Original Article

Potential Effectiveness of Flesh-Red Dragon Fruit Juice in The Improvement of Consequence Injury from Lead Acetate-Induced Neurotoxicity in Rats

Aml F. Elgazar[1], Mostafa A. Shalaby[2], Eman S. Ibrahem[3]
1. Department of Nutrition and Food Sciences, Faculty of Home Economics, Helwan University, Egypt.
2. Pharmacology Department, Faculty of Veterinary Medicine, Cairo University, Egypt
3. Food and Feed Regional Center, Agricultural Research Center, Giza, Egypt
Email: dr_aml_fawzy@yahoo.com
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Abstract

Fifty-adult male rats were indiscriminately divided into five groups, of similar number: negative control group (group 1), and the groups from 2 to 5 received orally 100 mg/kg b.wt of Pb once a day for 14 days. Then, group 2 was maintained as an untreated positive control group, while the groups 3, 4 and 5 received daily orally Red Dragon Fruit Juice (RDFJ) at a dose of 10, 20 and 30%, respectively. The results exhibited that the fleshy-fruit was higher in its content of phenolics, followed by flavonoids and anthocyanins. As a comparison to untreated positive control group, treated rats with oral administration of RDFJ have a significant ($P< 0.05$) decrease in serum activity of AST, ALT and ALP enzymes, and the levels of urea nitrogen, uric acid, creatinine and MDA. In addition to the increase in serum activity of catalase (CAT) and GSH-Px enzymes and levels of total antioxidant norepinephrine, dopamine and 5-hydroxytryptamine. Several degradations in cerebral tissue of untreated rats with the brain toxicity were detectable, while an august amendment in the rats treated with the RDFJ, especially with the higher level (30%). Lastly, routine consumption of RDFJ may be profitable to prevent the consequence injury from lead toxicity due to it's a wide range content of flavonids, phenolic and anthocyanins as bioactive compounds.

Keywords: Neurotoxicity**,** Pitaya, Oxidative Stress**,** Lead Poisoning**,** Neurotransmitters-Rats

INTRODUCTION

Lead (Pb) is a highly poisonous heavy metal that is a widespread environmental pollutant and is used in many industries and products as gasoline industries, paint and pigment materials, acid battery, etc. (**Wang *et al.*, 2016**). It can directly or indirectly contaminate foods and also enter the human body out of different channels as the respiratory and/or digestive tracts, skin, and mucous membranes (**Teerasarntipan *et al.*, 2020**).

Oxidative stress, which increases cellular material damage, existence the most likely action mechanism of lead toxicity (**Mitra and Sharma 2019**). Furthermore, chronic exposure to Pb may be related to various diseases such as reproductive troubling, birth defects, lower attention and autism spectrum disorder in children, brain and neural disturbance, high blood pressure, and liver and/or kidney impairment (**Jaishankar *et al.*, 2014**). The nervous system is the most virtually responsive organ to Pb, where, it most affects the peripheral nervous system of adults and the central nervous system of children, especially in the growing stages (**Elrasoul *et al.*, 2020**). The conventional treatment manner of lead toxicity depends on the incorporation of intercalating agents with several vitamins. Even so, the use of these agents for a long duration or at large doses once at a time can cause damage to liver and kidney cells (**Li *et al.*, 2020**).

Dragon fruit is an orbital fruit that has an oval-shaped fruit with different skin and pulp colors as pink skin with red or white pulp, yellow skin with white pulp and red skin with red pulp (**Muniz *et al.*, 2019**). It is a fruit of different tropical cactus species plants of the genus *Hylocereus*, family *Cactaceae*, known as red pitaya or pitahaya, and cultivated worldwide due to its commercial and health benefits (**Mercado-Silva 2018**). Dragon fruit pulp and peels have higher contents of water and fibers and several nutrients including vitamins, minerals, and antioxidants (**Perween *et al.*, 2018**). Further, the Dragon fruit edible pulp and peels are rich in phytochemical compounds and thus have the possibility to

use as herbal medicine or natural colors (**Mahdi *et al.,* 2018**). As much, as flesh and seeds of the fruit have an impressive content of fatty acids such as linoleic, oleic, and palmitic acid (**Jerônimo *et al.,* 2015**).

Many Asian countries are well-known for using dragon fruit in folk medicine in the prevention or treatment of several diseases (**Sofowora *et al.,* 2013**). Additionally, previous researchers reported that Dragon fruit has antidiabetic (**Poolsup *et al.,* 2017**), antimicrobial and antiviral (**Afandi *et al.,* 2017**), anti-obesity and hypolipidaemic activity (**Suastuti *et al.,* 2018**), and anti-inflammatory (**Eldeen *et al.,* 2020**). The potential health effects of dragon fruit may be related to its content of powerful antioxidants such as polyphenol, steroids, anthocyanin, betalains, and triterpenoids (**Guimarães *et al.,* 2017**).

To ameliorate the quality of the patient's life with lead poisoning and minimize the possibility hazard to peoples at high risk for lead exposure, it is insistent to discover new methods for the prevention and/or treatment of lead poisoning depending on natural treatment. Accordingly, the existing study was directed to explore the utilization of Red Dragon Fruit Juice as natural antioxidants source to prevent the **consequence** effect for toxicity of lead acetate (Pb) in the experimental rats

MATERIALS AND METHODS

Dragon Fruit and Preparation of Juice Extract Fresh and healthy Dragon fruit (red skin with red pulp as displayed in Picture 1) was purchased from a local fruit shop in Cairo, Egypt.

