



## **Betanin and Allicin Ameliorate Adriamycin-Induced Cardiotoxicity in Rats by Ameliorating Cardiac Ischemia and Improving Antioxidant Efficiency**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors OAHA and GSAEA supervised the project. Author GSAEA performed light microscopy imaging and analyzed histology results. Author WSAT performed the experiments, analyzed the data and wrote the manuscript with support from authors OAHA and GSAEA. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aims:** To investigate the protective effects of betanin and allicin against adriamycin (ADR)-induced cardiotoxicity.

**Study Design:** Experimental animal model.

**Place and Duration of Study:** King Abdul-Aziz University, Jeddah, Saudi Arabia; 10 days.

**Methodology:** Adult female Wistar rats were allocated to the following groups (n = 10 per group): Control, received water, a standard diet for 10 days and i.p normal saline on day 8; ADR, intraperitoneal injection with 15 mg/kg ADR as a single dose on day 8; ADR+BE, betanin (20

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mg/kg) administration followed by i.p. injection of ADR (15 mg/kg); ADR+ALL, allicin (20 mg/kg) administration followed by i.p. injection of ADR; and ADR+BE+ALL, equal volumes of betanin and allicin followed by ADR (15 mg/kg). Hemodynamic characteristics of the cardiovascular system and electrocardiography were evaluated. Blood samples were obtained to assess cardiac enzymes; cardiac homogenates were processed to analyze oxidative and antioxidant parameters and low-grade inflammatory indicators. Histopathological evaluation of heart tissues was also conducted.

**Results:** Rats pre-administered betanin and allicin were protected from ADR-associated ischemia based on the significant ( $P < .05$ ) shortening of QT, QT<sub>C</sub> interval, QRS, and T peak Tend interval compared with the ADR group. Betanin and allicin pre-treatment significantly decreased the ADR-induced elevated serum creatine kinase-MB and lactate dehydrogenase levels. ADR-elevated cardiac oxidative parameters, along with the serum concentrations of the tumor necrosis factor-alpha and the cardiac transforming growth factor-beta, were significantly inhibited by betanin and allicin. Histopathological findings confirmed the biochemical results. Betanin and allicin reduced ADR-induced heart damage by inhibiting several pathways, including those of oxidative stress and inflammation.

**Conclusion:** Betanin and allicin may be promising cardioprotective agents owing to their antioxidant and cytoprotective properties and could thus be used as adjuvant treatment for cancer therapy.

*Keywords: Adriamycin; allicin; betanin; cardiotoxicity; electrocardiography.*

## 1. INTRODUCTION

Cancer medications can cause side effects and organ toxicity imposing a significant burden on patients' health outcomes [1-3].

Adriamycin (ADR; doxorubicin) is a chemical therapy against cancer (cytotoxic or antineoplastic), prescribed to treat several human carcinomas, including ovarian and breast cancers [4,5]. The successful use of ADR has induced harmful effects, with cardiotoxicity being the most prominent, especially in patients administered large doses. The main toxicity of the anti-cancer drugs (anthracyclines) that they offer is the cardiotoxicity that starts shortly after the first dose. One explanation why the heart is deemed the most susceptible against DOX damage is attributed to the high energy expenditure and high mitochondrial density inside the heart. Numerous findings indicate that about 25% of the patient receiving adriamycin will develop cardiac dysfunction after treatment; however, developing congestive heart failure occurs in 1–4% of the patient [6-9].

Although the mechanism by which ADR induces cardiotoxicity remains unclear, key factors may be involved, such as the disruption of oxidative stress and antioxidant protection mechanisms [10-13].

The toxicity of anticancer drugs remains a significant barrier to their safe use. Protective

protocols and regimens have been tried, using synthetic drugs to avoid ADR-related adverse effects without reducing its clinical benefit. However, they have limited therapeutic effects and produce side effects [14,15,16]. Medicinal plants containing several highly effective anti-inflammatory, antioxidant, and anti-carcinogenic components [15,16,17, 18,19] have been used to treat several diseases.

Betanin is a glycosidic water-soluble red pigment and is the bioactive constituent of red beetroot. [20-23] Its consumption has several health benefits, such as protection against ventricular disruption and ischemic injury [24,25]. Allicin (diallyl thiosulfate) is a major constituent of garlic [26-29]. Daily allicin administration decreases systemic blood pressure and protects rats from coronary endothelial and heart hypertrophy [19,30].

Therefore, in several studies, Betanin and allicin separately showed protective effects against ADR-induced cardiotoxicity [25,31], but little is known regarding their combined effects. We hypothesized that betanin and allicin may exert cardioprotective effects. The experimental study evaluated the protection of betanin and allicin in Wistar rats against ADR-mediated cardiotoxicity by examining the hemodynamic, electrochemical, biochemical, and histopathological improvements in ADR-related cardiac toxicity.

## 2. METHODOLOGY

### 2.1 Chemicals and Reagents

ADR, betanin, and allicin (5%) were purchased from Sigma-Aldrich (St. Louis, MO, USA), Kasei Tokyo Chemical Industry Co., Ltd. (Japan), and Qingdao BNP Bioscience Co. Ltd (China), respectively. Creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), and malondialdehyde (MDA) for lipid peroxidation reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) enzymes, and transforming growth factor (TGF- $\beta$ 1) and tumor necrosis factor (TNF)- $\alpha$  were obtained from Bioassay Technology Laboratory (Shanghai, China).

### 2.2 Animals

6 weeks female Wistar rats (180-220 g) were obtained from King Abdul-Aziz University, KSA. They were kept in clear cages made of polypropylene and with good ventilation (3–4 rats in each cage), under constant environmental conditions of 22°C, 50–60% humidity, with 12-hour day/night cycles.

### 2.3 Experimental Protocol

After two weeks of acclimation, rats were divided into five groups of 10 animals each: (i) Control group: rats received a standard diet; (ii) ADR group: rats received a single dose of ADR (15 mg/kg intraperitoneally) on day 8 [32,33]; (iii) BE-ADR group: rats received betanin (20 mg/kg) before and after ADR infusion, which occurred on day 8 [31]; (iv) ALL-ADR group: rats received allicin (20 mg/kg) for 10 days and ADR infusion (15 mg/kg) [34]; and (v) BE-ALL-ADR group: rats received an equal volume of betanin and allicin (20 mg/kg) before and after ADR infusion.

Bodyweight gains were recorded throughout the experiment. Animals were anesthetized with xylazine (10 mg/kg) + ketamine (100 mg/kg) [35]. Hemodynamics and electrocardiography (ECG) were recorded. To obtain the serum, blood samples were collected from the vena cava, centrifuged for 10 min at 3,000 rpm, and stored at -20°C. Thereafter, the thoracic cavity was opened, and the heart was excised, washed with saline, dried on clean filter paper, and weighed. The hearts were divided longitudinally in two. One half was fixed in 10% neutral buffered formalin (NBF) for histopathological analysis. The other half was stored at -80°C for the assay of

antioxidant parameters and low-grade inflammatory indicators.

