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# Study Comparison between Alcoholic Extract of Broccoli, Folic Acid and Ferrous Sulfate Against Damage of the Cardiovascular System Induced by Lead-acetate in Rabbits

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## Abstract

Anemia is a most common blood disorder that contributes to Cardiovascular diseases. It is associated with red blood cell dysfunction and compensatory vascular and cardiac endothelial nitric oxide synthase activity enhancement. Present study aimed to investigate the role of broccoli, folic acid, and ferrous sulfate in treating cardiovascular damage induced by lead acetate. Twenty-five female local rabbits 5 months in age with a verge of weighing 1500 Kg were divided into 5 randomized groups: 1$^{st}$ as a control group (C) and the other four groups 2$^{nd}$(lead acetate ),3$^{rd}$(T1F),4$^{th}$(T2FS),5$^{th}$(T3B) were exposed to lead acetate 10 mg/kg/Bw/daily for 4 weeks , the second group continued to receive a lead for 8 weeks, while 3$^{rd}$,4$^{th}$, and 5$^{th}$ treated with alcoholic extract of the broccoli, folic acid and ferrous sulfate respectively. Blood samples were taken after the 4$^{th}$ and 8$^{th}$ weeks of the experimental period to examine Troponin I, platelets count, and prothrombin time. Heart tissue obtained to examine the histopathological changes. The results after the 4$^{th}$ week in the 2$^{nd}$,3$^{rd}$, 4$^{th}$ and 5$^{th}$ groups demonstrated a significant elevation ($p \leq 0.05$) in Troponin I, and platelets count while at the 8$^{th}$ week of the treatments, the mean values of Troponin I and platelets count reduced significantly($p \leq 0.05$) . In conclusion, the treatments of the alcoholic extract of *Brassica olecera,* folic acid, and ferrous sulfate confirmed the superiority of the *Brassica olecer*a in treating the pb-acetate adverse effects on the cardiovascular system, which contributed to its phytochemical and nutrient value.

**Keywords:** Anemia, Troponin I, Platelets, Prothrombin time.

## 1. Introduction

Plants in the family *Brassicaceae olecerea* are among the oldest cultivated plants known to man. (1). Dietary consumption of broccoli has prompted researchers to investigate various biological effects, including gastroprotective, antimicrobial, antioxidant, anticancer, hepatoprotective, cardioprotective, anti-obesity, anti-diabetic, anti-inflammatory, and immunomodulatory effects**.** It is an incredibly nutrient-dense herb, full of bioactive phytochemicals, including glucosinolates, phenolic compounds, and flavonoids, as well as antioxidants that are good for your diet (2). In addition, broccoli contains a lot of indol-3-carbinol. These elements found in broccoli are thought to be quite prevalent since they have a wide range of antioxidant qualities and advantages. Oleic and linoleic acids predominated in broccoli sprouts, while caproic, stearic, and oleic acids were present in the flowers (3).

Lead (Pb) is a metal that harms human health and is an environmental pollutant with no known biological role in organisms (4).Its harmful effects can manifest in very low doses, affecting all systems and organs and raising concerns worldwide (5,6).Exposure to Pb through polluted air, food and water represents a risk factor for developing cardiovascular diseases and other related diseases such as atherosclerosis, anemia, and hypertension. The main mechanisms by which Pb induce harmful effects are oxidative damage, apoptosis, inflammation and dyshomeostasis of essential cations(7,8).Exposure to lead can negatively impact many organ systems, including CVDs (10).Folic acid, also termed folate, is an essential vitamin for health at all ages since it participates in the biosynthesis of nucleotides, amino acids, neurotransmitters, and certain vitamins. Solution of folic acid increases lead ions' reduction current peaks while decreasing the oxidation current peak. Folic acid collaborates closely with vitamin B12 to help the body make red blood cells and maintain healthy iron metabolism (11). Iron supplementation may have different formulas for anemia; ferrous sulfate is the most common medicine preparation(12). Ferrous sulfate replenishes iron, an essential component in hemoglobin, myoglobin, and various enzymes**.** Iron plays a cardinal role in cellular metabolism and energy production by forming vital mitochondrial proteins (13,14).Given that cardiomyocytes have high energy demands, it is unsurprising to postulate that iron defeciency contributes to the severity of CVD and cardiomyocyte dysfunction. Lead and various chemical, physiological, and behavioral abnormalities influenced its prominent role in the rise in CVD deaths. Yet,the role of broccoli, folic acid, and ferrous sulfate approach to Pb adverse effects treatment was the aim of the present study.

