http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2021.599.611

Research Article
Ameliorative Effect of Kiwifruit (*Actinidia deliciosa*) against Lead-Induced Oxidative Stress in Wistar Albino Rats

[1,2]Eman F. El Azab

[1]Department of Clinical Laboratory Sciences, College of applied medical sciences, Jouf University, Kingdom of Saudi Arabia
[2]Department of Biochemistry, Faculty of Science, Alexandria University, Egypt

Abstract

Background and Objective: Lead is defined as a severe adverse metal that induces neurological, renal, haematological and hepatic dysfunctions. It stimulates oxidative stress to disrupt the antioxidative enzyme mechanism, organ structure and lipid membranes of the cell. Kiwifruit (*Actinidia deliciosa*) is amongst the world's most valuable fruits due to its various pharmacological characteristics and health benefits. The present research was intended to observe the antioxidant efficiency of kiwifruit ethanolic extract on lead toxicity in the hepatic, renal, brain and blood tissues in rats. **Materials and Methods:** Twenty-four adult Wister albino rats were classified into 4 groups with 6 rats within each group. The rats in group I functioned as normal control. Animals within group II, III and IV were given three intraperitoneal doses of lead acetate (25 mg kg^{-1} b.wt., liquefied in distilled H_2O as a vehicle) on the day 7th, 14th and 21st of the experiment. Group III and IV were the treatment groups and were treated with a daily oral dose of kiwifruit extract (250 and 500 mg kg^{-1} b.wt., respectively) for 28 days. **Results:** The protective impact of kiwifruit was observed in the improvement in antioxidant enzyme activity [Catalase (CAT), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx) and Glutathione Reductase (GR)] and decreased level of Lipid Peroxidation (LPO) in the liver, brain and kidney tissues. Additionally, *Actinidia deliciosa* has a great effect on increasing acetylcholine esterase activity in the brain and also, improved the delta-aminolevulinic acid dehydratase activity in the blood. **Conclusion:** Kiwifruit emerged as an effective factor for the alleviation of lead-induced oxidative damage in cells.

Key words: Lead, kiwifruit, oxyhemoglobin, caffeic acid, ellagic acid, acetylcholinesterase, neurotoxicity

Citation: El Azab, E.F., 2021. Ameliorative effect of kiwifruit (*Actinidia deliciosa*) against lead-induced oxidative stress in wistar albino rats. Pak. J. Biol. Sci., 24: 599-611.

Corresponding Author: Eman F. El Azab, Department of Clinical Laboratory Sciences, College of applied medical sciences, Jouf University, Kingdom of Saudi Arabia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

One of the earliest known environmental contaminants is inorganic lead and evidence of lead poisoning (Plumbism) can be traced to Roman times. At least five thousand years ago, lead production began of lead toxicity resulted from this period. Lead, which is a light, grey-blue heavy metal[1,2], is a major cause of poisoning in domestic animals worldwide. Lead is a toxic metal in both organic (tetraethyl lead) and inorganic forms (lead acetate and lead chloride) present in the atmosphere[3] and has been used in pharmaceuticals, carvings, barrels, ammunition and, more recently, in alloys used to weld chemical reagent storage materials[2].

It is a multi-organ toxicant[4], characterized by enlarged levels of lead in blood and other pivotal vital tissues of the body attained via air, food and drinking water[5]. Lead poisoning threatens hugely significant organs causes many undesirable effects in the body causing dysfunctions in the liver[6], brain[5,7] and kidney[7].

One of the important mechanisms suggested for lead toxicity is oxidative stress that has been greatly included in the overstated accumulation of Reactive Oxygen Species (ROS) within the cell[7]. Besides, ROS plays a noteworthy role in the oxidation of cell lipids, proteins and DNA leading to cellular damage[8]. ROS targets the cell membrane triggering oxidation of lipid bilayer liberating Malondialdehyde (MDA) as the end product[9].

Furthermore, lead attaches to the sulfhydryl groups existing in the most susceptible targets of antioxidants and antioxidant enzymes[10] and further negatively impacts the levels of glutathione by inactivating antioxidant enzymes associated with glutathione. Lead also causing inactivation to Superoxide Dismutase (SOD) and Catalase (CAT)[7,9]. Lead also induces apoptosis[10] and down-regulated antioxidant enzyme activity in blood and other body tissues[11]. Certain deleterious effects of lead involve diminishing haemoglobin synthesis by suppressing different main enzymes involved in the pathway of heme synthesis and also decreasing the life span of circulating erythrocytes by raising cell membrane fragility causing anemia[12].

Many natural products have been in use for a long time to minimize metal-related toxicity. To reduce the adverse symptoms of lead poisoning, consumption of polyphenols, flavonoids, vitamins and other bioactive compounds has been mentioned[7]. These natural products have a significant role in lead-related toxicities, as they are documented to massively reduce oxidative stress by suppressing the pro-oxidative and increased expression of anti-oxidative factors within the cells affected[10].

Kiwifruit (*Actinidia deliciosa*) is amongst the world's most valuable fruits because of its flavour and health benefits[13]. Generally, a significant number of phenolic constituents from kiwifruit extracts have been recognized[14]. The fundamental bioactive components of kiwifruit are distinguished by phenolic constituents and the ascorbic acid is responsible for its numerous bioactivities[15]. In comparative experiments with apples, oranges, pineapples, peaches, red grapefruit, bananas and other fruits, elevated levels of total phenolic compounds and greater antioxidant capacity are observed in kiwifruits[16]. Moreover, only one kiwifruit can accommodate 85% of our regular requirements for ascorbic acid[13].

Consumption of kiwifruits is commonly believed to have a preventative impact on cardiovascular disease, obesity and anti-diabetes[17]. Surely, kiwifruit has been clarified to have diverse biological activities, such as anti-inflammatory[15], antioxidants and anti-proliferative[16,18].

There is also no detailed study of the effectiveness of kiwifruit in different tissues such as the brain, liver and kidney for lead-induced oxidative stress. Accordingly, the research study was developed to investigate the antioxidant effectiveness of *Actinidia deliciosa* ethanolic extract on lead toxicity in adult albino rats' blood, brain, hepatic and renal tissues.

MATERIALS AND METHODS

Study site: The study was done out between May 2019 - May 2020 at the Faculty of Science, Alexandria University, Egypt.

Chemicals: Lead acetate (99.8%), Elman's reagent [5, 5'-dithiobis-(2-nitrobenzoic acid], 1-chloro-2, 4-dinitrobenzene], diethylene triamine pentaacetic, pyrogallol and Thiobarbituric Acid (TBA) were received from Sigma-Aldrich, Germany. Tetramethoxypropane and superoxide dismutase were imported from Sigma Company, USA. Perchloric acid and Dimethyl Sulfoxide (DMSO) were purchased from Diamond Company, Germany. Bovine egg albumin, Dithiothreitol (DTT) and Picrylsulfonic acid (2.4.6-trinitrobenzene sulfonic acid, TNBS) was purchased from Oxford, India. Moreover, all other materials were of the highest purity and obtained from Tata Chemicals Ltd, India. Also, all the materials for the analysis of kidney functions and liver functions were taken from Bioassay Systems, USA.

Fruit extract preparation: *Actinidia deliciosa* (kiwifruit) was isolated from the peeling skin, then homogenized by a high-speed hand blender. The fruit ethanolic extract was

prepared with 70% ethanol (1:1 w/v). Consequently, the filtrates were concentrated using a rotary vacuum evaporator (Buchi CH-9230, Germany) and thereafter lyophilized (Xiangyi, FD-10 Bench-top, China) and stored at -80°C until use. During the experiment, the required amount of kiwifruit extract powder was dissolved in distilled water.

Animals: Total 24 male Wister albino rats (130-150 g) were procured from National Research Centre's animal house, Giza, Egypt. The animals were housed in clean polypropylene cages bedded with husk under standard conditions of temperature (25°C), humidity (50-60%) and 12 hrs light/dark cycle at the Animal House of Faculty of Medicine and Alexandria University, Egypt. Rats have been having a normal diet and water during this week. The experiments were done rendering to the prescribed guidelines of the Institutional Animal Ethical Committee, the study protocol and all procedures including the use of laboratory animals were approved (Approval code No: II-M-22-19).