### 2.4 Cardiac Function Measurement

#### 2.4.1 Hemodynamic Recording

Animals were anesthetized using the procedures mentioned above, and their body temperature was kept at 37, through the use of controlled heating pads. The pressure catheter (Millar Devices, Houston, TX) was implanted into the right carotid artery and inserted into the left ventricle. Signals were recorded after a stabilization period (5 Min) and the catheter was linked to a Power Lab with Lab Chart software (v8.0, AD Instruments, Bella Vista) [36].

#### 2.4.2 Electrocardiography (ECG)

ECG was recorded as previously described [37].

#### 2.4.3 Assay of cardiac enzyme activities

LDH and CK-MB serum levels were measured using enzyme-linked immunosorbent kits (Bioassay Science Laboratory Kit, China) at 450 nm, according to the manufacturer's instructions.

### 2.5 Tissue Homogenate Preparation and Assay of Oxidative and Antioxidative Markers

Rat heart sections were homogenized. The amounts of lipid peroxidation marker (MDA) and oxidative enzyme activity (CAT, SOD, and GSH) were measured. TGF- $\beta$ 1 and TNF- $\alpha$  were assessed using a spectrophotometer at 450 nm [38-40].

### 2.6 Histopathological Analysis

NBF-fixed heart halves were sectioned into 5  $\mu$ m slices and stained with hematoxylin and eosin (H and E) for general architecture, and Masson's Trichrome (MT) stain for fibrous tissue [41]. The slides were examined and photographed using a digital camera (Olympus 20) connected to an Olympus light microscope (Olympus BX61, USA).

### 2.7 Statistical Analysis

Data are presented as mean  $\pm$  standard error of the mean (SEM). One-way analysis of variance (ANOVA) accompanied by Newman-Keuls' post-

hoc evaluation was performed using Prism 8 (GraphPad Software, San Diego, CA, USA). A  $P$  value of  $< .05$  was considered statistically significant.

### 3. RESULTS

#### 3.1 Effect of ADR versus Betanin, Allicin, and its Combination on Heart and Body Weight Gain

Bodyweight gain and heart weight values were lower in ADR-treated rats than in control rats ( $P < .05$ ; Table 1). Bodyweight gain was more rapid in ADR-treated rats pretreated with allicin than in ADR-treated rats and BE-ALL-ADR-treated rats. Little change in body weight gain, but significantly higher heart weight was observed in BE-ADR-treated rats compared to ADR-treated rats ( $P < .05$ ). Allicin did not affect the heart weight, but the combination treatment increased the heart weight as much as betanin treatment, compared to ADR treatment.

#### 3.2 Effect of ADR versus Betanin, Allicin, and their Combination on ECG Parameters

ADR caused gradual prolongation of QT interval, QRS complex, ST, and T peak-Tend time interval, achieving a peak and statistical significance ( $P < .05$ ; Fig. 1) indicating cardiac ischemia. Pre-treatment with betanin, allicin, or their combination removed ADR-associated ischemia, as seen from major shortening in QT, QTc, QRS, and T peak-Tend intervals, compared with ADR ( $P < .05$ ). Substantial expansion ( $P < .05$ ) in PR length suggested a link between cardiac toxicity and atrioventricular (AV) delay symptoms. ADR infusion markedly affected atrial conductivity, as evidenced by the substantial improvement in PR interval and duration. The PR length dropped significantly ( $P < .05$ ) when

betanin, allicin, and their combination were administered to ADR-treated rats, suggesting an ameliorating AV delay, and influencing P-wave duration.

#### 3.3 Effect of ADR versus Betanin, Allicin, and their Combination on Cardiac Hemodynamic Parameters

ADR infusion significantly influenced the heartbeat compared to control rats (Fig. 2). ADR gradually increased both diastolic and systolic duration, achieving a plateau and statistical significance ( $P < .05$ ). Pre-treatment with betanin, allicin, and their combination exerted no substantial effect on the heart rate, and a non-significant decrease in systolic and diastolic duration compared to ADR.

#### 3.4 Effect of ADR versus Betanin, Allicin, and their Combination on Cardiac Enzyme Levels

ADR administration substantially improved serum LDH and CK-MB concentrations compared to control ( $P < .05$ ; Fig. 3). BE-ADR treatment significantly lowered serum LDH and CK-MB concentrations. There were no significant differences among LDH and CK-MB concentrations in BE-ALL-ADR and control groups. ALL-ADR treatment significantly reduced serum LDH and CK-MB concentrations compared to ADR treatment ( $P < .05$ ).

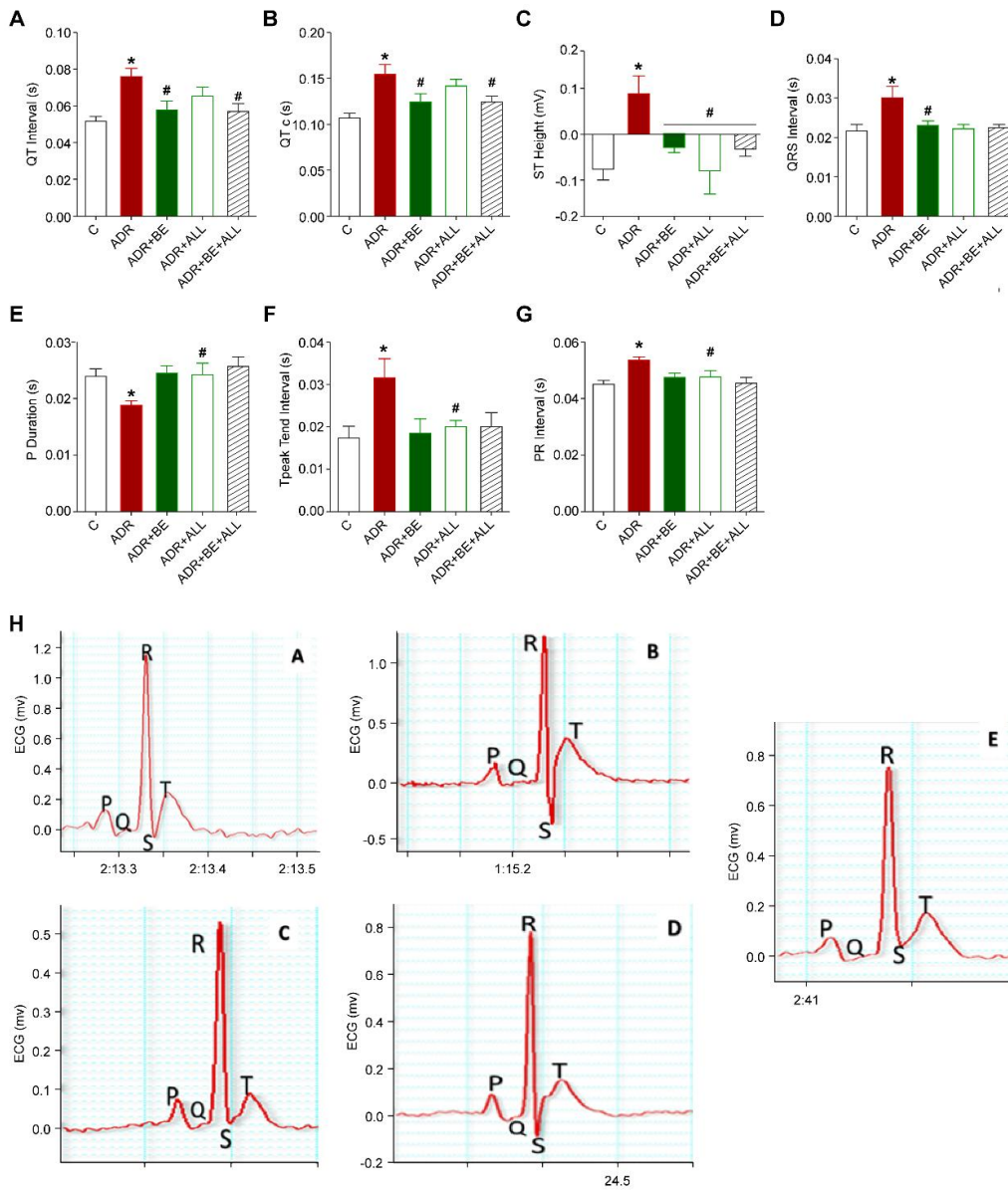
#### 3.5 Effect of ADR versus Betanin, Allicin, and their Combination on TNF- $\alpha$ and Cardiac TGF- $\beta$ 1 in Rats