The concentrate juice extract was prepared as outlined by **Dhumal *et al.,* (2015)**. In brief steps, fresh Dragon fruit (DF) was carefully scrubbed with flowing water to get free of any dirt or external bodies and peeled to separate the outside skin from the fruit flesh. The separate whole pulp was cut into small pieces and beaten up in a squeezer to get a homogeneous juice. The homogeneous juice (1-L) was concentrated at 40°C in a digital rotary evaporator (model: KA 8031901 RV 10). After that, the concentrated juice extract was taken for the preparation of three different concentrations (10, 20, and 30%), then embarked in separate receptacles and retained in the refrigerator at 5 ºC for use.

Picture (1) Whole dragon fruit

Ingredients and Formulation of Purified Basal Diet (AIN-93M) For the preparation of a purified basal diet, the basic ingredients (casein, fiber, vitamins and mineral mixtures, choline chloride, L-cysteine and Tert-butylhydroquinone) were bought off from El-Gomhoriya Co., for Trad., Drugs and Chem. Egypt. Pure soybean oil was obtained from the Agriculture Research Center, Giza, Egypt. Cornstarch and dextrinized corn starch were obtained from the Egyptian Manufacturing of Starch and Glucose, Co., SAE, Cairo, Egypt. Sucrose (table sugar) was obtained from a local market.

Lead acetate and Kits White crystalline lead acetate powder, diethyl ether alcohol and the other used chemicals were bought from Sigma Chem., Co., Cairo branch, Egypt. While, kits for biochemical tests were bought off from the Gamma Trade Co., for Pharmac., and Chem., Dokki, Cairo - Egypt.

The Valuation of Total Flavonoids, Phenolics, and Anthocyanins Contents in The Red-Flesh Dragon Fruit The total flavonoids content was estimated using colorimetric methods as outlined by **Zhishen *et al.,* (1999)**. Catechin was employed as a standard for the standardization curve and the total flavonoids content of the extract was expressed as mg Catechin equivalents per 100 grams of sample.

The total phenolics content in flesh Dragon fruits was evaluated as mentioned by **Chun *et al.,* (2003)** using Gallic acid as a standard for the standardization curve. The total phenolics content was expressed as mg Gallic acid equivalents/100 grams of sample.

The total anthocyanin content in the Dragon fruit sample was quantified by the pH differential method as mentioned by **Lee *et al.*, (2005).**
Thereafter, all components were mixed together to fulfill the desirable adequate dietary intake for keeping the health state of rats as confirmed by **Reeves *et al.*, (1993)**. Concisely, each 1 kg diet consists of 140g casein (85% protein), 465.70 g cornstarch, 155 g dextrinized cornstarch, 40g soybean oil, 100g sucrose, 50g fiber, 10g vitamin mixture, 35g mineral mixture, 2.5g choline chloride, 1.8 g L-cysteine and 0.008g Tert-butylhydroquinone.

Grouping of Rats and Induction of Brain Toxicity Fifty healthy adults' male Sprague Dawley rats weighing (200 ± 6 g) were obtained from experimental animal's house at Food and Feed Regional Center, Agriculture Research Center, Giza, Egypt and housed at the same lab. Rats were separated into five groups of equal number (n=10 rats). All groups were fed on basal diet for a week as adaptation period. After the acclimatizing period, the first group represented the health control animals and received 2ml of distilled water orally for 2 weeks. The second, third, fourth and fifth groups were given lead acetate at a dose of 30 mg/kg of b. wt oral using gastric tube for two weeks to induce brain toxicity according to the methods of **Sujatha *et al.*, (2011)**. Then, the groups were named in line with the type of treatment as follows:
Group (1) Negative control group was retained as normal rats and given 2ml of distilled water during the experimental period.
Group (2) Positive control group was maintained as untreated rats with lead toxicity and given 2ml of distilled water.
Group (3) The treated lead toxicity group by giving orally the red-flesh Dragon fruit juice (RDFJ) at a dose of 10% of concentrated juice.
Group (4) The treated lead toxicity group by giving orally the RDFJ at a dose of 20% concentrated juice.
Group (5) The treated lead toxicity group by giving orally the RDFJ at a dose of 30% concentrated juice.

Computation the Body Weight Gain, Relative Body Weight Gain and Feed Consumed The alteration in body weight was calculated by weighing the rats at the initial of the experimental period (IBW) and at the end of the experimental period (FBW) (6 weeks). Then, subtracting FBW from IBW, and the result was expressed as the body weight gain (BWG) (BWG= FBW-IBW). Relative body weight gain (RBWG%) was calculated as dividing the weight gained by the weight at the beginning of the experiment multiplied by 100 (RBWG%= BWG/IBW X 100). The overall amount of food consumed (FC) per day for each rat was recorded depending on the daily amount consumed for each group.

Blood Collection and Serum Separation After the sixth week of starting the experiment, rats were prohibited from the diet, except water for about 12 hours. Thereafter, rats were anesthetic with diethyl ether, and by using a 5 cm syringe, the heart was punctured and the blood samples drawn and relocated into a centrifuge tube and left at room temperature until complete coagulation. To get the serum, the coagulated blood samples were centrifuged for 15 mins. at 3000 rpm. Then, the separated pure serum specimens were taken by an automatic pipette, drawn into the clean covered Eppendorf pipe, and kept at -20°C in a deep freeze until used for biochemical inspection.

