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Protective Role of Berberine-Loaded Bovine Serum Albumin Nanoparticles Towards β-Cyfluthrin-Induced Cardiotoxicity in Male Rats

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Abstract

β-CYFLUTHRIN(β-CYF), a class II Pyrethroid, is an insecticide used worldwide in agriculture, the current study was intended to explore the prospect of β-cyfluthrin to persuade oxidative stress and biochemical perturbations in cardiac tissue and the effect of *Berberine* (BBR) and Berberine-Loaded BSA (Bovine Serum Albumin) Nanoparticles (BNPs) in alleviating its cardiotoxicity. Male Wistar rats (n = 40) were randomly allocated into eight groups of five animals each. Group I served as the control, while Group II received berberine (BBR, 10 mg/kg) and Group III received berberine nanoparticles (BNP, 5 mg/kg). Group IV was administered β-cyfluthrin (β-CYF, 15 mg/kg BW), whereas Groups V and VI were given β-CYF (15 mg/kg) followed by BBR (10 mg/kg) or BNP (5 mg/kg), respectively. Group VII received bovine serum albumin (1 mL), and Group VIII received corn oil (1 mL). All treatments were administered once daily by oral gavage for 15 days. Upon β-cyfluthrin exposure, a significant elevation was observed in lipid peroxidation (MDA) and a reduction in the protective activities of antioxidant enzymes like Total Antioxidant Capacity (TAC) and superoxide dismutase (SOD), This was accompanied by increase cardiac biomarkers (LDH, CPK, CK-MB) in the serum. Berberine Nanoparticles and Berberine administration pointedly Significant declined the serum and tissue quantity of cardiac enzyme and oxidative stress biomarkers in β-cyfluthrin treated rats and restored heart tissue integrity. The findings indicate that oxidative stress plays a crucial role in heart damage caused by cyfluthrin, BNPs is a prevailing antioxidant, can mitigate β-cyfluthrin's negative impact on rat heart tissue.

Keywords: β-cyfluthrin, Antioxidant, Berberine nanoparticles, Oxidative stress, cardiotoxicity.

Introduction

The Heart Disease and Stroke Statistics Report states that between 2006 and 2016, the number of heart disease deaths increased by 14.5%, leading to approximately 17.6 million deaths worldwide. [1, 2]. Worldwide, natural contact is one of the chief causes of CVD, These days, studies show that exposure to pesticides causes cancer and a number of illnesses affecting the kidney, heart, and brain system.[3, 4].Pesticides are a broad class of chemicals in contemporary agriculture that used to control weeds and pests and increase crop yields. But a lot of these chemicals can harm plants, animals, and the environment. Among these, synthetic pyrethroids are a more recent class of insecticides that are thought to be less harmful than conventional ones[5].

Pyrethroids (PY) are synthetic pesticides widely employed in various settings. Their main mechanism of action, which underlies their insecticidal effectiveness, is binding to voltage-gated sodium channels in insect neurons, delaying their inactivation and resulting in prolonged nerve stimulation. Pyrethroids, however, can also be

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hazardous to humans and other vertebrates.[6] , As highly toxic to insects, yet minimally harmful to mammals, pyrethroids are broadly used for insect control in both agricultural and indoor environments. [7] β-Cyfluthrin is a widely used synthetic pyrethroid, a photostable type II pyrethroid that is utilized in personal hygiene as well as agriculture to combat *Lepidoptera*, *Coleoptera*, and *Hemiptera* on cotton, fruits, vegetables, grains, and other crops[8, 9]. Occupational exposure may occur among workers handling and the general public may be exposed through pest control operations or by consuming tainted food or water[10, 11] According to reports, pyrethroids cause oxidative damage, metabolic alterations, and an increase in Oxygen radicals (ROS). [12-14] Thus, the production of free radicals is one way of pyrethroids toxicity.[15, 16] pyrethroids exposure was associated with higher odds of coronary heart disease (CHD)[17].

Berberine (BBR) is a natural compound found in certain Chinese medicinal herbs, particularly *Berberis vulgaris*. that we selected for this study, an isoquinoline alkaloid with a variety of therapeutic uses, such as antiviral, antimicrobial, anti-inflammatory, antitumor, and antidiarrheal effects. Additionally, it helps treat a variety of skin and eye disorders[18], BBR is additionally reported to have advantageous impacts on hypertension, cardiac arrhythmias, and congestive heart failure [18], It also has, antioxidative, anti-inflammatory, antiarrhythmic, and anti-tumor properties [19-23]. BBR demonstrates a number of biologically beneficial qualities, hepatoprotective in action[24], anti-inflammatory potential[25], antioxidant and antidiabetic potency [26], antibacterial activity [27], antidiarrheal action [28], strong antitumor activity against various cancers, including hepatocellular carcinoma and human colon cancer [22, 29, 30] BBR exhibits strong inotropic effects and reduces peripheral vascular resistance. Berberine also lowers ventricular filling pressures by lowering pulmonary venous and systemic pressures.[31] Berberine has been demonstrated to improve cardiac muscle contractility in experimentally induced rat, BBR also encourages the production of nitric oxide (NO), which relaxes arterial walls, It boosts blood flow, reduces blood pressure, and aids in preventing atherosclerosis.[32-34]. Recently, Nanotechnology has drawn a lot of attention, it can increase a drug's bioavailability and therapeutic efficacy while reducing its toxicity. Berberine has been added to a number of delivery systems based on nanoparticles in order to overcome this difficulty and to enhance its absorption and general therapeutic potential[35]. Bovine serum albumin (BSA) has the ability to bind medications noncovalently and effectively transport them to target site [36, 37]. Therefore, the study aimed to examine the efficacy of berberine loaded on BSA nanoparticles on β-Cyfluthrin induced cardiotoxicity. That study highpoints the Crucial role of nanotechnology for delivery of BBR and increased it`s effectiveness.

Material and Methods

Chemicals Preparations

β-cyfluthrin PESTANAL*, analytical standard (Cat#46003) was Acquired from SIGMA - ALDRICH. with 99.8% purity the commercial product.

Berberine (BBR) loaded on BSA Nanoparticles (BSA, ≥99%), was synthetized in medicinal technology center medical institute research Alexandria university.

Synthesis of Bovine Serum Albumin Nanoparticles (BSA) and Berberine-Loaded Bovine Serum Albumin Nanoparticles (BNPs)

BSA Nanoparticles and Berberine-loaded BSA Nanoparticles (BNPs) using a slightly modified desolvation method. [38], To create BSA Nanoparticles, 200 mg of bovine serum albumin (BSA) was first dissolved in 2 mL of Milli-Q water. This mixture was then stirred at 500 rpm for 10 minutes at room temperature (25 °C). Following this, 8 mL of ethanol was slowly added drop by drop (1 mL/min) to the BSA solution while continuously stirring at the same speed and temperature until the solution turned milky. Next, 235 µL of 8% (v/v) glutaraldehyde was added to the mixture to act as a crosslinker. The reaction was allowed to stabilize by continuous stirring for 24 hours. Afterward, the resulting nanoparticles were purified through three cycles of centrifugation at 10,000 rpm for 10 minutes. Each time, the collected pellets were redispersed in 1 mL of Milli-Q water and sonicated for 5 minutes at room temperature. The final solution was then stored overnight at -80 °C before being lyophilized (freeze-dried) for future use. To create (BNPs), 20 mg of BBR (Berberine) was dissolved in 8 mL of absolute ethanol. Separately, 200 mg of BSA (bovine serum albumin) was dissolved in 2 mL of Milli-Q water. The BBR solution was then added drop by drop (1 mL/min) to the BSA solution while continuously stirring at 500 rpm. The remaining steps mirrored the synthesis procedure for the BSA NPs. Both the synthesized BSA NPs and BNPs were then lyophilized and stored at 4 °C until ready for use.

Characterization of BNPs

The hydrodynamic size distribution of the particles was assessed at 25 °C using dynamic light scattering (DLS) with a Zetasizer NanoS90 (Malvern Analytical, UK). Both lyophilized BSA nanoparticles and Berberine-BSA Nanoconjugates (BNPs) were analyzed to determine their size and morphological characteristics. Zetasizer measures the hydrodynamic diameter of the particles using the Dynamic Light Scattering technique (DLS). BBR standard curve was

constructed by preparing different concentration of BBR and measuring their absorbance at 340 nm, by UV- 1900i spectrophotometer SHIMADZU Japan, BNPs was separated from aqueous suspension, The mixture then underwent centrifugation at 15,000 rpm for 30 minutes, after which. The supernatant was collected. The absorbance of free unloaded BBR in supernatant was determined by spectrophotometric method at 340 nm. Transmission electron microscopy (TEM; JEOL-100 CX, JEOL Ltd., Tokyo, Japan) was utilized at 10 kV acceleration voltage. The samples were sonicated for 10 minutes using a bath sonicator to ensure proper dispersion of the nanoparticles. Then, 10 µL of each sample was located onto a 300-mesh copper grid that had been coated with a carbon film. (Patch No. NC0205992, Ted Pella, Inc., Redding, CA, USA) and allowed to air-dry . The grids coated with the samples were then stained using a negative staining technique with 2% uranyl acetate. (Patch No. 6159-44-0, Ted Pella, Inc., Redding, CA, USA), The samples were air-dried at room temperature before being examined with a transmission electron microscope (TEM). To obtain their Fourier-transform infrared (FTIR) spectra, pure BBR, BSA nanoparticles, and BNPs were analyzed using a Nicolet iS50 FTIR spectrophotometer (Thermo Scientific, Waltham, MA, USA). The spectral range for these measurements was 4000–400 cm^{-1}

Animals and Experimental Design

The experiment was conducted with 40 adult's male albino rats that weight 160–200 g were acquired from the animal house in the Faculty of Agriculture, Alexandria University, Egypt.

Animals in the study were maintained and fed under standard laboratory conditions. A week prior to dosing, the animals were acclimatized to their environment. Every effort was made to minimize their suffering. They were housed in stainless-steel cages within a clean, ventilated room, maintained at 40–60% relative humidity and 25°C. Throughout the experiment, the animals received a standard rodent diet and had ad libitum (free access to) water.

After 15 day of acclimation, the animals were assigned randomly to eight groups: group 1 served as control, was given 1 ml normal saline, groups 2 received Berberine given at a 10 mg/kg dose orally) [39, 40] , groups 3 Berberine Nanoparticles 5 mg/kg dose orally) [41] , whereas group 4 β-cyfluthrin (15 mg/kg BW, 1/25 LD_{50}) [42]. Group 5 received a dose β-cyfluthrin (15 mg/kg BW) followed by dose of B (10 mg/kg), Groups 6 received a dose of β- Cyfluthrin followed by dose of BN (5 mg/kg),NP was administered with a time gap of 1 hour was maintained between doses, Groups 7 received 1 ml Bovine serum albumin and Groups 8 received 1 ml corn oil, Animals in the study received daily oral doses of the tested compounds via gavage for 15 days

Sample Preparation

At the experiment's conclusion, animals were fasted overnight, The animals were put under anesthesia with ketamine hydrochloride (30 mg/kg b/w, intramuscular injection), and euthanized by cervical dislocation Blood samples were obtained from the retro-orbital venous plexus from each group. Blood was collected into plain tubes and allowed to coagulate for 10 minutes in a slanted position. Following this, the tubes were centrifuged at 1200 *g* for 20 minutes to obtain the serum, which was then stored at -80 °C for later biochemical analysis. Immediately after the animals were euthanized, their heart tissue was removed and rinsed with physiological saline. Heart tissue was quickly divided out part was used for biochemical assay (oxidative stress parameters), The biochemical assay was made in homogenate/PMS by using a double-beam ultraviolet (UV)-spectrophotometer (UV-1800, Shimadzu) stigations. The remaining portion of the heart was immediately preserved in 4% paraformaldehyde (PFA) for subsequent histopathological and immunohistochemical examinations.

Serum Biochemical Assay

The conserved serum was used for the assessment of cardiac indicator. Blood serum lactate dehydrogenase (LDH) was distinguished according to [43]. Serum creatine kinase-MB (CK-MB) were assessed using the techniques of [44] and creatine kinase (CPK) levels [45]

Estimation of oxidative stress and Antioxidant Enzyme Activity

An antioxidant enzyme activity status was measured. Estimations of total antioxidant capacity (TAC)[46] ,superoxide dismutase (SOD) assessed by [47] method and Malondialdehyde (MDA) level was measured according to Mihara and Uchiyama [48], in heart tissue by preparing a 10% homogenate.

Histopathological examination

Morphological examination was done for heart of rats animals. Tissue samples, five from each group, were initially preserved in 10% neutral buffered formalin for 2-5 days. To prepare them further, the samples underwent dehydration using increasing concentrations of ethyl alcohol, from 50% up to absolute alcohol. Following this, xylene was used in three changes to clear the samples. Finally, they were paraffin-impregnated by placing them in a hot oven with melted paraffin wax, also in three changes, at 56°C. First, the processed tissue samples were embedded in paraffin wax to form blocks. These blocks were then cut into very thin sections, measuring 5-7 µm thick, using a rotary microtome.

These thin sections were mounted onto glass slides that had been coated with egg albumin-glycerin. To ensure proper adhesion, the slides were dried in an electrical incubator at 45°C for 30-60 minutes. Finally, the sections were stained with Hematoxylin and Eosin (H&E), allowing for a general examination of the organ's structure based on[49]. Micrographs of the sections were captured using a digital camera (Leica EC3, Leica, Germany) attached to a Leica DM500 microscope.

