Effect of Maternal Panadol Extra Exposure on Embryonic Heart of Rat's Offspring and Possible Protective Effect of Vitamin D

Original Article

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ABSTRACT

Introduction: Excessive use of paracetamol with caffeine in Panadol extra during pregnancy increase the risk of spontaneous abortion. Vitamin D stimulates cardiomyocyte proliferation and protects against injury.

Aim of Work: To investigate the effect of maternal exposure to large doses of Panadol extra on the development of the embryonic hearts of rat's offspring and possible protective effects of vitamin D.

Material and Methods: Forty-five albino rats; 30 adult females and 15 adult males were used in this study. The pregnant rats were divided into Group I as a control group, Group II (treated group; Panadol extra) received the maximum daily dose (4000mg paracetamol/ 520 mg caffeine) / kg/ day and Group III (protective group; Panadol extra + vitamin D) received previously mentioned dose of Panadol extra plus vitamin D at a dose of 400 IU/kg/day. All the treatments were given by oral gavage from the 1st day of pregnancy until day 19. Pregnant rats from all groups were sacrificed on day 19 of pregnancy and their pups were extracted & weighted, then sacrificed. Blood samples were taken for biochemical assessment. Sections from the hearts were stained (H&E, Eosin & Mallory's trichrome stain), Immunohistochemical (alpha-smooth muscles) for histopathological and morphometric studies.

Results: Group (II) revealed changes of blood gases as PH, increase in PCO2, decrease PO2, high lipid profile, and increase Creatinine kinase-MB. There were different histopathological changes as cardiomyocyte damage, necrosis, and inflammation with increased fibrosis by Mallory stain. The morphometric results revealed an enlarged size of cardiac cells, decreased proliferation, and maturation. All these results were greatly improved in the group (III) when concomitant administration of vitamin D in the last protective group.

Conclusion: Prolonged and excessive maternal use of Panadol extra greatly affect heart development. Vitamin D has a potential effect on cardiac regeneration and development.

Received: 25 October 2020, **Accepted:** 07 January 2021

Key Words: Heart; histopathology; panadol Extra; pregnancy; vitamin D.

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ISSN: 1110-0559, Vol. 44, No.4

INTRODUCTION

The gestational period is a physiologic phenomenon; it is characterized by many changes as, mental, hormonal, and physical changes that may involve a wide variety of discomforts[1] as back pain, leg spasms, and other complaints enforcing the pregnant to use analgesics[2].

Acetaminophen is a non-steroidal anti-inflammatory drug and considered one of the most broadly used worldwide[3]. It acts as an antipyretic and analgesic prescribed for different pain states with effective antipyretic action[4]. Caffeine can reduce pain sensation through its direct effect by central or peripheral inhibition for adenosine receptors' actions which in turn control pain signaling on sensory afferents[5].

Acetaminophen with caffeine is a combination in which the caffeine is probably can augment the efficacy of acetaminophen. However, this combination may be more subjected to different side effects[6]. Administration of extensive amounts of paracetamol with caffeine could lead to liver damage[7], renal medullary necrosis[8], and increase the occurrence of rebound headache[9]. If this combination is used during pregnancy; it may increase the risk of low birth weight infants or may lead to spontaneous abortion either due to the effect of paracetamol reduce prostacyclin manufacture during pregnancy[10] or through caffeine ingestion toxic metabolites[11].

The embryonic rat heart begins to develop as a nearly bending tube consisting of an external layer of the myocardium and an internal layer of endocardium at embryonic day (E) 9.5[12] and becomes a fully mature organ at the embryonic day of (E) 16[13].

Recent searches found that Vitamin D has wide extraskeletal functions after administration including the regulation of cell proliferation and differentiation, the enunciation of the immune system, influence on pancreatic β-cell function, and may has a role in the regulation of cardiac contractility and hypertrophy[14].

 DOI: 10.21608/ejh.2021.46953.1377

It has been observed recently; an increase in the use of analgesics during pregnancy without medical consulting. As there are very limited studies on the effect of large and prolonged use of Panadol extra during pregnancy on the fetal heart; so here we seek to establish for the first time the effect of maternal exposure to large doses of Panadol extra as a common analgesic on the development of embryonic hearts of rat's offspring and possible protective effects of vitamin D.

MATERIALS AND METHODS

Chemicals

1. Panadol Extra: (Acetaminophen + Caffeine; 500mg / 65mg). Was gained from Glaxo Smith kline (GSK), dissolved in distilled water and orally administered by gastric intubation. The maximum daily dose was given (4000mg paracetamol/ 520 mg caffeine) / kg/ day[15].
2. Vitamin D: Cholecalciferol (Vitamin D3) was obtained via Sigma-Aldrich Co., USA as a powder. It was taken in a dose of 400 IU/kg/day by oral gavage after dissolving in Corn oil[16].
3. Distilled water: purchased from a local market, which is used as a solvent.

Animals

Forty-five albino rats; 30 adult females and 15 adult males, weighing 200-250g, were utilized for mating. These rats were bought from the Laboratory Animal Unit, Faculty of Veterinary, Suez Canal University. They were lodged in good ventilated wide cages with stainless steel ends and wood splinters for bedding. The temperature was maintained at 24±2°c. The animals were good nitrified by an ordinary diet and water.

The experiment was performed according to the "Guide for the Care and Use of Laboratory Animals" (Institutes of laboratory Animal Research)[17] and followings the guidelines of the Institutional Animal Care and Use Committee (IACUC), Faculty of Medicine, Suez Canal University, Egypt.

Experimental design

Two female rats were housed with one male rat in each cage. Then, vaginal smears were obtained to detect the occurrence of pregnancy. Microscopic detection of the sperms in the vaginal smears confirms the first day of gestation (GD 1). The pregnant female rats were divided into equal three groups; each one has (n= 10 rats) as follows:

Group I (control group): which kept without any treatment to measure the normal values for comparisons with other study groups.

Group II (treated group; Panadol extra alone): Each pregnant rat received the maximum daily dose (4000mg paracetamol/ 520 mg caffeine) / kg/ day.

Group III (protective group; Panadol Extra + vitamin D): Each rat received the previously mentioned dose of panadol extra plus vitamin D at a dose of 400 IU/ kg/day.

All the treatments were given by oral gavage from the 1st day (GD 1) of pregnancy until day 19 (GD 19).

Pregnant rats from all groups were sacrificed under sodium pentobarbital anesthesia[18] on day 19 of pregnancy and their pups were extracted & weighted, then sacrificed by decapitation. The chest of the pups was opened and blood samples were taken directly from the heart by a puncture for biochemical assessment then the hearts were extracted, rapidly dissected, delivered, rinsed with cold normal saline, and kept in formalin 10% for histopathological (Hematoxylin and Eosin & Mallory's trichrome stain), Immunohistochemical (alpha-smooth muscles) and morphometric studies.

Heart Characteristics

The total body weight (BW) and heart weight (HW) of each pup were measured and the heart weight to body weight ratio (HW/BW) was calculated to assess the hemodynamic function of the heart (cardiac enlargement[19].

Biochemical assessment

The blood samples were taken from the hearts of pups via cardiac puncture from all groups. Plasma was obtained after centrifugation at 3500 rpm for 16 minutes. The plasma was collected into ordinary test tubes and stored at -20°C for analysis of the followings:-

1. Blood gas analysis: (PH, PCO2, and PO2), were assessed according to the method of Kaczmarczyk and Reinhardt[20].
2. Lipid profile: The plasma concentration of triglycerides and total cholesterol level were measured using spectrophotometric methods. The laboratory kit reagents (Randox Laboratory Ltd) were used for all the biochemical analysis of the study and their absorbance gradation were read utilizing of a UV-Vis spectrophotometer (DREL 3000 HACH)[21].
3. Cardiac marker (creatinine kinase-MB (CK-MB): An enzyme marker for cardiac muscle damage. It was assessed by standard enzymatic kits according to the method of Gerhardt &Waldenström[22].

Histological study

Specimens of hearts for histological examination were fixed in 10% neutral formol saline, and processed in ascending grades of alcohol (50%, 70%, 90% and 95%), the samples were dehydrated each for one hour. Then, in absolute alcohol (100%), two changes one hour for each. After dealing with xylene, the cardiac tissue samples were fixed in soft paraffin wax at 50 ºC for two hours then, embedded in hard paraffin at 60 ºC for another two hours[23]. Sections of 5 µm thick paraffin sections were prepared

for Hematoxylin & eosin stain (for studying the general histopathological features) and, Mallory's trichrome stain (for assessing the presence of fibrosis in cardiac tissues; collagen fibers were stained blue with Mallory's trichrome stain)[23].

Immunohistochemical study

Anti- alpha-smooth muscle; primary mouse monoclonal antibody kit (IgG2a) was used (IgG2a specific; Southern Biotechnology) in this study for detection of distribution and quantitative evaluation of actin isoforms during heart development. The heart tissue slices at first incubated with a secondary biotinylated donkey antibody then exposed to the streptavidin-biotin-peroxidase reaction. Tissue specimens were stained with hematoxylin. All processes were achieved at ordinary room temperature. Sections were examined microscopically for detection of the presence of positive α-SMA cardiomyocytes as a marker of cardiac differentiation and development[24].

Morphometric Study

Stained sections with H & E, Mallory's trichrome, and anti- α-SMA antibody stains were morphometrically analyzed using the ImageJ program (version 1.48v; National Institutes of Health)[25]. The microscopic images were calibrated to convert the measurement units (pixels) into actual micrometer units. Various fields were chosen and 5 readings were obtained from each slide for measuring the following:-

1. Cardiomyocyte cross-sectional area: Sectional area of at least 50 cardiac cells, whose nuclei were clearly recognized in the center of the cardiac cells, were measured[26]. Hematoxylin and Eosin stained transverse sections ×400 magnification were used through manual drawing a line around the perimeter of cardiomyocytes showed visible central nuclei. The average mean of cross sectional areas was used as an indicator of cardiac cell size.
2. The percentage of binucleated cardiomyocytes. To assess the proliferative capacity of injured cardiomyocytes[27].
3. Percent of the area of fibrosis: for evaluation of interstitial fibrotic areas (Area percentage of the interstitial collagen fibers). 5 random fields of view from Mallory stain stained myocardial sections were manually selected from each group and imaged with an objective lens magnification at x 20. The images were converted to RGB stacks providing grayscale pictures then the red-blue contrast bands were converted to absolute values. From the resulting values, the red-blue contrast band was subtracted and a threshold of 50 – 400 was selected to highlight blue collagen fibers[28].
4. Percent of alpha-smooth muscle: a marker for cardiomyocyte differentiation and development. Through analyzing the tool of the ImageJ program, all areas of α-SMA expression in the interstitium, as well as the perimeter of the histological slide, were measured per square millimeter (mm2) after grid application at 20x magnification[29].

Statistical analysis

Data from all groups were expressed as mean ± standard deviation (X ± SD). SPSS program version 21 (Chicago, USA) was used. One way analysis of variance (ANOVA) was used for statistically significant difference between groups, followed by Fisher's least significant difference multiple comparison test LSD test for multiple comparisons between the different study groups. *P- value* was < 0.05; is considered significant and highly significant when was < 0.001[30].

RESULTS

The Heart characteristics

The gross heart morphology of both the control and the protective rat hearts showed normal heart shapes, both atria appeared slightly brownish in color with clearly demarcated apex. The group that received Panadol extra (group II) appeared to had ventricular enlargement in comparison to other groups. The body weight of the treated group (II) by Panadol extra showed a statistically significant decrease in comparing to the control group (*P-value* < 0.05) but there was no significant difference in the body weight of the protective group (III) in comparing to the control group (Figure 1A). The heart weight of rats treated with Panadol extra (group II) showed a significant increase in comparing to the control group (*P-value* < 0.05) but also no significant difference in the heart weight of the protective group (III) in comparing to the control group (Figure 1B). There was a high statistically significant increase in the heart to body weight ratio in the treated group (II) (*P-value* < 0.01), and a significant increase in the protective group (III) (*P-value* < 0.05) in comparison to other groups (Figure 1C). All the data of body weight, heart weight, and the heart weight to body weight ratio in study groups are presented in (Table 1).

Biochemical results

The PH of the blood of the group (II) exposed to Panadol extra showed a significant decrease in its level in comparing to the control group (*P-value* < 0.05) but no difference between the protective group (III) and the control group. The value of PCO2 was increased in the protective group (II) (*P-value* < 0.05) but was a highly significant increase in the treated group (II) (*P-value* < 0.01) in comparison with the control group. There was a significant decrease in the level of PO2 in the protective group (III) (*P-value* < 0.05) but was a highly significant decreased in the treated group (II) (*P-value* < 0.01) in comparison with the control group and in comparing to the protective group (III) (*P-value* < 0.05). The serum triglyceride level showed a high statistical significant increase in the treated group (II) (*P-value* < 0.01) in comparison with the control group

greater than the protective group (III) (*P-value* < 0.05). No significant difference in the serum cholesterol level in the treated or the protective groups in comparing the control group. The serum creatinine kinase-MB (CK-MB) was increased in the protective group (II) (*P-value* < 0.05) but was a highly significant increase in the treated group (II) (*P-value* < 0.01) in comparison with the control group (I). All data of blood gas analysis, lipid profile, and cardiac marker levels of study groups were presented in (Table 2).

