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Original Article

Protective Effect of Curcumin Against Isoproterenol-Induced Myocardial Infarction in Adult Male Albino Rat

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ABSTRACT

Background: Myocardial Infarction (MI) is one of the most common cardiovascular diseases. Curcumin has been used in traditional Chinese and Indian medicine to treat a variety of diseases. It is safe and effective due to its anti-oxidant and anti-inflammatory properties.

Aim of the Work: This study was conducted to investigate the possible protective role of curcumin oral administration in experimental MI induced by isoproterenol (ISO) using biochemical and histological techniques.

Materials and Methods: This study was carried out on thirty-two male adult albino rats, divided into four equal groups. Group I was the normal control group. Group II was a sham control group that was divided into two subgroups: IIa and IIb, four rats each; they received distilled water and curcumin respectively. Group III received ISO. Group IV received both curcumin and ISO. Animals of all groups were sacrificed one day after the last dose of ISO. The serum cardiac marker enzymes Creatine Kinase (CK) and its myocardial isoenzyme (CK-MB) were estimated. Specimens from the left ventricle were subjected to light and electron microscopic studies.

Results: There was significant increase in serum CK and CK-MB in ISO-treated group compared to the control and sham control groups. Treatment with curcumin before ISO decreased significantly these enzymes. Isoprotrenol administration resulted in foci of separation and fragmentation of the muscle fibers. There was no distinction between the dark and light bands and obscured Z lines. Some of the fragmented cardiomyocytes lost their nuclei while others revealed pyknotic or distorted nuclei. Mononuclear cell infiltration, areas of haemorrhage, severe edema and cellular homogenicity were observed. Mitochondria appeared variable in size and shape. They were swollen and less in number as compared to the control sections. Administration of curcumin before ISO showed sound organization of the muscle fibers similar to that of control sections and minimal mononuclear cell infiltration. The mitochondria were of similar number to the control group but they were swollen and showed ruptured membranes and cristae.

Conclusions: curcumin administration minimized elevation of plasma cardiac enzyme markers CK and CK–MB following experimental myocardial injury by ISO administration and preserved the normal histological architecture of the myocardium. This beneficial effect of curcumin was mostly related to its antioxidant and anti-inflammatory properties.

Key words: Myocardial infarction- isoproterenol- curcumin- antioxidants.

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INTRODUCTION

The heart, an organ that repetitively contracts and relaxes, has advanced differential development. Its principal cells, the cardiomyocytes, actively divide and proliferate in the embryonic phase by maintaining their cell-dividing capacity after differentiation *(Kaneda & Fukuda, 2009)*. However, after birth, their regenerative/dividing capacity rapidly reduces. Cardiomyocytes show maladaptive hypertrophy in response to various stimuli, such as myocardial infarction, leading to heart failure *(Hosoda et al., 2010)*.

Myocardial Infarction (MI) is one of the most common cardiovascular diseases. Morbidity and mortality due to myocardial infarction are now reaching epidemic proportions throughout the world, accounting for 16.7 million deaths per year worldwide *(Yusuf et al., 2001)*. With changing lifestyles in developing countries, myocardial infarction is making an increasingly important contribution to mortality statistics *(Farvin et al., 2004)*. It is estimated that by the year 2020, up to three quarters of deaths in developing countries will result from non-communicable diseases and that MI will top the list of killers *(Ganesan & Anandan, 2009)*.

After MI, the acute loss of myocytes leads to an increased load to the heart and to the onset of a cascade of biochemical signaling processes that induce the remodeling of the infarcted zone and of the remote noninfarcted myocardium. Ventricular remodeling is complex and includes hypertrophy, which counterbalances the increased wall stress and attenuates progressive dilation. Nevertheless, heart failure is often the final result of this process after large infarcts *(Swynghedauw, 1999; Sutton & Sharpe, 2000)*. Heart failure is accompanied by excessive free radical production *(De-Jong et al., 2000)*.

Following infarction, the peri-infarct region of the heart shows hypertrophy with fibrosis, necrosis and infiltration of inflammatory cells. Alterations to cardiac morphology and function follow infarction, with increased end-diastolic and end-systolic volumes and decreased ejection fractions *(Tyler et al., 2006; Stuckey et al., 2008)*.

Isoproterenol (ISO) or Isoprenaline (3′, 4′-dihydroxyphenyl and isopropylaminoethanol hydrochloride), a synthetic catecholamine and β-adrenergic agonist, has been found to cause severe stress in the myocardium resulting in infarct-like necrosis of the heart muscle. It is similar to those observed in myocardial infarction in man. Isoproterenol-induced myocardial infarction serves as a well-standardized model to study the beneficial effects of many drugs and cardiac function *(Geng et al., 2004)*. Isoproterenol is also well-known to generate free radicals and to stimulate lipid peroxidation, which may be a causative factor for irreversible damage to the myocardial membrane *(Kakreja & Hess, 1992)*. Isoproterenol-induced myocardial infarction results in increased lysosomal hydrolases activity that may be responsible for tissue damage and infarcted heart *(Ravichandran et al., 1991)*. Intracellular release of lysosomal enzymes following myocardial ischemia may directly, or through activation of the complement pathway, results in cell injury and death. Isoproterenol-induced myocardial necrosis involves membrane permeability alterations that bring about loss of function and integrity of myocardial membranes *(Todd et al., 1980)*.

Natural products have been the starting point for the discovery of many important modern drugs. This fact has led to chemical and pharmacological investigations and general biological screening programs for natural products all over the world *(Nagle & Zhou, 2006)*.

Curcumin (diferuloylmethane) is a polyphenol responsible for the yellow color of turmeric, a curry spice. The yellow-pigmented fraction of turmeric contains curcuminoids, which are chemically related to its principal ingredient, curcumin. The major curcuminoids present in turmeric are demethoxycurcumin (Curcumin II), bisdemethoxycurcumin (Curcumin III) and the recently identified cyclocurcumin *(Kiuchi et al., 1993)*. Curcumin has relatively poor bioavailability when taken orally, but also low toxicity *(Hsu & Cheng, 2007)*. Curcumin has been used in traditional Chinese and Indian medicine to treat a variety of diseases *(Goel et al., 2008)*.

It has been suggested that curcumin possesses a myriad of beneficial activities and clinical trials have been conducted in patients with cancer, rheumatoid arthritis, cystic fibrosis, inflammatory bowel disease, psoriasis, pancreatitis and other disorders *(Mall & Kunzelmann, 2005; Hsu & Cheng, 2007; Miriyala et al., 2007)*. Curcumin is safe, well-tolerated and an efficacious chemo-preventive agent, mainly because of its anti-oxidant and anti-inflammatory properties *(Anand et al., 2007)*.