Immunohistochemical Techique

Heart samples were collected from rats and preserved in a 4% paraformaldehyde solution in PBS (phosphate-buffered saline), with a pH of 7.4.for 2 days at 4°C After being fixed, the samples were embedded in paraffin blocks, Then, sections were cut to the desired thickness (3-5 µm) using a microtome and placed onto positively charged gelatin-coated slides. The slides were then dried by incubating them at 45°C for several hours, after which they were ready for the immunohistochemical localization of TNF-α and PCNA. The samples were stained immunohistochemically at Medical Research Institute, Alexandria University, Alexandria; Egypt. Immunostaining of paraffin-embedded heart samples was done as follow; inactivation of endogenous peroxidase using 0.3% H_2O_2 in Methanol, the antigen retrieval was done according to the antibody used (Table). The sections were blocked for an hour using PBS containing 5% bovine serum albumin. Afterward, they were incubated with the primary antibody overnight at 4°C in a humid chamber.

After washing by PBS, the sections were then incubated with a biotinylated secondary antibody for 30 minutes at room temperature. Next, the ABC complex (Vector Laboratories, USA) was applied for an hour at room temperature. The color was then developed using a DAB solution (Sigma-Aldrich, country). Lastly, the sections were counterstained with Mayer's hematoxylin, rinsed with distilled water, air-dried, and then mounted with Entellan (Merck, country). They were then photographed using a [microscope brand and country to be inserted]. In control experiments, the elimination of either the primary or secondary antibody, or the ABC complex, completely abolished all staining, meaning no positive signals were observed. The intensity of the brown color, indicating the staining, was quantified using ImageJ software (NIH).

Statistical analysis

One-way ANOVA used to evaluate the data followed by Tukey's multiple comparison test. Ns = non-significant, *P < 0.05, **P < 0.001, and ***P < 0.0001. Error bars represent mean ± SD of five rats in each group by using GraphPad prism 7 software.

Results

Characterization of Nanoparticles

Transmission electron microscope (TEM)

We used Transmission Electron Microscopy (TEM) to examine the morphology (shape and structure) of the prepared formulations Fig. (1). TEM micrographs showed that BSA NPs and BBR loaded on BSA NPs (BNPs) were formed as discrete spherical vesicles with sizes below 100 nm. Plain BSA NPs appeared as spheres. However, BNPs particles were larger in size than their corresponding plain BSA NPs. This increase in size indicated that the drug was successfully encapsulated Fig. (1A) shows plain BSA NPs with an typical size of 6.21 nm with an increase in size after loaded of BNPs the average size reached 29.87 nm Fig. (1B).

Zeta potential analysis (Surface charge)

Zeta potential *(ζ)* of both plain BSA NP and BNPs were determined using Zetasizer, the zeta potential of BSA NPs was - 4.6 mv, the zeta potential of BNPs was – 3.21 mv Fig (2).

Determination of drug encapsulation efficacy (EE)

By calculation of total paclitaxel concentration and the concentration of the free paclitaxel which was not encapsulated into the liposomes, The drug encapsulation efficacy of the prepared BSA NPs loaded BBR (EE) was 80 %. The procedure was repeated three times and the result represent the mean of the produced values.

Fourier transnormal infrared spectroscopy (FTIR)

We used Fourier-transform infrared (FTIR) spectroscopy to assess any chemical changes that occurred within the nanoparticle formulations. To verify the production of (BNPs), FT-IR spectroscopy was employed In order to further confirm BSA NPs coupling with BBR. BSA-NPs and BNPs were analyzed using FTIR. FTIR analysis is used to investigate intermolecular interactions, through shift or broadening of the bands in the spectra. In Fig. (3), FTIR spectra corresponding to the BSA NPs, the basic characteristics peaks of BSA NPs at 3431.2 cm−1 that represent (O-H stretch and N-H, overlap), 1630.2 cm−1 that represent (NH2 deformation, C-C), 1388.5, 1317.4, 1274.7, 1170.4 and 1099.3, 1014 cm−1 that represent (C-O stretch), 952.4 cm−1 that represent (C-H stretch) , FTIR spectra corresponding to the BNPs, the basic characteristics peaks of BNPs at 3421.8 cm−1 that represent (O-H stretch and N-H, overlap), 2914.6 cm−1 that represent C–H stretching (alkanes), 1549.1, 1544,9, 1454.8 cm−1 that represent (NH2 deformation, C-C), 1383.7, 1322, 1274.7, 1170.4, 1118.3 cm−1 that represent (C-O stretch), 928.7 cm−1 that represent (C-H stretch) (1) As expected, the FT-IR spectrum of BSA NPs (A) without any significant change and BNPs (B) showed the basic characteristic peaks of the protein, and.

However, the peaks at 3431.2 in (A), 3421.8 in (B) cm-1 (amide A, related to N–H stretching), 2978.75 cm-1 (amide B, N–H stretching of NH3+ free ion), 1630.2 in (A) cm-1 1549.1, 1544,9, in (B) (amide I, C=O stretching), 1454.8 cm−1 in (B) (amide II, related to C–N stretching and N–H bending vibrations), and 1388.5, 1317.4 in (A) and 1383.7, 1322 cm-1 (CH2 bending groups), [2] Bronze et al. 2017. A protein structure is determined using amide I, II, III, and (A) IR absorption peaks [3]. Ultimately, the findings indicate that BNPs /BSA nanoparticles were synthesized.

Serum Biochemical Assay

The results explains that rats receive BNPs only, BBR only, BSA and CO showed no alteration in serum levels of LDH, CBK and CK-MP when contrasted with control, Fig.(4). However, rats that receive β-cyfluthrin (β- CYF) induce Significant increase LDH, CBK and CK-MP levels contrasted with control group. Meanwhile the group that receive β-Cyf + (BNP) shows non-significant drop in serum levels of LDH, CBK and CK-MP in comparison to β-Cyf intoxicated rats. β- CYF + BBR group shows non-significant reduction LDH, CBK and CK-MP levels compared to β-Cyf intoxicated rats but less effective than the β-Cyf + (BNP) group Fig.4.

Effect of β-Cyfluthrin and Berberine nanoparticles on of oxidative/antioxidant status

The results show Significant decreased SOD and TAC activity in β-cyfluthrin (β-CYF) intoxicated group, with elevated MDA level contrasted with control group. thought the group that receive (β-CYF) + (BNPs) shows restoration of SOD and TAC levels with reduction in MDA levels in comparison to β-Cyf intoxicated rats. β-CYF + BBR group shows increased SOD and TAC with moderate reduction in MDA activity compared to β-CYF intoxicated group but less effective than the nanoparticle form. The rats that received BNPs only, BBR only groups, BSA (Bovine Serum Albumin) and CO (Corn oil) groups show normal SOD and TAC activity nearly as control group. Fig.5.

Histopathological study

Histopathological examination of rat heart demonstrates regular branching of the striated muscle myofibers, centrally located oval nuclei with normal histological structure and regular interstitial space contain vascular supply as showing in Fig. 6. BBR, BNPs, BSA and CO were showing normal cardiac muscle structure and improvement was notice in regularity, striation and nuclei arrangement, Fig. 7, 8, 9,10.

β-CYF treated group showing muscular degeneration with market congestion of blood capillaries, vacuolation of myocytes, apoptotic nuclei, sever intermuscular edema and destruction of muscular striation, Fig.11 β-CYF and BBR treated showing apparently normal striated myofibers, mild vacuolated and apoptotic myocytes as in Fig. 12. β-CYF and BNPs treated group showing restoration in muscular normal arrangement, Berberine nano particle make recovery for degenerated tissue and mild toxicity was seen as showing in Fig.13.

Immunohistochemical examination.

Tumor necrosis factor-alpha (TNF-α) is an inflammatory cytokine that plays a role in heart inflammation. It's a pleiotropic cytokine, meaning it can have various effects, and is produced by several immune cells, notably macrophages and monocytes. TNF-α has the ability to activate multiple signaling pathways involved in processes like inflammation, cell proliferation, and programmed cell death (apoptosis). Normal heart tissue shows no reactivity by TNF-α which regular branching, arrangement of cardio myofibers shows in Fig. (14, 15, 16, 17, 18). β-cyfluthrin shows intense reaction with TNFα as apoptotic, necrotic nuclei of cardiomyocytes, inflammatory cells infiltration and degeneration with market perivascular congestion intense brown color was seen (Fig. 19). groups received β-CYF and BBR, β-CYF and BNPs shows moderate reaction by TNF-α which regeneration in cardiomyocytes occurs and decrease inflammation in cardiac muscle cell, lymphocytes infiltrations as mild brown color was seen in Fig. (20 and 21).

Anti-Proliferative cellular nuclear antigen (PCNA) who's finding is analogous to assessing cell proliferation. Control animal with normal expression of PCNA in heart cells nucleus and strong reactivity was seen indicate higher proliferations in improved cardiomyocytes and interstitial cells (Fig. 23, 24, 25, 26, 27). **β-CYF** injured heart showing numerous positive nuclei (brown staining) in fibroblast, macrophages within inflamed interstitial area indicated active proliferation, positive reaction in some endothelial cell and cardiomyocytes limited reaction (Fig. 28). **BBR and BNPs** treated toxic group showing reduced PCNA expression in inflammatory, fibroblast cell infiltration, cardiomyocytes show mild regenerative nuclear PCNA positively and preservation of myocardial fibers was seen in Fig.29 and 30.

Discussion

Prolonged exposure to pesticides is one factor contributing to cardiac complications.[50] ,In the present study aimed to investigate β-Cyf-induced cardiotoxicity and its decisive Principle of cardiotoxicity and the cardioprotective Consequences of BNP. Pyrethroid is one of the most often used environmental insecticides worldwide. However, evidence from animals indicates that it may have a negative impact on the cardiovascular system, namely in relation to inflammation and oxidative stress.[17].

However, BNPs effectively countered the toxic effects because of its strong antioxidant and anti-inflammatory characteristics. The size of the nanoparticles and their anti-inflammatory and antioxidant characteristics are thought to be the most crucial factors in their effectiveness. Smaller particles achieve greater permeation. [51] A smaller size range is indicated by a lower PDI value. The stability of colloidal nanoparticles dispersion is predicted by the surface charge. The value of the zeta charge is regarded as an evaluation of surface charge. When the zeta potential value is greater than 20, the formulations are electrostatically stabilized.[52] .

An imbalance between oxidant and antioxidant systems leads to oxidative stress, where oxygen-derived free radicals cause injury to critical cellular structures, representing an important mechanism of toxicity.[53] Reactive oxygen species formation which upset the cellular redox equilibrium and cause oxidative stress, is one of the main processes behind pyrethroid toxicity. Important biological constituents like lipids, proteins, and DNA are harmed by this stress. [54] Increased lipid peroxidation (as raised levels of malondialdehyde [MDA]) and decreased antioxidant defence mechanisms, such as glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) activity, are common indicators of pyrethroid-induced oxidative stress in the cardiac muscles [6, 54]. Oxidative imbalance caused by β-cyfluthrin exposure included suppression of the enzymatic antioxidant defence system in addition to an increase in LPO and a decrease in GSH [55]. Small variations in the level of these enzymatic antioxidants can have a significant impact for how well the lipids, proteins, and DNA within cells can withstand damage from oxidation. enzymatic antioxidant defence offer an amazing array of defence processes that are impactful in keeping ROS under optimal concentrations to neutralize free radicals and prevent oxidative damage to cells.[56, 57] The impact of β-cyfluthrin on antioxidant function is consistent with several investigations that documented notable alterations in various rat organs exposed to pyrethroids.[15, 58, 59], In addition alterations in physiochemical resources, membrane fluidity influences ion exchange characteristics and signal transduction, ultimately resulting in membrane remodeling.[60, 61].

In the recent study , Upregulated quantities of MDA were directly shown in heart tissues after β-Cyf administration , that ensure membrane impairment and cellular seepage[62], greater intracellular concentration of glutathione, a thiol-containing compound with reducing properties. maintains the redox state in the cells [63] In situations where the disease advances by producing ROS and redox imbalance, which strains the structural and functional stability of cells and ultimately results in their demise, depletion of GSH concentration is widely documented. [64] Also, β-CYF drastically reduced TAC and SOD, with significantly increasing lipid peroxidation, confirming its oxidative toxicity. [65] Research on herbal medicine for treatment of variety of disorders is steadily increasing, mostly because phytochemicals are recurrently shown to be less adverse effects than synthetic medications.[66]

BNPs treatment dramatically reduced tissue levels of cardiac oxidative indicators in rats exposed to β-CYF. It is thought to be a strong antioxidant by balancing oxidative indicators, which may mitigate the adverse effects on rat cardiac tissue.[34, 41] recent study show, Co-treatment groups (β-CYF + BBR, β-CYF + BNP) provided functional protection, (BNPs) and (BBR), effectively restores SOD and TAC activity, and reduces MDA levels, indicating a protective antioxidant effect. with CYF + BNP being the most potent treatment.

