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TOXIC EFFECT OF CAFFEINE ON THE CARDIAC MUSCLE TISSUE OF ALBINO RATS

Aly Khan[1*], Nabeel Mohammad Ghanem[2], Shagufta Ambreen Sheikh[3], Samina Waheed[4] and Tanveer Abbas[5]

[1]*CDRI, Pakistan Agricultural Research Council, University of Karachi, Karachi-75270, Pakistan*
[2]*Department of Medical Laboratories, Faculty of Applied Sciences, Turba Br. Taiz University, Taiz Yemen*
[3]*Microbiology Section, PCSIR Laboratories, Karachi*
[4]*Department of Zoology, University of Karachi, Karachi-75270, Pakistan*
[5]*Department of Microbiology, University of Karachi, Karachi-75270, Pakistan*
[*]Corresponding author: aly.khan@hotmail.com

ABSTRACT

Use of caffeine and other energy increasing compounds have become very popular amongst younger generation over the last few decades. In United States of America caffeine is considered to be one of the most important stimulants which may result in metabolic disorders, cardiovascular diseases, Parkinson disease etc. Caffeine three treatments were tested to observe histological changes, 3 mg/0.5 mL; 9 mg/3 mL and 125 mg/3 mL of distilled water on the cardiac muscles of albino rats. Untreated albino rats were kept as control.

The rats were anesthetized with chloroform 3 mL. In dead and anesthetized rats an incision was made in the abdomen and incised up to neck. Gross sections of heart muscular tissues was observed, and the tissues were taken and cut in longitudinal and transverse pieces and selected for tissue processing, block making, sectioning, mounting, staining, microscopic observation and photography.

The healthy cardiac muscles of albino rats showed normal and centrally arranged nucleus, the cardiac muscle fibres were arranged properly and connective tissue appeared normal. Several histopathological alterations were observed in cardiac tissue of treated albino rats. The cardiac muscle tissue of albino rats treated with 3 mg/0.5 mL of caffeine showed formation of spaces due to shrinkage of muscle fibres. The nucleus became more obvious due to the shrinkage of muscle fibres. In rats treated with 9 mg/3 mL of caffeine showed the hyaline degeneration of the arterial wall. At the same time there were spaces seen in between the muscles indicating atrophy. In rats treated with 125 mg/3 mL of caffeine heart tissue showed atrophy and condensation of tissue muscles fibres surrounding vascular area. The arterial wall showed hyaline degeneration.

Damaged heart tissue observed in the present study was due to different doses of caffeine administrated to the albino rats.

Keywords: Caffeine, cardiac muscle tissue, albino rats, histopathology.

INTRODUCTION

Caffeine a methyl xanthine alkaloid which is a natural compound with stimulant effects and decreases drowsiness. It is found in many drinks such as energy drinks, coffee, soft drinks and tea. Some chocolates also contain caffeine. It is generally safe in low amounts but high levels of use may cause anxiety, digestive issues, insomnia, muscle breakdown, high blood pressure, rapid heart rate, fatigue and addiction (Spritzler, 2023).

O'Neill and Eisner (1990) reported that if the exposure to caffeine was prolonged, the increase of systolic along with contraction was for a short time. Later on removal of caffeine systolic contraction decreased to a level below contract after recovering.

Kendall *et al*. (2014) suggested in their study that caffeine increases heart rate, metabolic rate, fat oxidation, respiratory center outputs and diuresis.

Keisler and Armsey (2006) reported that acute caffeine may cause hyperventilation and cardiac arrhythmia. Pasaoglu *et al*. (2011) reported that nitric oxide (NO) levels in treated and untreated rats with caffeine showed no significant difference.

Kimura (2016) recorded changes in cardiac morphology and function due to high dose of caffeine *in utero* exposure in the FI generation CD-1 mice are characteristics seen in dilated cardiomyopathy in human which include dilated ventricular cavity and systolic dysfunction. Fredholm (1995) suggested that caffeine is one of the most common non-selective adenosine receptor antagonist.

