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The effect of acute Carbon Monoxide intoxication on cardiac necrosis in rats: in relation to Adiponectin levels

El efecto de la intoxicación aguda por Monóxido de Carbono en la necrosis cardíaca en ratas: en relación con los niveles de Adiponectina

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ABSTRACT

In order to investigate the effects of acute CO poisoning and subsequent oxygen therapy on cardiac necrosis in rats, with a specific focus on adiponectin levels, twenty–one male Wistar albino rats were divided into three groups (Control, CO, CO+O_2). The Control group was placed in a container and exposed to room air for 30 min. Acute CO poisoning was induced in the CO group and CO+O_2 group by exposing the rats to CO gas for 30 min. Following CO exposure, the CO+O_2 group received oxygen therapy for 30 min, while the CO group did not receive any additional intervention. The animals were euthanized by cardiac puncture under anesthesia, following the approved ethical procedures. Carboxyhemoglobin (COHb), serum levels of creatine kinase (CK), creatine kinase myocardial band (CK–MB), C–reactive protein (CRP) and lactate dehydrogenase (LDH), as well as cardiac and serum adiponectin levels were measured. CO poisoning caused necrosis in cardiac tissue however, oxygen therapy alleviated the negative effect of CO on cardiac injury. COHb and LDH levels in CO group were increased, whereas both cardiac and serum adiponectin levels were decreased (all, $P<0.05$). There were no changes in CK, CK–MB, CRP levels among groups (all, $P>0.05$). Oxygen therapy decreased COHb, but increased both cardiac and serum adiponectin levels (all, $P<0.05$). Adiponectin and LDH may serve as potential biomarkers for early diagnosis of cardiac necrosis caused by acute CO poisoning. The assessment or quantification of adiponectin can also be useful for the early prognosis of cardiac necrosis after oxygen therapy.

Key words: Adiponectin; carbon monoxide poisoning; heart; oxygen therapy; rat

RESUMEN

Con el objetivo de investigar los efectos de la intoxicación aguda por monóxido de carbono (CO) y la terapia de oxígeno subsiguiente en la necrosis cardíaca en ratas, con un enfoque específico en los niveles de adiponectina, veintiuna ratas albinas Wistar machos fueron divididas en tres grupos (Control, CO, CO+O_2). El grupo de control fue colocado en un recipiente y expuesto al aire ambiente durante 30 min. Se indujo intoxicación aguda por CO en el grupo CO y el grupo CO+O_2 al exponer a los ratas a gas CO durante 30 min. Después de la exposición al CO, el grupo CO+O_2 recibió terapia de oxígeno durante 30 min, mientras que el grupo CO no recibió ninguna intervención adicional. La eutanasia de los animales se realizó mediante punción cardíaca bajo anestesia, siguiendo los procedimientos éticos aprobados. Se midieron los niveles de carboxihemoglobina (COHb), creatina quinasa (CK), banda de creatina quinasa miocárdica (CK–MB), proteína C–reactiva (CRP) y lactato deshidrogenasa (LDH), así como los niveles de adiponectina cardíaca y sérica. La intoxicación por CO causó necrosis en el tejido cardíaco; sin embargo, la terapia de oxígeno alivió el efecto negativo del CO en la lesión cardíaca. Los niveles de COHb y LDH en el grupo de CO aumentaron, mientras que tanto los niveles de adiponectina cardíaca como sérica disminuyeron (todos, $P<0.05$). No se observaron cambios en los niveles de CK, CK–MB y CRP entre los grupos (todos, $P>0,05$). La terapia de oxígeno disminuyó los niveles de COHb, pero aumentó tanto los niveles de adiponectina cardíaca como sérica (todos, $P<0.05$). La adiponectina y LDH pueden servir como biomarcadores potenciales para el diagnóstico temprano de la necrosis cardíaca causada por la intoxicación aguda por CO. La valoración o cuantificación de adiponectina también puede ser útil para el pronóstico temprano de la necrosis cardíaca después de la terapia de oxígeno.

Palabras clave: Adiponectina; intoxicación por monóxido de carbono; corazón; terapia de oxígeno; rata

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INTRODUCTION

Carbon monoxide (CO) is a gas that has no color, odor, or taste, and it is produced as a by–product of combustion processes. This gas originates from many industrial and domestic sources and exposure can lead to serious health problems [1]. CO poisoning can occur during the combustion of combustible materials, particularly in enclosed environments, and can have serious consequences in humans and animals [2, 3].