Serum TNF- $\alpha$  and cardiac TGF- $\beta$ 1 concentrations significantly increased in ADR-treated rats compared to control ( $P < .05$ ); Fig. 4). BE-ADR treatment substantially decreased serum TNF- $\alpha$  and cardiac TGF- $\beta$ 1

**Table 1. Effect of adriamycin (ADR) versus betanin (BE), allicin (ALL), and their combination on body weight gain and heart weight of cardiotoxic and control rats**

| Experimental groups | Body weight gain (%) <sup>a</sup> | Heart weight (g) <sup>a</sup> |
|---------------------|-----------------------------------|-------------------------------|
| Control             | 2.955 ± 0.27                      | 0.70 ± 0.02                   |
| Adriamycin          | -5.928 ± 1.42 *                   | 0.52 ± 0.02 *                 |
| ADR+BE              | -5.711 ± 0.60                     | 0.63 ± 0.02 #                 |
| ADR+ALL             | -1.329 ± 0.24 #                   | 0.52 ± 0.008                  |
| ADR+BE+ALL          | -0.2333 ± 0.68 #                  | 0.61 ± 0.011                  |

<sup>a</sup>Data are represented as mean ± standard error of mean ( $n = 10$ ), \* and # represent  $P < .05$  as determined by one-way analysis of variance followed by the post-hoc Newman-Keuls test between Adriamycin vs. control and Adriamycin vs. treated groups, respectively



**Fig. 1. Effect of Adriamycin versus betanin, allicin, and their combination on cardiac electrocardiographic parameters**

(A) QT, (B) QTc, (C) ST height, (D) QRS, (E) P duration, (F) T-peak to Tend, (G) PR interval, and (H) representative cardiac electrocardiogram recordings of cardiotoxic and control rats. Data are shown as mean  $\pm$  standard error of mean ( $n = 10$ ) and were evaluated using one-way analysis of variance followed by post-hoc Newman-Keuls tests to compare the control and cardiotoxicity group values ( $P < .05$ ). ADR, Adriamycin; BE, betanin; ALL, allicin; C, control

concentrations more than the reduction caused by ADR treatment ( $P < .05$ ). ALL-ADR treatment significantly decreased serum TNF- $\alpha$ , while cardiac TGF- $\beta$ 1 concentrations decreased slightly. However, BE-ALL-ADR treatment effectively diminished serum TNF- $\alpha$  and cardiac TGF- $\beta$ 1 levels compared to ADR ( $P < .05$ ).

### 3.6 Effect of ADR versus Betanin, Allicin, and their Combination on Oxidative Stress and Antioxidant Enzyme Levels

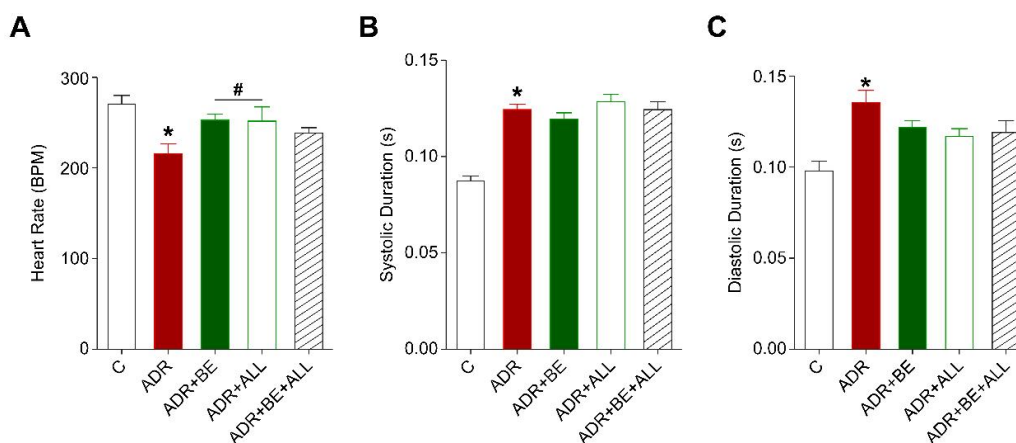
The MDA level was significantly higher ( $P < .05$ ) in ADR-induced cardiac toxicity in rats than in

control rats (Fig. 5). MDA levels were sharply reduced ( $P < .05$ ) with administration of betanin, allicin, and their combination, suggesting a reduction in oxidative stress. SOD, CAT, and GSH levels were significantly decreased ( $P < .05$ ) in ADR-treated rats, reflecting the inhibition of antioxidant enzymes, which was improved by betanin, allicin, and combination administration.

