PROTECTIVE POTENTIALS OF SPIRULINA PLATENSIS AGAINST BENZO [A] PYRENE-INDUCED CARDIOTOXICITY IN ADULT ALBINO RATS

Nahla M. Ibrahim[1], Heba Mohamed Abdel-aziz[2], Dalia Ahmed Selim Ali Abdou[1], Waleed Ahmed Badawy Abdelwahab[3], Noura Mohammed Qenawy[4], Hend Sameh[5], Mai M. Eldaly[5], Samar Samy Sakr[1]

[1]Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine Zagazig University, Egypt.
[2] Department of Histology and Cell Biology, Faculty of Medicine, Zagazig University, Egypt.
[3] Department of Anatomy and Embryology, Faculty of Medicine, Misr University for Science and Technology.
[4] Department of Anatomy and Embryology, Faculty of Medicine, Zagazig University, Egypt.
[5]Department of Medical Biochemistry, Faculty of Medicine, Zagazig University, Egypt.

ABSTRACT

Background: Benzo[a]pyrene (B[a]p) is regarded as a polycyclic aromatic hydrocarbon that results due to partial combustion of organic materials. B[a]p has variable probable toxic health effects on humans and that makes it an issue of concern to the public health. Spirulina Platensis is a type of cyanobacteria that is multicellular and filamentous, and it has gained considerable popularity in the field of medicine. **Aim of the work:** was to assess the potential protection by Spirulina against toxic effects of B[a]p in rats' heart tissues. **Material and Methods:** Fifty adult male albino rats have been categorized into 5 equal groups; Negative control, Positive control (10 mL/kg corn oil), Spirulina Platensis (300 mg/kg), Benzo[a]pyrene (50 mg/kg), and Benzo[a]pyrene + Spirulina Platensis groups. All treatments were given twice per week. After four weeks, rats had been sacrificed, NADPH oxidase-2 (NOX-2), malondialdehyde (MDA), nitric oxide (NO), reduced glutathione (GSH), superoxide dismutase (SOD), along with cytokines of inflammation; tumor necrosis factor-alfa (TNF-α) and interleukin-6 (IL-6) were measured in the heart. Determination of cardiac Toll-like receptor 4 (TLR4) by real-time polymerase chain reaction (RT-PCR) was also done. The left ventricular cardiac tissues were stained by both hematoxylin and eosin and Mallory trichrome stains, and the immunohistochemical expression of Connexin 43 (Cx43) was evaluated. **Results:** B[a]p-treated rats showed an elevation of oxidative and inflammatory markers, and increased expression of cardiac TLR4. Co-administration of Spirulina with B[a]p mitigated all the measured parameters. Histopathology and immunohistochemical staining showed that the B[a]p developed histological damage and immunohistochemical changes in the left ventricular tissues and these changes were alleviated by Spirulina co-administration. **Conclusion:** Administration of Spirulina produced positive impact on oxidative and inflammatory markers of the heart, along with ameliorating the histopathological and immunohistochemical findings induced by B[a]p. **Recommendations:** Spirulina Platensis is a suggested agent for protection against cardiotoxic effects of B[a]p. More studies are required to investigate cardio-protective potential as well as safe and effective doses in humans.

Keywords: *Spirulina, Benzo[a]pyrene, Cardiac toxicity, Rats.*

Corresponding author: *Dr. Nahla Mohammed Ibrahim Hassan*
Email: drnahlaibrahimforensic@gmail.com
ORCID: 0009-0004-7238-0812

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are the furthermost detected pollutants in different environmental samples all over the world ***(Zheng et al., 2024)***. There are 16 APH compounds, including Benzo[a]pyrene (B[a]p), registered by the Environmental Protection Agency ***(Manoj et al., 2017)***. It is generated from the pyrolysis of organic composites (e.g., wood and fossil fuel) where incomplete combustion takes place under high temperature and no or low oxygen ***(Hussein and Mona, 2016)***. In tobacco smoke, B[a]P is three times as much as the mainstream smoke ***(Bukowska et al., 2022)***.

Elevated levels of B[a]P elaborate while the food is being processed like heating, smoking, and drying of animal products, in addition to food frying, grilling, and roasting. Meanwhile, lower levels have been detected in some wheat, roasted coffee, tea leaves, cocoa beans, vegetable oils and fats ***(El said et al., 2016; Zelinkova and Wenzl, 2015)***. According to the European Commission, the accepted B[a]p concentrations in food had been set at five µg/kg in meat and smoked fish, two µg/kg in oils and fats, and 1 µg/kg in different cereals ***(European Commission, 2005)***. In Egypt, dangerous levels of B[a]p originated in the charcoal grilled Kebab (21-25 µg/kg) and Kofta (60-70 µg/kg) ***(El said et al., 2016)***.

Rapid absorption of B[a]P occurs by inhalation, oral exposure, and dermal contact. However, humans are exposed to B[a]P mostly via ingestion of contaminated food or inhalation of polluted air ***(Bukowska et al., 2022)***.

B[a]P is lipophilic, and this enhances its rapid absorption through the biological membranes, then it binds to the aryl hydrocarbon receptor (AHR) to be bioactivated. In liver, the first biotransformation phase of B[a]P depends on cytochrome P_{450} family particularly CYP1A1 which starts a chain of reactions that ends by the formation of the carcinogenic deoxyguanosine-DNA adducts ***(Jacques et al., 2010; Manoj et al., 2017)***. Instead, dihydhrodiol dehydrogenases enzyme can metabolize B[a]p into quinines with excess production of nitrogen species and reactive oxygen (RNS & ROS) ***(Sangeeta et al., 2018)***.

Benzo[a]pyrene demonstrated carcinogenic, mutagenic, and developmental effects in experimental animals. Recently, cardiac toxicity of B[a]p has attracted great attention, and a strong correlation has been established between B[a]p exposure and cardiovascular diseases ***(Wang et al., 2021; Dračínská et al, 2021)***.

Nowadays, great attention is directed towards the usage of natural products as protective agents to combat pathological conditions of different causes. The main mechanism of such protection is based on free radicals' elimination, redox balance restoration, and detoxification of carcinogen ***(Shahid et al., 2016)***.

Spirulina is a widely distributed cyanobacterial algae inhabiting most of the marine environments and appears as a water surface green scum. Interestingly, Spirulina can be grown easily in water, harvested, and processed for healthy purposes ***(Oruç et al., 2023)***. It is enriched with minerals, vitamins, fatty acids, and lipids and had hopeful advantageous effects on health of humans ***(Mahdieh et al. 2020; Abdullah et al., 2024)***. Besides, spirulina has demonstrated a positive effect on the redox system, enzymatic antioxidants, lipid peroxidation, and DNA damage ***(Abdullah et al., 2024)***.

THE AIM OF THE WORK

The cardiotoxicity of B[a]p has been previously evaluated in few studies; however, the molecular mechanism of cardiac toxicity is up till now to be studied. So, this work intended to investigate the biochemical, histological, and immunohistochemical changes on heart of albino rats induced by B[a]p in addition to the potential protection by Spirulina.

MATERIAL AND METHODS

Chemicals:

Both of B[a]p and the dried powder of Spirulina were brought from Sigma-Aldrich Chemicals Co., St. Louis, MO, USA, while corn oil was bought from local market in Egypt.

Animals*:*

In the current experiment, 50 healthy adult male albino rats (180-200 grams body weight) with ages ranging between 10-12 weeks were used. They were gotten from breeding animal house in Faculty of Medicine, University of Zagazig. The existing investigation was undertaken within the rules of Zagazig University IACUC Committee and in adherence to the international rules regulating animal research *(ZU-IACUC/3/F/363/2023)*.

Experimental groups:

Prior to the experiment, the rats were given two weeks to become acclimatized to the conditions of the laboratory. We divided the rats in a random manner into 5 groups, with ten animals in each of the groups.

