graduate students in the field of food Hygiene at the HIPH. The courses include: Food contamination, sanitation in food premises, food additives and contaminants, food technology, food standard and food legislation, and application of HACCP system. She supervised thesis for Master and Doctor Degree in the field of Public Health (Food Hygiene and Control, Nutrition and Food Analysis). She can be contacted at email: eglalagsalem@hotmail.com.

Ossama Abdullatef is currently Associate Professor in the Pharmaceutical Chemistry Department, Faculty of Pharmacy, Pharos University in Alexandria (PUA). He got his Ph. D. Degree in Chemistry (Electrochemistry), Faculty of Science, Alexandria University. He worked as research assistant for Kuwait university, Faculty of science for 4 years from 1997 to 2001.He worked as teacher assistant for Faculty of science Alexandria University from 2003 to 2009. He worked as part time lecturer for Arab Maritime and transportation Academy in college of Engineering and College of Navigation for 4 years from 2006 to 2010. He is member of the Egyptian Corrosion Society. He can be contacted at email: ossama_abdullatef@yahoo.com.

Samar M Aborhyem is a lecturer in Food analysis, Nutrition Department, High Institute of Public Health (HIPH), Alexandria University, she is the executive manager of Central laboratories, HIPH, and she is the head manager of Institutional Animal Care and Use Committee (IACUC), HIPH, Alexandria university. In addition to being a member of many committees in the University. Her research interests are mainly focused on the usage of herbal medicine and functional food in combating various chronic diseases, quantification of different chemical environmental contaminants facing humanity such as pesticide residues, heavy metals, acrylamide, transfat, and aflatoxins, and studying their risk. Formulation of new products from different herbal extracts to boost health and treat some diseases. She managed different projects and had several international peer reviewed publications. She can be contacted at email: samaraborhyem@yahoo.com.