Study of dihydroxyacetone effect on acute aluminum phosphide cardiotoxicity in albino rats.

Fayrouz Ibrahem Nour Elden[1], Ola Sweilum[1], Reda AA Abo-Elsoud[2], Ahmed MM Gaafar[2], Nermeen Mohamed Nooreldien [3], Amany Tawfik Elfakhrany[4], Situhom El Sayed El Agamy[1], Amira Mohamed Elseidy[1*]

KEYWORDS
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ABSTRACT

Aluminum phosphide (ALP) toxicity is a prevalent public health problem that induces severe damage in heart tissue. Dihydroxyacetone (DHA) is a simple saccharide naturally produced in the body it is used as antidote against toxins caused mitochondrial death due to its ability to improves mitochondrial function and ATP depletion. The current study investigated the possible cardio protective role of DHA in ALP- intoxicated male albino rats. Rats were divided into four groups: control, DHA, ALP, and combined (ALP+DHA) groups. Arterial blood pressure (ABP), heart rate (HR), electrocardiogram (ECG) and survival time were recorded. Biochemical analysis of blood, histological study and immunohistochemistry assessment of heart tissues were done. ALP- intoxicated rats showed significant decrease in ABP, HR, survival time, catalase with ECG changes, and significant increase in serum levels of cardiac biomarkers, {troponin I and creatine kinase-MB (CK-MB)}, malondialdehyde (MDA), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) compared to the control group. DHA co administration with ALP resulted in significant improvement in ABP, HR, and ECG parameters; significant decrease of cardiac biomarkers, MDA, TNF-α; and significant increase of survival time and catalase compared with the ALP- intoxicated rats. Immunohistochemistry showed upregulation of BAX, HIF-1 α and iNOS in ALP intoxicated rats. DHA coadministration with ALP group showed downregulation of BAX, HIF-1 α and iNOS. DHA has a potential protective role against cardiac damage caused by acute ALP toxicity, therefore, its administration in cases of ALP poisoning together with other supportive therapies can improve their outcome.

Introduction

Aluminum phosphide (ALP), also referred to as a rice pill, is a highly toxic pesticide. It is typically made up of dark grey or dark yellow crystals with an aroma resembling rotting fish or garlic. It is sold as compressed discs, powder sachets, solid pellets, or tablets (Agrawal et al., 2010). ALP is frequently used to protect agricultural products from pests and insects due to its ability to maintain quality and quantity of agricultural products (Bameri et al., 2021). Farmers still prefer to use it because it is economical, highly effective in controlling insects, simple to use, does not impair seed viability, and leaves a minimum residue on stored food (Aminjan et al., 2019).

Immediately after ingestion, and due to contact with gastric hydrochloric acid, ALP tablet releases a very toxic colorless and odorless gas called phosphine (PH 3), with a

(1) *Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine - Menoufia University*
(2) *Medical Physiology Department, Faculty of Medicine - Menoufia University*
(3) *Anatomy and Embryology Department, Faculty of Medicine Menoufia University, Badr University, Cairo.*
(4) *Pharmacology Department, Faculty of Medicine - Menoufia University.*
**** Corresponding author:** Amira Mohamed Elseidy Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine- Menoufia University. E-mail address: amiraelseidy@gmail.com Tel: 01004256955*

pungent garlic aroma when it is exposed to air (Anbalagan et al., 2021). ALP has also been identified as a potential chemical terrorism agent due to the release of this gas after exposure to air moisture (Bogle et al., 2006).

Phosphine is a general toxin that affects numerous organs, with a negative impact on the cardiovascular system through direct myocardial tissue injury. Additionally, it might result in hepatotoxicity, disseminated intravascular coagulation, and renal failure (Abdel Wahab et al., 2020).

Aluminum phosphide is one of the major causes of poisoning in the developing countries such as Egypt due to unregulated and unrestricted accessibility. Its widespread use caused a dramatic increase in incidence of unintentional poisonings and suicides with high mortality rate (Abdel Wahab et al., 2020). In Egypt, ALP poisoning is quite prevalent and highly lethal with 100% mortality rates in some poison control centers. Factors that affect its prognosis include: the dosage, whether the tablet is new or expired, and the interval between exposure and start of supportive treatment (Hashemi-Domeneh et al., 2016). There is currently no known specific antidote for ALP toxicity. The primary goals of management are to control developed toxic symptoms and avoid the anticipated consequences (Darwish et al., 2020).

Dihydroxyacetone (DHA), also known as glycerone, is a simple saccharide which is naturally produced in the body in the form of phosphate by glycolysis pathway and is a very safe and effective antidote against the toxicity induced by lethal mitochondrial toxins as cyanide due to its ability to improve mitochondrial function and ATP depletion (Heidari et al., 2023). The current research was designed to study the possible protective role of DHA on cardiac toxicity induced by acute ALP-poisoning in male albino rats.

Materials and Methods:

The current experiment was carried out in the Physiology Department, Faculty of Medicine, Menoufia University. The protocol of research was accepted by the Ethical Committee at Menoufia University (IRB; 3/2023 FORE 4).

Animals

Twenty-four adult male albino rats weighed 180-220 grams were used in this experiment**.** Rats were housed in plastic cages with free access to water and food 14 days before starting the experiment for acclimatization. Rats were fasted for food 24 hours prior to the experiment. The substances of experiment were given orally by gavage.

Rats were divided into four groups (6 rats /group):

Group 1: (Control) was given a single dose (1 ml) of corn oil and 1 mL 5 % dextrose.

Group 2: Dihydroxyacetone (DHA) group, (Liverpool, United Kingdom) was given a single therapeutic dosing of DHA (50 mg/kg in 1 mL 5 % dextrose **(**Ahmadi et al., 2018).

Group 3: Aluminum phosphide (ALP) group was given the LD_{50} of ALP as a single dose (10 mg/kg) dissolved in corn oil (Kakavandi et al., 2022).

Group 4: Combined (ALP + DHA) group was given ALP (10 mg/kg) followed by DHA (50mg/kg) after 30 minutes (Ahmadi et al., 2018).

Experimental design:

Rats of all groups were subjected to recording of arterial blood pressure (ABP), heart rate (HR), and electrocardiography (ECG), then rats in groups 3 and 4 were kept

alive and the survival time was measured, while rats in groups 1 and 2 were anesthetized and killed by cervical dislocation. Immediately after death, blood samples were taken from abdominal aorta and heart for biochemical analysis. Finally, the heart of each rat was dissected out for histological examination.

Methods:

A. Recording of ABP, HR, and ECG:

Arterial blood pressure, HR, and ECG were recorded using a non-invasive method, the Biopac apparatus (Biopac Lab System, MP36R Unit and Acknowledge 5 Software, California, USA). The pump was attached to the rat tail that inflated the cuff to occlude the artery automatically. When the pump reached the inflation point, the pump deflated the cuff slowly, providing a linear drop in the pressure. The inflation and deflation cycles were controlled by a single push button, then systolic, diastolic, mean arterial blood pressure (MABP) and HR were recorded by using the apparatus software (Joshi et al., 2012). Rats were subjected to ECG recording. Disk electrodes were applied to the palmer surface of front limbs and left hind limb, while the grounded electrode was fixed on the right hind limb. For measurement of PR interval, QRS duration, and ST segment, Lead II was recorded and analyzed (Salem et al., 2023).