Acute CO poisoning leads to serious health problems especially when the exposure occurs through inhalation [4]. Although the pathogenesis of acute CO poisoning is not fully understood, the primary mechanism involves hypoxic stress resulting from carbon monoxide binding to hemoglobin, which inhibits oxygen transport to tissues. This condition causes severe damage, particularly to organs with high oxygen demands such as the heart and brain [5].

Although the effects of CO on human health have been extensively studied, the effect of CO on the cardiovascular system is poorly understood. How CO affects cardiac necrosis and how this effect is mediated has attracted the attention of the scientific community. Myocardial toxicity from CO exposure can result in permanent myocardial damage and increase both short–term and long–term mortality [6]. Normobaric and hyperbaric oxygen therapies are very important in CO–poisoned patients, as they prevent complications and shortens the duration of hospitalization. Normobaric oxygen therapy may have favorable effects on the prognosis and provide significant economic benefits [7].

Myocardial enzymes are key clinical indicators of myocardial cell damage [8]. Creatine kinase (CK) and creatine kinase myocardial band (CK–MB) are intracellular enzymes primarily found in the myocardium, skeletal muscle, and brain [9]. Lactate dehydrogenase (LDH) plays a crucial role as an enzyme in the anaerobic metabolic pathways within the heart muscle and various other organs [10]. Consequently, LDH levels can also rise in pathological conditions such as myocardial infarction, hematological diseases, and circulatory failure associated with hypoxia [10].

Adiponectin is an adipokine that has recently been discovered to play an important role in cardiac health [11]. Adiponectin is the most abundant peptide hormone mainly secreted by adipocytes that acts on specific receptors in various tissues including heart through autocrine, paracrine, and endocrine signaling mechanisms [7]. Epicardial adipose tissue, located on the surface of the ventricles and the apex of the heart, is capable of secreting adiponectin and directly influencing heart function [12]. Adiponectin is secreted by various other cells and tissues, such as cardiomyocytes and endothelial cells [11]. It contributes to cardiovascular protection by enhancing lipid metabolism, protecting vascular endothelial cells, and preventing the formation of foam cells and the proliferation of vascular smooth muscle cells [11]. Additionally, adiponectin exhibits anti–inflammatory, antiapoptotic properties, mitigates hypertrophy, enhances angiogenesis, and inhibits the development of interstitial fibrosis [13]. Accordingly, adiponectin should play a protective role against cardiovascular diseases [11]. However, there are also some contradictory results that high adiponectin levels have been observed in patients with cardiovascular conditions [14].

While alterations in cardiac enzyme levels are critical for evaluating myocardial damage in acute carbon monoxide poisoning, these indicators do not possess adequate specificity to definitively diagnose myocardial injury, particularly in patients with skeletal muscle necrosis or multiple organ failure [15]. Consequently, the identification of different biomarkers for diagnosing cardiac injury in acute CO poisoning is of great importance. It is hypothesized that serum and cardiac adiponectin levels decrease with cardiac injury induced by CO poisoning and that oxygen therapy increases adiponectin levels in both serum and heart tissue.

Therefore, the aim of this study is to explore the impact of acute CO poisoning and subsequent normobaric oxygen therapy on cardiac necrosis in rats (*Rattus norvegicus*), with a focus on adiponectin levels and other biochemical biomarkers. The assessment of adiponectin levels may be valuable for evaluating the cardiac necrosis and the cardiac effects of oxygen therapy, thereby helping to optimize therapeutic strategies.

MATERIALS AND METHODS

Ethical Statement

This experimental study was approved by the University Experimental Animals Local Ethics Committee and was conducted following ethical rules (Ethical approval no:3 date: 26/01/2023).

Animals

Twenty–one male Wistar albino rats (8–10 weeks old; 300–350 g) were maintained in steel cages under standard conditions (22–25°C, 12 h day/night cycle). Standard rat chow was provided in steel containers and tap water was provided in glass bottles. All groups were given food and water *ad libitum* and no feed or water restrictions were applied to the rats during the study. All groups of animals were part of larger experimental design previously described in our earlier work, although different tissues were analyzed in the current study [16].