### 3.7 Histological Results

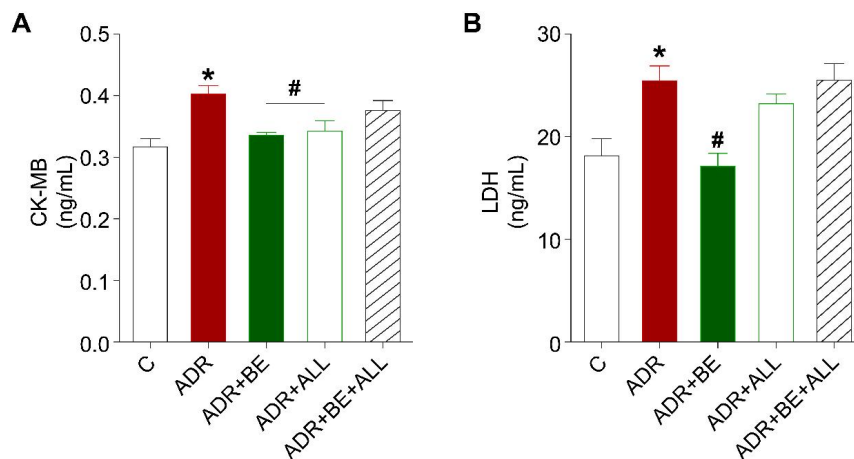
H and E staining of the control ventricular wall revealed typical architecture, with branching and

anastomosing cardiac fibers in various directions (Fig. 6). The transversely cut cardiac muscle fibers contained acidophilic cytoplasm with one or two oval vesicular nuclei, usually centrally located. Cross striation and intercalated discs were detected in the longitudinally cut fibers. Between the cardiac fibers, the interstitial tissue contained distinct nuclei of fibroblasts with blood capillaries. MT staining showed a small amount of fibrous tissue, which appeared bluish among the cardiac muscle fibers and across blood vessels.



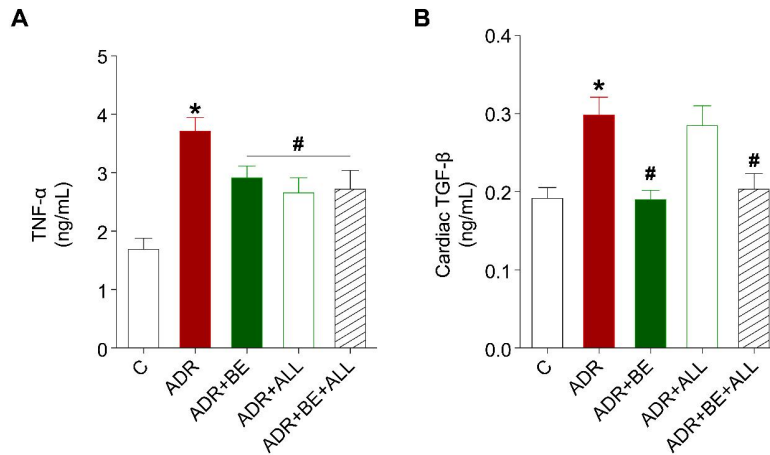
**Fig. 2. Effect of Adriamycin versus betanin, allicin, and their combination on heart rate (A) and cardiac cycles [systolic (B) and diastolic (C) durations] in cardiotoxic and control rats**

Data are shown as mean  $\pm$  standard error of mean ( $n = 10$ ) and were evaluated using one-way analysis of variance followed by post-hoc Newman-Keuls tests for comparisons of control and cardiotoxicity group values ( $P < .05$ ). ADR, Adriamycin; BE, betanin; ALL, allicin; C, control

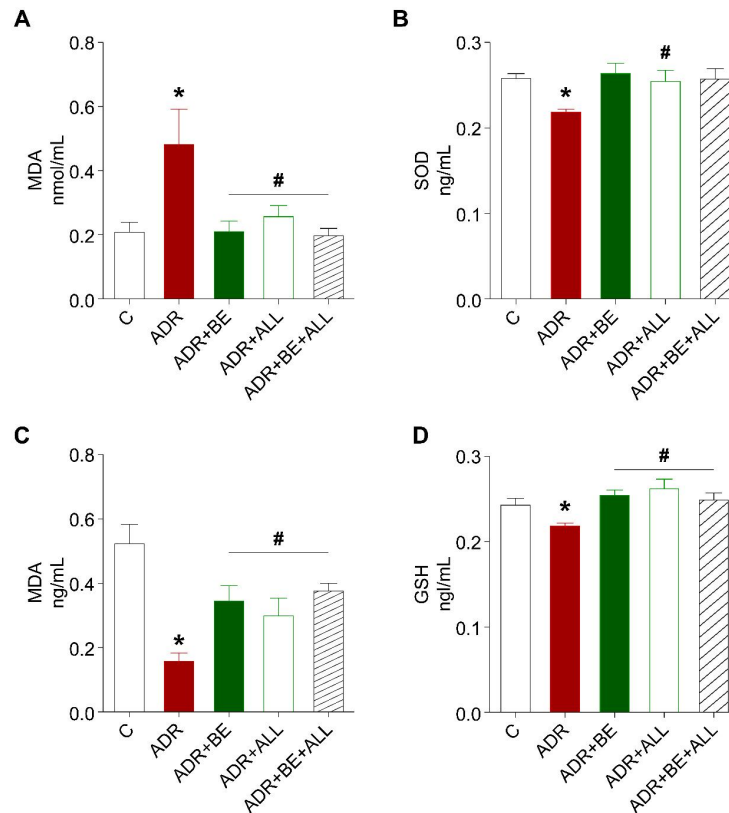


**Fig. 3. Effect of Adriamycin versus betanin, allicin, and their combinations on CK-MB (A) and lactate dehydrogenase (B) in cardiotoxic and control rats**

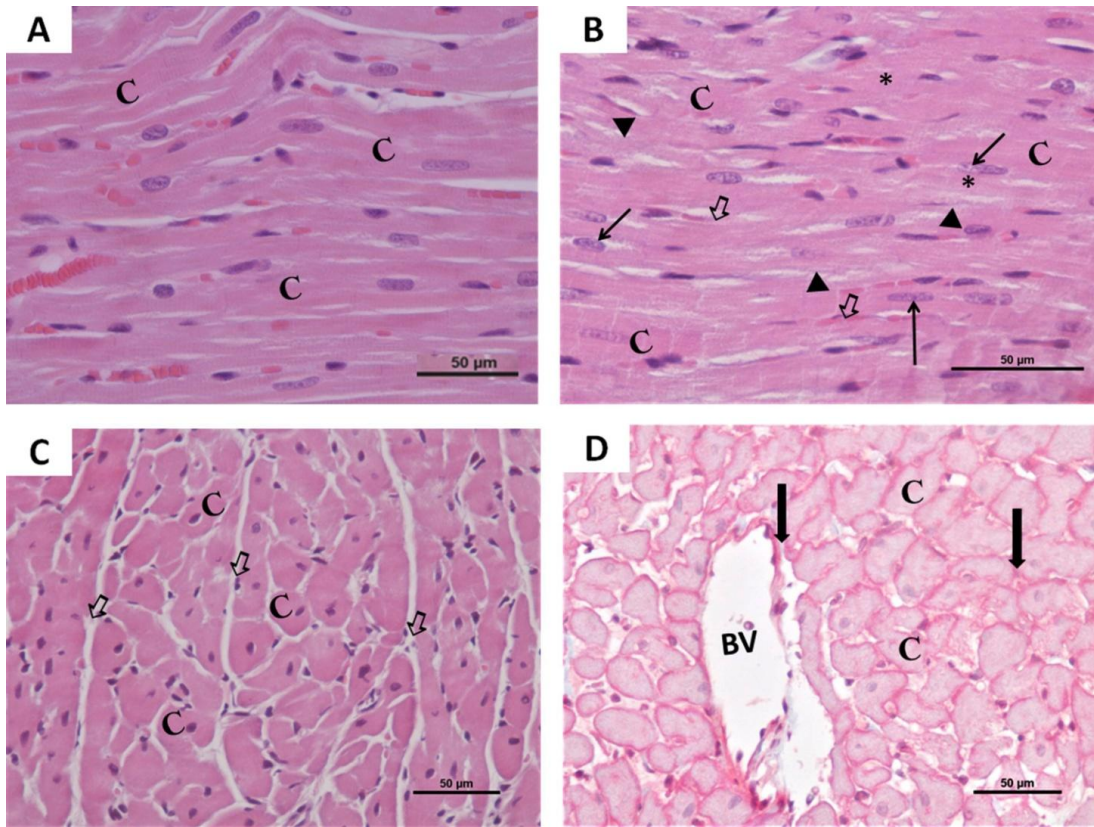
Data are shown as mean  $\pm$  standard error of mean ( $n = 10$ ) and were evaluated using one-way analysis of variance followed by post-hoc Newman-Keuls tests for comparisons of control and cardiotoxicity group values ( $P < .05$ ). ADR, Adriamycin; BE, betanin; ALL, allicin; C, control; LDH, lactate dehydrogenase; CK-MB, creatine kinase-MB