## 2.Materials and Methods

### 2.1. Plant extract preparation

*Brassica olecerae* flowers were cleaned and carefully washed under tap water, it chopped into small pieces and dried at a temperature of 45 °C. The dried flowers were ground to a thick powder shape. Fifty grams of powder was blended into 250 ml of ethanol 70% in a clean, tightly sealed conical flask incubated in the water bath (37 °C). After 24 hours, the mixture was blended for one hour with a magnetic stirrer. Then, after drying, scrape it off and put it in the refrigerator for further use (15). The dosage of Brassica extract is cited as 300mg/kg B.W. according to (16).

**2.2.Experimental Design**

This study was carried out by using 25 female local rabbits weighing between 1250 and 1750 Kg. Animals were kept in cages (5 animals/cage) at room temperature with regulated lighting for 24 hours in the animal house of the physiology, Biochemistry, and pharmacology department at the College of veterinary medicine, University of Baghdad. Throughout the trial, the animals had unrestricted access to water and pellet. Animals were divided into 5 groups as follows: 1$^{st}$ control group (C), 2$^{nd}$ (lead acetate ) group which was exposed to lead acetate at the dose of 10mg/kg /orally daily for 8 weeks,3$^{rd}$ group( T1F),4$^{th}$ group(T2FS) ,and 5$^{th}$ group (T3B)which were exposed by lead acetate 10 mg/kg/orally for 4 weeks and after induction of anemia treated with folic acid 0.07mg/kg , ferrous sulfate 33mg/kg , *Brassica olecerea. ethanolic* extract 300mg/kg/Bw. Blood samples were taken after the 4$^{th}$ week of the experimental period to examine Troponin I, Vascular endothelial growth factor (VEGF), platelets count , and prothrombin time. At the end of the 8$^{th}$ week of the experiment euthanized animals, blood samples and tissue of heart, and aortic samples were obtained.

**2.3. Cardiac marker**

Serum troponin I was determined (Elecsys Troponin I STAT kit- Germany) by using cobas e 411 immunoassay analyzer .$^{1}$st incubation: 30 µL of the sample, two biotinylated monoclonal anti-cardiac troponin I antibodies, and two monoclonal anti-cardiac analyzers. troponin I antibodies labeled with a ruthenium complex) react to form a sandwich complex, 2nd incubation: After adding streptavidin-coated microparticles, the complex becomes bound to the solid phase via biotin interaction. Results are determined via a calibration curve instrument specifically generated by $^{2nd}$point calibration and a master curve provided via the reagent barcode or e-barcode. The analyzer automatically calculates the analyte concentration of each sample (either in µg/L or ng/mL).

**2.4. Coagubility Markers**

Coagubility markers contain total platelets count and prothrombin time.

The total platelets count in fresh blood sample were determined by automated hematology (Genex, G002158,USA), and the prothrombin time was determined using the BIOLABO France kit .Pre-incubate Working Reagent for at least 15 minutes at 37°C and gently mix. Plasma 0.1 mL Incubate for 2 minutes at 37°C Working reagent (homogenized) 0.2 mL The automatic countdown timer will start immediately after working reagent addition and stop when the clot is formed.

**2.5. Histopathological study**

Histopathological examination was done from Hematoxiline and eosin-stained paraffin fixative samples (17). and analyzed by a histopathological specialist.

**2.6. Statistical Analysis**

The Statistical Analysis System- SAS (2018) program was used to detect the effect of different treatments and time in study parameters. Two ways analysis was used (ANOVA). Least

Significant Difference (LSD) post hoc tests were performed to assess significant differences among means (P ≤0.05) is considered statistically significant (18).

## 3.Results

### 3.1. Cardiac marker (troponin I)

In **Table 1.** showed the changes in mean values of serum in experimental groups. The troponin I concentrations in the lead acetae group after 4 and 8 weeks had increased significantly (p≤0.05) when compared to control group. Meanwhile, treatment with broccoli, folic and ferrous sulfate for 4 weeks after induction of anemia significantly declined (p≤0.05) in T1F,T2FS and T3B groups compared with lead acetae group, and the cardiac marker return to the semi-normal level of control at the 8 weeks of the experimental period .