Experimental design: A total of 24 adult Wister albino rats were distributed into four groups of six rats each. Animals were allowed to acclimatize for 1 week before the experiment started. The rats in group I functioned as control and were taken *ad libitum* feed and water Animals in group II, III and IV were given three intraperitoneal doses of lead acetate (25 mg kg^{-1} b.wt., dissolved in distilled water as a vehicle) at the day 7th, 14th and 21st of the experiment. LD_{50} of lead acetate is 150 mg kg^{-1} b.wt.[7]. Group III and IV were the treatment groups and were treated with an oral dose of kiwifruit extract 250 and 500 mg kg^{-1} b.wt., respectively daily for 28 days[19].

Sample collection: On the 30th day of the experiment animals were sacrificed under general anaesthetic conditions using the nose cone method of isoflurane (a mixture of 30% v/v isoflurane in propylene glycol) and the blood was extracted by cardiac puncture, some of the blood into heparinized tubes and were immediately used for the determination of haematological parameters and the other part centrifuged for 10 min at 3000×g using PLC-05 series centrifuge (Gemmy Industrial Corp, Taiwan), the serum was stored at -80°C until the assays were completed. The kidney, liver and brain were removed, then immediately washed with cold 0.9% NaCl and homogenized in cooled phosphate buffer (0.1M, PH 7.4) using a homogenizer and then centrifuged at 1500×g for 15 min to detach the nuclear debris. Finally, the Post-Mitochondrial Supernatant (PMS) was reserved at -80°C for biochemical analysis.

Haematological study: The percentage volume of Packed Cells (PCV) was calculated using the hematocrit method as designated by Alexander and Griffiths[20]. The haemoglobin (Hb) concentration in all blood samples was valued in all samples was measured using Drabkin's reagent (purchased from Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) accepted to the cyanmethemoglobin method with an absorption maximum at 540 nm, as referenced by Kratz *et al.*[21] Red Blood Cell (RBC) and White Blood Cell (WBC) counts were quantified using the visual method of Bond and Richards-Kortum[22].

Delta-Amino Levulinic Acid Dehydratase (ALAD) activity: The delta-Amino Levulinic Acid Dehydratase (ALAD, EC 4.2.1.24) activity was quantified no later than 10 hrs after the blood sample collection rendering to the reference of Berlin and Schaller[23]. The reaction mixture consisted of 250 µL of ALA (5.0 mM, melted in distilled water), 50 µL of 20 mM DTT, 100 µL of sodium phosphate buffer (0.25 M, pH 7.4) and 100 µL of PMS. The test tubes were brood at 37°C for 30 min and then 500 µL 10% TCA, containing 0.1 M HgCl was added to the mixture. All tubes were centrifuged at 1500×g for 10 min and 500 µL of the supernatant was collected. This supernatant was further mixed with an identical volume of freshly prepared 2 N modified Ehrlich's reagent (purchased from Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). After 15 min, the absorbance was estimated at wavelength 555 nm using a spectrophotometer (Alpha-1502 UV/Visible Spectrophotometer, Thomas Scientific Company, USA). The results were expressed as nanomol porphobilinogen (PPB) formed per hr mL^{-1}.

Estimation of Lipid Peroxidation (LPO): LPO was valued using the method of Ohkawa *et al.*[24]. Briefly, in two test tubes, 50 µL from homogenate or homogenizing buffer (blank) was added separately to 50 µL of Sodium Dodecyl Sulfate (SDS) (8.1%) followed by 375 µL of acetic acid (20% containing 0.27% M HCl, pH 3.5), 375 µL of Thiobarbituric Acid (TBA) (0.8%) and 150 µL of distilled H_2O. In a bath of boiling water, the experiment tubes were incubated for 45 min. After cooling reaching room temperature, 250 µL of distilled H_2O was applied to each mixture followed by 1.25 mL of n-butanol/pyridine mixture. Using a vortex, all tubes were collected well, then for 10 min, centrifugation at 3000×g. The absorption of the pink colour produced by the organic layer was read against the blank at wavelength 532 nm. The concentrations of LPO were determined using the standard curve of Tetramethoxypropane (TMP) and articulated as nmol MDA mg^{-1} protein.

Estimation of anti-oxidative enzyme status

Assay for superoxide dismutase activity: Superoxide Dismutase activity (SOD, EC 1.15.1.1) has been quantified using the Marklund and Marklund procedure[25]. In summary, 1000 µL of reagent 1 (240 mg Tris-HCl was dissolved in 70 mL water, add 39.3 mg Diethylene Triamine Pentaacetic Acid (DTPA), mix and adjust pH to 8.2 with 1 M HCl) was added to 10 µL of reagent 2 (25.2 mg pyrogallol was dissolved in 10 mL 10 mM HCl), blend well and after that 20 µL of tissue, the supernatant was added. After that, the samples were incubated at room temperature for 10 sec. Then, the change in the absorbance per minute of the established colour was recorded at 420 nm against blank using a Biotech Photometer Ultraspec 1000 (Labexchange Company, Burladingen, Germany). The activities of SOD in the samples were determined as (ng mL^{-1}) from the standard curve of SOD. The specific activities were expressed in ng min^{-1} mg^{-1} protein.

Catalase activity: Catalase activity (CAT; EC 1.11.1.6) quantified by the procedure of Aebi[26]. About 100 µL of the sample was added to 1900 µL of 50 mM phosphate buffer (pH 7.0). Total 1000 µL of 30 mM Hydrogen Peroxide (H_2O_2) was added and a change in absorbance was followed for 3 min at 15 sec intervals using Ultraspec 1000 (Biotech Photometer from Labexchange Company, Burladingen, Germany) at wavelength 240 nm. CAT activity was stated as µmoles of H_2O_2 oxidized per min per milligram protein.

Glutathione Reductase (GR) activity: Glutathione reductase activity (GR, EC 1.6.4.2); was measured in kidney and liver tissues by the methods of Smith *et al.*[27]. GR activity has documented the upsurge in the absorbance in the existence of oxidized glutathione (GSSG) and DTNB (5, 5-dithiobis-2-nitro benzoic acid). The mixture of reactions composed of 500 µL of 0.2 M potassium phosphate buffer (pH 7.5) having 0.1 mM EDTA, 250 µL of 3 mM DTNB, 50 µL of 2 mM NADPH, 50 µL of enzyme extract and 1450 µL final volume of distilled water. By adding 50 µL of 2 mM GSSG, the response was initialized. At 25°C, the raise in absorbance at wavelength 412 nm was observed by a spectrophotometer (Alpha-1502 UV/Visible Spectrophotometer, Thomas Scientific Company, USA) over 5 min. GR activity has been described as a unit g^{-1} tissue.

Glutathione peroxidase activity: Glutathione Peroxidase (GPx) activity was calculated by the methods of Yoshida *et al.*[28]. About 0.1 mL of 1 mM EDTA, 0.1 mL of 1 mM sodium azide, 0.01 mL of 1 mM H_2O_2, 1.49 mL phosphate buffer (0.1 M, pH 7.4), 0.1 mL of 1 mM GSH (reduced glutathione), 0.1 mL of 0.02 mM NADPH and 0.1 mL PMS were mixed together. NADPH oxidation was valued at wavelength 340 nm by a UV Ultrospec 1000 spectrophotometer (Biotech Photometer from Labexchange Company, Burladingen, Germany). The enzyme activity was calculated as nmoles of NADPH oxidized min^{-1} mg^{-1} protein.

Estimation of acetylcholinesterase levels: Acetylcholinesterase (AChE) activity assay was done in brain tissues using Ellman *et al.*[29] methods. Briefly, 330 µL of phosphate buffer (0.1 M, pH 7.8), 20 µL of DTNB (0.0076 M) as a chromogenic factor and 20 µL of supernatant were loaded into a 96-well microplate. AChE activity estimation was started by an addendum of 10 µL acetylthiocholine iodide solution (0.076 M). Substrate hydrolysis was estimated by two controls, one tube without acetylthiocholine and another blank without the sample. Absorption of the 2-nitro-5-thiobenzoate anion, produced from the reaction was estimated at 405 nm every 60 sec for 10 min at 25°C by Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer (USA). In supernatant, the enzyme activity was stated as nmol per mg protein.

Protein estimation: Protein estimation of each sample of liver, kidney, brain PMS and blood was done by the Lowry method[30]. Bovine serum albumin (6.0 g dL^{-1}) was used as standard and protein concentration in each sample was measured at 750 nm by a spectrophotometer.