**Fig. 4. Effect of Adriamycin versus betanin, alicin, and their combination on low-grade inflammation [(A) TGF-β1 and (B) TNF-α] in cardiotoxic and control rats**  
 Data are shown as mean ± standard error of mean (n = 10) and were evaluated using one-way analysis of variance followed by post-hoc Newman-Keuls tests for comparisons of control and cardiotoxicity group values (P < .05). ADR, Adriamycin; BE, betanin; ALL, alicin; C, control



**Fig. 5. Effect of Adriamycin versus betanin, alicin, and their combination on oxidative stress and antioxidant enzyme levels in cardiotoxic and control rats**  
 (A) malondialdehyde (MDA), (B) superoxide dismutase (SOD), (C) catalase (CAT), and (D) glutathione (GSH). The data are represented as mean ± standard error of the mean (n = 10) and were evaluated using one-way analysis of variance followed by post-hoc Newman-Keuls tests for comparisons of control and cardiotoxicity group values (P < .05). ADR, Adriamycin; BE, betanin; ALL, alicin; C, control



**Fig. 6. Photomicrographs of the ventricular walls of control rat hearts**

*In longitudinal sections (A, B), normal, branched, and anastomosing cardiac muscle fibers (C) show transverse striations (\*), intercalated discs (upward arrows), and vesicular oval nuclei (triangles); narrow interstitial spaces were occupied by fibroblasts with elongated oval nuclei (open right-pointing arrows). In transverse sections (C, D), centrally located nuclei (triangles) of cardiac muscle cells (C) are surrounded by narrow interstitial spaces with minimal amounts of collagenous fibers (open right-pointing arrows). Staining was performed using hematoxylin-eosin (A–C) and Masson's trichrome (D). Scale bars = 50 µm. BV, blood vessels*

In ADR-treated rats (Fig. 7), H and E staining of the ventricular wall revealed degenerative changes in the cardiac muscle fiber producing disintegration and disarrangement. Some fibers lost their nuclei, while others showed pyknotic nuclei. Some fibers appeared vacuolated with pale acidophilic cytoplasm, or showed foci of hyaline and eosinophilic material. The cardiac muscle fibers were widely separated by inflammatory cell infiltrates. Red blood cell extravasation was observed around the dilated blood vessels, which appeared congested. The MT-stained sections showed a marked increase in fibrous tissue via the blood vessels and among the cardiac fibers.

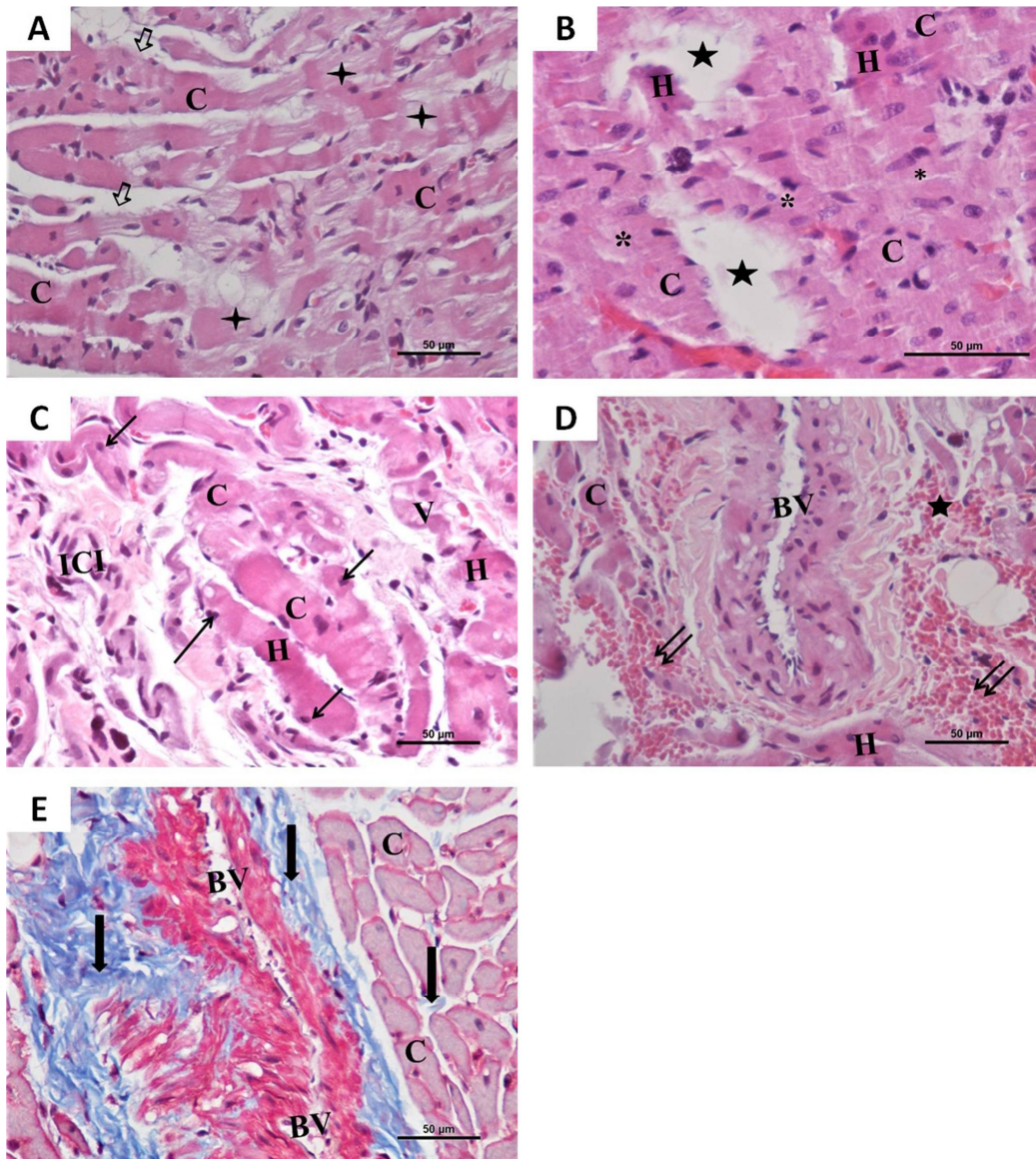
In BE-ADR-treated rats (Fig. 8), H and E analysis of the ventricular wall showed ameliorated of the degenerative changes observed in ADR-treated rats. There was diminished separation and