Light microscopic results

1) H&E stain

Examination of sections of rat cardiac muscle of the control rats (group I) showed elongated branching cardiac cells with cross striations exhibited oval vesicular centrally located nuclei with acidophilic sarcoplasm (Figure 2).

Cardiac tissues of rats of the group (II) which their mothers were treated with panadol extra alone showed many histopathological changes, especially in the cardiac cells. There were disorganized cardiac cells with wide intercellular spaces in between. Necrotic cardiac cells were seen with focal areas of total degeneration. Cardiomyocytes with deeply stained homogenous acidophilic sarcoplasm and deeply stained (pyknotic) nuclei but others showed pale acidophilic cytoplasm and small nuclei. Congestion and dilatation of blood vessels & capillaries with interstitial edema were seen. Marked cellular infiltrations were common in many sections (Figures 3 A,B,C,D).

The cardiac tissues of rats of the group (III) which their mothers were treated with Panadol extra concomitant with vitamin D showed a picture greatly resemble the control group especially for cardiomyocytes. Normally longitudinal or transverse cardiomyocytes with slightly wide intracellular spaces. Binucleated cardiomyocytes were found. Some cardiomyocytes showed flat & pale nuclei with pale cytoplasm were present, but others showed deeply stained homogenous acidophilic sarcoplasm and deeply stained (pyknotic) nuclei. Cellular infiltration was greatly decreased or totally absent. Very few areas of necrosis or degenerated cardiac cells. Shrunken fibroblasts were observed. Also, congested blood capillaries and exudation were greatly decreased or absent (Figures 4 A, B,C,D).

Mallory trichrome stain

On examination of sections of heart tissues stained with Mallory trichrome stain for detection of the presence of collagen tissues, it was noticed that the cardiac tissues of the control group showed few fine collagen fibers in the endomysium between the cardiac muscle fibers (Figure 5). The cardiac tissue section from rats of the treated group (II) showed an apparent increase in the amounts of the collagen fibers between the cardiac muscle fibers or deposited in focal areas and excessively deposited around congested blood vessels (Figures 6 A,B). The last group which was exposed to both Panadol extra with vitamin D group (III) showed a moderate amount of collagen fibers between the cardiac muscle fibers or around congested blood vessels with small focal areas of collagen fiber deposition (Figures 7 A,B).

Immunohistochemical results

Alpha-smooth muscles actin immune-stained sections of the cardiac tissues of the control group (I) showed weak positive immunoreaction in the endomysium and perimysium connective tissues and around small blood capillaries (Figure 8). The cardiac tissues from rats of the group (II) showed variations in the positivity of immunoreaction either strong in the endomysial & perimysium connective tissues or very strong in other areas and in the walls of dilated blood vessels small capillary (Figures 9 A,B). The cardiac tissues from rats of the protective group (III) showed a moderate positive immunoreaction around blood vessels, around the wall of small capillary, and in the endomysial & perimysial connective tissues (Figures 10 A,B).

Morphometric measures

1. Myocyte cross-sectional area

To assess the effect of maternal Panadol Extra administration on the offspring's myocyte, we measured cross-sectional area of cardiomyocyte in H and E stained myocardial transverse sections. Our data showed that, while Panadol extra exposed rats developed (group II) a highly significant increase in the cardiomyocyte cross-sectional area compared to the control group (233.2 ± 3.7 μm² and 85.7 ± 2.9 μm², respectively mean ± standard error of the mean, (*P-value* < 0.001), concomitant administration of vitamin D significantly improve the size of cardiomyocytes compared to the group received Panadol extra alone (157.4 ± 5.66 μm² and 233.2 ± 3.7 μm², respectively, mean ± SEM, *P-value* < 0. 01). However, the cross-sectional area of myocytes in vitamin D (protective group III) rats remained significantly higher than that of the control group (Figures 11 A,B,C,D).

2. Percentage of binucleated cardiomyocytes

To assess the protective effect of maternal concomitant vitamin D with Panadol extra administration as an inductive to the proliferation of cardiac myocyte, we measured the percent of binucleated cardiomyocytes in H and E stained transverse sections. The percent of binucleated cardiomyocytes in Panadol extra-exposed rats (group II) showed a significant decrease in the percent of binucleated cardiomyocytes compared to the control group (5.3 ± 0.7 % and 8.2 ± 1.09 %, respectively mean ± standard error of the mean, (*P-value* < 0.05). Concomitant administration of vitamin D increase the proliferation of the injured cardiomyocytes leading to a highly significant increase in the percent of binucleated cardiomyocytes (22.5 ± 4.6 %) compared to the control group and that group received Panadol extra alone (group III) (*P-value* < 0.001) (Figures 12 A,B,C,D).

3. Percent of interstitial fibrotic area

When assessing the area percentage of the interstitial collagen fibers deposition; the group that exposed to Panadol extra alone (group II) showed a high statistically significant increase in the percent of interstitial collagen fibers in comparing to the control group (8.6 ± 1.03 and 1.8 ± 0.06 respectively mean ± standard deviation) (*P-value* < 0.001). But after concomitant administration of vitamin D (group III), the percent of interstitial collagen fibers were significantly decreased (*P-value* < 0.01) in comparing the group exposed to Panadol extra alone (4.4 ± 1.01 and 8.6 ± 1.03 respectively mean ± standard deviation) (Figure 13).

4- Percent of positive α-SM cardiomyocytes expression

The percent of positive expression of α- SM cardiomyocytes showed a highly significant increase (*P-value* < .001) in the group that exposed to Panadol extra alone (group II) in comparing to the control (22.7±4.08 and 5.2±0.82 respectively) or in the group that exposed to Panadol extra concomitant with vitamin D (12.5±2.9; *P-value* < .01) which showed a significant decrease (*P-value* < .05) in the percent of positive expression of α- SM cardiomyocytes in comparing to the treated group (II) (Figure 14).

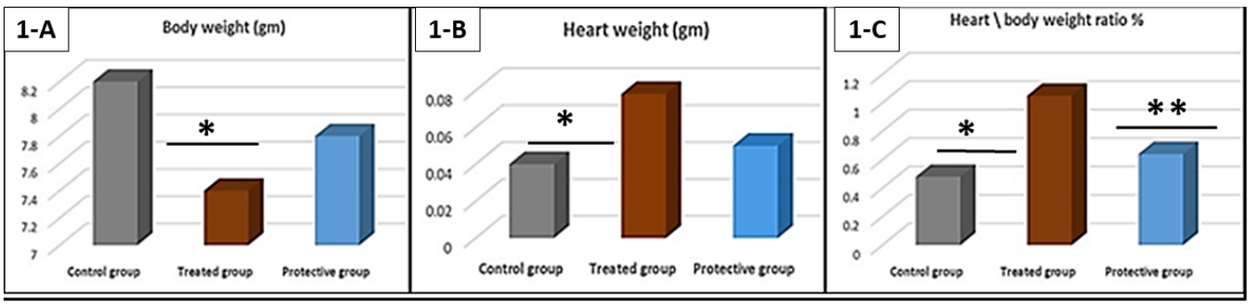

Fig. 1: A: The difference in the body weight of the study group. B: The difference in the heart weight of the study group. C: The difference in heart to body weight ratio of the study group. **P-value* < 0.05 in comparing to control group; ***P- value* < 0.05 in comparing to control & treated groups

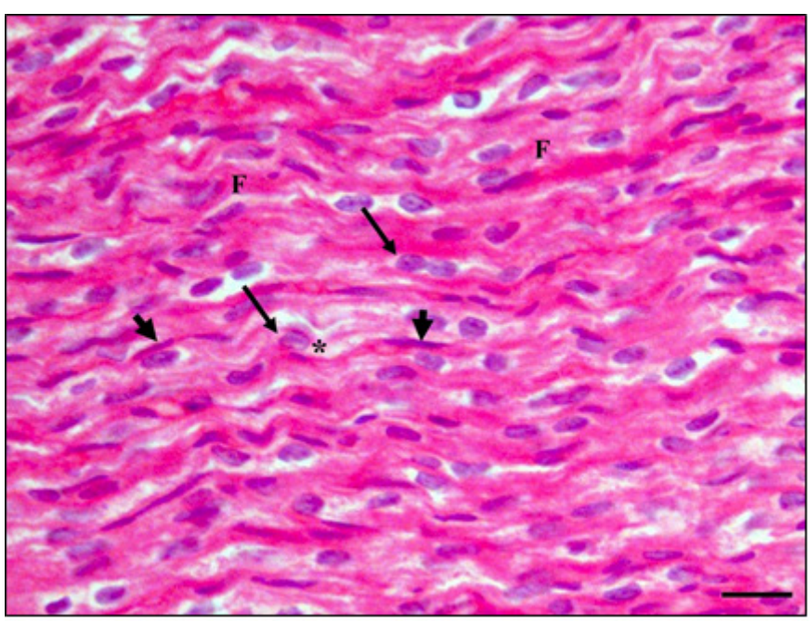

Fig. 2: A photomicrograph of cardiac muscles from the control rats group (I) showing normal elongated branching and anastomosing muscle fibers (F) having central oval vesicular nuclei (arrows) and acidophilic sarcoplasm (star). Flat dark nuclei of the fibroblasts of the connective tissue of endomysium (arrow heads) are noticed. (H&E, × 400)

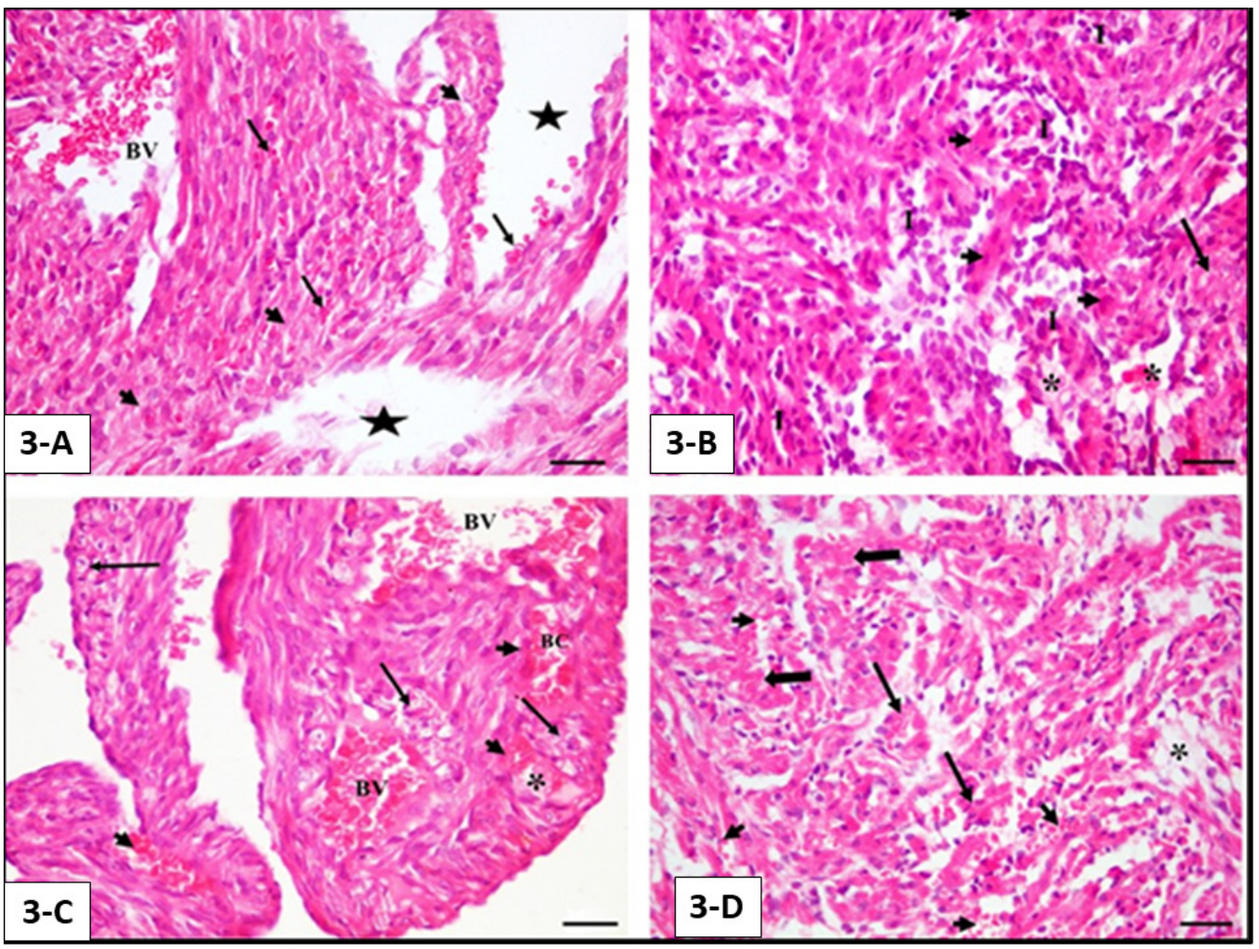

Fig. 3: A photomicrograph of cardiac muscles from rats of treated group (II; Panadol extra) showing; A: Focal areas of degenerated cardiac muscle fibers (Stars), multiple extravasted red blood cells (arrows), necrosis of cardiomyocytes (arrow heads), congested & dilated blood vessels (BV). B: the cardiac tissues showed focal areas of marked cellular infiltrations (I), cardiomyocytes with deeply stained homogenous acidophilic sarcoplasm and deeply stained (pyknotic) nuclei (arrow heads), areas of necrotized cardiac cells (arrows) and interstitial congestion & edema (Stars). C: marked congested blood vessels (BV) & capillaries (BC) and between the cardiac cells (arrow heads), some cardiac cells shows pale acidophilic cytoplasm and small nuclei (arrows). D: Marked disorganized cardiac cells with wide intercellular spaces in between, necrotized cardiac cells (thick arrows), interstitial extravasted blood (arrow heads) & edema (Stars). (H&E, × 400).