B. Biochemical analysis:

Blood samples were collected from all rats into serum separator tubes and were allowed to coagulate, then centrifuged for 5 min at 2,500 relative centrifugal forces (RCF). The serum was removed for biochemical analysis. According to the manufacturer's instructions, cardiac biomarkers (troponin I and CK-MB) and inflammatory cytokines (TNF-α and IL-6), were measured using ELISA kits from Glory Science Co., Ltd, USA. Oxidative stress markers malondialdehyde (MDA) and catalase were measured by colorimetric kits from Abcam Company.

C. Histological study

The heart from each rat was fixed in 10% formalin and processed. Paraffin sections in a thickness of 5-6 micron were obtained and were stained by hematoxylin and eosin (H&E)

D. Immunohistochemical study (IHC)

Heart Sections were deparaffinized, hydrated, washed in 0.1M phosphate buffer saline (PBS).

IHC staining was performed using polyclonal rabbit antibodies for Bcl-2 associated X protein (BAX) as an indicator for apoptosis (Cat# IW-1000), and anti-inducible nitric oxide synthase (iNOS) as an indicator for oxidative stress (Cat. #RB-9242-R7), while monoclonal mouse antibodies were used for hypoxia inducible factor (HIF -1α) (Cat. #MS-1164-P1ABX).

Sections were incubated for 1 hour at room temperature with diluted primary antibodies for BAX (1:200) iNOS (1:100), and HIF -1α (1:200). The slides were stained by (H&E) according to the company instructions. Breast carcinoma was used as a positive control for (BAX), Lung tissue is considered positive control for iNOS while human squamous cell carcinoma served as positive controls for (HIF -1α) (Kiernan, 2015).

E. Morphometric study

For immunohistochemical assessment of the percent of Bax, iNOS and HIF -1α positive cells, Image J version 1.47v software

(National Institutes of Health, USA) was used. Sections from at least six animals/experimental group were examined. Five non-overlapping fields (400x) per section were randomly captured by a Leica Microscope DML B2/11888111 equipped with a Leica camera DFC450**.**

Statistical analysis

Results were expressed as mean ± standard deviation (SD). Analysis of Variances (ANOVA) and T test were used for statistical analysis of the different groups, using Origin® software and the probability of chance (p values). P values < 0.05 were considered significant. P values < 0.001 were considered highly significant

Results

A. Arterial blood pressure, heart rate and ECG results:

Arterial blood pressure, heart rate and ECG parameters in all studied groups are shown in table (1) and figures (1 and 2). There was a highly significant decrease in values of systolic, diastolic, MABP and HR in ALP group compared to control and DHA groups ($P < 0.001$). Combined group (ALP+DHA) showed a highly significant increase in systolic, diastolic, MABP and HR compared to ALP group ($P < 0.001$). A highly significant prolongation in PR interval, QRS duration, and ST elevation interval ($P < 0.001$) was shown in ALP group compared to control and DHA groups. The combined group (ALP+DHA) showed a significant decrease in PR, and QRS duration and ST elevation compared to ALP group ($P < 0.05$). There was insignificant difference in arterial blood pressure, heart rate and ECG in DHA group compared to control group ($P > 0.05$).

Table (1): Statistical analysis of arterial blood pressure, heart rate and ECG in all studied group (n: 24).

Parameters	Control	DHA	ALP	(ALP+ DHA)
Systolic (mmHg)	107 ± 6	103.2± 2.7	66.5^{ab}± 3	86^{abc}± 4.2
Diastolic(mmHg)	73.5± 8	77.8± 4.7	40.2^{ab}± 5.7	56.8^{abc}± 7.7
MABP (mmHg)	84.6± 5.6	86.3± 3.6	49^{ab}± 4.3	66.4^{abc}± 6.1
Heart rate (beat/minute)	338.5± 5.2	327.2±8.4	106.7^{ab}± 7.2	196.3^{abc}± 9.5
PR interval (millisecond)	35.17± 2.3	33.5 ± 1.9	68.5^{ab}± 1.9	57.8^{abc}± 2.9
QRS (second)	0.04± 0.02	0.042 ±0.02	0.09^{ab}± 0.05	0.072^{abc}± 0.04
ST (μv)	23.7 ±3.3	26.6 ± 3.2	104.7^{ab}±3.9	88.8^{abc} ± 4.4

Data are expressed as mean ±SD (n=6 in each group). n: number. The marks [a], [b] and [c] indicate that values are significantly different on comparing the control, to DHA, ALP, and ALP+ DHA groups respectively. DHA: dihydroxyacetone, ALP: Aluminum phosphide, MABP: Mean arterial blood pressure.

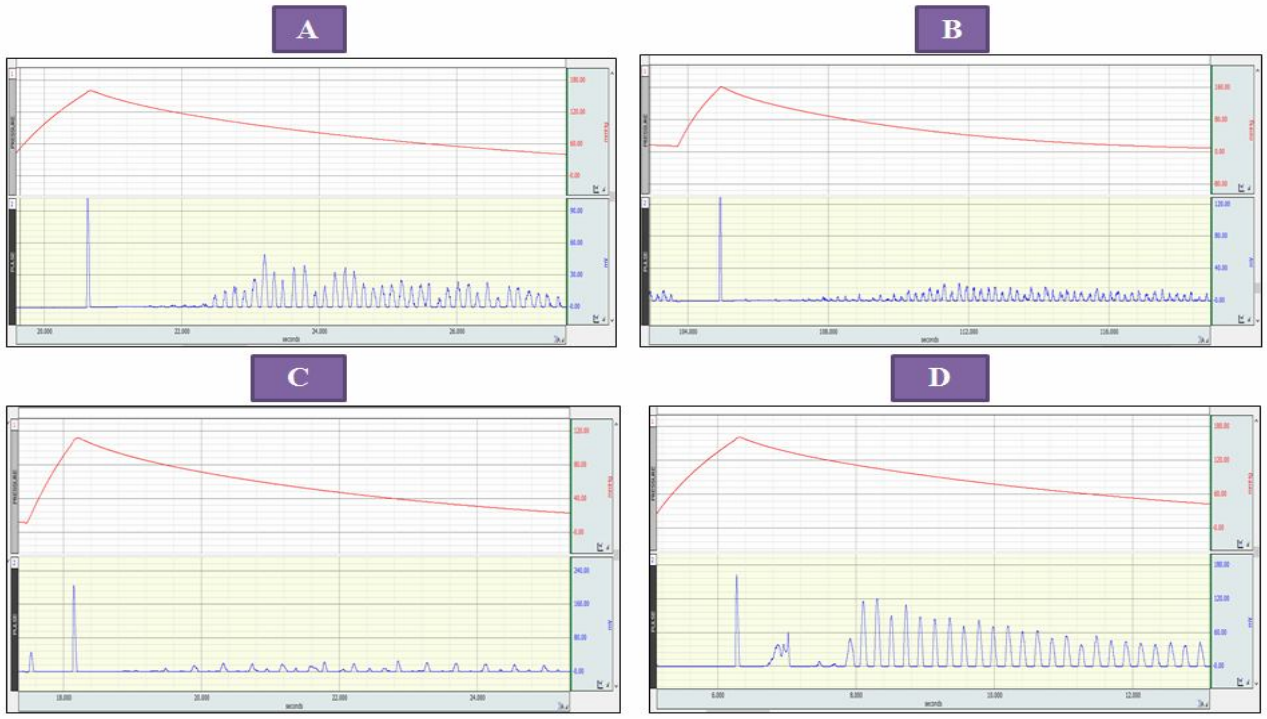


Fig. (1): Recorded arterial blood pressure and heart rate via biopac Acknowledge System in; (A) control, (B) DHA group, (C) ALP group, and (D) combined (ALP+DHA).