Determination of liver and kidney function markers: For evaluation of liver function, the concentrations of γ-glutamyl transferase (GGT), Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), bilirubin and albumin were analyzed using standard kits accepted to the manufacturer's guidelines by the methods of Szasz[31], Moss[32], Bergmeyer and Bernt[33], Gambino[34] and Doumas *et al.*[35], respectively. Serum urea and creatinine were analyzed for assessing kidney function using commercially available kits according to Wybenga *et al.*[36] and Slot[37] methods, respectively.

Statistical analysis: Using the Statistical Program for Social Sciences software (SPSS) 20, all the data obtained were subjected to statistical analysis. All the study results have been displayed as Mean±SE. Using One Way Analysis of Variance (ANOVA), the value of $p<0.05$ was considered as a criterion for statistical significance.

RESULTS

Effect of kiwi extracts on some haematological parameters: The data in Table 1 shows that administration of lead caused a significant surge in WBC 3528.50±80.36 $\times 10^9$ L^{-1} by 28.17% when compared to the normal control group 2534.23±85.42$\times 10^9$ L^{-1} ($p<0.05$). While the PCV level of the lead-induced group (28.75±1.94%) was significantly ($p<0.05$) lower than that of the control (41.48±1.79%) and both concentration of kiwi extract treated groups (36.04±0.31 and 40.17±2.03%, respectively). On the contrary, the RBC of the lead-induced group was 3.31±1.62×1012 L^{-1} that significantly ($p<0.05$) lower than the control (3.88±1.79×1012 L^{-1}). However, lead-induced groups treated with two doses of kiwi extract (3.56±1.24, 3.86±1.58×1012 L^{-1}, respectively) had a significantly higher RBC than the lead-induced group ($p<0.05$).

Similarly, the hemoglobin level of the lead-induced group was 9.02±0.67 g dL^{-1} that significantly ($p<0.05$) lower than that of the control (14.12±0.85 g dL^{-1}) and both concentration of kiwi extract treated groups (11.84±0.31 and 13.95±0.64 g dL^{-1}, respectively). In the same way, the percentage volume of packed cells (PCV) of the lead-induced group was 28.75±1.94% that significantly ($p<0.05$) lower than the control (41.48±1.79%). However, lead-induced groups treated with two doses of kiwi extract 36.04±0.31, 40.17±2.03%, respectively) had a significantly higher RBC than the lead-induced group ($p<0.05$).

Effect of kiwi extracts on delta-aminolevulinic acid dehydratase (ALAD) activity: The results on the blood delta-ALAD activity are shown in Fig. 1. There was a significant ($p<0.05$) decrease in the delta- ALAD activity to 0.267±0.121 nanomol porphobilinogen (PBB) formed h^{-1} mL^{-1} in the lead-exposed rats when compared to normal control (0.452±0.201 nanomol PBB formed h^{-1} mL^{-1}). Treatment with kiwi extract (group 3 and group 4) restored the activity of the enzyme (0.446±0.190 and 0.46±0.211 nanomol PBB formed h^{-1} mL^{-1}, respectively) when compared to the lead-exposed group ($p<0.05$).

Effect of kiwi extracts on lipid peroxidation (LPO) in different tissues: A noteworthy upturn ($p<0.05$) in the LPO levels was detected in all of the hepatic, renal and brain tissues of the lead-intoxicated group (17.36±3.15, 12.56±2.64 and 20.31±3.49 nmol MDA mg^{-1} protein of tissue, respectively) compared to the control group (5.86±2.14, 3.97±1.84 and 7.28±2.32 nmol MDA mg^{-1} protein of tissue, respectively) as represented in Fig. 2. Moreover, kiwi extracts treatment indicated a significant drop in the hepatic, renal and brain LPO levels compared to the lead-intoxicated group. The lead-intoxicated rats treated with the kiwi extract (500 mg kg^{-1} b.wt.) showed a decreasing in the hepatic, renal and brain LPO levels (6.23±2.05, 4.02±1.94 and 8.03±2.37 nmol MDA mg^{-1} protein of tissue, respectively) less than rats treated with 250 mg kiwi extract kg^{-1} b.wt. (9.28±2.24, 7.26±2.51 and 11.45±3.02 nmol MDA mg^{-1} protein of tissue, respectively) and also displayed no significant difference with the control group. However,

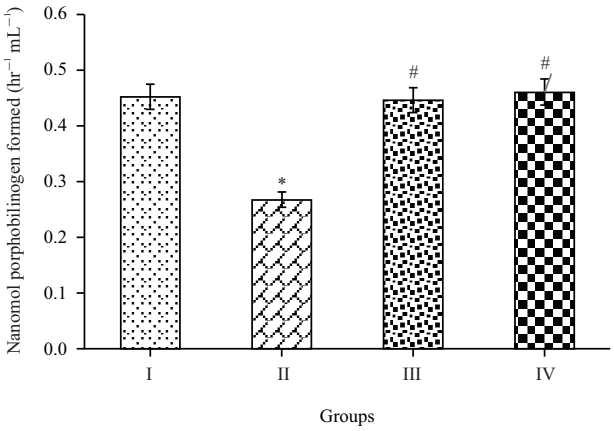

Fig. 1: Delta-aminolevulinic acid dehydratase (ALAD) activity in the blood of all groups

ALAD levels were expressed as nanomol of porphobilinogen (PBB) formed hr^{-1} mL^{-1}. Values are expressed in Mean±SD, each group contains 6 animals (n = 6). *Significant with a normal control group and #Significant with the lead group, ($p<0.05$). Where: Group I: Control group, Group II: Lead-intoxicated group. Group III: Lead-intoxicated treated with kiwi extract (250 mg kg^{-1} b.wt.) group, Group IV: Lead-intoxicated treated with kiwi extract (500 mg kg^{-1} b.wt.) group

Table 1: Effect of kiwi extracts on some hematological parameters

Animal groups	WBC ($\times 10^9$ L^{-1})	RBC ($\times 10^{12}$ L^{-1})	Hemoglobin (g dL^{-1})	PCV (%)
I	2534.23±85.42	3.88±1.79	14.12±0.85	41.48±1.79
II	3528.50±80.36*	3.31±1.62*	9.02±0.67*	28.75±1.94*
III	2869.23±63.41*#	3.56±1.24*#	11.84±0.31*#	36.04±0.31*#
IV	2509.72±74.92#	3.86±1.58#	13.95±0.64#	40.17±2.03#

Values are expressed in Mean±SD, Each group contains 6 animals (n = 6), *Significant with a normal control group and #Significant with the lead group, ($p<0.05$), where: Group I: Control group, Group II: Lead-intoxicated group, Group III: Lead-intoxicated treated with kiwi extract (250 mg kg^{-1} b.wt.) group, Group IV: Lead-intoxicated treated with kiwi extract (500 mg kg^{-1} b.wt.) group, WBC: White blood cell, RBC: Red blood cell, PCV: Percentage volume of packed cells

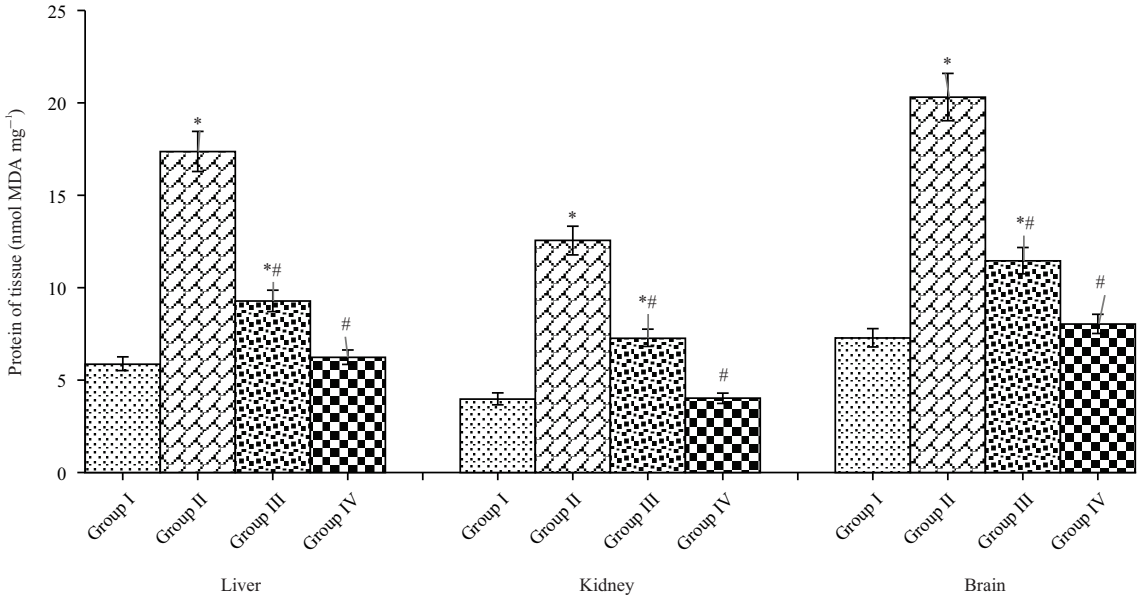

Fig. 2: Effect of kiwi extracts on lipid peroxidation (LPO) in different tissues

LPO levels were expressed as nmol MDA mg^{-1} protein of tissue. Values are expressed in Mean±SD, each group contains 6 animals (n = 6). *Significant with a normal control group and #Significant with the lead group ($p<0.05$). Where: Group I: Control group, Group II: Lead-intoxicated group, Group III: Lead-intoxicated treated with kiwi extract (250 mg kg^{-1} b.wt.) group, Group IV: Lead-intoxicated treated with kiwi extract (500 mg kg^{-1} b.wt.) group