Li et al. (2008) showed that rodents treated with oral curcumin were markedly resistant to cardiac hypertrophy produced by banding of the aorta, which mimics the cardiac enlargement seen in patients with high blood pressure. They added that even when treatment was begun two weeks after the induction

of pressure overload, curcumin was beneficial and the transition to heart failure was reduced.

Although the MI is one of the most common and killer diseases, its effect on the structure of the heart is still unclear. Also, only few previous studies have mentioned the use of curcumin as a protective measure against MI. Therefore, the aim of the current study was to clarify the effect of MI on the structure of the myocardium and to find out the possible protective role of concomitant administration of curcumin.

MATERIALS AND METHODS

Drugs And Chemicals

- Curcumin [1, 7-bis (4-Hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione] and Isoproterenol hydrochloride (Sigma Chemical Company, St. Louis, MO, USA) were purchased from Sigma Company, Cairo, Egypt.
- All other chemicals were of analytical grade.

Animals And Treatments

This study was carried out on thirty-two male adult albino rats, weighing 180-210 g. They were obtained from the Animal House, Faculty of Medicine, Cairo University. Rats were housed in stainless steel cages under normal hygienic conditions and allowed water and food (laboratory chow) ad libitum throughout the study. They were divided into four equal groups (eight rats each):

- **Group I (normal control group):** Received no medications.
- **Group II (sham control group):** Was divided into two subgroups:

- Subgroup II-a (distilled water-treated subgroup): Four rats, received 1 ml/kg distilled water subcutaneously on 14th and 15th day at an interval of 24 hours between the two doses.

- Subgroup II-b (curcumin-treated subgroup): Four rats, received curcumin at dose of 200 mg/kg suspended in 1ml 0.5% carboxy methylcellulose. It was administered orally by a gastric gavage daily for 15 days.

- **Group III (ISO-treated group):** Received freshly prepared 85 mg/kg Isoproterenol hydrochloride (dissolved in distilled water) subcutaneously on 14th and 15th day at an interval of 24 hours between the two doses *(Panda & Naik, 2008)*.

- **Group IV (ISO and curcumin-treated group):** Received curcumin at dose of 200 mg/kg suspended in 1ml 0.5% carboxy methylcellulose. It was administered orally by a gastric gavage daily for 15 days. They received also 85 mg/kg ISO (dissolved in distilled water) subcutaneously on 14th and 15th day at an interval of 24 hours between the two doses**.**

Rats of all groups were sacrificed (by decapitation) one day after the last dose of ISO.

Albino rat was selected as an animal model of MI in the present study due to the great similarity to humans in the physiopathological alterations that occur after the infarction *(Zornoff et al., 2009)*. Male albino rats were chosen in the present work as the cardiovascular system is influenced by female sex hormones *(Ostadal et al., 2009)*. The rats used were picked of weight about 200 gm as rats of this weight represent young adults with less mortality than old ones *(Zornoff et al., 2009)*.

Biochemical Estimation

Trunk blood is collected from the site where the animal is decapitated and allowed to clot for 30 min at room temperature. The serum was separated by centrifugation at 2500 rpm at 30 °C for 15 min. Then serum cardiac marker enzymes Creatine Kinase (CK) and its myocardial isoenzyme (CK-MB) were estimated in the Chemical Pathology Laboratory at Cairo University by using standard kits *(Panda & Naik, 2008)*.

Histological Examination

The anterior wall of the thorax was opened by a midline incision. The heart was removed and profusely washed by saline. The left ventricle was dissected and inner one third of its wall was taken off *(Kemi et al., 1996)*. A small specimen was selected for electron microscopy and the rest of the left ventricle was dehydrated and embedded in paraffin. Sections of 5µm-thickness were cut, subjected to Hematoxylin and Eosin technique and exam-

ined by light microscopy *(Bancroft & Gamble, 2002)*. The specimen selected for electron microscopy was fixed in fresh 3% glutaraldehyde at 4°C for four hours, washed in 0.15 M phosphate buffer, pH 7.4, for two hours (two changes), postfixed in 1% osmium tetroxide for one hour at 4°C, dehydrated and embedded in epoxy resin. Serial semithin sections were cut at 1µm-thickness by Joel UMTP-6M ultramicrotome, stained with 1% toluidine blue and examined by light microscope. For electron microscopy, ultrathin sections (0.1 µm thick) were prepared using the same ultramicrotome and stained with uranyl acetate and lead citrate *(Hayat, 2000)*. The sections were examined by transmission electron microscope (Joel TEM-1400 Japan) and photographed under different magnifications by CCD Camera AMT Image capture.

Statistical Methods

The statistical analysis was performed on the biochemical results using the arithmetic mean (X), Standard Deviation (SD) and student t-test. All statistical analyses were done on an IBM personal computer using the statistical software (Statistics for Windows version 5). Results were considered significant when probability (p) was ≤0.05, highly significant when (p) ≤0.01 and very highly significant when (p) ≤0.001 *(Mould, 1989)*.

Table 1: Values (mean±SD) of serum cardiac enzymes CK and CK-MB in the different groups.

	Control group	Distilled water-treated subgroup	Curcumin treated subgroup	ISO-treated group	ISO & curcumin treated group
CK (0-195 u/l)	151±33*	164±75*	165±76*	901±175**	233±68*
CK-MB (0-24 u/l)	18±8•	17±11•	17±11•	294±34□	55±15□

For CK: ** is significantly different from other groups * at *P <0.05*.
For CK-MB: Different symbols are significantly different from each other at *P <0.05*.

RESULTS

Biochemical Results

There was a significant increase *(P <0.05)* in serum Creatine Kinase (CK) in ISO-treated group (Group III) compared to the control and sham control groups (Groups I and II). Curcuminm administration before ISO (Group IV) decreased significantly *(P <0.05)* CK enzyme elevated by ISO alone but did not reach to normal values. There was no significant difference between concomitant administration of curcumin and ISO (Group IV) and control and sham control groups. There was a significant increase in serum creatine kinase myocardial isoenzyme CK-MB *(P <0.05)* in ISO-treated compared to the control and sham control groups. Treatment with curcumin and ISO (Group IV) decreased significantly CK enzyme elevated by ISO alone *(P <0.05)*, but was still significantly higher *(P <0.05)* than control and sham control groups (Table 1).

Histological Results

Light Microscopy

Sections of the left ventricular wall of Group I (control group) revealed the standard architecture of the myocardium with branching and anastomosing cardiac muscle fibers running in different directions (Figs. 1, 2). Cardiomyocytes presented central oval vesicular nuclei. Cardiac muscle cells exhibited the intercalated disks representing specialized end-to-end junctions (Fig. 2).

Sections of the left ventricular wall of Group II (sham control group) showed no observable differences from Group I.

