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Pharmaceutical Sciences **Research Article……!!!**

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EFFECT OF CRUDE CAFFEINE AND CAFFEINE CONTAINING SOFT DRINKS ON ISOLATED FROG HEART

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Keywords:

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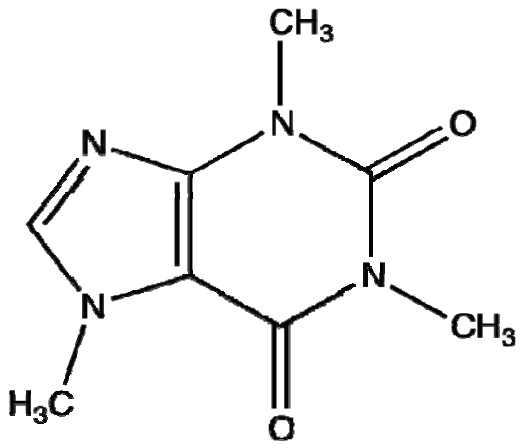
ABSTRACT

Aim: This research paper attempts the effect of crude caffeine and caffeine containing soft drinks on isolated frog heart. Materials and Methods: Preparation of caffeine by extraction method from tea powder, Isolation of frog heart by perfusion technique, Frog Ringer's Solution, Force of contraction, Heart rate, Cardiac output, caffeine intoxication were estimated by using crude caffeine and caffeine containing soft drinks. Results: Caffeine is a common ingredient of soft drinks, such as cola, originally prepared from kola nuts. Soft drinks typically contain about 10 to 50 milligrams of caffeine per serving. By contrast, energy drinks, such as Red Bull, can start at 80 milligrams of caffeine per serving. The results show that caffeine in low concentrations exhibited as a CVS stimulant and in high concentrations it induces the arrhythmias (irregular heart beat) with bradycardia and Caffeine antagonized the action of coca-cola and Thums up by inducing regular heart beats which is of functional properties of heart. Conclusion: caffeine acts as stimulant in lower doses and acts as toxic that means systolic block followed by arrhythmias with bradycardia (decreased heart rate) in higher doses, and caffeine containing soft drinks (Thums up and Coca-Cola) are inducing arrhythmias with bradycardia. finally noted that caffeine in low concentrations exhibited as a CVS stimulant and in high concentrations, induces the arrhythmias with bradycardia.

INTRODUCTION

There are few people who are not aware of the stimulating effect that caffeine provides. We have a choice and choose caffeinated beverages for a reason. Caffeine is considered the most commonly used psychoactive drug in the world. Approximately 90% of adults consume it on a daily basis, and research is being done on its health benefits and consequences. Global consumption of caffeine has been estimated at 120,000 tonnes per year, making it the world's most popular psychoactive substance. This amounts to one serving of a caffeinated beverage for every person every day. Beverages containing caffeine, such as coffee, tea, soft drinks, and energy drinks, enjoy great popularity in North America, 90% of adults consume caffeine daily. Caffeine is a bitter, white crystalline xanthine alkaloid. Caffeine is found in varying quantities in the seeds, leaves, and fruits of some plants, where it acts as a natural pesticide that paralyzes and kills certain insects feeding on the plants. It is most commonly consumed by humans in infusions extracted from the seed of the coffee plant and the leaves of the tea bush, as well as from various foods and drinks containing products derived from the kola nut.



Structure of Caffeine

Systematic (IUPAC) name of caffeine
1,3,7-trimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione
3,7-dihydro-1,3,7-trimethyl-1*H*-purine-2,6-dione