Table 2: Effect of kiwi extracts on anti-oxidative enzyme profile in hepatic tissue of all experimental groups

Animal groups	SOD (units mg^{-1} protein)	CAT (nmol of H_2O_2 released min^{-1} mg^{-1} protein)	Gpx (µ moles of GSH oxidized min^{-1} mg^{-1} protein)	GR (µg GSSG utilized min^{-1} mg^{-1} protein)
I	7.87±0.67	30.47±1.48	320.29±3.45	300.53±5.54
II	4.92±0.52*	10.22±1.39*	167.25±5.86*	162.47±4.85*
III	6.14±0.61*#	25.48±1.26*#	285.67±3.69*#	240.53±4.94*#
IV	7.29±0.58#	28.67±1.64#	316.82±4.17#	295.32±7.26#

Values are expressed in Mean±SD, Each group contains 6 animals (n = 6), *Significant with normal control group, #Significant with the lead group, ($p<0.05$), where: Group I: Control group, Group II: Lead-intoxicated group, Group III: Lead-intoxicated treated with kiwi extract (250 mg kg^{-1} b.wt.) group, Group IV: Lead-intoxicated treated with kiwi extract (500 mg kg^{-1} b.wt.) group, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase, GR: Glutathione reductase

the lead-intoxicated rats treated with the kiwi extract (250 mg kg^{-1} b.wt.) still revealed an elevation in the concentrations of LPO compared to the control group ($p<0.05$).

Effect of kiwi extracts on antioxidative enzyme profile in hepatic tissue: A significant ($p<0.05$) falling in CAT activity was noticed in the lead-intoxicated group (10.22±1.39 nmol of H_2O_2 released min^{-1} mg^{-1} protein) as to normal control (30.47±1.48 nmol of H_2O_2 released min^{-1} mg^{-1} protein) in liver homogenates. Treatment of lead-intoxicated rats with kiwi extracts at 250 and 500 mg kg^{-1} b.wt. Displayed a significant upsurge in hepatic CAT activity in comparison to the lead-intoxicated group (25.48±1.26 and 28.67±1.64 nmol of H_2O_2 released min^{-1} mg^{-1} protein, respectively) as represented in Table 2.

Additionally, a substantial ($p<0.05$) dropping in GR activity was noticed in the lead-intoxicated group (162.47±4.85 µg GSSG utilized min^{-1} mg^{-1} protein) as to normal control (300.53±5.54 µg GSSG utilized min^{-1} mg^{-1} protein) in liver homogenates. Kiwi extracts treatment also at both doses revealed a significant upsurge in GR enzyme activity (240.53±4.94 and 295.32±7.26 µg GSSG utilized min^{-1} mg^{-1} protein, respectively) as compared to the lead-intoxicated group in which GR activity was significantly ($p<0.05$) diminished (Table 2).

Also, a significant ($p<0.05$) reducing in GPx activity was noticed in the lead-intoxicated group (167.25±5.86 µ moles of GSH oxidized min^{-1} mg^{-1} protein) as to normal control (320.29±3.45 µ moles of GSH oxidized min^{-1} mg^{-1} protein) in liver homogenates. However, a momentous ($p<0.05$) upturn in the activity of GPx in kiwi extracts treatment at both doses (285.67±3.69 and 316.82±4.17 µ moles of GSH oxidized min^{-1} mg^{-1} protein, respectively) when measured to the lead group (Table 2). Moreover, the lead-intoxicated group displayed a substantial diminution in the activity of SOD

Table 3: Effect of kiwi extracts on anti-oxidative enzyme profile in renal tissue of all experimental groups

Animal groups	SOD (units mg^{-1} protein)	CAT (nmol of H_2O_2 released min^{-1} mg^{-1} protein)	Gpx (µ moles of GSH oxidized min^{-1} mg^{-1} protein)	GR (µg GSSG utilized min^{-1} mg^{-1} protein)
I	5.64±0.74	38.42±1.96	215.24±4.82	201.26±5.37
II	2.81±0.63*	16.74±2.53*	97.63±5.74*	83.28±4.82*
III	3.96±0.94*#	28.36±1.68*#	189.23±4.61*#	181.27±3.41*#
IV	4.91±0.85#	35.87±2.38#	212.95±5.67#	195.78±4.92#

Values are expressed in Mean±SD, Each group contains 6 animals (n = 6), *Significant with a normal control group and #Significant with the lead group, ($p<0.05$), where: Group I: Control group, Group II: Lead-intoxicated group, Group III: Lead-intoxicated treated with kiwi extract (250 mg kg^{-1} b.wt.) group, Group IV: Lead-intoxicated treated with kiwi extract (500 mg kg^{-1} b.wt.) group, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase, GR: Glutathione reductase

Table 4: Effect of kiwi extracts on anti-oxidative enzyme profile in brain tissue of all experimental groups

Animal groups	SOD (units mg^{-1} protein)	CAT (nmol of H_2O_2 released min^{-1} mg^{-1} protein)	Gpx (µ moles of GSH oxidized min^{-1} mg^{-1} protein)	GR (µg GSSG utilized min^{-1} mg^{-1} protein)
I	39.87±3.68	21.51±1.58	50.71±2.63	42.26±3.27
II	11.79±2.71*	8.05±1.93*	17.05±2.24*	16.08±2.76*
III	23.11±3.57*#	16.26±1.72*#	39.83±3.11*#	27.06±3.23*#
IV	34.28±4.76#	18.93±2.01#	47.15±2.57#	38.46±3.09#

Values are expressed in Mean±SD, Each group contains 6 animals (n = 6), *Significant with a normal control group and #Significant with the lead group, ($p<0.05$), where: Group I: Control group, Group II: Lead-intoxicated group, Group III: Lead-intoxicated treated with kiwi extract (250 mg kg^{-1} b.wt.) group, Group IV: Lead-intoxicated treated with kiwi extract (500 mg kg^{-1} b.wt.) group, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase, GR: Glutathione reductase

(4.92±0.52 units mg^{-1} protein) in comparison with the control animals (7.87±0.67 units mg^{-1} protein) in liver tissue homogenates ($p<0.05$). But a remarkable ($p<0.05$) increase in SOD activity was observed in groups treated with kiwi extracts (6.14±0.61 and 7.29±0.58 units mg^{-1} protein, respectively) when compared to the lead intoxicated group (Table 2).

Effect of kiwi extracts on antioxidative enzyme profile in renal tissue: The result of Table 3 shows the levels of SOD, CAT, GPx and GR in the kidney tissue for the four experimental groups. The quantitative data in the table displayed a substantial ($p<0.05$) drop in the SOD, CAT, GPx and GR activities in the lead-intoxicated group (2.81±0.63 units mg^{-1} protein, 16.74±2.53 nmol of H_2O_2 released min^{-1} mg^{-1} protein, 97.63±5.74 µ moles of GSH oxidized min^{-1} mg^{-1} protein and 83.28±4.82 µg GSSG utilized min^{-1} mg^{-1} protein, respectively) when compared with their respective control group (5.64±0.74 units mg^{-1} protein, 38.42±1.96 nmol of H_2O_2 released min^{-1} mg^{-1} protein, 215.24±4.82 µ moles of GSH oxidized min^{-1} mg^{-1} protein and 201.26±5.37 µg GSSG utilized min^{-1} mg^{-1} protein, respectively).

