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# Cardioprotective Effect of *Portulaca Oleracea L.* (Purslane) against Clozapine-Induced Cardiotoxicity in Rats

**Eman Hassan[1*] and Enas Ali[1]**

*[1]Department of Biochemistry and Nutrition, Faculty of Women for Arts, Science and Education, Ain Shams University, Egypt.*

***Authors' contributions***

*Both authors participated in all parts of the research. Both authors read and approved the final manuscript*

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## ABSTRACT

**Aim:** To investigate the protective role of purslane (regla) on clozapine-induced toxicity in rats.
**Methods:** Fifty male Sprague–Dawley rats were divided into five groups (10 rats per group) as follows: Group1 (control group): Rats injected daily with buffered saline for 21 days. Group2 (cardiotoxic group): Rats injected with clozapine (15mg/kg body weight) for 21 days. Group3: Rats injected daily with clozapine (15mg/kg body weight) for 21 days + 10% of regla water seed extract. Group4: Rats injected daily with clozapine (15mg/kg body weight) for 21 days + 10% of regla dry leaves water extract. Group5: Rats injected daily with clozapine (15mg/kg body weight) for 21 days + 10% of regla juice.
**Results:** Serum marker enzymes as creatinine kinase, lactate dehydrogenase, AST and troponin T were estimated, as well as inflammatory markers; E-selectin, myeloperoxidase (MPO) and CRP. Caspase-3 and MDA were also estimated. The result showed that purslane seeds, dry leaves extract or juice have ameliorative effect on clozapine-induced cardiac toxicity in rats through decreasing serum CK, LDH, AST and Troponin enzyme activities, serum E-selectin, CRP, myeloperoxidase, caspase-3 and MDA levels compared to cardiotoxic group with purslane juice being the most effective treatment.

**Corresponding author: Email: Eman.Sharaf@women.asu.edu.eg;*

**Conclusion:** It can be concluded that purslane juice, dry water extract or seeds extract can be used for the protection of clozapine induced toxicity.

*Keywords: Cardiac toxicity; purslane; clozapine; rats.*

## 1. INTRODUCTION

Clozapine, a tricyclic dibenzodiazepine, belongs to the class of second-generation antipsychotics that are often called a typical antipsychotic. It has a strong affinity for D4-dopami-nergic receptors [1] and potent serotonergic, noradrenergic, histamine and cholinergic M2 receptor blocking activity. It differs from traditional antipsychotic drugs in that it has a relatively weak D2-receptor activity. However, some toxic effects of clozapine caused the Food and Drug Administration (FDA) to restrict its use, requiring close monitoring conditions and reserving the medication for treatment-resistant schizophrenia unresponsive to conventional antipsychotics. Among these adverse effects are agranulocytosis that occurs in about 1% of patients, venous thromboembolism and seizures [2]. A commonly reported and serious adverse effect of clozapine is its potential to induce cardiotoxicity and myocarditis. Myocarditis has been reported in many clinical case reports in young schizophrenic patients on clozapine therapy, without previous cardiovascular diseases. Myocardial ischemia, which may result from a clozapine-induced increase in catecholamine release, can lead to cell injury with the release of ROS. Cell injury in the ischemic area also causes infiltration of neutrophils, which produce oxidants and cytokines. Certain cytokines such as tumor necrosis factor-a (TNF-a) trigger the mitochondrial release of ROS. In addition, an increase in ROS have been detected in various animal models of heart failure. An increase in oxidative stress due to increased production of ROS, a relative deficit in the endogenous antioxidant reservoir, or both can cause myocarditis, contractile dysfunction and cardiomyopathy. Moreover, oxygen free radical damage has been implicated as a precipitating event in ischemia, overload, or drug-induced heart failure.

*Portulaca oleracea* L. (*P. oleracea*) is a commonly found species and a medicinal food for human consumption. Diverse compounds have been isolated from *Portulaca oleracea*, such as flavonoids, alkaloids, polysaccharides, fatty acids, terpenoids, sterols, proteins vitamins and minerals. *Portulaca oleracea* possesses a wide spectrum of pharmacological properties such as neuroprotective, antimicrobial, antidiabetic, antioxidant, anti-inflammatory, antiulcerogenic, and anticancer activities [3]. *Portulaca oleracea L.* (Portulacaceae) (P. oleracea), commonly known as purslane, is listed by the World Health Organization as one of the most used medicinal plants and has even been termed as “global panacea”. Research indicates that purslane is nutritious than the major cultivated vegetables due to its shoot that is a rich source of omega-3-fatty acids, α-tocopherols, ascorbic acid, β-carotene and glutathione [4]. These findings due to the anti-oxidative properties of purslane which derive from the following pharmacologically active substances, including: 28% flavonoids; 8% terpenoids; 6–12% organic acids; and > 0.5% proanthocyanidins defined as flavonoid-based polymers [5]. Based on these issues and concerns, the present study was designed to investigate the protective effect of purslane on cardiac toxicity in rats.