Isoproterenol administration (Group III) resulted in many alterations in sections of the left ventricular wall. The muscle fibers showed foci of separation and fragmentation (Figs. 3-7) or appeared wavy (Figs. 4, 5). Areas of haemorrhage (Figs. 4-6), severe edema (Fig. 5) and cellular homogenicity (Fig. 6)

were observed. Some of the fragmented cardiomyocytes lost their nuclei while others revealed pyknotic nuclei (Fig. 7) and mononuclear cell infiltration (Figs. 3, 6, 7). Infiltration was observed in the form of neutrophils, lymphocytes and monocytes (Fig. 7).

Administration of curcumin before ISO (group IV) showed the sound organization of the muscle fibers similar to that of the control sections and with minimal mononuclear cell infiltration (Figs. 8-10).

Electron Microscopy

Ultrathin sections of the left ventricular wall of group I revealed the usual histological structure of the myofibrils (Figs. 11, 12) with nuclear chromatin evenly dispersed and cisternae of sarcoplasmic reticulum (Fig. 11). Alternating dark and light bands, regular Z lines appeared in the middle of I bands (Fig. 12). Many mitochondria were seen between the myofibrils (Figs. 11, 12).

Ultrathin sections of the left ventricular wall of group II (sham control group) showed no observable differences from Group I.

Isoproterenol administration (Group III) resulted in many electron microscopic changes in the ultrathin sections of the left ventricle. There was distortion of the nuclei (Fig. 13). The myofibrils appeared severely interrupted and fragmented (Fig. 13) or disrupted (Figs. 14-16). There was no distinction between dark and light bands (Figs. 13, 15). The Z lines appeared obscured (Figs. 13, 15) or focally preserved (Fig. 14). Mitochondria appeared variable in size and shape (Figs. 13-15). They were swollen (Figs. 13, 16) and less in number as compared to control sections (Figs. 14-16). Severe separation (Figs. 13, 15, 16) and vacuolations (Fig. 16) appeared between the myofibrils and mitochondria.

The ventricular wall ultrathin sections from rats of group IV which received curcumin before ISO obviously showed regular arrangement of the myofibrils with alternating dark and light bands. Areas of disrupted fibrils appeared. Regular Z lines appeared in the middle of I bands (Fig. 17). The number of mitochondria was similar to that of the control group (Fig. 17), but they were swollen and showed ruptured membranes and cristae (Figs. 17, 18).

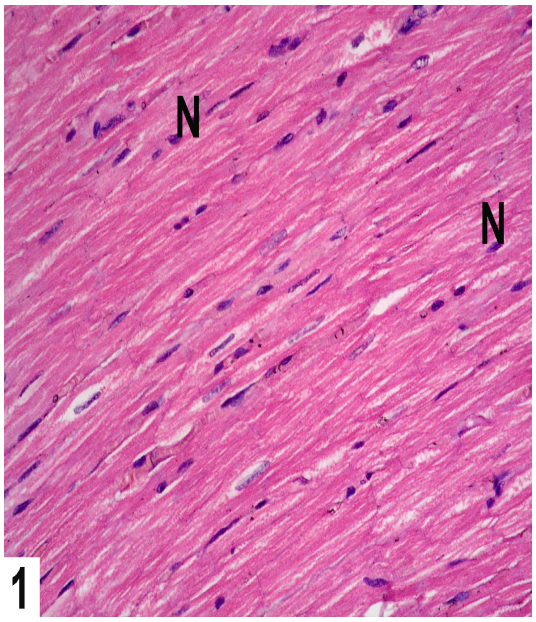

Fig. 1: A photomicrograph of a section of myocardium of a control albino rat (Group I) exhibiting the usual appearance of the myocardium with branching and anastomosing cardiac muscle fibers running in different directions. Cardiomyocytes have central oval nuclei (N). Hx. & E.; X 400

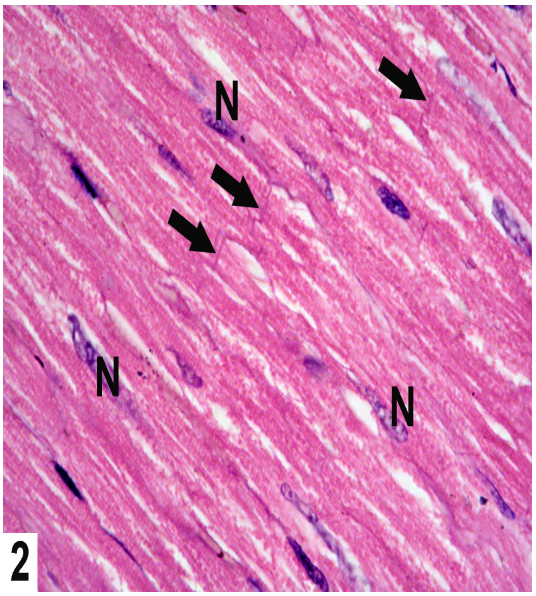

Fig. 2: A photomicrograph of a section of myocardium of a control albino rat (group I) showing the standard architecture of the myocardium with branching and anastomosing cardiac muscle fibers running in different directions. Cardiomyocytes have central oval vesicular nuclei (N). Intercalated disks (arrow) can be observed representing specialized end-to-end junctions. Hx. & E.; X 1,000

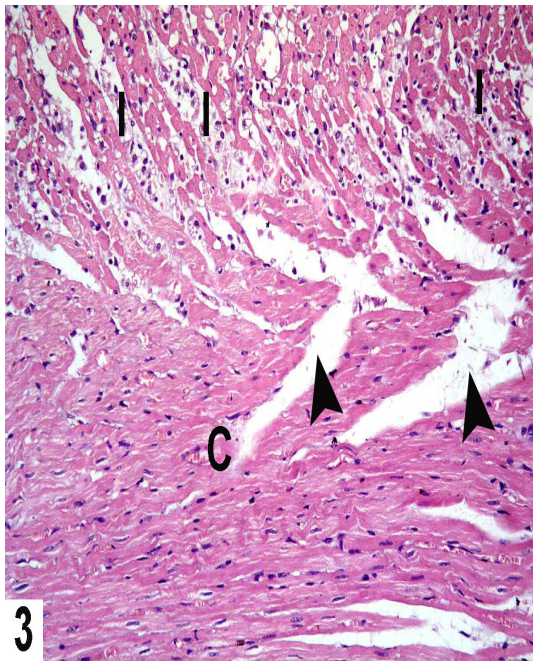

Fig. 3: A photomicrograph of a section of myocardium of an albino rat from Group III (ISO-treated group) displaying separation and fragmentation of muscle fibers (arrowhead) and mononuclear cell infiltration (I). A blood capillary (C) can be seen.
Hx. & E.; X 200