Caffeine is a common ingredient of soft drinks, such as cola, originally prepared from kola nuts. Soft drinks typically contain about 10 to 50 milligrams of caffeine per serving. By contrast, energy drinks, such as Red Bull, can start at 80 milligrams of caffeine per serving. The industry leader Red Bull started selling in 1987 in Austria. Today 2.5 billion cans are sold every year in more than 130 countries. The caffeine in these drinks either originates from the ingredients used or is an additive derived from the product of decaffeination or from chemical synthesis. Guarana, a prime ingredient of energy drinks, contains large amounts of caffeine with small amounts of the bromine and theophylline in a naturally occurring slow-release excipient. Most energy drink brands target male 20-somethings, consistent with the fact that the average age of the caffeine users is 21. In order to attract market share, many companies are using “taboo” names like Pimpjuice, Jolt, Reboot and Bawls. More than 500 energy drinks were launched worldwide the year of 2008. As more companies join the growing energy drink market, they are vying for the dollars of teenagers with promises of increased endurance and legal highs. Nutritionists warn that these drinks, which are loaded with caffeine and sugar, can hook kids on an unhealthy jolt-and-crash cycle. The caffeine in these drinks comes from multiple sources, making it hard to tell exactly how much caffeine they contain. Some contain B vitamins, which when taken in mega doses can cause rapid heartbeat, and numbness and tingling in the hands and feet. A new study found that a surprising number of poison center calls are from young people getting sick from too much caffeine. During three years of reports to a Chicago poison control center, the researchers found 265 cases of caffeine abuse. Caffeine in sodas aims to addict, according to a new study.

Product	Serving size	Caffeine per serving
Coca-Cola Classic	355 ml	34 mg
Guaraná Antarctica	350 ml	30 mg
Jolt Cola	695 ml	280 mg
Mountain Dew	355 ml	54 mg
Red Bull	250 ml	80 mg
MDX	236 ml	47 mg
Diet MDX	236 ml	50 mg
Amp Energy Drink	236 ml	71 mg
Atomic Rush	207 ml	100 mg
Pepsi	236 ml	25 mg
Diet Pepsi	236 ml	24 mg
Surge	236 ml	53 mg

Table 1: Caffeine Content in soft drinks and energy drinks

The purpose of this project is to explain the various effects caused by the caffeine containing soft drinks.

MATERIALS AND METHODS

Materials:

Apparatus: Beakers, separating funnel, frog board, venous cannula, Marriott's bottle, Starling heart lever, kymograph, rotating drum.

Chemicals: Chloroform, lead acetate solution, frog Ringer's solution.

Experimental animals: Frogs (*Rana tigrina*)

The experiment was carried out based on the following methods.

- Preparation of chloroform extract of caffeine
- Preparation of frog Ringer's solution
- Isolation of frog heart by perfusion technique
- Administration of crude caffeine and caffeine containing soft drinks like Coca-Cola and Thums up directly to the isolated frog heart
- Record the various physiological parameters like heart rate, cardiac output and force of contraction

Methodology:

Preparation of caffeine by extraction method from tea powder

Introduction: Extractions of certain solids can be performed by utilizing the different chemical properties of various solvents. The initial solvent used in the extraction of caffeine is water. Caffeine is sparingly soluble in water at ambient temperatures but highly soluble in water at 100^{o}C. The boiling of coffee beans and tea leaves dissolves caffeine and other materials to produce coffee and tea beverages. We will take advantage of the solubility properties of caffeine in water to create an aqueous solution of caffeine at room temperature. First, the caffeine will be dissolved from tea leaves by boiling them in water. The solution will be allowed to cool to room temperature. And then is treated with lead acetate solution to remove the tannins present in the solution. The caffeine will remain in solution and must be extracted with another solvent.

The solubility of caffeine in chloroform is quite high at room temperature. Therefore, when chloroform is added to the aqueous caffeine solution, the caffeine is transferred to

the chloroform. The chloroform- caffeine mixture can then be separated by utilizing the different densities of chloroform and water. Because chloroform is much denser than water and insoluble in it, the chloroform will form a layer under the water and can be separated from it. The chloroform layer will be separated by using separating funnel and then the chloroform layer is transferred in to the china dish. The caffeine is then crystallized on a china dish.

Procedure:

Part 1: Dissolution of Caffeine in Water

1. Weigh about 100g of tea powder and transferred in to the beaker.
2. Add 125ml of distilled water to the beaker.
3. Boil the water containing the tea powder on a boiling water bath for 15-20 minutes while stirring occasionally.
4. After the boiling period is over, remove the beaker from the heat and allow to cool 15 minutes or on ice until cooled.After the solution has cooled, filter the solution through regular filter paper to remove any solid particles.
5. Treat the above filtrate with lead acetate solution until the tannins are to be precipitated.
6. Then filter the tannins.

