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A study of the effect of Caffeine on some selected haematological parameters using rat model

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Abstract

The current study was conducted at the College of Pharmacy, University of Basrah in the period extended from 1/3/2018 of 10/4/2018.

Caffeine is a chemical compound that may be interfere with many organs functions in the body, it may cause endocrine dysfunction in diagnosis of many clinical cases.

Twenty four male rats are used in the study, they divided into 4 groups (n=6) and gavaged daily for 40 days as follows: control group (animals received 0.5 ml of distilled water), caffeine (25mg/kg) treated group, caffeine (50 mg/ kg) treated group and caffeine (100mg/ kg) treated group. At the end of the study, the animals were sacrificed and the blood samples were collected directly from the heart by using 5 ml disposable syringe, one ml of blood collected in **ETDE** tube for hematological parameters measurement as soon as possible. The results show a significant elevation of red blood cell (RBC) count, hemoglobin and platelets count in some caffeine treated group in comparison to control group, also significant depletion in white blood cell (WBC) count in comparison to control group.

Aim of the study

To evaluate the possibility of hematological parameters change (WBC, RBC, hemoglobin and platelets count) after caffeine administration in males rats.

Introduction

Coffee, an infusion of ground, roasted coffee beans, is reported to be among the most widely consumed beverages in the world. Although coffee is lauded for its aroma and flavor, its caffeine content likely plays a role in its popularity. In fact, coffee is a complex chemical mixture reported to contain more than a thousand different chemicals, including carbohydrates, lipids, nitrogenous compounds, vitamins, minerals, alkaloids and phenolic compounds (Linus Pauling Institute et al, 2006).

The structure of caffeine is similar to adenosine in the body (figure 1).

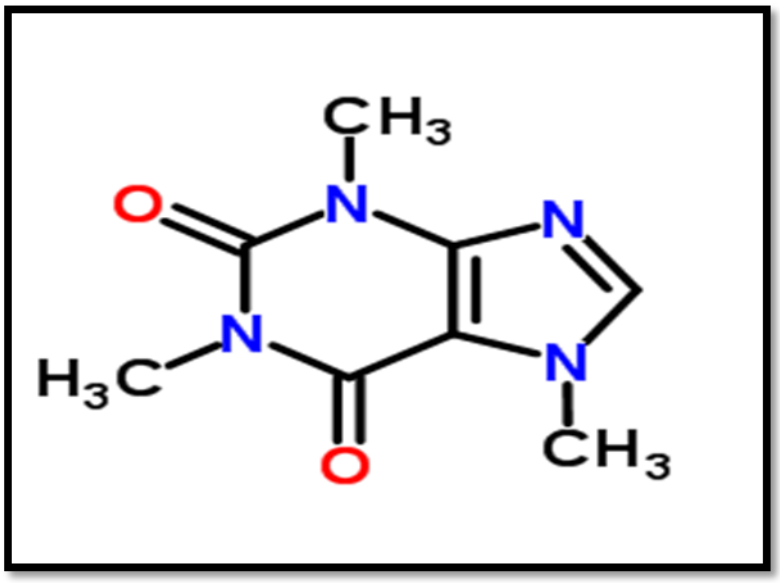

Figure (1): methyl xanthine (Linus Pauling Institute, 2006).

The caffeine bind to adenosine cell membrane receptors found in the heart, brain, smooth muscle, adiposities, and skeletal muscle. Caffeine can stimulateously affect a wide number of tissue in the body , it stimulates the CNS and increase the release of epinephrine, caffeine has been shown to increase heart rate, metabolic rate, respiratory center output and fat oxidation, it also decrease perception of pain and fatigue.

A main benefit of caffeine effect on performance has been linked to increase fat oxidation, causing increased serum free fatty acid levels and therapy sparing muscle glycogen. Another new mechanism that shows how caffeine effect substrate utilization during exercise, caffeine has been shown to decrease plasma potassium levels, this is significant because during exercise, potassium is transported out of the

muscle cells. As the intracellular potassium level fall and extracellular levels rise, motor unit activation decrease leading to a decrease in muscular force output. caffeine delays the outflux of potassium from muscle cells, which delays the onset of skeletal muscle fatigue, allowing an athlete to maintain motor unit force for a longer period of time, the half life of caffeine is about 4 to 6 hours, with the mean time to reach peak plasma concentration between 30 to 60 minutes.

Caffeine metabolism

Most of the caffeine is metabolized by CYP450 liver enzyme called CYP1A2, this means that the body uses CYP1A2 to demethylate caffeine molecules, transforming them into other substances, chiefly paraxanthine which are ultimately excreted in the urine.