inflammatory cell infiltrate between cardiac fibers. Congestion and hemorrhaging were less frequent. The MT-stained sections showed marked regression of fibrous tissue among the cardiac fibers and via the blood vessels.

In ALL-ADR-treated rats (Fig. 9), H and E staining of the ventricular wall revealed decreased ADR-induced histopathological alterations: small patches of cellular degeneration, wide intercellular spaces, and congested blood vessels within the cardiomyocytes. There were significant declines in the mass of fibrous tissue in cardiac muscle and around the congested blood vessels.

In BE-ALL-ADR-treated rats (Fig. 10), H and E analysis of the ventricular wall presented an almost healthy appearance; a small amount of fibrous tissue was observed using MT staining.





**Fig. 7. Photomicrographs of the ventricular walls of Adriamycin-treated rat hearts**

(A) Longitudinal sections showing disintegration (four-pointed stars) and disarrangement of the cardiac muscle fibers (C). Wide interstitial spaces between the fibers contain the nuclei of fibroblasts (open right-pointed arrows). (B) Longitudinal section with empty areas (five-pointed stars) between disarranged cardiac muscle fibers (C) that have lost their striation (\*). Some fibers show hyaline degeneration (H). (C) Transverse sections with widely separated and inflammatory cell infiltrates between the cardiac muscle fibers (C). Some fibers appear vacuolated (V) with pale acidophilic cytoplasm or show hyalinization foci (H). Pyknotic nuclei (arrow) can be noted on many fibers. (D) Transverse section with red blood cell extravasation (double arrows) around the dilated and thickened blood vessels (BV), which appear congested. Some cardiac muscle fibers (C) showed hyaline degeneration (H). (E) Transverse section with a marked increase in fibrous tissue (thick arrow) around the blood vessels (BV) and among the cardiac muscle fibers (C). (A–D) Hematoxylin-eosin  $\times 400$  magnification; (E) Masson's trichrome staining,  $\times 400$  magnification. Scale bars = 50  $\mu\text{m}$

#### 4. DISCUSSION

Cancer patients receiving ADR can suffer from its life-threatening cardiotoxicity, limiting its use in

cancer treatment. Most chemotherapeutic agents destroy cancer cells through complex mechanisms, such as free radical production, DNA damage, and apoptosis, which are also the

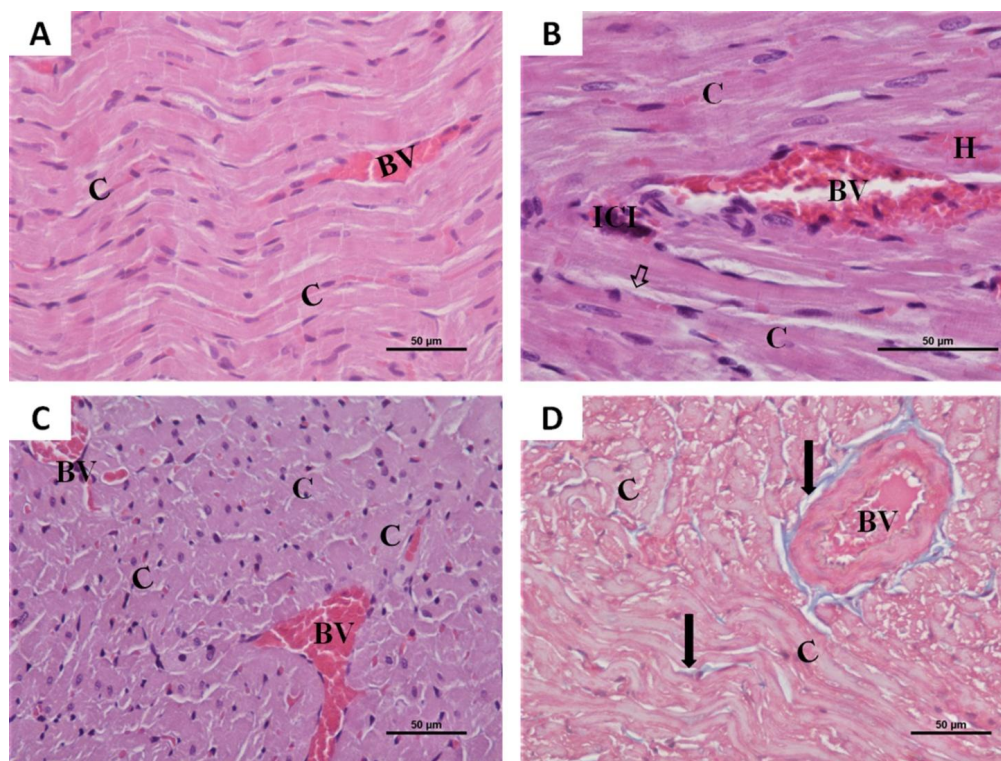
major causes of cardiac injury [42,43]. Various pathways were explored to determine methods to avoid fatal cardiotoxicity [41].

Synthetic antioxidants cannot decrease cardiotoxicity and increase the survival of ADR-treated patients. However, vegetable intake increases bioactive antioxidant phytochemicals, which combat oxidative stress [43]. Natural ingredients and herbs have been utilized to attenuate the toxic effects of ADR on the heart [44]. We examined the protective role of betanin and allicin, individually and in combination, against ADR-induced cardiotoxicity in rats.

ADR-treated rats pretreated with betanin and allicin had increased body weight gain and heart weight, relative to ADR-treated rats. Lower heart weight was also due to myocardium degeneration, necrosis, and atrophy following ADR exposure. Histopathological analyses indicated myocardial necrosis with focal areas of fibrosis.

ADR produced a sharp increase in cardiac enzymes, such as CK-MB and LDH, while betanin and allicin pretreatment substantially reduced their levels. Allicin pre-treatment lowered the serum levels of inflammatory markers and heart injury biomarkers, reflecting its anti-inflammatory and membrane-stabilizing effect. Our outcomes agree with those of a previous study [45] demonstrating the normalization of CK-MB and LDH elevated levels in groups that consumed beetroot juice before ADR injection [45].