Biochemical Tests
Serum AST, ALT and ALP enzymes were evaluated using Sandwich-Ab-ELISA-Elabscience Kits (E-EL-R0076, E-EL-R4325 and E-EL-R1109, respectively, in line with the mentioned instructions by **Young (2000)** for AST and ALT assay and **Friedman and Young, (1997)** for ALP assay.
Colorimetric Sandwich ELISA (EIABUN, A22181 and EIACUN) Kits were used for the measurement and identification of urea nitrogen (BUN), uric acid (UA) and creatinine (Cr) in the rat serum samples as described by **Mitrovic *et al*., (2012), Tietz *et al.*, (2005)** and **Needleman *et al.*, (1992)**, respectively.
The serum concentrations of lipid peroxidation as expressed by Malondialdehyde (MDA) were assayed colorimetrically using guidance of **Rio *et al.*, (2005)**.
The serum activities of CAT and GSH-Px enzymes were identified colorimetrically using commercial Invitrogen™ kits (EIACATC and EIAGSHC, respectively) at 560 and 412 nm, respectively, in line with the instruction kits by **Glorieux and Calderon (2017)** and **Chu *et al.*, (1993)**. OxiSelect™ (STA-360) kit was used to measure total antioxidant capacity (TAC) based on a reduction of copper I I (Cu^{+2}) to copper I (Cu^{+}) by antioxidants like uric acid at 490 nm as described by **Trachootham *et al.*, (2008)**.
The serum concentrations of Norepinephrine (NE), Dopamine (DA) and 5-hydroxytryptamine (5-HT) were colorimetrically tested using commercial ELISA Kit (KA1877, E-EL-0046 and MBS725497, respectively) instruction manual at 450nm as referred by **Bada *et al.*, (2012), Liu *et al.*, (2019)** and **Berger *et al.*, (2009),** respectively.

2**.9. Cerebrum Histopathological Screening** The histopathological screening process for the cerebrums of all rats was carried out as referred procedures by **Kier (1990)**. Briefly, cerebrum samples were carefully washed in an isotonic solution, dried on a filter paper and immersed in buffered formalin (10%). Afterwards, the fixed cerebrum specimens

were dehydrated in a graded ethyl alcohol from 50 to 100%. Subsequently, specimens were cleared by Xylol, immersed in paraffin bulk, sliced to 6 μm thickness and colored with Hematoxylin (HX) and eosin (E) for the inspection.

2.10. Statistical analysis of Data All data on chemicals and biochemical inspection were statistically analyzed using the SPSS computerized Statistics program for Windows, version 22.0. Descriptive data was compared using a one-way variance (ANOVA) test. Comparison of differences between groups was expressed as Mean ± Standard Division (SD) at $p<0.05$ (**Snedecor and Cochran (1980).**

RESULTS

Table (1) presents the discovery of the total content of flavonoids, phenolics and anthocyanins in the red-flesh Dragon fruit. Information proved that red-flesh Dragon fruit possess a higher content of phenolics (390.25±0.55), followed by flavonoids (215.52±0.85), and anthocyanins (90.17±0.75).

Table (1) Total content of flavonoids, phenolics and anthocyanins in the red-flesh Dragon fruit

Types	Concentrations as Mean ± SD
Total flavonoids (mg CE/100g)	215.52±0.85
Total phenolics (mg GE/100g)	390.25±0.55
Total anthocyanins (mg/100g)	90.17±0.75

The results of the effect of the oral administration of the different levels (10, 20 and 30%) of red-flesh Dragon fruit juice (RDFJ) on FC, FBW, BWG and RBWG (%) in the normal control rats and those treated with Lead acetate (Pb) to induce brain toxicity are recorded in Table **2**. The results found that the treated rats with Pb alone (positive control group) had a significant ($p<0.05$) decrease in FBW, BWG, RBWG (%) and no significant change in FC, compared to the normal control group. While, treating rats with the brain toxicity by oral administration of 10, 20 and 30% of RDFJ results in a significant increment in FBW, BWG and RBWG (%), and no considerable change in FC, compared to the non-treated rats with brain toxicity. It was also noted that the improvement rates in FC and body weight augmented with rising the taken RDFJ concentration.

Table (2) The effectiveness of RDFJ on FI, FBW, BWG and RBWG% in negative group and Pb-toxicity groups.

Groups Parameters	NCG	PCG	Treated rats with the RDFJ		
			LDG (10%)	MDG (20%)	HDG (30%)
FC (g)	21.43±0.29	20.96±0.19	21.59±0.71	21.75±0.66	21.95±0.52
IBW (g)	206.43±0.39	206.00± 0.42	205.57± 0.58	205.57± 0.41	206.57± 0.56
FBW(g)	296.86±0.07 ^{a}	275.00±0.29 ^{c}	282.43±0.40 ^{b}	295.82±0.39 ^{a}	295.86±0.19 ^{a}
BWG (g)	90.43±1.16 ^{a}	69.00± 1.27 ^{d}	76.86±0.79 ^{b}	90.25±1.26 ^{a}	90.29±1.16 ^{a}
RBWG (%)	43.90±0.38^{a}	33.50±0.43^{c}	37.39±0.13^{b}	43.60±0.22^{a}	43.71±0.42^{a}

NCG: Negative control group; **PCG**: Positive control group; **RDFJ:** Red-flesh Dragon fruit juice; **LDG**: Low dose group; **MDG**: Medium dose group; **HDG**: High dose group**; FC:** Food Consumed; **IBW:** Initial Body Weight; **FBW:** Final Body Weight; **BWG:** Body Weight Gain; **RBWG:** Relative Body Weight Gain; Data are expressed as the mean ± SD; Mean values with different superscript letters at the same row are significantly different at $P < 0.05$.