## 2. MATERIALS AND METHODS

### 2.1 Plant Materials

#### 2.1.1 Plant juice

Purslane leaves free of obvious defects were collected from local farm in Egypt during August 2020. An aqueous juice of the purslane herbs prepared by mashing in a proportion of 1:5 (w/v) and Left for about 24 h. After Mashing, the resulting crude extract was filtered and the filtrate was kept at 20°C for future use.

#### 2.1.2 Seed extract

One liter of boiled distilled water was added to 100 g of grinded purslane seeds, cooled and filtered. The extract was then concentrated to the desired volume according to [6].

#### 2.1.3 Dry leaves extract

Purslane leaves were collected and washed thoroughly in tap water to remove adhering dust, and the excess water was drained off. Twenty-five g of shade dried leaves of the plant was

finely powdered and extracted by maceration with (3×250 mL) of water at ambient temperature for 12 h with continuous stirring. The process was repeated twice. The aqueous extracts were filtrated with No. 1 Whatman filter paper and concentrated to dryness with a rotary evaporator at 50 ± 1°C to give solid residues. The yield was calculated to be 16.71 ± 0.7%. The dried extracts were kept in the dark at 4ºC prior analysis.

## 2.2 Animals and Experimental Design

Fifty male Sprague–Dawley rats weighing 170-200 g were obtained from the animal house of the National Research Institute, Elddoki, El-Giza, Egypt. All Rats were allowed acclimatization period of 7 days in an ambient temperature of 25-32°C on light/dark cycle of 12/12 hours. At the end of the acclimatization period, the experimental animals were divided into five groups (10 rats per group) as follows: Group1 (control group): Rats injected daily with buffered saline for 21 days. Group2 (cardiotoxic group): Rats injected with clozapine (15mg/kg body weight) for 21 days. Group3: Rats injected daily with clozapine (15mg/kg body weight) for 21 days + 10% of regla water seed extract. Group4: Rats injected daily with clozapine (15mg/kg body weight) for 21 days + 10% of regla dry leaves water extract.
Group5: Rats injected daily with clozapine (15mg/kg body weight) for 21 days + 10% of regla juice.

## 2.3 Blood Collection and Tissue Preparation

After the end of the experimental period, all animals were being fasted for 12 h then sacrificed under sodium pentobarbital anesthesia at a dose of 50 mg/kg b.wt (diluted to <10 mg/ml in saline) and given to rats by intra-peritoneal injection [7]. Blood samples were collected from the portal vein. Whole blood was collected in clean, dry tubes, left to coagulate at room temperature then centrifuged 15 minutes at 4000 rpm and they were kept in Eppendorf tubes and stored at -20ºC until the biochemical assay. At the same time, the heart from each rat was washed with normal saline and then kept fixed in neutral buffered formalin until the histopathological examination.

### 2.3.1 Determination of serum caspase-3

Caspase-3 was determined according to the method described by [8] Colorimetric kits were used to determine caspase-3 activity as recommended by the manufacturer (R&D Systems). The principle of the test is that degradation of a specific enzyme substrate by caspase-3 releases p-nitroaniline (pNA). Absorbance of pNA is measured by spectrophotometry at 405 nm, and pNA absorbance of different groups was compared with that of control group.

### 2.3.2 Determination of serum aspartate transaminase (AST) activity and lipid peroxidation

Aspartate transaminase (AST) activity in serum was assayed according to the method of [9], Serum lipid peroxidation was evaluated on the basis of Malondialdehyde (MDA) level using the method described by [10].

### 2.3.3 Determination of serum E-Selectin

E-Selectin was determined according to [11]. This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for rat E-Selectin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any E-Selectin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for rat E-Selectin is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of E-Selectin bound in the initial step. The color development is stopped and the intensity of the color is measured.