Adverse Effects

Caffeine usage may cause anxiety, heart palpitations, trembling, nervousness and facial flushing, these adverse effects are usually dose related, more side effect were reported when subject consumed greater than 6 to 9 mg/kg body weight. Two to three cups of coffee provide about 5 mg/kg body weight of caffeine, lethal half dose of caffeine is 150 to 200 mg/kg body weight (roughly 100 cups of coffee). Acute caffeine toxicity can cause hematemesis, hyperventilation, hyperglycemia, ketonuria, hypokalemia, metabolic acidosis and cardiac arrhythmia (brian D . busconi, Anthony A. schepsis 2006).

Effect of caffeine on blood

Some study mentioned that there is a synergistic effect of caffeine in addition to exercise to increase the circulating red and white cell counts because of their mobilization from blood storage sites (Adriana Bassini-Cameron et al 2007).

Caffeine and exercise interaction has the potential to affect the lymphocyte response during activity. What is less apparent is the consequence with regard to lymphocyte subsets and the apoptotic or migratory response during the post-exercise period in caffeine-familiar and caffeine-naïve individuals.

(James W Navalta et al 2016) mentioned that caffeine supplementation affected the CD4+ lymphocyte response in naïve individuals differently than familiar individuals. Whereas caffeine ingestion had no pre-exercise effect on familiar individuals, it

significantly increased the percentage of apoptotic helper T lymphocytes prior to the treadmill run. Caffeine consumption significantly increased CD4+ apoptotic cells in both familiar and naïve participants post exercise, and the response persisted to at least 1 h post exercise. Cellular migration was also differentially affected by caffeine ingestion with the familiar group showing significantly reduced CD4+/CX_3CR1+ percentages at 1 h following the exercise

Caffeine crosses the erythrocyte membrane and interacts with the two extreme conformational states of haemoglobin (the T and the R-state within the framework of the simple two states allosteric model) with different binding affinities. By promoting the high affinity state (R-state), the caffeine–haemoglobin interaction does enhance the pentose phosphate pathway. This is of benefit for red blood cells since it leads to an increase of NADPH availability. Moreover, caffeine effect on band 3, mediated by haemoglobin, results in an extreme increase of the anion exchange, particularly in oxygenated erythrocytes. This enhances the transport of the endogenously produced CO_2 thereby avoiding the production of dangerous secondary radicals (carbonate and nitrogen dioxide) which are harmful to the cellular membrane.

Furthermore caffeine destabilizes the haeme–protein interactions within the haemoglobin molecule and triggers the production of superoxide and met-haemoglobin. However this damaging effect is almost balanced by the surprising scavenger action of the alkaloid with respect to the hydroxyl radical. These experimental findings are supported by *in silico* docking and molecular dynamics studies and by what we may call the “caspase silence”; in fact, there is no evidence of any caspase 3 activity enhancement; this is likely due to the promotion of positive metabolic conditions which result in an increase of the cellular reducing power.

Effect of caffeine on blood pressure

In subjects who irregularly consuming caffeine, slight rise in blood pressure is the most consistent acute effect of caffeine ingestion. It has been found to trigger release of epinephrine from meduloadrenal hyperfunction (R. macrae, R.j. Clarke, 1988).

Caffeine supplementation, stress hormones & immunological variables

(Adriana Bassini-Cameron et al 2007) was referred that ingesting of 6 mg of caffeine 1h before endurance exercise consistently rises plasma adrenaline concentration,

compared with a placebo treatment caffeine supplementation does not alter the number of circulating neutrophils following exercise or neutrophil production of reactive oxygen species.

The number of circulating CD3/CD56 NK cells is greater compared with a placebo treatment, whereas changes in the number of activated NK cells expressing CD69 are variable after exercise and caffeine ingestion.

Changes in the total number of circulating lymphocytes after exercise and caffeine intake are also variable, the numbers of circulating CD4 T helper cells and CD8 T cytotoxic cells are greater after exercise and caffeine intake compared with a placebo treatment.

Caffeine encourage stress hormones such as epinephrine and norepinephrine to be released and this affects blood flow and the amount
of oxygen in the blood.

Material and methods

Experimental Animals

Twenty four adult male rats (350±15 g body weight) were housed (4 rats/cage) under optimum identical conditions (12/12 light, dark cycle, 22 ± 2 C˚) where in these are allowed free access to pelleted rat chow and tap water. This experiment was carried out on 24 adult rats, all were received treatment or DW (control) for 40 days; they were divided into four groups each group which include 6 males, first group(G1) rats; they were dosed orally by gavage with distal water in a similar volume as treated group. Second group(G2); rats in this group were dosed by gavage with caffeine 25 mg/kg. Third group(G3) they were dosed by gavage with caffeine 50 mg/kg. Fourth group(G4) they were dosed by gavage with caffeine 100 mg /kg). At the end of the study the blood samples were collected from inferior vena cava of the heart of sacrificed animals. Then the blood sample were drops directly from the heart by using 5 ml disposable syringe 1 ml of blood collected in **ETDE** tube for hematological parameters measurements as soon as possible.