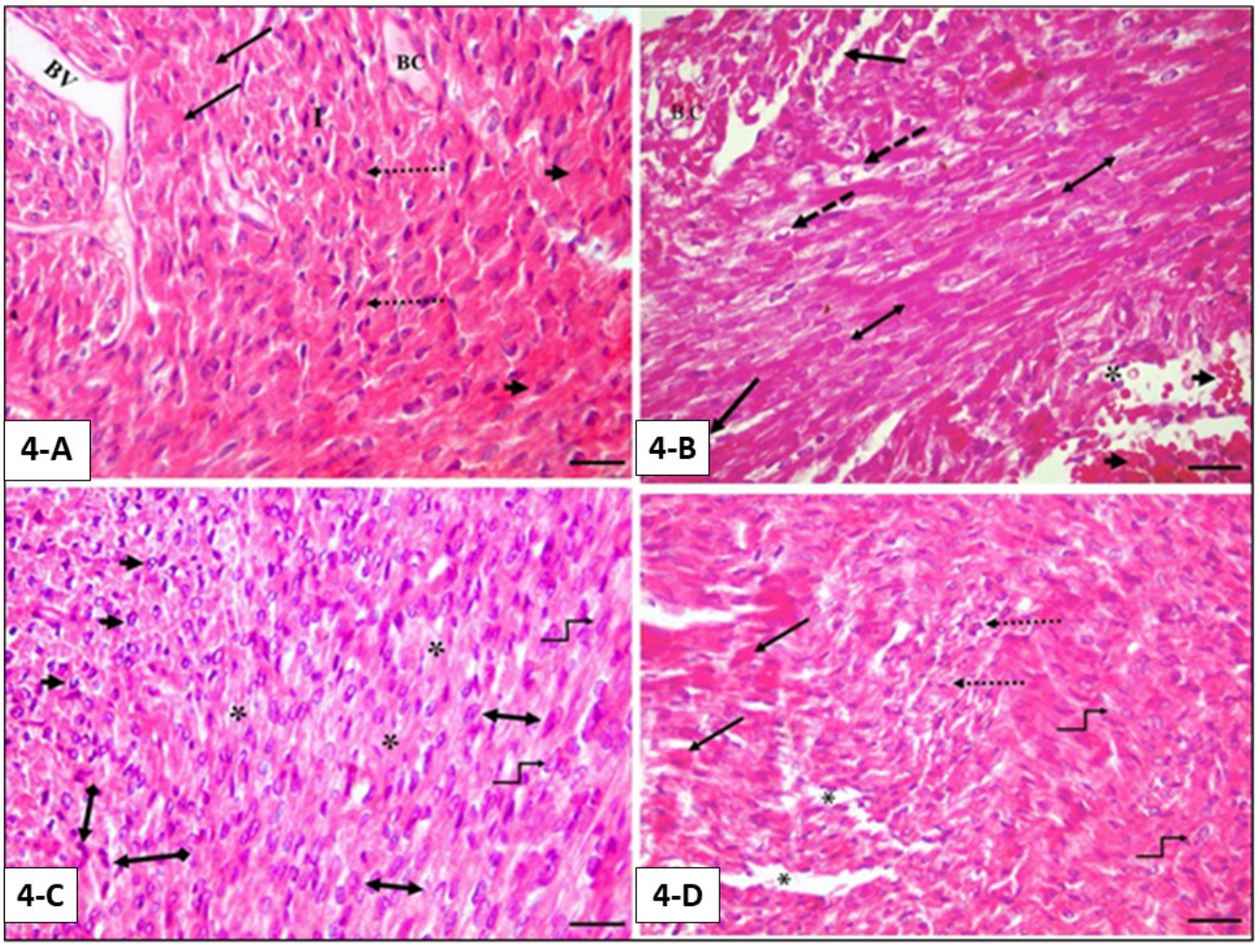

Fig. 4: A photomicrograph of cardiac muscles from rats of protective group (III) Panadol extra + vitamin D showing; A: Normal longitudinal cardiac cells (arrow heads), normal transverse cardiac cells (detached arrows), perivascular necrosis (arrows), focal area of cellular infiltration (I), normal blood vessels & capillaries (BC). B: Normal elongated cardiac cells (double heads arrows), intra-cardiac cellular spaces (arrows), shrunken fibroblast cells (detached arrows), congested blood capillaries (CB) and extravasted blood (arrow heads) with exudation (star). C: normal longitudinal (double heads arrows) & transverse (arrow heads) cardiac cells, cardiac cells with flat & pale nuclei (elbow arrows) with focal degeneration of cardiac cells (stars), binucleated cardiomyocytes (pointed arrows). D: Normal longitudinal cardiomyocytes (elbow arrows), transverse cardiomyocytes (detached arrows), slightly wide intracellular spaces (stars) and cardiomyocytes with deeply stained homogenous acidophilic sarcoplasm and deeply stained (pyknotic) nuclei (arrows). (H&E, × 400).

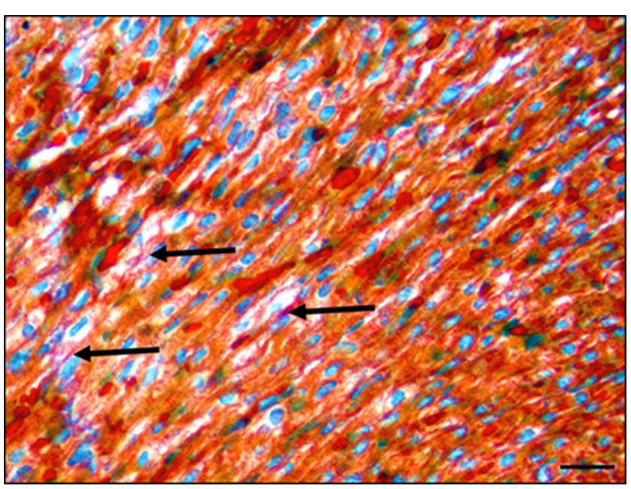

Fig. 5: A photomicrograph of control group I showing few fine collagen fibers in the endomysium between the cardiac muscle fibers (arrows). (Mallory's trichrome stain, × 400).

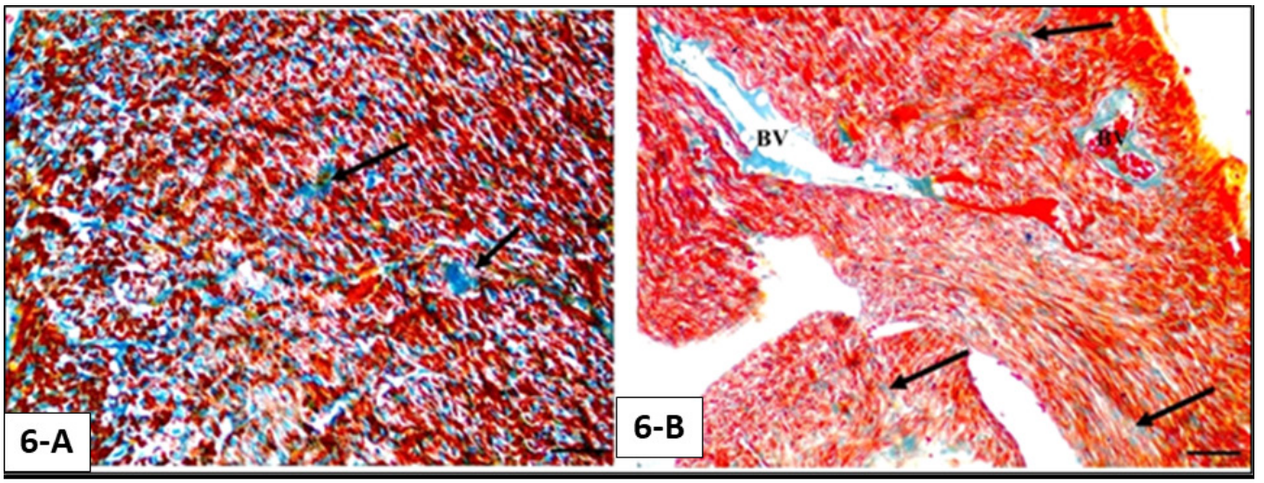

Fig. 6: A photomicrograph of cardiac muscles from rats of treated group (II; Panadol extra) showing; A: an apparent increase in the amounts of collagen fibers between the cardiac muscle fibers (blue color) with focal areas of excessive increase in collagen fiber (arrows). (Mallory's trichrome stain, × 200). B: excessive deposition of collagen fibers around congested blood vessels (BV) and between cardiac cells (arrows). (Mallory's trichrome stain, × 100).

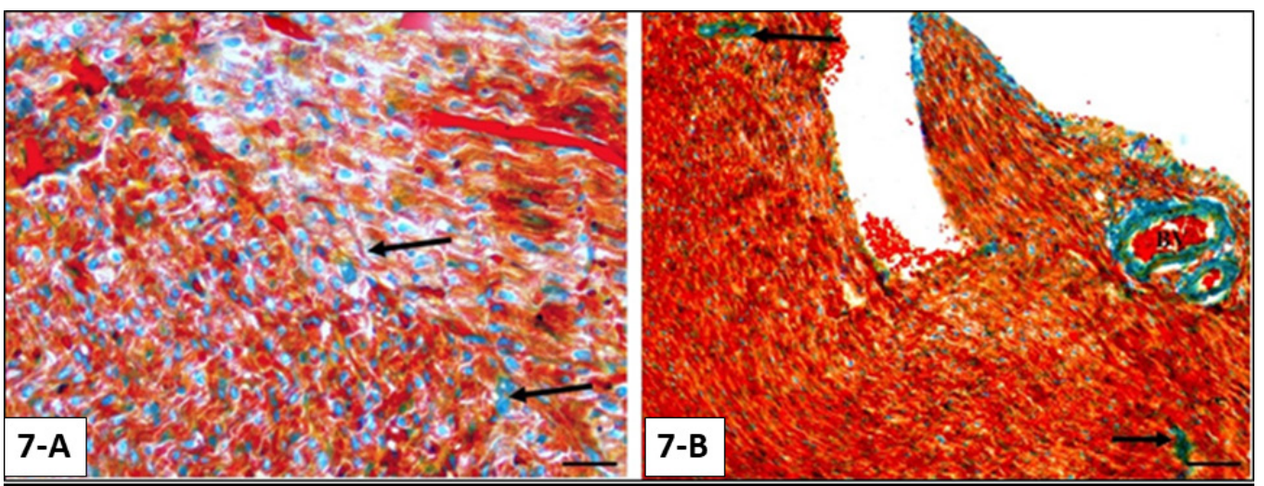

Fig. 7: A photomicrograph of cardiac muscles from rats of protective group (III) Panadol Extra + vitamin D showing; A: moderate amount of collagen fibers between the cardiac muscle fibers (arrows). (Mallory's trichrome stain, × 200). B: around congested blood vessels (BV) with focal areas of increase in collagen fiber (arrows) (Mallory's trichrome stain, × 100)

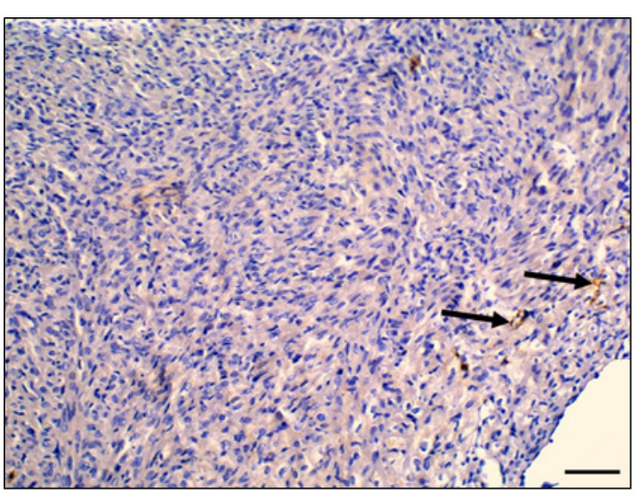

Fig. 8: A photomicrograph of control group showing weak positive immunoreaction in the endomysium and perimysium connective tissues (faint brown color) and around small blood capillaries (arrows). (Immunostaining SMA, X 200)

