



Research Article

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Possible Effect of Natural Honey on Isoproterenol Induced Acute Myocardial Infarction in Adult Male Albino Rats



Marwa A Omar*, Fatma El-Nabawia, A El-Safty, Wael B El-kholy and Abeer E El-Mehi

Department Anatomy and Embryology, Faculty of Medicine, Menoufia University, Menoufia, Egypt

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*Corresponding author: Marwa Abd El-Rasoul Omar, Department Anatomy and Embryology, Faculty of Medicine, Menoufia University, Shebin El-Kom City, Menoufia Governate, Egypt, Tel: 002-01062023766; Email: marwao1433@gmail.com

Abstract

Objective: This study was undertaken to evaluate the effect of natural honey in acute myocardial infarction.

Background: Being a global health problem, there is an increasing demand to use natural cheap products to decrease the incidence and financial burden of acute myocardial infarction. This work was designated to evaluate the effect of natural honey before and after induction of acute Myocardial Infarction (AMI).

Methods: Blood samples were collected and myocardial specimens were processed for ordinary histological as well as semithin sections. Quantitative immunohistochemical assessments of the expression of Caspase-3 and Troponin T were performed.

Results: Our results showed that AMI caused edema and dilated congested blood vessels in the myocardium. Moreover, there was a significant increase in creatine kinase MB and a significant decrease in catalase activity. There was significantly increased expression of caspase-3 and reduced Troponin T color intensity. Oral administration of honey before, and not after, AMI induction revealed improvement in all parameters towards normal.

Conclusion: The results of our work added to the known beneficial effects of natural honey being a cardio protective agent in acute myocardial infarction.

Keywords: Acute myocardial infarction; Rat; Heart; Isoproterenol; Natural honey

Abbreviations: AMI: Acute Myocardial Infarction; ISO: Isoproterenol; SD: Standard Deviation; ANOVA: Analysis of Variance

Introduction

Acute myocardial infarction (AMI) is the main cause of death worldwide [1]. It is the acute condition of necrosis of the myocardium that occurs due to an imbalance between myocardial demands coronary blood supplies [2]. The accumulations of free radicals have been implicated in the pathophysiology of AMI [2]. Ischemia occurring during AMI is a potential cause of increased free radicals [3]. Myocardial injury in rat was induced using Isoproterenol (ISO), a synthetic β -adrenergic agonist, as a result of causing disturbance in the balance between production of free radicals and antioxidative defense system [4]. Honey, a natural liquid produced by honey bees, has received a considerable attention recently because it contains different substances that are believed to have a therapeutic role in cardiovascular diseases [5].

Materials and Methods

Materials

- Isoproterenol hydrochloride (Sigma-Aldrich chemical Co., St. Louis. Mo. (USA). It was available in the form of white powder, bottle 500mg.
- Sider Mountain Honey (The Egyptian Co. (13 Dr. Mohamed Kamel Hussein St, Elnozha Elgedida, Cairo, Egypt). It was available in the form of a brown viscid liquid, glass container 180g.
- CK-M and Catalase enzyme kits (Bio Systems S.A. Costa Brava Co., Barcelona (Spain).
- Caspase-3 and Troponin T immune marker (Thermo Scientific Co., 93-96 Chadwick Road, Astmoor, Cheshire, UK).

Animals

This study included adult male albino rats of Sprague-Dawley strain weighting about 200 g. They were housed in the animal house of the Faculty of Medicine, Menoufia University ($25 \pm 2^\circ\text{C}$, 50% humidity and 12 h light/dark illumination). They had free access of diet and tap water. Strict care and hygiene were provided to keep them in normal and healthy conditions. All experimental procedures were conducted in accordance with the international regulations on care and use of laboratory animals.

Experimental design

After an acclimatization period for about one week, rats were divided into four groups as follows:

Group I (control group): included 6 rats that were kept without any treatment until the end of the study and served as a control for all studied groups.

Group II (Honey treated group): included 6 rats that received a daily dose of 0.8g/kg.bw of natural honey dissolved in distilled water orally by gavage for one month [6].