The obtained results in Table **3** illustrate the effect of giving RDFJ orally on the serum activity of liver enzymes (AST, ALT, ALP) as a measurable of liver function in normal control rats and those treated with Pb alone. Tabulated results established that Pb resulted in liver detriment as detected by the significant ($P<0.05$) increase in serum activities of AST, ALT and ALP enzymes, comparable to normal control rats. In comparison, treating rats with brain toxicity by oral administration of the three different levels of RDFJ caused a significant ($p<0.05$) lowering in the serum activity of AST, ALT and ALP enzymes, compared with the positive control group.
There was also a notable difference in the level of enzyme activity between treated groups with RDFJ, as the improvement rate increased with increasing juice concentration taken.

Table (3) The effectiveness of RDFJ on serum activity of liver enzymes in negative group and Pb-toxicity groups.

Groups Parameters	NCG	PCG	Treated rats with the RDFJ		
			LDG (10%)	MDG (20%)	HDG (30%)
AST (μ/L)	39.64±0.95^{e}	117.51±1.22^{a}	85.87±2.81^{b}	62.90±1.07^{c}	45.40±1.35^{d}
ALT (μ/L)	46.93±1.40^{e}	146.64±2.22^{a}	116.82±1.31^{b}	94.34±0.77^{c}	48.76±0.94^{d}
ALP (μ/L)	60.43±1.51^{e}	118.83±2.01^{a}	98.90±1.58^{b}	81.40±1.74^{c}	68.54±1.21^{d}

NCG: Negative control group; **PCG**: Positive control group; **RDFJ:** Red-flesh Dragon fruit juice; **LDG**: Low dose group; **MDG**: Medium dose group; **HDG**: High dose group; **AST**: Aspartate transaminase; **ALT**: Alanine transaminase; **ALP**: Alkaline phosphatase; Data are expressed as the mean ± SD; Mean values with different superscript letters at the same row are significantly different at $P < 0.05$.

As an appraisal of kidney function results in Table **4** demonstrated the effect of giving orally RDFJ on the serum levels of BUN, UA and Cr in normal rats and those with brain toxicity caused by Pb. In comparison to normal rats, oral administration by Pb induced kidney dysfunctions as found out by the significant increase in serum concentrations of BUN, UA and Cr. On the other hand, oral giving of Pb for 14 days and then giving RDFJ for four consecutive weeks resulted in a significant amelioration in the serum levels of BUN, UA and Cr, compared with those giving Pb alone. The better results in kidney function were observed in rats treated with the highest level (30%) of RDFJ, compared to the other two levels (10 and 20%), as well as the positive control group.

Table (4) The effectiveness of RDFJ on serum levels of BUN, UA and Cr in negative group and Pb-toxicity groups.

Groups Parameters	**NCG**	**PCG**	**Treated rats with the RDFJ**		
			LDG (10%)	**MDG (20%)**	**HDG (30%)**
BUN (mg/dl)	40.47±1.50^{e}	75.90±0.65^{a}	65.05±2.66^{b}	51.40±2.23^{c}	45.34±0. 57^{d}
UA (mg/dl)	1.25±0.08^{e}	1.78±0.05^{a}	1.65±0.04^{b}	1.43±0.04^{c}	1.33±0.22^{d}
Cr (mg/dl)	0.39±0.02^{e}	1.00±0.02^{a}	0.79±0.01^{b}	0.70±0.01^{c}	0.63±0.02^{d}

NCG: Negative control group; **PCG**: Positive control group; **RDFJ:** Red-flesh Dragon fruit juice; **LDG**: Low dose group; **MDG**: Medium dose group; HDG: High dose group; **BUN**: Blood urea nitrogen; **UA**: Ureic acid; **Cr**: Creatinine; Data are expressed as the mean ± SD; Mean values with different superscript letters at the same row are significantly different at $P < 0.05$

Table 5 constitutes the effect of RDFJ on serum levels of MDA and TAC, and the antioxidant enzymes activities (CAT and GPx) in the treatment of rats from brain toxicity caused by Pb. Our results proved that oral administration of Pb for two weeks of the experimental period gave rise to a significant ($p<0.05$) rise in serum MDA level, and a decline in CAT and GPx activities and TAC levels, in comparison to normal rats. However, oral administration of Pb, followed by the three levels (10, 20 and 30%) of RDFJ for four weeks, significantly reduced serum levels of MDA and increased activity of the tested antioxidant enzymes and levels of TAC, compared to positive control rats.

The results also showed that the superior betterment of the tested parameters was increased with increasing the concentration of juice taken.

The concluded results in Table **6** summarized that non-treated rats with brain toxicity have a significant ($p <0.05$) decrease in serum Norepinephrine (NE), Dopamine (DA) and 5-Hydroxytryptamine (5-HT), as compared to that of the normal rats. In contrast, oral administration with 10, 20 and 30% of RDFJ for the treatment of brain toxicity in rats yielded about a significant ($p<0.05$) increase in the serum NE, DA and 5-HT levels, in comparison to treated rats with Pb alone. As shown, the get better improvement in the serum levels of the tested parameters was found in the treated group with the highest level (30%) of RDFJ, followed by those treated with 20 and 10%, compared to the positive rats.

Table (5) The effectiveness of RDFJ on serum levels of MDA, the activity of CAT and GP-x enzymes and TAC concentration in negative group and Pb-toxicity groups.