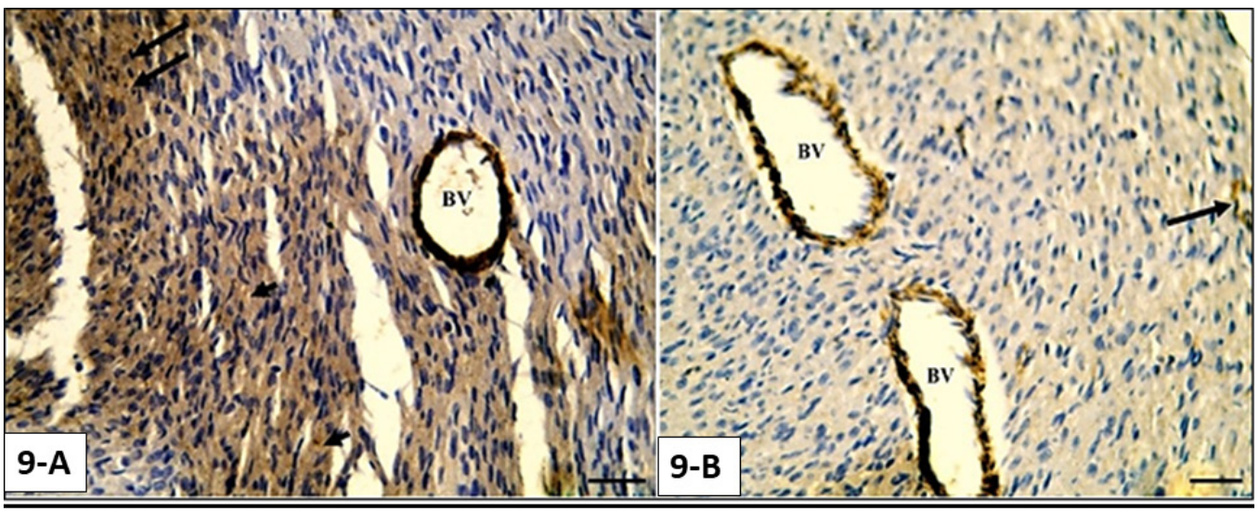

Fig. 9: A photomicrograph of cardiac tissues from rats of treated group (II; Panadol extra) showing; A: a strong positive immunoreaction in the endomysial & perimysium connective tissues (arrow heads) and very strong in another areas (arrows) and in the walls of blood vessels (BV). B: very strong positive stain around dilated blood vessels (BV) and around small capillary (arrow). (Immunostaining SMA, X 200)

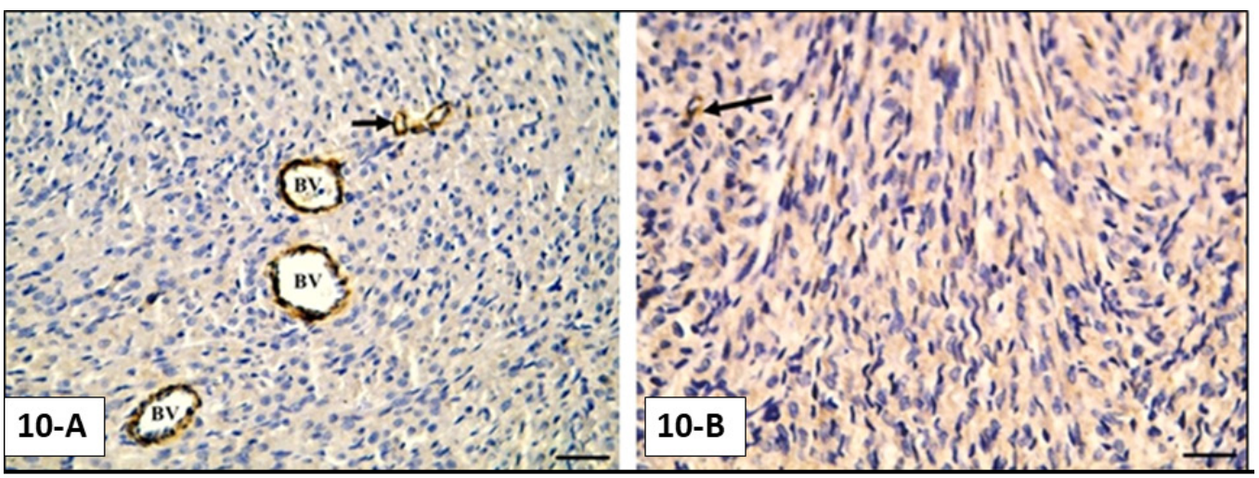

Fig. 10: A photomicrograph of cardiac tissues from rats of protective group (III) Panadol extra + vitamin D showing, A: moderate positive immunoreaction around blood vessels (BV) and around small capillary (arrow). B: moderate positive immunoreaction in the endomysial & perimysial connective tissues (arrow, light brown color). (Immunostaining SMA, X 200)

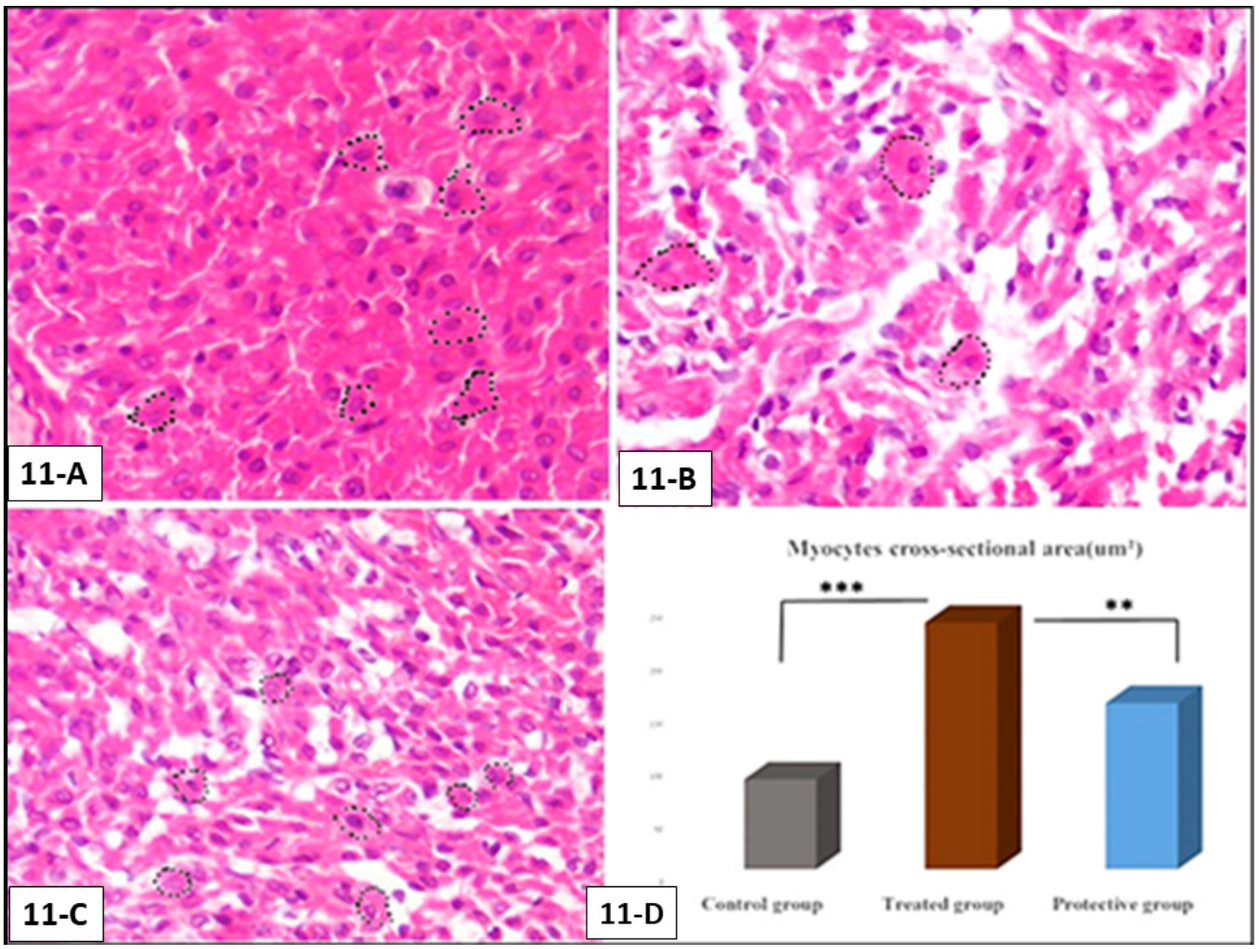

Fig. 11: Panadol extra -induced hyper trophy of cardiomyocyte. Representative micrographs of the transverse sections of the heart myocardium of (A) control group, (B) Panadol extra exposed group (II) showing increased cardiomyocyte cross-sectional area. (C) Protective group (III, vitamin D) showing decreased myocytes cross-sectional area compared to treated group II. (H & E), dashed area, representative example for cross-sectional area measurement, scale bar = 20 µm. (D) Bar chart showing quantitative measurements of myocytes cross-sectional area among the experimental groups. Data are presented as mean ± standard error of mean, ****P-value* < 0.001 in comparing to control group, ***P-value* < 0.01 in comparing to Panadol extra exposed group.

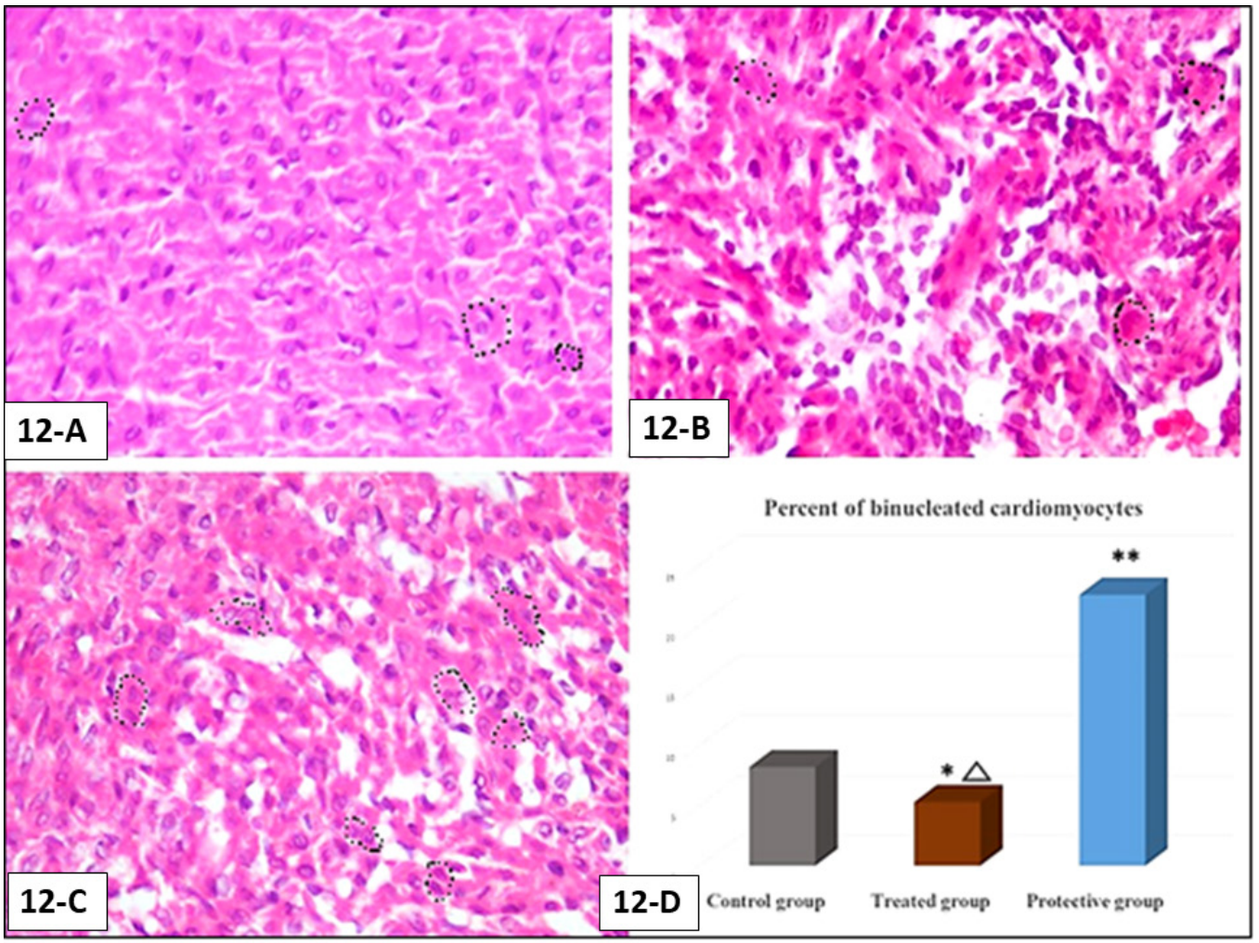

Fig. 12: Concomitant administration of vitamin D with Panadol extra induce cardiomyocytes proliferation. Representative micrographs of the transverse sections of the heart myocardium of (A) control group, (B) Extra panadol exposed group (II) showing decrease in percent of binucleated cardiomyocyte. (C) Concomitant administration of vitamin D high significantly increase in percent of binucleated cardiomyocyte compared to Panadol extra - exposed group & control group. (H&E), dashed area, representative example for cross-sectional area measurement, scale bar = 20 µm. (D) Bar chart showing quantitative measurements of in percent of binucleated cardiomyocyte among the experimental groups. Data are presented as mean ± standard error of mean, **P-value* < 0.05 in comparing to control group, ***P-value* < 0.001 in comparing to control group & Panadol extra exposed group, Δ *P-value* < 0.001 in comparing to vitamin D group (III).