Experimental Groups and Study Design

The rats were equally divided into three groups, with seven in each: 1. Control, 2. CO intoxication (CO), 3. CO intoxication treated with 100% normobaric oxygen with reservoir mask. The control group of animals was placed in a container and allowed to breath room air for 30 min. CO intoxication was induced according to our previous study [16]. Briefly, the rats in CO and CO+O_2 groups were individually placed in a 45×30×30 cm sized transparent plastic container and exposed to CO at the concentration of 4000 ppm with the flow rate of 3 L·min^{-1} CO for 30 min [17] using a 10 L CO cylinder (Habaş Company, İzmir, Türkiye). The detector (Dräger X–am® 5000 brand, 0–10000 ppm, Germany) was used to determine the concentration of CO gas. Thereafter, the animals in CO+O_2 group received 100% normobaric oxygen with the flow rate of 15 L·min^{-1} for 30 min as described in our previos study [16]. Oxygen was administered by inhalation with a reservoir mask (PLUSMED, Chinese) which is connected to the oxygen gas cylinder with a flow meter.

Blood and tissue collection

At the end of study, all animals were eutanized by cardiac puncture under midazolamine intraperitoneal injection anesthesia. Thereafter, the abdomen of each animal was opened with midline incision. Blood samples were taken from the heart's left ventricle using blood gas syringes (BERİKA Technology Medical, Türkiye) and were immediately used to measure carboxyhemoglobin (COHb) levels (ABL800 Radiometer, Denmark) [16].

Additionally, blood samples for serum adiponectin and other biochemical biomarkers were collected into a yellow biochemistry tube (BD Vacutainer®, USA) for the measuring of adiponectin, CK, CK–MB, LDH and C–reactive protein (CRP).

The heart from each group of animals was excised and longitudinally bisected into right and left halves along the septum. The right part was kept at -80°C (Binder UF V 500 Standard Model, Germany) until the analysis of adiponectin. Samples from the left part were fixed in 10% neutral buffered formalin for furher histolopathological examinations.

Serum biochemical biomarkers

Blood samples collected from each animal were centrifuged (Megafuge STPlus, Thermo Scientific, Waltham, MA) at 1500 g for 10 min.

Serum supernatants were collected to sterile tubes, and analyzed for levels of CK, CK–MB, LDH and CRP in a biochemical autoanalyzer (Architect c8000; Abbott, Wiesbaden, Germany) using ultraviolet spectrophotometric, colorimetric, and enzymatic methods. CK and CK–MB levels of these compounds were measured using ultraviolet spectrophotometric methods. Ultraviolet light absorption was used to determine the concentration of these compounds. LDH levels were determined using enzymatic methods. The enzymatic method involves measuring the chemical reactions catalyzed by specific enzymes to determine the concentration of the compound. CRP levels were determined using colorimetric methods. In this method, the color change resulting from the reaction between CRP and chemical compounds is measured to determine the CRP levels [18]. Serum samples were kept at -80°C for further analysis of serum adiponectin levels.

Measurement of cardiac and serum adiponectin levels

For the analysis of cardiac adiponectin, the homogenates were prepared from the right heart tissue stored at -80°C. Heart tissues were removed from the freezer, placed in a glass tube with phosphate buffer solution (PBS) at a ratio of 1:10 (w/v, pH:7.2), and homogenized on ice with a tissue homogenizer (Bandelin, UW 2070, Sigma, St. Louis, MO) at 30000 g for 60 s. The homogenates were placed in centrifuge tubes and centrifuged at 4200 g for 10 min at 4°C. The supernatants were transferred to an eppendorf tubes, and the precipitates were discarded. Serum samples were also removed from freezer. Adiponectin levels in both tissue supernatants and serum samples were measured by enzyme–linked immunosorbent assay (ELISA) using specific ELISA kits (Sun Red, cat. no: 201-11-0759) according to the manufacturer's instructions [19]. The supernatants were read spectrophotometrically at 450 nm using a microplate reader (Biochrom, Anthos Zenyth 200).

Histomorphological examination

The left heart tissue samples of the sacrificed animals were fixed with 10% neutral formalin. After fixing, all tissues were washed under tap water, dehydrated with ascending–graded ethanol series and embedded in paraffin wax. The 5–µm sections were cut and deparaffinized in xylene solution. Thereafter, the sections were hydrated in a descending ethanol series, stained with hematoxylin and eosin (H&E) and visualized under a light microscope (ZEISS Axiolab 5, Germany). The pathological findings to assess myocardial necrosis were categorized into four grades according to injury severity: Grade 0: normal histological appearance, Grade 1: scattered necrotic cells, Grade 2: one or two necrotic foci, and Grade 3: more than two necrotic foci [20].