Groups Parameters	**NCG**	**PCG**	**Treated rats with the RDFJ**		
			LDG (10%)	**MDG (20%)**	**HDG (30%)**
MDA (nmol/ml)	9.70±0.75^{e}	32.60±0.95^{a}	20.74±0.48^{b}	16.89±0.45^{c}	10.80±0.64^{d}
CAT (µ/ml)	135.06±0.55^{a}	73.81±0.45^{d}	98.77±0.49^{c}	108.44±0.69^{b}	136.11±0.85^{a}
GPx (µ/ml)	63.34±1.03^{b}	25.29±0.93^{e}	43.44±0.99^{d}	51.54±0.96^{c}	65.73±0.67^{a}
TAC (nmol/ml)	1132.10±1.48^{a}	788.64±2.07^{e}	836.56±0.85^{d}	985.58±1.08^{c}	1106.40±1.67^{b}

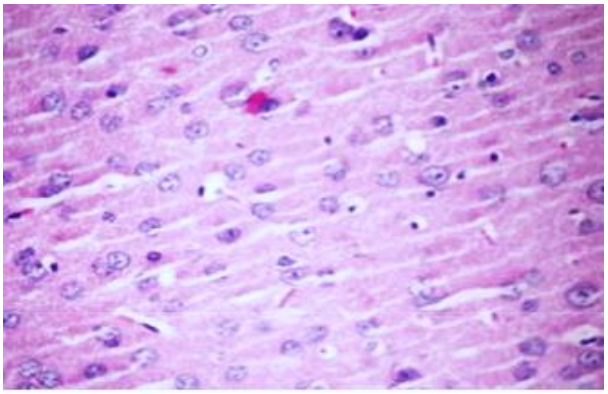
NCG: Negative group; **PCG**: Positive group; LDG: **RDFJ:** Red-flesh Dragon fruit juice; **LDG**: Low dose group; **MDG**: Medium dose group; **HDG**: High dose group; **MDA**: Malondialdehyde; **CAT**: Catalase; **GPx**: Glutathione Peroxidase; **TAC**: Total antioxidant capacity; Data are expressed as the mean ± SD; Mean values with different superscript letters at the same row are significantly different at $P < 0.05$

Table (6) The effectiveness of RDFJ on serum levels of NE, DA and 5-HT in normal rats and those with brain toxicity

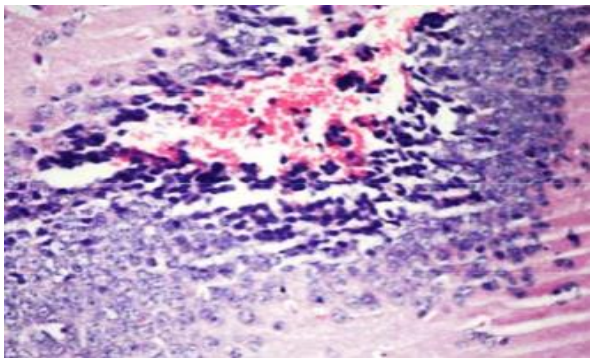
Groups / Parameters	NCG	PCG	Treated rats with the RDFJ		
			LDG (10%)	MDG (20%)	HDG (30%)
NE (µg/ml)	343.86±0.90[a]	165.00±1.73[e]	177.14±1.77[d]	295.14±1.95[c]	330.86±1.77[b]
DA (µg/ml)	147.00±2.94[a]	76.00±2.38[e]	97.14±2.34[d]	117.77±1.51[c]	140.54±1.30[b]
5-HT (µg/ml)	197.71±2.63[a]	92.57±3.05[e]	106.43±1.62[d]	156.86±1.77[c]	194.29±1.11[b]

NCG: Negative group; **PCG**: Positive group; **RDFJ:** Red-flesh Dragon fruit juice; **LDG**: Low dose group; **MDG**: Medium dose group; **HDG**: High dose group; **NE**: Norepinephrine; **DA**: Dopamine; **5-HT**: 5-Hydroxytryptamine ; Data are expressed as the mean ± SD; Mean values with different superscript letters at the same row are significantly different at $P < 0.05$

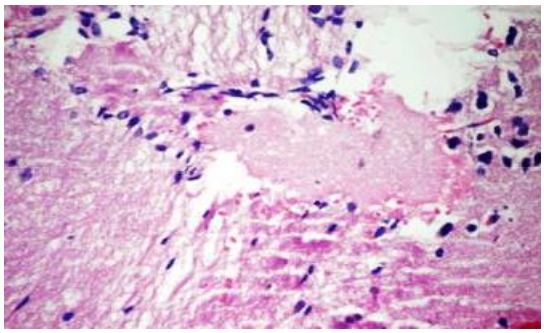
The histopathological inspections of cerebrum sections of the negative control group showed apparently normal anatomy with no histological malformation in cerebrum tissues (Picture **2**). Even so, cerebrum sections of untreated intoxication rats (positive control group) implicate several noteworthy alterations distinguished by focal hemorrhages area with leukocyte cell infiltration (Picture **3**) as well as focal proliferation of glia cells (gliosis) and atrophied neurons in the focal area (Picture **4**). Further, as shown in Picture **5**, cerebrum sections from intoxicated rats that were treated with 10% of red-flesh Dragon fruit juice (RDFJ) had degenerated glia cells. Additionally, microscopic inspection of cerebrum sections of intoxicated rats that were treated with 20% of RDFJ showed a large focal hemorrhagic area as shown in Picture **6**. Whilst, cerebrum sections of intoxicated rats that were treated with 30% of RDFJ revealed no histopathological changes (Picture **7**).



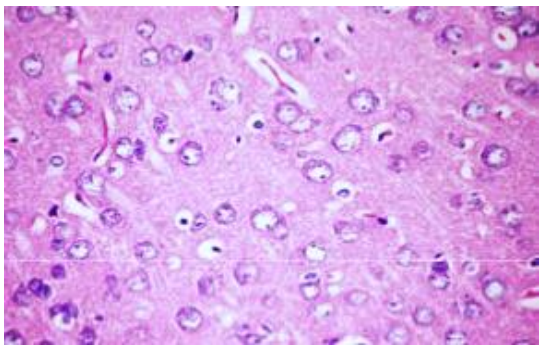
Picture (2) Photomicrograph of cerebrum samples from the negative rats showing no histological malformation in cerebrum tissues (H and E X 400).