Group III: (Isoproterenol treated group): included 6 rats that received a single intraperitoneal injection of 1ml of 0.9% normal saline daily for one month in addition to an intraperitoneal injection of isoproterenol hydrochloride (85 mg/kg.bw dissolved in 1 ml of 0.9% normal saline) once daily on the 29th & 30th days at an interval of 24 hours [3]. They were sacrificed 24 hours after the last dose.

Group IV: (Isoprotrenol and Honey treated group): included 12 rats subdivided into 2 subgroups (6 each) as follows:

a. Subgroup IVa: received a daily dose of 0.8g/kg.bw of natural honey dissolved in distilled water orally by gavage for one month in addition to an intraperitoneal injection of isoproterenol hydrochloride (85mg/kg.bw dissolved in 1 ml of 0.9% normal saline) once daily on the 29th& 30th days at an interval of 24 hours. They were sacrificed 24 hours after the last dose.

b. Subgroup IVb: received an intraperitoneal injection of isoproterenol hydrochloride (85mg/kg.bw dissolved in 1 ml of 0.9% normal saline) once daily for 2 days at an interval of 24 hours then received a daily dose of 0.8g/kg.bw of natural honey dissolved in distilled water orally by gavage for one month.

Preparation of Honey

100g of pure natural honey was added into 625ml of distilled water, and a homogenous solution was obtained by continuous mixing and shaking with the help of a machine. The concentration of honey in the final solution was 0.8g/5mL and this solution was stored at room temperature. The final concentration of the solution was adjusted according to the body weight of each rat (0.8g per kg of rat body weight). Honey was given orally by

gavage to each rat in the desired group. All rats were observed for physical activity. Any cases of death were recorded.

At the end of the study, rats were anaesthetized by diethyl ether inhalation. Blood samples were collected from the retro-orbital sinus using heparinized microcapillary tubes and then collected in clean test tubes. Serum was separated by centrifugation at 3000 rpm for 15 minutes and used within 48 hours for estimation of: CK-MB and Catalase, Rats were then sacrificed by cervical dislocation. The thorax of each animal was opened and the heart was dissected out and washed with physiological saline. The heart tissue near the apex was subdivided transversally into two parts for histological and immunohistochemical studies.

Methods

Histological studies: The specimen for histological studies was further subdivided into two parts:

- The first part was used for preparation of semithin sections as follows: It was cut into transverse sections 1-2 mm in diameter using blocked razor plate on wax sheet immersion. Tissue was post fixed in 2% glutaraldehyde (in 0.1 M sodium Cacodylate buffer) over night and then further processed. Tissue was rinsed in sodium cacodylate buffer, stained with 2% aqueous uranyl acetate and lead citrate, dehydrated with graded ethanol up to 100%, rinsed with propylene oxide and embedded in 100% Epon. Semithin sections (0.5-1 μm) were stained with toluidine blue stain. This was done at the Faculty of Medicine, Tanta University, Egypt.
- The remaining part of each specimen was cut into small pieces that were fixed in 10% neutral formaline for 24 h then dehydrated in ascending grades of alcohol, cleared and embedded in paraffin. Sections of 3-5 μm thick were cut by microtome and subjected to: Haematoxylin and Eosin stain (Hx&E): to study the general structure of the heart.

Immunohistochemical study: Immunohistochemical reactions were carried out using antibodies to cTnT, and caspase-3 using a standard avidin-biotin-peroxidase system.

- Caspase-3: used for detection of apoptosis.
- Troponin T: for staining of I bands of myocardium.

Morphometric assessment: Data were obtained using Image J analyzer version 1.4308, National institute of health, USA. The following parameters were measured:

- The percentage of the surface area of positive Caspase-3.
- The color intensity of Troponin T.

Statistical analysis: Statistical Analysis was performed for the biochemical and the morphometric results. Data were expressed as mean (\bar{x}) \pm standard deviation (SD) from 5 rats in

each group by Analysis of variance (ANOVA) followed by post hoc test for statistical analysis of the different groups using graph pad 4 instant software and graph pad prism version 6.03 (San Diego, CA, U.S.A). P-value of >0.05 was considered statistically non-significant, <0.05 & <0.001 were considered statistically significant.

Results

General results: Animals of group I, II and IVa showed no change in their physical activity and food intake. Animals of group III and group IVb showed marked decrease in their physical activity and food intake. They were lethargic. One rat died in each of group III and group IVb but there was no mortality among groups I, II and IVa.