### 2.3.4 Determination of serum troponin T

Troponin T was determined according to [12]. A rabbit anti-cTnI polyclonal antibody is used for solid phase immobilization (on the microtiter wells). A goat anti-cTnT peptide-specific polyclonal antibody is conjugated to horseradish peroxidase (HRP) and used for detection. Samples (serum or plasma) and calibrators (200 µL) are pipetted into the microtiter wells and incubated for 2 hours on a plate shaker. After washing the wells, 100 µL of diluent and 100 µL of HRP-conjugated anti-cTnT are pipetted into each of the microtiter wells. The plate is incubated for one hour on a plate shaker. During this step, cTnT becomes sandwiched between the solid phase and HRP-conjugated antibodies.

The wells are then washed to remove unbound HRP conjugated antibodies. TMB, an HRP substrate (100 µL), is then added and incubated for 20 minutes, resulting in the developmnt of a blue color. The color development is stopped by the addition of 1N HCl (100 µL), changing the color to yellow, and absorbance at 450 nm is measured. The concentration of cTnT is proportional to the absorbance at 450 nm and is derived from a calibration curve.

#### 2.3.5 Determination of CK-MB and LDH Activities

Creatinine kinase (CK-MB) activity was estimated in serum according to the method of [13] using diagnostic kit. The increase in absorbance at 340 nm is measured spectrophotometrically to calculate CK-MB level as (U/L). LDH activity was determined using diagnostic kit. The increase in absorbance was measured spectrophotometrically at 340 nm at 1-min intervals for 3 min. Serum total LDH activity was calculated as (U/L) according to the method of [14].

### 2.4 Histopathological Examination

heart was dissected and fixed at 10% neutral formalin. Fixative tissues were processed and stained with hematoxylin and eosin (H&E) and examined under a light microscope.

### 2.5 Statistical Analysis

The data were presented as means ± SE. One-way analysis of variance (ANOVA) was performed using the statistical package for social science (SPSS) version 16 to compare all treated groups. Differences were considered to be significant when ($p < 0.05$).

## 3. Results

### 3.1 Serum Marker Enzymes

As shown in Table (1), there were significant elevations in serum CK, LDH, AST and Troponin enzyme activities ($p < 0.05$) in cadiotoxic rats compared with control group, in addition there were significant decrease in serum CK, LDH, AST and Troponin T enzyme activities ($p < 0.05$) in cadiotoxic rats+purslane seeds, cariotoxic rats+purslane leaves and cardiotoxic rats+purslane juice compared with cadiotoxic group. Purslane juice treated group being the more effective than purslane seeds or purslane leaves extracts.

### 3.2 Inflammatory Biomarkers

Table (2) showed that, clozapine-induced toxicity in rats caused significant increase ($P<0.05$) in serum E-selectin**,** CRP and myeloperoxidase compared with control group. Treatment of intoxicated rats by purslane seeds, dry leaves and purslane juice caused significant decrease in serum E-selectin, CRP and myeloperoxidase levels compared to cardiotoxic group with purslane juice being the most effective treatment.

### 3.3 Serum Caspase-3 and MDA Levels

As shown in Table (3), there were significant elevations in serum caspase-3 and MDA levels ($p < 0.05$) in cadiotoxic rats compared with control rats. Purslane treatment either seeds extract, dry seeds extract or juice caused significant decrease in serum caspase-3 and MDA levels. Purslane dry leaves extract is the most effective treatment in reducing caspase-3 level, while purslane juice is the most effective in reducing MDA level.

**Table 1. Effect of purslane consumption (seeds extract, dry leaves extract or juice) on serum cardiac enzymes**

| Parameters / Groups | Creatine kinase (U/l) | LDH (U/l) | AST (U/l) | Troponin |
|---|---|---|---|---|
| Control group | 47.93±1.97 [a] | 80.90±2.15 [a] | 40.51±1.26 [a] | 384.36±18.26 [a] |
| Cardiotoxic group | 208.05±8.00 [e] | 217.11±4.89 [e] | 75.38±4.21 [e] | 1618.36±85.31 [e] |
| Cardiotoxic+purslane seeds extract | 144.06±7.31 [c] | 137.03±5.90 [c] | 56.53±2.30 [c] | 1412.66±35.79 [d] |
| Cardiotoxic+purslane dry leaves extract | 159.15±5.66 [d] | 168.30±4.86 [d] | 62.91±1.85 [d] | 1358.11±6.77 [c] |
| Cardiotoxic+purslane juice | 107.81±4.26 [b] | 106.13±8.53 [b] | 49.08±1.63 [b] | 1260.52±32.32 [b] |

*Values are represented as mean ± SD, there was no significant difference between means have the same letter in the same column (P<0.05)*