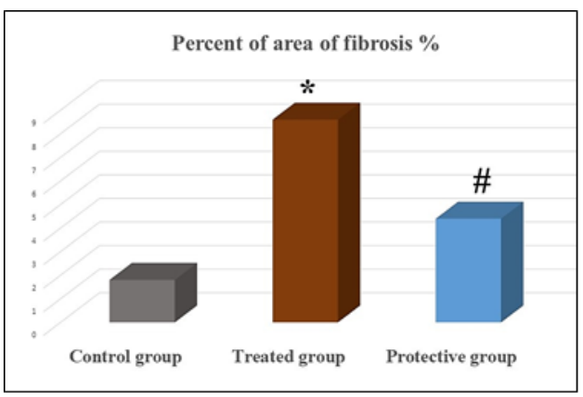

Fig. 13: Bar graph showing % fibrosis in the study groups. Each bar represents mean ± S.E.M. **P-value* < .001 versus control group; #*P-value* < .01 versus treated group (II).

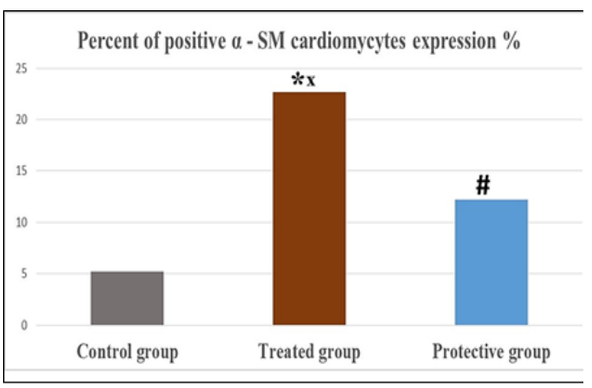

Fig. 14: Bar graph showing % of positive α - SM cardiomyocytes expression in the study groups. Each bar represents mean ± S.E.M. **P-value* < .001 versus control group; ˣ *P-value* < .01 versus protective group (III), # *P-value* < .05 versus group (II)

Table 1: Mean ± SD of body weight, heart weight and the heart weight to body weight ratio in study groups

	Control group (I)	Treated group (II)	Protective group (III)
Body weight	8.2±0.07	5.4±0.06*	7.8±0.03
Heart weight	0.04±0.002	0.088±0.003*	0.05±0.003
Heart \ body weight ratio %	0.48±0.05	1.05±0.2*	0.64±0.04**

**P value* < 0.05 in comparing to control group
***P value* < 0.05 in comparing to control & treated groups

Table 2: Mean ± SD of blood gas analysis, lipid profile and cardiac marker levels (enzyme creatinine kinase-MB (CK-MB) of study groups

	Control group (I)	Treated group (II)	Protective group (III)
PH	7.4±1.02	5.03±1.08[a]	7.03±0.72
PCO2 (mmHg)	35.1±3.02	85.3±6.2[b]	42.6±4.2[a]
PO2(mmHg)	98.5±6.5	65.5±8.3[b,c]	78.7±7.2[a,d]
Triglyceride level(mg\dl)	42.3±4.04	75.2±7.2[b]	53.3±5.3[a]
Total cholesterol level(mg\dl)	53.4±5.7	58.3±6.3	55.6±4.8
Creatinine kinase-MB (CK-MB) (IU\L)	40.3±4.02	84.3±6.32[b]	57.3±6.01[a,d]

a: *P value* < 0.05 in comparing to control group
b: *P value* < 0.01 in comparing to control group
c: *P value* < 0.05 in comparing to protective group
d: *P value* < 0.05 in comparing to treated group

DISCUSSION

In recent years, the safety of paracetamol and caffeine in pregnancy has come under increasing inquiry. Both paracetamol and caffeine and their metabolites cross the placenta[31,32] and experience different pharmacokinetic / pharmacodynamics processes in neonates than in adults[33]. As cardiovascular diseases are the most cause of mortality and morbidity at young age worldwide[34] we investigate the effects of maternal use of Panadol extra on the heart of their offspring and the possible protective of vitamin D.

In the present study, results of fetal heart characteristics revealed the treated group (II) by Panadol extra showed a significant decrease in body weight following an excessive overdose of acetaminophen. The saturation of the conjugation metabolism pathways of acetaminophen occurred in the liver, resulting in more amount of this drug being oxidized into the toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI), by the cytochrome P450 (CYP450) enzyme, CYP2E1. Following a high therapeutic dose, this highly reactive metabolite is detoxified by conjugation with glutathione which was immature in fetuses, resulting in a build-up of a lot of toxic metabolites that accumulated in most of the developing fetal tissues and fitting well the development and so low body weight[35]. The high lipid solubility and low molecular weight characteristics of caffeine metabolites can cross through the placenta easily resulting in high blood level of these toxic metabolites in both mother and fetus, but as the fetus has no enzymes to metabolize them, so; accumulation of caffeine metabolites in the fetal tissues influencing organs' development, and decreasing the body weight[36]. The resulting significant increase in the heart weight and heart \body weight ratio of this group (II) indicate fetal cardiac enlargement as an ordinary response to maternal or fetal hypertension induced by large & prolonged doses of both acetaminophen[37] and caffeine[38].

The rat's body weight was greatly improved to mimic the control group after concomitant maternal administration of vitamin D with Panadol extra group (III) due to proliferative actions of vitamin D on most of fetal tissues as skin, skeletal tissues, parathyroid gland, immune system, and pancreas as well as the small intestine and colon[39]. Moreover, vitamin D helps keep normal levels of glucose and regulate calcium homeostasis[40], so; improving the rat body weight. Our results were in accordant with a previous study[41]. Better heart weight and heart\body weight ratio in the same group (III) as receptors vitamin D are well expressed in vascular smooth muscle cells, endothelial cells, and cardiomyocytes exerting beneficial effects on them[42]. Vitamin D plays an important role in lowering hypertension by regulating the renin-angiotensin system and decreasing the coagulation according to previous studies[43,44].

Analysis of results of the blood gases of rats' offspring exposed to by Panadol extra group (II) revealed tissues hypoxia, hypoventilation, or acidosis as indicated by significant decreased PH, increased pCO2, and decreased pO2 blood levels. These changes are attributed to the effect of prolonged and high doses of maternal paracetamol and caffeine. The toxic metabolites of paracetamol in Panadol extra, N-acetyl-p-benzoquinone imine, prevents electron transmission to the mitochondrial respiratory chain and thus blocks aerobic respiration of developing fetal tissues[45]. Acetaminophen metabolites could cause oxidative damage to red blood cells through oxidative stress which increases the intracellular reactive oxygen species (ROS). So, the iron

component of hemoglobin is changed decreasing oxygen transport to body cells resulting in tissue hypoxia[46]. Excess metabolites of caffeine ingestion in Panadol extra enter the fetal circulation, but are metabolized slowly as the placenta or the fetus itself has cytochrome P450 which metabolize it, leading to additional toxic metabolites resulting in tissue hypoxia[47]. Blood gases levels were greatly improved after administration of vitamin D in the group (III) caused by the protective effect of Vitamin D3 through inhibition of ROS generation improving tissue oxygenation[48]. Previous studies reported that vitamin D3 supplementation recovers blood acidosis through regulation of renal bicarbonate reabsorption[49,50].

The lipid profile (total triglycerides and cholesterol) of this study revealed no changes in the level of total cholesterol in all study groups, but there was a significant increase in level of triglycerides in the group (II) that are exposed to Panadol extra. This can be attributed to the high level of acetaminophen which inhibits myeloperoxidase enzyme action decreasing the oxidation of both low- and high-density lipoproteins by HOCl resulting in high serum level[51]. The caffeine components of Panadol extra have thermogenic effects and can stimulate fat oxidation, either through sympathetic activation of the central nervous system or through its xanthine metabolites that make mitochondrial reactive oxygen species (ROS)[52] increasing fat oxidation. Serum triglycerides level showed a significant decrease in the protective group (III) through the regulatory action of vitamin D via increasing the activity of lipoprotein lipase in adipose tissues. Vitamin D3 has a lowering action on fatty acid absorption through the formation of insoluble calcium–fatty complexes in the intestine resulting in decreased absorption of fat, chiefly saturated fatty acids, so reducing the level of high-density lipoprotein[53]. Also, vitamin D could activate calcium reabsorption which in turn increases the transformation of cholesterol to bile acids due to its ability for bind with it[54]. Our results are in accordance with others who regard there was an inverse relation between vitamin D deficiency and lipid profile[55].

Cardiac marker level (enzyme creatinine kinase-MB (CK-MB) is a biomarker measured to evaluate the heart function or cardiac injury in the study groups. It was noticed a highly significant increase in the level of CK-MB in treated group (II) by Panadol extra than in the protective group by vitamin D which still increased in comparing control group (I). Armour and Slater reported similar results and described direct toxic myocarditis from autopsy from paracetamol toxicity case with evidence of myocardial injury being present in the endocardium[56]. Previous results explained increased cardiac enzyme marker in paracetamol overdoses by the presence of a metabolic enzyme (Cytochrome p450s) in the heart and suggested the possibility of metabolic activation of paracetamol metabolites; N-acetyl-p-benzoquinoneimine. One explanation indicates the direct toxic effect of paracetamol on the heart tissue suggests that myocardial injury occurs due to a similar mechanism in hepatic damage, as the toxic metabolite of paracetamol; N-acetyl-p-benzoquinoneimine, cannot be modulated in neonates due to deficient glutathione could act as a direct toxin on the myocardium. Acetaminophen could bind with different kinds of proteins in both the liver and cardiac tissue resulting in the change of the protein structure and function, potentially triggering cytokine release and tissue injury[57]. Another explanation; cardiac injury resulting in ischemia caused by acetaminophen that can deplete sulfhydryl groups, which interferes with nitric oxide production, leading to coronary ischemia. This sulfhydryl deficit interferes with endothelium-derived vascular relaxing factor leading to functional coronary ischemia[58] resulting in myocardial infarction, heart failure, and elevated enzyme marker. Excess caffeine intake in Panadol extra during pregnancy had been shown to increase intracellular calcium levels, renin activity, plasma catecholamines, adenylate cyclase and cyclic adenosine monophosphate levels inhibiting adenosine A1 and A2 receptors; different mechanisms can postulate atrial fibrillation (AF) that mediate heart dysfunction, and elevated its enzyme markers[59]. Prolonged caffeine metabolites potentiate stress-related neuronal changes as hyperactivity of intrinsic autonomic ganglia on the heart maintains an electrical activation which can initiate and maintain AF[60]. Persons possessing a slow metabolic profile especially during pregnancy have been shown to have a high prevalence of hypertension and myocardial infarction and so, elevated cardiac markers[61]. Metabolites of caffeine act on specific enzymes in the heart tissues. These heart enzymes are concerned the propagating the intensity of the heart's contractility, as they produce a stimulated action similar to that of adrenaline resulting in congestive heart failure[62]. It was found that the enzymes that metabolize caffeine metabolites act very slowly during pregnancy, allowing higher accumulations in blood and tissues so, a higher risk for adverse cardiovascular effects in both mother and fetus[63].

Zhao *et al*., reported that vitamin D3 was linked with improvements in cardiac function, so, increasing left ventricular ejection fraction and inhibits ventricular function restoration[64]. Several studies[65] have shown that vitamin D acts as an adverse regulator of the renin-angiotensin-aldosterone system and moderates myocardial extracellular matrix turnover, improving cardiac functions supporting the beneficial effects of vitamin D in improving cardiovascular diseases.

Regarding light microscopic examination of H&E sections of rat cardiac tissues of the group (II) which their mothers were treated with Panadol extra showed many histopathological changes as disorganization of cardiac cells with wide intercellular spaces in between, necrosis or totally degenerated cardiac muscle fibers. Congestion and dilatation of blood vessels & capillaries with interstitial hemorrhage and edema. Marked cellular infiltrations were common in many sections. Khabazian Zadeh *et al*.,

stated that the cardio toxic effect of paracetamol is due to a highly reactive metabolite, N-acetyl-p- benzoquinone imine could bind with cardiac cell macromolecules resulting in disturbance of protein structures and functions and releasing oxidative stress that decrease cellular ATP resulting in cardiac cell death, necrosis and apoptosis[58]. Same results obtained in the liver by Hinson *et al* who stated that a higher rate of oxidative stress caused by acetaminophen toxicity due to a higher synthesis of NAPQI leads to hepatic cell death, leukocytic infiltration, and necrosis. Paracetamol metabolites were found to have a sensitized reactions that leads to pericarditis[66,67]. Cr *et al*., and his worker suggested that the pathological characters of apoptosis (cell shrinkage, chromatin condensation, and margination, and apoptotic body formation) with massive necrosis were morphological criteria of toxic doses of acetaminophen. These reactive metabolites lead to congestive changes caused by the accumulation of red blood cells, microvascular injury, and endothelial cell damage[68].

Previous research to explain the toxicity of oxidative stress in acetaminophen overdose focused on iron mediated toxicity. The mediated-iron initiates cellular superoxide formation and its fragmentation to form higher hydrogen peroxide[69]. This means the reduction of peroxide by ferric ions forming a highly reactive hydroxyl free-radical which in turn oxidizes the lipids leading to lipid peroxidation as well as oxidation of proteins, and nucleic acids, agents cause tissue damage, necrosis and interstitial hemorrhage[70,71].

Cover *et al*., reported that acetaminophen toxicity occurred with activation of interstitial macrophages increasing both pro-inflammatory and anti-inflammatory cytokines. Cytokines have a significant role in inflammation, immunity, cell proliferation, and differentiation. Tumor necrotic factor (TNF-α) could increase oxidative stress through formation of reactive oxygen species and reactive nitrogen species, also, could activate other inflammatory cells[72]. Bessems and Vermeulen, and another reported that the increased oxidative stress, possibly associated with alterations in calcium metabolism, initiation of signal transduction responses, mitochondrial permeability transition, loss of mitochondrial membrane potential and loss of the ability of the mitochondria to synthesize ATP which in conjunction with inflammatory events causes necrosis[73,74].