Part 2: Transfer of Caffeine from Water to Chloroform

Caution: Use Chloroform under hood with proper ventilation. Do Not Breath Fumes.

1. Transfer the filtrate obtained from step 7 above to a 500ml separating funnel. Add 100ml of chloroform and extract the caffeine for three times.
2. Allow the chloroform to settle to the bottom. Carefully drain the chloroform layer into a china dish. Dispose of the aqueous top layer...

Isolation of frog heart by perfusion technique

Drugs may influence the rate (chronotropy) and force (inotropy) of contraction of the heart. An increase in the heart rate is called positive chronotropic response, while a negative chronotropic response is decrease in the heart rate. Similarly an increase in the force of contraction is called positive inotropic response and decrease in the force of

contraction is called negative inotropic response. The myocardial contraction of normal heart takes place according to Starling's law of heart. According to this law force of systolic contraction is directly proportional to the fibre length in diastole. Since systolic contraction represents cardiac output and the fibre length in diastole indicates venous pressure, the law indicates that cardiac output (i.e. stroke volume) is directly related to venous return or venous pressure during diastole. When the cardiac musculature fails to obey this relationship as in failing heart (i.e. congestive heart failure) there will be decrease in stroke volume (cardiac output), incomplete emptying of the ventricles during systole and enlargement of heart size due to residual blood in the heart at the end of systolic contraction. When the heart is in this state, i.e. inability to contract to physiological normal it is said to be a hypodynamic heart. Experimentally hypodynamic heart can be produced by perfusing the heart with Ringer containing less quantity of calcium as this bivalent ion is essential for myocardial contraction.

Table 2: Frog Ringer's Solution composition:

Chemicals	Quantity for 1000ml
Sodium bicarbonate	1.19 gm
Sodium chloride	7.25 gm
Calcium chloride	0.265 gm
Potassium chloride	0.225 gm
Dextrose	1.80 gm
Magnesium chloride	0.324 gm

Sodium bicarbonate: act as a buffer.

Sodium chloride: to maintain isotonicity, excitability, contractility of the preparation.

Calcium chloride: to maintain the contractility of the preparation.

Potassium chloride: to maintain ionic balance.

Dextrose: to provide energy.

Magnesium chloride: to stabilize preparation and hence to reduce spontaneous activity.

Procedure

- Pith the frog and pin it to the frog board.
- Give a midline incision on the abdomen.
- Remove the pectoral girdle and expose the heart.

- Carefully remove the pericardium and put a few drops of frog ringer over heart.
- Trace the inferior venacava, put a thread around it and give a small cut in order to insert the venous cannula which is in turn connected to a perfusion bottle containing frog ringer.
- Insert the cannula in the vein and tie the thread to assure the cannula in place.
- Give small cut in one of the aortae for the perfusate to come out.
- Adjust a proper venous pressure of 2-4 cm by altering the height of perfusion bottle. The effective venous pressure is the height in cms from level of the venous cannula and the ringer level in the perfusion bottle. Use of Marriott's bottle helps in attaining the constant pressure.
- Start the perfusion by opening the screw clamp attached to the tube.
- Pass a thin pin hook through the tip of the ventricle, and with the help of a fine thread attached to the hook, tie it to the free limb of the lever, which is fixed to a stand.
- Adjust proper tension and magnification by altering the height of the lever.
- Record the normal contraction of the heart on the rotating drum or smoked drum.
- Prepare the stock solution of caffeine by dissolving 100 mg of caffeine in 1ml of boiled water then from that prepare secondary stock solutions in different concentrations by serial dilutions.
- Inject increasing doses of the stock solution of caffeine in a sequential order and note the heart rate and cardiac output.
- Keep at least 2 min gap between administration of each dose of caffeine. The drug is administered by injecting the drug into the perfusion tube very close to the venous cannula.
- Take precautions to avoid any leakage of the drug from the tube, and the injection of air bubbles. Label and fix the tracing with the fixing solution.

Experiment No. 1

Effect of Caffeine and Coca-Cola on isolated frog heart

Method: The frog heart was isolated as mentioned in the methodology.