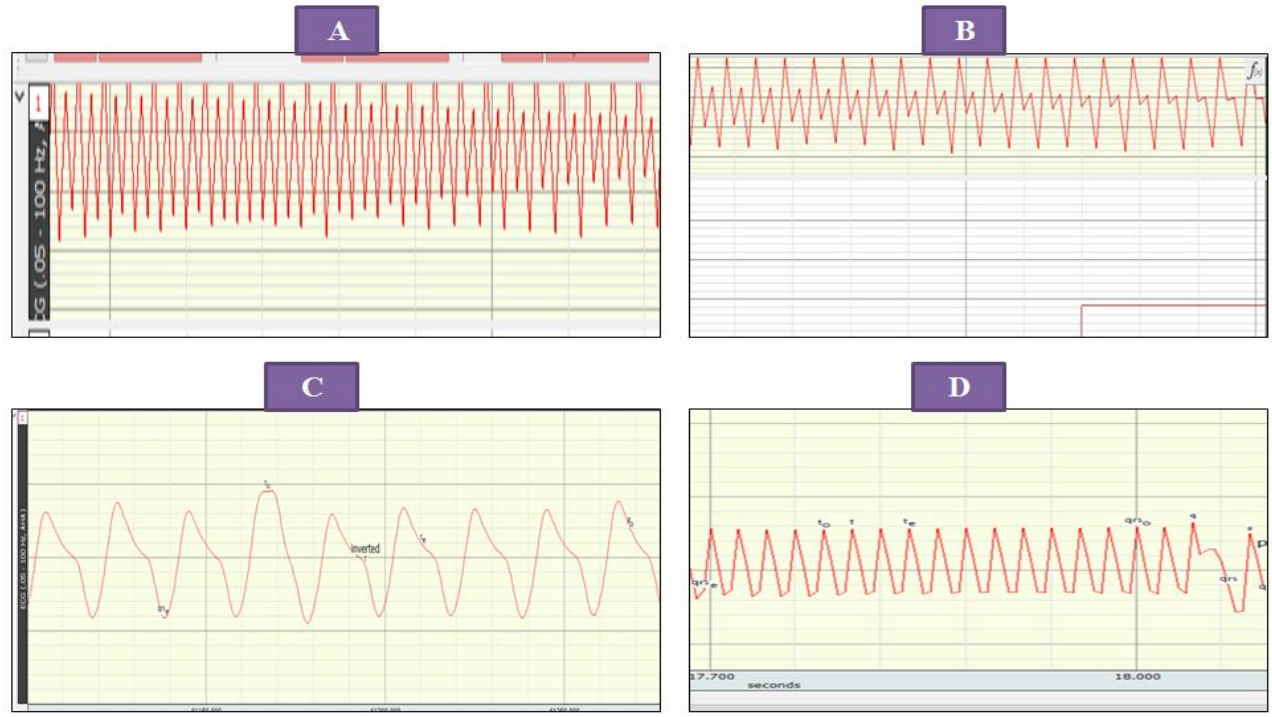


Fig. (2): Recorded ECG via biopac Acknowledge System in; (A) control, (B) DHA group, (C) ALP group, and (D) combined (ALP+DHA).

B. Biochemical results

Table 2 showed serum levels of cardiac biomarkers, oxidative stress, and inflammatory markers in all studied groups. There was a highly significant increase in cardiac troponin I and CK-MB levels in ALP group compared to control and DHA groups ($P < 0.001$).

Combined group (ALP+DHA) showed a highly significant decrease in troponin I and CK-MB levels compared to the ALP group ($P < 0.001$). No significant difference was observed between the control and DHA group ($P > 0.05$). There were highly significant increase of oxidative stress and inflammatory markers, MDA, TNF-α and IL-6 levels in ALP group compared to control and Dihydroxyacetone groups ($P < 0.001$). Combined group (ALP+DHA) showed a highly significant decrease in MDA, TNF-α and IL-6 levels compared to the ALP group (($P < 0.001$). No significant difference in MDA, TNF-α and IL-6 levels was observed between control and Dihydroxyacetone group ($P > 0.05$).

C. Survival time

Gradual decrease in activity; eventually, very reduced activity, occasional standing, and finally death was reported. These symptoms delayed and the mean value of survival time in the ALP intoxicated animals after ingestion of DHA was significantly higher than the rats intoxicated with ALP ($P < 0.001$) as shown in table (2).

Table (2): Statistical analysis of serum levels of cardiac biomarkers, survival time, oxidative stress, and inflammatory markers in all studied groups (n:24).

Parameters	Control	DHA	ALP	ALP + DHA
Troponin 1 (pg/ml)	4.9± 0.6	4.8 ± 0.5	47.17 ^{ab}± 9.3	21.1 ^{abc} ± 1.97
CPK-MB (U/L)	145 ± 17.6	146.17 ± 17.9	374.17 ^{ab} ± 49.7	207.17 ^{abc} ± 16
Survival time (minutes)			44.2 ± 3.6	78.3 ^{c} ±10.3
MDA (nmol/ml)	4.5 ± 0.46	4.25 ± 0.53	76.7^{ab} ± 11	24.2 ^{abc} ± 7.2
Catalase (U/ml)	155.6 ± 4.3	152.2 ± 9.3	35.33^{ab}± 5.1	55.5^{abc}± 5.4
IL6 (pg/ml)	35.8 ± 2.6	33.5 ± 2.07	110.7 ^{ab}± 5.4	64 ^{abc} ± 2.8
TNF-α (pg/ml)	13.37 ± 1.5	12.8 ± 1.2	85.7 ^{ab}± 3.5	58 ^{abc} ± 2.4

Data are expressed as mean ±SD (n=6 in each group). n: number, the marks ^{a}, ^{b} and ^{c} indicate that values are significantly different on comparing control to DHA, ALP, and ALP+DHA groups respectively. DHA: dihydroxyacetone, ALP: Aluminum phosphide, CK-MB: creatine kinase-MB, MDA: malondialdehyde, IL-6: interleukin-6, TNF-α: tumor necrosis factor-alpha.

D. Histopathological results

Figure (3) shows the heart section of control group, with longitudinal arrangement of cardiac muscle fibers. Acidophilic sarcoplasm, central oval vesicular nuclei, and dark, elongated fibroblast nuclei in the endomysium were observed in cardiac myocyte. DHA group showed normal cardiac striation, cardiac myocytes with acidophilic sarcoplasm and central oval vesicular nuclei. Few small blood capillaries are seen in intercellular. The ALP group showed disruption of the myocardial fibers and small dark stained nuclei with perinuclear spaces. Many cytoplasmic vacuoles appear between the muscle fibers with massive hemorrhage. There were infiltrations of inflammatory cells that cause thickening of blood vessel walls, some fibers showed focal areas of less acidophilic sarcoplasm. Combined group (ALP+DHA) showed restoration of the muscle fibers with acidophilic cytoplasm and central oval vesicular nuclei and minimal hemorrhage between the muscle fibers.

