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Impact of Binary CoO/CuO Nanoparticles on the Activities of Creatine Kinase (CK-MB) of Myocardial Infarction Patients

Marwa Saad Mohammed[1]*, Nada Nadhim Ahmed[1], Fatin Fadhel Al-kazazz[1], Ahmed Mahdi Rheima[1] and Mohammed F. Al Marjani[2]
[1]Department of Chemistry, College of Science, Mustansiriyah University, Baghdad, Iraq
[2]Department of Microbiology, College of Science, Mustansiriyah University, Baghdad, Iraq
*Corresponding author (e-mail: marwoasaad15@gmail.com)

Nanoparticles have emerged as effective tools in various applications, including medicine, due to their ability to overcome organic barriers and enhance targeted therapy. This study investigates the effect of cobalt oxide/copper oxide (CoO/CuO) divalent nanoparticles on creatine kinase-MB (CK-MB) levels in the serum of patients with myocardial infarction. The CoB/CuO divalent nanoparticles were produced via photoluminescence irradiation and characterized using XRD, TEM, SEM, and EDX techniques, confirming their crystalline structure and average size, which ranged from 16 to 35 nm. Patients with myocardial infarction had significantly higher serum CK-MB levels (30.49 ± 1.35 U/L) compared to the control group (17.28 ± 4.06 U/L; $P < 0.0001$). In this study, the activity of CoO/CuO nanoparticles was found to be concentration-dependent in inhibiting CK-MB activity, with inhibition reaching up to 57% at 0.05 M. These results indicate that CoO/CuO binary nanoparticles have a modulating effect on CK-MB activity, confirming their potential for developing nanoparticle-based therapeutic approaches for myocardial infarction.

Keywords: Creatine Kinase (CK-MB), myocardial infarction (MI), binary CuO\CoO nanoparticles

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Blood and heart vessel conditions, such as thrombosis, coronary heart disease (CHD), peripheral arterial disease, and cerebrovascular disease, are called cardiovascular diseases (CVDs). Apart from being the primary cause of mortality globally, additional risk variables, like increased longevity, sedentary ways of living, poor diets, and related metabolic illnesses, also contribute to the prevalence of CVDs [1, 2]. Heart attacks, also known as myocardial infarction (MI), are brought on by coronary heart disease, which damages the blood vessels that supply the myocardium, the heart muscle. Infracted heart muscle is an area with either no or so little flow that cardiac muscle function cannot be maintained. The total procedure is known as a myocardial infarction. Mainly caused by oxidative stress and atherosclerosis [3]. A typical acute occurrence in CVDs is a myocardial infarction (MI), which happens when the tissue is injured through the cessation of blood flow. This results in the cessation of oxygen and nutrient supply and the build-up of toxic compounds [4]. Generally speaking, after unstable atherosclerotic plaques burst, MI progresses to coronary thrombosis and myocardial ischemia-reperfusion (I/R) damage [5]. Acute myocardial ischemia raises intracellular and mitochondrial calcium levels because anaerobic respiration replaces aerobic metabolism in cells. Cell enlargement, rupture, and apoptotic, necroptotic, autophagic, and necrotic processes also contribute to cell death. Reperfusion exacerbates the damage by reactivating the electron transport chain, producing excessive reactive oxygen species (ROS) in cardiomyocytes, and infiltrating proinflammatory neutrophils into the wounded tissue upon oxygen restoration [1]. The most prevalent sign of acute MI is chest discomfort, which is frequently characterized as a constriction, pressure, or squeezing sensation. The most prevalent MI symptoms in women are fatigue, weakness, dyspnea, and irregular sleep patterns. Angiotensin-converting enzyme (ACE) inhibitors, calcium channel blockers, nitrates, and diuretics can all be used to treat it [3].