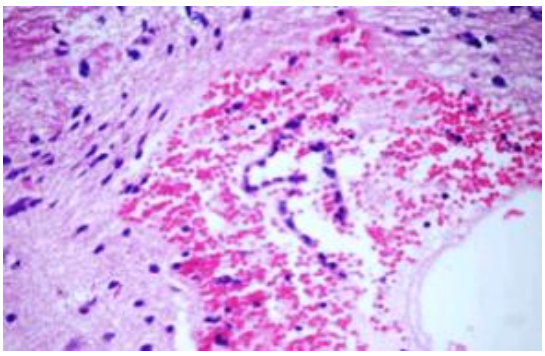
Picture (3) Photomicrograph of cerebrum sections of rats from the positive control group showing focal hemorrhages area with leukocyte cell infiltration (H and E X 400)



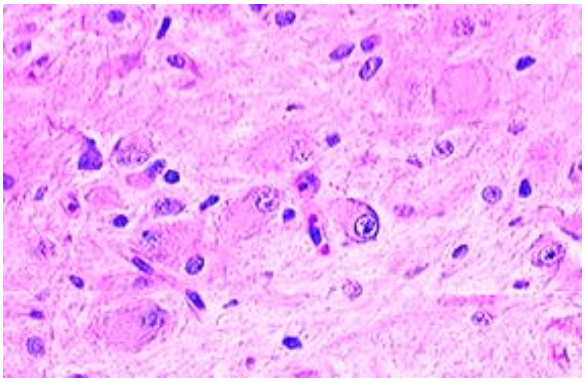
Picture (4) Photomicrograph of cerebrum sections of rats from the positive control group showing focal proliferation of glia cells (gliosis) and atrophied neurons in the focal area (H and E X 400).



Picture (5) Photomicrograph of cerebrum sections of intoxicated rats with Pb and treated with 10% of RDFJ showing degenerated glia cells (H and E X 400).



Picture (6) Photomicrograph of cerebrum sections of intoxicated rats with Pb and treated with 20% of RDFJ showing a large focal hemorrhagic area (H and E X 400).



Picture (7) Photomicrograph of cerebrum sections of intoxicated rats with Pb and treated with 30% of RDFJ showing apparently normal histological tissues (H and E X 400).

DISCUSSIONS

Lead (Pb) is a mightily venomous heavy metal that persists in the environment and human and animal bodies. Pb poisoning induces an assortment of disruption in neurological and vital organ systems (liver, kidney, brain and lungs), and other biological body functions (**Bauchi *et al.*, 2016**). Previous studies showed that when exposed to lead poisoning, a tiny amount of Pb is secreted in urine and the remnant amount accumulates in various tissues of the body, which results in structural alteration that can remain yet after lowering its level in the blood (**Flora *et al.*, 2012** and **Ibrahim *et al.*, 2012**). The existing study was directed to explore the potential effectiveness of red-flesh Dragon fruit juice (RDFJ) in the improvement of consequence injury from lead acetate (Pb) -induced brain toxicity in rats. Additionally, the total contents of flavonoids, phenolics and anthocyanins were detected in flesh fruit.

The total content analysis of red-flesh Dragon fruit (RDF) proved that it possesses a higher content of phenolics, followed by flavonoids and anthocyanins. Despite the fact that the content and average amounts of possibility change according to the situation, harvesting and market conditions, and maturity stage. The result was almost consistent with the results of **Dembitsky *et al.*, (2011)** and **Pasko *et al.*, (2021a)**.

Regarding the effect of Pb ingestion on body weight (BW). The acquired results demonstrate that receiving rats Pb orally at a dose of 30 mg/kg of b. wt. undergo a considerable decline in BWG and RBWG (%), compared to normal rats. The obtained effect in BW was in alignment with **Xia *et al.*, (2010)** who proved that exposing rats to Pb induced a notable lower in their body weight. **Ibrahim *et al.*, (2012)** stated that the final body weight of intoxicated rats with Pb was lowered significantly. **Ramah *et al.*, (2015)** also, confirmed that daily oral administration of Pb reduced BWG. Additionally, **Rezq *et al.*, (2018)** mentioned that Intraperitoneal injected rats with Pb resulted in a significant reduction in BWG and RBWG (%). The adverse effect of Pb on the body weight of rats may be related to its effect on the gastrointestinal tract, and consequently results in malabsorption of the nutritional elements, as mentioned by **Nabil *et al.*, (2012)**. In addition, **Hwang and Wang (2001)** disclosed that the reduction in body weight is caused potentially by the effect of Pb toxicity on zinc status by diminishing it in zinc-dependent enzymes that are required for many metabolic operations.

With respect to the effect of Pb on some biochemical analyses as indicative of hepatic and renal functions. The results evidence that receiving Pb rats orally caused a considerable increase in serum levels of AST, ALT and ALT enzymes, as well as MDA, BUN, UA and Cr, when compared to normal rates. These results agreed with **Asiwe *et al.*, (2022)** who mentioned that the oral administration of Pb (25 mg/kg of b.wt.) for two weeks caused a significant increase in serum ALT, AST, ALP, BUN, UA and Cr levels as indices of the defective in hepat-renal functions of rats. Likewise, the earlier studies by **Samuel *et al.*, (2017)** and **Rezq *et al.*, (2018)** informed that the serum levels of AST, ALP, ALT, globulins, bilirubin, UN and Cr were substantially increased in Pb-treated male Wistar rats.