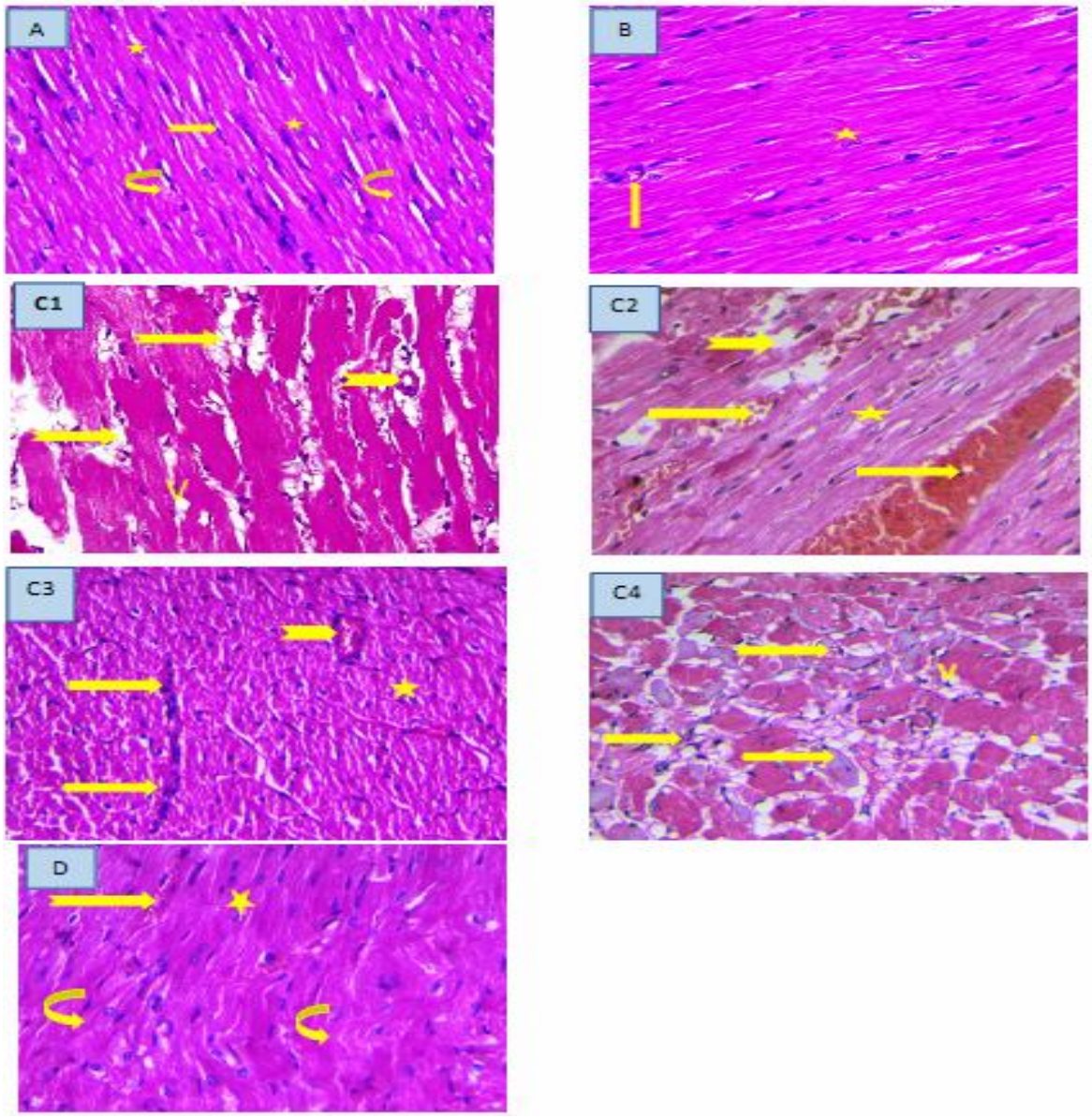


Fig. (3): photomicrograph of cardiac section (H&E X400): (A) control group, (B) DHA group, (C1, 2, 3, 4) ALP group, and (D) combined (ALP+DHA). Control and DHA groups showed longitudinal arrangement of cardiac muscle fibers. Cardiac myocytes appear with acidophilic sarcoplasm (curved arrows) and central oval vesicular nuclei (*). Dark elongated nuclei of fibroblast in the endomysium (notched arrow) normal blood vessel (arrow). ALP group (C1) showed disruption of cardiac muscle fibers (notched arrows) multiple vacuolations between the fibers (V), thickened wall blood vessel (chevron) (C2) showed massive hemorrhage (arrows) and perinuclear space (stars). (C3) showed small dark stained nuclei with perinuclear spaces (stars). Inflammatory cells (arrow), thickened wall blood vessel (chevron) (C4) showed multiple focal areas of less acidophilic sarcoplasm (arrows), multiple Vacuolations between the muscle fibers (□) and inflammatory cell infiltration (notched arrows) are seen. (D) Group showed restoration of the muscle fibers with acidophilic cytoplasm (curved arrows) and central oval vesicular nuclei (stars). Minimal hemorrhage between the muscle fibers (notched arrows).

E. Immunohistochemical results:

Figure (4) shows BAX -immunostained cardiac sections. The control and DHA groups showed negative reactions in heart muscle cells, whereas the ALP group showed a strong positive immunoreaction. The combined ALP+DHA group exhibited modest BAX expression in the cells.