**Table 1**. Comparative of alcoholic extract of *Brassica oleracea var. Italica,* with folic acid and ferrous sulfate in Troponin I (ngl/ml) against lead acetate.

| | **Troponin I (ngl/ml)** | |
|---|---|---|
| Groups | Before treatment | After 4Weeks of treatments |
| Control | 0.172 ±0.01 Ac | 0.339 ±0.16 Ac |
| Lead acetate | 5.83 ±0.33 B a | 8.95 ±0.58 A a |
| T1F | 5.00 ±0.48 A b | 2.97 ±0.45 B b |
| T2FS | 6.21 ±0.35 A a | 2.61 ±0.59 B b |
| T3B | 5.77 ±0.66 A a | 1.69 ±0.19 B b |
| LSD | 1.288 | |

Means with a different small letter in the same column are significantly different (P≤0.05), Means with a different capital letter in the same row are significantly different (P≤0.05 ),C=control group, lead acetate group= Animals were exposed by lead acetate , T1F = Animals were exposed lead acetate with folic acid, T2FS =:Animals were exposed lead acetate with ferrous sulfate, T3B = Animals were treated orally with alcoholic extract of the *Brassica oleracea* .

### 3.2. Platelet count and prothrombin time

in **Table 2** illustrate the mean values of control and treated groups .There are significant ( p≤0.5) increments of platelet count and a significant decrease (p≤0.05 ) in Serum prothrombin time affected by lead acetate when compared with control group. On the contrary, platelets significantly (p≤0.05**)** decreased in T1F, T2FS, T2B treatment in comparison with the control and lead acetate groups. On the other hand, the result of prothrombin time also was observed significant (p≤0.05) elevation in different treatment groups, T1F, T2FS and T3B compared to lead acetate and control groups.

**Table 2**. Comparative of alcoholic extract of *Brassica oleracea var. Italica,* with folic acid and ferrous sulfate in platelets counts(10^3/Ul) and prothrombin time (sec) against lead acetate**.**

| | **Platelets count (10^3/Ul)** | | **Prothrombin time ( sec)** | |
|---|---|---|---|---|
| **Groups** | Before treatment | After 4Weeks of treatments | Before treatment | After 4Weeks of treatments |
| Control | 283.40 ±1.40 A b | 283.40 ±1.29 A b | 23.40 ±0.24 A a | 23.40 ±0.24 A a |
| Lead acetate | 428.00 ±32.58 B b | 675.40 ±61.17 A a | 17.40 ±0.67 A b | 10.20 ±0.86 B c |
| T1F | 626.80 ±72.95 A a | 303.40 ±20.41 B b | 17.60 ±0.51 B b | 21.00 ±0.54 A b |
| T2FS | 610.80 ±57.95 A a | 280.80 ±2.51 B b | 17.00 ±0.54 B b | 22.00 ±0.45 A ab |
| T3B | 602.20 ±70.02 A a | 281.00 ±1.41 B b | 17.20 ±0.66 B b | 22.60 ±0.51 A ab |
| LSD | 116.07 | | 1.755 | |

Means with a different small letter in the same column are significantly different ($P \leq 0.05$), Means with a different capital letter in the same row are significantly different ($P \leq 0.05$ ),C=control group, lead acetate group= Animals were exposed by lead acetate , T1F = Animals were exposed lead acetate with folic acid, T2FS =:Animals were exposed lead acetate with ferrous sulfate, T3B = Animals were treated orally with alcoholic extract of the Brassica oleracea.

### 3.3. Histopathological Changes

The histopathological examination of the myocardium section of the control group, illustrated in **Figure 1.**, revealed the normal appearance of myofibers of the heart. Most histopathological observations of the heart showed moderate diffused vascular degeneration related to fat droplets within myofibers, necrosis of the myocardium, and marked splitting of myofibers (**Figure 2.**), T1Fand T2FS myocardium section showed no pathological changes and were similar to those of control group (**Figure 3.** and **4**). In contrast, most heart section showed moderate diffused vascular degeneration related to fat droplets within myofibers and necrosis of myocardium and marked splitting of myofibers. Afew sections of the myocardium showed a normal appearance (**Figure 5.**).