However, the lead-intoxicated treated with kiwi extract (250 mg kg^{-1} b.wt.) group showed an upturn in the SOD, CAT, GPx and GR activities values in the renal tissue (3.96±0.94 units mg^{-1} protein, 28.36±1.68 nmol of H_2O_2 released min^{-1} mg^{-1} protein, 189.23±4.61 µ moles of GSH oxidized min^{-1} mg^{-1} protein and 181.27±3.41 µg GSSG utilized min^{-1} mg^{-1} protein, respectively) when measured to a lead-intoxicated group and the antioxidative enzyme values still lower than the control group. While the lead-intoxicated treated with kiwi extract (500 mg kg^{-1} b.wt.) group showed better recovery tissue (4.91±0.85 units mg^{-1} protein, 35.87±2.38 nmol of H_2O_2 released min^{-1} mg^{-1} protein, 212.95±5.67 µ moles of GSH oxidized min^{-1} mg^{-1} protein and 195.78±4.92 µg GSSG utilized min^{-1} mg^{-1} protein, respectively) than animals treated with 250 mg kg^{-1} b.wt., of kiwi extract in the renal tissue. The antioxidative enzyme values in group 4 (500 mg kg^{-1} b.wt., of kiwi extract) were almost restored to control levels, showing maximum protection (Table 3).

Effect of kiwi extracts on antioxidative enzyme profile in brain tissue: The present study displays that lead-intoxicated treatment causes obvious declines in enzymatic (SOD, CAT, GPx and GR) antioxidant molecules when compares with the control group ($p<0.05$) in brain tissue as represented in Table 4. A significant ($p<0.05$) descending in CAT activity was noticed in the lead-intoxicated group (8.05±1.93 nmol of H_2O_2 released min^{-1} mg^{-1} protein) as to normal control (21.51±1.58 nmol of H_2O_2 released min^{-1} mg^{-1} protein) in brain homogenates. Treatment of lead-intoxicated rats with kiwi extracts at (250 and 500 mg kg^{-1} b.wt.) protruded out a significant improvement in brain CAT activity in comparison to the lead-intoxicated group (16.26±1.72 and 18.93±2.01 nmol of H_2O_2 released min^{-1} mg^{-1} protein, respectively).

Moreover, a considerable ($p<0.05$) reducing in GR activity was detected in the lead-intoxicated group (16.08±2.76 µg GSSG utilized min^{-1} mg^{-1} protein) compared to normal control (42.26±3.27 µg GSSG utilized min^{-1} mg^{-1} protein) in brain homogenates. Also, both doses of kiwi extracts treatment revealed a significant improvement in GR enzyme activity (27.06±3.23 and 38.46±3.09 µg GSSG utilized min^{-1} mg^{-1} protein, respectively) as compared to the lead-intoxicated group (Table 4).

Table 5: Effect of kiwi extracts on liver function markers

Animal groups	GGT (U L^{-1})	ALT (U L^{-1})	AST (U L^{-1})	ALP (IU L^{-1})	Albumin (g dL^{-1})	Serum protein (g dL^{-1})
I	147.87±4.68	14.56±4.46	32.77±4.91	76.72±3.65	4.08±0.26	7.95±0.68
II	361.79±5.61*	89.73±3.72*	123.19±4.23*	236.63±4.91*	2.26±0.34*	4.07±0.71*
III	203.21±4.57*#	48.26±4.21*#	56.38±3.37*#	115.08±3.47*#	3.15±0.42*#	6.51±0.38*#
IV	142.28±4.36#	11.08±4.34#	28.02±4.38#	73.71±4.12#	4.01±0.19#	7.87±0.57#

Values are expressed in Mean±SD, Each group contains 6 animals (n = 6), *Significant with a normal control group and #Significant with the lead group, ($p<0.05$), where: Group I: Control group, Group II: Lead-intoxicated group, Group III: Lead-intoxicated treated with kiwi extract (250 mg kg^{-1} b.wt.) group, Group IV: Lead-intoxicated treated with kiwi extract (500 mg kg^{-1} b.wt.) group, GGT: γ-glutamyl transferase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase

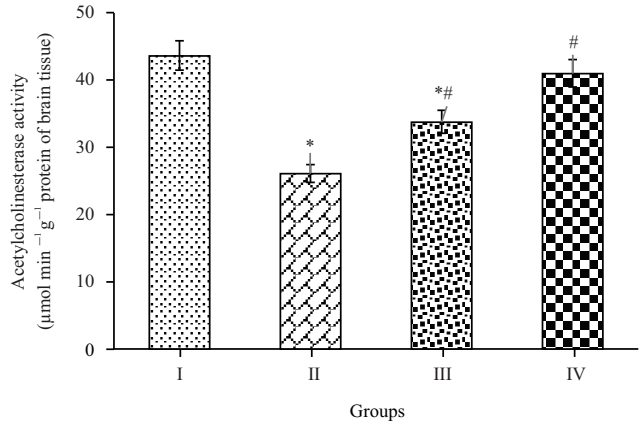

Fig. 3: Effect of kiwi extracts on acetylcholinesterase (AChE) activity in brain tissue

AChE activity was expressed as a μmol^{-1} min^{-1} g^{-1} protein of brain tissue. Values are expressed in Mean±SD, each group contains 6 animals (n = 6). *Significant with a normal control group and #Significant with the lead group ($p<0.05$). Where: Group I: Control group, Group II: Lead-intoxicated group, Group III: Lead-intoxicated treated with kiwi extract (250 mg kg^{-1} b.wt.) group, Group IV: Lead-intoxicated treated with kiwi extract (500 mg kg^{-1} b.wt.) group

Similarly, a momentous ($p<0.05$) diminishing in GPx activity was observed in the lead-intoxicated group (17.05±2.24 μ moles of GSH oxidized min^{-1} mg^{-1} protein) as to normal control (50.71±2.63 μ moles of GSH oxidized min^{-1} mg^{-1} protein) in liver homogenates. Conversely, a significant ($p<0.05$) upturn in the activity of GPx in kiwi extracts treatment at both doses (39.83±3.11 and 47.15±2.57 μ moles of GSH oxidized min^{-1} mg^{-1} protein, respectively) when measured to the lead group (Table 4). Furthermore, the lead-intoxicated group proved a significant reduction in the activity of SOD (11.79±2.71 units mg^{-1} protein) in comparison with the control animals (39.87±3.68 units mg^{-1} protein) in brain tissue homogenates ($p<0.05$). While lead-intoxicated rats treated with two doses of kiwi extracts significantly improved the activity of SOD (23.11±3.57 and 34.28±4.76 units mg^{-1} protein, respectively) when compared to the lead intoxicated group ($p<0.05$) in a dose-dependent manner.

Effect of kiwi extracts on acetylcholinesterase (AChE) activity in brain tissue: Lead administration highly affected brain function; significantly dropped AChE activity was demonstrated in the brain from the lead-intoxicated group (26.09±3.08 μmol min^{-1} g^{-1} tissue) in comparison with the normal control group (43.61±3.56 μmol min^{-1} g^{-1} tissue) (Fig. 3).

Treatment of lead-intoxicated rats with kiwi extracts at both doses (250 and 500 mg kg^{-1} b.wt.) induced a significant increase in neural AChE activity to 33.74±2.76 and 40.98±2.87 μmol min^{-1} g^{-1} tissue, respectively (Fig. 3).

Effect of kiwi extracts on liver function markers: The effect on liver function parameters is shown in Table 5. Liver-specific enzymes viz GGT, ALT, AST and ALP showed a significantly ($p<0.05$) increased activity in the lead-intoxicated group (361.79±5.61, 89.73±3.72, 123.19±4.23 and 236.63±4.91, respectively) compared to the control group (147.87±4.68, 14.56±4.46, 32.77±4.91 and 76.72±3.65 U L^{-1}, respectively). However, kiwi extracts significantly ($p<0.05$) decreased GGT, ALT, AST and ALP levels in treatment groups compared to the lead intoxicated group.

Additionally, the serum concentrations of total protein and albumin dwindled in the animals treated with lead acetate only (4.07±0.71 and 2.26±0.34 g dL^{-1}, respectively) compared to control rats (7.95±0.68 and 4.08±0.26 g dL^{-1}, respectively). Moreover, kiwi extracts increased the levels of serum total protein and albumin significantly ($p<0.05$) in animals treated with kiwi extracts at 250 and 500 mg kg^{-1} b.wt., when compared to the lead-intoxicated group.