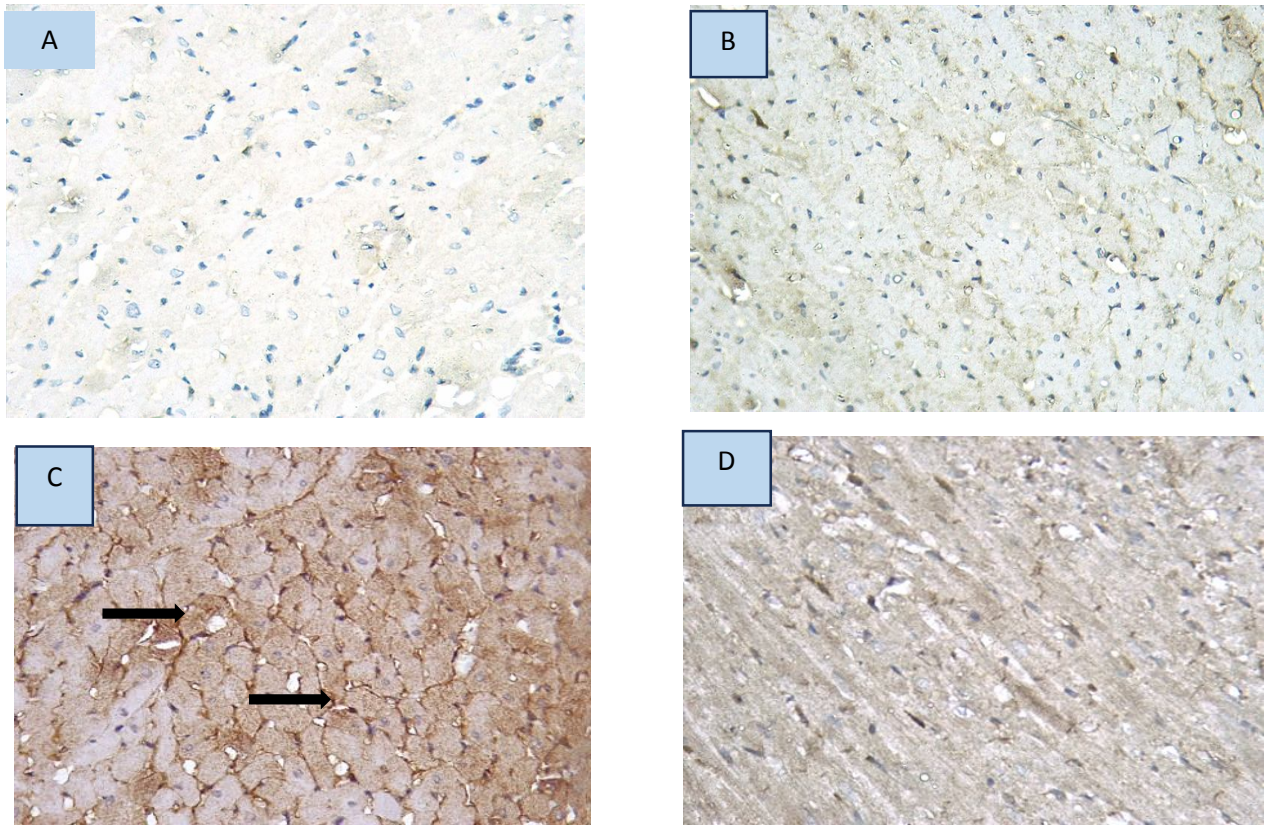


Fig. (4): Representative photographs of BAX immunostaining of the heart: (A) control group, (B) DHA group, (C) ALP group, and (D) combined (ALP+DHA). Control and DHA groups showed negative expressions. ALP group showed marked expression of BAX (arrow), compared to control group while combined (ALP+DHA) showed mild expression of BAX, compared to ALP group ((Immunohistochemical stain of BAX x 400)

Figure (5) shows iNOS -immunostained cardiac sections. The control and DHA groups exhibited negative reaction in heart muscle cells, whilst the ALP group revealed an intense positive immunoreaction. The combined ALP+DHA group demonstrated slight iNOS expression in the cells.

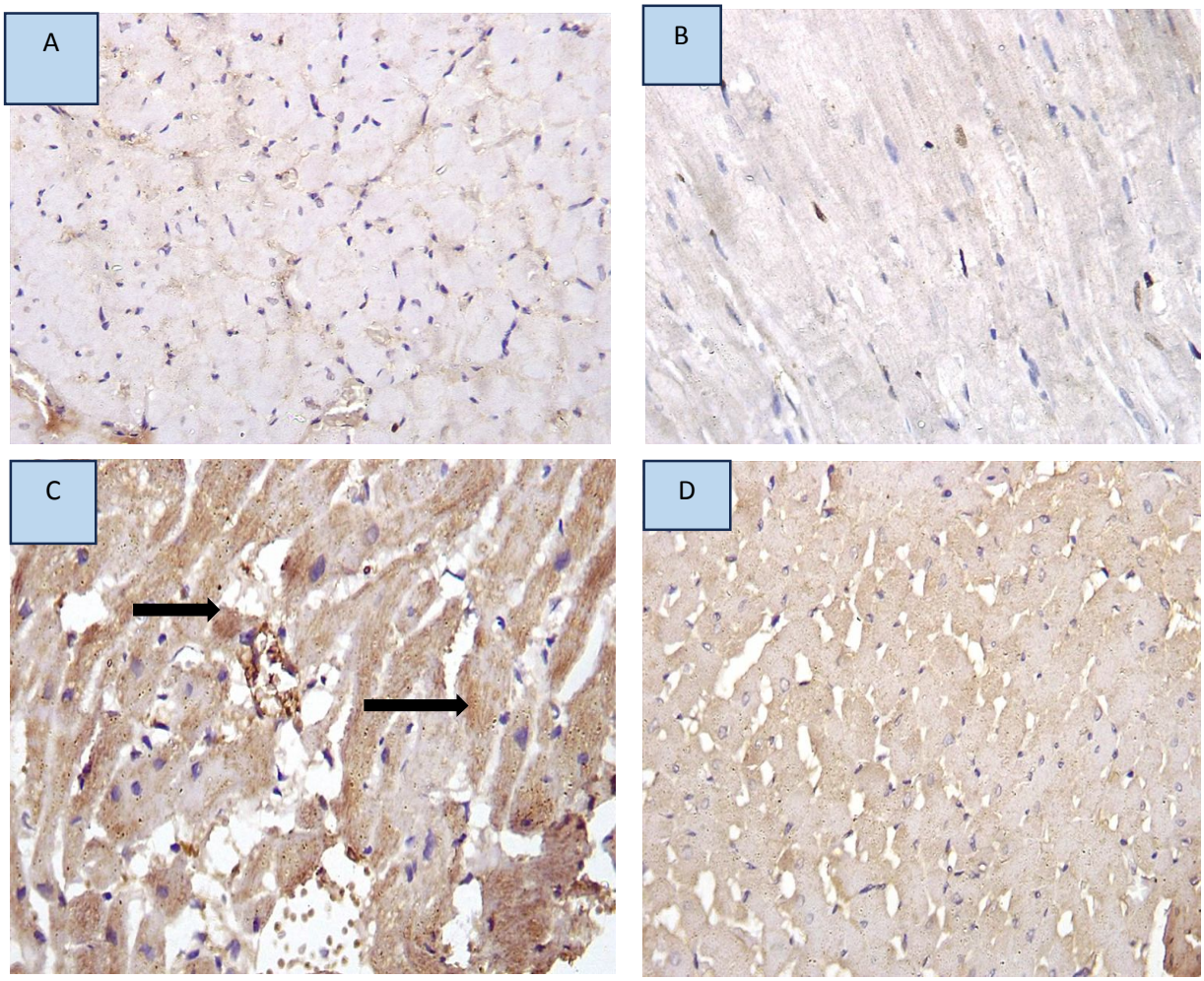


Fig. (5): Representative photographs of iNOS immunostaining of the heart. (A) control group, (B) DHA group, (C) ALP group, and (D) combined (ALP+DHA) group. Control and DHA groups showed negative expression. ALP group showed marked expression of iNOS, compared to control group (arrow) while combined (ALP+DHA) showed mild expression of iNOS compared to ALP group (Immunohistochemical stain of iNOs x 400)

Figure (6) shows HIF-1α - immunostained cardiac sections. The control and DHA groups had negative reactions in the cardiac muscle cells, whereas the ALP group had a strong positive reaction in both the cytoplasm and nucleus. The combined ALP+DHA group demonstrated mild expression of HIF-1α in both the cytoplasm and nucleus of cardiac muscle cells.