Statistical analysis

SPSS 26 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis and graphics. A Shapiro–Wilks test was used to confirm the normality of the distribution in each group. Since the distributions were normal, statistics for continuous variables, including the mean and standard deviation (SD), were calculated. ANOVA test was employed to detect differences in the continuous data and Tukey's test used for comparisons between groups. Pearson's correlation analysis was applied to detect the positive and negative relationships between the data. While $P<0.05$ values are considered statistically significant, $P<0.001$ indicates a stronger association, emphasizing the robustness of the results in certain comparisons [21]. The use of these two significance levels was determined by the magnitude of the observed differences and the need to emphasize different degrees of statistical confidence. This two–level approach was used for a more detailed interpretation of the data.

RESULTS AND DISCUSION

Levels of blood COHb and serum biochemical biomarkers

Blood COHb levels and serum levels of CK, CK–MB, LDH and CRP are shown in TABLE I.

For the current study, the experimental conditions were identical to those in our previous study, which focused on liver damage, where the same cohort of animals was used and the same protocols

TABLE I
The levels of blood carboxy haemoglobin and serum biochemical biomarkers in all study groups

Parameters	Control (n=7)	CO (n=7)	CO+O_2 (n=7)
COHb (%)	0.71 ± 0.34^{a}	90.27 ± 1.36^{b}	34.03 ± 7.57^{c}
CK–MB (pg·mL^{-1})	218.43 ± 184.62^{a}	225.85 ± 120.58^{a}	241.01 ± 186.98^{a}
LDH (u·L^{-1})	304.57 ± 134.08^{a}	1129.43 ± 345.68^{b}	$901.71 \pm 508.94^{b,d}$
CK (u·L^{-1})	589.86 ± 125.14^{a}	651.43 ± 359.04^{a}	621.43 ± 189.36^{a}
CRP (mg·L^{-1})	0.11 ± 0.02^{a}	0.11 ± 0.02^{a}	0.12 ± 0.01^{a}

Data are represented as mean ± standard deviation. Different superscripts in the same row indicate statistically significant differences. $^{a-b, a-c, b-c}$ indicate significant differences at $P<0.001$. $^{a-d}$ indicates significant differences at $P<0.05$. CO: Carbon monoxide intoxication group; CO+O_2: Carbon monoxide intoxication treated with 100% normobaric oxygen group; COHb: Carboxyhemoglobin; CK–MB: Creatine kinase myocardial band; LDH: Lactate dehydrogenase CK: Creatine kinase; CRP: C–reactive protein

were followed [16]. Given that COHb is the only indicator of CO poisoning [22], we employed the COHb data from the same cohort of animals used in our previous study [16]. COHb level was significantly higher in CO group than in that of the control group (P<0.001). COHb level was significantly decreased in CO+O_2 group compared to CO group (P<0.001). However, COHb level was still significantly higher in CO+O_2 than control group (P<0.001).

There were no significant differences in CK, CK–MB and CRP levels among the groups (all, P>0.05). LDH level was significantly increased in CO group compared to control group (P<0.001). There were no significant differences in LDH levels between CO and CO+O_2 (P>0.05). LDH level was still significantly higher in CO+O_2 group than control group (P<0.05). LDH levels were positively correlated with COHb (R=0.649; P=0.001).

CO poisoning mainly results from the formation of COHb, which prevents oxygen delivery to tissues, and causes hypoxia [23]. In patients with CO intoxication, COHb levels at the moment of the intoxication are significant predictors of the late appearance of myocardial infarction [24]. Neurological and structural damage may occur in cardiac tissue as hypoxia intensifies [6, 25]. CO poisoning induces a wide range of cardiac pathologies through mechanisms involving tissue hypoxia and direct cellular injury [26]. In this study, CO group had significantly higher COHb level compared to the control. Oxygen (O_2) treatment significantly reduced the COHb level in CO+O_2 group, however, it was still significantly higher than control group. This might be due to shorter duration of oxygen therapy, which was only 30 min [16]. In addition to the shorter duration of oxygen therapy, another known factor contributing to this phenomenon is the more stable binding between hemoglobin and carbon monoxide. This stable binding prolongs the presence of CO in the bloodstream, even with short–term exposure, and may impact the recovery process [3, 27].