Increasing serum levels of AST, ALT and ALP enzymes in the Pb-treated rats indicate damage to the structural integrity of liver function. As demonstrated by **Gaskill *et al.*, (2005)** and **Abdel-Kader *et al.*, (2011)**, this effect may be due to the escape of these enzymes from the liver cytosol into the bloodstream as evidence of the occurrence of necrosis in hepatocellular. Additionally, the elevation in AST, ALT and ALP activities is usually caused by rising free radical obstetrics, alteration in the liver tissue and microsomal membrane fluidity (**Ibrahim *et al.*, 2012**). Also, **Abdel-Moneim *et al.*, (2015)** indicated that Pb disrupts the cytoskeleton, causing membrane split and lysis and consequent in a rise in the level of AST, ALT and ALP in the blood.

The kidney is apt to the effect of toxic agents that can cause renal damage and/or failure. **Staessen *et al.*, (1990)** revealed that severe or continued exposure to high doses of lead induces impairment in the function of the kidney tubules, which manifests in hyperphosphaturia, amino-aciduria and glycosuria, and therefore toxic stress on the kidney which develops into chronic nephritis. In the same context, **Acharya *et al.*, (2003)** reported that lead is deposited mostly in the kidney tubules and is considered to be the principal reason for its deleterious effects on the kidney cortex. As is obvious by **Jabeen *et al.*, (2010)**, lead acetate exerts its toxic effects via the generation of reactive oxygen species on various organ systems. Additionally, **Jose and Novoa, (2002)** indicated that the kidney is extremely susceptible to damage resulting from reactive oxygen species, probably due to the abundance of polyunsaturated fatty acids in the composition of renal lipids. As well, reactive oxygen species are implicated in glomerulosclerosis and nephritic fibrosis. As confirmed by **Tohma *et al.*, (2017),** the hepatic and renal cells suffer from unrestrained lipid peroxidation due to the fact that they are the most affected organs that result from consumption and/or aspiration exposure of lead.

With regard to the effect of lead administration on oxidation processes and the level of activity of antioxidant enzymes. Our results evidence that receiving Pb rats orally caused considerable increase in serum MDA level, and decreases in serum activities of antioxidant enzymes (CAT and GPx) and TAC. Belonging to our results, we concur to an approximately extent with some earlier studies that commented that lead has been shown to drive oxidative damage to cell membrane lipids (**Flora *et al.*, 2012**) and causes an enormous rise in lipid peroxidation as quantified by a notable increase in MDA concentrations (**Laamech *et al.*, 2017**). The results are also in agreement with **Elgawish and Abdelrazek, (2014)** who exhibit that the injection of lead acetate caused a significant diminishing of serum SOD and

CAT levels, while increasing MDA levels. As well, **Samuel *et al.,* (2017)** and **Rezq *et al.,* (2018)** observed that the serum levels of GSH, SOD, GSH-PX and TAC were significantly reduced, while MDA increased in lead acetate-treated rats.

The discovered increment in serum levels of MDA, decline in the activities of antioxidant enzymes (CAT and GPx), and the level of TAC prove that the lead acetate generated oxidative stress and dominated the antioxidant system as a main mechanism of brought about lead toxicity. Oxidative stress stands for an unbalance in the midst of the free radical's production and the biological system's capacity to promptly detoxicate the passive intermediates or remedy the resulting damage of free radicals (**Flora, 2011**). The lead-induced oxidative stress mechanism involves an unbalance between the creation and expulsion of the reactive oxygen sorts in tissues and cellular parts, causing damage to membranes, proteins and DNA (**Patra *et al.,* 2011**). According to the influence of lead, the beginning of oxidative stress appears on account of two diverse pathways operative at identical time. Firstly, the reactive oxygen portions generation such as hydroxyl radicals, hydrogen peroxide and superoxide radicals, as well as the production of lipid peroxides and nitric oxide (**El-Nekeety *et al.,* 2009**). Secondarily, the antioxidant reserves turn weakened with co-occurrence reduction in antioxidant enzyme activity (**Abdel-Moniem *et al.,* 2010**). Additionally, lead targets the sulfhydryl groups, and can also substitute the zinc ions that serve as important cofactors for the antioxidant enzymes and deactivate them (**Flora *et al.,* 2007**).

From the existing results in table (6) it is evidenced that administration of Pb induced a significant decrease in serum levels of NE, DA and 5-HT hormones, as an indication of a brain malfunction. It was also confirmed by pathological examination, which confirmed the presence of several noteworthy alterations distinguished by focal hemorrhage area with leukocyte cell infiltration and focal proliferation of glia cells (gliosis), and atrophied neurons in the focal area. Our results were somewhat in agreement with **Gill *et al.,* (2003)** who discovered that lead exposure modifies the level of NE and DA. Furthermore, **Rezq *et al.,* (2018)** noted that the administration of lead acetate caused a significant decrease in the level of NE, DA and 5-HT in the brain. **Rocha and Trujillo (2019)** also indicated that Pb brought about a significant decline in the DA and 5-HT concentration in the brain. In the same context **Highab *et al.,* (2020)** discovered lead acetate-treated rats had markedly lower levels of NE and 5-HT in both serum and the brain. Previous studies have outlined that lead exerts its neurotoxic consequences through interference with Ca2+ calmoduline mediated neurotransmitter release, whichever is ultimately accountable for behavioral deterioration (**Bouton *et al.,* 2001**). Also**, El-Masry *et al.,* (2011)** reported that lead acetate intoxication encourages an oxidative stress circumstance in the rat brain that might be the key mechanism implicated in neurotoxicity.