**Table 2. Inflammatory biomarkers**

| Parameters / Groups | E-selectin | CRP (ng/ml) | Myeloperoxidase |
|---|---|---|---|
| Control group | 384.36±18.26[a] | 6.83±0.18 [a] | 78.21±8.09 [a] |
| Cardiotoxic group | 1618.36±85.31[e] | 11.18±0.42 [d] | 161.08±7.06 [d] |
| Cardiotoxic+purslane seeds extract | 1412.66±35.79[d] | 8.88±0.40 [b] | 124.53±2.67 [c] |
| Cardiotoxic+purslane dry leaves extract | 1358.11±6.77[c] | 10.02±0.43[c] | 110.68±2.26 [b] |
| Cardiotoxic+purslane juice | 1260.52±32.32 [b] | 8.57±0.41 [b] | 115.18±1.52 [b] |

*Values are represented as mean ± SD, there was no significant difference between means have the same letter in the same column (P<0.05)*

**Table 3. Serum caspase-3 and MDA levels**

| Parameters / Groups | Caspase-3 | MDA (µM) |
|---|---|---|
| Control group | 1.41±0.17[a] | 1.28±0.09 [a] |
| Cardiotoxic group | 5.37±0.21[d] | 2.18±0.12[e] |
| Cardiotoxic+purslane seeds extract | 4.13±0.21[c] | 1.74±0.09[c] |
| Cardiotoxic+purslane dry leaves extract | 3.79±0.15[b] | 1.87±0.07[d] |
| Cardiotoxic+purslane juice | 4.02±0.15[c] | 1.62±0.05[b] |

*Values are represented as mean ± SD, there was no significant difference between means have the same letter in the same column (P<0.05)*

### 3.4 Histopathological Examination of Cardiac Tissue

Histopathological studies of cardiac sections of clozapine-treated animals showed evidence of myocarditis and myocardial cellular infiltration in cardiac sections of clozapine-treated rats compared to control rats. These changes are in the form of focal subendocardial fibrosis with marked interstitial oedema and perinuclear vacuolation. Inflammatory lesions were found in both the left and right ventricles, primarily in the myocardium below the endocardium of the left ventricle, in the posterior papillary muscle of the left ventricle and in the septum, consistent with myocarditis. Treatment of toxic rats with regla juice or seeds extract showed a marked improvement of cardiac legions (Fig.1-11).