Negative control group: received tap water and regular food.

Positive control group (Corn oil): orally gavaged with 10 mL/kg corn oil (which is the Benzo[a]pyrene solvent), administered two times/week for a period of four weeks.

Spirulina Platensis group: orally gavaged with Spirulina (300 mg/kg) dissolved in distilled water, administered two times/ week for a period of four weeks ***(Simsek et al., 2009)***.

Benzo[a]pyrene group: orally gavaged with B[a]p (50 mg/kg) which represents 1/20 of LD_{50} ***(Audra et al., 2007)*** dissolved in corn oil (10 ml/kg), administered two times/ week for a period of four weeks ***(Sunil et al., 2019)***.

Benzo[a]pyrene+Spirulina Platensis group: orally gavaged with Spirulina (300 mg/kg), administered 30 minutes before administration B[a]p (50 mg/kg), two times / week for four weeks.

Sample size:

Assuming that mean±SD of GSH in negative control group versus group III (benzo(a) pyrene) was (9.33±0.48) versus (0.25±0.1) ***(Elsayed et al., 2023)***. So, the sample size was calculated to be 50 rats using open epi CI 95%. Power of test 80%.

Specimen collection and preparation:

After 4 weeks, all rats were sacrificed by the means of intraperitoneal injection of 100 mg/kg ketamine-xylazine. A ventral midline incision had been done, hearts were excised, washed in saline and frozen in -80 for biochemical analysis and gene expression. $1cm^3$ specimen from left ventricle was taken by sharp razors for histological and immunohistochemical studies.

The samples of the heart were homogenized in ten percent w/v ice-cold phosphate buffer (0.01 M, pH 7.4). Then, the homogenates were centrifuged (3000 rotations per minute for twenty min). The supernatant was analyzed for NOX-2, MDA, NO, GSH, SOD, TNF-α, and IL-6. Also, TLR4 gene was detected by RT-PCR in cardiac tissue samples that had been preserved at -80°C.

Determination of oxidative-related markers and inflammatory cytokines in cardiac tissues:

The Rat ELISA Kit for NOX-2 (Bioassay Technology Laboratory, Shanghai, China) has been utilized as per the instructions of the manufacturer for NOX-2 quantitative estimation.

Griess method has been employed to chemically determine NO, an indicator of nitrosative stress. The assay's principle involves the conversion of No2 to No3 followed by the development of color in acidic medium with a Griess reagent ***(Sastry et al., 2002)***. The outcomes are measured in nmol/g tissue via a colorimetric method at 540 nanometers in accordance with the manufacturer's instructions (Biodiagnostic, Cairo, Egypt).

Marklund and Marklund (1974) presented a colorimetric method of assessing the activity of SOD at 420 nanometers, as per the instructions of the manufacturer kit, which relies on restricting the autoxidation of pyrogallol by super oxide dismutase, the results of which have been stated as U/g tissue (Biodiagnostic, Cairo, Egypt).

According to ***Buege and Aust (1978)***, MDA was estimated by means of a colorimetric method, as per the guidelines of the manufacturer kit, using spectrophotometric measurement of color at 534 nanometers, to evaluate the thiobarbituric acid reacting substance and the outcomes had been shown as nmol/g tissue (Biodiagnostic, Cairo, Egypt).

In ***(1979), Moron et al.*** described the reaction between Ellman's reagent and thiol groups of GSH which results in formation of the yellow 5-thio-2-nitrobenzoic acid. This product was then assessed by spectrophotometer at 412 nanometers by means of a colorimetric method as per the directions of the manufacturer kit and demonstrated as nmol/g tissue (Biodiagnostic, Cairo, Egypt).

More recently, ***Al-Taher et al. (2020)*** determined both of TNF-α and IL-6 by using TNF-α and IL-6 rat ELISA kits that were purchased from Daxing Industry Zone (Beijing, China), and their concentrations were shown as pg/ml, as prescribed by the manufacturer.

Cardiac TLR4 gene expression using RT-PCR:

The instructions of the manufacturer have been followed to isolate total RNA from cardiac homogenate utilizing the RNeasy Mini Kit (Qiagen).

The absorbance proportion (260/280 nanometer) has been utilized to evaluate the quality of total RNA, and it varied between 1.8 and 2.0 for all formulations. The QuantiTect Reverse Transcription Kit has been utilized to produce cDNA. Utilizing five microliters of cDNA, ten pmol/uL of every primer, in addition to 10 microliters of SYBR Green 2x Master Mix Green (QuantiTect SYBR Green PCR Kits, Qiagen), the gene expression investigation has been conducted using qRT-PCR. The RT-qPCR was done utilizing Mx3005P (Stratagene, CA, state).

Thermal cycling has been undertaken under the following circumstances: Denatured at ninety-five degrees Celsius for five minutes, followed by forty cycles of fifteen seconds at ninety-five degrees Celsius, annealing at sixty degrees Celsius for thirty sec., and elongation at 72°C for thirty sec. 2-ΔΔCt technique has been utilized to design relative expression, and data have been normalized against GADPH transcript levels ***(Livak and Schmittgen, 2001)***.

Sequences of the TLR4 primers ***(Schultz et al., 2007)***: Forward primer sequence is 5-AATCCCTGCATAGAGGTACTTCCTAAT-3 and Reverse primer sequence is 5-CTCAGATCTAGGTTCTTGGTTGAATAA G-3. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers: Forward primer sequence is 5′-GTCGGTGTGAACGGATTTG-3′ and Reverse primer sequence is 5′-CTTGCCGTGGGTAGAGTCAT-3′ ***(Wimmer et al., 2018)***.

Histological and immunohistochemical examination:

Specimens were processed by paraffin techniques and cut into 5-7 µm paraffin sections ***(Bancroft and Gamble 2008)***.

Sections were submitted to:

a. Haematoxyin and eosin (H&E Stain) according to ***Kiernan (2001)***.

b. Mallory's trichrome stain for collagen fibers following ***Bancroft and Gamble (2008)***.

c. Immunohistochemical staining through the utilization of the avidin-biotin peroxidase complex technique ***(Suvarna et al., 2012)***.

Anti-Connexin 43 (Cx43) antibody as a marker for gap junctions of intercalated discs. It is a ready-to-utilize rabbit monoclonal antibody (Santa Cruz Biotechnology, Inc, Europe (C-20) sc 6560 Ab). The sections were de-paraffinized using xylene and subsequently rehydrated with a series of decreasing concentrations of alcohol. To inhibit endogenous peroxidase activity, the sections were immersed in hydrogen peroxide for duration of 15 min. The sections were kept for 60 min. with two drops of the primary antibody. Incubate the slides for 10 min. with two drops of biotinylated secondary antibody, followed by an additional 10 minutes with two drops of streptavidin-peroxidase.

Diaminobenzidine (DAB) Plus chromogen was utilized in order to visualize the reaction. Counterstaining of the slides was performed using Mayer's hematoxylin, followed by dehydration through a series of increasing alcohol concentrations, purification with xylene, and subsequent mounting.

Morphometric Study: by "Leica Qwin 500C" image analyzer computer system (Leica Imaging System Ltd, Switzerland) at Analysis Unit of Human Anatomy and Embryology Department.

Light microscope has been used to examine the slides and the parameters were measured in 10 non-overlapping high-power fields (x400) selected in a random manner for each section using the binary mode:

a. The mean area % of collagen fibers distributions in mallory's trichrome stained sections.

b. The mean area % of Cx43, positive immunoreactivity in immunostained sections.

Statistical Analysis:

Utilizing the Statistical Package for Social Science (SPSS) version 27.0 (IBM, 2020), we were able to conduct computerization and statistical analysis on the data that was collected. The mean±SD (Standard deviation) was utilized to illustrate quantitative data. ANOVA F-test with post hoc Tukey test was utilized to estimate the variance between various categories. Significant results are expressed as P value <0.05, while highly significant results are expressed as P value <0.001.

