

# Effect of nicotine-caffeine interaction on rats coronaries and cardiac muscle

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## Abstract

The influence of nicotine and caffeine interaction at dose levels approximating human consumption on histology of cardiac muscles and coronaries was investigated in rats. Adult male rats were sorted into four groups, GI: control and injected subcutaneously with 2 ml normal saline. GII: received subcutaneous nicotine injection in a dose of (10 mg/kg/body weight). GIII: received daily intravenous injection of caffeine (100 mg/kg). GIV: received a combination of nicotine and caffeine in similar dose and route. Nicotine leads to marked congestion, damage in cardiac muscles, increase in collagen fibers around thickened coronaries. The effect of caffeine produced little change and congestion in cardiac fibers and slight increase in collagen fibers around coronaries. In group 4 caffeine prevent the increase in collagen fibers around coronaries but did not protect cardiac muscles against congestion and damage by nicotine. The present study indicated that nicotine causing structural damage and changes in the histological profile of the cardiac muscles and coronaries of rats and caffeine could modify nicotine effect on coronary walls but ischemic changes in cardiac muscles seemed to be irreversible.

## Introduction

Nicotine and caffeine represent two of the most common pharmacologically active substances used by human. These are responsible for 80% of all diagnoses of cerebrovascular and coronary heart diseases [1-3]. Coronary endothelial dysfunction and development of atherosclerotic changes and risk of cardiac complication and deaths were reported in heavy smokers [4,5]. Caffeine is classified as an addictive substance and consumed all over the world to relieve sense of fatigue, improve mood concentration, insomnia, and increased heart rate [6-8]. This study aimed to document the effect of nicotine-caffeine interaction on histology of coronaries and cardiac muscle of adult male rats.

## Materials and methods

### Pharmacological agents

Nicotine tartrate and caffeine were obtained from Sigma Chemical Co. , Jeddah, Saudi Arabia.

### Animal treatment

**Animals and experimental design:** The present study was conducted at King Fahd Medical Research Center, King Abdulaziz University, and Jeddah, Saudi Arabia on a total number of 24 apparently healthy albino male rats, weighted from 200 to 250 grams. Rats had free access to commercial rat pellets and tap water for one week before experiment. The study was performed according to animal care ethics recommended by the University Committee. These animals were allocated into 4 groups (n=6) as follow:

Group I: control and injected subcutaneously with 2ml normal saline.

Group II: Nicotine group received daily subcutaneous nicotine/saline (10mg/kg/body weight).

Group III: caffeine treated group (received daily intraperitoneally caffeine anhydrous 100mg/kg/ body weight).

Group IV: caffeine and nicotine treated groups given in the same doses and by the same routes as GI & GII. After 4 weeks animals were euthanized by cervical dislocation under deep ether anesthesia. The heart was perfused with normal saline followed by 10% neutral buffered formalin. Cross sections from left ventricles at different levels from the apex were re-fixed in 10% neutral buffered formalin for paraffin processing. 5 micron thick sections were stained by haematoxylin and eosin for general structure. Masson Trichrome was used for collagen fibers. Slides prepared from all groups were examined and photographed [9].

## Results

### Effect on coronaries

In GI (control group) showed that rat cardiac muscles of left

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ventricle possess normal structure described in literature. The nuclei of muscles are oval and vesicular. The fiber cytoplasm is homogeneously stained but the cross striation are ill defined. Capillaries among the fibers are thin wall and compressed. Coronaries showed normal ordered layers. The inner intimal layer showed smooth intact endothelial cells Media was mainly made of elastic fibers with few smooth muscles and collagen fibers. Those two layers' merge together to make one intima-media layer. Adventitia or the most outer layer consisted from loose collagen fibers and perivascular tissue is scanty and contains thin fibers and few connective tissue cells. Caffeine on the other hand produced no change in coronary wall thickness. Slight capillary congestion was observed but cardiac muscles did not show any histological changes. In animals administrated combination of nicotine and caffeine, there was no change in coronary wall thickness but cardiac fibers still showed histological changes observed in nicotine group (Figure 1).

### Masson stain and collagen

Masson trichrome stain was used to demonstrate the changes in collagen content around coronaries and any fibrotic changes in cardiac muscles. Nicotine leads to increase in collagen fibers around thickened coronaries. The effect of caffeine showed slight increase in collagen fibers around coronaries. In group 4 caffeine prevent the increase in collagen fibers around coronaries (Figure 2).