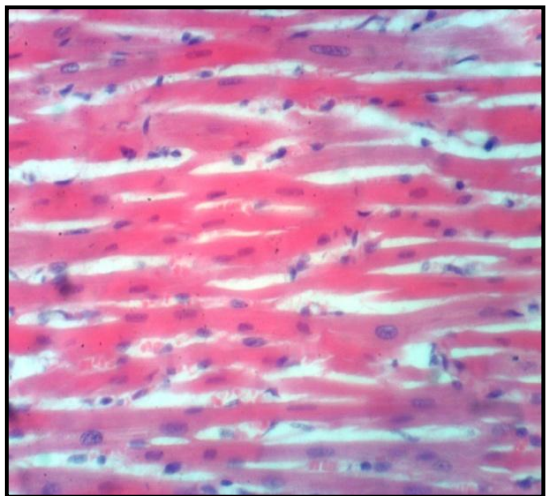

**Fig. 1. Heart of rat from group 1 showing normal cardiac myocytes (H & E X 400)**

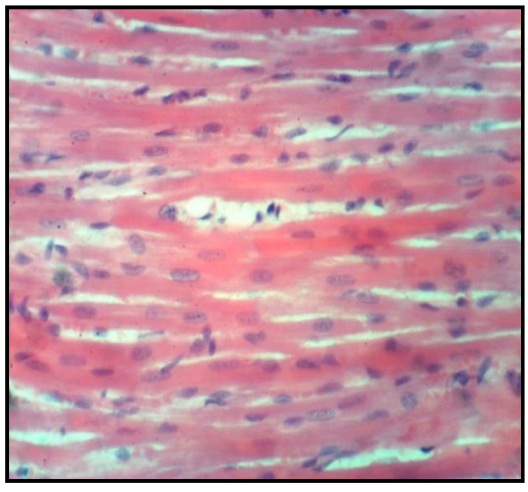

**Fig. 2. Heart of rat from group 1 showing normal cardiac myocytes (H & E X 400)**

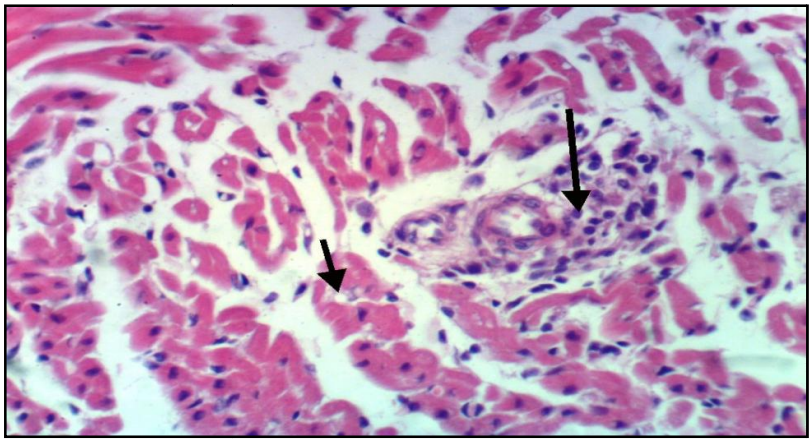

**Fig. 3. Heart of rat from group 2 showing vacuolation of cardiac myocytes and perivascular inflammatory cells infiltration (H & E X 400)**

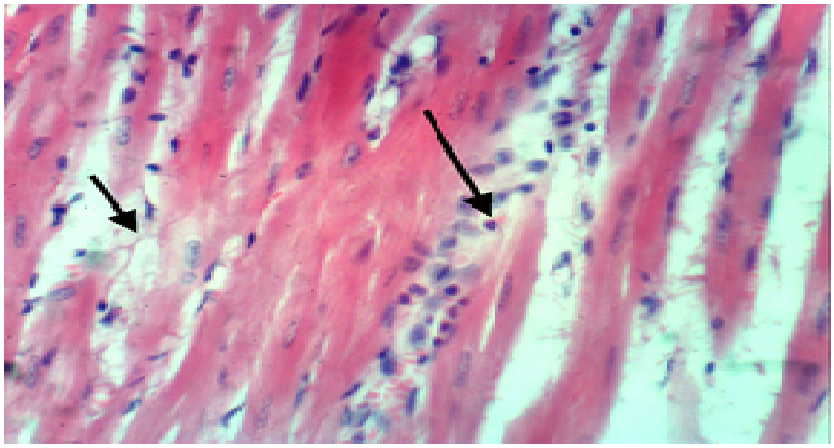

**Fig. 4. Heart of rat from group 2 showing intermuscular oedema associated with inflammatory cells infiltration (H & E X 400)**

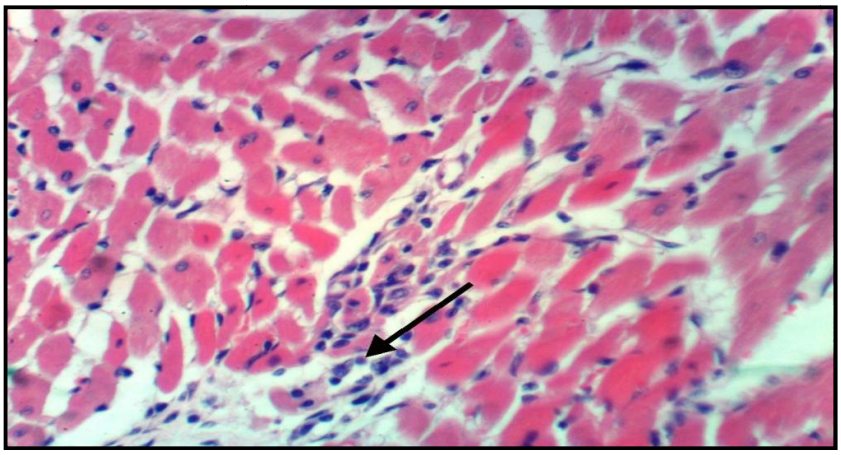

**Fig. 5. Heart of rat from group 2 showing focal necrosis of cardiac myocytes associated with inflammatory cells infiltration (H & E X 400)**