RESULTS

Results revealed no statistically significant difference among negative control and positive control (corn oil) groups in any of the biochemical parameters investigated, as well as histological and immunohistochemical analyses. So, the negative control was used for statistical comparison with the other groups.

Results of oxidative-related markers and inflammatory cytokines in cardiac tissues

Both GSH level and SOD activity were reduced in the group that was given B[a]p, while there was an increase in the levels of NOX-2, NO, and MDA in the heart. This was contrary to the control and Spirulina groups. Unlike the B[a]p-treated group, combination with spirulina resulted in a significant decline in the amounts of NOX-2, MDA, and NO, in addition to augmented level of GSH and SOD activity **(Table 1)**.

A significant elevation in levels of TNF-α and IL-6 was detected in B[a]p-treated group compared to the group that served as control. Also, no significant difference was noted for Spirulina group versus control. Contrary, a significant diminution in these inflammatory cytokines was found in rats that were treated with spirulina along with B[a]p, in comparison to the group that was given B[a]p alone **(Figures 1 and 2)**.

Results of cardiac TLR4 gene Expression:

When compared to normal control rats, no significant difference was noted for Spirulina group. Meanwhile, rats treated with B[a]p demonstrated a noteworthy elevation in TLR4 mRNA expression. The expression of TLR4 mRNA was significantly decreased by Spirulina therapy; however, it was significantly greater than that of the control group **(Figure 3)**.

Table (1): Results of oxidative-related parameters in cardiac tissues.

Group / Variable	Negative Control group	Positive control group	Spirulina group	Benzo[a] pyrene group	Benzo[a] pyrene + Spirulina group	F	P
NOX-2 (ng\ml tissue)	1.68±0.07	1.66±0.02	1.69±0.05	5.99±0.28 a,c	3.21±0.09 a,b	1,871.64	**<0.001 ****
NO (nmol/g tissue)	780.35±50.4	778±48.9	785.1±56.05	3712±163 a,c	1777.65±28.55 a,b	2,291.08	**<0.001 ****
GSH (nmol/g tissue)	1260.38±11.20	1258±10.88	1265.7±11.33	488.77±34.53 a,c	768.21±50.25 a,b	1,588.51	**<0.001 ****
SOD (U/g tissue)	5458.1±263.57	5457.9±262	5459.7±257.14	5032.72±34.45 a,c	4878.3±304.03 a,b	13.245	**<0.001 ****
MDA (nmol/g tissue)	27.33±1.74	27.22±1.44	27.48±2.34	59.02±3.92 a	29.83±0.03 a,b	373.97	**<0.001 ****

*Data demonstrated as mean ± SD (Standard deviation), **: Highly significant (P-value less than 0.001), Post hoc: Tukey test, a: Significant versus control group, b: Significant versus B[a]p group, c: significant versus B[a]p + Spirulina group.*
NOX-2=NADPH oxidase-2, NO=nitric oxide, GSH=reduced glutathione, SOD=superoxide dismutase, MDA=malondialdehyde,

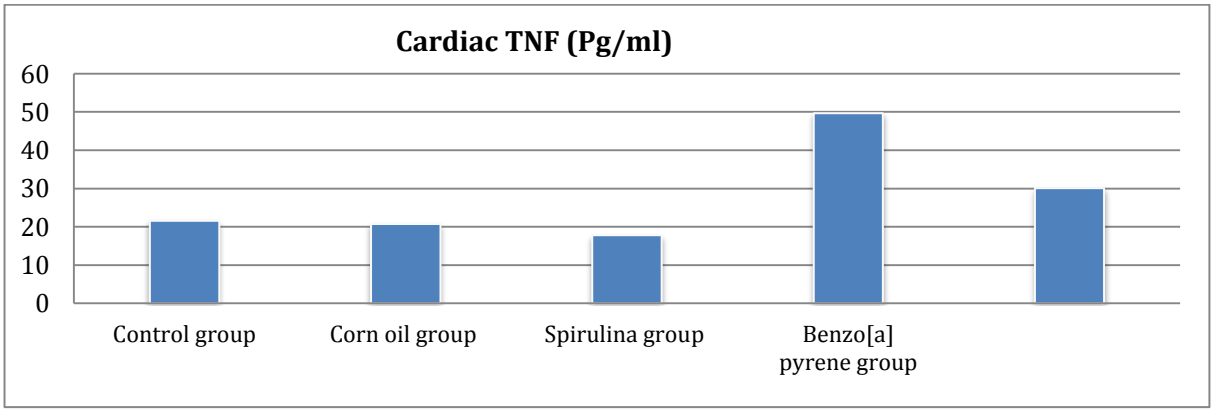

Figure (1): Bar chart comparing groups as regards the mean values of TNF-α level.

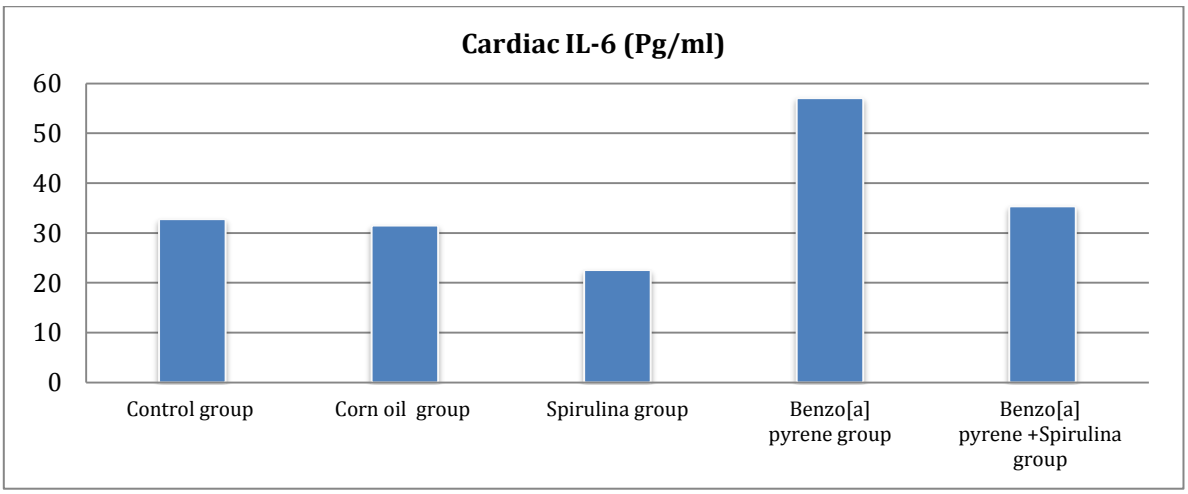

Figure (2): Bar chart comparing groups as regards the mean values of IL-6 level.

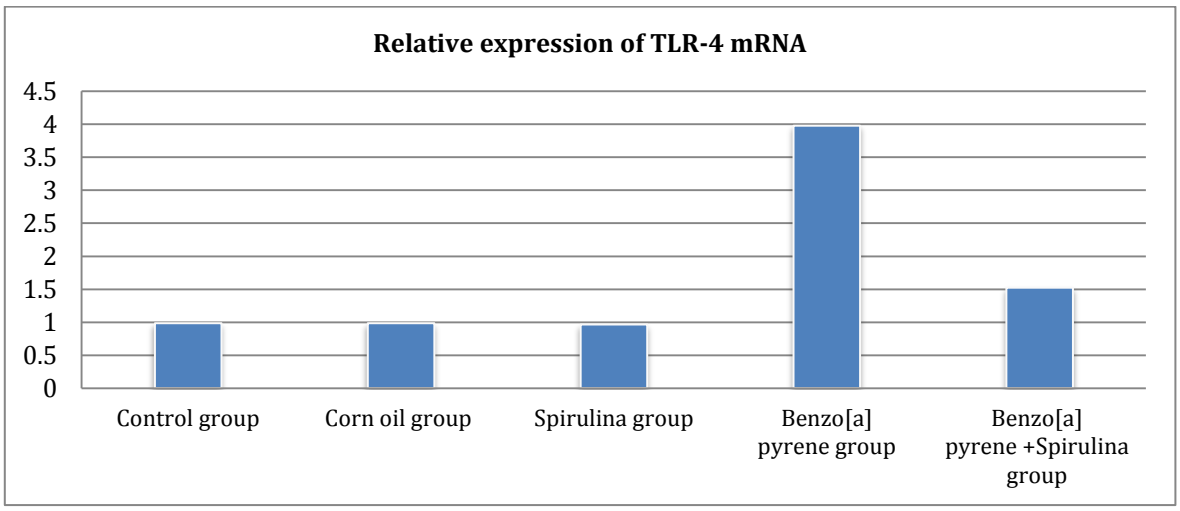

Figure (3): Bar chart comparing groups as regards the mean values of cardiac TLR4 mRNA Expression.