This study was designed to investigate the effect of caffeine on cardiac muscle tissue of albino rats.

MATERIALS AND METHODS

Male albino rats obtained from Animal house, University of Karachi were used for this study. The rats were housed in plastic cages in a well-ventilated animal house with 12 h light and dark cycle. Human care was received to all the animals according to standard guidelines. The study protocol was accepted by the Zoology department committee on Animal and local ethics. The rats were fed with pelleted rat chow and distilled water.

The compound used in the experiment was purchased from Lahore Pharma (Caffeine citrate). The treatments tested to observe histopathological changes were 3 mg/0.5 mL; 9 mg/3 mL and 125 mg/3 mL of caffeine in distilled water. There were three replicates of each treatment. Control albino rats were given distilled water.

The dosages to the male albino rats via oral route through gastric feeding needle. Feeding needle with a ball tip was used to prevent introduction of needle into trachea and prevent trauma of oral cavity.

As soon as the animal died, cardiac tissue were quickly removed, washed in distilled water and frozen in refrigerator. Later on, incision was made in the abdomen and incised up to neck in both anesthetized with chloroform (3 mL) animals and dead animals. Gross section of heart muscular tissue were observed, selected for processing, block making, sectioning, mounting, staining with hematoxylin and eosin, microscopic examination and finally photography.

RESULTS

The tested animals were continuously monitored after treatment. Rats treated with acute dose of caffeine showed marked changes. After 90 minutes the rats started getting convulsion with high snoring and difficulty in breathing. Moreover, the body developed bluish color, especially on extremities i.e. forelimbs and hind limbs. The animals treated remained unconscious for 30 minutes and then died.

After dissecting the gross examination of the animals showed marked pathological changes. Tissue of healthy untreated rats showed no abnormalities (Fig. 1). In rats treated with (3 mg/0.5 mL) the cardiac tissue showed shrinkage of muscles leaving, large cavities in between. Due to shrinkage the nuclei of muscle fibre became prominent (Fig. 2). Condensation of muscle fibre was also observed. At a relatively higher dose (9 mg/3 mL) the blood vessels affected, losing their normal structure of various layers and converted into indistinct tissue with numerous chromatin particles. This indicated hyaline degeneration of heart vessel (Fig. 3).