Biomarkers are essential endpoints for ACS's diagnosis, risk stratification, and prognosis assessment. Among these biomarkers [6]. Creatine kinase MB (CK-MB) is a crucial biomarker in diagnosing acute M [7]. CK comprises three isozymes (CK -MM, CK -MB, and CK -BB), of which CK-MB is myocardial-specific [8, 9]. Thus, myocardial injury is reflected in circulating CK-MB activity. The myocardium releases CK-MB into the bloodstream when it sustains injury. In contrast to the straightforward incidence of MI, CK-MB activity is a continuous variable connected with the extent and timing of myocardial damage [8, 10]. The gold standard for diagnosing MI was creatine kinase MB (CK-MB). Nevertheless, routine measurement for diagnostic purposes is not advised by the current European Society of Cardiology (ESC) guidelines [6, 11]. In muscle cells, creatine kinase converts creatine and adenosine triphosphate (ATP) to creatine phosphate and adenosine diphosphate (ADP), and the CK-MB isoenzyme makes up 20% of the total CK pool in the myocardium. CK-MB begins to rise four to six hours after an MI, offering the chance of early re-infarction

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identification [11, 12]. During the first 6 h after an MI, CK-MB has an NPV of 97% and a 91% sensitivity [12].

Materials that are purposefully created to be smaller than 100 nm in at least one dimension are known as engineered nanomaterials. Because of its small size, nanotechnology can be used to create novel physicochemical qualities used in coatings, electronics, food goods, and cosmetics [13, 14]. Nanomedicine has tremendous prospects for improving the diagnosis and treatment of human diseases [13, 15]. With clinical uses ranging from contrast agents in imaging to carriers for medication and gene delivery into tumours, nanomaterials are essential players in contemporary medicine [16, 17]. Among metallic nanoparticles, copper, cobalt, and their binary mixtures possess great significance in various industrial applications, which stem from their outstanding physical and chemical properties [18, 19]. These findings suggest that binary CoO/CuO nanoparticles exert a ability modulatory effect on CK-MB interest, indicating their promise in developing nanoparticle-based therapeutic techniques for myocardial infarction.

EXPERIMENTAL

Chemicals and Materials

Subjects

The study samples consisted of 30 healthy individuals as a control group and 30 patients with myocardial infarction (MI) dysfunction attending Baghdad Teaching Hospital/ medical, July – November general information of each patient, such as age, sex, and aetiology.

Serum Sampling: - Venous blood 5ml was taken from healthy donors and patients. Blood samples were centrifuged at 3000 rpm for 10 min after coagulation. Serum was thus separated and stored at -20ºC until being used. Serum levels of CK-MB enzyme were assayed by spectrophotometric kits obtained from (LINEAR CHEMICALS) according to the provided assay procedure.

Synthesis of Binary CuO\CoO Nanoparticles

The photo-irradiation process created Binary copper-cobalt oxide nanoparticles [20, 21]. A 1:1 molar ratio of copper and cobalt solutions was utilized to produce binary CuO\ CoO NPs. As a result, 50 mL (2 mmol) of Cu $(NO_3)_2$ and 50 mL (2 mmol) of Co $(NO_3)_2$ were combined for 15 minutes at 25 °C while being magnetically stirred. A 100 mL (4 mmol) polyethene glycol solution was added gradually to this binary solution. After 30 minutes of photocell radiation exposure, the solution was cooled in a closed wood box at 5 °C. After the irradiation, the solution was mixed with 60 mL of 1 N NaOH. When obtained, deionized water was used to clear the dark brown precipitate (all procedures involved centrifuging and decanting). After drying at 110 °C in an oven for two hours, the precipitate was calcined for three hours at 450 °C. A black powder, or binary CuO\CoO NPs precipitate, was created.

Binary CuO/CoO Nanoparticle Characterization

The CuO\CoO nanoparticles have been characterized using a variety of methods. Utilizing a Cu-Kα radiation (λ=0.154 nm) source in 2θ (10° to 80°), the specimens' composition was examined using an X-ray diffraction (XRD) Model D-5000. Sample morphologies were studied using the TEM model Jeol JSM-6010LV at 500 X and 60 kX magnification with a 5 kV accelerating violation. The CuO/CoO nanoparticles were isolated in the deionized and sonic water for about 15 minutes, according to the TEM examination. FE-SEM using the Jeol JSM-6010LV EDX model. To find the constituent elements' particle size and proportions, 20 µL was lowered over a 300-mesh Cu grid and allowed to dry at room temperature.

Determination of Human Serum CK-MB Level

The interest of human serum creatine kinase-MB (CK-MB) changed into measured in the presence and absence of binary CuO/CoO nanoparticles (NPs) to evaluate their capacity inhibitory effect.