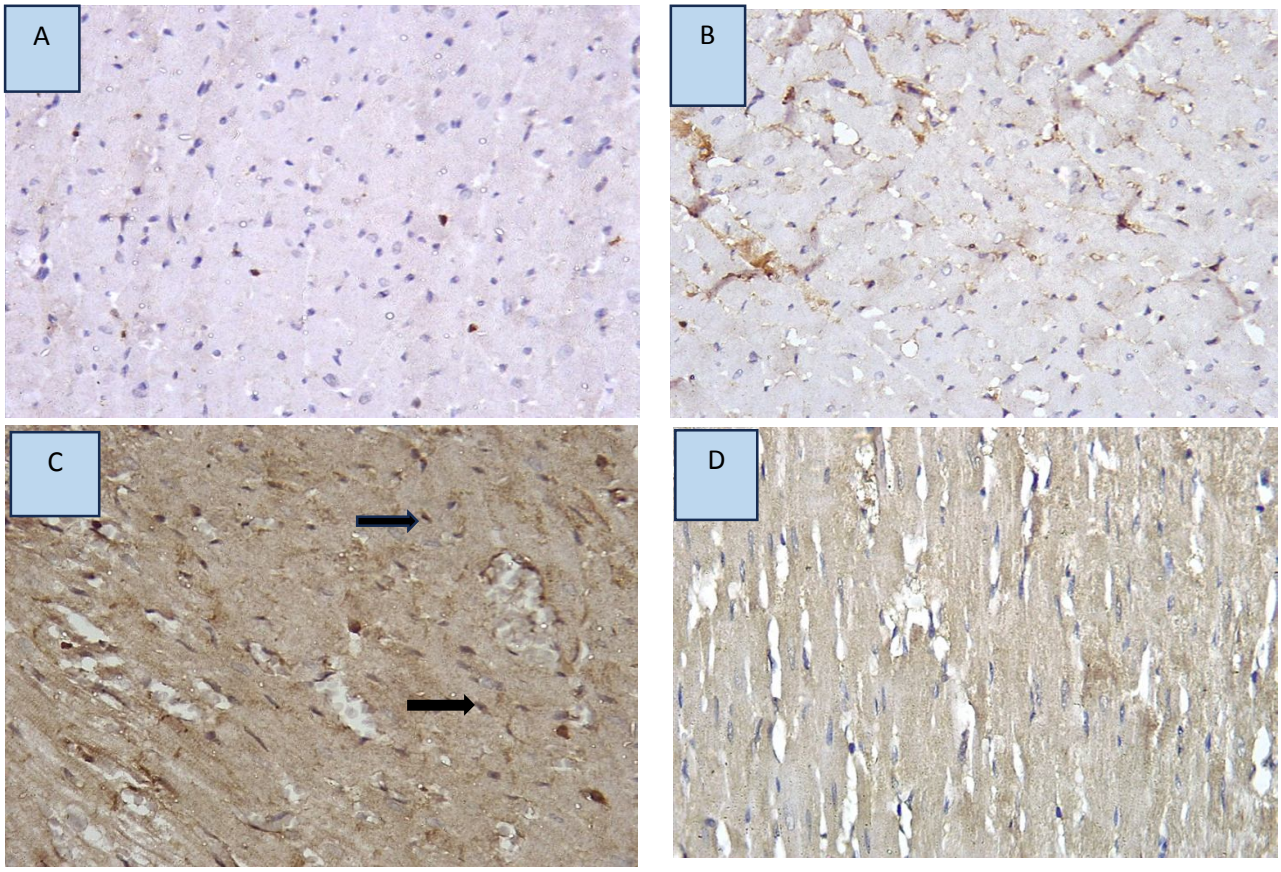


Fig. (6): Representative photographs of HIF-1 α immunostaining of the heart. (A) control group, (B) DHA group, (C) ALP group, and (D) combined (ALP+DHA) group. Control and DHA groups showed negative expressions. ALP group showed marked expression of HIF-1 α in both the nucleus and cytoplasm of cardiomyocytes (arrow) compared to control group while combined (ALP+DHA) showed mild expression of HIF-1 α compared to ALP group (Immunohistochemical stain of HIF-1 α x 400)

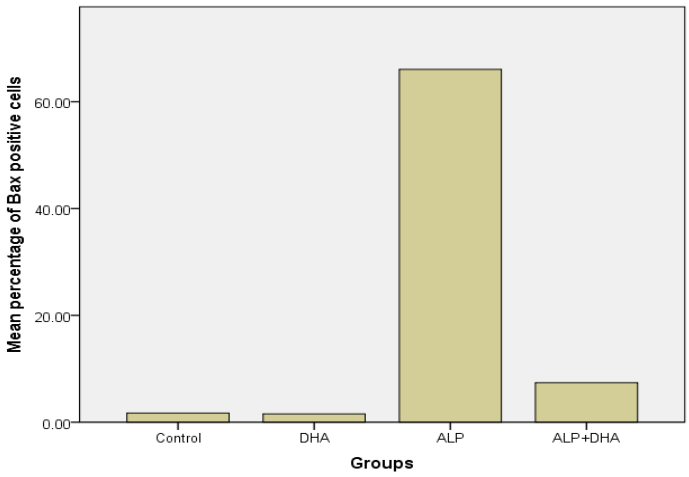
F. Morphometric and statistical results

Data in table (3) and histogram (1) show that ALP group exhibited a highly significant increase (P* .001) in the mean percentage % of BAX (apoptotic marker) positive cells in the cardiac sections in comparison with control group. The combined (ALP+DHA) group showed a significant decrease in the mean percentage of BAX in comparison with ALP group (P* .0.05).

Table (3): Mean number of BAX immune-positive cells in the different studied groups (n: 24).

Groups	% of Bax positive cells $\bar{x}$ ±SD	P value
Control	1.61±0.84	
DHA	1.45±1.01	P1>0.05
ALP	66.05±5.31	P2 <0.001
ALP+DHA	7.39±2.31	P3 <0.05

Percentage (%) of Bax positive cells, $\bar{x}$ = mean, **SD**= standard deviation n: number. **P value**> 0.05 non-significant, < 0.05 significant. P1: comparison between DHA & Control, P2: comparison between ALP& Control, P3: comparison between ALP + DHA & ALP, DHA: dihydroxyacetone, ALP: Aluminum phosphide



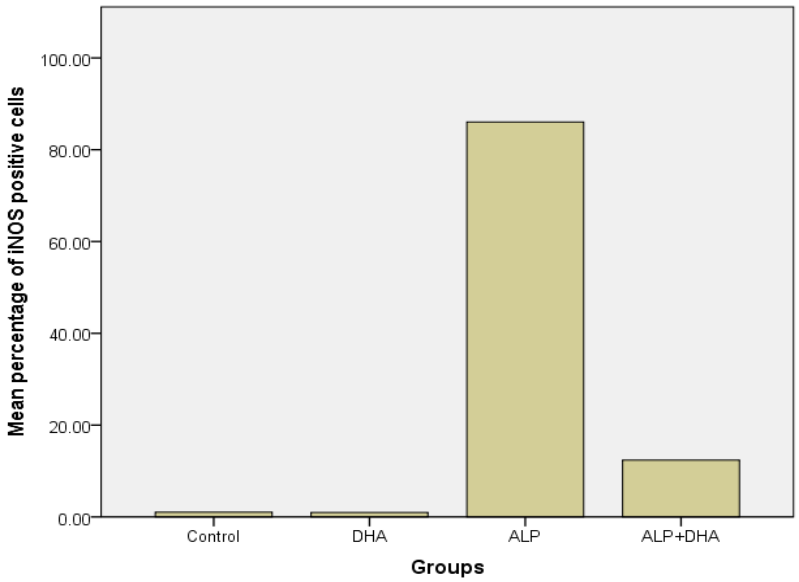
Histogram (1): Mean percentage (%) of Bax positive cells in different groups.