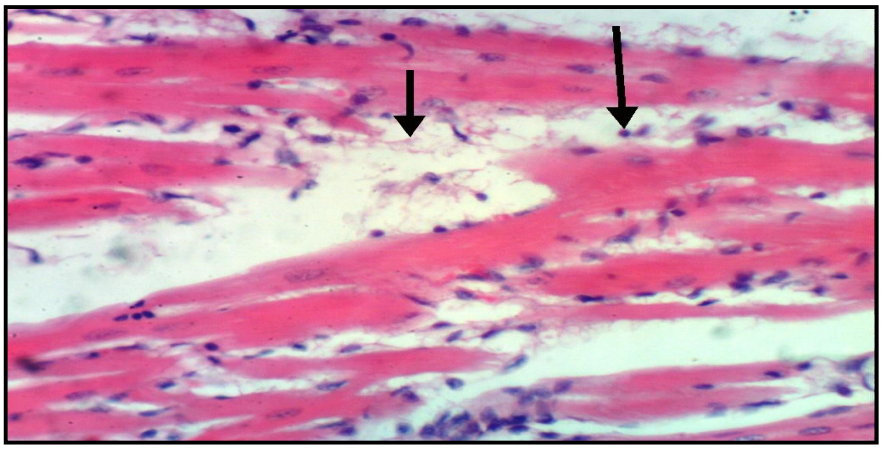

**Fig. 6. Heart of rat from group 3 showinginteramuscular oedema associated with few inflammatory cells' infiltration (H & E X 400)**