Effect of kiwi extracts on kidney function markers: The result of Fig. 4 illustrates the influence of kiwi extracts on parameters of renal function. There was a substantial upsurge ($p<0.05$) in serum creatinine and BUN (Blood Urea Nitrogen) concentration in the group intoxicated with lead (6.97±0.45 and 27.56±0.62 mg dL^{-1}, respectively) compared to the normal control group (1.08±0.38 and 9.22±0.41 mg dL^{-1}, respectively). However, kiwi extracts significantly reinstated

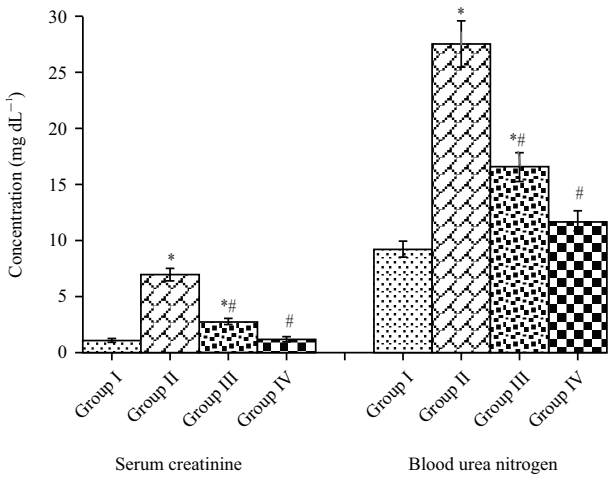

Fig. 4: Effect of kiwi extracts on serum creatinine and BUN in lead-treated rats

Values are expressed in Mean±SD, each group contains 6 animals (n = 6). *Significant with a normal control group and #Significant with the lead group ($p<0.05$). Where: Group I: Control group, Group II: Lead-intoxicated group, Group III: Lead-intoxicated treated with kiwi extract (250 mg kg^{-1} b.wt.) group, Group IV: Lead-intoxicated treated with kiwi extract (500 mg kg^{-1} b.wt.) group

the high level of serum creatinine and BUN. A significant diminution in serum creatinine and BUN concentration was noticed in groups treated with both doses of kiwi extracts (250 and 500 mg kg^{-1} b.wt.) as compared to the lead-intoxicated group (Fig. 4).

DISCUSSION

The current investigation was aimed to test the efficiency of *Actinidia deliciosa* (kiwifruit) ethanolic extract against lead-induced oxidative stress in extremely important organs such as the brain, liver and kidney of Wistar Albino male rats. The tissue accumulation of lead in the brain, liver and kidney caused an upsurge in oxidative stress liberating more ROS which in sequence resulted in the generation of lipid peroxidation and a decline in the delta-ALAD activity in the blood is an indicator of lead toxicity[38].

Dwindled erythrocytes count, haemoglobin and PCV values in rats were drastically reduced by lead exposure[39]. Also, Pratush *et al.*[40] reported that low-level exposure to inorganic lead in the workplace may have adverse effects on definite types of blood cells, decrease haemoglobin content and cause decreased erythrocytes lifespan and function. Erythrocytes also have a high lead affinity to fix ninety-nine percent of the lead in the bloodstream[9]. The lead devastating influence on the membrane of the erythrocytes may initiate hemolysis or a substantial decline of blood iron, which appears to be the explanation for decreasing PCV and Hb concentrations[9]. As a result of lead toxicity, this may be due to the massive inflammatory reaction upon which WBCs are a significant component. This overall result is aligned with those outlined by Ugwuja *et al.*[41].

Within the current study, the consequence of lead on erythrocyte and its inhibition on the heme biosynthetic process may correlate to the significantly low levels of PCV, RBC and haemoglobin in the lead-induced group compared with the other groups. Kiwi extract was proved to reverse the negative effects of lead toxicity. Being existed in the kiwi extract, caffeic acid proves the highly noteworthy effect of the kiwi extract in improved the haematological parameters. Colina *et al.*[42] confirmed that caffeic acid has a protective effect in vitro study on human RBCs.

Moreover, Obafemi *et al.*[9] mentioned that lead owns a major influence on the pathway of heme synthesis by downregulating three main enzymes that participated in biosynthesis, in particular delta-Amino Levulinic Acid Dehydratase (ALAD). ALAD contains a sulfhydryl moiety that makes it vulnerable to an assault by the lead. Lead stops ALAD activity and the suppression of ALAD leads to a strong pro-oxidant overproduction of 5-aminolevulinic acid (ALA). Superoxide radical and hydrogen peroxide are produced by ALA levels, react with oxyhemoglobin, triggering its oxidation[43]. Ascorbic acid in kiwi extract has an antioxidative effect on lead toxicity by reviving ALAD activity[44]. Ademuyiwa *et al.*[44] pronounced that a daily dose of 500 mg ascorbic acid for two weeks inverted the upshot of the lead on suppression of ALAD activity. In our experiment, we also noticed that kiwi extract was able to improve ALAD activity significantly in a dose-dependent manner. Also, the decline in ALA production through its strong anti-oxidant properties, hence declining the oxidative damage in the erythrocyte.

Lead promotes oxidative damage through increasing substantial elevation in MDA levels which signpost an increased cellular LPO and propagation of ROS[9]. In the current study, lead acetate triggered a considerable rise in LPO clarified by a risky elevation MDA content in kidney, liver and brain homogenates, which is consistent with previous researches[5,9]. However, the kiwi extract significantly diminished LPO in the liver, kidney and brain organs which may be owing to the components of the high antioxidant in the kiwi ethanolic extract that has free radical scavenging properties as syringic acid[45], cinnamic acid[46], ellagic acid[47] and caffeic acid[48].

The endogenous antioxidant enzymes system combat the free radicals produced through oxidative stress. In the current investigation, the activities of SOD, CAT, GR and GPx were declined by the influence of lead toxicity in the hepatic, brain

and kidney tissues, consequently making tissues more feasible to oxidative stress as spotlighted in many studies[5,9]. Lead has a strong affinity to bind with sulfhydryl functional groups of these enzymes disrupting their activities[7,9].

Our study found that treatment with kiwi extract restored the activities of the anti-oxidative enzymes which were diminished by lead toxicity through emphasizing the scavenging properties of the kiwi extract against ROS. Ellagic acid as a component of kiwi extract[49] was known to counteract DNA damage prompted by the high oxidative due to lead poisoning. A recent study done by Amirahmadi *et al.*[50] confirmed that ellagic acid has antioxidant properties on the neurotoxicity of lead-exposed rats by an improvement of superoxide dismutase, catalase and glutathione peroxidase activities and inhibition on lipid peroxidation.

The primary focus for lead toxicity is the nervous system. Several studies documented that lead toxicity can immediately disturb the activity of acetylcholinesterase (AChE) activity[51]. Also, Liu *et al.*[52] stated that lead-induced neurotoxicity in rats diminishing the AChE activity. Kiwi extract is a powerful antioxidant that can reduce neurotoxicity caused by lead intoxication by enhancing the AChE activity. The current results are in agreement with Akomolafe *et al.*[53], who revealed that caffeic acid exhibited improved AChE activity.

A substantial rise in serum urea and creatinine levels had been seen in lead-administered rats to the extent that kidney function parameters are concerned, as was also observed in previous studies[7,54]. Oxidative stress contributes to accelerated protein degradation, which raises serum ammonia levels and urea concentrations[7]. Induced free radical damage to cells leading to disruption of the epithelium of the renal cell brush border that makes them impermeable to urea and creatinine[54]. Thus, the elevation in the kidney markers owing to minimize or no tubular absorption of creatinine and urea into the renal tubules[7].

In the present study, treatment with kiwi extract considerably declined the levels of creatinine and BUN. This may be owing to the powerful antioxidant potential of kiwi extract, which counters the oxidative insults generated in the kidneys as an effect of lead acetate-induced toxicity, indicating the nephroprotective potential of kiwi extract in lead intoxication. A previous study by Mahmoud[55] also reported that *Actinidia deliciosa* improves nephrotoxicity caused by gentamicin in albino rats and increases kidney function by significantly reducing urea and creatinine levels.

