

# Ameliorating effect of *Brassica oleracea* (broccoli) extract on the myocardial damage induced by polycystic ovary syndrome in the adult female albino rat

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## Abstract

Polycystic ovary syndrome (PCOS) is one of the most common and complex hormonal disorders affecting the females in the childbearing period. It is associated with several comorbidities recognized as cardiovascular risk factors. This study investigated, for the first time to our knowledge, the myocardial structural changes in experimental PCOS rat model and the ameliorating effects of *Brassica oleracea* (broccoli) extract. Forty adult female rats were divided into four groups; control, broccoli extract treated, PCOS and PCOS treated with broccoli extract. PCOS was induced by a single intramuscular injection of estradiol valerate (EV) (16mg/kg). Four weeks after EV injection, broccoli extract (1g/kg dissolved in distilled water) was given orally once daily for 4 weeks. At the end of the experimental period, blood samples were collected. The myocardial specimens were processed for biochemical, histological and immunohistochemical studies. PCOS group showed oxidative stress and dyslipidemia. The myocardium exhibited many degenerative changes with significant increase in the cross-sectional area of cardiac myofibers and collagen deposition. Significant upregulation of androgen receptor, caspase-3, and glucose transporter 1 (GLUT1) with downregulation of desmin and heat shock protein 70 (Hsp70) immunoreaction was also noted. Broccoli extract exerted cardioprotective effects in PCOS through antioxidant and antiandrogen activities. The results of this work may throw more light on the myocardial damage associated with PCOS and provide a new insight into the possible use of broccoli extract to alleviate these effects.

**Keywords:** Myocardium, PCOS, Broccoli, Histopathology, Immunohistochemistry, Biochemistry

## Introduction

Polycystic ovary syndrome (PCOS) is the most widely recognized endocrinopathy affecting females of reproductive age with a prevalence rate of 6-10% [1]. It is no longer considered as a straightforward disorder constituting predominantly the domain of gynecologists, it is currently perceived as a metabolic issue that put the affected women at a higher risk of developing type 2 diabetes and heart diseases than the general population. Women with PCOS are exposed to cardiovascular risk factors and the syndrome may elucidate a significant proportion of heart disease diagnosed in women [2,3].

The etiology of PCOS is uncertain, but hyperinsulinemia and

hyperandrogenemia constitute the two principal features of this disease [4]. These characteristics can have significant associations with oxidative stress and greater cardiovascular risk [5]. Abnormal production of free radicals increases the stress on the cellular level with induction of changes in the molecular pathways that in turn underpins the pathogenesis of several diseases including cardiovascular disease (CVD) [6-8].

Glucose is a considerable source of metabolic energy for mammalian cells and can control gene transcription, enzyme activity, and hormone secretion [9]. Heart consumes more energy than other organs [10]. The lipid bilayer of plasmalemma is impermeable for glucose. In this way, glucose transport over

the plasma layer is mediated by means of glucose transporters (GLUTs). Insulin triggers glucose uptake by increasing the translocation of these transporters [11,12].

Heat shock proteins (Hsps) include several classes of functionally related families of proteins found in all organisms under normal physiological conditions. However, many types of environmental stress factors have been shown to stimulate synthesis of such proteins and hence Hsps are also called stress proteins [13,14]. They were found to have important roles in protecting cells against cellular stressors including heat [15,16], hypoxia [17], bright illumination [18] and oxidative pressure [19]. Moreover, Hsp70 was considered to have important impact in protection against stress-induced heart cell damage such as ischemia-reperfusion injury and cardiac infarctions [20].

Broccoli, a member of the Brassicaceae family, is rich in vitamins, minerals, dietary fiber, flavonol glycosides, hydroxycinnamic acids and sulphur-containing compounds [21,22]. Sulforaphane (SFN), a naturally-occurring sulphur-containing compound, abundant in broccoli and cabbage, has antioxidant and anti-inflammatory properties. So, it has beneficial effects in CVD [23]. In addition to the antioxidant, anticancer and anti-inflammatory activities of broccoli, it was postulated that it has an antiandrogen activity [24].

Currently, plant extracts are being widely used in the treatment of some female reproductive disorders [25,26]. A recent study by Nofal et al. [27] has concluded that broccoli extract presented beneficial effects on hormonal indices in a rat model of PCOS. Also, it exerted strong antioxidant potentials, promoted healthy follicles and regained the pleura of follicles. So, we were encouraged to study its possible cardioprotective effects in PCOS.

Taken together, this work was designed, for the first time to the best of our knowledge, to investigate the reflection of the main etiological factors of PCOS on the histological structure of the myocardium and whether broccoli extract can exert cardioprotective effects.