Myocardial necrosis occurs during moderate and severe CO poisoning, with changes in the electrocardiography and biomarkers [25]. Although CO exposure leads to alterations in cardiac biomarkers, the prognostic value of these indirect indicators of myocardial following CO intoxication remains unclear. Myocardial enzymes such as CK, CK–MB, CRP and LDH are used as the primary clinical indicators for assessing myocardial cell injury [8, 28]. In this study, no significant changes were found in serum CK, CK–MB, and CRP levels among the control, CO, and CO+O_2 groups. Similarly, no significant changes in CK and CK–MB have been reported in children at early time points of CO poisoning [29]. It has been reported that serum CK and CK–MB levels usually occur 4-6 hours (h) after the onset of myocardial damage, peak at 24 h, and return to baseline within 48-72 h [30]. Similar to these results, it has also been shown that CRP levels do not increase in patients with severe CO poisoning [31]. However, it has been shown that both CK–MB, and CRP levels have been increased in acute CO poisoning patients [5, 28]. In an other sudy, patients with acute poisoning, CRP levels have been observed to be at their lowest on the first day and peaking on the third day before decreasing thereafter [32]. The biomarkers were measured 30 min after CO exposure to capture the early effects of acute CO poisoning. This time point was chosen based on the understanding that the half–life of CO in ambient air is 4-5 h, but with 100% oxygen therapy, it is reduced to 1 h, allowing for the observation of early effects within this timeframe [33]. Since we measured these biomarkers at 30 min after CO exposure, acute CO poisoning migth have not caused any significant changes in CK, CK–MB and CRP levels compared to the control group. However, in this study, the LDH level was significantly increased in the CO group compared to the control group. The increase in LDH levels in the CO group compared to the control group is an expected result, given the toxic mechanism of action of carbon monoxide. Carbon monoxide binds to hemoglobin, reducing oxygen delivery to tissues and resulting in cellular hypoxia. This hypoxic condition promotes the release of enzymes like LDH, which are typically elevated in response to cell damage [27]. Moreover, carbon monoxide poisoning may result in rhabdomyolysis, a potentially life–threatening condition that is characterized by the breakdown of skeletal muscle and the release of muscle cell contents, including enzymes like LDH, into the bloodstream. This process further supports the elevation of LDH levels in the CO group, as rhabdomyolysis can be a direct consequence of CO toxicity [4]. Similar to our results, LDH levels have been reported to increase in rabbits exposed to acute CO poisoning [34]. In clinical studies, LDH levels have also been found to be elevated in patients with severe, acute CO poisoning [35]. The rise in LDH levels may occur earlier than CK, CK–MB, and CRP levels, which are more specific to muscle damage or inflammation and may take longer to become elevated. In this study, oxygen therapy did not cause any significant change in LDH levels in CO–poisoned rats. This might be due to the shorter duration of oxygen therapy, which was only 30 min.

Cardiac and serum adiponectin levels

TABLE II shows the adiponectin levels in the cardiac tissues and serum of all groups. The cardiac adiponectin level in CO group was significantly lower than that in the control group (P<0.001). However, cardiac adiponectin level in CO+O_2 group was significantly increased compared to CO group (P<0.05). Moreover, there were no significant differences between control and CO+O_2 levels (P>0.05).

TABLE II
Cardiac and serum adiponectin levels in all study groups

	Control (n=7)	CO (n=7)	CO+O_2 (n=7)
Cardiac Adiponectin (ng·mL^{-1})	7.99 ± 1.53^{a}	3.47 ± 1.82^{b}	6.16 ± 1.61^{a,c}
Serum Adiponectin (ng·mL^{-1})	5.64 ± 1.05^{a}	3.36 ± 0.32^{b}	4.42 ± 0.47^{d}

Data are represented as mean ± standard deviation. Different superscripts in the same row indicate significant differences. $^{a–b}$ indicates significant differences at P<0.001. $^{b–c, a–d, b–d}$ indicate significant differences at P<0.05. CO: Carbon monoxide intoxication group; CO+O_2: Carbon monoxide intoxication treated with 100% normobaric oxygen group

Serum adiponectin level in CO group was significantly lower than that in the control group (P<0.001). However, serum adiponectin level in CO+O_2 group was incresed significantly compared to CO group (P<0.05). There was a significant difference between control and CO+O_2 groups (P<0.05). Moreover, there was a positive correlation between cardiac and serum adiponectin levels (R=0.686; P=0.001) (FIG. 1). This positive correlation may suggest a systemic regulation of adiponectin levels during acute stress conditions, as both cardiac and serum adiponectin levels are known to respond to inflammatory and oxidative stress pathways [36].