Caffeine mediated a cyclic adenosine monophosphate activation resulting in elevated levels of cyclic adenosine monophosphate, and protein kinase A, which results in increasing calcium elimination from the cytoplasm, elevated cyclic guanosine monophosphate and stimulation of protein kinase G, via nitric oxide, all of them increase levels of reactive oxygen species (ROS) causing tissue damage, necrosis and mediate inflammatory infiltration[75]. High levels of metabolized caffeine toxic metabolites result in potent diffuse vasoconstriction of the coronary arterial system with decreased myocardial perfusion that would lead to cardiovascular complications, such as myocardial ischemia and myocardial necrosis[76].

Our histopathological result was changed greatly after concomitant protection by vitamin D administration group (III) that, the cardiac tissues showed a picture greatly resemble the control group. Cardiomyocytes showed flat & pale nuclei with pale cytoplasm signifies recovery by the action of vitamin D. It is found that vascular smooth muscle cells, endothelial cells, and cardiomyocytes, expressed vitamin D producing 1α-hydroxylase, which converts 25-hydroxy vitamin D to calcitriol, which inhibits vascular smooth muscle cell proliferation, regulate the renin-angiotensin system, decrease coagulation, and exhibit anti-inflammatory properties[77]. Our results were in following Han *et al*., who suggested that vitamin D promotes cardiomyocyte proliferation and different ion during tissue growth, development, homeostasis, and injury-induced regeneration in zebrafish[78]. Vitamin D increased embryonic cell proliferation and size as indicated by elevated the number of cells with mitotic figures as binucleated cells[79].

Cellular infiltration in this group was greatly decreased or totally absent, so necrosis and degeneration of cardiac cells decreased. This improvement is caused by vitamin D which can modify the innate and adaptive immune responses. Studies on the tumor cells revealed that vitamin D exerts important regulatory effects on the key molecular pathways involved in inflammation[80]. Several mechanisms explain how vitamin D affects the inflammatory microenvironment including modifying the interaction between immune and tumor cells controlling the levels of cytokines, inhibiting NF-κB signaling pathway, up-regulating MKP5, and interfering with the prostaglandins pathway and immune cells (macrophages, neutrophils, B cells, and T cells)[44,81]. As a result of anti-inflammatory properties, and vascular smooth muscle cell proliferative effect of vitamin D; the picture of congested blood capillaries, exudation and interstitial hemorrhage were decreased or absent in the group (III). Our results are similar to previous results of[82,83].

Regarding results of the examination of sections of heart tissues stained with Mallory trichrome stain in this study, the cardiac tissues from rats exposed to Panadol extra group (II) showed an apparent increase in the amounts of the collagen fibers between the cardiac muscle fibers or deposited in focal areas and excessively deposited around congested blood vessels. Similar findings have been also found from Sagor and Mohib[84]. Literature also discovered that once there is any necrosis or injury observed inside the organ, local fibroblast tissues are activated and secrete collagen as well as extracellular matrix[85,86]. Overdose of acetaminophen metabolite inhibits mitochondrial oxidative phosphorylation that further stops ATP synthesis resulted an increase of Ca++ level, decreases mitochondrial membrane permeability triggering oxidative stress that further induces immune cell infiltration, higher extracellular matrix production and cellular damage which finally activate fibroblast proliferation, and collagen deposition[87].

A decreasing amount of collagen fibers to be restricted around congested blood vessels or between cardiomyocytes in the group (III) denotes the antifibrotic action of vitamin D. Artaza and Norris and others reported that; vitamin D would increase the expression of specific antifibrotic agents that in turn would ameliorate the progression of chronic diseases. When vitamin D was added to the culture of mesenchymal stem cells; they found that its lower expression of TGFB1, and plasminogen activator inhibitor, two well-known profibrotic factors. Also, vitamin D could decrease expression of collagen I, III and other collagens isoforms; and increased expression of several antifibrotic factors such as TGFB1 antagonist, MMP8 a collagen breakdown inducer and follistatin, an inhibitor of the profibrotic factor myostatin resulting in decreasing profibrotic signaling pathway, and gene expression, so, decrease the collagen deposition[88]. Recent studies indicate that calcitriol induces anti-fibrotic hepatocyte growth factor expression, which in turn blocks the myofibroblast activation and matrix production in interstitial fibroblasts[89]. Furthermore, in *vivo* and in *vitro* studies demonstrate that active vitamin D effectively blocks tubular epithelial to mesenchymal transition (EMT), which plays a central role in the development of interstitial fibrosis[90]. The active vitamin D can prevent TGFβ1-mediated biochemical and functional pro-fibrotic changes in primary cardiac fibroblasts. A reverse relationship between vitamin D status and cardiac fibrosis in end stage heart failure support an inhibitory role for vitamin D in cardiac fibrosis[91].

Three types of α-muscle actin are consecutively expressed during in *vivo* cardiac development; α-Smooth muscle actin is the first and briefly expressed, followed by α-skeletal and finally α-cardiac actin[92]. α -SMA marks the onset of cardiomyocyte differentiation, and as development continues, it is sequentially replaced by α-SKA and α-CAA isoforms. α-muscle actin isoform is expressed initially from the beginning of development at day 8 (E.8), elevated at E.13, then greatly decreased or approximately absent at day 17[93]. We use α-smooth muscles actin immune-staining as a marker of cardiac differentiation and development in this study. The cardiac tissues from rats of the group (II) which were exposed to Panadol extra showed strong positive immunoreaction either in the endomysial & perimysium connective tissues or in the walls of dilated blood vessels small capillary. As noticed previously from results of H&E and Mallory stains of this group which showed that, the paracetamol toxicity leads to inflammations, necrosis & degeneration of cardiomyocytes with excess collagen release and extracellular matrix and other effects caused by paracetamol and caffeine toxic metabolites and their free radicals in heart tissues would lead to undifferentiating cardiomyocytes at this stage and impaired development & turnover to mature cardiomyocytes resulting in the strong positive expression of α-smooth muscles actin immune-staining and increase of its percent by morphometric measuring of this group. Our results are in accordance with previous results of Thiele *et al*. who found delayed development to most organs of the rats after prenatal acetaminophen exposure and elevated α-smooth muscles actin in skeletal and cardiac muscles[94] and with Ninh *et al*., who noticed a high expression of α-smooth muscles actin in heart tissues after prenatal alcohol exposure[95].

The cardiac tissues from rats of the protective group (III) by vitamin D showed a decrease in the amount of positivity of immunoreaction to be restrictive around large blood vessels, in the wall of small capillary and little in the endomysial & perimysial connective tissues. Also, the percent of positive expression of α - SM was greatly decreased by morphometric measuring of this study due to antifibrotic, proliferative, and anti-inflammatory actions of vitamin D resulting in differentiation and processing of development of cardiomyocytes. Vitamin D is associated with cardiovascular health through activating its receptor that targets genes related to cardiovascular disease. Vitamin D deficiency is also linked to fibrosis and immune T-cell activation. Besides, vitamin D prevents liver fibrosis by interfering with fibrosis markers TGF-β1, collagen I, and α-SMA[96,97]. Results of Hlaing *et al*., indicate that vitamin D supplementation could prevent and/or improve cardiovascular disorders that are linked with abnormal cardiac differentiation and there was a good relationship between vitamin D status and cardiac development[98].

Regarding the morphometric results of this study, we measure the degree of cardiac enlargement by identifying the size and width of cardiomyocytes in a cross-sectional area. Our data showed that the cardiomyocyte size and so the cardiomyocyte cross-sectional area was a highly significant increase in Panadol extra exposed rats (group II) compared to other groups but these measures were greatly improved after concomitant administration of vitamin D. This increase in cardiomyocyte size is due to the effect of both paracetamol and caffeine toxicity, both paracetamol and caffeine metabolites and their ROS free radicals that lead to vasoconstriction, hypoxia, increased blood pressure \ volume and overloaded heart resulting in its enlargement[99,100]. Stress and inflammation caused by excess paracetamol and caffeine metabolites are another mechanisms for cardiac enlargement[101].

When measuring the percent of binucleated cardiomyocyte as an indicator for cardiomyocyte proliferation in this study; we found that the group that was received extra panadol (group II), showed a significant decrease in the percent of the binucleated cardiomyocyte. It is well known that several factors such as hypoxia, glucocorticoids, hypertension, and hypertrophic stimuli increase the degree of cardiomyocyte multinucleation or regeneration[102]. As previously noted, all these factors resulted from toxic metabolites and ROS activity of both acetaminophen and caffeine overdose. Jin and Park revealed in their *vitro* study that acetaminophen was toxic to cardiomyocytes, and many critical genes responsible for cardiomyocytes proliferation were affected. Mainly caused by oxidative stress, DNA damage, and apoptosis. Suppressed genes included those associated with cell proliferation, myocardial contraction, and cell shape

control[57]. Also, caffeine-induced apoptosis through p53, Bax, and caspase-3 activation polymerase cleavage responsible for the proliferation of many cell types as brain and heart[103]. But when concomitant administration of vitamin D, it increases the proliferative capacity of the injured cardiomyocytes leading to a high significant increase in the percent of the binucleated cardiomyocyte. Yao *et al*., demonstrate in their study that the vitamin D receptor is the original endogenous self-defensive and cardioprotective receptor against cardiac damage, through lowering oxidative stress, and inhibiting apoptosis and autophagy dysfunction-mediated cell death[104]. The previous study demonstrates that the vitamin D receptors signaling system has direct on the heart tissues that can suppress the pro-hypertrophic calcineurin/NFAT/MCIP 1 pathway identifying its anti-hypertrophic activity demonstrating the useful effects of vitamin D in the cardiovascular system[105].

The area percentage of the interstitial collagen fibers deposition in this work was greatly increased in the group that exposed to extra Panadol alone but after concomitant administration of vitamin D, the percent of interstitial collagen fibers were significantly decreased. The interstitial collagen fibers deposition developed in the group of Panadol extra in response to a loss of cardiomyocytes as previously confirmed in our histopathological results. It is considered to be reparative fibrosis which stimulated by myocyte necrosis which was resulted from injury and cell death caused by acetaminophen and caffeine metabolites[106]. Same results of collagen deposition and fibrosis were noticed in liver tissues after long-term acetaminophen treatment in mice[107]. After vitamin D administration in the group (III); the percentage was significantly decreased due to proliferative, anti-inflammatory, and anti-fibrotic effect of vitamin D. The antifibrotic effects of various vitamin D analogs in *vitro* and its probability for the treatment of liver fibrosis[108].

It could be concluded that prolonged and excessive maternal use of Panadol extra greatly affects the heart development as noticed from disturbance of the biochemical marker, histopathological changes, and morphometric abnormalities which greatly improved after concomitant administration of vitamin D. According to the result of this study, it is essential to survey the effect of Panadol extra on the fetus during pregnancy and the recommendation for the necessary use of vitamin D.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Davis DC. The Discomforts of Pregnancy. J. Obstet. Gynecol. Neonatal Nurs. 1996; 25(1):73–81.
2. Babb M, Koren G, Einarson A. Treating pain during pregnancy. Can. Fam. Physician. 2010; 56(1):25–27.
3. Brune K, Renner B, Tiegs G. Acetaminophen/paracetamol: A history of errors, failures and false decisions. Eur. J. Pain. 2015;19(7):953–965.
4. Seegers AJ, Jager LP, Zandberg P, van Noordwijk J. The anti-inflammatory, analgesic and antipyretic activities of non-narcotic analgesic drug mixtures in rats. Arch. Int. Pharmacodyn. Ther. 1981;251(2):237–254.
5. Baratloo A, Rouhipour A, Forouzanfar MM, Safari S, Amiri M, Negida A. The Role of Caffeine in Pain Management: A Brief Literature Review. Anesthesiol. Pain Med. 2016; 6(3).
6. Uddin MS, Wali MW, Mamun AA, Asaduzzaman M, Amran MS, Rashid M. Assessment of Risk Involved in the Combination Medicine of Paracetamol and Caffeine. J. Adv. Med. Pharm. Sci. 2015;5(3):1–8.
7. Cameron MD, Wen B, Roberts AG, Atkins WM, Campbell AP, Nelson SD. Cooperative binding of acetaminophen and caffeine within the P450 3A4 active site. Chem. Res. Toxicol. 2007;20(10):1434–1441.
8. Cai Q, Dmitrieva NI, Michea LF, Rocha G, Ferguson D, Burg MB. Toxicity of acetaminophen, salicylic acid, and caffeine for first-passage rat renal inner medullary collecting duct cells. J. Pharmacol. Exp. Ther. 2003;306(1):35–42.
9. Evers S, Jensen R, European Federation of Neurological Societies. Treatment of medication overuse headache--guideline of the EFNS headache panel. Eur. J. Neurol. 2011;18(9):1115–1121.
10. Czeizel AE, Dudás I, Puhó E. Short-term paracetamol therapy during pregnancy and a lower rate of preterm birth. Paediatr. Perinat. Epidemiol. 2005;19(2):106–111.
11. Hahn KA, Wise LA, Rothman KJ, Mikkelsen EM, Brogly SB, Sørensen HT, Riis AH, Hatch EE. Caffeine and caffeinated beverage consumption and risk of spontaneous abortion. Hum. Reprod. Oxf. Engl. 2015;30(5):1246–1255.
12. Anatomy and Embryology of the Laboratory Rat. Alemanha: BioMed Verlag; 1986.
13. Marcela SG, Cristina RMM, Angel PGM, Manuel AM, Sofía D-C, Patricia DLR-S, Bladimir R-R, Concepción SG. Chronological and Morphological Study of Heart Development in the Rat. Anat. Rec. 2012; 295(8):1267–1290.
14. Al-Solami KM, Al-refaie Z, Awad H, Rasool M. Potential Protective Effect of Vitamin D on Cardiac Extrinsic pathways of apoptosis in Male Rats Fed with high fat diet. Genet. Mol. Res. 2019; 18(1).
15. Waris N, Kausar R, Waris N, Naz F, Raza A. Effect of Extra Panadol on Blood and Behavior of Rats. J. Bioanal. Biomed. 2017; 09(04).