## Materials and Methods

### Chemicals

Estradiol valerate (EV), a product of (Bayer Weimar GmbH und Co. KG Weimar- Germany a subsidiary of: Baer pharma AG, Germany), was obtained from El-Ezaby pharmacy- Cairo in the form of tablets, each containing 2 mg of EV. The tablets were crushed and dissolved in distilled water to be given by intramuscular injection.

### Plant extract

Broccoli was purchased from the market. The plant material was authenticated and extracted in Botany Department, National Research Center, El-Doki, Cairo, Egypt. Fresh broccoli florets were cleaned with tap water to remove the dirt adhering to them and were spread on tissue paper to absorb excess surface water. The plants were dried and powdered in a mechanical

grinder. The powdered material was extracted successively in 80% ethanol using a Soxhlet apparatus at 45°C for 24 h. The extract was concentrated in vacuo and kept in a vacuum desiccator for complete removal of solvent [28].

### Animals

Forty healthy adult female albino rats at an average weight 180-200 g were obtained and housed at Theodor Bilharz Research Institute Animal House, Cairo, Egypt. The rats were kept in metallic cages at room temperature to keep them at healthy conditions at light/dark cycle for 12 h with free access to a standard palletized diet and water. The experiment was carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). All procedures were conducted in accordance with the guidelines approved by the Committee of Animal Research Ethics, Faculty of Medicine, Menoufia University.

### Induction of PCOS

Polycystic ovary syndrome (PCOS) was induced by a single intramuscular injection of estradiol valerate (EV) (16 mg/kg) [29] dissolved in 0.2 ml distilled water. During the experiment, the estrous cycle phases were monitored by the analysis of relative proportion of leukocytes, epithelial and cornified cells. The rats were allowed 4 weeks to establish PCOS. Cessation of cyclicity, which was shown by the persistent cornification of vaginal smears, was used as a criterion for selection of the PCOS group [30].

### Experimental design

All rats were checked daily for ovarian cycle using vaginal smear for determination of the estrous cycle phases. Vaginal contents were swapped using an ear cotton rod soaked in normal saline (NaCl 0.9%). Vaginal swap was placed on a glass slide. Unstained material was observed under the light microscope [31]. Rats with regular cycle were chosen for the experiment.

After one week of adjustment to the new environment, the rats were divided equally into four experimental groups (10 rats each);(i) control, (ii) broccoli extract treated (1g/kg dissolved in distilled water given once daily orally by gavage for 4 weeks), (iii) PCOS induction and (iv) PCOS treated with broccoli extract (4 weeks after EV injection, broccoli extract was given at the same dose, route of administration and duration as group ii).. At the end of the experiment, the rats were anesthetized by inhalation of pentobarbital overdose (200mg/kg), blood samples were collected from retroorbital venous plexus. A midline thoraco-abdominal incision was performed, and the heart of each rat was dissected out and washed with physiological saline. Specimens from the left ventricular tissue near the apex were taken. Each specimen was divided into two parts: one part was fixed in 10% neutral buffered formalin for light microscopic study and the second part was immediately frozen at -70°C to be used for tissue

biochemical study.

### Evaluation methods

#### Biochemical analysis

Serum levels of total testosterone, total cholesterol (TC), Triglycerides (TG), High density lipoprotein (HDL) and low density lipoprotein (LDL) were analyzed using commercially available kits.

Cardiac tissue was homogenized in 5-10 ml cold buffer (i.e. 50 mM potassium phosphate, pH 7.5. 1 mM EDTA). Homogenate was centrifuged at 10000 × g for 15 minutes at 4°C. The supernatant was used for the measurement of tissue superoxide dismutase (SOD), according to the methods of Kono [32]. Meanwhile, the concentration of malondialdehyde (MDA) was measured as an index of lipid peroxidation [33].

#### Histological and immunohistochemical studies

Cardiac tissue samples were processed routinely by embedding in paraffin and sectioned into 5 µm thick sections to be stained with hematoxylin & eosin for routine histological assessment and Mallory trichrome stain for detection of collagen deposition.

For immunohistochemical study, the paraffin sections on poly-L-lysine coated slides were deparaffinized and rehydrated. Endogenous peroxidase was blocked by inserting the sections in 3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The microwave antigen retrieval procedure was performed. The sections were incubated with primary anti androgen (rabbit monoclonal, Abcam, 1:500), anti HSP70 antibody (mouse monoclonal, Abcam, 1:100), anti caspase-3 antibody (rabbit polyclonal, Lab Vision, USA, 1:500), anti desmin (mouse monoclonal, Thermo Fisher Scientific Industries, 1:100) and anti GLUT-1 antibody (rabbit monoclonal, Abcam, 1:250). After that, biotinylated goat-polyvalent secondary antibody was applied. The sections were then incubated in preformed streptavidin peroxidase and finally the prepared DAB substrate chromogen (3,3'-diaminobenzidine tetrahydrochloride) was applied and the slides were counterstained with hematoxylin to be examined under light microscope.