Biochemical results: (Figures 1a & 1b)

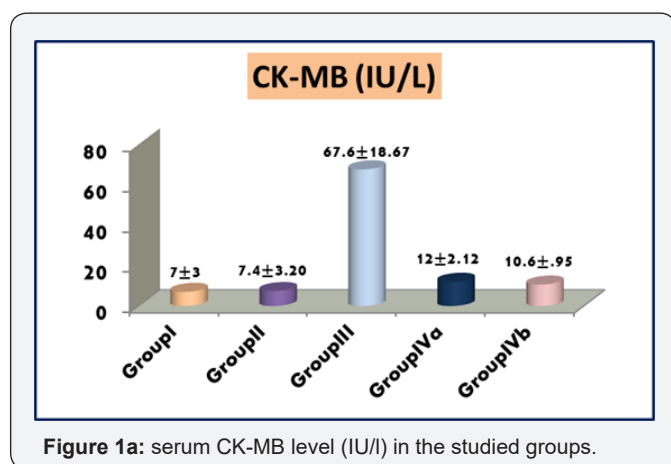


Figure 1a: serum CK-MB level (IU/L) in the studied groups.

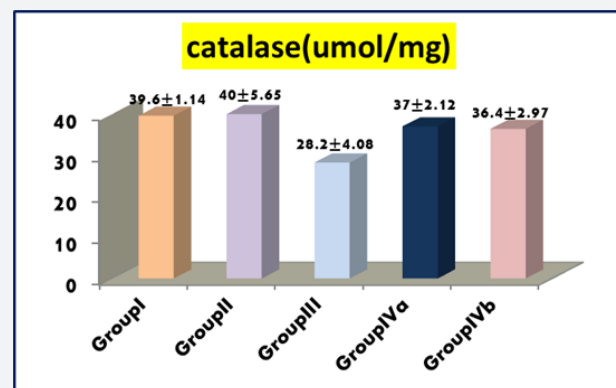


Figure 1b: serum Catalase level (umol/mg) in the studied groups.

A. As regards serum CK-MB, No statistically significant difference was detected between groups I, II, IVa and IVb but there was a significant difference between them & group III being higher in group III (Figure 1a).

B. As regards serum Catalase, no statistically significant difference was detected between groups I, II, IVa and IVb but there was a significant difference between them & group III being lowered in group III (Figure 1b).

Histological and immunohistochemical results: On correlating the histological and immunohistochemical results of group I and group II, there was no significant differences. So group I was considered as the reference one.

Histological results: (Figure 2)