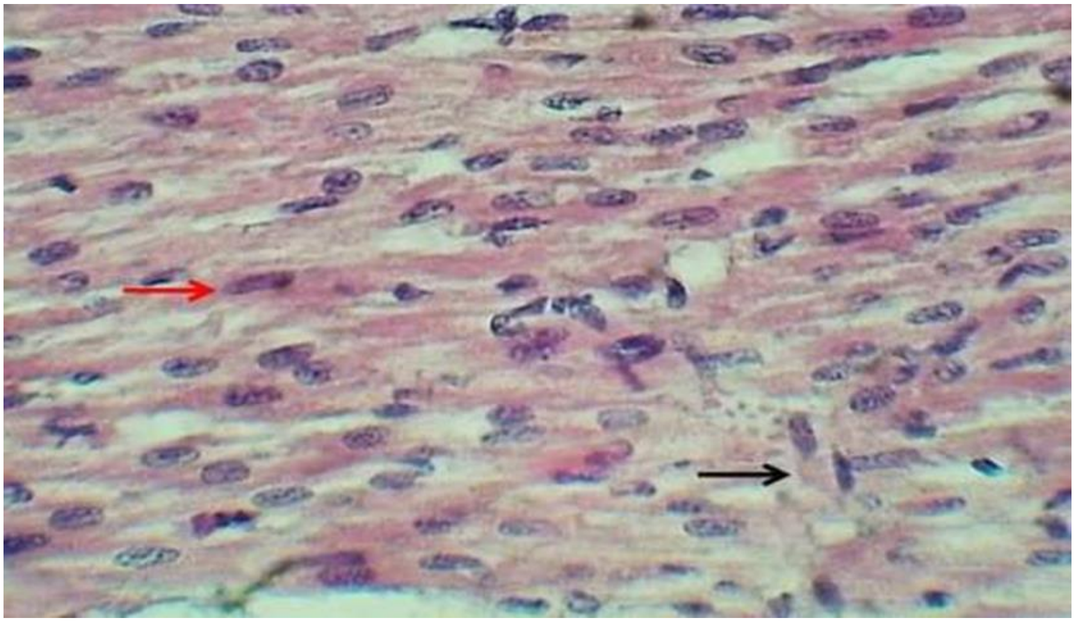

**Figure 1**. Light microscopic image for heart section of myofibers Control group shows: Normal appearance of nucleus of myofibers (Red arrow) & intercalated disc (black arrow).( H&E stain.400x.)

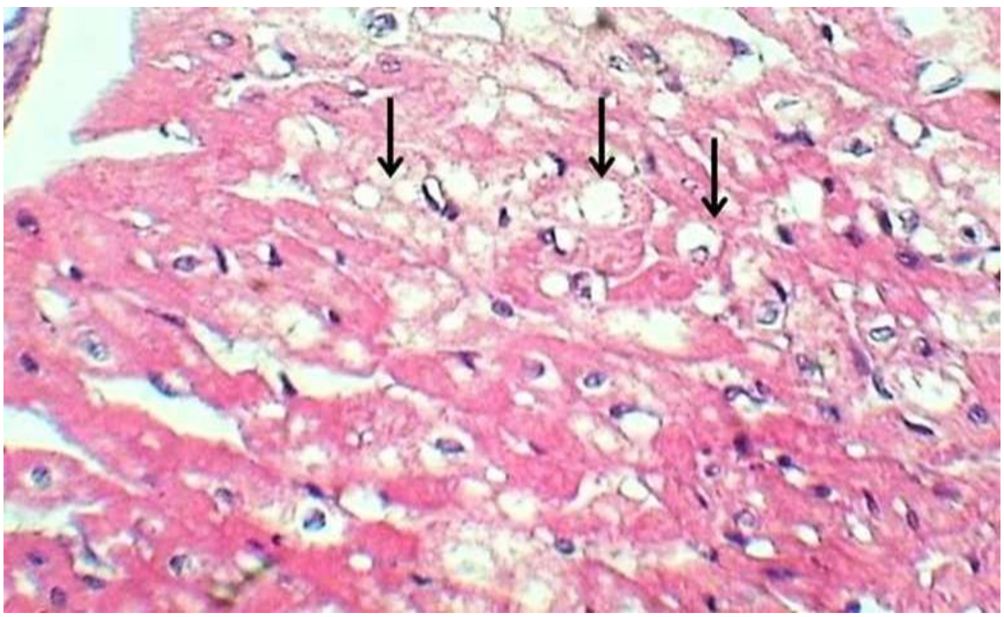

**Figure 2**.Light microscopic image for section of myofibers lead group shows: fat droplets in myofibers with mild necrosis (a dark arrow). H&E stain.400x