#### Quantitative assessment

Using Image J software, version K 1.45, the following parameters were measured:

1. The cross-sectional area of cardiac myocytes and area % of collagen deposition.
2. The area % of caspase-3, HSP70, desmin and GLUT 1 immunoreaction.
3. The number of androgen receptor positive cells.

For each parameter, ten non-overlapping fields (40 x) for every specimen were examined.

#### Statistical analysis

The data were collected, tabulated and analyzed by SPSS (statistical package for social science) version 23.0 on IBM

compatible computer (SPSS Inc., Chicago, IL, USA). The obtained data were analyzed using one way-ANOVA followed by post hoc Bonferroni test. A p value of <0.05 was considered statistically significant and P value >0.05 was considered non-significant.

### Results

There was a non-significant difference between the control group and the broccoli extract treated group throughout all the examined parameters. So, they were pooled in a one "control" group.

#### Biochemical results

##### Serum testosterone level and lipid profile

Estradiol valerate induced PCOS caused significant increase in serum level of total testosterone, triglyceride (TG), total cholesterol (CH), low density lipoprotein (LDL) and significant decrease in high density lipoprotein (HDL) as compared to the control group. Polycystic ovary group treated with broccoli extract exhibited a significant decrease in the serum level of total testosterone, TG, CH and LDL and a significant increase in the level of HDL compared to PCOS group (Table 1).

**Table 1. Serum testosterone level and lipid profile of the different groups.**

Serum level	Control	PCOS	PCOS treated with broccoli extract
Total testosterone (ng/dl)	3.61±0.48	117.18±4.34 a***	18.27±2.10 b***
Triglyceride (mg/dl)	61.10±3.07	137.10±4.48 a***	70.00±3.39 b***
Total cholesterol (mg/dl)	79.50±3.43	119.20±5.13 a***	87.80±2.69 b***
Low density lipoprotein (mg/dl)	33.90±3.84	59.00±4.47 a***	40.80±2.48 b***
High density lipoprotein (mg/dl)	40.50±3.97	16.70±2.05 a***	31.90±2.68 b***

a\*\*\*P<0.001 PCOS vs control.

b\*\*\*P<0.001 PCOS treated with broccoli extract vs PCOS.

#### Tissue malondialdehyde and superoxide dismutase levels

The myocardium of the PCOS group showed a significant increase in the malondialdehyde (MDA), level and a significant decrease in the level of superoxide dismutase (SOD) compared to that of the control group. On contrary, the PCOS group treated with broccoli extract revealed a marvelous beneficial effect via a significant decrease in the MDA and a significant increase in the SOD level within the myocardium when compared to that of the PCOS untreated group (Table 2).

#### Histological results

##### Hematoxylin and eosin (H&E)

Hematoxylin and eosin (H&E) stained sections of the different experimental groups were examined under light microscope. The control group showed organized branching and anas-



**Table 2. Tissue level of MDA and SOD in the different groups.**

Tissue Level	Control	PCOS	PCOS treated with broccoli extract
MDA (nmol/mg)	1.25±0.17	5.99±0.54 a***	2.72±0.31 b***
SOD (U/mg)	38.35±1.41	9.65±0.77 a***	32.16±1.28 b***

a\*\*\*P<0.001 PCOS vs control.

b\*\*\*P<0.001 PCOS treated with broccoli extract vs PCOS.

tomosing cardiac muscle fibers with acidophilic sarcoplasm, single, oval and centrally located nuclei of cardiomyocytes, and intact intercalated discs. Moreover, flat dark nuclei of the fibroblasts were observed in the interstitial tissue (Figure 1a). On the other hand, PCOS group displayed disruption of the cardiac structure in the form of fragmented degenerated fibers with rarified vacuolated sarcoplasm. In addition, widely separated myofibers with homogenous highly acidophilic sarcoplasm (hyalinized), myocytolysis, congested dilated blood vessels, extravasation, pyknotic nuclei and inflammatory infiltrate were noted (Figures 1b-1e). These changes were ameliorated in the PCOS group treated with broccoli extract except for slight separation of some muscle fibers (Figure 1f).

### Statistical results of cross-sectional area

Statistically, there was a significant increase (P<0.001) in the cross-sectional area (µm<sup>2</sup>) of the cardiomyocyte of the PCOS group compared to that of the control group (271.41±11.56 vs 31.38±2.07). The PCOS group treated with broccoli extract revealed a dramatic decrease (P<0.001) in the cross-sectional area (µm<sup>2</sup>) of the cardiomyocyte compared to that of the PCOS group (41.95±1.80 vs 271.41±11.56) (Figure 2).