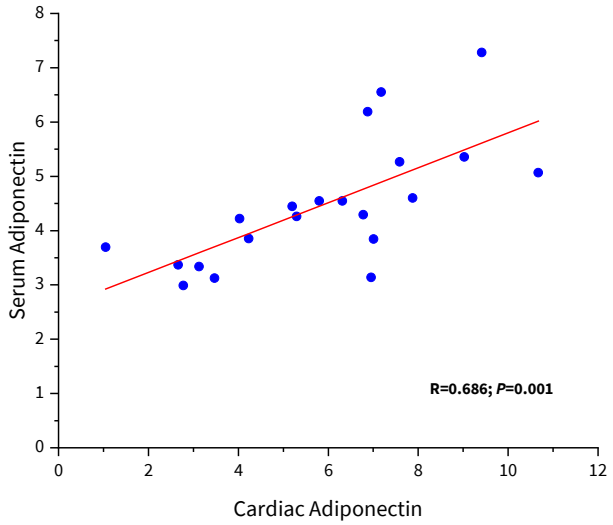

FIGURE 1. Correlation between cardiac and serum adiponectin levels. R: Pearson correlation coefficient. Significant correlation, level of significance *P*<0.001

Cardiac adiponectin levels were inversely correlated with COHb and LDH (R= -0.780; *P*=0.001 and R= -0.662; *P*=0.001, respectively) (TABLE III). Similarly, serum adiponectin levels were inversely correlated with both COHb and LDH (R= -0.823; *P*=0.001 and R= −610; *P*=0.003, respectively) (TABLE III). This inverse correlation may be explained by the anti–inflammatory and cytoprotective roles of adiponectin, which are likely downregulated in the presence of increased oxidative stress and cellular damage, as indicated by elevated COHb and LDH levels. Further studies are needed to explore this hypothesis in detail [36].

Adiponectin is an adipokine secreted by adipocytes, cardiomyocytes and endothelial cells as well as pericardial and perivascular tissues, and directly affect the function of the cardiovascular system [11]. However, the relationship between adiponectin levels and the incidence of cardiovascular diseases is controversial [37]. Some clinical studies support the idea that populations with higher adiponectin levels are less likely to suffer from cardiovascular disease [13]. In contrast, higher adiponectin levels have been associated with an increased risk of cardiovascular diseases such as heart failure and myocardial infarction [37, 38].

To the best of current knowledge, no studies have been conducted on adiponectin levels in animals or humans exposed to CO intoxication. Moreover, no research has been found regarding the correlation between cardiac and serum adiponectin levels in CO–induced cardiac injury. This study showed that there was a positive correlation between cardiac and serum adiponectin levels.

In our study, both cardiac and serum adiponectin levels were decreased significantly in CO group, but both were increased following oxygen treatment. Moreover, both adiponectin levels were inversely correlated with LDH and COHb levels. These findings suggest that the adverse effects of CO on cardiac health may be related to a decrease in adiponectin levels, and oxygen therapy may ameliorate the negative effects of acute CO poisoning on cardiac tissue by elevating adiponectin levels.

Macroscopic and Histomorphological Observations

The macroscopic appearance of heart tissues in all groups of animals has been shown in FIG. 2. In all groups of animals, the anatomical structure, general size and shape of the heart and the thickness of ventricular wall were in normal limits. Additionally, there was no discoloration in the control group. Diffuse congestion was detected in all animals of CO group compared to control. The degree of congestion was less in all animals of CO+O_2 group compared to CO group.

The histological observations and the degenerative grading scores in all groups are shown in FIG. 3 and TABLE IV, respectively. The heart tissue of the control group had normal histological structure. CO poisoning group had more than two necrotic foci in heart tissue, whereas CO+O_2 group had only scattered necrotic cells. The degenerative grading score in CO group was higher than that in control group (*P*<0.001). The degenerative score in the CO+O_2 group was significantly lower than that in the CO group (*P*<0.05), but it remained significantly higher than that of the control group (*P*<0.001) [21].