### Effect on cardiac muscle

Longitudinal section (LS) of cardiac muscles of controlled rats (G1) showed normal cardiac fibers. Nicotine (GII) showed shrinkage and irregularity of cardiac fibers, caffeine(GIII): produced slight shrinkage in cardiac fibers with mild congestion of interfibrillar capillaries, combination of nicotine and caffeine (GIV) showed that caffeine not protect against nicotine induced histological cardiac changes (Figure

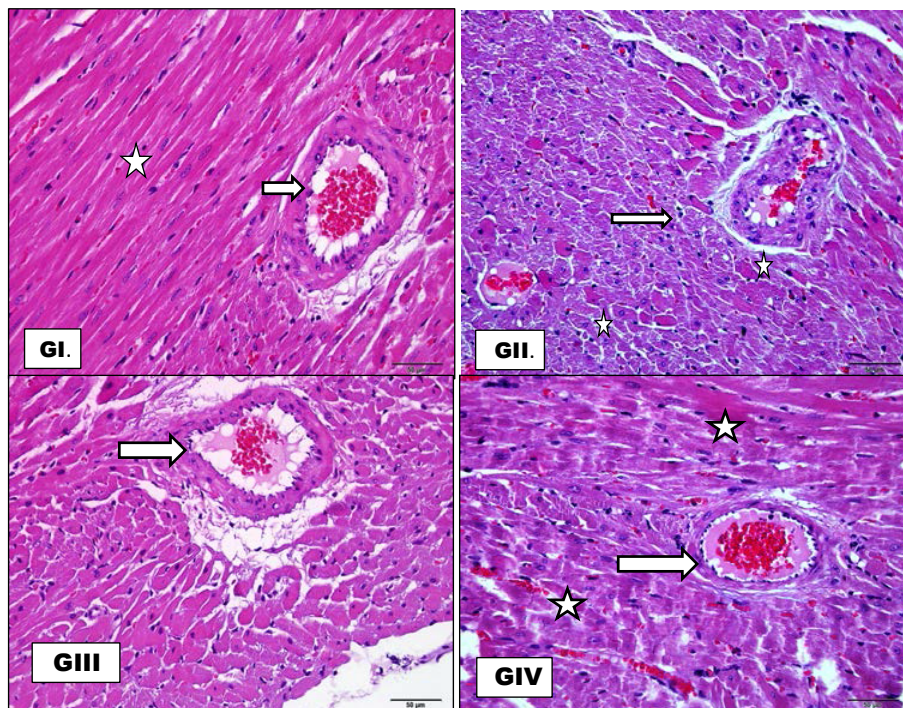
3). Cross section (CS) of cardiac muscles show GI showed regular and normal sized cardiac fibers .GII (nicotine) showed marked vascular congestion( white stars).nearby muscles looked darker and degenerated (black stars) GIII (caffeine) showed no apparent changes in cardiac fibers, while administration of Caffeine in combined with nicotine (GIV) did not protect cardiac muscle from nicotine effect (Figure 4).