16. Shamardl HA, El-Ashmony SM, Kamel HF, Fatani SH. Potential Cardiovascular and Renal Protective Effects of Vitamin D and Coenzyme Q10 in l-NAME-Induced Hypertensive Rats. Am. J. Med. Sci. 2017; 354(2):190–198.

17. Guide for the care and use of laboratory animals, (2011), 8th Edition. Institute for Laboratory Animal Research; Division on Earth and Life Studies. National research council of the national academies, The National Academies Press. Washington, D.C. (2011)pp: 382-387.

18. Kato H, Dokai M, Katagiri R, Arima A, Ooshima Y. Investigation for methods of anesthesia and euthanasia for rat fetuses in developmental toxicity studies. Congenit. Anom. 2013;53(1):46–48.

19. Nakayama M, Tad H, Asaumi Y, Shimokawa H. Identification and visualization of stimulus-specific transcriptional activity in cardiac hypertrophy in mice. Int J Cardiovasc Imaging. 2014; 30:211–219.

20. Kaczmarczyk G, Reinhardt HW. Arterial blood gas tensions and acid-base status of Wistar rats during thiopental and halothane anesthesia. Lab. Anim. Sci. 1975;25(2):184–190.

21. Aberare OL, Okuonghae P, Mukoro N, Dirisu JO, Osazuwa F, Odigie E, Omoregie R. Triglycerides, total cholesterol, high density lipoprotein cholesterol and low density lipoprotein cholesterol in rats exposed to premium motor spirit fumes. North Am. J. Med. Sci. 2011; 3(6):277–280.

22. Gerhardt W, Waldenström J. Creatine kinase B-subunit activity in serum after immunoinhibition of M-subunit activity. Clin. Chem. 1979;25(7):1274–1280.

23. Bancroft, J. D. (2008). Theory and practice of histological techniques. 8th Edition, Philadelphia, PA: Churchill Livingstone Elsevier Health Sciences. 2008 (8): 274–280.

24. Clement S, Stouffs M, Bettiol E, Kampf S, Krause K-H, Chaponnier C, Jaconi M. Expression and function of -smooth muscle actin during embryonic-stem-cell-derived cardiomyocyte differentiation. J. Cell Sci. 2007;120(2):229–238.

25. Dimiter P and Verstreken K. Automated Segmentation and Morphometry of Cell and Tissue Structures. Selected Algorithms in ImageJ. Computer Science. Molecular Imaging.2012,183-208.

26. Helms SA, Azhar G, Zuo C, Theus SA, Bartke A, Wei JY. Smaller cardiac cell size and reduced extra-cellular collagen might be beneficial for hearts of Ames dwarf mice. Int. J. Biol. Sci. 2010;6(5):475–490.

27. Zebrowski DC, Jensen CH, Becker R, Ferrazzi F, Baun C, Hvidsten S, Sheikh SP, Polizzotti BD, Andersen DC, Engel FB. Cardiac injury of the newborn mammalian heart accelerates cardiomyocyte terminal differentiation. Sci. Rep. 2017; 7(1):8362.

28. Schipke J, Brandenberger C, Rajces A, Manninger M, Alogna A, Post H, Mühlfeld C. Assessment of cardiac fibrosis: a morphometric method comparison for collagen quantification. J. Appl. Physiol. 2017; 122(4):1019–1030.

29. Kern S, Feng H-Z, Wei H, Cala S, Jin J-P. Up-regulation of alpha-smooth muscle actin in cardiomyocytes from non-hypertrophic and non-failing transgenic mouse hearts expressing N-terminal truncated cardiac troponin I. FEBS Open Bio. 2013;4:11–17.

30. Emsley R, Dunn G, White IR. Mediation and moderation of treatment effects in randomised controlled trials of complex interventions. Stat. Methods Med. Res. 2010;19(3):237–270.

31. Levy G, Garrettson LK, Soda DM. Evidence of Placental Transfer of Acetaminophen. Pediatrics. 1975; 55(6):895–895.

32. Dlugosz L, Belanger K, Hellenbrand K, Holford TR, Leaderer B, Bracken MB. Maternal caffeine consumption and spontaneous abortion: a prospective cohort study. Epidemiol. Camb. Mass. 1996;7(3):250–255.

33. Pacifici GM, Allegaert K. Clinical Pharmacology of Paracetamol in Neonates: A Review. Curr. Ther. Res. Clin. Exp. 2014; 77:24–30.

34. Virani Salim S., Alonso Alvaro, Benjamin Emelia J., Bittencourt Marcio S., *et al*. Heart Disease and Stroke Statistics, A Report From the American Heart Association. Circulation. 2020; 141(9):e139–e596.

35. Holt MP, Ju C. Mechanisms of drug-induced liver injury. AAPS J. 2006; 8(1):E48–E54.

36. Yadegari M, Khazaei M, Anvari M, Eskandari M. Prenatal Caffeine Exposure Impairs Pregnancy in Rats. Int. J. Fertil. Steril. 2016;9(4):558–562.

37. Rebordosa C, Kogevinas M, Bech BH, Sørensen HT, Olsen J. Use of acetaminophen during pregnancy and risk of adverse pregnancy outcomes. Int. J. Epidemiol. 2009;38(3):706–714.

38. Van der Hoeven T, Browne JL, Uiterwaal CSPM, van der Ent CK, Grobbee DE, Dalmeijer GW. Antenatal coffee and tea consumption and the effect on birth outcome and hypertensive pregnancy disorders. PLoS ONE. 2017;12(5).

39. Theodoropoulos C, Demers C, Delvin E, Ménard D, Gascon-Barré M. Calcitriol regulates the expression of the genes encoding the three key vitamin D3 hydroxylases and the drug-metabolizing enzyme CYP3A4 in the human fetal intestine. Clin. Endocrinol. (Oxf.). 2003; 58(4):489–499.

40. Olt S. Relationship between vitamin D and glycemic control in patients with type 2 diabetes mellitus. Int. J. Clin. Exp. Med. 2015; 8(10):19180–19183.

41. Wen J, Hong Q, Wang X, Zhu L, Wu T, Xu P, Fu Z, You L, Wang X, Ji C, Guo X. The effect of maternal vitamin D deficiency during pregnancy on body fat and adipogenesis in rat offspring. Sci. Rep. 2018;8(1):365.

42. Zittermann A, Koerfer R. Protective and toxic effects of vitamin D on vascular calcification: clinical implications. Mol. Aspects Med. 2008;29(6):423–432.

43. Li YC, Kong J, Wei M, Chen Z-F, Liu SQ, Cao L-P. 1,25-Dihydroxyvitamin D3 is a negative endocrine regulator of the renin-angiotensin system. J. Clin. Invest. 2002;110(2):229–238.

44. Wong MSK, Delansorne R, Man RYK, Vanhoutte PM. Vitamin D derivatives acutely reduce endothelium-dependent contractions in the aorta of the spontaneously hypertensive rat. Am. J. Physiol. Heart Circ. Physiol. 2008;295(1):H289-296.

45. Shah AD, Wood DM, Dargan PI. Understanding lactic acidosis in paracetamol (acetaminophen) poisoning. Br. J. Clin. Pharmacol. 2011;71(1):20–28.

46. Van Swelm RPL, Laarakkers CMM, Blous L, Peters JGP, Davidson ENB, van der Kraan PM, Swinkels DW, Masereeuw R, Russel FGM. Acute Acetaminophen Intoxication Leads to Hepatic Iron Loading by Decreased Hepcidin Synthesis. Toxicol. Sci. 2012; 129(1):225–233.

47. Willson C. The clinical toxicology of caffeine: A review and case study. Toxicol. Rep. 2018; 5:1140–1152.

48. Won S, Sayeed I, Peterson BL, Wali B, Kahn JS, Stein DG. Vitamin D Prevents Hypoxia/Reoxygenation-Induced Blood-Brain Barrier Disruption via Vitamin D Receptor-Mediated NF-kB Signaling Pathways. PLoS ONE. 2015;10(3).

49. Hulter HN. Effects and interrelationships of PTH, Ca2+, vitamin D, and Pi in acid-base homeostasis. Am. J. Physiol. 1985;248(6 Pt 2):F739-752.

50. Kumar R, Tebben PJ, Thompson JR. Vitamin D and the Kidney. Arch. Biochem. Biophys. 2012; 523(1):77–86.

51. Parthasarathy S, Raghavamenon A, Garelnabi MO, Santanam N. Oxidized Low-Density Lipoprotein. Methods Mol. Biol. Clifton Nj. 2010;610:403–417.

52. Shirali S, daneghian sevana, Hosseini SA, Ashtary-Larky D, Daneghian M, Mirlohi M-S. Effect of Caffeine Co-Ingested with Carnitine on Weight, Body-Fat Percent, Serum Leptin and Lipid Profile Changes in Male Teen Soccer Players: a Randomized Clinical Trial. Int. J. Pediatr. 2016; 4(10).

53. Christensen R, Lorenzen JK, Svith CR, Bartels EM, Melanson EL, Saris WH, Tremblay A, Astrup A. Effect of calcium from dairy and dietary supplements on faecal fat excretion: a meta-analysis of randomized controlled trials. Obes. Rev. Off. J. Int. Assoc. Study Obes. 2009;10(4):475–486.

54. Vaskonen T, Mervaala E, Sumuvuori V, Seppänen-Laakso T, Karppanen H. Effects of calcium and plant sterols on serum lipids in obese Zucker rats on a low-fat diet. Br. J. Nutr. 2002;87(3):239–245.

55. Saedisomeolia A, Taheri E, Djalali M, Moghadam AM, Qorbani M. Association between serum level of vitamin D and lipid profiles in type 2 diabetic patients in Iran. J. Diabetes Metab. Disord. 2014; 13:7.

56. Armour A, Slater SD. Paracetamol cardiotoxicity. Postgrad. Med. J. 1993; 69(807):52–54.

57. Jin S, Park K. Acetaminophen Induced Cytotoxicity and Altered Gene Expression in Cultured Cardiomyocytes of H9C2 Cells. Environ. Health Toxicol. 2012;27:e2012011.

58. KhabazianZadeh F, Kazemi T, Nakhaee S, Ng PC, Mehrpour O. Acetaminophen poisoning-induced heart injury: a case-based review. DARU J. Pharm. Sci. 2019; 27(2):839–851.

59. Rashid A, Hines M, Scherlag BJ, Yamanashi WS, Lovallo W. The effects of caffeine on the inducibility of atrial fibrillation. J. Electrocardiol. 2006; 39(4):421–425.

60. Frost L, Vestergaard P. Caffeine and risk of atrial fibrillation or flutter: the Danish Diet, Cancer, and Health Study. Am. J. Clin. Nutr. 2005;81(3):578–582.

61. Cornelis MC, El-Sohemy A, Kabagambe EK, Campos H. Coffee, CYP1A2 Genotype, and Risk of Myocardial Infarction. JAMA. 2006;295(10):1135–1141.

62. Geleijnse JM. Habitual coffee consumption and blood pressure: an epidemiological perspective. Vasc. Health Risk Manag. 2008;4(5):963–970.

63. Bakker R, Steegers EAP, Raat H, Hofman A, Jaddoe VWV. Maternal caffeine intake, blood pressure, and the risk of hypertensive complications during pregnancy. The Generation R Study. Am. J. Hypertens. 2011;24(4):421–428.

64. Zhao J-D, Jia J-J, Dong P-S, Zhao D, yang X-M, Li D-L, Zhang H-F. Effect of vitamin D on ventricular remodelling in heart failure: a meta-analysis of randomised controlled trials. BMJ Open. 2018;8(8).

65. Danik JS, Manson JE. Vitamin D and Cardiovascular Disease. Curr. Treat. Options Cardiovasc. Med. 2012; 14(4):414–424.

66. Hinson JA, Roberts DW, James LP. Mechanisms of Acetaminophen-Induced Liver Necrosis. Handb. Exp. Pharmacol. 2010;(196):369–405.

67. Ralapanawa U, Jayawickreme KP, Ekanayake EMM, Dissanayake AMSDM. A study on paracetamol cardiotoxicity. BMC Pharmacol. Toxicol. 2016;17.

68. Cr G, Jd L, Dm D, H C, Sk D, P Z, M B, Dr G, Mk G, Dl L. Exaggerated hepatotoxicity of acetaminophen in mice lacking tumor necrosis factor receptor-1. Potential role of inflammatory mediators. Toxicol. Appl. Pharmacol. 2003;192(2):119–130.

69. Corcoran GB, Todd EL, Racz WJ, Hughes H, Smith CV, Mitchell JR. Effects of N-acetylcysteine on the disposition and metabolism of acetaminophen in mice. J. Pharmacol. Exp. Ther. 1985;232(3):857–863.