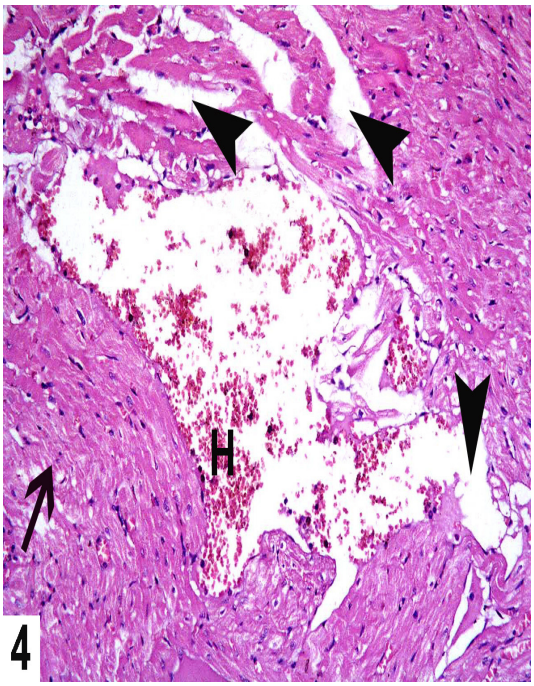

Fig. 4: A photomicrograph of a section of myocardium of an albino rat from group III (ISO-treated group) showing separation and fragmentation of muscle fibers (arrowhead) and haemorrhage (H). Areas of wavy muscle fibers (arrow) can be observed.
Hx. & E.; X 200

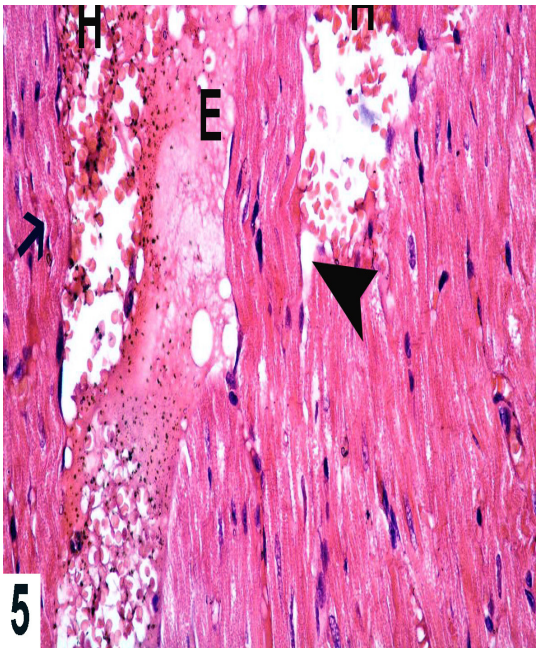

Fig. 5: A photomicrograph of a section of myocardium of an albino rat from Group III (ISO-treated group) exhibiting separation and fragmentation of muscle fibers (arrowhead). Some muscle fibers appear wavy (arrow). Areas of haemorrhage (H) and severe edema (E) can be seen.
Hx. & E.; X 400

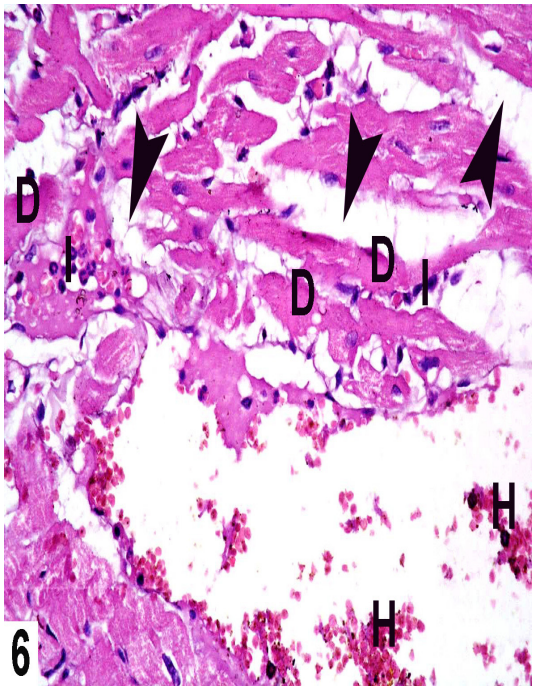

Fig. 6: A photomicrograph of a section of myocardium of an albino rat from Group III (ISO-treated group) showing separation and fragmentation of muscle fibers (arrowhead) and mononuclear cell infiltration (I). Areas of cellular homogenicity (D) and haemorrhage (H) can be seen.
Hx. & E.; X 400

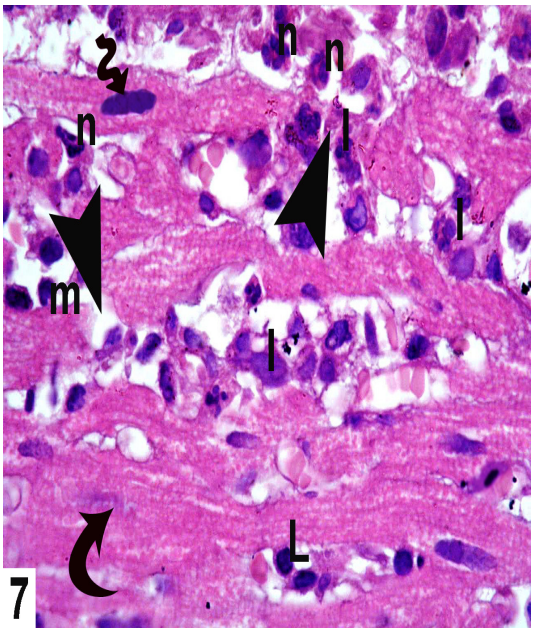

Fig. 7: A photomicrograph of a section of myocardium of an albino rat from group III (ISO-treated group) displaying separation and fragmentation of muscle fibers (arrowhead). Some of the fragmented cardiomyocytes lose their nuclei (curved arrow) while others have pyknotic nuclei (spiral arrow). Mononuclear cell infiltration (I) is observed in the form of neutrophils (n), lymphocytes (L) and monocytes (m).
Hx. & E.; X 1,000

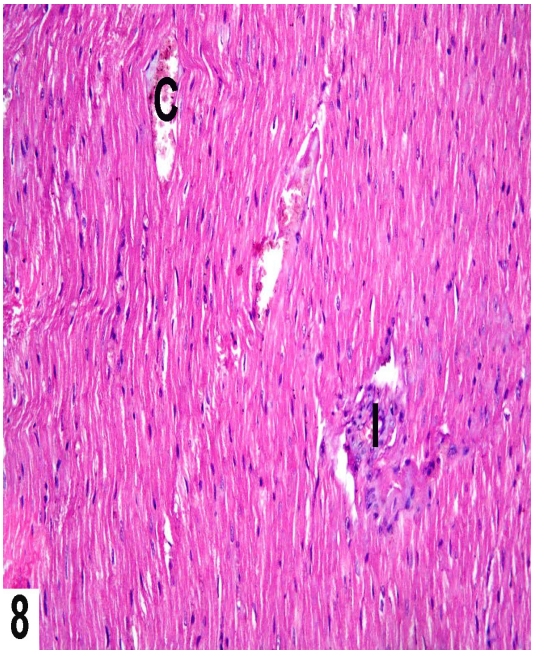

Fig. 8: A photomicrograph of a section of myocardium of an albino rat from group IV (ISO and curcumin-treated group) presenting the sound organization of the muscle fibers with minimal mononuclear cell infiltration (I). Blood capillaries (C) can be seen.
Hx. & E.; X 200