Histological and immunohistchemical results:

a- Light microscopic analysis of standard H&E stained sections from both the control and the Spirulina Platensis groups showed a typical organization of cardiac muscle fibers, exhibiting clearly defined striations and branching patterns. The cardiac myocytes displayed a standard histological architecture, distinguished by centrally located oval nuclei and acidophilic striated sarcoplasm. Elongated nuclei of interstitial cells, along with the intercalated discs situated between them were noted **(Figures 4a and 4b)**.

B[a]p group revealed an abnormal arrangement of cardiac muscle fibers, characterized by a separation of myocytes and the presence of extensive interfibrillar spaces. Certain myocyte cells exhibited intensely acidophilic sarcoplasm and darkly stained nuclei **(Figure 4c)**. Moreover, Benzo[a]pyrene + Spirulina Platensis group showed noticeable conservation of cardiomyocyte morphology. Myocytes appeared with central oval vesicular nuclei and intact intercalated disc in-betweens. Some cells had deep acidophilic sarcoplasm and wide interfibrillar spaces were seen **(Figure 4d)**.

b- Light microscopic examination of mallory-trichrome stained sections of the control and Spirulina Platensis groups to evaluate myocardial fibrosis. Few numbers of blue-stained fibers were observed between the cardiac muscles in the control and Spirulina Platensis groups (**Figures 5a and 5b)**.

B[a]p group exhibited numerous, blue-stained collagen fibers interspersed among cardiac muscle tissues and regions surrounding the blood vessels **(Figure 5c)**.

While Benzo[a]pyrene + Spirulina Platensis group demonstrated some collagen fibers deposited between cardiac myocytes **(Figure 5d)**. There was a markedly significant rise in the area percentage of collagen fibers among the cardiac muscles in B[a]p group versus control, spirulina and Benzo[a]pyrene + Spirulina Platensis groups. However, control, spirulina and Benzo[a]pyrene + Spirulina Platensis groups showed no significant difference **(Figure 7)**.

c- Light microscopic examination of immunohistochemical stained sections of control group and Spirulina Platensis group demonstrated multiple positive Cx43 immunoreactivity in intercalated discs between cardiomyocytes **(Figures 6a and 6b)**.

B[a]p group showed markedly decreased Cx43 immunoreactions **(Figure 6c).** While in the Benzo[a]pyrene + Spirulina Platensis group, they showed largely preserved Cx43 immunoreactions **(Figure 6d)**.

There was a highly significant elevation in area percent of Cx43 positive immunoreactivity at intercalated disc of cardiac fibers in B[a]p group compared to control, spirulina and Benzo[a]pyrene + Spirulina Platensis groups. However, control, spirulina and Benzo[a]pyrene + Spirulina Platensis groups showed no significant difference **(Figure 8)**.

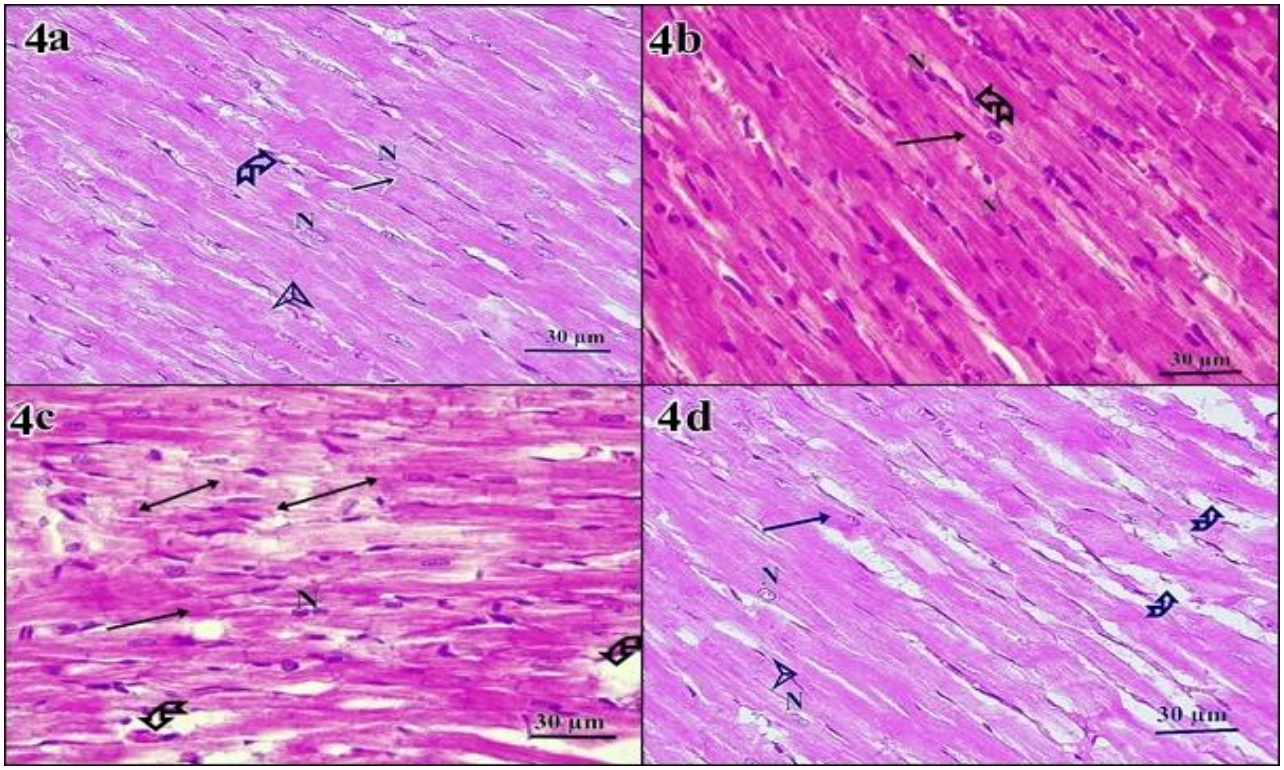

Figure (4): *A photomicrograph of* ***H&E*** *stained sections in longitudinal segments of left ventricular tissues:*
Fig. 4a: *The control group shows cardiac myocyte has an acidophilic striated sarcoplasm* ***(arrow)****, central oval vesicular nuclei* ***(N)*** *and an intercalated disc* ***(arrowhead)*** *between cardiac muscle fibers. Elongated nuclei of interstitial cells* ***(curved arrow)*** *are observable* ***(H&E X400, Scale bar 30 µm)****.*
Fig. 4b: *Spirulina Platensis group displaying cardiac myocytes have acidophilic striated sarcoplasm* ***(arrow)*** *and central oval vesicular nuclei* ***(N).*** *Elongated nuclei of interstitial cells are observed in the interfiber space (****curved arrow)*** ***(H&E X400, Scale bar 30 µm)****.*
Fig. 4c: *Benzo[a]pyrene group showing separation of myocytes* ***(double-headed arrows)*** *and wide interfiber spaces* ***(curved arrows)****. Darkly stained nuclei* ***(N)*** *and deeply acidophilic sarcoplasm* ***(arrow)*** *are observed in some myocytes* ***(H&E X400, Scale bar 30 µm)****.*
Fig. 4d: *Benzo[a]pyrene + Spirulina Platensis group showing myocytes has central oval vesicular nuclei* ***(N)*** *and intact intercalated disc in-betweens* ***(arrowhead)****. Some myocyte cells have deeply acidophilic sarcoplasm* ***(arrow)*** *and wide interfiber spaces* ***(curved arrows)*** *are also detected (****H&E X400, Scale bar 30 µm)****.*