### Mallory trichrome (area% of collagen deposition)

Mallory trichrome stained sections of the different experimental groups revealed a significant increase (P<0.001) in the area % of collagen deposition within the myocardium of the PCOS group stained sections compared to the control group (49.72±1.08 vs 17.21±0.91). On the other hand, the area % of collagen deposition was significantly decreased (P<0.001) in the PCOS group treated with broccoli extract compared to that of PCOS (17.42±1.00 vs 49.72±1.08) (Figures 3 and 4)

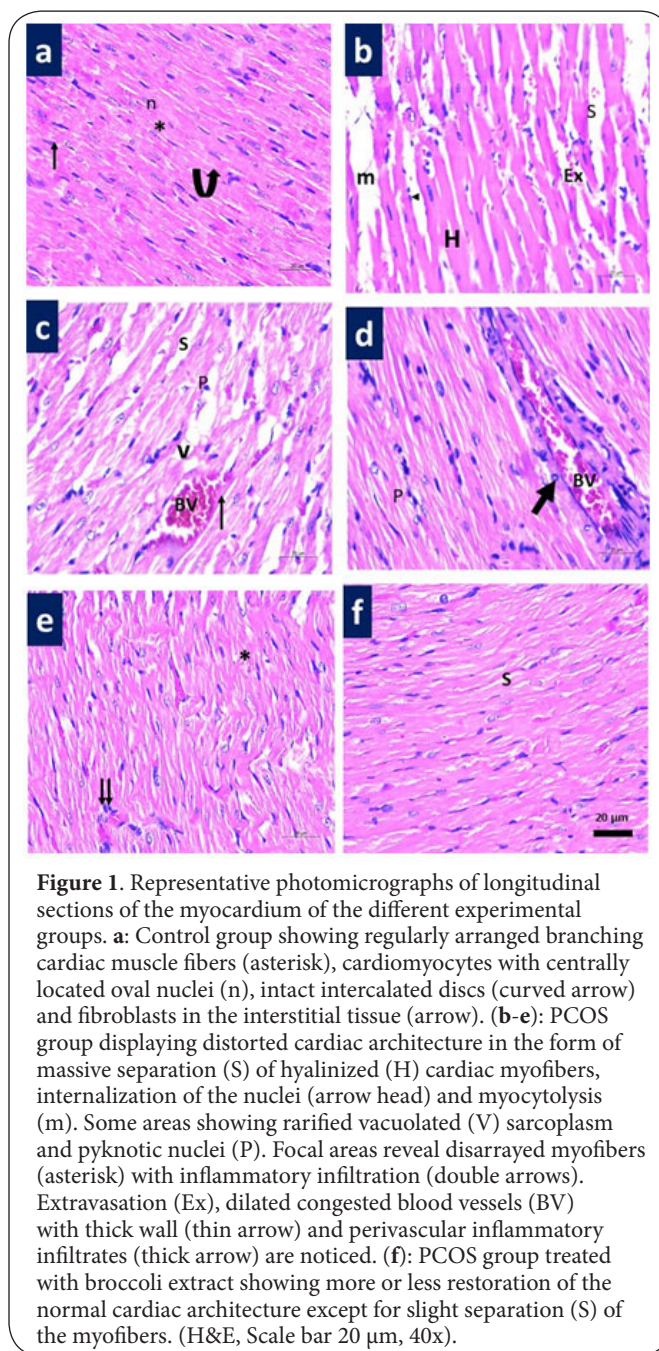
### Immunohistochemical results

#### Androgen receptor immunoreaction

The number of positive immunoreactive androgen receptor was significantly upregulated (P<0.001) in the myocardium of PCOS group compared with the control group (38.20±2.57 vs 4.00±1.15). In contrast, a significant downregulation (P<0.001) was observed in PCOS group treated with broccoli extract compared with that of PCOS group (5.30±1.33 vs 38.20±2.57) (Figure 5a-5c and Figure 6).