ROS induce myocardial damage that mediated by opening of the MPTP, acting as a neutrophil chemoattractant.[67] Which induce intracellular Ca^{2+} overload and cell membrane damages due to lipid peroxidation, persuading enzyme denaturation and eventually oxidative damage to DNA. The production of cytokines, activated complement, and the chemoattractant ROS led to gather of neutrophils in the infracted cardiac tissue after myocardial reperfusion. [68, 69]

A study on β-cyfluthrin, indicated increase serum cardiac markers (e.g., CK-MB and LDH), with histological signal of myocardial inflammation, further confirming the role of pyrethroids in inducing cardiac injury and inflammation [70]

The results express significant increase in LDH, CPK and CK-MB the markers of cardiac injury; was observed due to intoxication of β-cyfluthrin group versus control, so, it might be determined that elevated cardiac-specific serum indicators as result of increased cellular leak in bloodstream, likely due to membrane disruption [71, 72]. BNPs treatment led to a reduction in cardiac enzyme concentration in serum and improved myocardial structural damage[41] recent results indicated that coadministration of BNPs and BBR with β-cyfluthrin decrease the serum concentration of that cardiac markers , previous work shown that BNPs treatment reduced these particular indicators in the co-administered groups primarily via controlling membrane stability through their antioxidant power.[73, 74] However, the BNP and BBR -alone treated group exhibited no observable changes in comparison to the control group.

Exposure to pyrethroids has been shown to activate both oxidative stress pathways and pro-inflammatory signaling cascades, collectively contributing to tissue damage and the subsequent activation of immune cells.[75] Histopathological

analyses of cardiac tissue exposed to pyrethroids frequently show degeneration of heart muscle fibers, interstitial oedema, and mononuclear cell infiltration.[76]

Histopathological consequences revealed distorted cardiac tissue in β-cyfluthrin group showing muscular degeneration with market congestion of blood capillaries, vacuolation of myocytes, apoptotic nuclei, sever intermuscular edema and destruction of muscular striation, These findings align with previous studies.[76, 77].β-cyfluthrin and Berberine treated showing apparently normal striated myofibers, mild vacuolated and apoptotic myocytes. β-cyfluthrin and berberine nano particle treated group showing restoration in muscular normal arrangement, Berberine nano particle make recovery for degenerated tissue, as reported earlier[41, 78].

Environmental contaminants, as pyrethroid can induce the secretion of inflammatory cytokines[79-81]. It is well recognized that oxidative stress, which toxicants can activates sensitive transcription factors, which in turn cause inflammatory reactions.[82]. Cyfluthrin increased significantly gene expressions of inflammatory marker (TNFα) [83] In a recent study, it was suggested that these transcription factors might have been activated by the increased oxidative stress induced by β-Cyf, producing the increased TNF-α and PCNA amount which clearly seen by immunohistochemistry. PCNA is a key marker of cell proliferation and DNA repair. In the heart, upregulation would suggest increased cardiac cell turnover or reparative activity, possibly in response to injury. Many investigations have shown that BBR can improve endothelial dysfunction by regulating the balance between reactive oxygen species (ROS) and nitric oxide (NO). [84] in the present study, upon concurrent treatment with BNP, it reversed the expression of TNF-α that result consistent with [85, 86] BBR has anti-proliferative and anti-inflammatory possessions through lowering the countenance of proliferating cell nuclear antigen (PCNA) Our findings align with an earlier report. [86, 87]

Conclusion

In conclusion, results of this study showed that giving a sub-lethal dose orally of β-Cyfluthrin interrupt the cellular antioxidant defenses and encourage inflammation of rat`s cardiac tissue. Berberine and Berberine Nano, are antioxidant or anti-inflammatory compounds, show a protective duty in eliminating the harmful effects of inflammation and oxidative stress, which can also cause cardiac damage. Therefore, because of its anti-inflammatory and free radical scavenging qualities, berberine and berberine nano may be safe, natural medications to employ in β-Cyf-induced cardiotoxicities.

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Declaration of Conflict of Interest

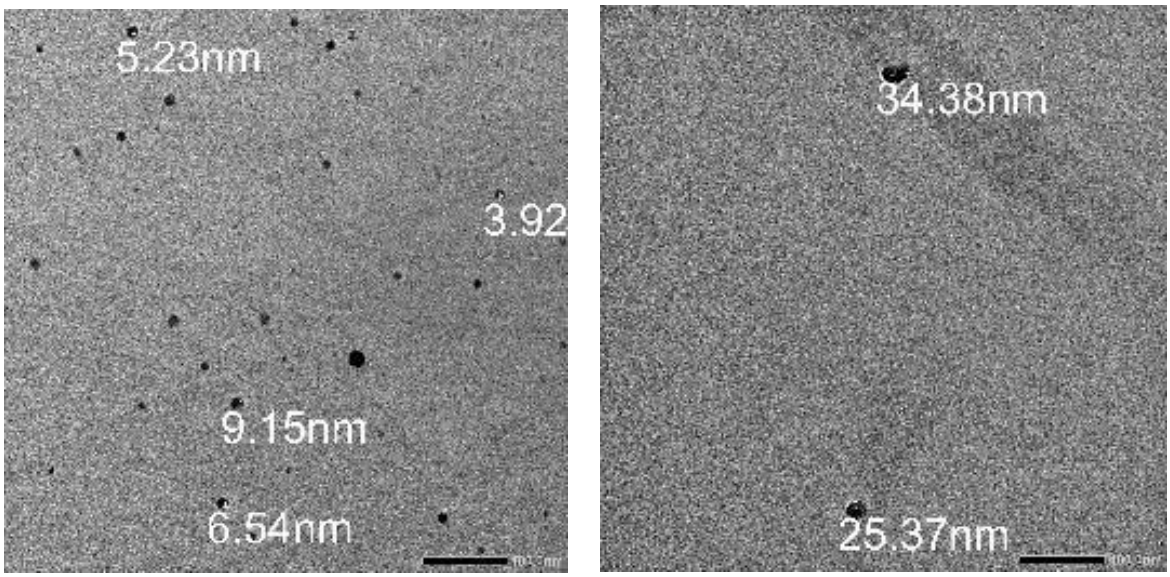
The authors claim to have no conflicts of interest. for this study.

Ethical of approval

All experiments were conducted in accordance with the standard Guidelines of Faculty of Veterinary Medicine, Damanhour University under all hygienic conditions using recoded published methods (Approval Number: DMU/VetMed-2025/018).

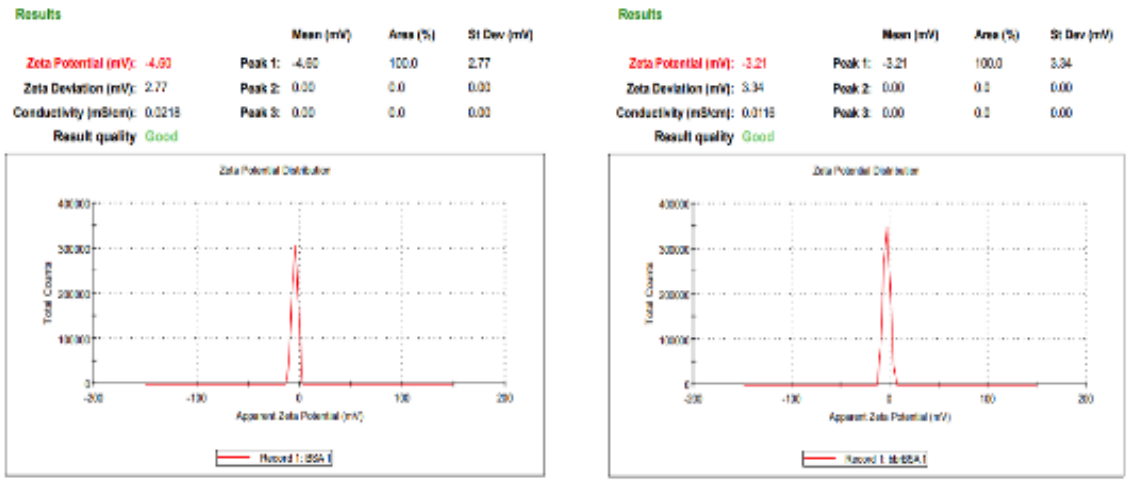
TABLE 1 . Below is a list detailing the antibodies used, their respective antigen retrieval methods, incubation periods, and working dilutions.

Primary AB	Source	Dilution of 1ry AB	Secondary AB	Dilution of 2ry AB	Antigen retrieval
Tumor necrosis factor alfa (TNF- α), human	Sigma-Aldrich Chemie GmbH	1:200	Anti-human	1:50	CB (citrate buffer) at 105°C for 20 min or 37°c overnight
Goat polyclonal anti-proliferative cell nuclear antigen (PCNA) Human	sc-9857, Santa Cruz, CA, USA)	1:2000	Anti-human	1:200	10 mM citrate buffer (pH 6.0) at 95°C, 20 min



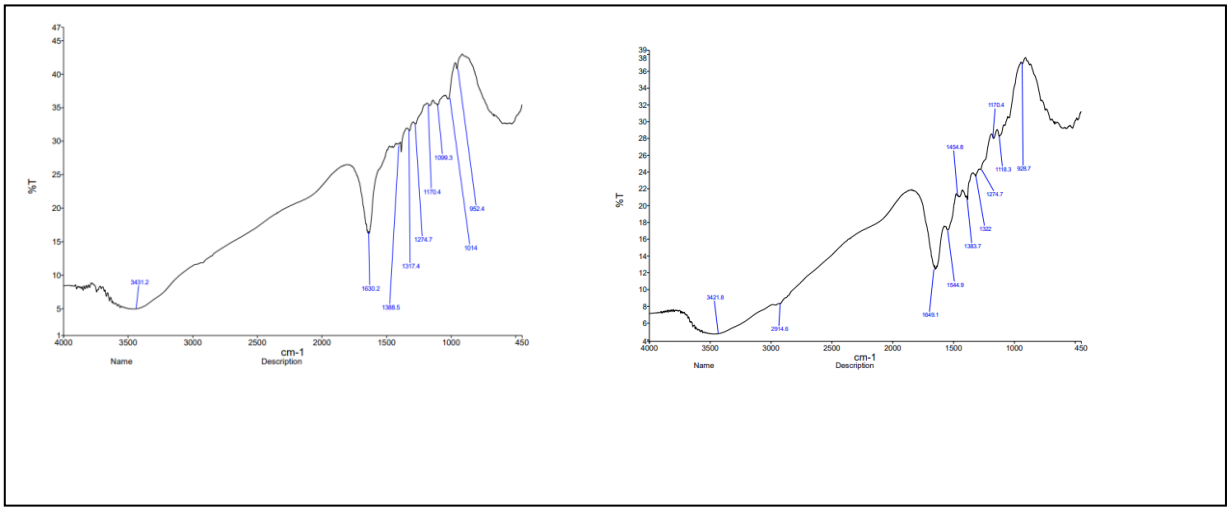
(A) BSA NP **(B)BNPs**

Fig 1. TEM image of BSA NP(A) and BNPs (B)



(A) **(B)**

Fig 2. Zeta potential distribution of BSA NPs (A) and BNPs (B) by Zetasizer



A **B**

Fig. 3. A. FTIR of BSA NPs. B. FTIR of BNPs

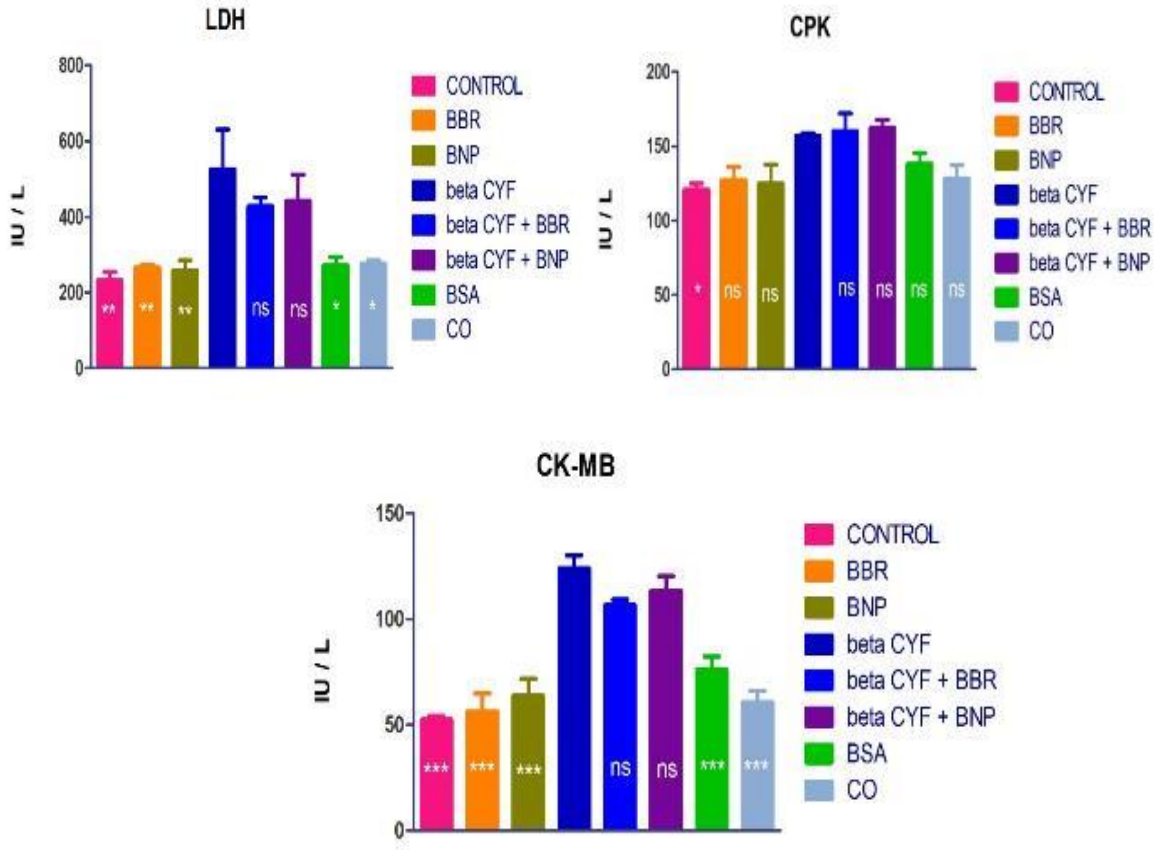


Fig. 4. Representative Changes in LDH, CBK and CK-MP levels in serum of male rats in different experimental groups (Control, BBR, BNP β- CYF β- CYF treated BBR; β- CYF treated BNP, BSA and CO groups). Data were investigated with a one-way ANOVA followed by Tukey's multiple comparison test. Ns = non-significant, *P < 0.05, **P < 0.001, and ***P < 0.0001. Error bars represent mean ± SD of five rats in each group.. BBR; Berberine. BNP; Berberine nanoparticle. β-CYF; β-cyfluthrin.