With regard to the potential effectiveness of red-flesh Dragon fruit juice (RDFJ) in the improvement of consequent injury from intoxicated by lead acetate (LA) in rats. The obtained results showed that RDFJ exhibited enhanced BWG, and hepatic and renal functions as well as diminished oxidative stress as stated by the lowering level of MDA and rising activity of CAT and GPx. In addition, showed significant improvement in the serum concentration of NE, DA and 5-HT hormones and pathological improvement of brain cells. The get better improvement in the serum concentrations of the tested parameters was found in the treated group with the highest level (30%), followed by those treated with 20 and 10% of RDFJ, compared to the positive rats. There was an agreement between our results and the results of **Ramli *et al.,* (2014)** who demonstrated that red Dragon juice treatment reduced serum ALP and ALT levels. The reduction of AST, ALT and ALP after an intervention is probably related to the decrease in the deposition of fat and the degree of liver cell necrosis as reported by **Wang *et al.,* (2012)** and **Mihir *et al.,* (2019)** also demonstrate that Dragon fruit possesses an antioxidant and hepatoprotective potential against Acetaminophen-induced liver injury. This was confirmed by **Cauilan (2019)** who suggested that the hepatoprotective activity of Dragon fruit may be related to its abundant content of antioxidants such as flavonoids, alkaloids, triterpenes and glycosides. **Abdulshahed *et al.,* (2020)** showed that the aqueous extract of Dragon fruit significantly protects from the sodium nitrate-induced kidney toxicity in albino rats by increment of GSH and CAT enzymes and decreases MDA. The effect of dragon fruit enhances antioxidant defense due to the effective phytochemical bioactive compounds in the red pulp of Dragon fruit like polyphenols and flavonoids, as well as vitamins A, C and E **(Armutcu *et al.,* 2018)**. **Swarup *et al.,* (2010)** pointed out that the dragon fruit extract significantly increased serum levels of SOD and TAC and decreased MDA in diabetic rats.

The biological activity of dragon fruits might be due to their high antioxidant properties, tied to their betacyanins, phenolics and flavonoids substances (**Pasko *et al.,* 2021b**). Therefore, the consumption of dragon fruits has favorable effects against inflammation and other oxidative stress-related disorders (**Verona-Ruiz *et al.,* 2020**). The different identified types of polyphenols in dragon fruit like flavones, flavonols, flavanones, tannins and phenolic acids (**Al-Mekhlafi *et al.,* 2021**) have been displayed to exhibit many health benefits due to their antioxidant properties and have significant effects on the first pass metabolic process (**López-Yerena *et al.,* 2020**). Flavonoids possess very strong antioxidant ability and can discourage damage resulting in free radicals by direct clearance of reactive oxygen species (ROS) and activating antioxidant enzymes (**Nijveldt *et al.,* 2001**). In addition, flavonoids act as metal-chelating activity (**Ferrali *et al.,* 1997**), inhibit oxidative stress (**Heim *et al.,* 2002**), and increase antioxidant activity (**Yeh *et al.,* 2005**).

Youdim and Joseph (2001) reported that the consumption of flavonoid-rich fruits and vegetables or their extracts has performance- enhancing cerebral function either by protecting susceptible neurons, enhancing the extent of neuronal function, or encouraging neural regeneration. As well, the neuroprotective capability of flavonoids has been shown in oxidative stress studies.

Phenolic compounds also showed distinct bioactivities in neuroprotective (**Jayasena *et al.*, 2013**), due to their effect as scavengers of hydroxyl radicals and superoxide radicals that generate neurotoxicity (**Dhanalakshmi *et al.*, 2015**).

The red dragon fruit extract contains anthocyanin pigments which serve as antioxidants and suppressed free radical production (**Jaafar *et al.*, 2009**). Consequently, anthocyanins can prevent the event of lipid peroxidation and suppress the production of MDA (**Rusip *et al.*, 2022**). Additionally, anthocyanins can predicament metal ions to establish a stable anthocyanin-metal complex (**Harahap *et al.*, 2019**).

In humans and animals, anthocyanins are absorbed as glycosides (**Paul *et al.*, 2002**), subsequently, traverse the blood-brain barrier, centralize and extended in brain areas, in particular, the cerebellum, cortex, hippocampus and the stratum (**Milbury and Kalt 2010**). Previous studies revealed that the consumption of anthocyanins was related to the decline of brain disturbance hazards (**Simone *et al.*, 2018**).

The established process by which anthocyanins possess neuroprotective effects is their capacity to suppress inflammation and oxidative stress induced in the brain by several risk factors (**Min *et al.*, 2011**). On the other hand, anthocyanins can prohibit neuronal degeneration by suppressing oxidative stress production and/or pro-inflammatory cytokines (Ali et al., 2018). This action occurs through the activation of brain-acquired neurotrophic factor, protein kinase B, cAMP-response component tying protein and extracellular signal-regulated kinase (**Williams *et al.*, 2008**).

CONCLUSION

In conclusion, the existing study was showed the effective effect of dragon fruit juice is exhibited by decreasing the toxic effect of lead acetate on the functions of both the liver and kidneys; and reducing oxidation rates by increasing the activity of antioxidant enzymes. It also showed substantial improvement in the Norepinephrine, dopamine and 5-Hydroxytryptamine levels. Therefore, the study suggested that the ordinary consumption of Red Dragon fruit or embedding it into diets may be profitable in preventing or protecting against lead toxicity due to its content of flavonoids, phenolics, and anthocyanins as bioactive compounds.

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