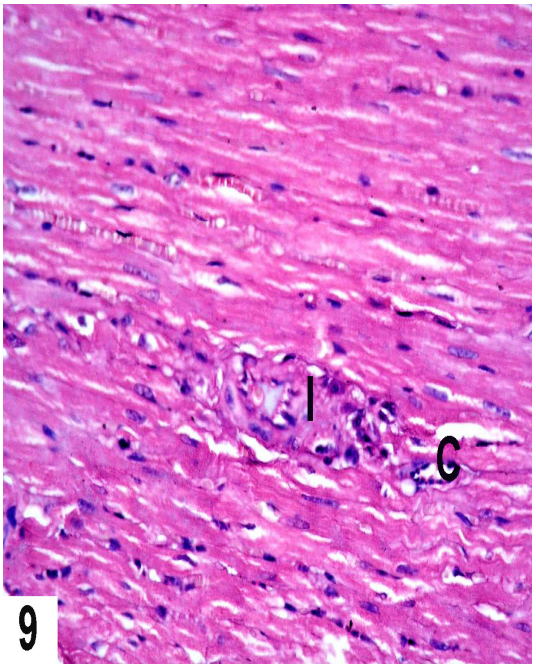

Fig. 9: A photomicrograph of section of myocardium of an albino rat from group IV (ISO and curcumin-treated group) showing the usual appearance of the muscle fibers with minimal mononuclear cell infiltration (I). A blood capillary (C) can be seen. Hx. & E.; X 400

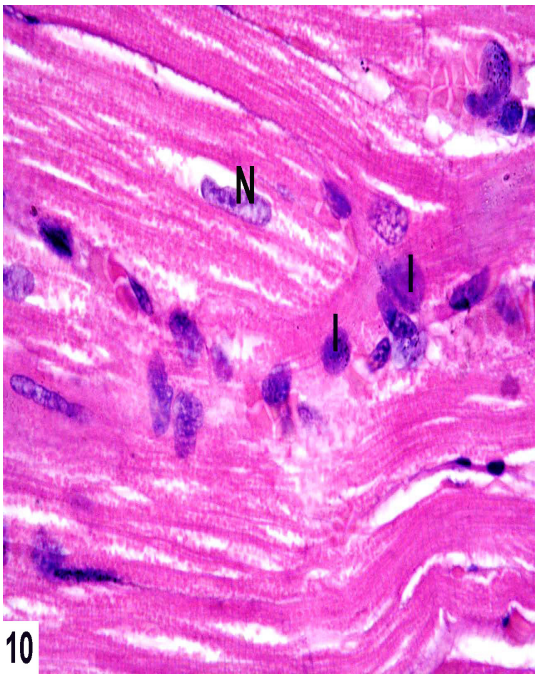

Fig. 10: A photomicrograph of a section of myocardium of an albino rat from Group IV (ISO and curcumin-treated group) showing the sound organization of the muscle fibers with central oval vesicular nuclei (N). Minimal mononuclear cell infiltration (I) can be seen. Hx. & E.; X 1,000

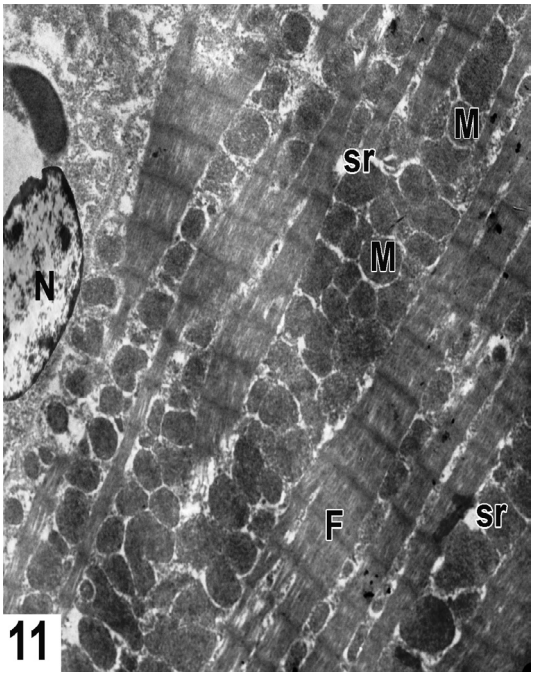

Fig. 11: An electron micrograph of the myocardium of a control albino rat (Group I) exhibiting the usual histological structure of the myofibrils (F) with many mitochondria (M) in between and the cisternae of sarcoplasmic reticulum (sr). The nucleus (N) shows evenly dispersed chromatin. TEM X 5,000

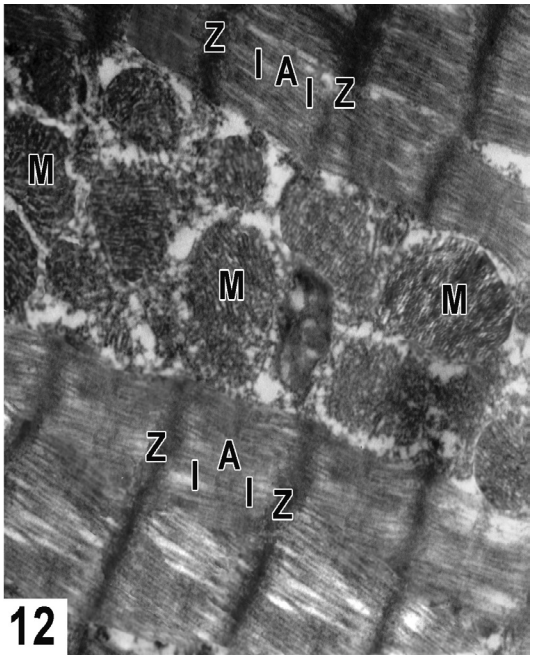

Fig. 12: An electron micrograph of the myocardium of a control albino rat (Group I) showing the usual histological structure of the myofibrils with alternating dark (A) and light (I) bands. Regular Z lines (Z) appear in the middle of the I bands. Many mitochondria (M) are seen between the myofibrils. TEM X 15,000