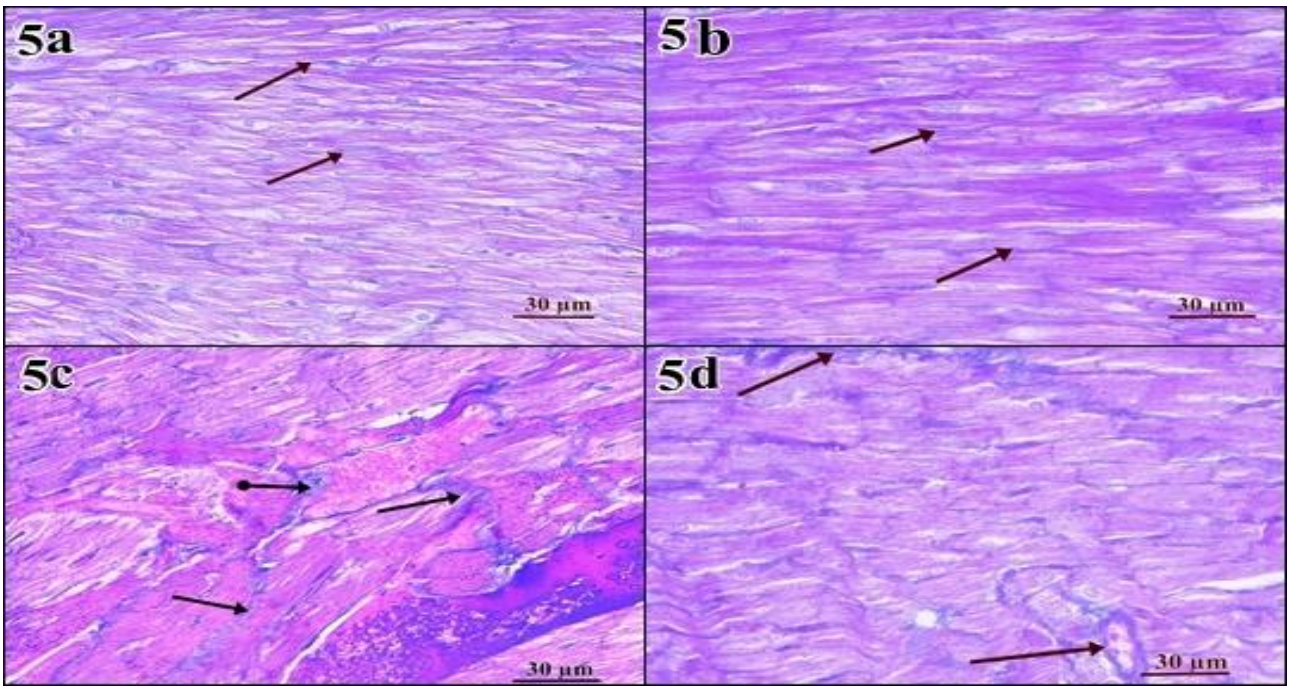

Figure (5): *A photomicrograph of* ***Mallory's trichrome*** *stained sections in longitudinal segments of left ventricular tissues:*

Fig. 5a*: Control group showing few blue stained collagen fibers* ***(arrows)*** *between the cardiac muscles* ***(Mallory's trichrome X400, Scale bar 30 µm)****.* ***Fig. 5b****: Spirulina Platensis group showing few blue stained collagen fibers* ***(arrows)*** *between the cardiac muscles* ***(Mallory's trichrome X400, Scale bar 30 µm)****.* ***Fig. 5c:*** *Benzo[a]pyrene group many blue stained collagen fibers* ***(arrows)*** *are located between the cardiac muscle and around blood vessels* ***(pointed arrow)*** ***(Mallory's trichrome X400, Scale bar 30 µm)****.* ***Fig. 5d:*** *Benzo[a]pyrene + Spirulina Platensis group showing some blue stained collagen fibers* ***(arrows)*** *between the cardiac muscles* ***(Mallory's trichrome X400, Scale bar 30 µm)****.*

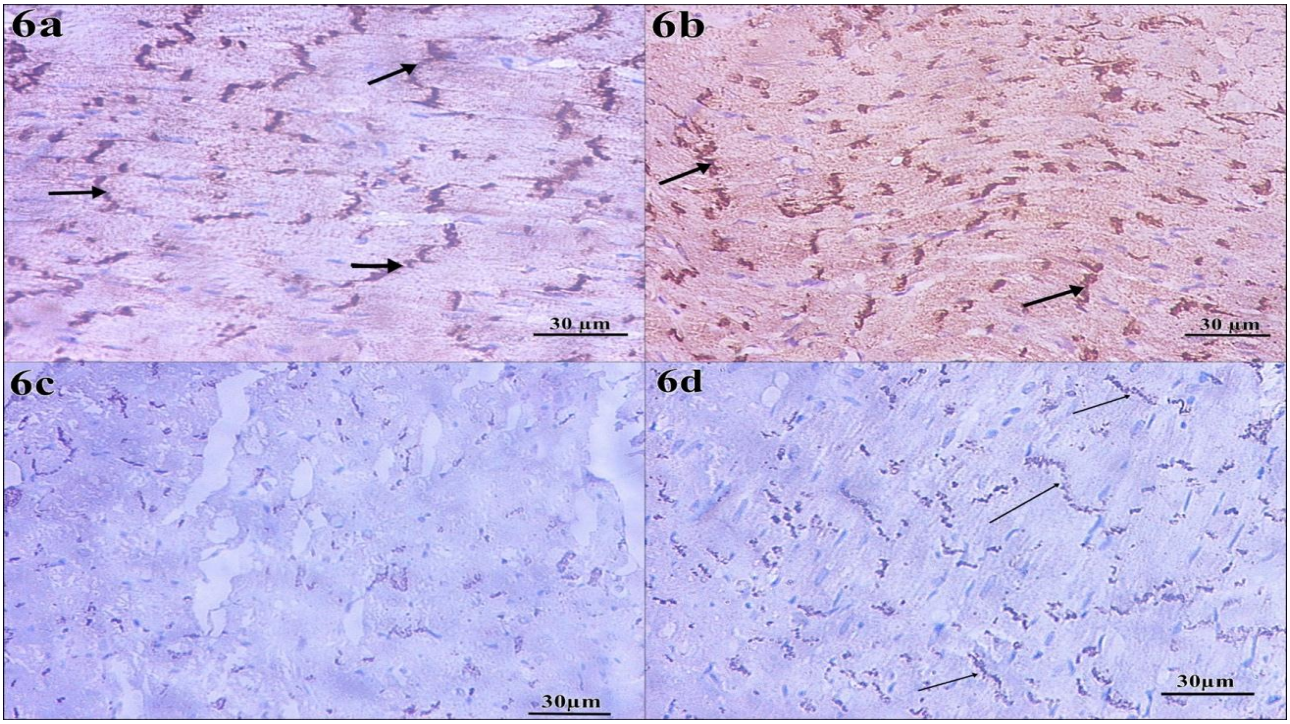

Figure (6): A photomicrograph of immunohistochemical stained sections in longitudinal segments of left ventricular tissues:

Fig. 6a: Control group showing multiple positive Cx43 immunoreaction at intercalated discs (arrows) between the cardiac muscle cells (Cx43 immunostaining, X400, Scale bar 30 µm). Fig. 6b: Spirulina Platensis group showing multiple positive Cx43 immunoreaction at intercalated discs (arrows) between the cardiac muscle cells (Cx43 immunostaining, X400, Scale bar 30 µm). Fig. 6c: Benzo[a]pyrene group showing markedly decreased Cx43 immunoreactions at intercalated discs (Cx43 immunostaining, X400, Scale bar 30 µm). Fig. 6d: Benzo[a]pyrene + Spirulina Platensis group showing largely preserved Cx43 immunoreactions (arrows) at intercalated discs (Cx43 immunostaining, X400, Scale bar 30 µm).