Drug: Caffeine

Caffeine containing soft drink: Coca-Cola

Procedure: The isolated frog heart has been administered with different concentrations of prepared stock solutions of caffeine and coca-cola and the responses were recorded on the kymograph paper. The doses of caffeine and coca-cola administered were 1 ng, 3 ng, 10 ng, 30 ng, 100 ng, 300 ng, 1 µg, 3 µg, 10 µg, 30 µg, 100 µg, 300 µg, 10 mg, 30 mg and 0.3 ml, 0.5 ml, 1 ml, which produced dose dependent force of contraction of heart.

Experiment No. 2

Effect of Coca-Cola and Caffeine on isolated frog heart

Method: The frog heart was isolated as mentioned in the methodology.

Drug: Caffeine

Caffeine containing soft drink: Coca-Cola

Procedure: The isolated frog heart has been administered with different concentrations of coca-cola along with caffeine and the responses were recorded on the kymograph paper. The doses of coca-cola and caffeine administered were,

coca-cola: 0.1 ml; diluted coca-cola: 0.1 ml, 0.2 ml, 0.3 ml, 0.5 ml, 1 ml; caffeine: 20 mg.

Experiment No. 3

Effect of Thumps up and Caffeine on isolated frog heart

Method: The frog heart was isolated as mentioned in the methodology.

Drug: Caffeine

Caffeine containing soft drink: Thums up

Procedure :The isolated frog heart has been administered with different concentrations of thums up along with caffeine and the responses were recorded on the kymograph paper. The doses of thums up and caffeine administered were,

diluted thums up: 0.4 ml, 0.8 ml, 1 ml; thums up: 0.1 ml, 0.2 ml, 0.3 ml, 0.6 ml, 1ml; caffeine: 1mg, 2mg, 3mg, 4 mg.

RESULTS AND DISCUSSIONS

We observed from the experiment no 1 that the 10 mg of caffeine showed the stimulant effect with increased heart rate. After two minutes the same dose of caffeine (10 mg) showed the stimulant effect followed by arrhythmias (irregular heartbeat) with bradycardia (A slow rhythm that is less than the normal beats/min). As the dose of caffeine is increased to 30 mg, initially stimulant effect observed, after few seconds the

heart is blocked at systole, and then arrhythmias are maintained with bradycardia (decreased heart rate). So from the above experiment finally noted that caffeine in low concentrations exhibited as a CVS stimulant and in high concentrations it induces the arrhythmias (irregular heart beat) with bradycardia.