#### HSP70 immunoreaction

There was a significant decrease (P<0.001) in the mean area%

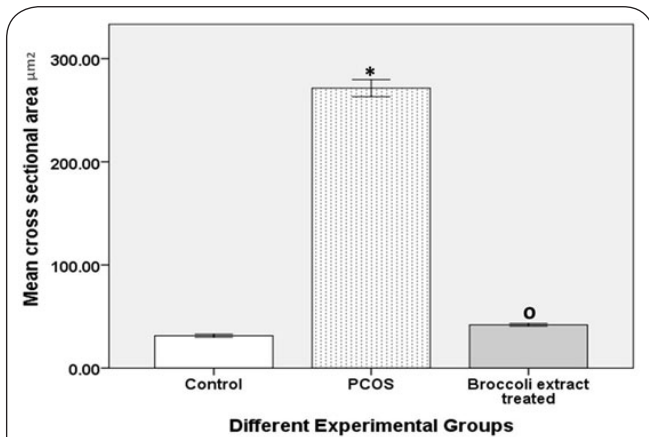


**Figure 1.** Representative photomicrographs of longitudinal sections of the myocardium of the different experimental groups. **a:** Control group showing regularly arranged branching cardiac muscle fibers (asterisk), cardiomyocytes with centrally located oval nuclei (n), intact intercalated discs (curved arrow) and fibroblasts in the interstitial tissue (arrow). **(b-e):** PCOS group displaying distorted cardiac architecture in the form of massive separation (S) of hyalinized (H) cardiac myofibers, internalization of the nuclei (arrow head) and myocytolysis (m). Some areas showing rarified vacuolated (V) sarcoplasm and pyknotic nuclei (P). Focal areas reveal disarrayed myofibers (asterisk) with inflammatory infiltration (double arrows). Extravasation (Ex), dilated congested blood vessels (BV) with thick wall (thin arrow) and perivascular inflammatory infiltrates (thick arrow) are noticed. **(f):** PCOS group treated with broccoli extract showing more or less restoration of the normal cardiac architecture except for slight separation (S) of the myofibers. (H&E, Scale bar 20 µm, 40x).

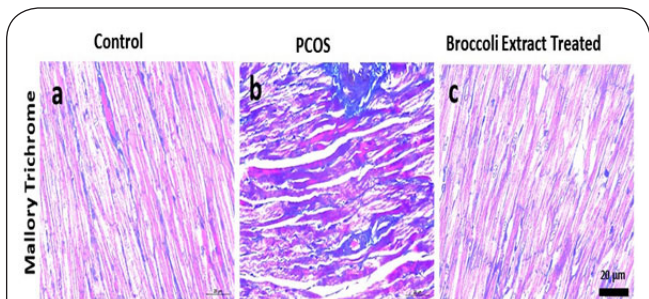
of HSP70 in the PCOS group compared with the control group (16.61±1.90 vs 44.56±2.96) that was dramatically increased (P<0.001) in PCOS group treated with broccoli extract compared to the PCOS group (37.00±1.87 vs 16.61±1.90) (Figure 5d-5f and Figure 6).

#### Caspase-3 immunoreaction

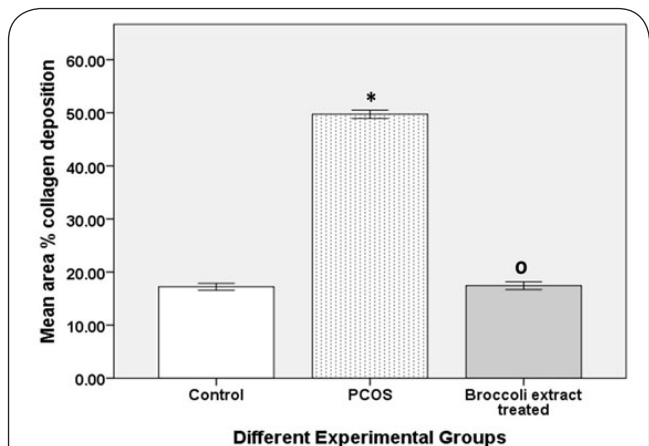
Moreover, in PCOS group, area % of caspase-3 immunoreaction, a marker for apoptosis, was significantly upregulated (P<0.001) compared with the control group (36.69±2.06 vs 5.05±0.51). On the other hand, a significant downregulation (P<0.001) in



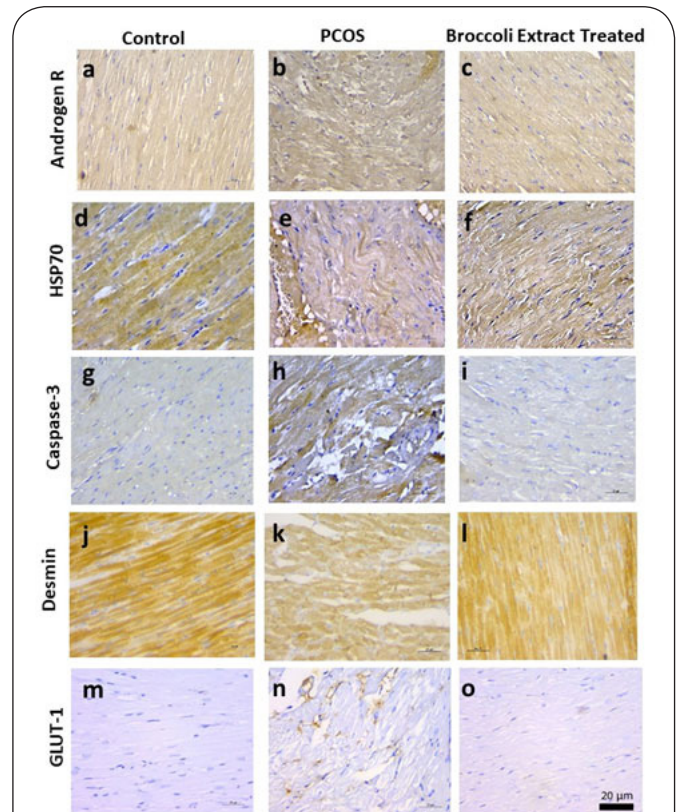
**Figure 2.** A histogram showing a significant increase ( $P<0.001$ ) in the cross-sectional area of the cardiomyocytes in PCOS group (\*) compared to the control group and a significant decrease ( $P<0.001$ ) in the PCOS group treated with broccoli extract (o) compared to the PCOS group.