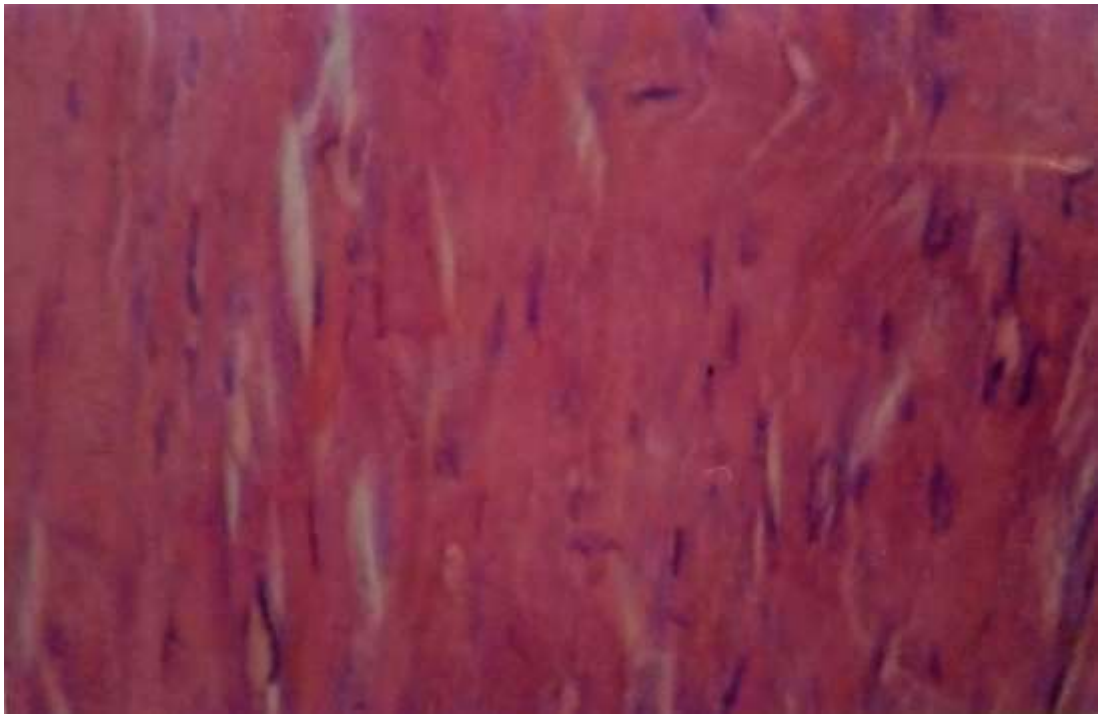

Fig. 1. Longitudinal section of cardiac muscle tissue of (untreated) healthy albino rat (x 200) (H & E stain).

Empty spaces of variable size were seen near the vessel which was due to atrophy of muscles. Muscular condensation was also observed and individual muscle fibres were not distinct. At an acute dose (125 mg/3 mL) of caffeine, blocking as well as obliteration of muscle was observed. A large vessel was completely blocked. The tissue surrounding this area showed atrophy and condensation of muscles (Fig. 4). The arterial wall showed hyaline degeneration. In other section pale and dark areas were observed. Yellowish-brown pigment lipofuscin tend to accumulate in many tissue. The muscle fibres are narrow and atrophic (Fig. 5). In pale areas, nuclei was hardly visible while in darker stained areas, eosinophilic condensed muscle fibres were seen. Empty spaces were prominent.

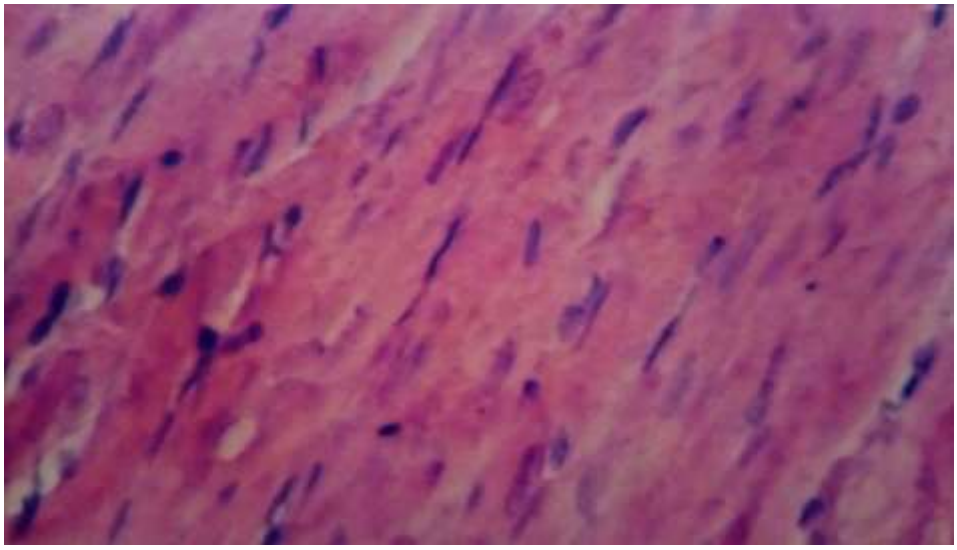

Fig. 2. Longitudinal section of cardiac muscle tissue of orally treated albino rats with 3 mg/0.5 mL of caffeine. Formation of spaces due to shrinkage of muscle fibres is obvious. Nuclei become more obvious due to shrinkage and condensation of muscle fibre (x 200) (H & E stain).