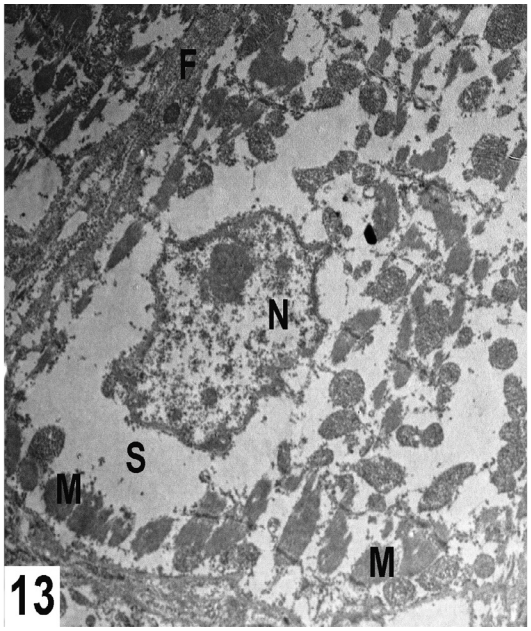

Fig. 13: An electron micrograph of the myocardium of an albino rat from group III (ISO-treated group) exhibiting distortion of the nucleus (N). The myofibrils (F) appear severely interrupted and fragmented with no distinction between dark and light bands and obscured Z lines. The mitochondria (M) appear variable in size and shape but mostly swollen. Severe separation (S) between the myofibrils and mitochondria can be seen. TEM X 5,000

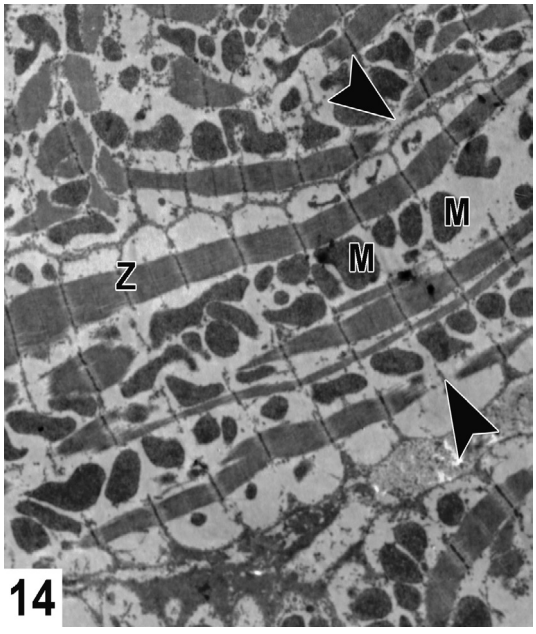

Fig. 14: An electron micrograph of the myocardium of an albino rat from group III (ISO-treated group) presenting disruption of myocardial fibers (arrowhead) with focal preservation of Z lines (Z). The mitochondria (M) appear variable in size and shape but less in number compared to control sections. TEM X 5,000

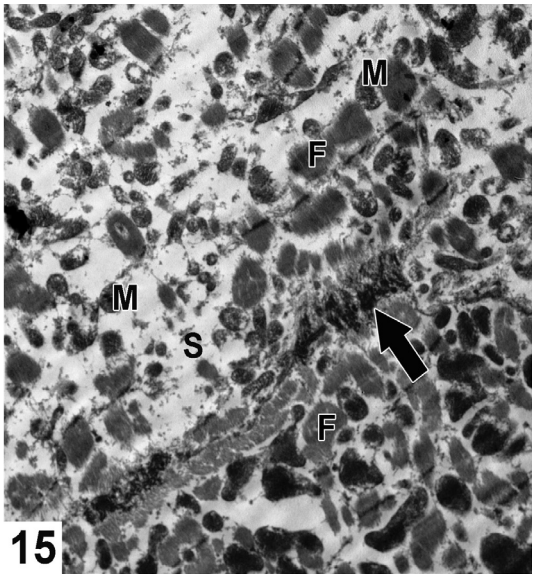

Fig. 15: An electron micrograph of the myocardium of an albino rat from Group III (ISO-treated group) exhibiting severe disruption of myocardial fibers (F) with no distinction between dark and light bands and obscured Z lines. The mitochondria (M) appear variable in size and shape and less in number compared to control sections. Severe separation between the myofibrils and mitochondria can be seen (S). Intercalated disks (arrow) can be observed. TEM X 5,000

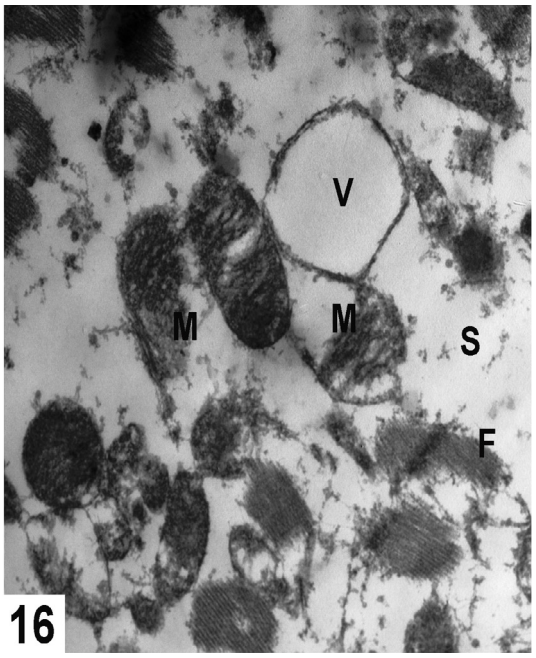

Fig. 16: An electron micrograph of the myocardium of rat from Group III (ISO-treated group) showing disruption of myocardial fibers (F). The mitochondria (M) appear swollen, ruptured and less in number compared to control sections. Severe separation (S) and a vacuole (V) between the myofibrils and mitochondria can be seen. TEM X 15,000

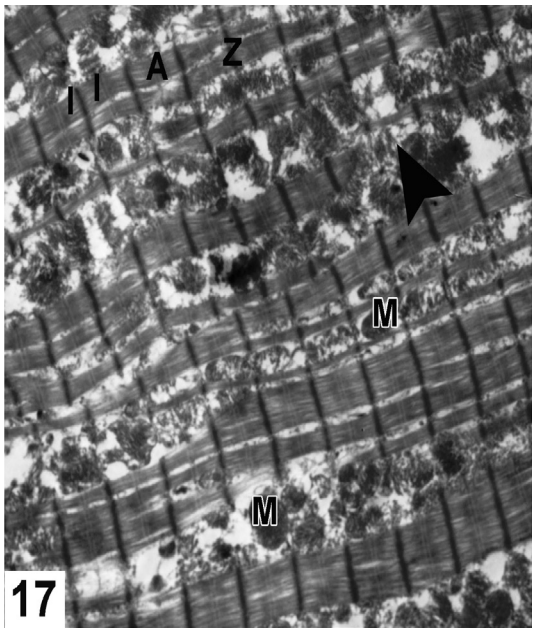

Fig. 17: An electron micrograph of the myocardium of an albino rat from Group IV (ISO and curcumin-treated group) exhibiting regular arrangement of the myofibrils with alternating dark (A) and light (I) bands. Regular Z lines (Z) appear in the middle of I bands. Areas of disrupted fibrils (arrowhead) appear. The mitochondria (M) are of number similar to control group but they are swollen and with ruptured membranes. TEM X 5,000