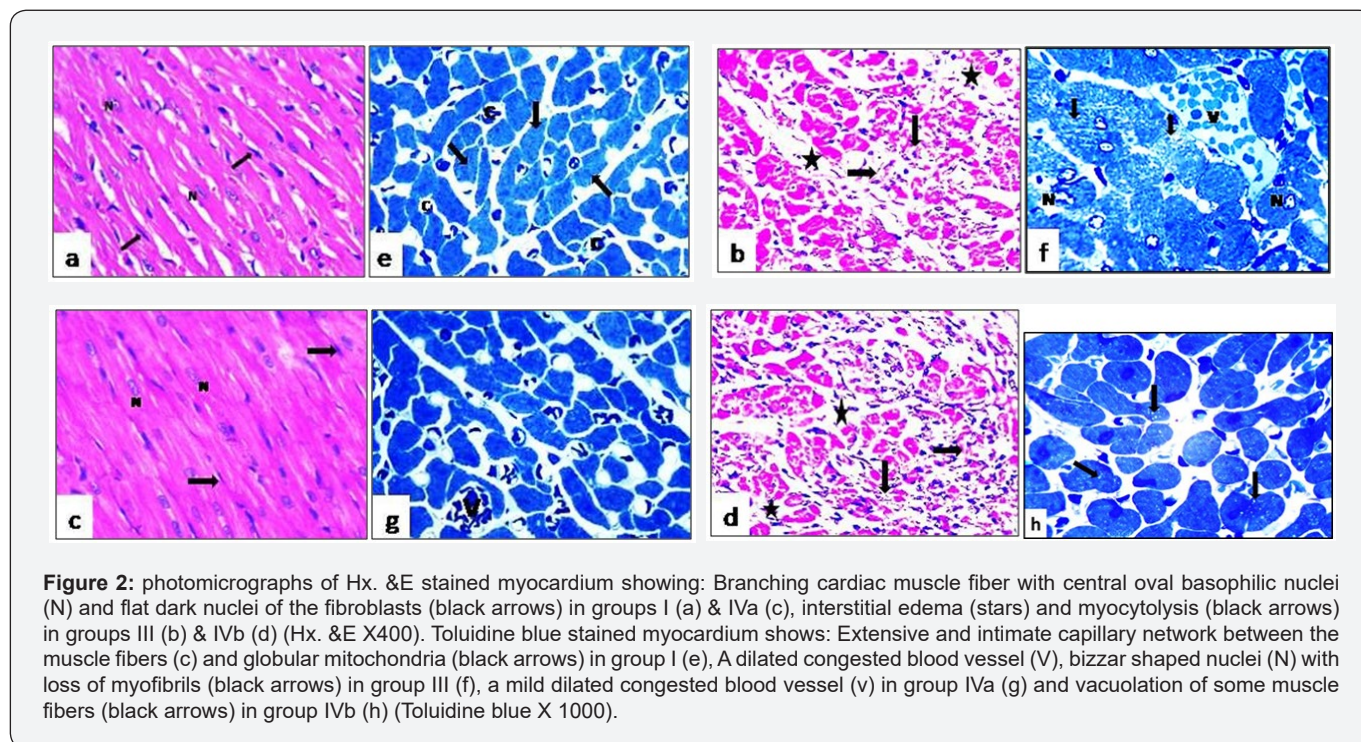


Figure 2: photomicrographs of Hx. & E stained myocardium showing: Branching cardiac muscle fiber with central oval basophilic nuclei (N) and flat dark nuclei of the fibroblasts (black arrows) in groups I (a) & IVa (c), interstitial edema (stars) and myocytolysis (black arrows) in groups III (b) & IVb (d) (Hx. & E X400). Toluidine blue stained myocardium shows: Extensive and intimate capillary network between the muscle fibers (c) and globular mitochondria (black arrows) in group I (e), A dilated congested blood vessel (V), bizzar shaped nuclei (N) with loss of myofibrils (black arrows) in group III (f), a mild dilated congested blood vessel (v) in group IVa (g) and vacuolation of some muscle fibers (black arrows) in group IVb (h) (Toluidine blue X 1000).

A. Hx. &E stain: Group I showed normal organized branching and anastomosing muscle fibers running in different directions, with centrally located oval basophilic nuclei. The flat nuclei of the connective tissue cells could be noticed in the interstitial tissue between the muscle fibers. Group III & Subgroup IVb showed interstitial edema and myocytolysis. Subgroup IVa was similar to group I.

B. Toluidine blue stain: Group I semithin sections showed an extensive and intimate capillary network between the muscle fibers. Globular mitochondria appeared as dark spots between the myofibrils. Group III showed a dilated congested

blood vessel, nuclei with peripherally marginated chromatin and loss of myofibrils. Group IVa showed a mild congested blood vessel with normal appearance of the muscle fibers. Group IVb showed vacuolation of some muscle fibers.

Immunohistochemical results (Figures 3 & 4): Groups I showed a negative Caspase-3 immune reaction and strong troponin T color intensity. Group III & IVb showed a positive Caspase-3 immune reaction in widespread areas and reduced troponin T color intensity. Group IVa showed a limited positive Caspase-3 immune reaction and strong troponin T color intensity (Figures 3 & 4).