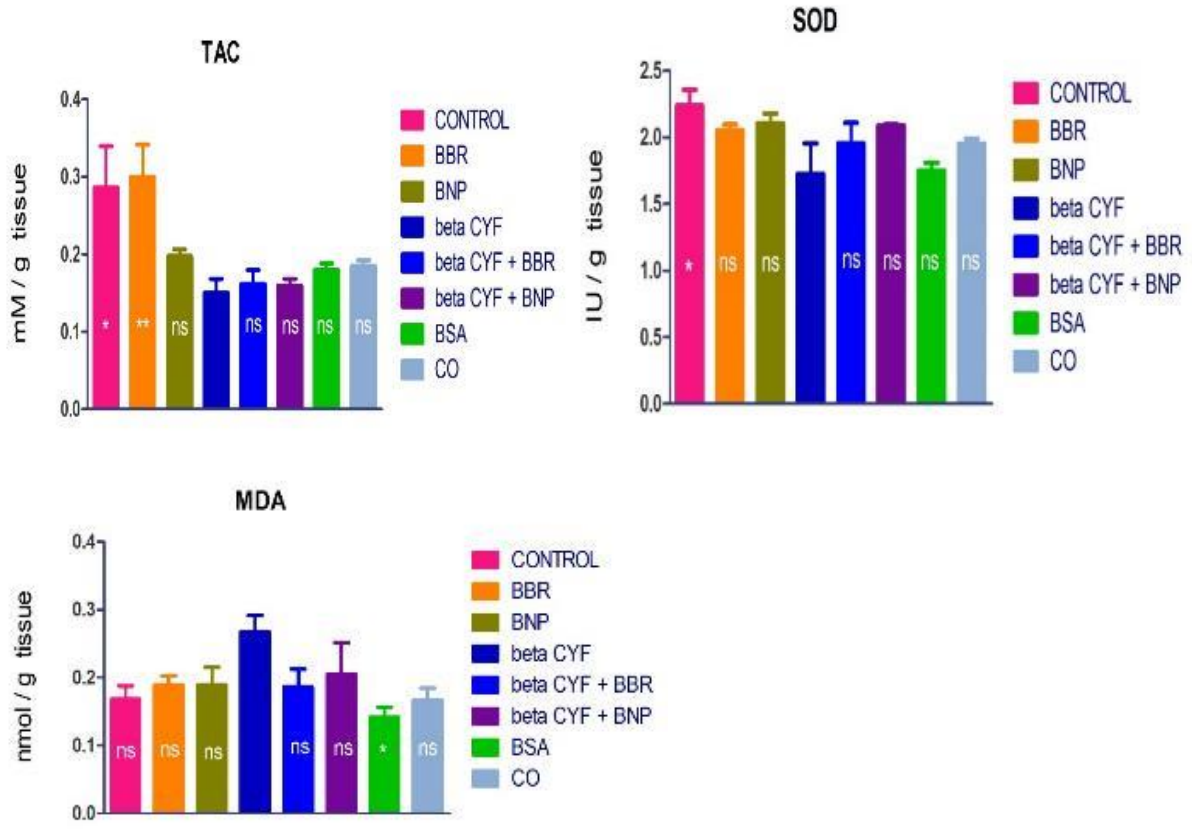


Fig. 5. Representative Changes in Total Antioxidant capacity (TAC) , Super oxide dismutase (SOD) and Malondialdehyde (MDA) levels in different experimental groups (Control, BBR, BNPs, β-CYF, β- CYF treated BBR; β- CYF treated BNPs, BSA and CO groups). Data were analysed with a one-way ANOVA followed by Tukey's multiple comparison test. Ns = non-significant, *P < 0.05, **P < 0.001, and ***P < 0.0001. Error bars represent mean ± SD of five rats in each group.. BBR; Berberine. BNPs; Berberine nanoparticle. β- CYF; β-cyfluthrin.

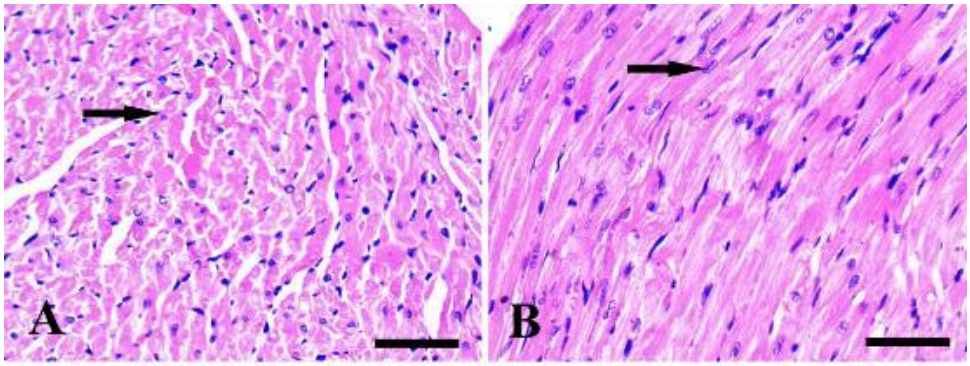


Fig. 6. Photomicrograph of control rat heart, Hematoxylin and eosin stain; 50 µm scale bar. A, normal atria structure with normal branched, striated and thin cardiomyocytes than ventricles (arrow). B, showing normal ventricle histological structure characterized by branching, acidophilic of the striated muscle cells (arrow).

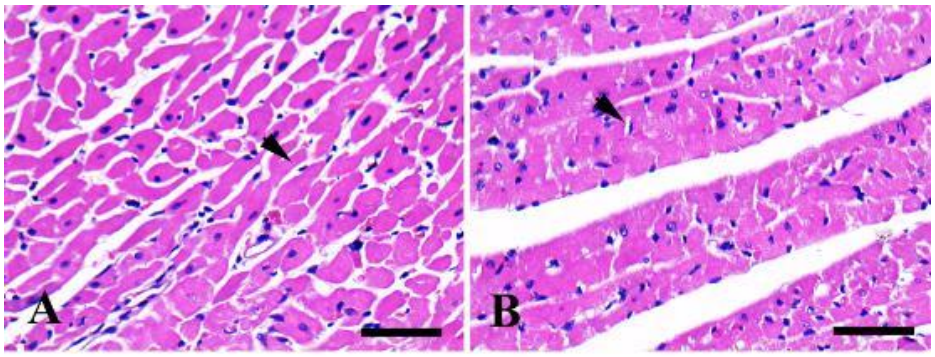


Fig. 7. Photomicrograph of rat heart, treated by BBR, Hematoxylin and eosin stain; 50 µm scale bar. A, normal atria structure with normal nuclei of cardiomyocytes (arrow head). B, normal striated muscular structure with regular situated nuclei (head arrow).

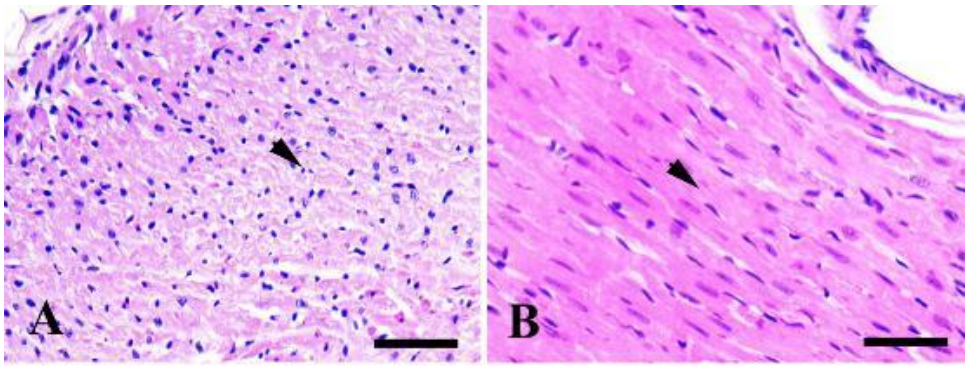


Fig. 8. Photomicrograph of rat heart, treated by BNPs, Hematoxylin and eosin stain; 50 µm scale bar. A, showing normal atria structure with normal cardiomyocytes (arrow head). B, showing normal muscular arrangement and branching striated myofibers (arrowhead).

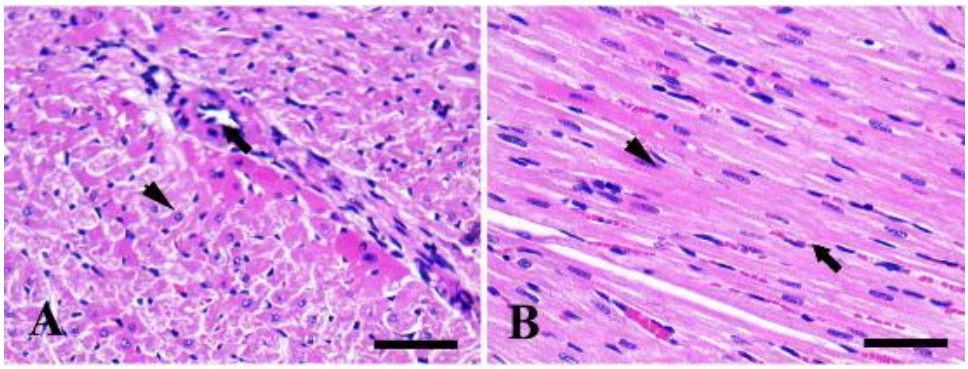


Fig. 9. Photomicrograph of rat heart, BSA treated group, Hematoxylin and eosin stain; 50 µm scale bar. A, showing atria structure with regular cardiomyocytes (arrow head) and blood supply in interstitial tissues (arrow). B, showing regular cardiac myofibers arrangement with centrally located nuclei (arrow head) and vascular supply in interstitial space (arrow).

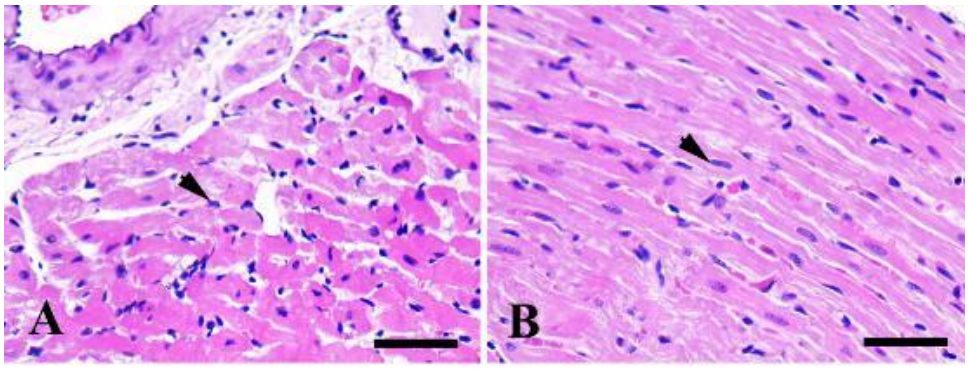


Fig. 10. Photomicrograph of rat heart, CO treated group, Hematoxylin and eosin stain; 50 µm scale bar. A, showing atria structure with normal structure of cardiomyocytes (arrow head). B, supplied group showing normal striated acidophilic myofibers (arrow head).

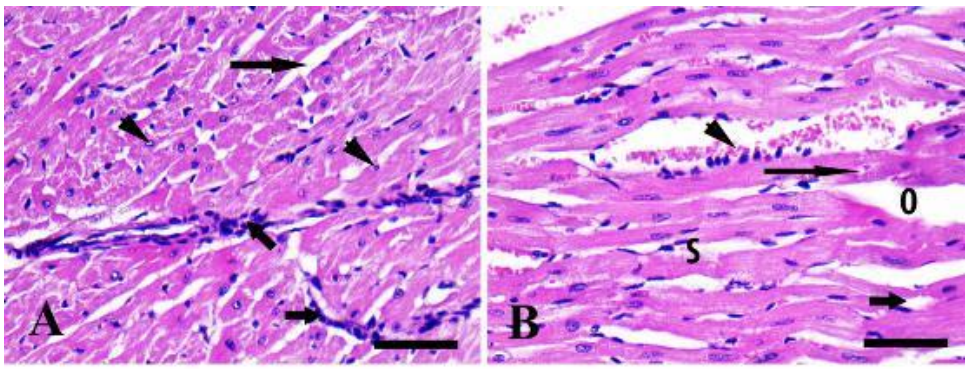


Fig. 11. Photomicrograph of rat heart, β-cyf treated group, Hematoxylin and eosin stain; 50 µm scale bar. A, showing atria structure with pycnotic, necrotic nuclei of cardiomyocytes (arrow head), sever inflammatory cells infiltration (short arrow) and intramuscular edema (long arrow). B, showing muscular degeneration with market congestion of blood capillaries with inflammatory cells infiltration (head arrow), vacuolation of myocytes (short arrow), apoptotic nuclei (long arrow), sever intermuscular edema (O) and destruction of muscular striation (S).