Fig. 3. Transverse section of the cardiac muscle tissue of orally treated albino rat with 9 mg/3 mL of caffeine showing hyaline (H) degeneration of arterial wall. There were spaces (S) in between muscles indicating atrophy (x 50) (H & E stain).

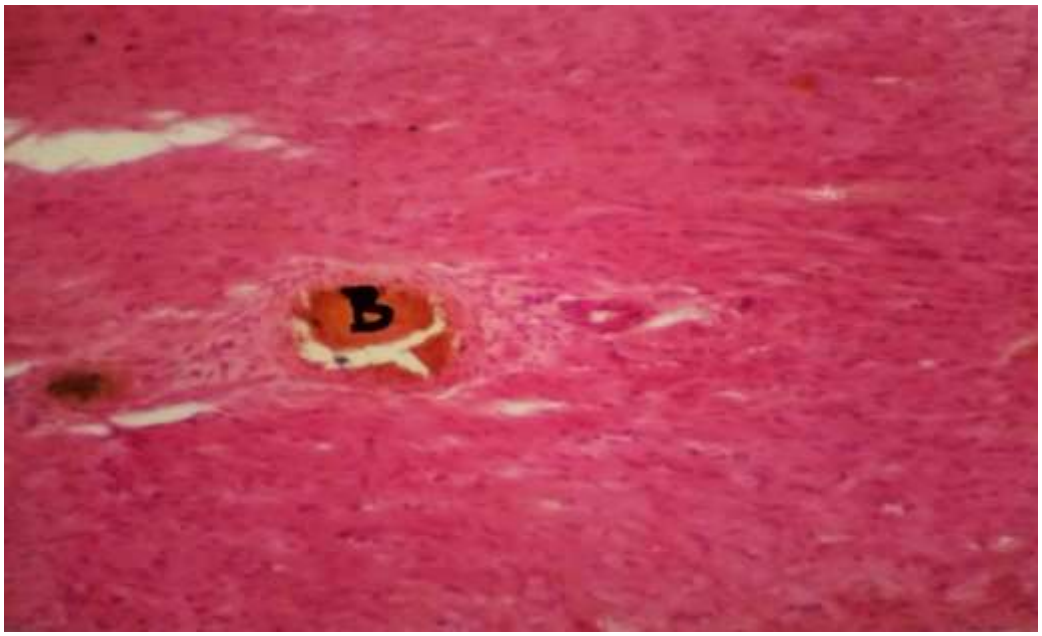

Fig. 4. Transverse section of cardiac muscle tissue of albino rats with 125 mg/3 mL caffeine showing muscle tissue blocking (B) and obliteration of vessel lumen (x 50) (H & E stain).