In this study, discoloration and diffuse congestion in the heart were detected in all animals in the CO group, however, O_2 therapy reduced the extent of congestion. Similarly, in both humans and animals, CO poisoning has been reported to be characterized by red discoloration of the skin, mucous membranes, cardiomyocytes, as well as neurological alterations, including necrosis in the brain [3, 39, 40]. Cardiac necrosis refers to damage and death of heart tissue and has serious consequences on cardiovascular health. In this study, necrotic foci were observed in the heart tissue of the CO group, however, early O_2 treatment alleviated this necrosis. Similarly, necrosis in cardiac tissue has been observed in a rat model following CO poisoning [41]. Furthermore, it has been reported that normobaric oxygen therapy can reduce the cardiac necrosis in patients with CO poisoning [42].

TABLE III
Correlation analysis of all parameters

Variables	Cardiac ADN (R ; P)	COHb (R ; P)	LDH (R ; P)	CK (R ; P)	CK–MB (R ; P)	CRP (R ; P)
Cardiac ADN	–	-0.780; 0.001*	-0.662; 0.001*	-0.221; 0.336	0.088; 0.703	0.111; 0.631
Serum ADN	0.686; 0.001*	-0.823; 0.001*	-0.610; 0.003*	-0.191; 0.406	0.071; 0.758	0.117; 0.615

R: Pearson correlation coefficient. *: Significant correlation, level of significance *P*<0.05. Cardiac ADN: Cardiac adiponectin; Serum ADN: Serum adiponectin; COHb: Carboxyhemoglobin; LDH: lactate dehydrogenase; CK: creatine kinase; CK–MB: creatine kinase myocardial band; CRP: C–reactive protein