**Figure 3.** Representative photomicrographs of the different experimental groups showing increased collagen deposition in the myocardium of the PCOS group (b) compared to that of the control (a) and PCOS group treated with broccoli extract (c) (Mallory Trichrome, Scale bar 20 µm, 40x).



**Figure 4.** A histogram revealing a significant increase ( $P<0.001$ ) in the collagen deposition within the myocardium of the PCOS group stained sections compared to the control group (\*) and a significant decrease ( $P<0.001$ ) in the PCOS group treated with broccoli extract compared to that of PCOS (o).



**Figure 5.** Representative immunostaining of rat myocardium of the different experimental groups revealing obvious increase of androgen receptor (a,b), Caspase-3 (g,h) and GLUT-1 (m,n) and decrease of HSP70 (d,e) and desmin (j,k) immunoeexpression in the PCOS group compared to control group. These changes were dramatically reversed with broccoli extract treatment (c,f,i,l&o). (Scale bar 20 µm, x 40).

PCOS group treated with broccoli extract compared to the PCOS group ( $7.86\pm 0.51$  vs  $36.69\pm 2.06$ ) was observed (Figure 5g-5i and Figure 6).

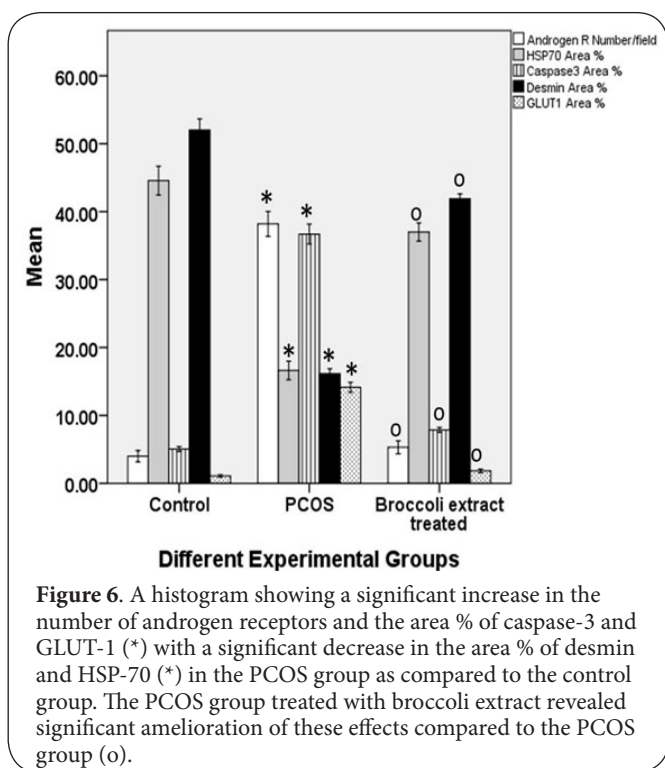
#### Desmin immunoreaction

Desmin area % immunoeexpression was significantly decreased ( $P<0.001$ ) in the PCOS rats compared with the control group ( $16.13\pm 1.01$  vs  $52.01\pm 2.30$ ) and this was dramatically increased ( $P<0.001$ ) in the PCOS group treated with broccoli extract compared to the PCOS group ( $41.88\pm 1.02$  vs  $16.13\pm 1.01$ ) (Figure 5j-5l and Figure 6).

#### GLUT-1 immunoreaction

A significant upregulation ( $P<0.001$ ) of GLUT-1 area % immunoreaction was noted in the myocardium of the PCOS group compared to that of the control group ( $14.13\pm 1.02$  vs  $1.09\pm 0.24$ ). On contrast, this expression was significantly decreased ( $P<0.001$ ) in the PCOS group treated with broccoli extract when compared to that of PCOS group ( $1.83\pm 0.35$  vs  $14.13\pm 1.02$ ) (Figure 5m-5o and Figure 6).





**Figure 6.** A histogram showing a significant increase in the number of androgen receptors and the area % of caspase-3 and GLUT-1 (\*) with a significant decrease in the area % of desmin and HSP-70 (\*) in the PCOS group as compared to the control group. The PCOS group treated with broccoli extract revealed significant amelioration of these effects compared to the PCOS group (o).