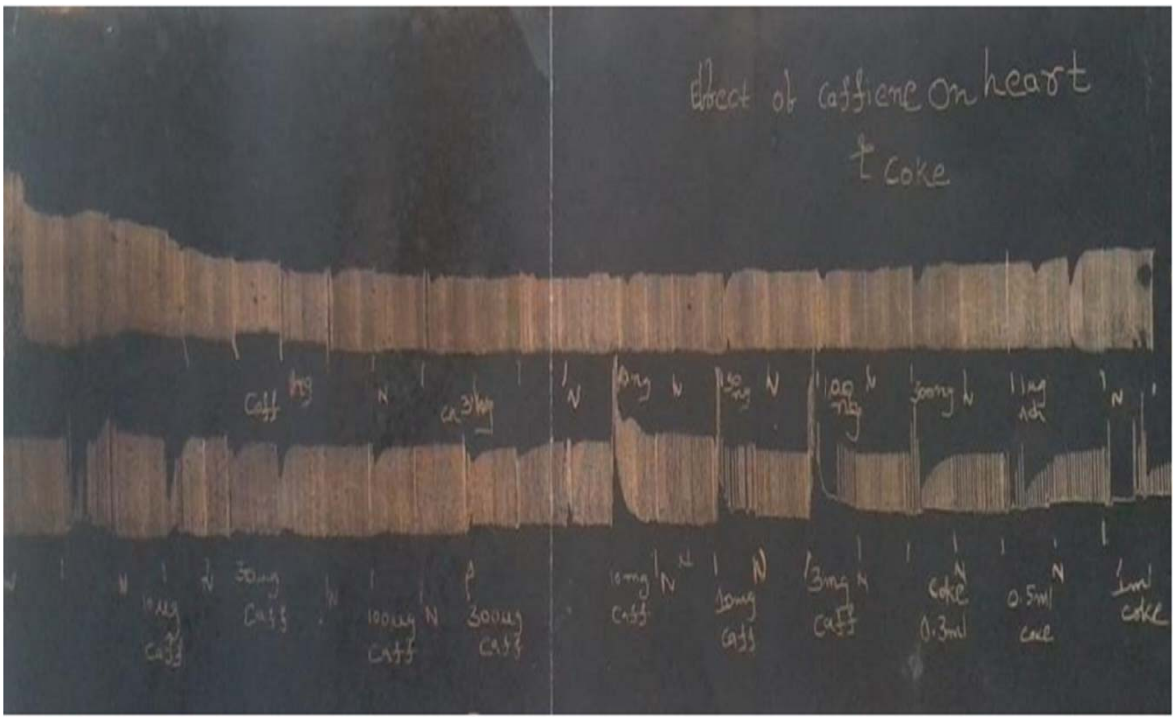


Figure 1: Effect of Caffeine and Coca-Cola on isolated frog heart

We observed from the experiment no 2 that 0.2 ml of diluted coca-cola started inducing arrhythmias with bradycardia (decreased heart rate), as the concentration increased by 0.3 ml distinct arrhythmias with bradycardia (decreased heart rate) have been observed. Caffeine antagonized the action of coca-cola by inducing regular heart beats which is of functional properties of heart. So from the above experiment finally noted that caffeine in low concentrations exhibited as a CVS stimulant

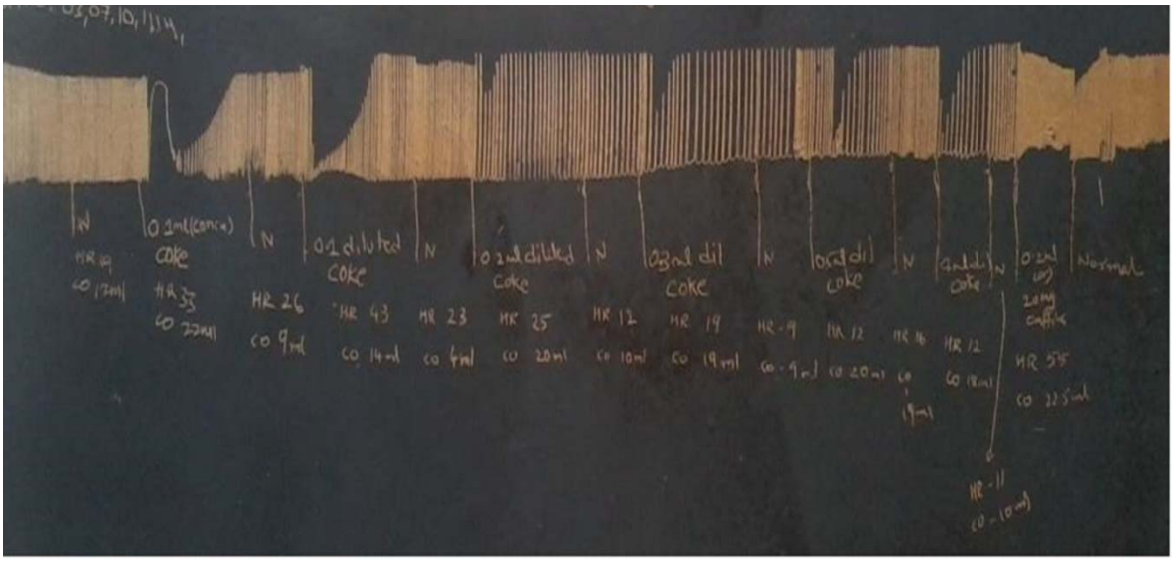


Figure 2: Effect of Coca-Cola and Caffeine on isolated frog heart

We observed from the above experiment no 3 that 0.1 ml of thums up (without dilution) started inducing arrhythmias with bradycardia (decreased heart rate). Caffeine antagonized the action of thums up by inducing regular heart beats which is of functional properties of heart. So from the above experiment finally noted that caffeine in low concentrations exhibited as a CVS stimulant and in high concentrations induces the arrhythmias with bradycardia (decreased heart rate).