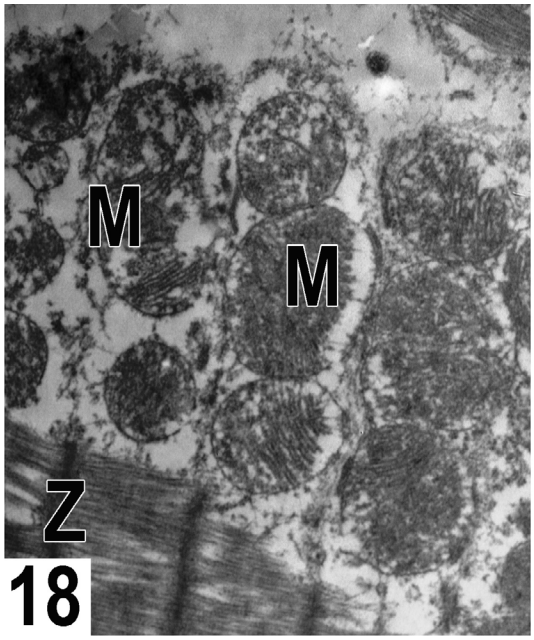

Fig. 18: An electron micrograph of the myocardium of an albino rat from Group IV (ISO and curcumin-treated group) showing myocardial fibers with regular Z lines (Z). The mitochondria (M) appear swollen with ruptured membranes and cristae. TEM X 15,000

DISCUSSION

Myocardial Infarction (MI) is one of the most common killer cardiovascular diseases *(Ganesan & Anandan, 2009)*. So the aim of this study was to evaluate the possible cardioprotective effect of curcumin due to its natural antioxidant and anti-inflammatory properties against experimental MI induced by ISO.

Isoproterenol injection used in the present work to obtain an animal model of acute MI is considered the method of choice. Other methods of experimental MI as coronary artery ligation have a number of technical difficulties associated with invasive procedure that requires a skilled operator. Removal of the pericardium, to access the heart, increases the susceptibility of infection *(Heather et al., 2009)*.

Isoproterenol-induced MI in the current study was confirmed biochemically by significant elevation of plasma cardiac enzyme markers Creatine kinase and Creatine kinase–MB isoenzyme. The choice of CK and CK–MB was after *Suchalatha and Shyamala-Devi (2004)* who suggested that the myocardium contained an abundant amount of diagnostic marker enzymes for MI and once metabolically damaged, it released its intracellular contents into the extracellular fluid and blood. Moreover, *Sabeena-Farvin et al. (2004)* found that the serum level of CK and CK–MB reflected the alterations in membrane integrity and/or permeability and this is the best way to estimate the extent of infarction.

Inner one third of the left ventricular wall was selected in the current work as it was mentioned by *Kemi et al. (1996)* that this area was the most sensitive region to hypoxia and ischemic conditions of the heart. They studied ISO as a cardiotoxic compound.

In the present work, light microscopic examination of the left ventricular wall of ISO-treated group revealed many alterations. Areas of wavy muscle fibers appeared in the present work. This finding was described previously by *Naik et al. (2007)* who found that the wavy fibers were seen one to three hours after irreversible ischemic injury. They added that this was the earliest change seen on routine H&E histological sections. These wavy fibers might be due to intercellular edema separating the dead myocytes as the surrounding normal contracting myocardium pulls on them. Foci of separation and fragmentation of the muscle fibers in the current study were observed by *Zhao et al. (2009)* who studied acute MI after ligation of left anterior descending coronary artery in rats.

Some of the fragmented muscle fibers in the present study lost their nuclei while others revealed pyknotic nuclei. These results are in correspondence with those of *Naik et al. (2007)* who mentioned that pyknotic nuclei took place and nuclei were lost 18 hours and 2-4 days after MI respectively. Moreover, *Ouyang et al. (2010)* found pyknotic nuclei on examining MI in human autopsy specimens.

The mononuclear cell infiltration, observed in the present work, was described previously by *Naik et al. (2007)* as neutrophilic infiltration. They added that this infiltration began after four to 12 hours of MI and peaked at two to four days after MI. Moreover, *Nef et al. (2007)* portrayed this infiltration on studying stress-induced cardiomyopathy. Furthermore, *Heather et al. (2009)* narrated this infiltration on examining cardiac hypertrophy after chronic ISO administration in rats. In the present study, hemorrhage was evident after ISO administration. This is in correspondence with the findings observed by *Gavira et al. (2006)* who compared the efficacy of surgical versus percutaneous administration of skeletal myoblasts in a swine model of chronic MI.

In the present study, severe edema and cellular homogenicity suggestive of hyaline degeneration were found. This edema and hyaline degeneration was explained by *Naik et al. (2007)* who found irreversible cellular injury after 20-24 minutes of myocardial ischemia. The cells appeared with leaky cell membranes and release of proteolytic enzymes and other molecules including troponin in the myocardial interstitium. They added also that the increased vascular permeability led to increased intercellular oncotic pressure and intercellular edema. The same was regarded by *Ouyang et al. (2010)* who examined MI in human autopsy specimens.

Isoprotrenol administration resulted in many electron microscopic changes in the ultrathin sections of the left ventricle. There was distortion of the nuclei. The myofibrils appeared

severely interrupted and fragmented or disrupted. There was no distinction between dark and light bands. The Z lines appeared obscured or focally preserved. The same findings were stated by *Zhao et al. (2009)* who mentioned that these ultrathin findings were found four hours and increased markedly six hours after experimental acute MI with ligation of left anterior descending coronary artery in rats.

In the present work, mitochondria appeared variable in size and shape. They were swollen and less in number compared to control sections. Similar findings were observed by *Laky and Parascan (2007)* who found small mitochondria in chronic MI. Moreover, *Tissier et al. (2009)* narrated wide-spread irreversible ischaemic damage to the mitochondria characterized by membrane rupture and amorphous densities after chronic coronary artery occlusion in rabbits. Furthermore, *Zhao et al. (2009)* described degenerated mitochondria with ruptured and disrupted cristae in acute MI in rats.

In the current study, severe separation and vacuolations appeared between the myofibrils and mitochondria. This finding was also observed by *Tissier et al. (2009)* who portrayed separation and intercellular edema in chronic MI in rabbits. Moreover, *Zhang et al. (2009)* found autophagic vacuoles in cardiac myocytes, 24 hours after coronary artery ligation in rats. They added that the number of these vacuoles in the region bordering the infarction was significantly greater as compared with that in the infarction and the normal regions.