Data in table (4) and histogram (2) shows that ALP group exhibited a highly significant increase (P*.001) in the mean % of iNOS (oxidative stress marker) immunoreactions in the cardiac sections in comparison with control group. Combined (ALP+DHA) group showed a highly significant decrease in comparison with ALP group (P* .001).

Table (4): Mean number of iNOS immune-positive cells in the different studied groups (n: 24).

Groups	% of iNOS positive cells $\bar{x}$ ±SD	P value
Control	1.04±0.07	
DHA	.96±0.01	P1>0.05
ALP	86.02±5.01	P2 <0.001
ALP+DHA	12.39±2.21	P3 <0.001

Percentage (%) of iNOS positive cells, $\bar{x}$ = mean, **SD**= standard deviation. n: number, **P value**> 0.05 non-significant, < 0.05 significant, P1: comparison between DHA& Control, P2: comparison between ALP& Control, P3: comparison between ALP + DHA& ALP, DHA: dihydroxyacetone, ALP: Aluminum phosphide.



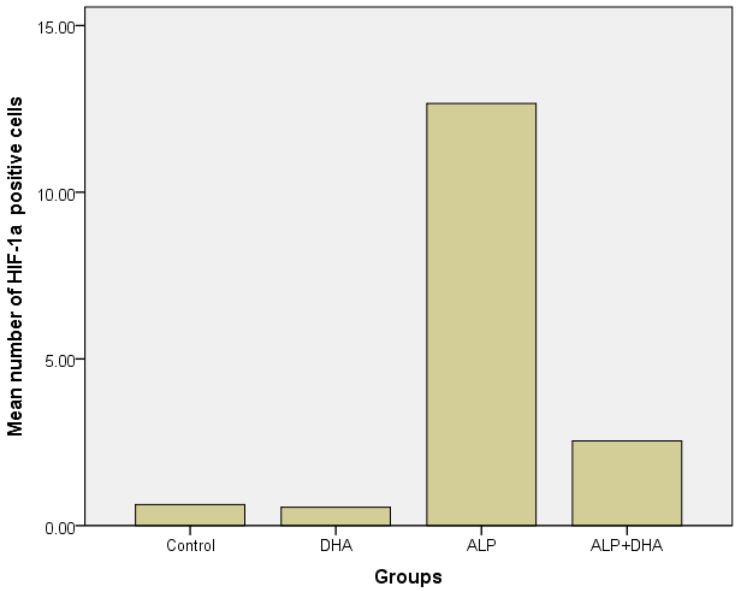
Histogram (2): Mean percentage of iNOS positive cells in different groups.

Data in table (5) and histogram (3) showed a highly significant increase in HIF-1α immunoreaction, (a marker for hypoxia), in ALP group compared with control group (P < 0.001). Combined (ALP+DHA group) showed a significant decrease in HIF-1α immunoreaction compared to ALP group (P < 0.05).

Table (5): Mean number of HIF-1 α immune-positive cells in the different studied groups (n: 24).

Groups	Number of HIF-1α positive cells $\bar{x}$ ±SD	P value
Control	0.63±0.28	
DHA	0.55±0.09	P1>0.05
ALP	12.66±2.37	P2 <0.001
ALP+DHA	2.54±1.13	P3 <0.05

Percentage of HIF-1 α positive cells, $\bar{x}$ = mean, **SD**= standard deviation. n: number. **P value**> 0.05 non-significant, < 0.05 significant, P1: comparison between DHA& Control, P2: comparison between ALP& Control, P3: comparison between ALP + DHA& ALP, DHA: dihydroxyacetone, ALP: Aluminum phosphide.



Histogram (3): Mean percentage (%) of HIF-1 α positive cells in different groups**.**

Discussion:

Aluminum phosphide toxicity is a prevalent public health problem that induces severe cardiac damage via oxidative stress and apoptosis. These changes can be confirmed by alternation of physiological functions (BP, HR, and ECG) and histological study of the cardiac tissue. The aim of this study was evaluation the possible role of DHA to ameliorate cardiotoxic effects (physiological, biochemical, and histological) produced in rats with acute ALP toxicity.

In the present study, rats intoxicated with ALP showed physiological changes in the form of decrease in the HR (bradycardia) and BP (hypotension), treatment of ALP-intoxicated rats with Dihydroxyacetone showed significant improvement in blood pressure and heart rate parameters. The present results agree with studies by Asghari et al. (2017), and Bameri et al. (2021). The mechanisms of decreasing blood pressure and heart rate are due to the ability of phosphine to reduce oxygen intake, blood flow, activity of cholinesterase enzyme, and direct cardiac myocytes toxicity resulting in hypotension. Phosphine gas could induce cardiogenic shock by metabolic acidosis and lactic acid accumulation through suppression of oxidative phosphorylation or tissue perfusion and its depressing influence on the contractility of the heart (Hosseini et al., 2020). In contrast to our results Ahmadi et al. (2018) reported tachycardia and hypotension in rats intoxicated with ALP and stated that tachycardia could be the cause of cardiac ischemia and left ventricular dysfunction in ALP toxicity. The cause of tachycardia may relate to the high dose of Aluminum phosphide (15 mg/kg) given to the animals.

In the present study, rats with ALP toxicity showed ECG abnormalities, in the form of prolongation of PR interval and QRS duration with ST segment elevation with inverted T wave. This is in accordance with Mohan et al. (2016), who reported that ALP induced myocardial infarction by oxidative stress and its direct toxic effect on myocardium causing change in membrane action potential which gives the ECG of myocardial ischemia. The mechanism by

which DHA can improve ABP, HR and ECG abnormalities may be due to its ability to improve tissue oxygenation and lactic acidosis via activation of cytochrome C cell which restores cell respiration (Ahmadi et al., 2018).

The current results showed that ALP - poisoning increased cardiac biomarkers, oxidative stress and inflammatory markers indicating myocardial degeneration. Elevation of serum cardiac biomarkers (troponin I and CPK-MB) in ALP group compared to control group in the present study agrees with Kalawat et al. (2016) who stated that troponin I and CPK-MB increased after ALP poisoning as ALP causes severe myocardial damage that could produce cardiovascular collapse and death. Levels of these biomarkers significantly decrease by DHA co-treatment indicating improvement of myocardial damage. It is suggested to be due to its ability to improve tissue oxygenation and lactic acidosis via activation of cytochrome c cell which restore cell respiration.