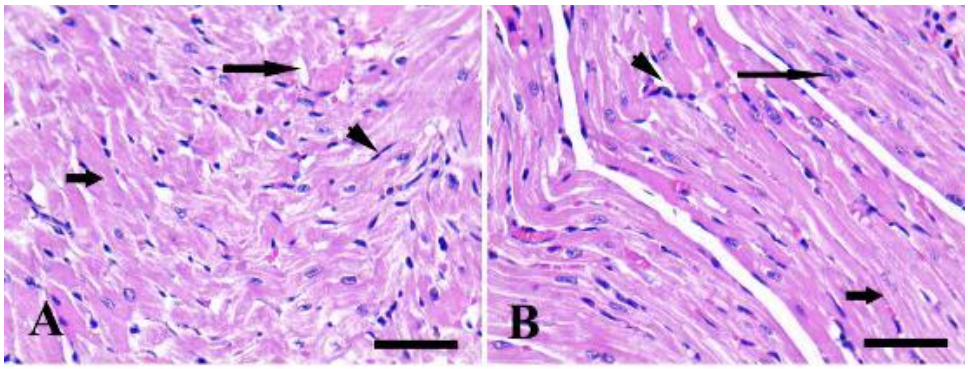


Fig. 12. Photomicrograph of rat heart, β-Cyf and BBR treated group, Hematoxylin and eosin stain; 50 µm scale bar. A, showing atria structure with regenerated cardiomyocytes (short arrow), decrease inflammatory cells infiltration (head arrow) and mild intramuscular edema (long arrow). B, showing apparently normal striated myofibers (arrow), mild vacuolated and apoptotic myocytes (head arrow) and mild congestion in blood capillaries (short arrow).

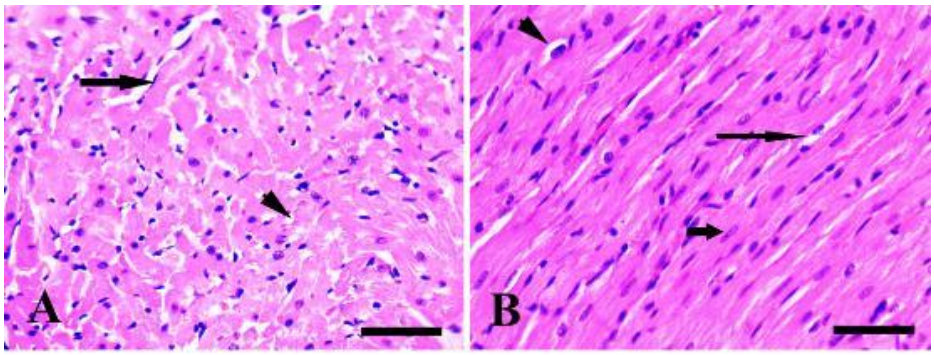


Fig. 13. Photomicrograph of rat heart, β-Cyf and BNPs treated group, Hematoxylin and eosin stain; 50 µm scale bar. A, showing atria structure with mild vacuolation, regeneration in cardiomyocytes (head arrow), and decrease intramuscular edema with congestion (long arrow). B, showing restoration in muscular normal arrangement (arrow), mild vacuolation (head arrow) and mil- intermuscular edema (long arrow).

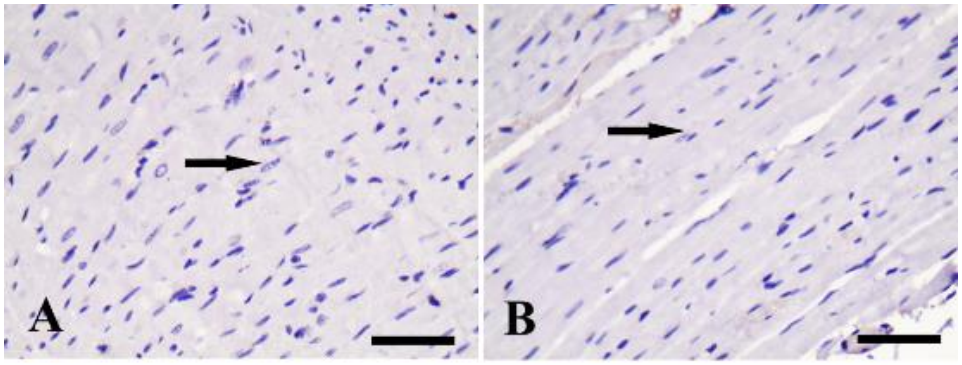


Fig. 14. Immunohistochemical study of rat heart stained by (tumor necrosis factor-alpha) TNF-α. A, B control group without treatment showing normal cardiomyocytes branching and no reactivity by TNF-α. Scale bar= 50 µm.

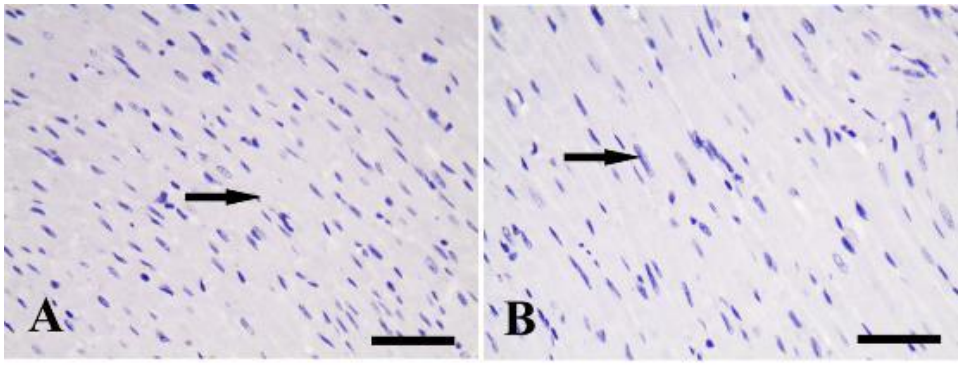


Fig. 15. Immunohistochemical study of rat heart stained by TNF-α. A, B BBR group treatment showing normal cardiomyocytes cells branching and no reactivity by TNF-α. Scale bar= 50 µm.

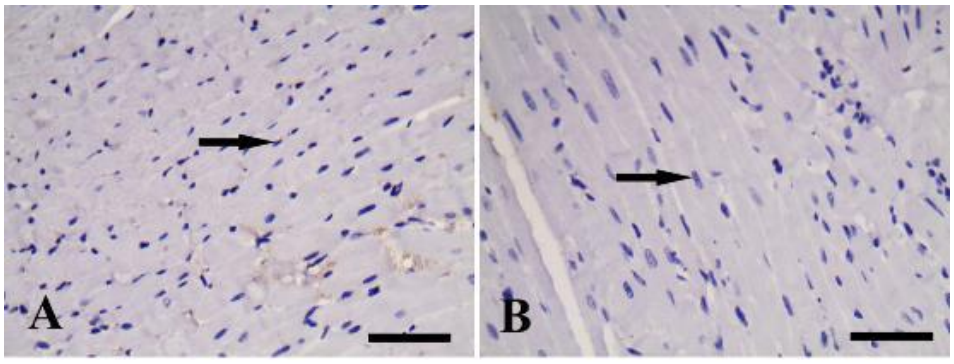


Fig. 16. Immunohistochemical study of rat heart stained by TNF-α. A, B BNPs group treatment showing normal, healthy cardiac muscle branching and no reactivity by TNF-α. Scale bar= 50 µm.

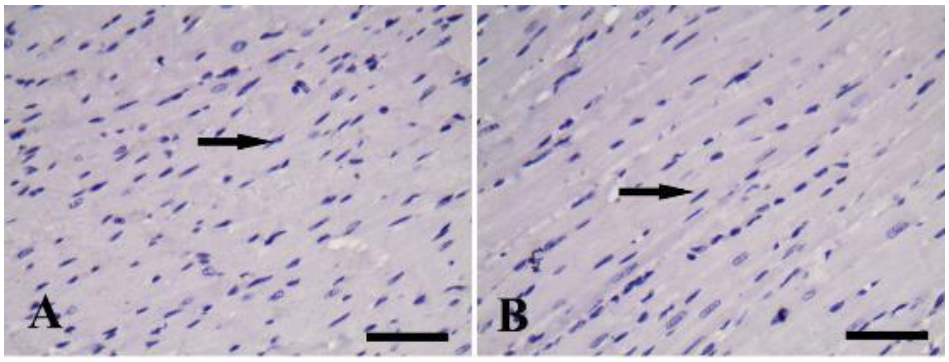


Fig. 17. Immunohistochemical study of rat heart stained by TNF-α. A, B BSA group treatment showing normal cardiomyofibers branching and no reactivity by TNF-α. Scale bar= 50 µm.

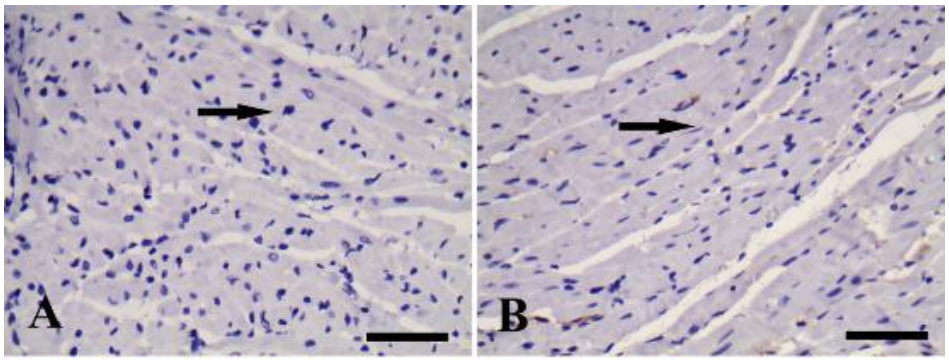


Fig. 18. Immunohistochemical study of rat heart stained by TNF-α. A, B, CO group treatment showing normal cardiomyocytes branching and no reactivity by TNF-α. Scale bar= 50 µm.

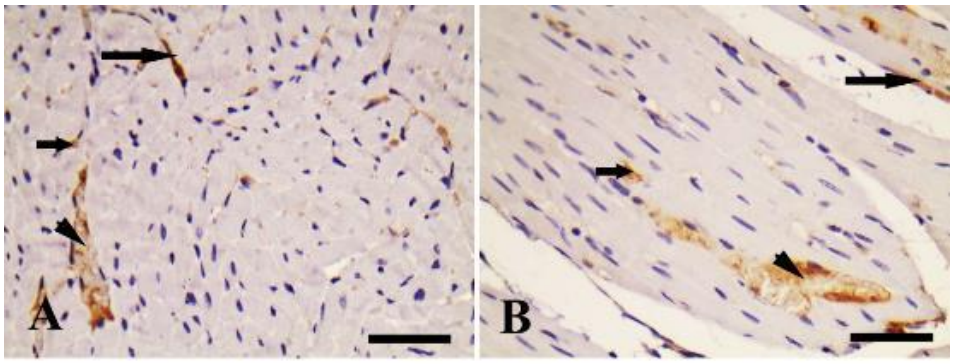


Fig. 19. Immunohistochemical study of rat heart stained by TNF-α. A, B β-CYF group treatment showing apoptotic, necrotic nuclei of cardiomyocytes (short arrows), inflammatory cells infiltration (long arrow) and degeneration with market perivascular congestion (head arrow) (brown color). Scale bar= 50 µm.

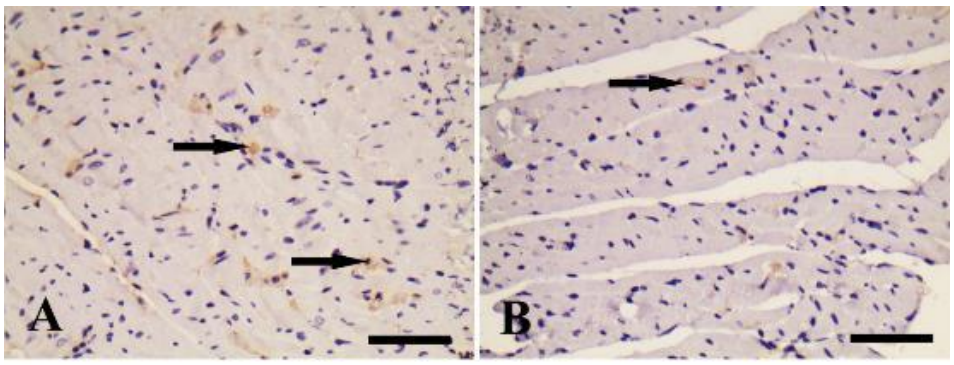


Fig. 20. Immunohistochemical study of rat heart stained by TNF-α. A, B β-CYF and BBR group treatment showing regenerate cardiomyocytes (arrows), decrease inflammation (brown color). Scale bar= 50 µm.