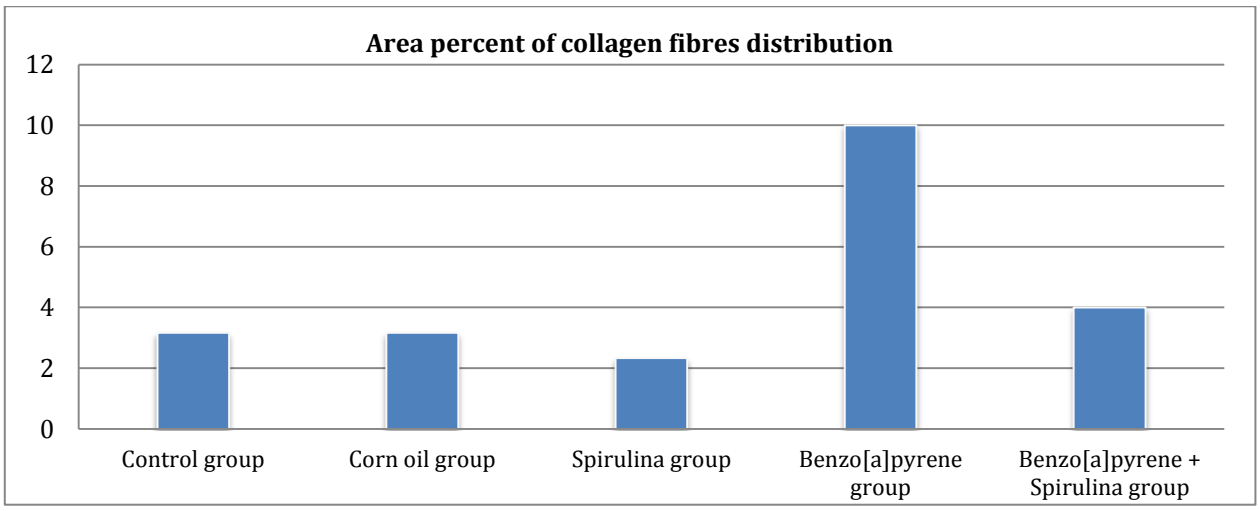

Figure (7): Bar chart comparing groups as regards the mean values of area % of collagen fibers distribution stained by Mallory trichrome

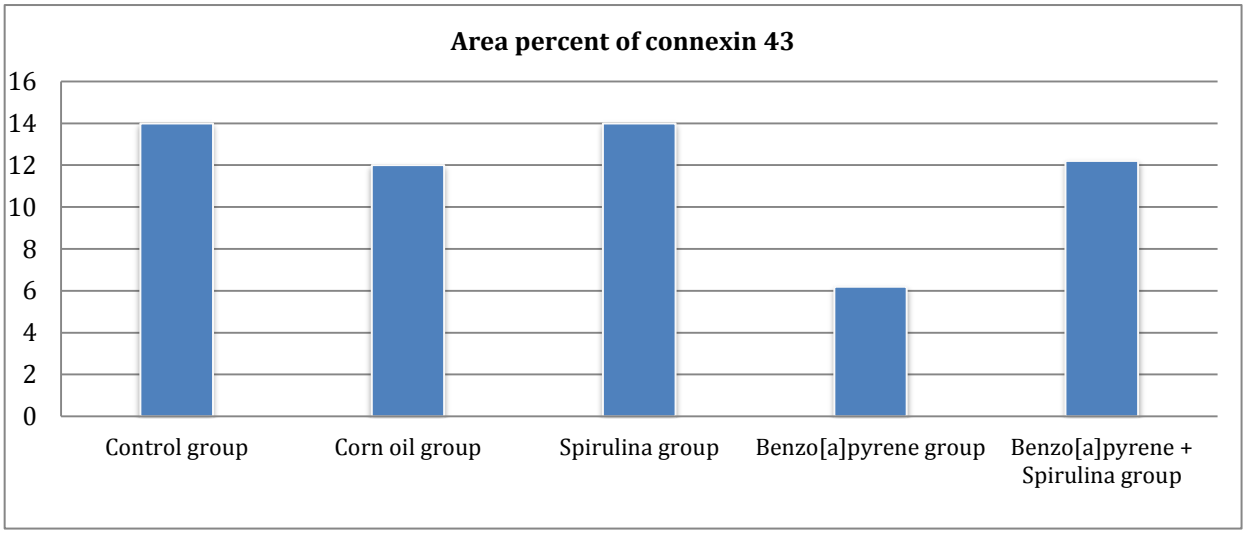

Figure (8): Bar chart comparing groups as regards the mean values of area % of connexin 43 immunoreactions

DISCUSSION

Cardiac toxicity resulting from high doses of B[a]p has been recorded previously in both animals and humans, manifesting as arrhythmias, hypotension, and cardiac arrest ***(Gentner, 2010; Fu et al., 2022)***. The latest outcomes indicate that B[a]p therapy induces damage to cardiac tissues, as supported by histological changes observed in the cardiac tissues.

The current investigation has provided evidence that Spirulina Platensis possesses a safeguarding impact on B[a]p-induced cardiotoxicity in albino rats. ***Vilahur et al. (2022)*** suggested that spirulina exerts cardioprotection via anti-oxidative, anti-inflammatory, and anti-apoptotic mechanisms. It has been indicated that Spirulina Platensis effectively diminished lipid peroxidation, nitric oxide, inflammatory markers, and improved the antioxidant enzymes within the heart tissue of B[a]p-treated rats. These findings propose that Spirulina Platensis has the ability to regulate the oxidative effect and inflammation triggered by B[a]p within the heart tissue.

The primary cause of the cardiotoxicity caused by B[a]p is its metabolism by cytochrome P450 enzymes, which produce reactive oxygen species (ROS) and electrophilic metabolites that have the potential to harm cellular macromolecules like lipids, proteins, and DNA ***(Chu et al., 2010)***. The oxidative stress mediated by B[a]p can negatively impact mitochondrial function, compromise membrane integrity,

and activate both apoptotic and necrotic pathways leading to cell death ***(Bin-Jumah et al., 2021)***.

Moreover, oxidative stress activates two important pathways, namely nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinases (MAPKs). They induce expression of certain cytokines (e.g., IL-1β, IL-6, TNF-α) which in turn worsen cardiac inflammation and exacerbate tissue damage ***(Saha et al., 2020)***.

The present study demonstrated the ability of B[a]p exposure to trigger oxidative damage in cardiac tissue, resulting in an imbalance between oxidants and antioxidants. This is evident through a notable rise in cardiac MDA and NOX-2 levels, accompanied by diminished cardiac GSH and SOD. Furthermore, B[a]p significantly elevates NO level. These findings are analogous to previous research conducted by ***Chandrashekar (2021); Guo et al. (2021); and Chenghao et al. (2022)*** who noted that malondialdehyde and nitric oxide levels have significantly risen, whereas the GSH level and SOD activity significantly decreased, following B[a]p treatment. In contrast, ***Bukowska et al. (2022)*** found that in rats treated with B[a]p, MDA and NO level decreased but GSH level and SOD activity increased.