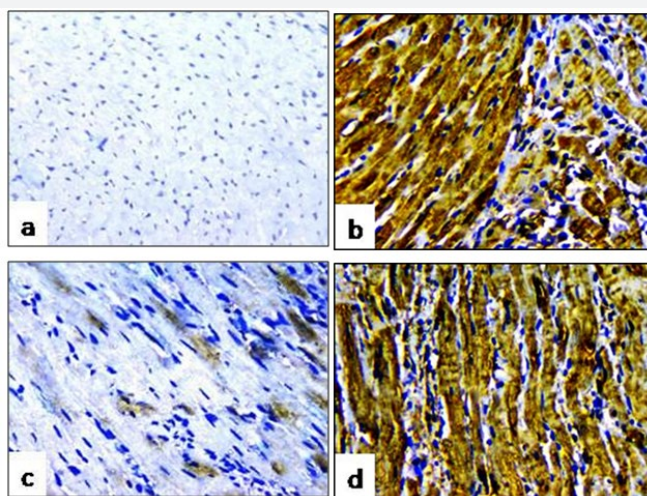


Figure 3: photomicrographs of caspase-3 stained myocardium showing: negative caspase-3 immune reaction in group I (a), positive immune reaction in widespread areas in groups III (b) & IVb (d) and a limited positive immune reaction in group IVa (c) (caspase-3 immune stain X 400).

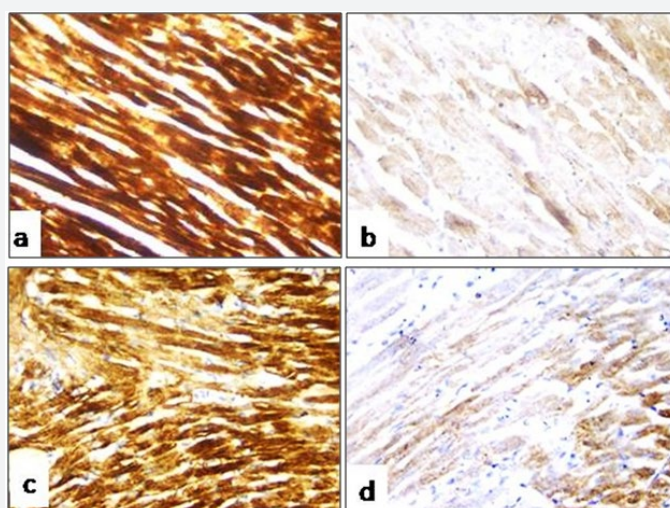


Figure 4: photomicrographs of Troponin T stained myocardium showing: strong color intensity in groups I (a) & IVa (c) but a marked decrease in color intensity in groups III (b) & IVb (d) (Troponin T immune stain X 400).

Morphometric results: (Figures 5a & 5b)

A. As regard Mean area% of Caspase-3, no statistically significant difference was detected between groups I, II and

IVa. Also there was no significant difference between groups III and IVb but a significant increase was detected between them and other groups (Figure 5a).

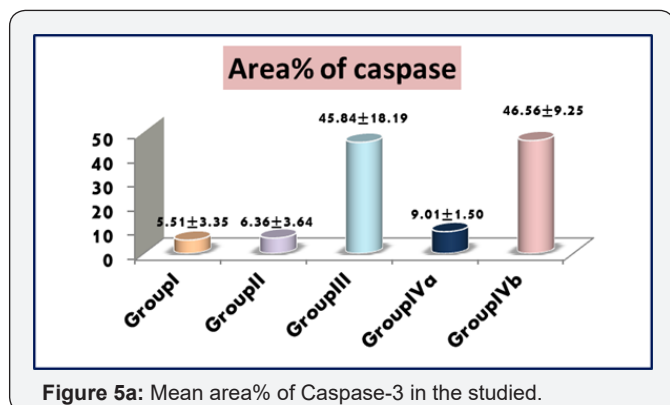


Figure 5a: Mean area% of Caspase-3 in the studied.

B. As regard Troponin T color intensity, no statistically significant difference was detected between groups I, II and IVa. Also there was no significant difference between groups III and IVb but a significant decrease was detected between them and other groups (Figure 5b).

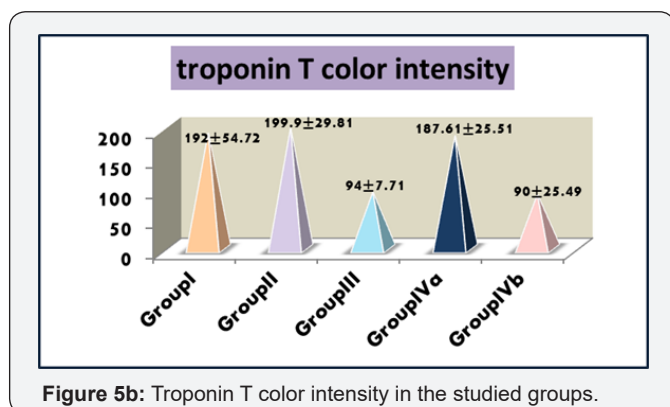


Figure 5b: Troponin T color intensity in the studied groups.