Degenerative changes in ISO-induced MI in the present work was explained by *Lowenstein (2004)* who attributed MI to oxidation of catecholamine and generation of excessive amounts of highly cytotoxic free radicals. They added that these reactive free radicals have the potential to injure the cardiomyocytes directly and may be also involved in triggering inflammatory cascades through the induction of cytokines regulating leucocyte trafficking. *Nguewa et al. (2005)* also suggested that following MI, Poly ADP-ribose polymerase (PARP) increased. This PARP has been synthesized in the nucleus and released into the cytoplasm where it led to Apoptosis Inducing Factor (AIF) release from mitochondria, which then translocates to the nucleus. Moreover, *Petermann et al. (2005)* found that PARP has a role in DNA repair, regulation of cytoskeletal organization, expression of various proteins and genes and apoptosis. Furthermore, *Erdelyi et al. (2005)* proposed that excessive activation of PARP in response to inflammation or oxidation, led to mitochondrial injury, cellular energy failure and death.

Administration of curcumin before ISO in the current study decreased significantly the serum marker enzymes CK and CK-MB which were elevated by ISO alone but still high as compared to the control group. Moreover, curcumin greatly minimized the degenerative changes induced by ISO alone. The muscle fibers exhibited the sound organization similar to that of the control sections and minimal mononuclear cell infiltration. Some cardiomyocytes revealed pyknotic nuclei. Electron microscopic examination showed regular arrangement of the myofibrils with alternating dark and light bands. Regular Z lines appeared in the middle of I bands. The mitochondria were of a number similar to the control group but they were swollen and with ruptured membranes and cristae. These findings are in correspondence to those of *Srivastava et al. (1985)* who examined the biochemical effect of curcumin on myocardial ischemia induced by the ligation of the left descending coronary artery in rats. These authors found an increase in blood level of glutathione (GSH), malonaldialdehyde (MDA), myeloperoxidase (MPO), superoxide dismutase (SOD), catalase (CAT) and lactate dehydrogenase (LDH). Moreover, *Manikandan et al. (2004)* investigated t he biochemical protective effect of curcumin against isoprenaline-induced myocardial ischemia in rat myocardium. They found that curcumin protected rat myocardium against ischemic insult.

The protective effect of curcumin against ISO-induced MI in the present study may be explained by its reduction of the oxidative stress *(Quiles et al. 2002)*. Moreover, *Mach (2005)* stated that inflammation and oxidant stress contributed to atherosclerosis. *Ansell (2007)* added that atherosclerosis affected lipoproteins, the walls of blood vessels and cellular membranes leading to myocardial ischemia, hypertension and re-stenosis after angioplasty. Furthermore, *Morimoto et al. (2008)* found that curcumin could protect against cardiac hypertrophy, inflammation and fibrosis.

In conclusion, curcumin preserved the normal histological architecture of the heart and minimized elevation of plasma cardiac enzyme markers CK and CK–MB following experimental myocardial injury by ISO administration. This beneficial effect of curcumin was mostly related to its antioxidant and anti-inflammatory properties. Curcumin proved to be both effective and safe. The results of the present investigation may trigger an interest in using curcumin to prevent MI in high risk individuals.

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التأثير الوقائي للكركم على احتشاء عضلة القلب المستحدث بواسطة الايزوبروترينول في ذكر الجرذ الأبيض البالغ

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ملخص البحث

يعتبر احتشاء عضلة القلب أحد أمراض القلب و الأوعية الدموية الأكثر شيوعاً. وقد كان الكركم يستعملَ في الطبِّ الصيني والهندي التقليدي لمُعَالَجَة عدة أمراضٍ. وهو آمنٌ و فعّالٍ بخواصه المانعِة للتأكسد والمضادة للالتهاب. وقد استهدفت هذه الدراسةِ تَوضيح الدورِ الوقائي المحتملِ لتعاطى الكركم عن طريق الفم على احتشاء عضلة القلب المستحدث بواسطة الايزوبروترينول باستخدام التقنيات الكيميائية الحيويةِ والهستولوجية.

وقد أجريت هذه الدراسةِ على إثنين وثلاثين جرذِ أبيض بالغِ ذكرٍ، تم تقسّيمَها إلى أربع مجموعاتِ متساويةِ. المجمّوعة الأولى مجموعة ضابطة طبيعية. المجموعة الثّانية مجموعة ضابطة زائفة. قد قُسّمت المجموعة الثّانية إلى مجموعتين فرعيتين: IIa & IIb (أربعة جرذانِ لكُلّ مجموعة) تم إعطائها ماءً مُقَطَّر وكركم على الترتيب. المجموعة الثّالثة: تم حقنها بالأيزوبروترينول. المجموعة الرّابعة: تم إعطائها كركم و ايزوبروترينول. تم التضحية بجرذان كُلّ المجموعات يوم واحد بعد الجرعةِ الأخيرةِ للأيزوبروترينول. وقد تم قياس الإنزيمات القلبيةً (CK & CK-MB) بالدم وتم فحص عينات مِنْ البطينِ الأيسرِ بالمجهرين الضوئي و الألكتروني.

في المجموعة المعالجة بالأيزوبروترينول مُقَارَنِة بالمجموعات CK & CK-MB وقد كان هناك زيادةُ ذات دلالة إحصائية في الإ الضابطة الطبيعية و الزائفة. وقد أدى إعطاء الكركم قبل الأيزوبروترينول إلى نَقص ملحوظ في هذه الإنزيماتِ.كما أدّى إعطاء الأيزوبروترينول إلى ظهور بؤرِ من التباعد وتجزؤِ لألياف العضلات بدون تمييزِ بين الشريطين الداكن و الفاتح وقد كان هناك طمسا . و قد فَقدتَ بعض الخلايا القلبية المُمَزَّقة أنويتها بينما ظهرت بعض الأنوية صغيرة داكنة أَو مشوهة. وقد كان هناك ارتشاحZلخطوط للخلايا أحادية النواةِ و مناطق للنزفِ وتورما شديدا و تجانسا خلويا. وقد بَدت الميتوكوندريا متباينة الحجمِ والشكلِ وظهرت منتفخة و أقل في العدد مقارنة بالمجموعات الضابطة. وأدى تعاطى الكركم قبل الأيزوبروترينول إلى تنظيم سوى فى الألياف العضليةً مشابها لقطاعات المجموعات الضابطة مع ارتشاح محدود للخلايا أحادية النواةِ و قد ظهرت بعض ألياف القلب بأنوية صغيرة داكنة و ظهرت الميتوكوندريا بعدد مشابه للمجموعة الضابطة و لكنها كانت منتفخة و بأغشية و أعراف محطمة. ويمكن استنتاج أن إعطاء الكركم قد قلل من زيادة دلالات انزيمات القلب (CK & CK-MB) بالدم بعد الأحتشاء التجريبى بعضلة القلب المستحدث بالأيزوبروترينول و هذا التأثير المفيد غالبا يرجع الى صفات الكركم المضادة للأكسدة و الألتهاب.