### Discussion

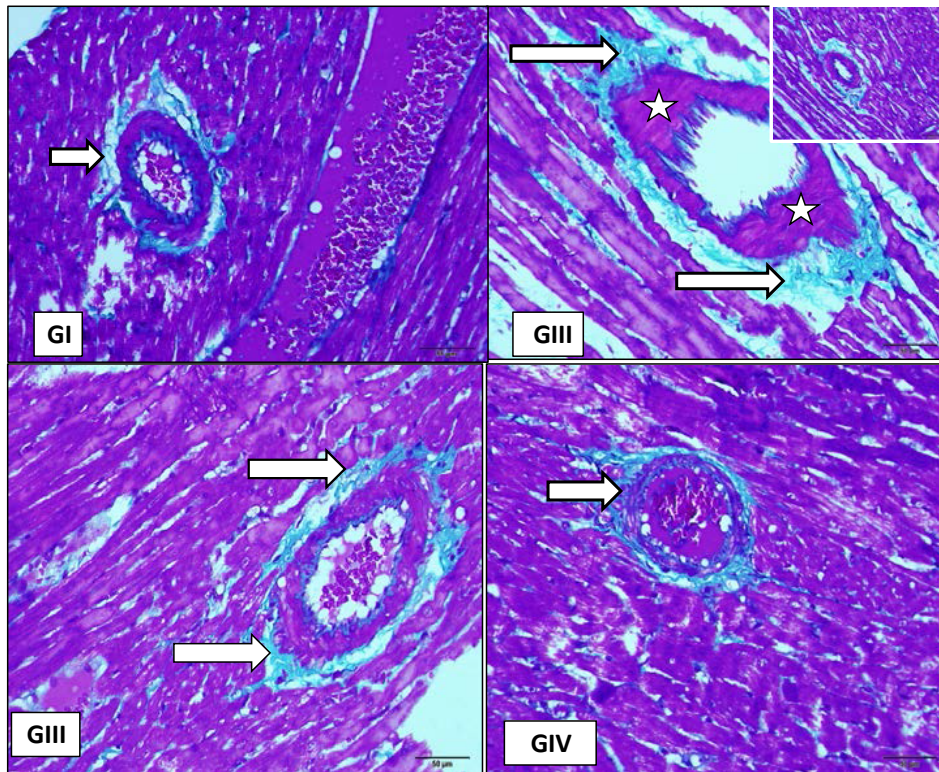
The smoking effects on human health are well known and its impact on vital organs and functions can leads to deaths in many cases especially when was accompanied by cardiovascular complication. Cardiac muscles with their coronaries were the most involved parts [10]. Few studies have examined the effects of tobacco on cardiac muscles and coronaries. The results of this research declared that nicotine have deleterious effects on the heart, and coronaries and structural damage to the histological profile It result in increase in coronary wall thickness and increase perivascular type I collagen deposition as observed by Masson trichrome stain. Cardiac muscles showed numerous foci of apoptotic fibers [11,12].

Damage of coronaries and cardiac muscles could be induced *via* free radicles and oxidative stress known to be induced by nicotine. Nicotine was known to result in sympathetic neural stimulation and systemic catecholamine release [13]. Czernin and Waldherr [14] reported an increase liberation of reactive oxygen upon exposure to nicotine with subsequent injury of vascular endothelium decrease coronary blood flow, increased cardiac metabolic demand and increase risk of myocardial ischemic changes and initiation of atherosclerotic changes. Based on such data one can explain the degenerative apoptotic changes in cardiac observed in nicotine injected rats.

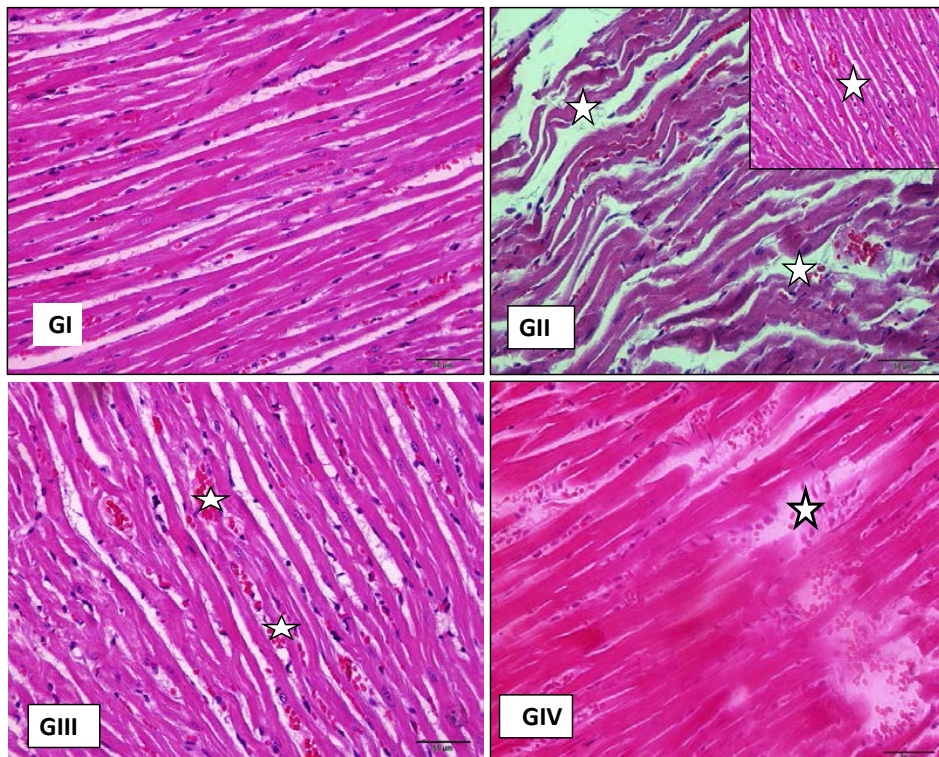
In the present study caffeine administration on the other hand produced no change in coronary wall thickness. or did not alter its