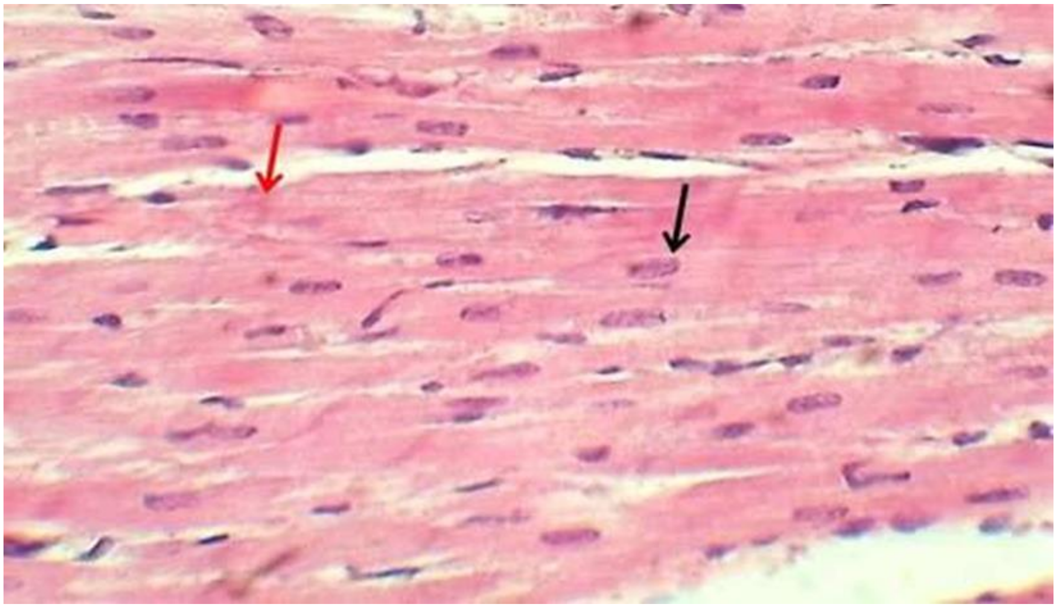

**Figure 3.** Light microscopic image for section of myofibers (T1pb-F) shows: Normal appearance of nucleus of myofibers (Black arrow), & intercalated disc (Red arrow). (H&E stain.400x.)

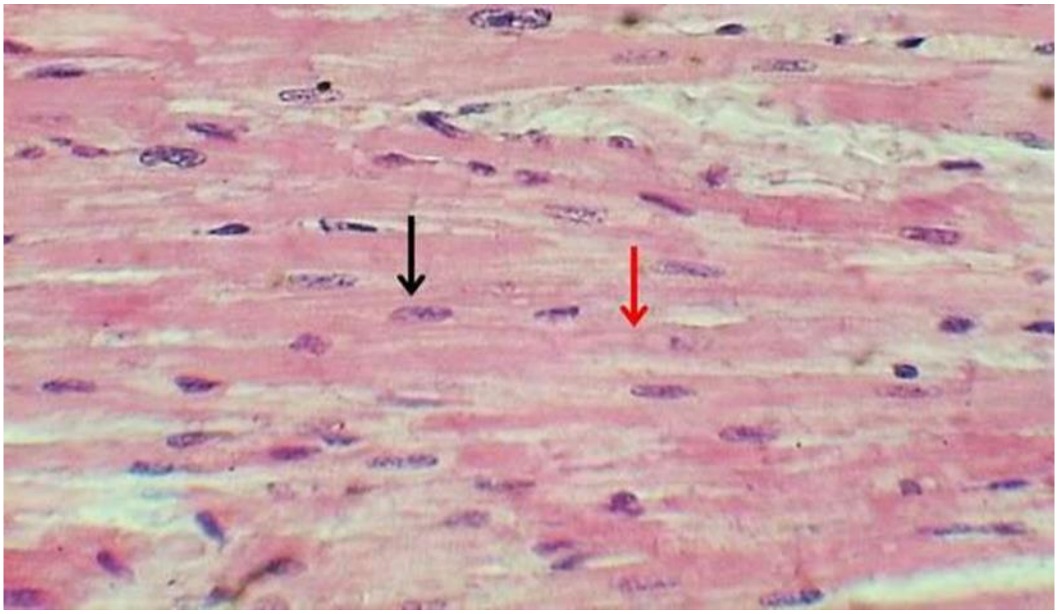

**Figure 4**. Light microscopic image for section of myofibers (T2pb-FS) shows: Normal appearance of nucleus of myofibers (Black arrow), & intercalated disc (Red arrow). H&E stain.400x**.**