## Discussion

Although cardiovascular disease (CVD) was already studied in women with PCOS or animal models of the syndrome, there's no data, up to our knowledge, regarding histopathological changes in the heart in such cases. Moreover, CVD outcomes of PCOS necessitate early intervention programs and preventative strategies to reduce mortality from cardiovascular events. Accordingly, this work was set to investigate, for the first time up to the best of our knowledge, the reflection of cardiovascular risk factors associated with PCOS on the histopathological alterations in the cardiac muscle of adult female albino rats and whether broccoli extract can ameliorate these effects.

In this work, we used a model in which PCOS was induced by a single intramuscular injection of estradiol valerate (EV) as employed by previous studies [29,34]. Estradiol valerate was reported to induce morphological changes in the ovaries like those of PCOS women. This corresponds to the persistent cornification of vaginal smears observed at the beginning of this study as well as the presence of many atretic follicles and cysts in ovarian specimens as reported previously [27].

Our results reproduced the findings of previous studies where PCOS induced group showed significant increase in serum level of triglyceride (TG), total cholesterol (CH), low density lipoprotein (LDL) and significant decrease in high density lipoprotein (HDL) [35,36]. Previous studies [37,38] attributed imbalances in lipid profile in PCOS to hyperandrogenemia as confirmed in our study by the significant increase in the

total testosterone level. Gardner et al. [39] postulated that small, dense LDL particles have been linked with a 3- to 7-fold increased relative risk of coronary artery disease. Dahlgren et al. [40]; Holte et al. [41] documented that early and prolonged exposure to dyslipidemia, i.e. lessened HDL and more levels of plasma triglycerides, LDL and total cholesterol [42], confer significant cardiovascular risk. Desai et al. [26]; Sasikala and Shamila [43] confirmed that dyslipidemia is a root cause of CVD in PCOS patients.

In the present work, PCOS animals exhibited significant oxidative stress in the form of depletion of the endogenous antioxidant SOD and increase of lipid peroxidation (MDA) in myocardial tissue. This was previously proposed by many researches and was implicated in the pathogenesis of the disease as well as its sequelae including CVD [44-46]. Elahi et al. [47] concluded that cardiovascular disease mechanisms are strongly linked to the production of reactive oxidant species and the dysregulation of oxidant-antioxidants pathways. They explained that the oxidation and nitration of cellular proteins, lipids and nucleic acids, and formation of aggregates of oxidized molecules underlie the loss of cellular function, cellular aging and the inability of cells to withstand physiological stresses. Oxidative stress causes depolarization of the inner mitochondrial membrane with subsequent release of cytochrome c into the cytosol leading to induction of caspase mediated apoptosis [48]. This was reflected in myocardial sections of PCOS group, in this study, as significant upregulation of caspase-3 immunoreaction. In line with this, Kuyucu et al. [49] has reported a significant increase in caspase-3 staining in uterine specimens of PCOS female rats.

Our results showed that PCOS is associated with obvious histomorphological changes in the myocardium in the form of loss of normal architecture with degenerated hyalinized myofibers, rarified vacuolated sarcoplasm and pyknotic nuclei. Degeneration of cardiac myocytes may occur because of increased protein degradation and decreased myofibrillar protein synthesis where accumulation of reactive free radicals might facilitate the release of lysosomal enzymes into the cytosol with subsequent oxidation of the protein backbone of myofibrils causing their fragmentation [50].

In this study, Hsp70 was found to be downregulated in myocardial specimens of the PCOS group. This may be explained by the results of Chen et al. [51] who found overexpression of Hsp70 level, in myocardial cells, at the acute stage that was decreased at the later stages of stress exposure. They attributed that to the tolerance to stress following chronic exposure or due to material deficiencies following long term stress. Similarly, Khan et al. [52] reported that Hsp70 and Hsp90 in the heart of broiler chickens were significantly upregulated after the exposure to heat stress for two hours and then downregulated rapidly with further exposure. They related the upregulation of these stress proteins in heart to their role as important biomarkers and protective proteins at the start of stress and that the low signals of Hsps indicate

that myocardial cell lesions may adversely affect the function of Hsps under stress conditions.

Hyperandrogenism was proved, in this work, by the significant increase of serum testosterone as well as androgen receptor (AR) immunostaining in PCOS group. Pournaderi et al. [53] explained that insulin resistance, associated with PCOS, results in compensatory hyperinsulinemia which stimulates the androgen synthesis in ovarian theca cells that is considered as the pathogenic cause of the hyperandrogenemia in PCOS. Previous researches have demonstrated the occurrence of cardiac hypertrophy with hyperandrogenism [54,55]. This was confirmed, in the current study, by the significant increase of the cross-sectional area of cardiac muscle fibers and the area percent of collagen deposition in PCOS group. Marsh et al. [56]; Basualto-Alarcón et al. [57]; Hou et al. [55] concluded that androgens can activate the intracellular AR pathway to induce hypertrophy in cardiomyocytes and skeletal muscle. Myocardial fibrosis was reported to be an important histopathological change during cardiac hypertrophy [58]. Excess deposition of fibrotic extracellular matrix by fibroblasts leads to stiffness and organ dysfunction and activated fibroblasts can directly cause hypertrophy of cardiomyocytes, damages mechano-electric coupling of cardiomyocytes and accelerates the risk of arrhythmias along with tissue hypoxia and loss of myocytes [59].