A pooled serum sample changed into prepared with the aid of combining 10 µL from each individual patient's pattern (n = 30), yielding a complete pooled volume of 300 µL. CK-MB activity was determined the usage of a colorimetric assay package (Linear Chemicals, Spain) following the producer's protocol.

For nanoparticle exposure, described concentrations of CuO/CoO NP s (0.1/2, zero.01, and zero.05 mg/mL) were added to aliquots of the pooled serum. The enzyme activity become then compared among nanoparticle-handled and untreated samples to assess the concentration-structured effect of CuO/CoO NPs on CK-MB activity.

Effect of CuO\CoO NPs on CK-MB Activity

The method described previously was used to study the effect of CuO/CoO NPs on CK-MB activity: 10 µL of CuO/CoO NPs were added to pool sera (sample test).

Due to the optimum concentrations of CuO\CoO on CK-MB, three different concentrations of CuO\CoO NPs (0.01, 0.05, 0.005) were used. The following formula was used to get the inhibition percentages [22].

%Inhibition=100-100x [activity of CK-MB in the presence of NPs/ activity of CK-MB in the absence of NPs]

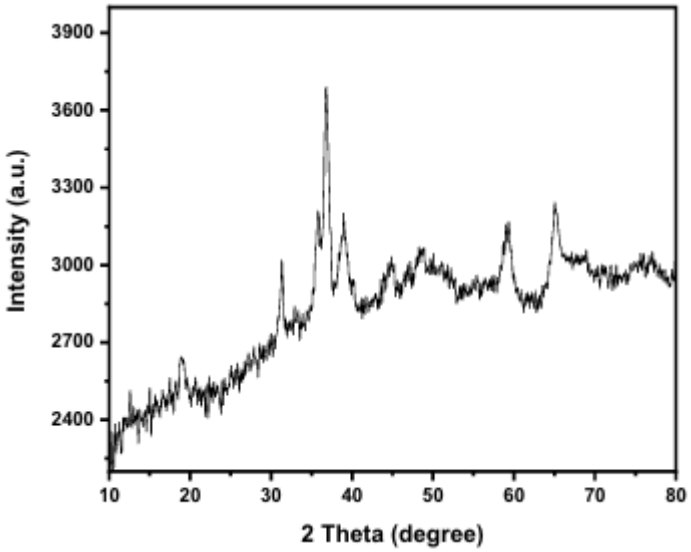

Figure 1. XRD pattern of binary Cuo\CoO NPs.

Statistical Analysis

The research data was analyzed using the Statistical Program for the Social Sciences (SPSS) (Statistical Microsoft Office, version 25). The differences in the mean values were assessed using the student t-test, and $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Characterization of Binary CuO\CoO Nanoparticles

XRD analysis was used to determine the binary CuO\CoO NPs' crystallographic phase and structure Figure 1. All of the diffraction peaks for the CuO sample are found to be monoclinic CuO phase (JCPDS: 48-1548) and free of impurity peaks. The diffraction peaks of the binary CuO\CoO NPs at 35.74, 38.96, 59.32, and 64.95 can be associated with the (002), (111), (113), and (311) crystal planes of the monoclinic CuO phase (JCPDS: 75-0393). 36.77, 44.7, 69.2, and 76.3 represent the (111), (200), (311), and (222) planes of the cubic CoO phase, respectively. The purity of the material is indicated by the lack of peaks for any impurities the samples' dimensions.

The TEM micrograph Figure 2a revealed predominantly round CuO/CoO nanoparticles with uniform morphology. The particle size distribution, predicted from multiple measurements throughout the TEM area, ranged among 25 and 35 nm, that's consistent with the crystallite size calculated the usage of the Scherrer equation. For clarity, representative particle dimensions had been indicated within the TEM photograph Figure 2b [23, 24].