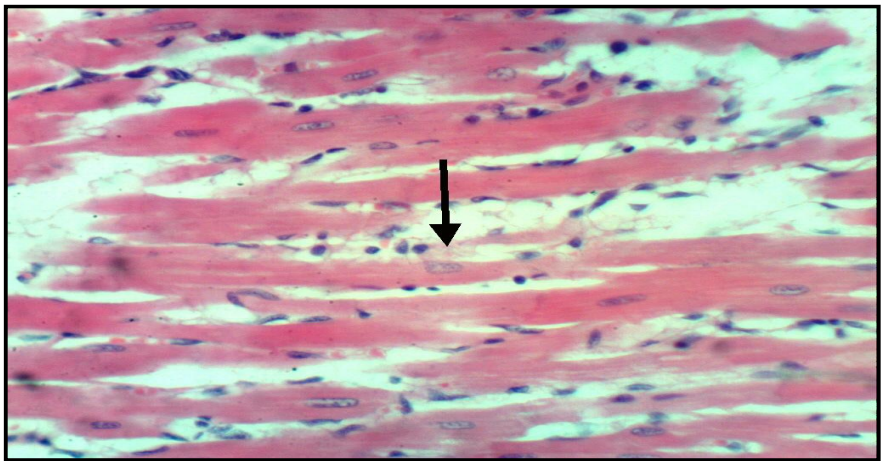

**Fig. 7. Heart of rat from group 3 showing slight interamuscular oedema (H & E X 400)**

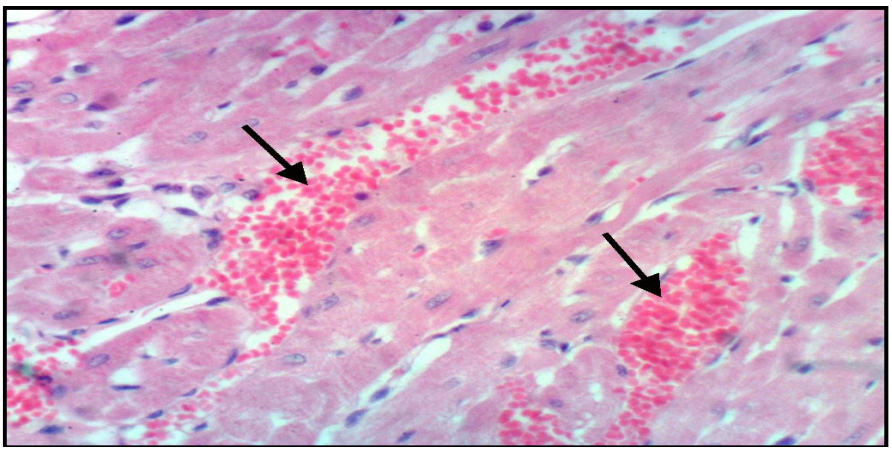

**Fig. 8. Heart of rat from group 4 showing dilatation and congestion of myocardial blood vessels (H & E X 400)**

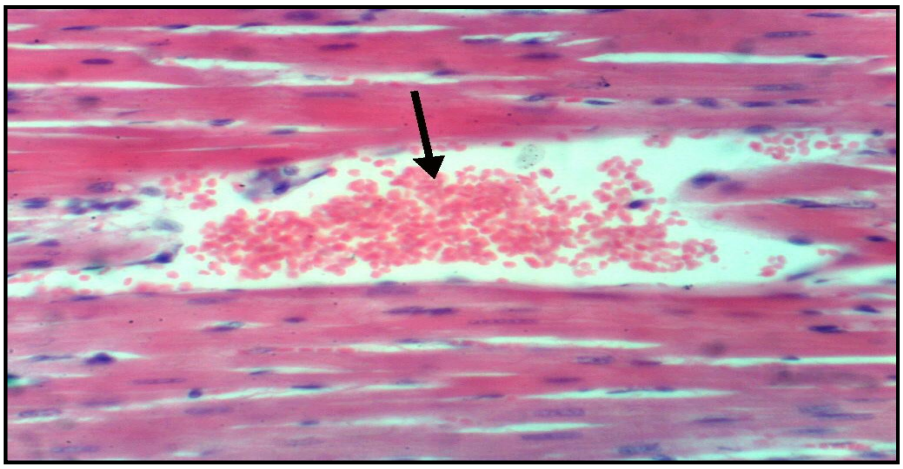

**Fig. 9. Heart of rat from group 4 showing dilatation and congestion of myocardial blood vessels (H & E X 400)**

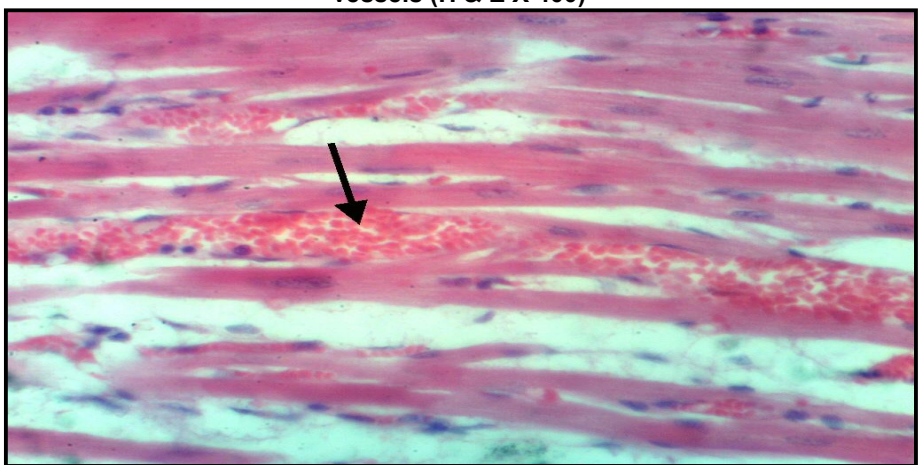

**Fig. 10. Heart of rat from group 5 showing dilatation and congestion of myocardial blood vessels (H & E X 400)**

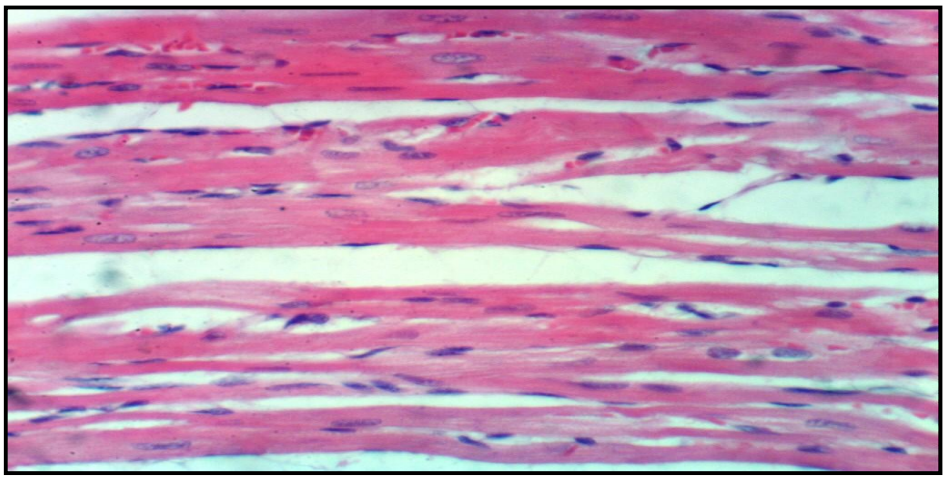

**Fig. 11. Heart of rat from group 5 showing no histopathological changes (H & E X 400)**

## 4. DISCUSSION

Clozapine is a typical antipsychotic drug selectively effective in the treatment of refractory schizophrenia. However, myocarditis, as a serious cardiotoxic effect, has in several case reports been associated with clozapine therapy. This cardiotoxic effect was confirmed by elevation in the activities of serum aspartate amino transferase (AST), lactate dehydrogenase (LDH) and creatinine kinase (CK-MB). Elevation of serum AST, LDH and CK-MB enzymes are considered important markers of early and late cardiac injury [2]. In the present study, the serum concentrations of AST, CK-MB and LDH increased significantly following clozapine administration. It believed that the increase of aminotransferase concentrations is due to their enhanced release from damaged or dead cells and not to an increased enzyme synthesis. AST is found in skeletal, cardiac muscle, brain and many other organs. LDH is also a general marker of tissue injury [15].