Hematological Parameters

The hematological tests were done in research laboratory- College of Pharmacy – University of Basra, by using auto hematology analyzer (Genex Inc., Florida USA).The blood parameters estimated by this instrument included: Total Erythrocytes count, total platelets count, Total Leukocytes count and Hemoglobin concentration.

Statistical Analysis

The data were expressed as mean ± Standard deviation (**SD)**. In addition to used **ANOVA** analysis in our experiment. Least significant difference (**LSD**) was used to test the differences among means for ANOVA indicated a significant ($P<0.05$), using computerized SPSS version 11.

Results

Effect of caffeine on red blood cells (RBC)

Red blood cells count was significantly increased ($P \leq 0.05$) in caffeine treated group 50 mg/kg and 100 mg/kg in comparison to the control group and 25 mg/kg caffeine treated group.

Effect of caffeine on hemoglobin concentration

The level of hemoglobin concentration was significantly increased in group treated with 100 mg/kg caffeine in comparison to control group.

Effect of caffeine on white blood cells (WBC)

White blood cells count was significantly decreased in all caffeine treated group in comparison with control group.

Effect of caffeine on platelets count

50 mg/kg and 100 mg/kg caffeine treated group were showed a significant increased in platelets count in comparison with control and 25 mg/kg caffeine treated group.

Table (1): effects of different doses of caffeine on WBC, RBC, hemoglobin (Hb) and platelets (PLT).

Parameters **Groups**	**RBC** $*10^6$**cell/m**^{3}	**Hb g/dl**	**WBC** $*10^3$**cell/mm**^{3}	**PLT**
G1: Control	**5.87±0.51 b**	**12.60±0.59 bc**	**10.53±0.75 a**	**431.50±48.55 b**
G2: 25mg/kg	**6.27±0.14 b**	**13.11±0.45 b**	**7.51±0.53 b**	**438.71±28.71 b**
G3:50 mg/kg	**6.58±0.24 a**	**13.66±0.38 b**	**6.96±0.71 b**	**561.83±49.55 a**
G4: 100 mg/ kg	**6.96±0.52 a**	**14.68±0.90 a**	**5.71±0.49 bc**	**552.66±56.71 a**
LSD	**0.68**	**1.01**	**1.25**	**114.50**

Discussion

Caffeine causes most of its biological effects via antagonizing all types of adenosine receptors (ARs): A1, A2A, A3, and A2B and, as does adenosine, exerts effects on neurons and glial cells of all brain areas. When acting as an AR antagonist, is doing the opposite of activation of adenosine receptors due to removal of endogenous adenosinergic tonus. Besides AR antagonism, xanthines, including caffeine, have other biological actions: they inhibit phosphodiesterases (PDEs) (e.g., PDE1, PDE4, PDE5), promote calcium release from intracellular stores, and interfere with GABA-A receptors. Caffeine, through antagonism of ARs, affects brain functions such as sleep, cognition, learning, memory, and modifies brain dysfunctions and diseases.

Effect of caffeine on platelets count may reflect its action on purinergic receptors—a proinflammatory action that appears to be mediated by adenosine monophosphate and protein kinase, or to be caused by release from the spleen (Horrigan LA et al 2006).

Caffeine consumption may lead to a reduction in platelet aggregability as a result of upregulation of the adenosine A2A receptors located on the platelet surface and is accompanied by increases in cAMP accumulation and decreases in aggregation and calcium levels after stimulation of adenosine A2A receptors by sensitization, in a time- and dose-dependent manner. The upregulation of adenosine A2A receptors caused by chronic intake of caffeine could be interpreted to indicate that endogenous adenosine has a tonic influence on human platelets, and the presence of the antagonist is counterbalanced by the upregulation of A2A receptors.

The mechanism behind the depletion in white blood cells count is not well defined, many studies have assessed the effect of caffeine supplementation during exercise on the lymphocyte response. Bishop et al. 2008, found that caffeine ingestion of 6 mg/kg increased the resting concentration of both CD4+ and CD8+ lymphocytes prior to exercise, activated subsets of both t-cell type (CD4+/CD69+, CD8+/ CD69+) were significantly greater than baseline.

Conclusion

Caffeine pure powder when administrated orally for 40 days in males rats precipitated a significant changes in blood parameters like decrease in white blood cells count and increase in red blood cells, hemoglobin and platelets count. This related to a specific mechanisms of caffeine on blood cells.

Reference

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