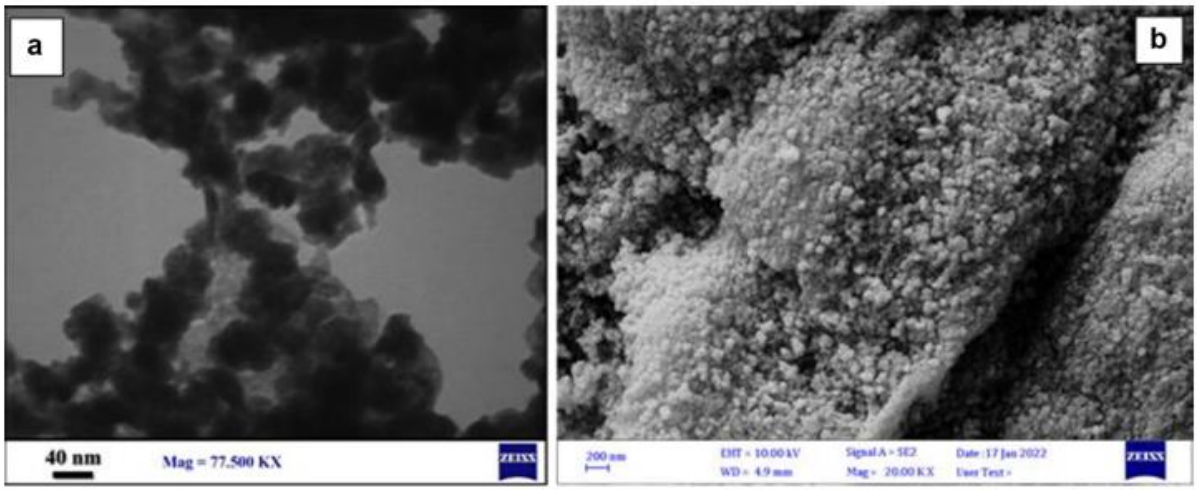

Figure 2. Binary CuO/CoO nanoparticle electron microscopy images (a) TEM and (b) SEM.

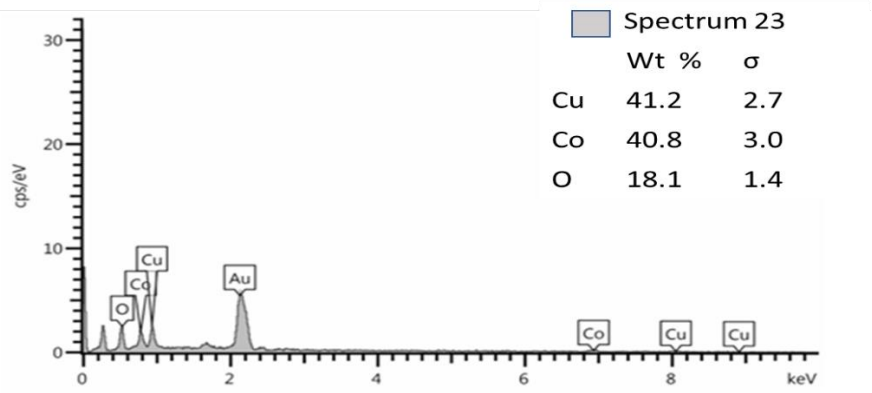

Figure 3. EDX of binary CuO\CoO nanoparticles.

Figure 3 displays the EDX analysis of CuO\CoO NPs that underwent a 450 °C calcination. The findings show that copper (Cu), cobalt (Co), and oxygen (O) are present in the goods. Additionally, they demonstrate that NPs are nearly stoichiometric. There are no extra elemental pollutants in EDX spectra. A uniform distribution of CoO and CuO with an atomic ratio of 1:1 is shown by the EDX result. This result supports the synthesis of pure CuO and CoO NPs. The XRD results and all of the other results concur well.

Figure 3 affords the EDX spectrum of the binary CuO/CoO nanoparticles calcined at 450 °C. The major peaks correspond to Cu, Co, and O, confirming the a success synthesis of the binary oxide with out primary contamination. However, minor peaks acting before the oxygen sign likely stand up from the copper grid used because the substrate for the duration of analysis or from heritage instrumental noise. These extra peaks do no longer represent overseas elements in the pattern, as their intensities had been negligible and not detected in repeated measurements. Overall, the atomic ratio of Cu to Co remained approximately 1:1, consistent with the intended stoichiometry of the synthesized nanoparticles.

Table 1 displays the standard deviation and means of age between different groups of patients, and the control groups were 48.40±5.076 years and 50.40±9.719 years, respectively. Indicated a non-significant difference (P>0.05) between the different groups.

Body mass index (BMI) is easily obtained during clinic questioning. The results in Table 1 show that the mean ± SD values estimated at the patients compared with the control groups were 19.257±2.374 Kg/m^2 and 19.290±2.443 Kg/m^2, respectively. These results did not show a significant difference (P>0.05) in BMI in both patients' groups as compared to the control group.