Aluminum phosphide increased oxidative stress and inflammatory markers, these results agreed with Farahani et al. (2016) who concluded that phosphine (PH_3) gas liberated after ingestion of ALP inhibits activity of cytochrome c oxidase and suppresses production of ATP. Aluminum phosphide could enhance production of reactive oxygen species (ROS) and inhibit antioxidants such as catalase and superoxide dismutase (SOD). Also, Anand et al. (2011) reported that mitochondrial dysfunction and electron flow chain interruption are the most probably causes of oxidative stress in ALP toxicity and PH_3 inhibits the antioxidant activity and induces peroxide radicles accumulation resulting in elevation of hydroxyl radicals like hydrogen peroxidation. Treatment of ALP toxicity by DHA improved oxidative stress and inflammatory markers. Similar results by Rashedinia et al. (2016) explained that DHA may restore mitochondrial respiration via activation of cytochrome c oxidase and increasing ATP production by glycolysis.

The histopathological findings in the present study showed that ALP toxicity induced severe damage in rats' heart tissue. This agrees with Shah et al. (2009), Taromsari, et al. (2010), Kalawat et al. (2016) and Liang et al. (2020). They observed congestion, myocardial contraction band necrosis, vacuolar degeneration, edema, and scattered inflammatory cells in the heart tissues in the ALP poisoned children and explained that the histopathological changes were due to phosphine gas liberated after ALP ingestion which induces myocardial contractility impairment and leads to circulatory failure and pulmonary edema.

Immunohistochemical analysis was conducted using activated BAX, iNOS and HIF-1 α to indicate the mechanism by which ALP induced damage to the heart muscle. BAX (Bcl-2 family) is a cytoplasmic pro-apoptotic protein. Cellular damage can induce changes in the structure and position of BAX, producing a heterodimer with Bcl-2, which inhibit the activity of Bcl-2 protein, resulting in damage of mitochondrial membrane and activation of apoptosis signaling pathway in the mitochondria (Haghi-Aminjan et al., 2018). In the present study, ALP group showed a highly significant increase in the BAX positive cells in comparison with control group. This is in accordance with studies by El Shehaby et al. (2021) who noticed that phosphine causes apoptosis and necrosis in cardiac cells. Increase in cytochrome c release, a decrease in mitochondrial membrane potential (MMP), and an increase in mitochondrial permeability transition pore (MPTP) opening are the

possible mechanisms of phosphine-induced apoptosis.

Several studies on animals reported that ALP can induce oxidative stress due to production of free radicals and inhibition of antioxidant defense system, which can explain that after receiving ALP cardiac myocytes showed an increase in oxidative stress (Hsu et al., 2000). These studies supported our finding that iNOS expression in cardiac muscle is raised in ALP group, while its expression is significantly reduced in combination group. This agreed with Changal et al. (2017), who reported that cardiac toxicity induced by ALP is due to generation of free radicals, while the combination with antioxidant therapy decreased production of free radicals leading to improvement of the heart specimens.

The HIF-1 α is a primary regulator for vital responses to hypoxia (Gurjar et al., 2011). Malekinejad et al. (2012) reported that nitric oxide (NO) production increased in hypoxic condition leading to upregulation of HIF-1α. In the current study, ALP group showed upregulation of HIF-1α due to ability of ALP to induce ischemia and hypoxia in the cardiac muscle. Expression HIF-1α in the combined (ALP+ DHA) group was significantly lower than ALP group. This improvement shows the ability of DHA to improve ischemia and hypoxia induced by ALP in the cardiac muscle.

Conclusion:

Aluminum phosphide toxicity induces cardiac damage via oxidative stress and apoptosis confirmed by alternation of physiological functions (BP, HR, and ECG), biochemical analysis and histological study of cardiac tissue. Dihydroxyacetone has a potential protective role against cardiac damage caused by acute ALP toxicity, by improvement of physiological, biochemical, and histological changes. The protective effects of DHA in ALP- induced cardiac disorders may be related to its ability to improve hypoxia, apoptosis and necrosis in cardiac cells. Therefore, its administration in cases of ALP poisoning together with other supportive therapies can improve their prognosis.

Declaration of conflicting interests

No conflicts of interest

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دراسة تأثير ثنائي هيدروكسي الأسيتون على السمية القلبية الحادة لفوسفيد الألومنيوم في الجرذان البيضاء

فيروز إبراهيم نورالدين [١] ، علا عبدالهادي منصور [١] ، رضا أبو الفتح أحمد أبو السعود [٢] ، أحمد محمد محمد جعفر [٢] ، نيرمين محمد نورالدين [٣] ، أماني توفيق الفخراني [٤] ، ستهم السيد العجمي [١] ، أميرة محمد الصعيدي [١]

[١] قسم الطب الشرعي والسموم الإكلينيكية- كلية الطب- جامعة المنوفية

[٢] قسم الفسيولوجيا الطبية- كلية الطب- جامعة المنوفية

[٣] قسم التشريح والأجنة- كلية الطب- جامعة المنوفية- جامعة بدر بالقاهرة

[٤] قسم الفارماكولوجيا- كلية الطب- جامعة المنوفية

تعد سمية فوسفيد الألومنيوم (ALP) مشكلة صحية عامة منتشرة تؤدي إلى أضرار جسيمة في أنسجة القلب. ثنائي هيدروكسي أسيتون (DHA) هو سكر بسيط يتم إنتاجه بشكل طبيعي في الجسم ، يستخدم كترياق فعال ضد السموم المسببة لموت الميتوكوندريا لقدرته على تحسين وظيفة الميتوكوندريا واستنفاد ATP تبحث الدراسة الحالية في الدور الوقائي المحتمل لـ (DHA) علي القلب في ذكور الجرذان البيضاء المتسممة بـ ALP. تم تقسيم الجرذان إلى أربع مجموعات: المجموعة الضابطة، مجموعةDHA ، مجموعةALP ، ومجموعة (ALP+DHA). تم تسجيل ضغط الدم الشرياني(ABP) ، ومعدل ضربات القلب(HR) ، وتخطيط القلب الكهربائي (ECG) ووقت البقاء على قيد الحياة. تم إجراء التحاليل الكيميائية للدم والدراسة النسيجية والكيمياء المناعية لأنسجة القلب.

أظهرت الجرذان المسممة بـ ALP انخفاضًا ملحوظًا في ضغط الدم الشرياني ومعدل ضربات القلب مع تغيرات تخطيط القلب ECG وزيادة ملحوظة في مستويات انزيمات القلب (troponin I, CPK-MB)، وعلامات الاجهاد التاكسدى مثل المالوندهايد (MDA) و دلالات الالتهاب مثل الانترليوكن ٦ IL-6 , و (TNF)مقارنة بالمجموعة الضابطة. أدت إضافة DHA مع ALP إلى تحسن كبير في ضغط الدم الشرياني ومعدل ضربات القلب وتحسن في تخطيط القلب ECG؛ مع تحسن كبير في المؤشرات الحيوية للقلب، وعلامات الاجهاد التاكسدى و دلالات الالتهاب ؛ وزيادة كبيرة في وقت البقاء على قيد الحياة مقارنة مع الجرذان المتسممة ب ALP. أظهر الفحص النسيجي المناعى زيادة BAX و HIF-1 α و iNOS في مجموعة ALP. أدت أضافة DHA مع ALP الي تقليل BAX و HIF-1 α و iNOS.

يحتمل أن يكون لثنائي هيدروكسي الأسيتون (DHA) دور وقائي ضد تلف القلب الناتج عن سمية ALPالحادة، وبالتالي فإن تناوله في حالات التسمم بـ ALP مع العلاجات الداعمة الأخرى يمكن أن يحسن الناتج المتوقع.