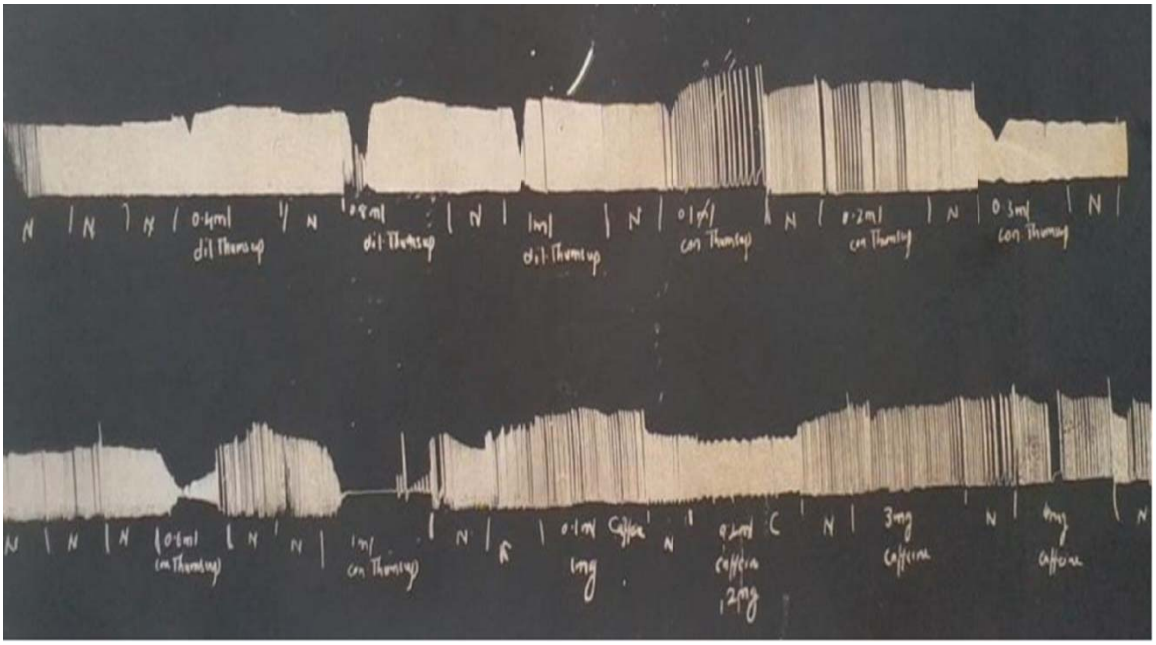


Figure 3: Effect of Thums up and Caffeine on isolated frog heart

CONCLUSION

There are few people who are not aware of the stimulating effect that caffeine provides. We have a choice and choose caffeinated beverages for a reason. Caffeine is considered the most commonly used psychoactive drug in the world. Approximately 90% of adults consume it on a daily basis. Caffeine is a common ingredient of soft drinks, such as Coca-Cola, Thums up. Soft drinks typically contain about 10 to 50 milligrams of caffeine per serving. By contrast, energy drinks, such as Red Bull, can start at 80 milligrams of caffeine per serving. Caffeine competitively inhibits different adenosine receptors and their associated G protein to make a person feel alert. A mild stimulant of the central nervous system, caffeine also stimulates cardiac muscle. High long-term consumption is associated with a lower risk of cardiovascular disease, increase in blood pressure. Caffeine also induces the cardiac arrhythmias (improper heart rate).

As we gone through detailed study of caffeine and caffeine containing soft drinks on isolated frog heart, we can conclude that caffeine acts as stimulant in lower doses and acts as toxic that means systolic block followed by arrhythmias with bradycardia (decreased heart rate) in higher doses, and caffeine containing soft drinks (Thums up and Coca-Cola) are inducing arrhythmias with bradycardia. Caffeine antagonized the actions of caffeine containing soft drinks by inducing regular heart beats which is of functional properties of heart. So from the above experiments finally noted that caffeine in low concentrations exhibited as a CVS stimulant and in high concentrations, induces the arrhythmias with bradycardia.

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