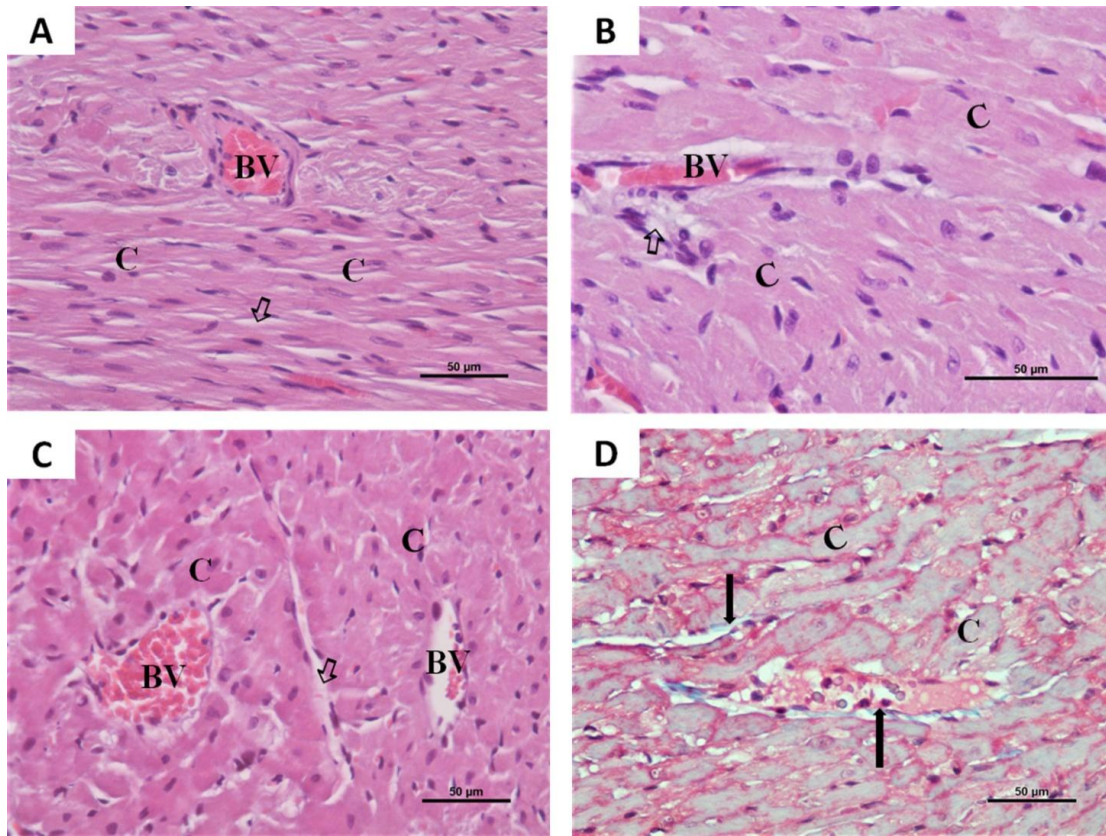
Serum TNF- $\alpha$  and cardiac TGF- $\beta$ 1 levels were significantly increased over controls in ADR-treated rats; allicin greatly diminished these parameters. TNF- $\alpha$  and TGF- $\beta$ 1 exert anti-inflammatory and immunomodulatory effects. This research supports earlier findings that ADR causes an immediate inflammatory response, resulting in serum TNF- $\alpha$  and IL-1 $\beta$  elevation [46,47].



**Fig. 8. Photomicrographs of the ventricular walls of the hearts of Adriamycin (ADR)-treated rats pre-treated with betanin**

*Betanin protected the cardiac musculature against the degenerative effects of ADR; the cardiac musculature shows a normal structure and arrangement of the muscle fibers in longitudinal sections (A, B). In transverse sections (C, D), neither hemorrhage nor deposition of collagenous fibers is evident between the cardiac musculature (upward arrows). (A–C) Hematoxylin-eosin staining; (D) Masson's trichrome staining.*

*Scale bars = 50  $\mu$ m. BV, blood vessels*



**Fig. 9. Photomicrographs of the ventricular walls of the hearts of Adriamycin-treated rats pre-treated with allicin**

*In longitudinal sections (A, B), the cardiac musculature (C) shows a nearly normal appearance with congested blood vessels (BV) and narrow interstitial spaces (open right-pointing arrows). In transverse sections (C, D), congested BV and few collagenous fibers (upward arrows) can be seen around BV and between the muscle fibers in (D). (A– C) Hematoxylin-eosin staining; (D) Masson's trichrome staining. Scale bars = 50 μm*

ADR-induced cardiotoxicity appears to be multifactorial. DNA/RNA injury, mitochondrial dysfunction, nitric oxide release, and increased inflammatory mediators were involved in cardiotoxicity. Mitochondria in cardiac muscle include cardiolipin, which has a strong affinity for ADR, resulting in aggregation inside the cardiac mitochondria, weakening the respiratory chain, and inducing apoptotic death [48,49].

SOD, CAT, and GSH levels significantly declined, indicating inhibition of antioxidant enzymes, which act against oxidative stress [50]. These enzymes protect cells against oxidative stress by detoxifying cardiac myocyte superoxide radicals and hydrogen peroxide. Owing to its lower antioxidant content, the heart was deemed the main target organ for ADR-induced oxidative stress [50].

Betain and allicin significantly decreased MDA levels, suggesting reduced oxidative stress, and significantly increased ADR-induced antioxidant enzyme suppression in cardiac tissue. Beetroots contain betalain pigments, which have strong antioxidant action. Beetroot antioxidants may reduce oxidative stress-mediated apoptosis in cardiomyocytes. Beetroot juice minimizes myocardial infarction and left ventricular contractile dysfunction after ischemic-reperfusion injury [51].