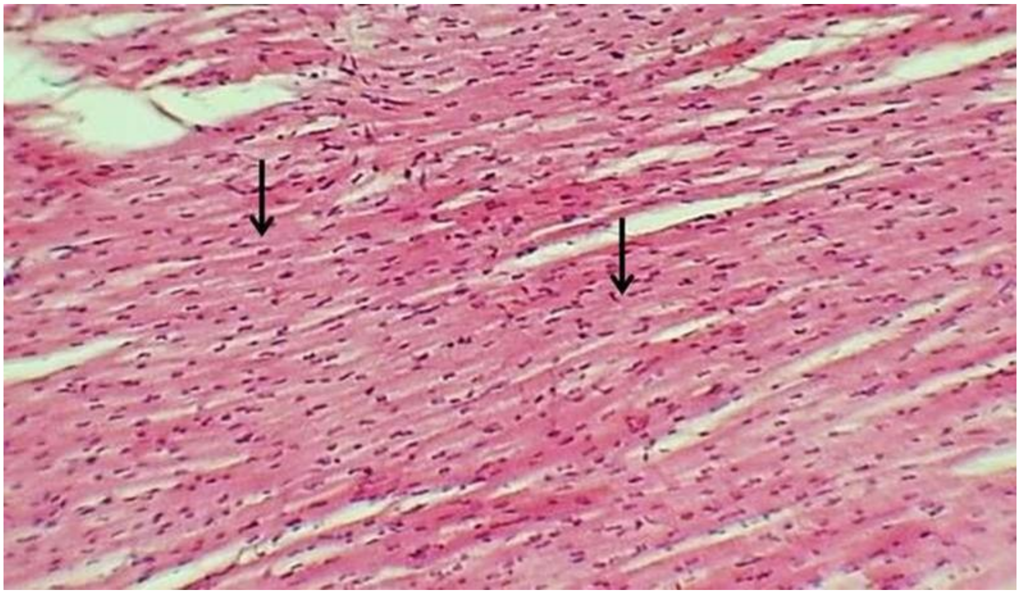

**Figure 5.** Section of the heart (T3B) shows myofibers' normal arrangement and appearance (Arrows). H&E stain.100x**.**

## 4. Discussion

The present study reveal that the adverse effects of lead acetate on the cardiovascular system. There are many defined lead toxicity mechanisms. However, it appears that lead accumulation-induced oxidative stress is the primary cause of the toxicity of Lead. (19). Troponin as a biomarker for myocardial disease has been well studied due to the high specificity and sensitivity of these markers with high detectability even in very low serum levels of asymptomatic CVD (20). It is suggested that Troponin I was identified as a predictable myocardium inflammation marker. (20). In the present study suggest that oxidative stress induced by Pb and ROS production is a mechanism for the pathophysiology of many diseases .This ROS contribute to systemic inflammatory processes that could be involved in the imbalance in myocardial oxygen supply and demand. Increased oxygen uptake, lowered perfusion pressure, extrinsic myocardial depression, and a subsequent release could result from troponin (21,22).Table 2 also shows the activation of the coagulation cascade increase in lead acetate group, (T1F), (T2FS) ,and (T3B) by stimulating the intrinsic pathway coagulation factors, which in turn results in a reduction in the coagulation time in the platelet aggregations after penetrating the platelet plasma membrane and stimulating a rapid and prolonged NO release**.** Therefore, endothelial myocardium is a marker of endothelial (dys) function probably associated with adverse cardiovascular outcomes. Platelets perform immunological tasks by interacting with leukocytes, which are drawn to the site of infection and inflammation and retained while creating an "immune thrombus" under high-shear stress conditions (23). In systemic inflammation, platelets are more metabolically and enzymatically active and have a larger prothrombotic potential. Mean platelet volume (MPV) controls platelet stimulation and production rate. Increased MPV has been observed in CRC, pancreatic cancer, and hepatocellular carcinoma (24) .Counts of thrombocyte and volumes are correlated. Reactivity and serves as a diagnostic indicator the presence of giant-cell arteritis