In the present study, lead toxicity caused diminishing in the hepatic function markers as an indicator of liver injuries. Lead toxicity caused an enlarged concentration of GGT, ALP, AST and ALT in the serum of rats as also described in other studies[7]. Additionally, in the current study, there was a decline in total protein and albumin levels, which is consistent with other studies that documented a reducing in total protein and albumin in rats in response to dose-dependent lead intoxication[6,7]. In our study, treatment with kiwi extract effectively reversed the alterations triggered by lead acetate by lessening GGT, ALT, AST and ALP and improving serum albumin and total protein, in line with previous studies[6,7].

Finally, the current study confirms that kiwifruit (*Actinidia deliciosa*) gives defence against lead-induced oxidative stress in different tissues as the blood, liver, kidney and brain in Wistar Albino male rats. To appreciate the protective efficiency of these agents in diminishing overall lead-induced toxicity, especially haematological toxicity, hepatotoxicity, nephrotoxicity and neuron toxicity, experimental antioxidant therapy in laboratory animals is being explored. If the experimental models have positive outcomes, it might be worth further study to use them in integration with the existing clinical interventions approved.

CONCLUSION

Kiwifruit (*Actinidia deliciosa*) markedly prevented lead-induced oxidative stress in blood, liver, kidneys and brain through increasing the enzymes of the antioxidant system and diminishing lipid peroxidation level. Also, kiwifruit has a potent effect on increasing acetylcholine esterase activity in the brain and also, improved delta-aminolevulinic acid dehydratase activity in the blood. Finally, the exact mechanism and various molecules participating in the defensive effects of *Actinidia deliciosa* against lead toxicity need to be studied deeply.

SIGNIFICANCE STATEMENT

This study proved that kiwifruit (*Actinidia deliciosa*) can be valuable as an antioxidant agent at the recommended dose (500 mg kg^{-1} b.wt.) against lead-induced oxidative stress in the blood, liver, kidneys and brain through improving the enzymes of the antioxidant system and diminishing lipid peroxidation level. As a consequence, this study will open a modern approach for the researchers to discover a safer and more efficient treatment for lead toxicity.

REFERENCES

1. Highab, S.M., M. Aliyu and B.Y. Muhammad, 2017. Effect of resveratrol on liver histopathology of lead-induced toxicity in wistar rats. J. Pharm. Res. Int., 20: 1-8.

2. Dökmeci, A.H., 2020. Environmental Impacts of Heavy Metals and their Bioremediation. In: Heavy Metals-Their Environmental Impacts and Mitigation, Nazal, M. (Ed.)., IntechOpen, New York.
3. Kushwaha, A., N. Hans, S. Kumar and R. Rani, 2018. A critical review on speciation, mobilization and toxicity of lead in soil-microbe-plant system and bioremediation strategies. Ecotoxicol. Environ. Saf., 147: 1035-1045.
4. Assi, M.A., M.N.M. Hezmee, A.W. Haron, M.Y.M. Sabri and M.A. Rajion, 2016. The detrimental effects of lead on human and animal health. Vet. World, 9: 660-671.
5. Kumar, V.M., A.K. Henley, C.J. Nelson, O. Indumati, Y.P. Rao, S. Rajanna and B. Rajanna, 2017. Protective effect of *Allium sativum* (garlic) aqueous extract against lead-induced oxidative stress in the rat brain, liver and kidney. Environ. Sci. Pollut. Res., 24: 1544-1552.
6. Chen, C., B. Lin, S. Qi, J. He and H. Zheng, 2019. Protective effects of salidroside on lead acetate-induced oxidative stress and hepatotoxicity in Sprague-Dawley rats. Biol. Trace Elem. Res., 191: 426-434.
7. Amin, I., I. Hussain, M.U. Rehman, B.A. Mir and S.A. Ganaie *et al*., 2020. Zingerone prevents lead induced toxicity in liver and kidney tissues by regulating the oxidative damage in Wistar rats. J. Food Biochem., 10.1111/jfbc.13241.
8. Sarker, S., D. Vashistha, M.S. Sarker and A. Sarkar, 2018. DNA damage in marine rock oyster (*Saccostrea cucullata*) exposed to environmentally available PAHs and heavy metals along the Arabian Sea coast. Ecotoxicol. Environ. Saf., 151: 132-143.
9. Obafemi, T.O., A. Onasanya, A. Adeoye, J.A. Falode and D.J. Daniel *et al*., 2019. Protective effect of methanolic and flavonoid-rich leaf extracts of *Synsepalum dulcificum* (Danielli) on lead-acetate-induced toxicity in Wistar albino rats. J. Applied Pharm. Sci., 9: 065-072.
10. Albarakati, A.J.A., R.S. Baty, A.M. Aljoudi, O.A. Habotta, E.K. Elmahallawy, R.B. Kassab and A.E.A. Moneim, 2020. Luteolin protects against lead acetate-induced nephrotoxicity through antioxidant, anti-inflammatory, anti-apoptotic and Nrf2/HO-1 signaling pathways. Mol. Biol. Rep., 47: 2591-2603.
11. Laamech, J., J. El-Hilaly, H. Fetoui, Y. Chtourou, H. Gouitaa, A. Tahraoui and B. Lyoussi, 2017. *Berberis vulgaris* L. effects on oxidative stress and liver injury in lead-intoxicated mice. J. Complementary Integr. Med., Vol. 14. 10.1515/jcim-2015-0079.
12. Dezfuly, Z.T., A. Aramoon, M. Alishahi, M. Halimi and R. Rahnama, 2020. The effects of lead toxicity on the hematological parameters common carp (*Cyprinus carpio*) at varying salinity levels. Iran. J. Toxicol., 14: 1-8.
13. Pérez-Burillo, S., M.J. Oliveras, J. Quesada, J.A. Rufián-Henares and S. Pastoriza, 2018. Relationship between composition and bioactivity of persimmon and kiwifruit. Food Res. Int., 105: 461-472.
14. Wang, Y., L. Li, H. Liu, T. Zhao, C. Meng, Z. Liu and X. Liu, 2018. Bioactive compounds and *in vitro* antioxidant activities of peel, flesh and seed powder of kiwi fruit. Int. J. Food Sci. Technol., 53: 2239-2245.
15. Peng, Y., S.B. Cordiner, G.M. Sawyer, T.K. McGhie, R.V. Espley, A.C. Allan and R.D. Hurst, 2020. Kiwifruit with high anthocyanin content modulates NF-κB activation and reduces CCL11 secretion in human alveolar epithelial cells. J. Funct. Foods, Vol. 65. 10.1016/j.jff.2019.103734.
16. Li, H.Y., Q. Yuan, Y.L. Yang, Q.H. Han and J.L. He *et al*., 2018. Phenolic profiles, antioxidant capacities and inhibitory effects on digestive enzymes of different kiwifruits. Molecules, Vol. 23. 10.3390/molecules23112957.
17. Podsedek, A., I. Majewska, M. Redzynia, D. Sosnowska and M. Koziołkiewicz, 2014. *In vitro* inhibitory effect on digestive enzymes and antioxidant potential of commonly consumed fruits. J. Agric. Food Chem., 62: 4610-4617.
18. Zuo, L.L., Z.Y. Wang, Z.L. Fan, S.Q. Tian and J.R. Liu, 2012. Evaluation of antioxidant and antiproliferative properties of three actinidia (*Actinidia kolomikta*, *Actinidia arguta*, *Actinidia chinensis*) extracts *in vitro*. Int. J. Mol. Sci., 13: 5506-5518.
19. Kassab, A.A. and W.M. Elwan, 2020. Kiwifruit extract attenuates tadalafil induced retinal injury in adult male albino rats: A histological and immunohistochemical study. Egypt. J. Histol., 43: 477-495.
20. Alexander, R.R. and J.M. Grifiths, 1993. Haemotocrit In: Basic Biochemical Methods. 2nd Ed., John Willey and Sons, Inc. Publications. New York, pp: 186-187.
21. Kratz, A., K.B. Lewandrowski, A.J. Siegel, K.Y. Chun, J.G. Flood, E.M. Van Cott and E. Lee-Lewandrowski, 2002. Effect of marathon running on hematologic and biochemical laboratory parameters, including cardiac markers. Am. J. Clin. Pathol., 118: 856-863.
22. Bond, M.M. and R.R. Richards-Kortum, 2015. Drop-to-drop variation in the cellular components of fingerprick blood: Implications for point-of-care diagnostic development. Am. J. Clin. Pathol., 144: 885-894.
23. Berlin, A. and K.H. Schaller, 1974. European standardized method for the determination of delta-aminolevulinic acid dehydratase activity in blood. Z. Klin. Chem. Klin. Biochem., 12: 389-390.
24. Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.
25. Marklund, S. and G. Marklund, 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem., 47: 469-474.
26. Aebi, H., 1984. Catalase *in vitro*. Meth. Enzymol., 105: 121-126.