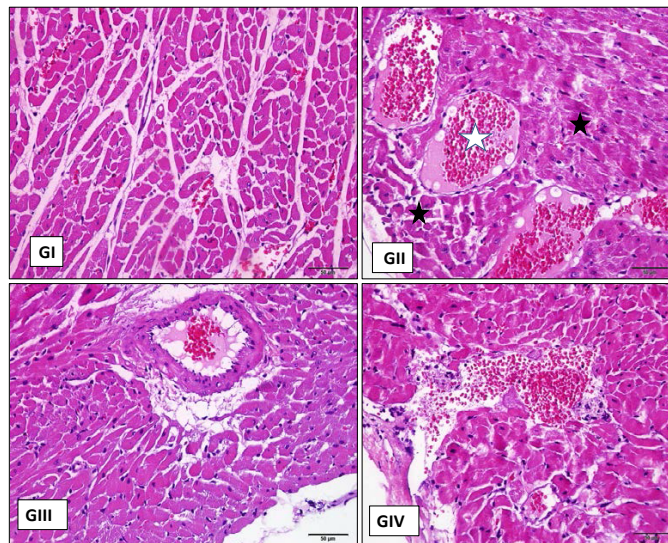
**Figure 1.** Cross section of coronaries, G1: control with normal wall thickness (white arrow) and well organized nearby cardiac muscle fibers ( star). GII:(nicotine) showing increased wall thickness (white arrows). Nearby cardiac fibers (thin black arrows) showed apoptotic changes (dark red shrunken fibers with dark pyknotic nuclei) or tiny lipid droplets (white stars). GIII: Caffeine produced no change in coronary wall thickness (white arrow) or cardiac fibers. GIV: Caffeine combined with nicotine protected coronary from the effect of nicotine but still some degeneration in cardiac muscles (White stars) (H&E x20).



**Figure 2.** Cross section in coronaries G1: control with normal wall thickness (white arrow), GII: Nicotine leads to increased wall thickness (white stars) and increase in collagen fibers around thickened coronaries (white arrows). GIII: caffeine result in slight increase in collagen fibers around coronaries (white arrows), GIV: Caffeine combined with nicotine prevents the increase in collagen fibers around coronaries (Masson Trichrome).



**Figure 3.** LS in cardiac muscles Shown: G1(control): normal cardiac fibers. GII: (nicotine) showed shrinkage and irregularity of cardiac fibers. (GIII): caffeine produced slight shrinkage in cardiac fibers with mild congestion of inter-fibrillar capillaries (white stars) GIV: combination of nicotine and caffeine showed that caffeine not protect against nicotine induced histological cardiac changes, (H&E stain x20).



**Figure 4.** Cross section to show GI: control with regular control and normal sized cardiac fibers .GII : (nicotine) showed marked vascular congestion (white stars) nearby muscles looked darker and degenerated ( black stars) GIII : caffeine with no apparent changes in cardiac fibers. GIV: administration of caffeine in combined with nicotine didnot protect cardiac muscle from nicotine effect (H&E stain x20).

structure, but it resulted in dilation of cardiac interfibrillar capillaries with no apparent histopathological changes [15,16]. Caffeine consumption when combined with nicotine is considered stimulants. In the present study, administration of caffeine together with nicotine result in preservation of coronary wall thickness but cardiac fibers still showed histological changes observed in nicotine group [17]. Higgins and Babu [18] demonstrated increased 10 percent in resting flow-mediated dilatation inside brachial artery after caffeine ingestion. Caffeine's effect on blocking adenosine-induced vasodilatation in the coronary arteries in normal healthy subjects.

Our findings suggest that nicotine leads to marked vascular congestion nearby muscles looked darker and degenerated while caffeine showed no apparent changes in cardiac fibers and sudden deaths were not recorded [13] while Nyska *et al.* [15] demonstrated acute hemorrhagic myocardial necrosis and sudden death of rats exposed to a combination of ephedrine and caffeine.

## Conclusion:

Caffeine could modify nicotine effect on coronary walls but ischemic changes in cardiac muscles seemed to be irreversible.

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