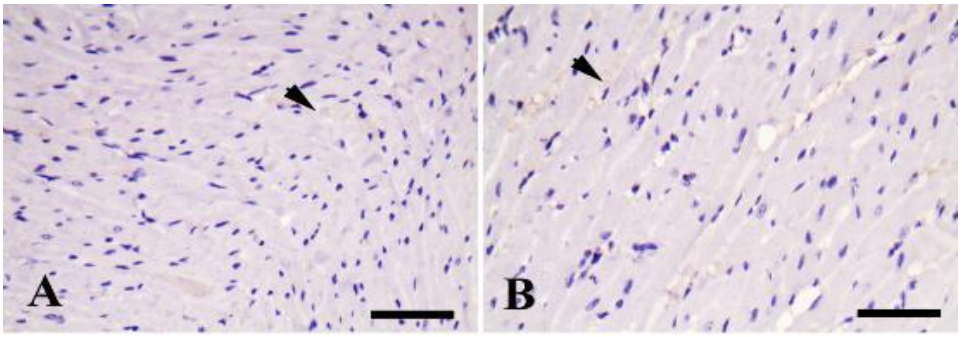


Fig. 21. Immunohistochemical study of rat heart stained by TNF-α. A, B β-CYF and BBR group treatment showing regenerated cardiomyocytes (arrows), mild inflammation (brown color) . Scale bar= 50 µm.

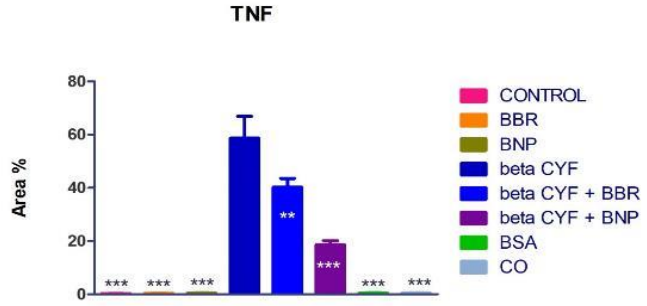


Fig. 22 . Immunohistochemical study of rat heart stained by TNF-α of rats heart in different experimental groups, (Control, BBR, BNP β- CYF β- CYF treated BBR; β- CYF treated BNP, BSA and CO groups). Data were evaluated with a one-way ANOVA followed by Tukey's multiple comparison test. Ns = non-significant, *P < 0.05, **P < 0.001, and ***P < 0.0001. Error bars represent mean ± SD of five rats in each group. BBR; Berberine. BNP; Berberine nanoparticle. β-CYF; β-cyfluthrin.

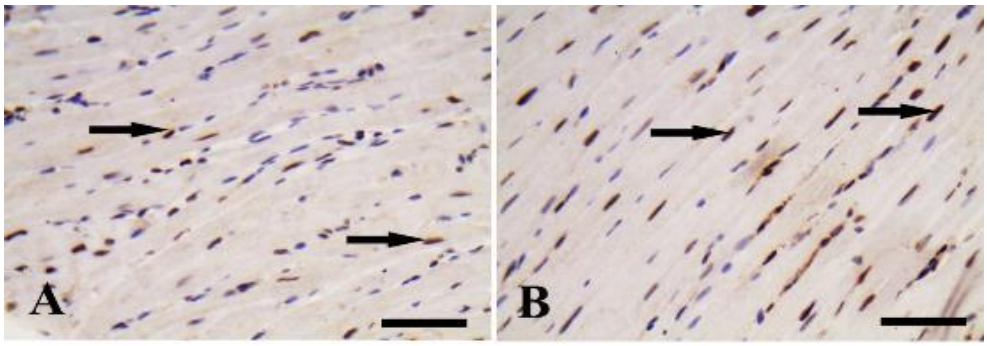


Fig. 23. Immunohistochemical (ICH) study of rat heart stained by proliferating cell nuclear antigen (PCNA) as PCNA is a indicator for cell proliferation). A, B Control group showing strong PCNA positive nuclei (brown color), (arrows) indicate higher proliferation activity. Scale bar= 50 µm

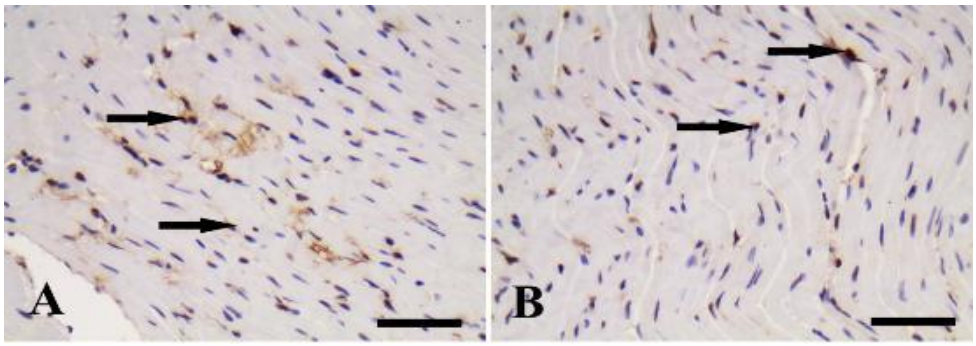


Fig. 24. Immunohistochemical study of rat heart stained by (PCNA). A, B, BBR group treatment showing normal reactivity in heart cells nucleus (arrows) (brown color). Scale bar= 50 µm.

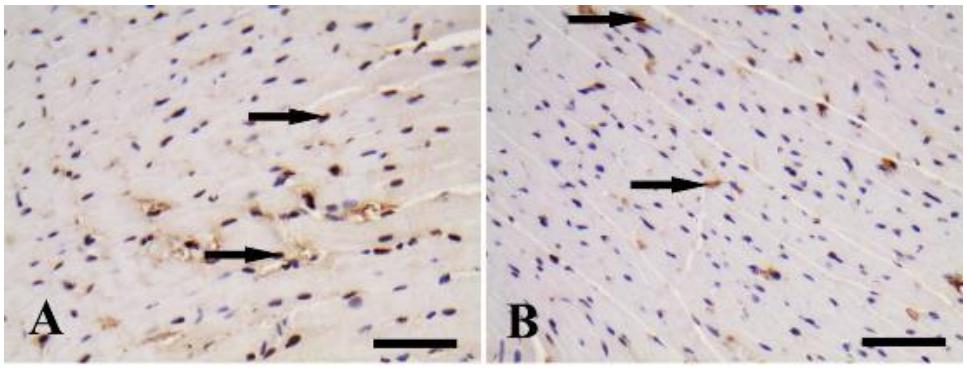


Fig. 25. Immunohistochemical study of rat heart stained by (PCNA). A, B BNPs group treatment showing normal reactivity in heart cell's nucleus (arrows) (brown color). Scale bar= 50 µm.

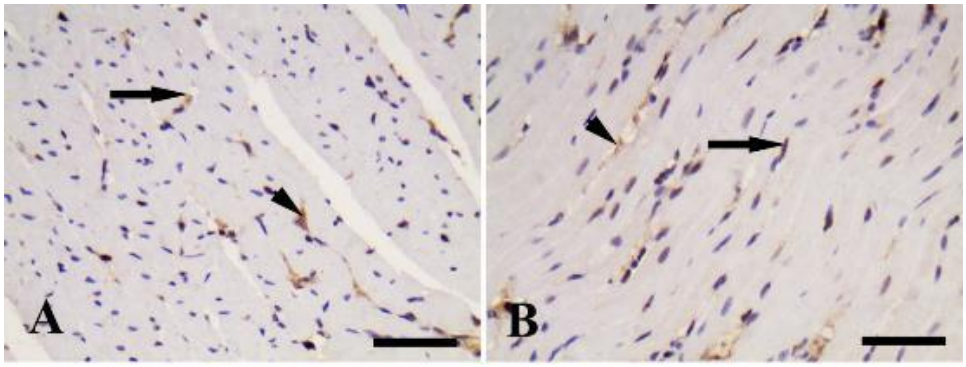


Fig. 26. Immunohistochemical study of rat heart stained by (PCNA). A, B, BSA group showing sparse PCNA positive as low proliferations, brown stained nuclei (arrows) (brown color) and interstitial tissue cell (arrow head) . Scale bar= 50 µm.

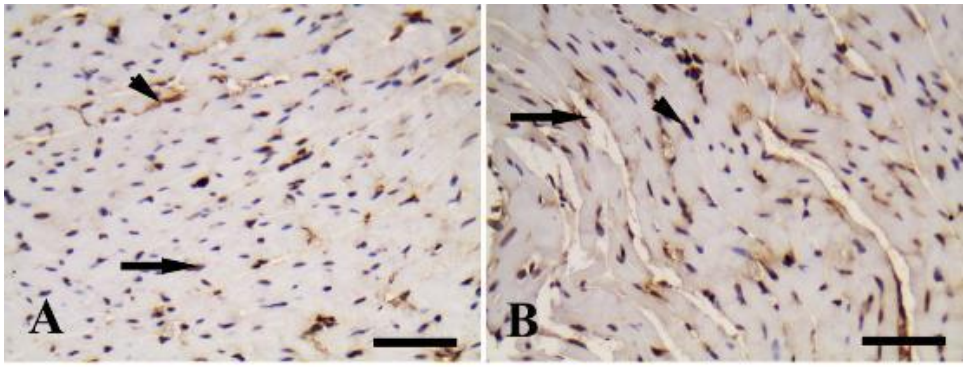


Fig. 27. Immunohistochemical staining for PCNA rat heart. A, B, CO group treatment showing normal reactivity in cardiac muscle nucleus (arrows) (brown color) and interstitial cells (arrowhead) 50 µm scale.

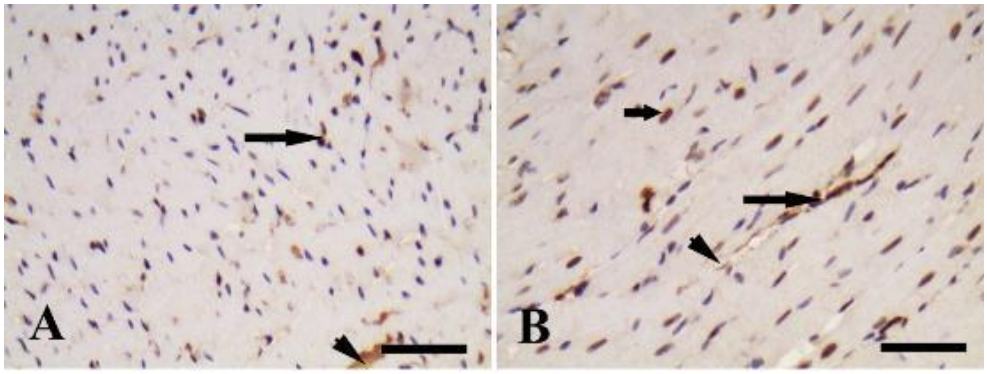


Fig. 28. Immunohistochemical study of rat heart stained by (PCNA). A, B β-CYF injured heart showing numerous positive nuclei(brown staining) in fibroblast, macrophages within inflamed interstitial area indicated active proliferation (long arrows), positive reaction in some endothelial cell (arrows head) and cardiomyocytes limited reaction (short arrow) . 50 µm scale.

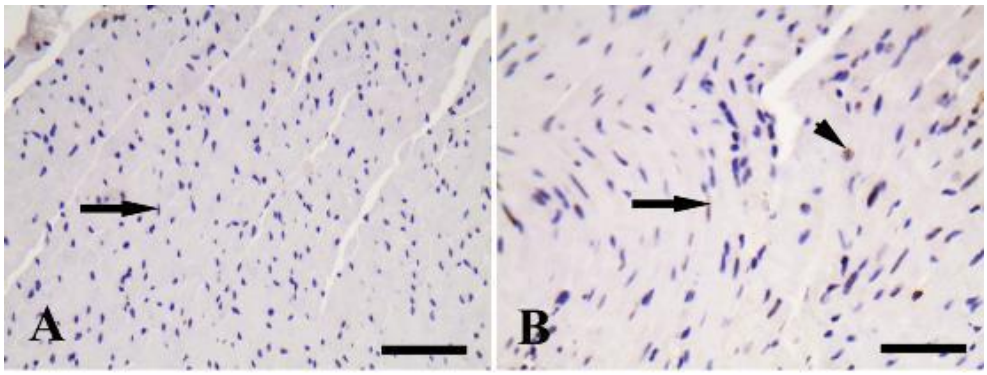


Fig. 29. Immunohistochemical study of rat heart stained by (PCNA). A, B β-CYF and BBR group treatment showing reduced PCNA expression in inflammatory cell infiltration (long arrows), and cardiomyocytes show mild regenerative nuclear PCNA positively (head arrow) . Scale bar= 50 µm.

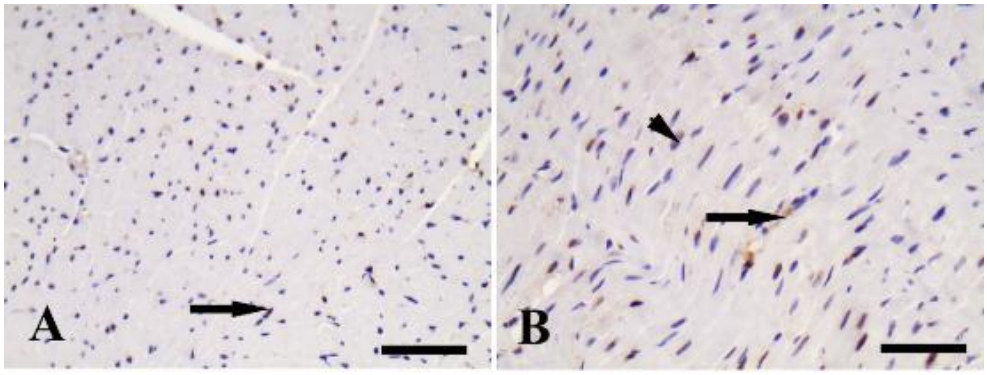


Fig. 30. Immunohistochemical staining of PCNA in rat heart. A, B Heart treated with Berberine nanoparticles demonstrating markedly reduced PCNA positively, with preservation of myocardial fibers (head arrow) and minimal fibroblast (long arrows). Scale bar= 50 µm.