(GCA) like temporal arterial inflammation in older adults. In regard to this, a new study was conducted found an elevated thrombocyte count can predict the GCA diagnosis regardless of absence of the usual altered of arteries. Platelets promote the activation of the coagulation cascade by adsorbing and boosting the intrinsic route coagulation factors. This increases the ratio of thrombocytes to lymphocytes, a useful measure indicating platelet alterations (25). Red blood cells' elevated viscosity, which creates a hypercoagulable state which, in turn, affects blood flow inside the vessels, has been linked to an increased probability of thrombosis (26).
Several histopathological changes were observed in the heart tissue of the anemic and treated groups. These changes in the cardiac section in the group of exposed pb include moderate diffused vascular degeneration-associated fat droplet within myocardial fibers and necrosis. A somewhat normal appearance of heart tissue can be detected in the exposed group, with irregular distribution of nuclei of myocytes of cardiac tissue. Cardiac marker are biomarker measured to evaluate heart function. In the context of myocardial infarction and atherosclerosis in animals exposed to Pb pollution, there are bulg of evidence about this. The Pb metal transport cell membrane via proteins acts as transporters, and intracellularly, it induces adverse effects on different cellular mechanisms (27). Pb has Ca ions on its biding sites of different Ca-binding proteins, particularly calmodulin Protein Kinase C (PKC) and calcium-dependent potassium channels. It can replace calcium in Ca2+-dependent signaling pathways (28). The disorder of Ca intracellular leads to dysfunction of mitochondria permeability, releasing apoptotic factors that lead to cell death and tissue necrosis (29). Inflammatory responses due to ROS generation, are the key events in the formation of atherosclerotic lesions. Interleukin 1 [IL-1], tumor necrosis factor-alpha (TNF-), chemokine monocyte chemoattractant factor 1 (MCP-1), growth factors like platelet-derived growth factor (PDGF), and adhesion molecules are among the cytokines and chemokines that the endothelial cells start to express. Broccoli is a vegetable that contains a variety of bioactive substances, such as vitamins, minerals, dietary fibers, plant proteins, and phytochemicals. Vegetables may have cardioprotective effects that include reducing blood pressure, altering lipid metabolism, regulating blood glucose, enhancing endothelial function, lessening myocardial damage, regulating related enzyme activities, gene expressions, and signaling pathways, as well as a few other CVD risk biomarkers(30). Additionally, broccoli contains tannins, which have an antiplatelet impact due to their antioxidant characteristics and the resultant reduction in ROS generation in platelets. According to (31). the inhibitory action of tannins on oxidative stress also entails an increase in the concentration of antioxidant enzymes and avoidance of stress-induced protein modification. Chemical and biological processes characterization among the active secondary metabolites. In particular employing in vitro experiments based on several pathways, ferrous ion chelating and radical scavenging activities were observed, with the latter being stronger than the former and only weak reducing power being shown. The antioxidant effects of *Brassica* leaf extract by identifying many phenolic components. It was proposed that phenolic chemicals, known to act as antioxidants through various chemical processes, including directly as reducing agents or hydrogen donors, may be partially responsible for the extract's antioxidant effects and indirectly as metal chelates (32). The

efficiency of folic acid therapy in decreasing CVD risk as measured by carotid intima-media thickness (33). .Ferrous sulfate has a higher margin of increase in hemoglobin levels and essential for good health, because it promotes the formation of red blood cells (34). It is incorporated in hem proteins and Fe-S cluster proteins, mediating mitochondrial oxidative phosphorylation.

## 5. Conclusion

The comparisons treatment between the alcoholic extract of *Brassica olecer*, folic acid and ferrous sulfate confirmed the superiority of the *Brassica olecera* in treating the Pb adverse effects on the cardiovascular system, which contributed to its phytochemical and nutrient value

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## Conflict of Interest

No conflicts of interest

## Funding

No funding

## Ethical Clearance

Before starting this study, at the College of Veterinary Medicine inside the local council on animal care and use obtained ethical approval.984/PG on 9/5/20.

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