Punetha et al. (2022) reported similar findings for B[a]p on human cells.

It is possible to infer that B[a]p may have initiated a response aimed at improving the antioxidant defense and minimizing oxidative damage. Additionally, the effect of B[a]p treatment on MDA, NO, GSH, as well as SOD levels could be influenced by various factors including dosage, duration, route, and frequency of exposure, as well as the specific species, tissue, and cell type involved.

The outcomes of the existing investigation are also parallel to ***Abdel-Daim et al. (2013)*** who investigated the cardiac effects of Spirulina platensis on rats exposed to myocardial injury caused by Deltamethrin, by increasing the GSH and SOD, as well as reducing the MDA level.

Spirulina platensis contains abundant natural antioxidants, including phycocyanin, polysaccharides, carotenoids, and phenolic acids, which possess the power to neutralize free radicals and hinder lipid peroxidation ***(Kumar et al., 2022)***.

The potential of Spirulina to protect against the oxidative effect mediated by B[a]p in the present study may be directly attributed to the inhibition of lipid peroxidation and scavenging free radicals. Additionally, it may indirectly enhance the activity of CAT and SOD, which are free radical scavengers. These beneficial effects are likely due to the abundant presence of antioxidants (e.g. c-phyocyanin), and other useful substances (e.g., carotenoids, vitamins and minerals) as reported in SP ***(Piovan, 2022)***.

Moreover, the inflammatory response can be regulated by Spirulina platensis through the reduction of the NF-κB and the MAPKs pathways, along with inhibiting the pro-inflammatory cytokine *production* ***(Abdel-Daim, 2013; Upasani and Balaraman, 2003)***. As a result, Spirulina has the potential to be employed for the prevention of cardiovascular disorders, particularly those caused by oxidative damage.

The formation of ROS in the heart is significantly influenced by NOX isoforms, which have been involved in a variety of cardiovascular diseases including atherosclerosis, cardiac arrhythmias, hypertension, and heart failure ***(Zhang et al., 2020)***. GSH a nonenzymatic antioxidant composed of three amino acids, has a direct antioxidant defense role by scavenging ROS as well as an indirect role by supporting the function of antioxidant enzymes ***(Franco et al., 2007)***. SOD, a crucial antioxidant enzyme at the forefront, converts superoxide radicals into hydrogen peroxide or molecular oxygen ***(Lewandowski et al., 2019)***. The antioxidant properties and/or ability of Spirulina to suppress the inducible NO synthase-induced nitric oxide bioactivation ***(Abdel-Daim et al., 2013)*** may account for its suppressive impact on lipid peroxidation, evaluated as MDA.

Several previous studies have reported similar findings in line with the existing study, demonstrating reduction of NO by Spirulina ***(Bin-Jumah, 2021; Wu et al., 2016)***.

NO is a short-lived gasotransmitter, derived from iNOS, and is produced in large amounts in response to inflammatory stimuli. It works by mediating and regulating the inflammatory process. The activated cells of inflammation generate ROS, with which NO rapidly interacts resulting in production of the peroxynitrite which promotes the proinflammatory and toxic effects on the cells ***(Korhonen et al., 2005)***.

Furthermore, the activation of NO can trigger the activation of NF-κB which regulates numerous genes of inflammatory response ***(Hierholzer et al., 1998)***. NF-κB is inadvertently bound to the inhibitor of κB (IκB), that limit its cytosolic transfer. Upon TLR activation, MyD88 signaling causes IκB kinase phosphorylation, which in turn leads to its degradation. This degradation enables IκB to access nucleus and initiate transcription of target genes which are associated with inflammation. In the development of specific immune cell types, such as macrophages, the genes controlled by NF-κB are essential, as they produce important cytokines, involving TNF-α as well as IL-6 ***(Baker et al., 2020)***.

The present investigation, the B[a]p induced group exhibited notable increases in MDA and nitric oxide levels, and diminished GSH and SOD in the cardiac tissues. In contrast, the Spirulina administration with B[a]p showed reduced NO and MDA levels, and a noticeable elevation in GSH and SOD levels when compared to B[a]p-treated group.

Moreover, results demonstrated that Spirulina platensis effectively inhibits the increase of NOX-2 induced by B[a]p. This was demonstrated by the significant difference between B[a]p-induced group and combined group, with an NOX2 elevation in the B[a]p group, followed by its decrease after treatment by Spirulina. This is in accordance with the findings of other investigations, which indicated a decrease in NOX-2 levels in rat primary microglia when treated with Spirulina platensis ***(Ziyaei, et al., 2023)***.

Likewise, ***Calella et al. (2022)*** reported that Spirulina platensis prevents the B[a]p-enhanced accumulation of NOX-2 in rats ***(Calella et al., 2022)***.

However, contrasting results have been reported in other studies, where Spirulina platensis supplementation had no impact or even exacerbated the B[a]P-mediated increase of NOX-2 in rats and mice ***(Araujo et al., 2020)***.

TLR4 is one member of the TLR family which is widely expressed in cardiac cells. TLR4 works as an initiator of the inflammatory response in many cardiac diseases such as myocarditis, myocardial infarction, as well as heart failure. It is responsible for activating the NF-κB pathway and generation of inflammatory cytokines which aggravate the myocardial injury. Accordingly, targeting the TLR4 dependent pathways seems to be beneficial in combating myocardial inflammation and protecting against myocardial damage ***(Yang et al., 2016; Al-Hassani et al., 2023)***.

This work verified the inflammatory response (TNF-α/IL-6) caused by B[a]P could serve as proof of the heightened expression of cardiac TLR4 mRNA. In our study, in the B[a]p group, the TNF-α, IL-6 and TLR4 mRNA significantly increased. Comparable outcomes were observed in several prior research conducted by ***Urschel et al. (2015)*** and ***Jiedong et al. (2022)*** who stated that exposure to B[a]P can elevate (TNF-α/IL-6) and cardiac expression of TLR4 mRNA in various animal models, including rats, mice, and zebrafish. These studies indicate that the elevated expression of cardiac TLR4 mRNA is a consequence of the oxidative stress in addition to inflammation triggered by B[a]P. Furthermore, it highlights that the cardiotoxicity caused by B[a]P is mediated via cardiac TLR4 signaling pathway.

Furthermore, Spirulina group demonstrated a noteworthy decline in TNF-α/IL-6 and cardiac TLR4 mRNA levels. Similarly, ***Su et al. (2021)*** conducted a study that also explored the protective properties of Spirulina against B[a]P cardiotoxicity by regulating the cardiac inflammatory response (TNF-α/IL-6) and cardiac expression of TLR4 mRNA. Their findings demonstrated that Spirulina platensis can diminish the concentrations of cardiac TNF-α, IL-6, as well as TLR4 mRNA in B[a]P-treated rats by

enhancing the antioxidant defense system and constraining the NF-κB signaling pathway.

However, the evidence regarding the effects of Spirulina on TLR4 expression remains inconclusive. Various studies have presented different outcomes, which can be ascribed to aspects such as the dosage and type of Spirulina administered, the duration of administration, the animal model or cell type utilized, and the method employed to measure TLR4 expression. For instance, certain investigations have observed an elevation in TLR4 expression in human monocytes and rats following Spirulina administration ***(Alessio and Francesco, 2020; Céline and Yuanqing, 2014)***. Conversely, other studies have reported no impact or even a decrease in TLR4 expression in mice, rats, and human macrophages ***(Liu et al., 2023; Escoubet-Lozach et al., 2011)***. Hence, further investigation is vital to explain the mechanisms as well as the consequences of Spirulina's regulation of TLR4 expression.