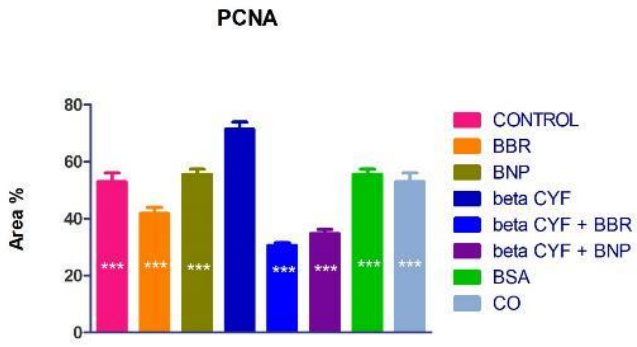


Fig. 31. Immunohistochemical study of rat heart stained by PCNA in rat heart in different experimental groups, (Control, BBR, BNP β- CYF β- CYF treated BBR; β- CYF treated BNP, BSA and CO groups). Data were evaluated with a one-way ANOVA followed by Tukey's multiple comparison test. Ns = non-significant, *P < 0.05, **P < 0.001, and ***P < 0.0001. Error bars represent mean ± SD of five rats in each group. BBR; Berberine. BNP; Berberine nanoparticle. β-CYF; β-cyfluthrin.

References

1. Benjamin, E. J., Muntner, P., Alonso, A., Bittencourt, M. S., Callaway, C. W., Carson, A. P. and et al. Heart disease and stroke statistics-2019 update: a report from the American Heart Association. *Circulation*, **139**(10), e56–e528 (2019).
2. World Health Organization. Global health estimates: deaths by cause, age, sex and country, 2000–2012. *WHO*, **9**, (2014).
3. Prüss-Üstün, A. and Corvalán, C. Preventing disease through healthy environments: towards an estimate of the environmental burden of disease. *World Health Organization*, (2006).

4. Wei, J., Liu, J., Zhang, L., Zhu, Y., Li, X., Zhou, G. and et al. Endosulfan induces cardiotoxicity through apoptosis via unbalance of pro-survival and mitochondrial-mediated apoptotic pathways. *Science of The Total Environment*, **727**, 138790 (2020).
5. Rajawat, N. K., Soni, I., Syed, F., Verma, R., John, P. and Mathur, R. Effect of β-cyfluthrin (synthetic pyrethroid) on learning, muscular coordination and oxidative stress in Swiss albino mice. *Toxicology and Industrial Health*, **35**(5), 358–367 (2019).
6. Marques, L. P., Joviano-Santos, J. V., Souza, D. S., Santos-Miranda, A. and Roman-Campos, D. Cardiotoxicity of pyrethroids: molecular mechanisms and therapeutic options for acute and long-term toxicity. *Biochemical Society Transactions*, **50**(6), 1737–1751 (2022).
7. Smith, T. M. and Stratton, G. W. Effects of synthetic pyrethroid insecticides on nontarget organisms. *Residue Reviews: Reviews of Environmental Contamination and Toxicology*, **93**, 93–120 (1986).
8. European Food Safety Authority (EFSA). Modification of the existing maximum residue levels for beta-cyfluthrin in various cereals. *EFSA Journal*, **14**(3), 4417 (2016).
9. US Environmental Protection Agency. Office of Pesticides and Toxic Substances, Office of Pesticide Programs, Pesticide fact sheet: Cyfluthrin. (1987).
10. Gorell, J. M., Johnson, C. C., Rybicki, B. A., Peterson, E. L. and Richardson, R. The risk of Parkinson's disease with exposure to pesticides, farming, well water, and rural living. *Neurology*, **50**(5), 1346–1350 (1998).
11. Food and Agriculture Organization of the United Nations. Manual on the development and use of FAO specifications for plant protection products. *Food & Agriculture Org.*, **1995**.
12. Livingstone, D. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Marine Pollution Bulletin*, **42**(8), 656–666 (2001).
13. El-Demerdash, F. M. Cytotoxic effect of fenitrothion and lambda-cyhalothrin mixture on lipid peroxidation and antioxidant defense system in rat kidney. *Journal of Environmental Science and Health, Part B*, **47**(4), 262–268 (2012).
14. Nazıroğlu, M. Role of selenium on calcium signaling and oxidative stress-induced molecular pathways in epilepsy. *Neurochemical Research*, **34**, 2181–2191 (2009).
15. Giray, B., Gürbay, A. and Hincal, F. Cypermethrin-induced oxidative stress in rat brain and liver is prevented by vitamin E or allopurinol. *Toxicology Letters*, **118**(3), 139–146 (2001).
16. Nasuti, C., Gabbianelli, R., Falcioni, M. L., Di Stefano, A., Sozio, P. and Cantalamessa, F. Dopaminergic system modulation, behavioral changes, and oxidative stress after neonatal administration of pyrethroids. *Toxicology*, **229**(3), 194–205 (2007).
17. Han, J., Zhou, L., Luo, M., Liang, Y., Zhao, W., Wang, P. and et al. Nonoccupational exposure to pyrethroids and risk of coronary heart disease in the Chinese population. *Environmental Science & Technology*, **51**(1), 664–670 (2017).
18. Mirhadi, E., Rezaee, M. and Malaekeh-Nikouei, B. Nano strategies for berberine delivery, a natural alkaloid of *Berberis*. *Biomedicine & Pharmacotherapy*, **104**, 465–473 (2018).
19. Derosa, G., D'Angelo, A., Bonaventura, A., Bianchi, L., Romano, D. and Maffioli, P. Effects of berberine on lipid profile in subjects with low cardiovascular risk. *Expert Opinion on Biological Therapy*, **13**(4), 475–482 (2013).
20. Battu, S. K., Repka, M. A., Maddineni, S., Chittiboyina, A. G., Avery, M. A. and Majumdar, S. Physicochemical characterization of berberine chloride: a perspective in the development of a solution dosage form for oral delivery. *AAPS PharmSciTech*, **11**, 1466–1475 (2010).
21. Huang, C. H., Huang, Z. W., Ho, F. M. and Chan, W. H. Berberine impairs embryonic development in vitro and in vivo through oxidative stress-mediated apoptotic processes. *Environmental Toxicology*, **33**(3), 280–294 (2018).
22. Parkin, D. M., Bray, F. I. and Devesa, S. S. Cancer burden in the year 2000: the global picture. *European Journal of Cancer*, **37**, 4–66 (2001).
23. Gaba, S., Saini, A. and Singh, G., Monga, V. An insight into the medicinal attributes of berberine derivatives: a review. *Bioorganic & Medicinal Chemistry*, **38**, 116143 (2021).
24. Domitrović, R., Jakovac, H. and Blagojević, G. Hepatoprotective activity of berberine is mediated by inhibition of TNF-α, COX-2, and iNOS expression in CCl_4-intoxicated mice. *Toxicology*, **280**(1–2), 33–43 (2011).
25. Kuo, C. L., Chi, C. W. and Liu, T. Y. The anti-inflammatory potential of berberine in vitro and in vivo. *Cancer Letters*, **203**(2), 127–137 (2004).
26. Li, Z., Geng, Y. N., Jiang, J. D. and Kong, W. J. Antioxidant and anti-inflammatory activities of berberine in the treatment of diabetes mellitus. *Evidence-Based Complementary and Alternative Medicine*, **2014**(1), 289264 (2014).
27. Dash, S., Kumar, M. and Pareek, N. Enhanced antibacterial potential of berberine via synergism with chitosan nanoparticles. *Materials Today: Proceedings*, **31**, 640–645 (2020).
28. Yue, S. J., Liu, J., Wang, W. X., Wang, A. T., Yang, X. Y., Guan, H. S. and et al. Berberine treatment-emergent mild diarrhea associated with gut microbiota dysbiosis. *Biomedicine & Pharmacotherapy*, **116**, 109002 (2019).
29. Wang, L. H., Yu, C. H., Fu, Y., Li, Q. and Sun, Y. Q. Berberine elicits anti-arrhythmic effects via IK1/Kir2.1 in the rat type 2 diabetic myocardial infarction model. *Phytotherapy Research*, **25**(1), 33–37 (2011).

30. Chen, H., Chen, Y., Tang, Y., Yang, J., Wang, D., Yu, T. and et al. Berberine attenuates spontaneous action potentials in sinoatrial node cells and the currents of human HCN4 channels expressed in *Xenopus laevis* oocytes. *Molecular Medicine Reports*, **10**(3), 1576–1582 (2014).

31. 31. Song, D., Hao, J. and Fan, D. Biological properties and clinical applications of berberine. *Frontiers of Medicine*, **14**, 564–582 (2020).

32. Xia, L.M. and Luo, M.H. Study progress of berberine for treating cardiovascular disease. *Chronic Diseases and Translational Medicine*, **1**(4), 231–235 (2015).

33. Li, Y., Chen, X., Liu, H., Luo, F. and Li, G. Effects of ginseng total saponins with berberine on plasma brain natriuretic peptide and Ca^{2+} concentration in experimental rats with chronic congestive heart failure. *Zhongguo Zhongyao Zazhi (China Journal of Chinese Materia Medica)*, **34**(3), 324–327 (2009).

34. Dong, S. F., Hong, Y., Liu, M., Hao, Y. Z., Yu, H. S., Liu, Y. and et al. Berberine attenuates cardiac dysfunction in hyperglycemic and hypercholesterolemic rats. *European Journal of Pharmacology*, **660**(2–3), 368–374 (2011).

35. Movahedi, F., Li, L., Gu, W. and Xu, Z. P. Nanoformulations of albendazole as effective anticancer and antiparasite agents. *Nanomedicine*, **12**(20), 2555–2574 (2017).

36. Bronze-Uhle, E., Costa, B., Ximenes, V. and Lisboa-Filho, P. Synthetic nanoparticles of bovine serum albumin with entrapped salicylic acid. *Nanotechnology, Science and Applications*, **2016**, 11–21 (2016).

37. Casa, D., Scariot, D., Khalil, N., Nakamura, C. and Mainardes, R. Bovine serum albumin nanoparticles containing amphotericin B were effective in treating murine cutaneous leishmaniasis and reduced the drug toxicity. *Experimental Parasitology*, **192**, 12–18 (2018).

38. Solanki, R., Patel, K. and Patel, S. Bovine serum albumin nanoparticles for the efficient delivery of berberine: preparation, characterization and in vitro biological studies. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **608**, 125501 (2021).

39. Xiong, C., Wu, Y. Z., Zhang, Y., Wu, Z. X., Chen, X. Y., Jiang, P. and et al. Protective effect of berberine on acute cardiomyopathy associated with doxorubicin treatment. *Oncology Letters*, **15**(4), 5721–5729 (2018).

40. Hao, G., Yu, Y., Gu, B., Xing, Y. and Xue, M. Protective effects of berberine against doxorubicin-induced cardiotoxicity in rats by inhibiting metabolism of doxorubicin. *Xenobiotica*, **45**(11), 1024–1030 (2015).

41. Hosseini, S. H., Nazarian, M., Rajabi, S., Jafari-Nozad, A. M., Mesbahzadeh, B., Samargahndian, S. and et al. Protective effect of berberine nanoparticles against cardiotoxic effects of arsenic trioxide. *Cardiovascular Toxicology*, **24**(12), 1311–1316 (2024).

42. Jebur, A. B., Nasr, H. M. and El-Demerdash, F. M. Selenium modulates β-cyfluthrin-induced liver oxidative toxicity in rats. *Environmental Toxicology*, **29**(11), 1323–1329 (2014).

43. Buhl, S. and Jackson, K. Optimal conditions and comparison of lactate dehydrogenase catalysis of the lactate-to-pyruvate and pyruvate-to-lactate reactions in human serum at 25, 30, and 37 °C. *Clinical Chemistry*, **24**(5), 828–831 (1978).

44. Szasz, G., Waldenström, J. and Gruber, W. Creatine kinase in serum: 6. Inhibition by endogenous polyvalent cations, and effect of chelators on the activity and stability of some assay components. *Clinical Chemistry*, **25**(3), 446–452 (1979).

45. Neumeier, D., Prellwitz, W., Würzburg, U., Brundobler, M., Olbermann, M., Just, H. J. and et al. Determination of creatine kinase isoenzyme MB activity in serum using immunological inhibition of creatine kinase M subunit activity: activity kinetics and diagnostic significance in myocardial infarction. *Clinica Chimica Acta*, **73**(3), 445–451 (1976).

46. Güney, Ş., Cumaoğlu, A., Öztürk, G., Akbulut, K. G. and Karasu, Ç. Comparison of melatonin effect on oxidant status and antioxidant capacity in liver and heart of young and aged rats. *International Journal of Gerontology*, **7**(1), 45–49 (2013).

47. Nishikimi, M., Rao, N.A. and Yagi, K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical and Biophysical Research Communications*, **46**(2), 849–854 (1972).

48. Mihara, M. and Uchiyama, M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Analytical Biochemistry*, **86**(1), 271–278 (1978).

49. Layton, C. and Suvarna, K. Bancroft's theory and practice of histological techniques. *Churchill Livingstone Elsevier*, London, (2013).

50. Georgiadis, N., Tsarouhas, K., Tsitsimpikou, C., Vardavas, A., Rezaee, R., Germanakis, I. and et al. Pesticides and cardiotoxicity: where do we stand? *Toxicology and Applied Pharmacology*, **353**, 1–14 (2018).