70. Blazka ME, Elwell MR, Holladay SD, Wilson RE, Luster MI. Histopathology of acetaminophen-induced liver changes: role of interleukin 1 alpha and tumor necrosis factor alpha. Toxicol. Pathol. 1996;24(2):181–189.

71. Wakeel RA, Davies HT, Williams JD. Toxic myocarditis in paracetamol poisoning. Br. Med. J. Clin. Res. Ed. 1987;295(6606):1097.

72. Cover C, Liu J, Farhood A, Malle E, Waalkes MP, Bajt ML, Jaeschke H. Pathophysiological role of the acute inflammatory response during acetaminophen hepatotoxicity. Toxicol. Appl. Pharmacol. 2006;216(1):98–107.

73. Bessems JG, Vermeulen NP. Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. Crit. Rev. Toxicol. 2001;31(1):55–138.

74. Almansori M, Alhammadi H, Almulhim F. Paracetamol overdose: Analysis of a sample from a tertiary hospital in Eastern Saudi Arabia. Saudi J. Med. Med. Sci. 2015;3(3):209.

75. Higgins JP, Babu KM. Caffeine Reduces Myocardial Blood Flow during Exercise. Am. J. Med. 2013; 126(8):730.e1-730.e8.

76. Nyska A, Murphy E, Foley JF, Collins BJ, Petranka J, Howden R, Hanlon P, Dunnick JK. Acute hemorrhagic myocardial necrosis and sudden death of rats exposed to a combination of ephedrine and caffeine. Toxicol. Sci. Off. J. Soc. Toxicol. 2005;83(2):388–396.

77. Campbell MJ, Trump DL. Vitamin D Receptor Signaling and Cancer. Endocrinol. Metab. Clin. North Am. 2017; 46(4):1009–1038.

78. Han Y, Chen A, Umansky K-B, Oonk KA, Choi W-Y, Dickson AL, Ou J, Cigliola V, Yifa O, Cao J, Tornini VA, Cox BD, Tzahor E, Poss KD. Vitamin D Stimulates Cardiomyocyte Proliferation and Controls Organ Size and Regeneration in Zebrafish. Dev. Cell. 2019;48(6):853-863.e5.

79. Cortes M, Chen MJ, Stachura DL, Liu SY, Kwan W, Wright F, Vo LT, Theodore LN, Esain V, Frost IM, Schlaeger TM, Goessling W, Daley GQ, North TE. Developmental Vitamin D Availability Impacts Hematopoietic Stem Cell Production. Cell Rep. 2016; 17(2):458–468.

80. Liu W, Zhang L, Xu H-J, Li Y, Hu C-M, Yang J-Y, Sun M-Y. The Anti-Inflammatory Effects of Vitamin D in Tumorigenesis. Int. J. Mol. Sci. 2018; 19(9).

81. Wöbke TK, Sorg BL, Steinhilber D. Vitamin D in inflammatory diseases. Front. Physiol. 2014; 5.

82. Danik JS, Manson JE. Vitamin D and Cardiovascular Disease. Curr. Treat. Options Cardiovasc. Med. 2012; 14(4):414–424.

83. Zittermann A. Vitamin D and disease prevention with special reference to cardiovascular disease. Prog. Biophys. Mol. Biol. 2006; 92(1):39–48.

84. Taher Sagor MA, Mohib MM. Fresh Seed Supplementation of Syzygium Cumini Attenuated Oxidative Stress, Inflammation, Fibrosis, Iron Overload, Hepatic Dysfunction and Renal Injury in Acetaminophen Induced Rats. J. Drug Metab. Toxicol. 2016; 7(2).

85. Salem M, Helal O, Adly N. Histological study of the effect of paracetamol on the seminiferous tubules of adult rabbits. Egypt. J. Histol. 2011;34:790–799.

86. Barnes JL, Gorin Y. Myofibroblast Differentiation During Fibrosis: Role of NAD(P)H Oxidases. Kidney Int. 2011; 79(9):944–956.

87. Schunke KJ, Coyle L, Merrill GF, Denhardt DT. Acetaminophen Attenuates Doxorubicin-Induced Cardiac Fibrosis Via Osteopontin and GATA4 Regulation: Reduction of Oxidant Levels. J. Cell. Physiol. 2013; 228(10):2006–2014.

88. Artaza JN, Norris KC. Vitamin D reduces the expression of collagen and key profibrotic factors by inducing an antifibrotic phenotype in mesenchymal multipotent cells. J. Endocrinol. 2009; 200(2):207–221.

89. HJ L, H L, C G, Y J, H M, M U, D N, M R, N S. Gene expression profiling changes induced by a novel Gemini Vitamin D derivative during the progression of breast cancer. Biochem. Pharmacol. 2006; 72(3):332–343.

90. Li Y, Spataro BC, Yang J, Dai C, Liu Y. 1, 25-dihydroxyvitamin D3 inhibits renal interstitial myofibroblast activation by inducing hepatocyte growth factor expression. Kidney Int. 2005; 68(4):1500–1510.

91. Meredith A, Boroomand S, Carthy J, Luo Z, McManus B. 1,25 Dihydroxyvitamin D3 Inhibits TGFβ1-Mediated Primary Human Cardiac Myofibroblast Activation. PLoS ONE. 2015; 10(6).

92. Clément S, Stouffs M, Bettiol E, Kampf S, Krause K-H, Chaponnier C, Jaconi M. Expression and function of α-smooth muscle actin during embryonic-stem-cell-derived cardiomyocyte differentiation. J. Cell Sci. 2007; 120(2):229–238.

93. Carrier L, Boheler K R, Chassagne C, de la Bastie D, Wisnewsky C, Lakatta E G, Schwartz K. Expression of the sarcomeric actin isogenes in the rat heart with development and senescence. Circ. Res. 1992;70(5):999–1005.

94. Thiele K, Solano ME, Huber S, Flavell RA, Kessler T, Barikbin R, Jung R, Karimi K, Tiegs G, Arck PC. Prenatal Acetaminophen Affects Maternal Immune and Endocrine Adaptation to Pregnancy, Induces Placental Damage, and Impairs Fetal Development in Mice. Am. J. Pathol. 2015; 185(10):2805–2818.

95. Ninh VK, El Hajj EC, Ronis MJ, Gardner JD. N-Acetylcysteine prevents the decreases in cardiac collagen I/III ratio and systolic function in neonatal mice with prenatal alcohol exposure. Toxicol. Lett. 2019; 315:87–95.

96. Lai C-C, Juang W-C, Sun G-C, Tseng Y-K, Jhong R-C, Tseng C-J, Wong T-Y, Cheng P-W. Vitamin D Attenuates Loss of Endothelial Biomarker Expression in Cardio-Endothelial Cells. Int. J. Mol. Sci. 2020; 21(6).

97. Meems LMG, Cannon MV, Mahmud H, Voors AA, van Gilst WH, Silljé HHW, Ruifrok WPT, de Boer RA. The vitamin D receptor activator paricalcitol prevents fibrosis and diastolic dysfunction in a murine model of pressure overload. J. Steroid Biochem. Mol. Biol. 2012;132(3–5):282–289.

98. Hlaing SM, Garcia LA, Contreras JR, Norris KC, Ferrini MG, Artaza JN. 1,25-Vitamin D3 promotes cardiac differentiation through modulation of the WNT signaling pathway. J. Mol. Endocrinol. 2014;53(3):303–317.

99. Chung W-S, Lin C-L. Increased risk of congestive heart failure in patients with acetaminophen poisoning: A nationwide cohort study. J. Appl. Toxicol. JAT. 2018;38(5):766–772.

100. Turnbull D, Rodricks JV, Mariano GF, Chowdhury F. Caffeine and cardiovascular health. Regul. Toxicol. Pharmacol. 2017;89:165–185.

101. Tsutsui H, Kinugawa S, Matsushima S. Oxidative stress and heart failure. Am. J. Physiol.-Heart Circ. Physiol. 2011; 301(6):H2181–H2190.

102. Paradis AN, Gay MS, Zhang L. Binucleation of cardiomyocytes: the transition from a proliferative to a terminally differentiated state. Drug Discov. Today. 2014; 19(5):602–609.

103. He Z, Ma W-Y, Hashimoto T, Bode AM, Yang CS, Dong Z. Induction of apoptosis by caffeine is mediated by the p53, Bax, and caspase 3 pathways. Cancer Res. 2003;63(15):4396–4401.

104. Yao T, Ying X, Zhao Y, Yuan A, He Q, Tong H, Ding S, Liu J, Peng X, Gao E, Pu J, He B. Vitamin D Receptor Activation Protects Against Myocardial Reperfusion Injury Through Inhibition of Apoptosis and Modulation of Autophagy. Antioxid. Redox Signal. 2015; 22(8):633–650.

105. S C, Cs L, Cl G, K O, Tt H, Y Z, Y Y, Dg G. Cardiomyocyte-specific deletion of the vitamin D receptor gene results in cardiac hypertrophy. Circulation. 2011; 124(17):1838–1847.

106. Cowling RT, Kupsky D, Kahn AM, Daniels LB, Greenberg BH. Mechanisms of cardiac collagen deposition in experimental models and human disease. Transl. Res. J. Lab. Clin. Med. 2019; 209:138–155.

107. Bai Q, Yan H, Sheng Y, Jin Y, Shi L, Ji L, Wang Z. Long-term acetaminophen treatment induced liver fibrosis in mice and the involvement of Egr-1. Toxicology. 2017;382:47–58.

108. Reiter FP, Ye L, Bösch F, Wimmer R, Artmann R, Ziesch A, Kanitz V, Mayr D, Steib CJ, Trauner M, Regel I, Gerbes AL, Mayerle J, Hohenester S, de Toni EN, Denk G. Antifibrotic effects of hypocalcemic vitamin D analogs in murine and human hepatic stellate cells and in the CCl 4 mouse model. Lab. Invest. 2019;99(12):1906–1917.

الملخص العربى

تأثير تعرض الأم لبانادول اكسترا على القلب الجنيني لنسل الجرذ البيضاء والتأثير الوقائي المحتمل لفيتامين د

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المقدمة: قد يؤدى الاستخدام المفرط للباراسيتامول مع الكافيين في البانادول اكسترا أثناء الحمل الى خطر الإجهاض التلقائي أو تغيرات مورفولوجية فى مختلف أعضاء الجنين خاصة منها القلب لانة اول عضو يتكون فى المراحل الاولى. وقد وجد ان يحفز فيتامين د لة وظائف متعددة غير تاثيرة على العظام مثل تكاثر خلايا عضلة القلب كما انة يحمي من الإصابة بالامراض المتعددة.

الهدف من العمل: التحقيق في تأثير تعرض الأمهات لجرعات كبيرة من بانادول إكسترا على القلوب الجنينية لنسل الفئران البيضاء والتأثيرات الوقائية المحتملة لفيتامين د

المواد والطرق: تضمن البحث خمسة وأربعون جرذًا ؛ تم استخدام ٣٠ أنثى بالغة و ١٥ ذكرًا وتمت عملية التزواج. تم تقسيم الجرذان الحوامل إلى المجموعة الأولى كمجموعة ضابطة ولم تتعرض الى اى نوع من العلاج ، اما المجموعة الثانية (بانادول إكسترا فقط) فقد تلقت الجرعة اليومية القصوى (٤٠٠٠ مجم باراسيتامول / ٥٢٠ مجم\ كافيين \ يوميا. بالنسبة للمجموعة الثالثة فقد تلقت الجرعة المذكورة سابقًا من بانادول إكسترا مع فيتامين د بجرعة ٤٠٠ وحدة دولية / كجم / يوم. تم إعطاء جميع العلاجات بالتطعيم الفموي من اليوم الأول للحمل حتى اليوم ١٩. تم التضحية بالفئران الحامل من جميع المجموعات في اليوم التاسع عشر من الحمل وتم استخلاص صغارها ووزنها ثم التضحية بها. تم أخذ عينات الدم للتقييم البيوكيميائي. وايضا تم اخذ عينات من الخلايا النسيجى لعضلة القلب وصبغها باستخدام صبغة الايوسين, والمالورى والصبغة الهيستوكيمائية لمعادل الفا فى خلايا عضلة القلب وذلك للفحص الهيستولوجى. كما تم عمل احصائية مورفومترية.

النتائج: أظهرت المجموعة الثانية تغيرات في غازات الدم مثل تغيرات في غازات الدم مثل التغير معدل لاقلوية الدم وانخفاض نسبة الاوكسجين وارتفاع نسبة ثانى اكسيد الكربون وارتفاع نسبة الهون عالية الاكسدة فى الدم. ايضا كانت هناك تغيرات نسيجية مرضية مختلفة مثل تلف خلايا عضلة القلب مع وجود خلايا التهاب مع زيادة التليف بواسطة صبغة مالوري.و أظهرت النتائج المورفومترية تضخم خلايا القلب وانخفاض تكاثرها ونضجها. ولكن وجد تحسن فى كل هذه النتائج بشكل كبير في المجموعة (الثالثة) عند تناول فيتامين د في مجموعة الحماية الأخيرة.

الخلاصة: إن استخدام الأم للبانادول اكسترا بشكل مفرط ولفترات طويلة يؤثر بشكل كبير على نمو القلب. ووجد ان فيتامين (د) له تأثير محتمل على تجديد القلب وتطوره.