Table 1. Demographic characteristics for patients and control groups under the study.

Parameter	Group	N	Mean ± SD	P-value
Age (Years)	Patient	30	48.40±5.076	NS*
	Control	30	50.40±9.719	
Gender • **Male** • **Female**	Patient	30	- F 15 (50%) - M 16 (53 %)	——
	Control	30	- F 15 (50%) - M 16 (53 %)	——
Weight (Kg)	Patient	30	76.067±12.362	NS*
	Control	30	75.633±12.794	
Height (cm)	Patient	30	166.33±7.350	NS*
	Control	30	165.700±7.312	
BMI (Kg/m^2)	Patient	30	19.257±2.374	NS*
	Control	30	19.290±2.443	

SD: Standard deviation, NS: Non-significant at P>0.05.

Table 2. t-test of serum CK-MB levels distributed among patients and control groups.

CK-MB (U/L)			
Group	**N**	**Mean ± SD**	**P-value**
Patient	30	30.489±1.353	0.0001
Control	30	17.281±4.061	

N: Number of subjects, SD: Standard deviation, CK-MB: Creatine Kinase.

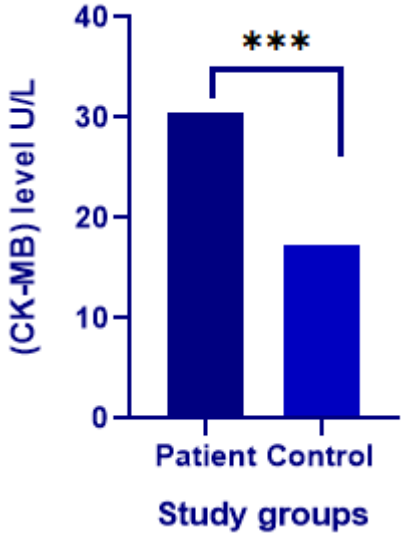

Figure 4. The mean ± SD value of CK-MB levels in sera of MI patients and control.

Table 2 shows the statistical analysis of CK-MB levels between patients and the control group. The results revealed that the highest mean ± SD of CK-MB was estimated in patients at 30.489±1.353 U/L, compared with the control group at 17.281±4.061 U/L.

On the other hand, there was a highly significant increase (P≤0.0001) in the serum levels of CK-MB in the group of patients compared with the control group, as seen in Figure 4.

Myocardial ischemia (MI) is diagnosed using biomarkers like CK-MB. Still, they can also be employed as a surrogate endpoint in clinical studies investigating novel treatments for MI to quantify the extent of the infarct. The size of an infarct can be determined using a variety of techniques, but there isn't much comparison data to show which technique better captures the association between infarct size and essential clinical outcomes like death [8, 10]. Previous studies have shown a relationship between CK-MB activity and the prognosis, MI time, left ventricular ejection fraction, and infarct size. Furthermore, elevated cardiac troponin or CK-MB mass above the 99th percentile upper reference limit (URL) for healthy persons is referred to as MI [8, 10].

The Impact of Binary CoO/CuO Nanoparticles on Creatine Kinase (CK-MB) Activity

The present study demonstrates that binary CuO/CoO nanoparticles drastically inhibit the activity of the myocardial enzyme creatine kinase-MB (CK-MB) in vitro. This location suggests a possible biochemical interaction among metallic oxide nanoparticles and cardiac enzymes which can impact mobile oxidative stability and strength metabolism.

Previous reports have shown that steel nanoparticles including CuO and CoO can engage with proteins via electrostatic and coordinative binding, main to conformational adjustments and partial lack of enzyme characteristic (22). The found inhibition of CK-MB pastime on this have a look at may also therefore end result from direct interplay

among the nanoparticles' floor metal ions (Cu^{2+} and Co^{2+}) and the thiol or histidine residues at the enzyme's active website. Such interactions can regulate the enzyme's tertiary structure, lowering its catalytic efficiency.

Another workable mechanism involves reactive oxygen species (ROS) era induced by means of steel oxide nanoparticles. Both CuO and CoO are redox-energetic oxides able to generating superoxide and hydroxyl radicals in aqueous media (13; 14) (18). Excess ROS can oxidize essential amino acid residues or coenzymes inside CK-MB, main to enzyme inactivation. This mechanism aligns with preceding findings that oxidative amendment of CK-MB is associated with decreased enzyme pastime in myocardial ischemia (3).