27. Smith, I.K., T.L. Vierheller and C.A. Thorne, 1988. Assay of glutathione reductase in crude tissue homogenates using 5,5'-dithiobis(2-nitrobenzoic acid). Anal. Biochem., 175: 408-413.
28. Yoshida, S., F. Kumakura, I. Komatsu, K. Arai, Y. Onuma, H. Hojo and M. Iwaoka, 2011. Antioxidative glutathione peroxidase activity of selenoglutathione. Angew. Chem., 123: 2173-2176.
29. Ellman, G.L., K.D. Courtney, V. Andres Jr. and R.M. Featherstone, 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7: 88-95.
30. Waterborg, J.H., 2009. The Lowry Method for Protein Quantitation. In: The Protein Protocols Handbook, Walker, J.M. (Ed.). 3rd Edn., Humana Press Inc., New Jersey, USA., ISBN: 978-1-58829-880-5 pp: 7-10.
31. Szasz, G., 1969. A kinetic photometric method for serum γ-glutamyl transpeptidase. Clin. Chem., 15: 124-136.
32. Moss, D.W., 1987. Diagnostic aspects of alkaline phosphatase and its isoenzymes. Clinical Biochem., 20: 225-230.
33. Bergmeyer H.U. and E. Bernt, 1974. Aminotransferases and related enzymes. In: Methods of Enzymatic Analysis, Bergmeyer, H.U., Academic Press, New York, pp. 735-763.
34. Gambino, S.R., 1967. Direct serum bilirubin and the sulfobromophthalein test in occult liver disease. JAMA, 201: 1047-1049.
35. Doumas, B.T., W.A. Watson and H.G. Biggs, 1997. Albumin standards and the measurement of serum albumin with bromcresol green. Clin. Chim. Acta, 258: 21-30.
36. Wybenga, D.R., J. Di Giorgio and V.J. Pileggi, 1971. Manual and automated methods for urea nitrogen measurement in whole serum. Clin. Chem., 17: 891-895.
37. Slot, C., 1965. Plasma creatinine determination: A new and specific Jaffe reaction method. Scand. J. Lab. Invest., 17: 381-387.
38. Rahman, Z. and V.P. Singh, 2019. The relative impact of toxic heavy metals (THMs) (arsenic (As), cadmium (Cd), chromium (Cr)(VI), mercury (Hg) and lead (Pb)) on the total environment: An overview. Environ. Monit. Assess., Vol. 191. 10.1007/s10661-019-7528-7.
39. Chwalba, A., B. Maksym, M. Dobrakowski, S. Kasperczyk, N. Pawlas, E. Birkner and A. Kasperczyk, 2018. The effect of occupational chronic lead exposure on the complete blood count and the levels of selected hematopoietic cytokines. Toxicol. Appl. Pharmacol., 355: 174-179.
40. Pratush, A., A. Kumar and Z. Hu, 2018. Adverse effect of heavy metals (As, Pb, Hg and Cr) on health and their bioremediation strategies: A review. Int. Microbiol., 21: 97-106.
41. Ugwuja, E.I., N. Vincent, I.C. Ikaraoha and S.R. Ohayi, 2020. Zinc ameliorates lead toxicity by reducing body Pb burden and restoring Pb-induced haematological and biochemical derangements. Toxicol. Res. Appl., Vol. 4. 10.1177/2397847320956562.
42. Colina, J.R., M. Suwalsky, M. Manrique-Moreno, K. Petit, L.F. Aguilar, M. Jemiola-Rzeminska and K. Strzalka, 2019. An *in vitro* study of the protective effect of caffeic acid on human erythrocytes. Arch. Biochem. Biophys., 662: 75-82.
43. Monteiro, H.P., D.S.P. Abdalla, O. Augusto and E.J.H. Bechara, 1989. Free radical generation during δ-aminolevulinic acid autoxidation: Induction by hemoglobin and connections with porphyrinpathies. Arch. Biochem. Biophys., 271: 206-216.
44. Ademuyiwa, O., R.N. Ugbaja, D.A. Ojo, A.O. Owoigbe and S.E. Adeokun, 2005. Reversal of aminolevulinic aciddehydratase (ALAD) inhibition and reduction of erythrocyte protoporphyrin levels in Vitamin C in occupational lead exposure in abeokuta Nigeria. Environ. Toxicol. Pharmacol., 20: 404-411.
45. Vo, Q.V., M.V. Bay, P.C. Nam, D.T. Quang, M. Flavel, N.T. Hoa and A. Mechler, 2020. Theoretical and experimental studies of the antioxidant and antinitrosant activity of syringic acid. J. Org. Chem., 85: 15514-15520.
46. dos Santos, D.M., M.P. Sanches, C.M. Poffo, A.L. Parize, G.J.S. Darelli and V.R. de Lima, 2020. Syringic and cinnamic acids antiradical/antioxidant activities as *R. ferruginea* extract components and membrane physico-chemical influence. J. Mol. Struct., Vol. 1220. 10.1016/j.molstruc.2020.128749.
47. Aslan, A., Y.T. Hussein, O. Gok, S. Beyaz, O. Erman and S. Baspinar, 2020. Ellagic acid ameliorates lung damage in rats via modulating antioxidant activities, inhibitory effects on inflammatory mediators and apoptosis-inducing activities. Environ. Sci. Pollut. Res., 27: 7526-7537.
48. Yilmaz, S., 2019. Effects of dietary caffeic acid supplement on antioxidant, immunological and liver gene expression responses and resistance of Nile tilapia, *Oreochromis niloticus* to *Aeromonas veronii*. Fish Shellfish Immunol., 86: 384-392.
49. Huang, C.H., J.J. Tao, G.L. Liao, M. Xie, X.Y. Qu, L. Chen and X.B. Xu, 2020. Dynamic changes of phenol and antioxidant capacity during fruit development of three *Actinidia* species (kiwifruit). Sci. Hortic., Vol. 273. 10.1016/j.scienta.2020.109571.
50. Amirahmadi, A., J. Babaei, R. Abrishami, M. Goudarzi and M. Kalantar, 2020. The effect of ellagic acid on the neurotoxicity of lead exposed rats. Toxicol. Environ. Health Sci., 10.1007/s13530-020-00073-3.
51. Richetti, S.K., D.B. Rosemberg, J. Ventura-Lima, J.M. Monserrat, M.R. Bogo and C.D.Bonan, 2011. Acetylcholinesterase activity and antioxidant capacity of zebrafish brain is altered by heavy metal exposure. Neurotoxicology, 32: 116-122.

52. Liu, C.M., G.H. Zheng, Q.L. Ming, J.M. Sun and C. Cheng, 2013. Protective effect of puerarin on lead-induced mouse cognitive impairment via altering activities of acetyl cholinesterase, monoamine oxidase and nitric oxide synthase. Environ. Toxicol. Pharmacol., 35: 502-510.
53. Akomolafe, S.F., A.J. Akinyemi, O.B. Ogunsuyi, S.I. Oyeleye, G. Oboh, O.O. Adeoyo and Y.R. Allismith, 2017. Effect of caffeine, caffeic acid and their various combinations on enzymes of cholinergic, monoaminergic and purinergic systems critical to neurodegeneration in rat brain-*in vitro*. NeuroToxicology, 62: 6-13.
54. Mailafiya, M.M., K. Abubakar, S.M. Chiroma, A. Danmaigoro and E.B.A. Rahim *et al*., 2020. Curcumin-loaded cockle shell-derived calcium carbonate nanoparticles: A novel strategy for the treatment of lead-induced hepato-renal toxicity in rats. Saudi J. Bio. Sci., 27: 1538-1552.
55. Mahmoud, Y.I., 2017. Kiwi fruit (*Actinidia deliciosa*) ameliorates gentamicin-induced nephrotoxicity in albino mice via the activation of Nrf2 and the inhibition of NF-κB (Kiwi and gentamicin-induced nephrotoxicity). Biomed. Pharmacother., 94: 206-218.