Histological analysis of sections stained with H&E from the B[a]P group in this study showed major alterations in the left ventricular tissues. These changes were characterized by a disorganization of cardiac muscle architecture, separation of myocytes, and enlarged interfibrillar spaces. Additionally, certain cells exhibited intensely acidophilic sarcoplasm and prominently stained nuclei. ***El-kader et al. (2020)*** described comparable results attributed to oxidative damage affecting DNA, proteins, and cellular lipids due to heightened free-radical production. Conversely, in the group administered Benzo[a]pyrene and Spirulina Platensis, the administration of Spirulina demonstrated a significant and effective preservation of the left ventricular cardiac tissues.

Connexin 43 (Cx43) is a gap junction protein that is widely present in the heart. It is essential for facilitating cell-to-cell communication and electrical conduction within the heart, in conjunction with ionic channels ***(yin et al., 2021)***.

Immunohistochemical analysis revealed that B[a]P group demonstrated a noteworthy reduction in Cx43 immunoreactivity. In contrast, the Benzo[a]pyrene + Spirulina Platensis group exhibited largely intact Cx43 immunoreactivity. A markedly significant rise in area percentage of Cx43 positive immunoreactivity had been observed at the intercalated discs of cardiac fibers in B[a]P group, in comparison to control, spirulina, and B[a]P + Spirulina Platensis groups.

Lee et al. (2021) proposed a significant role of oxidative stress in the expression of Cx43, while ***Fu et al. (2022)*** indicated that B[a]p induces cardiotoxicity by generating oxidative stress. Additionally, ***Su et al. (2021)*** noted that Spirulina Platensis exhibits protective effects against B[a]P-induced cardiotoxicity by modulating the inflammatory response in the heart.

Gao et al. (2014) indicated that fibroblasts constitute the predominant population of nonmyocytes and have a vital role in the synthesis of extracellular matrix proteins and cardioprotective factors. Consequently, in response to cardiac pathological disorders, these fibroblasts transform into myofibroblasts, resulting in myocardial fibrosis due to fibronectin and collagen being excessively produced.

Hazzaa et al. (2020) indicated that macrophages could enhance the levels of fibronectin and platelet-derived growth factor, both of which promote the proliferation of fibroblasts.

The B[a]p group exhibited a significant presence of blue collagen fibers interspersed among the tissues of cardiac muscle and surrounding blood vessels. In contrast, the Benzo[a]pyrene + Spirulina Platensis group showed a limited deposition of collagen fibers between the cardiac myocytes. A markedly significant rise in the area percentage of collagen fibers was observed in the cardiac muscles of the B[a]p group in comparison to control, spirulina, and Benzo[a]pyrene + Spirulina Platensis groups. This aligns with the findings of ***Shredah (2017)*** who attributed these results to lipid peroxidation induced by B[a]p, resulting in the excessive production of fibrogenic cytokines, ultimately leading to fibrosis.

Additionally, ***Coue et al. (2019)*** noted that spirulina supplementation offers protection to

mice against hepatic fibrosis through its anti-inflammatory properties.

CONCLUSION

To summarize, the current study demonstrated that Spirulina platensis plays a crucial role in protecting adult albino rats against B[a]p -induced cardiotoxicity. This protection is achieved by regulating oxidative stress and inflammation. The findings propose that Spirulina platensis holds promise as a natural remedy for preventing and treating cardiovascular complications resulting from exposure to B[a]p.

RECOMMENDATIONS

- Spirulina Platensis is recommended for protection against B[a]p cardiotoxicity.
- More studies are required to investigate the cardio-protective potential as well as safe and effective doses in humans.

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الإمكانات الوقائية للسبيرولينا بلاتنسيس ضد تسمم القلب الناجم عن البنزو[أ] بيرين في الجرذان البيضاء البالغة

نهله محمد ابراهيم[١]، داليا احمد سليم عبده[١]، هبه محمد عبد العزيز[٢]، وليد احمد بدوى عبد الوهاب[٣]، نورا محمد قناوي[٤]،هند سامح[٥]، مي الدالي[٥]، سمر سامي صقر[١]

[١]قسم الطب الشرعى والسموم الاكلينيكية، كلية الطب البشرى، جامعة الزقازيق، مصر.
[٢]قسم الهستولوجيا الطبية وبيولوجيا الخلية، كلية الطب البشرى، جامعة الزقازيق، مصر.
[٣]قسم التشريح والاجنة، كلية الطب البشرى، جامعة مصر للعلوم والتكنولوجيا، مصر.
[٤]قسم التشريح والاجنة، كلية الطب البشرى، جامعة الزقازيق، مصر.
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الملخص العربى

المقدمة: البنزو(أ) بيرين هو أحد المواد الكيميائية المنتشرة بيئيا في كل مكان و التي يتم إنتاجها من الاحتراق الغير مكتمل للمركبات العضوية فيما تعد السبيرولينا واحدة من أهم مكونات التغذية العلاجية والوقائية ومضادات الأكسدة في القرن الحالي.
الهدف من الدراسة: تم اجراء هذه التجربة لتقييم دور السبيرولينا الوقائي ضد التأثيرات السمية للبنزو(أ) بيرين على قلب ذكور الجرذان البيضاء البالغة.
المواد والطرق المستخدمة: تم تقسيم 50 من ذكور الجرذان البيضاء البالغة بالتساوي إلى ٥ مجموعات: مجموعة ضابطة سالبة, مجموعة ضابطة موجبه (زيت الذرة) ،مجموعة السبيرولينا ، مجموعة البنزو(أ) بيرين ومجموعه (السبيرولينا + البنزو(أ) بيرين). تم اعطاء زيت الذره والسبيرولينا وكذلك البنزو(أ) بيرين مرتين اسبوعيا ولمدة أربع اسابيع ، و بنهاية الأسبوع الرابع ، وبعد ٢٤ ساعة من آخر جرعة ، تم ذبح الفئران بشكل رحيم واجراء الفحوصات التالية : االنيتريك اوكسيد ، المالونديالدهيد، النيتريك اوكسيد٢ ، الجلوتاثيون ، سوبر اكسيد ديسميوتيز، عامل نخر الورم الفا ،الانترلوكين٦ . كما تم استخلاص الحامض النووي وتحليله كهربيا فى الأنسجة القلبيه .وكذلك تم الفحص المجهرى لأنسجة القلب بواسطة صبغات الهيماتوكسيلين والايوسين وصبغة مالورى ترايكروم وعمل الاختبارات الامينوهستوكيائية للكونيكسين (٤٣) على الانسجة.
النتائج: في المجموعة المعالجة بالبنزو(أ) بيرين ، كان هناك ارتفاع في المالونديالدهيد وانخفاض في االنيتريك اوكسيد ،النيتريك اوكسيد٢ ، الجلوتاثيون ،سوبر اكسيد ديسميوتاز في الانسجة القلبية وكذلك ارتفاع نسبه عامل نخر الورم الفا و الانترلوكين٦ . أيضا فقد كشف اختبار تجزئة الحمض النووي عن زيادة تجزئة الحمض النووي في الأنسجة القلبيه لمجموعة البنزو(أ) بيرين. كما أحدث البنزو(أ) بيرين تغييرات هستوباثولوجيه فى أنسجة القلب فيما لوحظ تحسن كافة هذا النتائج في المجموعة التي تعالج بالسبيرولينا مع البنزو(أ) بيرين .
الخلاصة: أدى إعطاء السبيرولينا إلى تحسن ملحوظ في أنسجة القلب وكذلك تقليل الإجهاد التأكسدي والالتهابات الناجمة عن البنزو(أ) بيرين.
التوصيات: توصي هذه الدراسة الى امكانية استخدام السبيرولينا للوقايه من التسمم القلبي الناجم عن البنزو(أ) بيرين وكذلك اجراء المزيد من الدراسات لبحث الامكانات الوقائية للسبيرولينا ضد التسمم القلبي في الانسان وكذلك التاكد من سلامتها و معرفة الجرعات الفعالة.
الكلمات المفتاحية: السبيرولينا ، البنزو(أ)البيرين ، تسمم القلب، الجرذان.