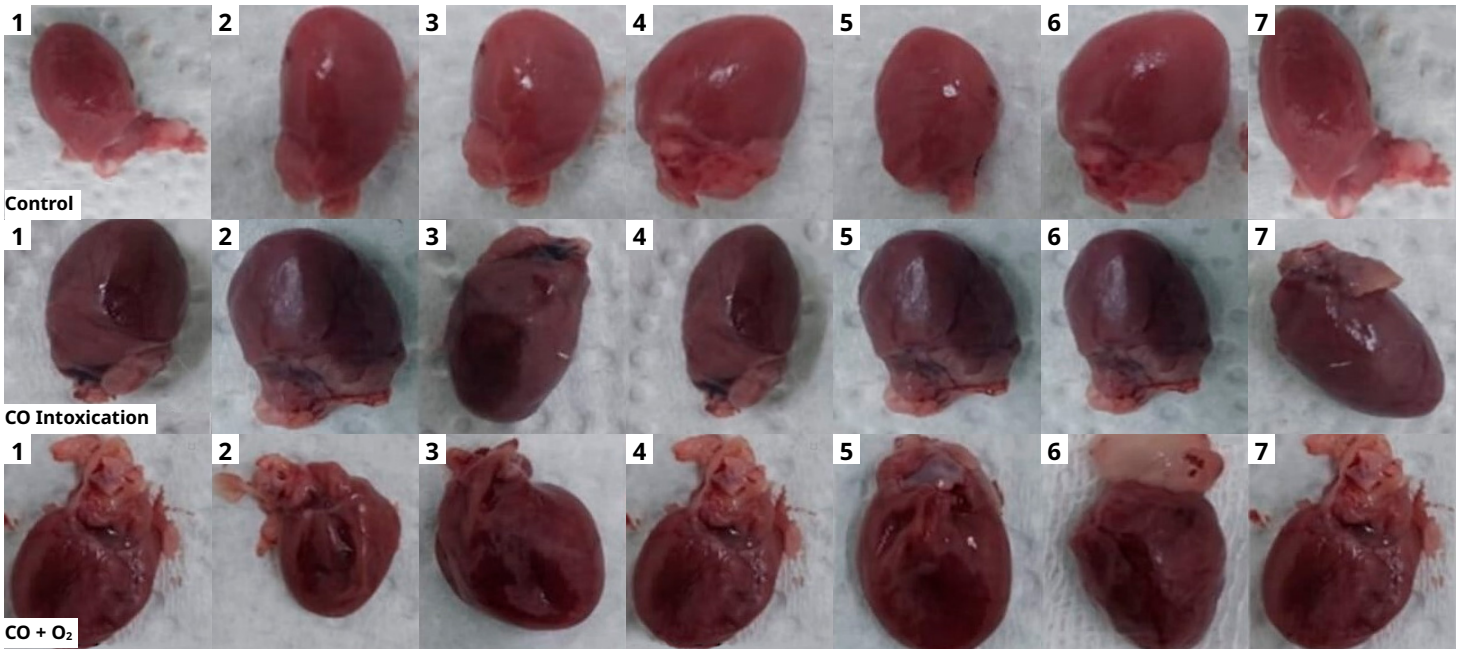

FIGURE 2. Macroscopic appearance of hearts in all rats according to groups. CO: carbon monoxide intoxication group; CO+O_2: carbon monoxide intoxication treated with 100% normobaric oxygen group

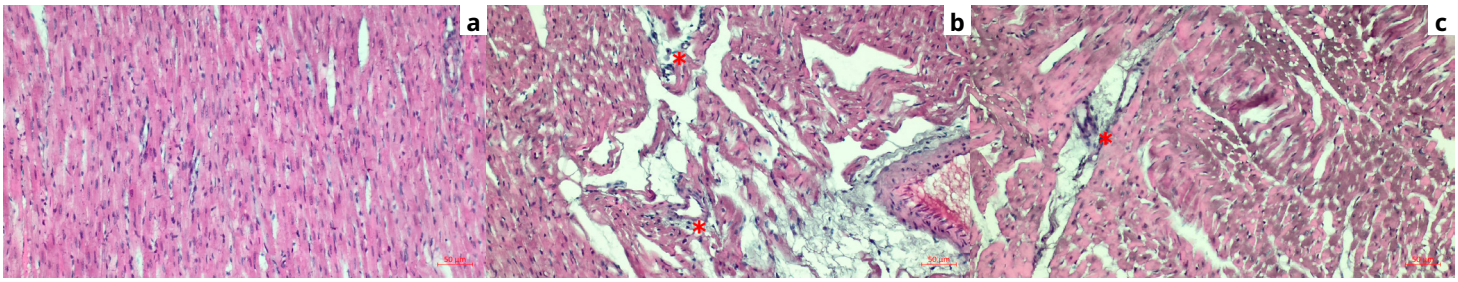

FIGURE 3. Histomorphological appearance of each study group. A: Control group: B: Carbon monoxide intoxication group C: Carbon monoxide intoxication treated with 100% normobaric oxygen group. *: Necrotic foci. Staining: hematoxylin and eosin; scale bar: 50 µm; magnification: 20 µm

TABLE IV
The degree of cardiac degeneration in all study groups

Groups	Heart Degeneration Grading
Control (n=7)	0.29 ± 0.49^{a}
CO (n=7)	2.71 ± 0.49^{c}
CO+O_2 (n=7)	1.86 ± 0.69^{b}

Data are represented as mean ± standard deviation. Different superscripts in the same column indicate significant differences. $^{a-b, a-c}$ indicate significant differences at $P<0.001$. $^{b-c}$ indicates significant differences at $P<0.05$. CO: Carbon monoxide intoxication group; CO+O_2: Carbon monoxide intoxication treated with 100% normobaric oxygen group

CONCLUSION

Exposure to CO induces cardiac necrosis and reduces the levels of both cardiac and serum adiponectin. Oxygen therapy may alleviate the negative effects of acute CO poisoning on cardiac injury by increasing both cardiac and serum adiponectin levels. It can be concluded that adiponectin and LDH may serve as potential biomarkers for early diagnosis of cardiac necrosis caused by acute CO poisoning. Adiponectin may also be of value in early prognosis of cardiac necrosis. However, more comprehensive studies are needed to establish the precise cut–off values of adiponectin and LDH to improve their diagnostic accuracy and clinical reliability as biomarkers for cardiac necrosis caused by CO poisoning.

Furthermore, adiponectin might be used as an adjunctive therapeutic agent in addition to oxygen therapy for CO–induced cardiac injury. The relationship between adiponectin and the molecular pathways involved in CO–induced cardiac necrosis—such as oxidative stress, inflammation, and apoptosis—requires further investigation.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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