51. Sager, T. M., Porter, D. W., Robinson, V. A., Lindsley, W. G., Schwegler-Berry, D. E. and Castranova, V. Improved method to disperse nanoparticles for in vitro and in vivo investigation of toxicity. *Nanotoxicology*, **1**(2), 118–129 (2007).

52. Makeen, H. A., Mohan, S., Al-Kasim, M. A., Attafi, I. M., Ahmed, R. A., Syed, N. K. and et al. Gefitinib loaded nanostructured lipid carriers: characterization, evaluation and anti- human colon cancer activity in vitro. *Drug Delivery*, **27**(1), 622–631 (2020).

53. Shalaby, S. E. and Abdel-Latif, A. M. Preventive action of ascorbic acid and β-carotene from beta-cyfluthrin insecticide toxicity on rats. *International Journal of PharmTech Research*, **9**(12), 243–250 (2016).

54. Muhammed, R. E., El-Desouky, M. A., Abo-Seda, S. B., Nahas, A., Elhakim, H. K. and Alkhalaf, M. I. The protecting role of Moringa oleifera in cypermethrin-induced mitochondrial dysfunction and apoptotic events in rat brain. *Journal of King Saud University – Science*, **32**(6), 2717–2722 (2020).

55. Jebur, A. B., El-Sayed, R. A. and El-Demerdash, F. M. *Ocimum basilicum* essential oil modulates hematotoxicity, oxidative stress, DNA damage, and cell cycle arrest induced by β-cyfluthrin in rat liver. *Frontiers in Pharmacology*, **12**, 784281 (2022).

56. Auten, R. L. and Davis, J. M. Oxygen toxicity and reactive oxygen species: the devil is in the details. *Pediatric Research*, **66**(2), 121–127 (2009).

57. Bal, R., Naziroğlu, M., Türk, G., Yilmaz, Ö., Kuloğlu, T., Etem, E. and et al. Insecticide imidacloprid induces morphological and DNA damage through oxidative toxicity on the reproductive organs of developing male rats. *Cell Biochemistry and Function*, **30**(6), 492–499 (2012).

58. Eraslan, G., Kanbur, M., Silici, S., Altinordulu, S. and Karabacak, M. Effects of cypermethrin on some biochemical changes in rats: the protective role of propolis. *Experimental Animals*, **57**(5), 453–460 (2008).

59. Ranjbar, A., Solhi, H., Mashayekhi, F. J., Susanabdi, A., Rezaie, A. and Abdollahi, M. Oxidative stress in acute human poisoning with organophosphorus insecticides: a case–control study. *Environmental Toxicology and Pharmacology*, **20**(1), 88–91 (2005).

60. Kumari, S., Varghese, A., Muraleedharan, D. and Menon, V. Protective action of aspirin in experimental myocardial infarction induced by isoproterenol in rats and its effect on lipid peroxidation. *Indian Journal of Experimental Biology*, **28**(5), 480–485 (1990).

61. Abbas, A. M. Cardioprotective effect of resveratrol analogue isorhapontigenin versus omega-3 fatty acids in isoproterenol-induced myocardial infarction in rats. *Journal of Physiology and Biochemistry*, **72**, 469–484 (2016).

62. Vijayakumar, R. and Nachiappan, V. *Cassia auriculata* flower extract attenuates hyperlipidemia in male Wistar rats by regulating the hepatic cholesterol metabolism. *Biomedicine & Pharmacotherapy*, **95**, 394–401 (2017).

63. Lalitha, G., Poornima, P., Archanah, A. and Padma, V. V. Protective effect of neferine against isoproterenol-induced cardiac toxicity. *Cardiovascular Toxicology*, **13**, 168–179 (2013).

64. Noeman, S. A., Hamooda, H. E. and Baalash, A. A. Biochemical study of oxidative stress markers in the liver, kidney and heart of high-fat diet induced obesity in rats. *Diabetology & Metabolic Syndrome*, **3**, 1–8 (2011).

65. Eraslan, G., Saygi, S., Essiz, D., Aksoy, A., Gul, H. and Macit, E. Evaluation of some oxidative stress parameters using vitamin E, proanthocyanidin and N-acetylcysteine against exposure to cyfluthrin in mice. *Pesticide Biochemistry and Physiology*, **88**(1), 43–49 (2007).

66. Wang, L., Li, H., Wang, S., Liu, R., Wu, Z., Wang, C. and et al. Enhancing the antitumor activity of berberine hydrochloride by solid lipid nanoparticle encapsulation. *AAPS PharmSciTech*, **15**, 834–844 (2014).

67. El Kazzi, M., Rayner, B. S., Chami, B., Dennis, J. M., Thomas, S. R. and Witting, P. K. Neutrophil-mediated cardiac damage after acute myocardial infarction: significance of defining a new target cell type for developing cardioprotective drugs. *Antioxidants & Redox Signaling*, **33**(10), 689–712 (2020).

68. Hausenloy, D. J. and Yellon, D. M. Myocardial ischemia–reperfusion injury: a neglected therapeutic target. *Journal of Clinical Investigation*, **123**(1), 92–100 (2013).

69. Abdulredha, A., Abosaooda, M., Al-Amran, F. and Hadi, N. R. Berberine protects the heart from ischemic reperfusion injury via interference with oxidative and inflammatory pathways. *Medical Archives (Sarajevo, Bosnia and Herzegovina)*, **75**(3), 174–179 (2021).

70. Türkan, F., Huyut, Z. and Atalar, M. N. The toxicological impact of some avermectins on human erythrocytes glutathione S-transferase enzyme. *Journal of Biochemical and Molecular Toxicology*, **32**(10), e22205 (2018).

71. Ajiboye, T. O., Akinpelu, S. A., Muritala, H. F., Ogunbode, S. M., Adeleye, A. O., Oladiji, A. T. and et al. *Trichosanthes cucumerina* fruit extenuates dyslipidemia, protein oxidation, lipid peroxidation and DNA fragmentation in the liver of high-fat diet-fed rats. *Journal of Food Biochemistry*, **38**(5), 480–490 (2014).

72. Baynes, J. W. Role of oxidative stress in development of complications in diabetes. *Diabetes*, **40**(4), 405–412 (1991).

73. Geetha, A., Sankar, R., Marar, T. and Devi, C. Alpha-tocopherol reduces doxorubicin-induced toxicity in rats—histological and biochemical evidences. *Indian Journal of Physiology and Pharmacology*, **34**(2), 94–100 (1990).

74. Gupta, M. and Singh, A. Studies of the antimicrobial activity and phytochemical properties of *Berberis lycium*. Book Rivers (2022).

75. Lopes-Ferreira, M., Farinha, L. R. L., Costa, Y. S. O., Pinto, F. J., Disner, G. R., da Rosa, J. G. S. and et al. Pesticide-induced inflammation at a glance. *Toxics*, **11**(11), 896 (2023).

76. Hussain, S., Ashafaq, M., Alshahrani, S., Siddiqui, R., Alam, M. I., Mohammed, M. and et al. Cardioprotective effects of nano-piperine against cypermethrin toxicity through oxidative stress, histopathological and immunohistochemical studies in male Wistar rats. *Natural Product Communications*, **18**(3), 1934578X231154029 (2023).

77. Kumar, S., Singhal, V., Roshan, R., Sharma, A., Rembhotkar, G. W. and Ghosh, B. Piperine inhibits TNF-α induced adhesion of neutrophils to endothelial monolayer through suppression of NF-κB and IκB kinase activation. *European Journal of Pharmacology*, **575**(1–3), 177–186 (2007).

78. Alsabeelah, N. and Kumar, V. Quality by design-based optimization of formulation and process parameters for berberine nanosuspension for enhancing its dissolution rate, bioavailability, and cardioprotective activity. *Journal of Food Biochemistry*, **46**(10), e14361 (2022).

79. Gangemi, S., Gofita, E., Costa, C., Teodoro, M., Briguglio, G., Nikitovic, D. and et al. Occupational and environmental exposure to pesticides and cytokine pathways in chronic diseases. *International Journal of Molecular Medicine*, **38**(4), 1012–1020 (2016).

80. Mwanga, H. H., Dalvie, M. A., Singh, T. S., Channa, K. and Jeebhay, M. F. Relationship between pesticide metabolites, cytokine patterns, and asthma-related outcomes in rural women workers. *International Journal of Environmental Research and Public Health*, **13**(10), 957 (2016).

81. Multhoff, G., Molls, M. and Radons, J. Chronic inflammation in cancer development. *Frontiers in Immunology*, **2**, 98 (2012).

82. Forrester, S. J., Kikuchi, D. S., Hernandes, M. S., Xu, Q. and Griendling, K. K. Reactive oxygen species in metabolic and inflammatory signaling. *Circulation Research*, **122**(6), 877–902 (2018).

83. Martínez, M. A., Rodríguez, J. L., Lopez-Torres, B., Martínez, M., Martínez-Larrañaga, M. R., Anadón, A. and et al. Oxidative stress and related gene expression effects of cyfluthrin in human neuroblastoma SH-SY5Y cells: protective effect of melatonin. *Environmental Research*, **177**, 108579 (2019).

84. Huang, Z., Cai, X., Li, S., Zhou, H., Chu, M., Shan, P. and et al. Berberine-attenuated monocyte adhesion to endothelial cells induced by oxidized low-density lipoprotein via inhibition of adhesion molecule expression. *Molecular Medicine Reports*, **7**(2), 461–465 (2013).

85. Liu, S. J., Yin, C. X., Ding, M. C., Wang, Y. Z. and Wang, H. Berberine inhibits tumor necrosis factor-α-induced expression of inflammatory molecules and activation of nuclear factor-κB via the activation of AMPK in vascular endothelial cells. *Molecular Medicine Reports*, **12**(4), 5580–5586 (2015).

86. Feng, X., Sureda, A., Jafari, S., Memariani, Z., Tewari, D., Annunziata, G. and et al. Berberine in cardiovascular and metabolic diseases: from mechanisms to therapeutics. *Theranostics*, **9**(7), 1923–1951 (2019).

87. Xu, R. X., Sun, X. C., Ma, C. Y., Yao, Y. H., Li, X. L., Guo, Y. L. and et al. Impacts of berberine on oxidized LDL-induced proliferation of human umbilical vein endothelial cells. *American Journal of Translational Research*, **9**(10), 4375–4385 (2017).

الدور الوقائي لجسيمات مصل الزلال البقري النانوية المحملة بمادة البربرين تجاه السمية القلبية التي يسببها البيتا سيفلوثرين في ذكور الجرذان

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الملخص

البيتا سيفلوثرين (β-CYF)، وهو أحد مبيدات الحشرات من فئة البيرثرويد ويُستخدم على نطاق واسع في الزراعة، وقد هدفت هذه الدراسة إلى استكشاف قدرته على إحداث **الإجهاد التأكسدي** والاضطرابات الكيميائية الحيوية في أنسجة القلب. كما هدفت إلى تقييم دور **البربرين (BBR)** والجسيمات النانوية المحملة بالبربرين والزلال البقري (BNPs) في التخفيف من سميته القلبية.تم تقسيم أربعين من ذكور جرذان الويستر بشكل عشوائي إلى ثماني مجموعات، كل منها تتكون من خمسة حيوانات.**المجموعة الأولى:** الضابطه.,**المجموعة الثانية:** تلقت البربرين (10 ملغ/كغم).**المجموعة الثالثة:** تلقت الجسيمات النانوية من البربرين (5 ملغ/كغم),**المجموعة الرابعة:** عُولجت بالبيتا سيفلوثرين (15 ملغ/كغم من وزن الجسم),**المجموعتان الخامسة والسادسة:** عُولجتا بالبيتا سيفلوثرين (15 ملغ/كغم)، ثم تلاها إعطاء البربرين (10 ملغ/كغم) أو الجسيمات النانوية من البربرين (5 ملغ/كغم) على التوالي,**المجموعة السابعة:** تلقت الزلال البقري المصل (1 مل).**المجموعة الثامنة:** تلقت زيت الذرة (1 مل).تم إعطاء جميع العلاجات عن طريق التغذية الفموية مرة واحدة يوميًا لمدة 15 يومًا.عند التعرض للبيتا سيفلوثرين، لوحظ ارتفاع كبير في **بيروكسيد الدهون (MDA)**، وانخفاض في نشاط الإنزيمات المضادة للأكسدة مثل **القدرة الكلية لمضادات الأكسدة (TAC)** وإنزيم **ديسموتاز الفائق (SOD)**. وقد صاحب ذلك ارتفاع في مؤشرات حيوية القلب (LDH و CPK و CK-MB) في مصل الدم.أدى إعطاء الجسيمات النانوية من البربرين والبربرين إلى انخفاض كبير في كمية الإنزيمات القلبية ومؤشرات الإجهاد التأكسدي في مصل الدم والأنسجة لدى الجرذان المعالجة بالبيتا سيفلوثرين، وأعادت أنسجة القلب إلى حالتها الطبيعية.تشير النتائج إلى أن **الإجهاد التأكسدي** يلعب دورًا حاسمًا في تلف القلب الناجم عن البيتا سيفلوثرين، وأن الجسيمات النانوية من البربرين (BNPs) هي **مضاد أكسدة قوي** يمكن أن يخفف من التأثير السلبي للبيتا سيفلوثرين على أنسجة قلب الجرذان.

الكلمات الدالة: البيتا سيفلوثرين، مضاد للأكسدة، الجسيمات النانوية من البربرين، الإجهاد التأكسدي، السمية القلبية.