Discussion

The present study was conducted to evaluate the possible effect of natural honey in a model of AMI induced by ISO in adult male albino rats using biochemical, histopathological, immunohistochemical, and morphometric methods. The dose of ISO used in this study (85 mg/kg administered by intraperitoneal injection at 24 h intervals for 2 successive days) was reported to induce AMI in rats. The mechanisms by which ISO causes MI involve its β adrenergic cardio stimulatory activity that increases cardiac oxidative metabolism and therefore decrease oxygen available to the myocyte. Also it involves complex biochemical and structural changes as mentioned by Zhang et al. [7].

Gurram et al. [8] reported that CK-MB serves as a diagnostic marker for MI which leaks out from the heart tissue to blood when cell membrane becomes permeable. In the present study, there was a significant increase in the level of CK-MB in the serum of ISO treated rats in comparison with the control group meaning that cardiac damage has occurred by ISO. Also subsequent to isoproterenol administration, there was a significant reduction in the activity of the antioxidant enzyme Catalase in the serum. This might be due to increase its utilization in the removal of

free radicals produced by the oxidative stress effect of ISO on myocardium. The biochemical results were further confirmed by histological studies that revealed interstitial edema and myocytolysis.

The occurrence of myocardial injury in rats treated with ISO has been explained by Padma et al. [9] who reported that ISO leads to overproduction of reactive oxygen species resulting in an inflammatory response and cardiac toxicity. Concerning the immunohistochemical results, this study demonstrated a great increase in the surface area of caspase-3 immune stain and a reduction in cTnT immune reactivity in ISO treated group. Also the morphometric study showed a significant increase in area % of positive Caspase-3 and a significant decrease in Troponin T color intensity when compared to the control group. These results were supported by Amin et al. [10] who mentioned that loss of cTnT as well as positive staining for caspase-3 occur very soon after ischemic injury.

In this study, rats treated with honey alone showed results similar to that of the control group which may indicate that natural honey is beneficial and safe in cardiac tissues. Using natural honey before ISO, on the biochemical level; caused a significant reduction in the elevated CK-MB to normal. This might be due to the protective effect of honey on myocardium, reducing the myocardial damage and thereby restricting the leakage of these enzymes. Also honey significantly reversed catalase level nearer to normal which might be due to the high level of this enzyme in honey.

The possible mechanisms through which honey supplementation restores antioxidant enzyme function was explained by Khalil et al. [5] who attributed it to the up regulation of the activity of Nrf2, a transcription factor that is released from its repressor (Keap1) under oxidative stress. Nrf2 induces the expression of the antioxidant response element of cytoprotective genes as well as induces free radical scavenging enzymes to eliminate the cytotoxic oxidants. There was improvement in histopathological and immunohistochemical findings which might be due to the antioxidant effect of honey.

Moreover Aman et al. [11] reported that oxidative stress provokes DNA fragmentation and apoptosis and that treatment with antioxidants inhibits them. Conversely, post ischemic administration of natural honey showed no improvements in comparison with ISO group. On the biochemical level, the return of CK-MB to baseline could be explained by the results of Kemp et al. [12] who reported that the CK-MB level returned to baseline 48–72 h after MI. Catalase level was found to return to normal which may be due to the high level of this antioxidant enzyme in honey. This study suggested that the dose of natural honey used was too small to overcome the excessive amount of free radicals generated through the process of infarction that had already started before using honey or this might be due to the very acute onset of the disease.

Based on the results presented, our study concluded that honey proved to have protective but not curative effect in case of AMI. We attributed this cardio protective effect to the antioxidant activity of honey making it working as a free radical scavenger. Further studies are needed to be carried out to isolate the potential chemical constituents of Honey to find out its mechanism of action in the treatment. More studies are recommended to evaluate the possible therapeutic effect of natural honey on myocardial infarction using other doses.

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