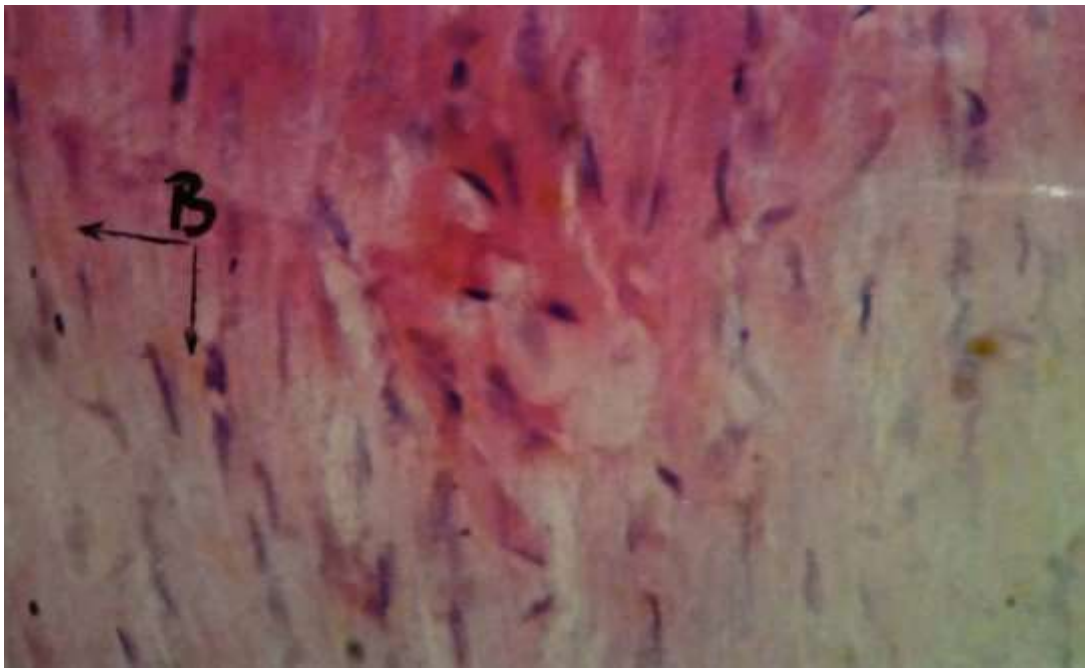

Fig. 5. Transverse section of cardiac muscle tissue of albino rats treated with 125 mg/3 mL of caffeine showing condition of brown atrophy (B). Yellowish-brown pigment lipofuscin granules tend to accumulate in many tissue sites and are present at the poles of nuclei (x 200) (H & E stain).

DISCUSSION

Caffeine being a stimulant of central nervous system in higher concentration affects cardiac muscles (Fredholm *et al.*, 1999; Burkard *et al.*, 2007). Furthermore the mechanism by which caffeine improves performance is still

debatable (Greer *et al*., 2000). When the albino rats were treated with a dose 3 mg/0.5 mL of caffeine, cardiac muscle tissue were affected. The histological section showed condensation and shrunken cardiac muscles. At a higher dose (9 mg/3 mL), blood vessels were affected, losing their normal structure of various layers and converted into indistinct muscle tissue with a number of chromatin particles. Muscular condensation was also observed and individual muscle fibres were not distinct. Moreover at acute dose (125 mg/3 mL), blocking of vessels and obliteration of vessel observed. Yellowish-brown pigment lipofuscin tend to accumulate in many tissues.

Al-Mozie'l *et al.* (2019) stated that in male rats treated with 25 mg/kg caffeine, it was observed muscle cells and congestion of blood vessel whereas those treated with 100 mg/kg caffeine had degeneration of myocardial muscle cells and congestion of blood vessels.

Cardiac muscle tissue is highly organized and is one of the three type of muscles found in the body. The other two are smooth muscle and skeletal muscle. Cardiac muscle tissue only are present in the heart and are capable of heart pumping and circulation of blood in the body. The cardiac muscle cells are tubular structure composed of chains of mycrofils which are rod like units within the cell (Saxton *et al*., 2023).

Earlier research conducted on effect of caffeine on cardiac muscle tissue are still unclear suggesting the typical daily doses of caffeine have yielded contradictory and ambiguous results (James, 1991). The effects of caffeine in different cardiac muscle tissue modifies the slow inward current (Ohba, 1973).

Vassalle and Lin (1979) studied the effect of caffeine on cardiac Purkinje fibres in peculiar in that in low concentration (ImM), caffeine initially increases force but later decreases force. The reason for the initial force increase seems to be facilitation of calcium release from the SR (Lin and Vassalle, 1983).

CONCLUSION

The present study determined that the three different dose had distinct effect, whereas high doses of caffeine to albino rats caused deleterious effect on cardiac muscle tissue with blocking and obliteration of muscle and arterial wall showed hyaline degeneration.

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