Another viable mechanism entails reactive oxygen species (ROS) era brought about by metal oxide nanoparticles. Both CuO and CoO are redox-lively oxides able to generating superoxide and hydroxyl radicals in aqueous media (13; 14) (18). Excess ROS can oxidize vital amino acid residues or coenzymes inside CK-MB, leading to enzyme inactivation. This mechanism aligns with previous findings that oxidative change of CK-MB is associated with reduced enzyme pastime in myocardial ischemia (3).

From a therapeutic perspective, although inhibition of CK-MB in vivo could indicate potential cytotoxicity at excessive nanoparticle publicity, controlled modulation of enzyme pastime may open avenues for nanoparticle-based totally cardioprotective or diagnostic techniques. Further mechanistic studies, along with enzyme kinetics, molecular docking, and redox assays, are encouraged to delineate whether or not inhibition arises more often than not from direct binding, ROS generation, or both.

The findings in Figure 5 demonstrate that the nanoparticles inhibited the activity of creatine kinase (CK-MB) in the serum of patients who experienced myocardial infarction. By comparing the CK-MB concentration with and without CoO/CuO, the inhibition percentages were determined, as shown in Equation (22):

%Inhibition=100-100x [CK-MB in the presence of NPs/ CK-MB in the absence of NPs]

A novel contact between nanotechnology and biotechnology, known as the "nano-bio interface," was developed by the emerging discipline of nanomedicine. Nanomaterials provide significant promise for the development of new therapeutic opportunities because of their unique physicochemical characteristics (25). In this part of the study, the level of CK-MB was investigated in the presence and absence of binary CoO/CuO Nanoparticles. the optimum binary CoO/CuO Nanoparticles concentration was determined for serum CK-MB samples. This study uses different concentrations (0.01, 0.05, 0.005) of binary CoO/CuO NPs on CK-MB. It's found that binary CoO/CuO NPs had an inhibitor role on CK-MB in pool serum from MI, and it's concluded that increased concentration led to increased inhibition. The highest assay of inhibition levels to binding sera CK-MB was achieved when the binary CoO/CuO NPs is 57% at (con. 0.005), while less level is 38% for (con.0.01), as shown in Figure 6.

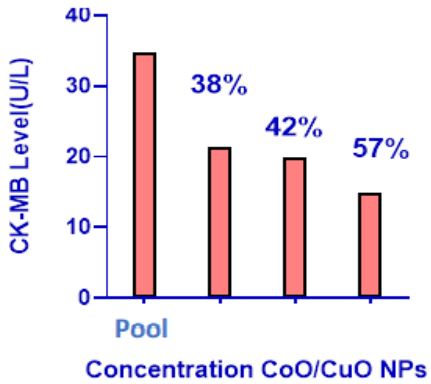

Figure 5. The CK-MB levels in sera of Pool patients MI, Pool patients with Binary CoO/CuO NPs.

CONCLUSION

This look at demonstrates that binary CuO/CoO nanoparticles appreciably inhibit the interest of creatine kinase-MB (CK-MB) in the sera of myocardial infarction (MI) sufferers in a attention-structured manner. Structural characterization showed that the synthesized nanoparticles possess nanoscale dimensions (sixteen–35 nm) and a nicely-described crystalline morphology.

The located inhibition of CK-MB can be attributed to floor interactions between the nanoparticles and the enzyme's active websites, as well as oxidative changes on account of reactive oxygen species (ROS) generated by way of the redox-energetic metal oxides. These findings suggest that binary CuO/CoO nanoparticles can modulate cardiac enzyme pastime through combined surface and oxidative mechanisms.

New therapeutic approaches are desperately needed to treat MI since the condition poses a significant risk to people's lives and health. In previous years, consideration was given to the effects of cellular and molecular mechanisms on the pathological processes of MI and the development of therapeutic techniques and treatment protocols. Promising clinical applications are incorporated into several current MI therapy regimens to aid in MI recovery (e.g., pharmacotherapy, gene therapy, protein therapy, cell therapy, and exosome therapy) (26; 27). The main finding of the present study is that Binary CoO/CuO Nanoparticles inhibit CK-MB activities, which in turn inhibit MI.

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