Glucose transporter 1 (GLUT1) immunoexpression was found, in this study, to be significantly upregulated in PCOS myocardial specimens. Similar results were reported by Corbould et al. [60] who found significant increase of GLUT1 expression in cultured myotubes from women with PCOS. In line with our results, Montessuit and Thorburn [61] observed increased expression of GLUT1 in hypertrophied hearts both in vitro and in vivo. This can be explained by the results of previous studies which found decreased long-chain fatty acid utilization in case of cardiac hypertrophy with a compensatory shift of metabolism towards glucose utilization [62-64]. Moreover, Stenbit et al. [65] suggested that upregulation of GLUT1 may serve as a compensatory mechanism for the decreased GLUT4 mRNA and protein observed in hypertrophied hearts of human patients and animal models. Liao et al. [66] demonstrated that increasing myocardial glucose uptake protects against the progression to heart failure and improves survival in mice with chronic pressure overload. However, initial compensatory response is followed by a process of deleterious remodeling in which the hypertrophied heart dilates and fails to contract effectively [67].

Desmin is a cytoskeleton intermediate filament protein that is exclusively expressed in muscle cells [68]. The desmin network, which connects the Z-disks in adjacent myofibrils and the myofibrils to nuclear envelope and sarcolemma, is critical for the structural integrity of cardiomyocytes [69]. Also, desmin might be involved in the regulation of gene expression, myofibrillogenesis and intercellular signaling [70] and be responsible for shape and tension of the cell membrane

and other organelles [71]. Changes of the cytoskeleton expressed as decreased or increased desmin immunostaining are known features related to myocardial tissue injury. These have been shown in experimental studies [72] and human heart diseases [73-75]. In this work, we found significant downregulation of desmin expression, in PCOS specimens, as compared to controls. Similar results were reported by Pawlak et al. [76]; Pawlak et al. [77] who studied patterns of desmin distribution in cardiomyopathy. Thornell et al. [78]; Capetanaki et al. [79]; Milner et al. [80] concluded that desmin deficiency leads to cardiomyocyte hypertrophy and cardiac dilatation with compromised systolic function.

Our study revealed that broccoli extract was able to act as a cardioprotective agent in PCOS female rats. Broccoli was proved to have antioxidant and antiandrogen activities [24,81,82]. This was reflected as the significant improvement observed in biochemical parameters as well as histopathological changes of the myocardium in PCOS group treated with broccoli extract. The effect of dietary broccoli was previously evaluated in the prevention of ischemia-reperfusion injury to the heart by Mukherjee et al. [83]. They found decreased infarct size and decreased apoptosis of cardiomyocytes with broccoli treatment suggesting cardioprotection. Similarly, and in line with our results, stroke-prone SHRsp rats were fed a diet containing broccoli sprouts from the age of 5 weeks [84]. These rats were found to have significantly higher tissue reduced glutathione (GSH) levels (aorta, heart, kidney) and lower blood pressure than their control-fed counterparts, which spontaneously developed oxidative stress, loss of tissue GSH and elevated blood pressure. Moreover, a recent study by Shawky et al. [82] reported that sulforaphane (SFN) showed insulin-sensitizing, hepatoprotective and vasculoprotective effects in fructose-fed rats. They explained these effects based on SFN-mediated antioxidant, dyslipidemia-improving and anti-inflammatory effects. SFN mediated antioxidant effects may have occurred through activation of the transcription factor; nuclear factor-erythroid 2-related factor 2 (Nrf2), which increases gene expression of endogenous antioxidant proteins such as SOD [85,86]. Moreover, quercetin, a flavonoid present in broccoli, was proved to have the potentials to alleviate the hormonal and metabolic disturbances occurring in PCOS [87].

## Conclusion

The findings of the current study can provide histopathological and immunohistochemical confirmation for the harmful effects of PCOS on the heart allowing a more comprehensive understanding of the underlying mechanisms. Moreover, broccoli extract intake was proved to offer cardioprotection through its antioxidant, antiandrogen and dyslipidemia-improving effects.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

Authors' contributions	AEE	MAF
Research concept and design	✓	✓
Collection and/or assembly of data	✓	✓
Data analysis and interpretation	✓	✓
Writing the article	✓	✓
Critical revision of the article	✓	✓
Final approval of article	✓	✓
Statistical analysis	✓	✓

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