Pre-treatment of ADR-overdose mice with allicin returned the levels of antioxidant enzymes (SOD, CAT, and GSH) and cardiac MDA to normal levels. Allicin has been implicated in acrylamide safety [52], and cyclophosphamide and gentamicin cytotoxicity [53]. The antioxidant activity of allicin can be regulated by upregulating

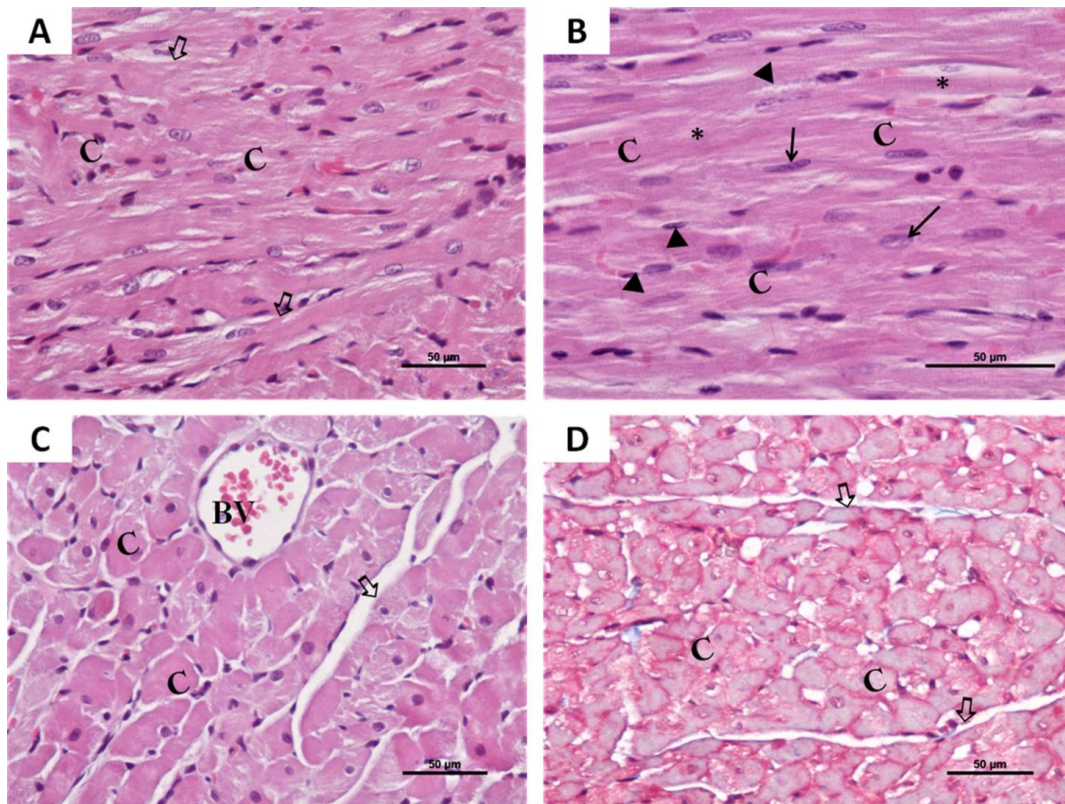
the expression of genes encoding detoxifying enzymes [54].

Recent cardiac hemodynamic parameters revealed that ADR infusion in rats greatly altered their heart rate compared to controls. ADR gradually increased the systolic and diastolic duration to a maximum. Prolonged ADR administration significantly reduces the heart rate. A decline in intracellular calcium-mediated the decreased excitability of SA node pacemaker cells.

Pre-treatment with betanin, allicin, and their combination showed no major effect on heart rate. Non-significant decreases in systolic and diastolic lengths occurred following ADR treatment. Dietary nitrate-rich beetroot juice

intake exerts positive effects in stable and hypertensive patients by reducing blood pressure [55].

ADR contributed to a gradual extension of the QT period, QRS complex, ST, and Tpeak-Tend periods, reaching a plateau. Betanin, allicin, and their combination prevented ADR-associated changes, as seen from the significant shortening of the abovementioned parameters compared with the ADR-treated rats. This large increase in PR length indicated a link between cardiac toxicity and AV delay signs, as demonstrated by the improved PR interval and P duration. ADR infusion greatly enhanced atrial conductivity. Betanin, allicin, and their combination significantly decreased PR duration, indicating amelioration of AV delay, and P-wave duration.



**Fig. 10. Photomicrographs of the ventricular walls of the hearts of Adriamycin-treated rats pre-treated with a combination of betanin and allicin**

(A) Longitudinal section showing a normal structure in the form of branched and anastomosing cardiac muscle fibers (C), similar to that of controls. Note the narrow interstitial spaces (open right-pointing arrows). (B) Longitudinal section of cardiac muscle fibers (C) with acidophilic cytoplasm and clear transverse striations (\*) and intercalated discs (triangles) that connect different fibers. Oval and vesicular nuclei are present (upward arrows). (C) Transverse section with central nuclei (upward arrows) of cardiac muscle fibers (C) and narrow interstitial spaces (open right-pointing arrows). (D) Transverse section of cardiac muscle fibers (C) with slight fibrous tissue (open right-pointing arrows) between and around the blood vessels (BV). (A–C) Hematoxylin-eosin staining; (D) Masson's trichrome staining. Scale bars = 50  $\mu$ m

Xu et al. stated that ECG alterations are among the most accurate parameters for the evaluation of ADR-induced cardiotoxicity [56]. They documented major ECG changes in ADR-treated rats, such as QT prolongation, ST intervals, and QRS complex expansion. Betanin, allicin, and their combination resulted in a preventive function reflected by heart rate regularization. QT and ST intervals and QRS complex results appeared normal. These findings are consistent with other observations showing the QR duration is a measure of ventricular activation sustained during toxicity with oxidative stress [56].

The deleterious influence of ADR on cardiac muscle was verified using histological analyses, which revealed several harmful morphological changes: disorganized, fractured muscle fibers with striation loss, inflammatory cell infiltration, hyalinization, vacuolization, myofibrillary degeneration, interstitial edema, vascular obstruction, hemorrhage, and focal fibrosis. Some nuclear degeneration, such as pyknotic or fading nuclei and perinuclear vacuolation, were observed. Related manifestations, including visible intracellular edema, focal myocardial fibrosis, perinuclear vacuolation, and myocardial necrosis, have previously been identified in numerous animal models, indicating that these modifications lead to ADR-induced cardiotoxicity [57].

Oral betanin and allicin pretreatment greatly decreased ADR-induced histopathological alterations in cardiac tissue, and restored the healthy appearance of the myocardium, possibly due to the strong antioxidant properties of these juices. Beetroot juice defends oxidative damage to DNA, lipids, and protein structures in cell culture experiments [58]. Betanin has hypolipidemic, anti-atherosclerosis, and anticancer effects [59]. Furthermore, beetroot juice consumption has been shown to prevent ventricular disruption and myocardial ischemia [60]. The positive effect of allicin on cardiovascular conditions, including stroke, coronary heart disease, and hypertension, is well established. Oral consumption of aged garlic extract maintained the histological composition and normalized animal cardiac tissue architecture. Allicin's defensive role was due to its antioxidant ability, which prevented lipid peroxidation and increased GSH in cells [61].

## 5. CONCLUSIONS

In conclusion, Betanin, allicin, and their combination minimized ADR-induced

cardiotoxicity by alleviating cardiac ischemia and increasing cardiac antioxidant capacity. This protective effect can be attributed to their antioxidant and anti-inflammatory mechanisms. Therefore, both betanin and allicin could be promising cardioprotective agents through their antioxidant and cytoprotective potentials. Consequently, they can be used as adjuvant treatment during administration of ADR in cancer therapy.

## DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

The Medical Science Ethics Committee approved all animal procedures and methods used in this study (Faculty of Medicine, King Abdul-Aziz University, Jeddah, Saudi Arabia, Reference No. 714-19).

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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