Among various hypotheses of clozapine-induced cardiotoxicity, the results of [16] proposed that clozapine induced myocarditis may result from a type IgE-mediated acute hypersensitivity reaction. This is confirmed by the onset of clozapine-induced myocarditis, which commonly includes peripheral eosinophilia and eosinophilic myocardial infiltrates [17].

The results of the present study are consistent with the results of [2] that showed an increase in cardiac MPO level, which is an index of neutrophil migration, in clozapine-treated animals. Activated eosinophils induce tissue injury and necrosis through the production and release of reactive oxygen metabolites and cytotoxic proteins (e.g., proteases and MPO) into the extracellular fluid [18]. The possible explanation of this hypothesis comes from the fact that clozapine undergoes bioactivation in myocardial tissue to a chemically reactive nitrinium ion metabolite, which stimulates cellular injury, lipid peroxidation and free radical production [19], which is in agreement with our results.

The present results showed an increase in expression of caspase-3 in cardiac tissues of clozapine-treated animals which is in accordance with the results of [2]. Caspase-3 is an important marker of apoptosis, and this finding indicates that the clozapine-induced cardiotoxicity can lead to apoptosis of cardiac cells. This can be attributed to the observed increase in oxidative stress with attenuation of antioxidant defenses and the consequent cellular and DNA damage.

Purslane is best used for human consumption as a green vegetable rich in minerals and Omega-3 fatty acids [20]. Omega-3 fatty acid is a precursor of a specific group of hormones (prostaglandins) and may offer protection against cardiovascular disease, cancers and a number of chronic diseases and conditions throughout the human life.

In our study, purslane treated rats showed an increase in the activities of antioxidant enzymes with a reduction in lipid peroxidation and free radical scavenging activity. Purslane has been described as a “power food of the future because of its high nutritive and antioxidant properties. Antioxidants work by neutralizing or scavenging free radicals by hydrogen donation before they are able to attack cells and other biological components [21]. Purslane has the propensity to attenuate oxidative stress by reversing the inhibition of Na+/K+ ATPase activity [22]. The decreased activity of the liver enzymes, ALT, AST, γ-GT and ALP in purslane treated group, indicates its protective role against liver damage [19].

The results of [2] showed that clozapine-induced cardiotoxicity was associated with marked elevation of myocardial levels of the lipid peroxidation product (MDA), with reduction of GSH content and activity of the antioxidant enzyme GSHPx. Therefore, these results give evidence for the concept that increased oxidative stress and weakness of antioxidant defenses play an important role in clozapine-induced myocarditis [23].

According to the present results, P. oleracea juice, dry leaves or seeds extract has antioxidant properties which possess cardioprotective properties with purslane juice being the most effective. According to the results of [24] purslane extract was found to reduce blood pressure in a dose-dependent manner but had no beneficial effect on tachycardia. These results demonstrated that P. oleracea hydro-alcoholic extract reduces blood pressure and tissue level of MDA. Purslane was found to contain high concentrations of β- carotene, B2, C and E and is very rich in magnesium, zinc and other trace elements; which act as antioxidants and have been found to be useful in preventing toxicant-induced tissue injury [25]. Also, the study of [26]

demonstrated that aqueous purslane juice possesses a potent hepatoprotective action upon CCl4-induced hepatic damage in rats and the ability of purslane juice to decrease oxidative stress in rat liver, as evidenced by the very highly significant decrease of lipid peroxidation product; and a very highly significant rise of endogenous antioxidants GSH, SOD and CAT This may be due to its antioxidant activity with its ability to scavenge free radicals and inhibit lipid peroxidation.

## 5. CONCLUSION

From the results of the present study, it can be concluded that, purslane seeds, dry water extract or juice have ameliorative effect on clozapine-induced cardiac toxicity in rats due to the anti-oxidative and anti-inflammatory properties of purslane.

## AVAILABILITY OF DATA AND MATERIALS

The datasets supporting the conclusions of this article are included within the article and its additional files.

## CONSENT

It is not applicable.

## ETHICS APPROVAL

The experiment was designed and conducted according to bioethics approved by the animal care use committee (IACUC) of Faculty of Science, Cairo University NO: 1307 in /3/5/2017.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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