Evaluation of cardiac biomarkers in albino rats consumed instant coffee and non-dairy creamer.

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Abstract: Instant coffee (Nescafé) and non-dairy creamer (Coffee- Mate) are the commonly consumed beverages all over the world. The present study aimed to evaluate the effects of consuming 1% instant coffee, 2% non-dairy creamer and a solution of 1% instant coffee plus 2% non-dairy creamer (v/v) on some cardiac biomarker parameters. The study was conducted on twenty four male albino rats for 30 days. Heart contents of reduced glutathione (GSH) and malondialdehyde (MDA) in addition to serum troponin, lactate dehyrogenase (LDH), creatine phosphokinase (CPK), lipid profile, glucose, insulin, triiodothyronine (T3) and thyroxine (T4) were investigated. All treatments significantly increased CPK, triglycrieds (TG), low density lipoprotein (LDL-c) but, significantly decreased GSH and insulin and significant increases of MAD, LDH, very low density lipoprotein (VLDL) and glucose regarding coffee consumption, VLDL, LDL/HDL and glucose after non-dairy creamer treatment in addition to MAD and LDL/HDL ratio after consumption of coffee plus non-dairy creamer were also shown. The obtained results showed that the consumption of instant coffee and non-dairy creamer showed an adverse effect on various biological markers of the heart and suggestive of increased cardiovascular disease risk.

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1.Introduction:

Coffee holds second position in consumption among all beverages after water, and people from all over the world consume approximately 500 billion cups annually (**Prakash *et al.,* 2002**). Seventy five per cent of the soft drinks that consumed regularly are coffee. Instant coffee is the extract of coffee beans, and is available in a remarkable variety of different types throughout the world (**Rojo Camargo, and Fatah, 1999**). Coffee is a chemical mixture reported to contain more than a thousand different molecular substances, including carbohydrates, lipids, nitrogenous and phenolic compounds, vitamins, minerals, alkaloids, caffeine, cafestol, kahweol, and chlorogenic acids. Caffeine, cafestol, kahweol, and chlorogenic acids may affect serum lipid profile (**Grzegorzewska *et al.,* 2009**). Coffee consumption has been shown to have adverse effects on various biological markers related to coronary heart disease risk, such as serum cholesterol (**Jee *et al.,* 2001**), blood pressure (**Noordzij *et al.,* 2005**), insulin resistance (**Keijzers *et al.,* 2002**), and plasma homocysteine (**Urgert *et al.,* 2002**). In contrast, high coffee consumption has not been associated with a high risk of coronary heart disease in the study of **Lopez-Garcia *et al.*(2006)**, and significantly reduces the incidence of type2 diabetes in study of **Salazar-Martinez *et al.* (2003)**.

Coffee is commonly consumed with a coffee creamer or lightener to soften the acidic taste (**Pordy, 1994**). The coffee cream consists of vegetable fat, sodium caseinate, stabilizers, sweetener, emulsifiers, flavor and color (**Ellinger, 1972**).

Recent years many studies have focused on the effect of coffee and caffeine on cardiovascular diseases. To the best of our knowledge, there is a scanty of literatures studied the double effect of both instant coffee and non-dairy creamer and their interaction on the cardiovascular disease. Millions of Egyptians have been consumed the powdered non-dairy creamer and coffee beverage, at least once a day. The present study aimed to evaluate the effects of coffee and non-dairy creamer on some cardiac biomarkers in male albino rats.

2. Material and Methods

Instant coffee (Nescafé classic) and non-dairy creamer (Coffee- Mate) are manufactured in Egypt by Nestlé Company and purchased from the local market. Instant coffee is an extract of coffee beans and, the ingredients of coffee mate are corn syrup solids, vegetable oil (hydrogenated palm kernel), sodium caseinate (a milk derivative), and less than 2% of dipotassium phosphate (moderates coffee acidity), mono- and diglycerides (prevents oil separation), sodium aluminosilicate, artificial flavor, annatto color. One percent instant coffee was prepared by dissolving 1 gm Nescafé classic in 100ml boiling water then leave to cool (**Noori *et al.*,2009**). Two percent non-dairy creamer was prepared by dissolving 2gm non-dairy creamer in 100 mL boiling water then, leave to cool. A solution of 1% instant coffee plus 2% non-dairy creamer v/v was prepared.

The volume of instant coffee, non-dairy creamer and a solution of instant coffee plus non-dairy creamer consumed by each rat were measured every morning. The mean intake of instant coffee, non-dairy creamer and a solution of instant coffee plus non-dairy creamer were 37.80±12.93 ml/ day/ rat, 34.62±10.43 mL/ day/ rat and 42.82±8.12 mL/ day/ rat, respectively.

Twenty four male albino rats (90-100gm) were purchased from the laboratory Animal House of the Institute of the Ophthalmology, Giza, Egypt. All animals were housed in stainless steel cages with wire mesh lid and allowed balanced standard diet and water *ad libitum* up to one week for adaptation. Rats were randomly divided into four groups of 6 animals each.

Group1: served as control. Group2: animals received 1% instant coffee. Group3: rats received 2% non-dairy creamer. Group4: rats received a solution of 1% coffee plus 2% non-dairy creamer. Thirty days later, all animals were fasted overnight then sacrificed and blood samples were collected, centrifuged to obtained serum. Heart tissues were dissected and blotted on filter paper. All samples were kept at- 20 ^{o}C pending biochemical analyses.

Activity of reduced glutathione (GSH) and levels of malondialdehyde (MDA) in the heart homogenates were estimated by the methods of **Jollow *et al.* (1974)** and **Ohkawa *et al.* (1979)** respectively. The activities of serum lactic dehydrogenase (LDH) and serum creatine phosphokinase (CPK) were determined according to method of **Wacker *et al.* (1956)** and **Buhl and Jackson (1978)**, respectively. Total troponin was measured by using ELISA (SPIN React kit).

Serum total cholesterol (TC), triglycerides (TG), and High Density Lipoprotein-Cholesterol (HDL-c) were analyzed by means of colorimetric enzymatic methods (Biodiagnostic kits), using PRIME-E automatic digital photometer at wave length 505nm. Very low density lipoprotein cholesterol (VLDL-c) and Low Density Lipoprotein-Cholesterol (LDL-c) were calculated according to the formula of **Friedewald *et al.* (1972)** as follows VLDL-c = TG / 5

LDL- cholesterol = Total cholesterol – (VLDL-c + HDL-c). Atherogenic index was calculated by dividing LDL-c by HDL-c according to **Castelli and Levitar(1977).**

Colorimetric determination of serum glucose was measured using SPIN React kit.

Serum levels of insulin were measured by using ELISA (SPIN React kit).

Levels of T_3 and T_4were determent using radioimmunoassay commercial kits (Institute of Isotopes Co.,LTD Budapest).

Statistical analysis

The obtained data were presented as means ± SD. One-way analysis of variance (ANOVA) was carried out. The statistical comparisons among the groups were performed with Duncan's test, using a statistical package program (COSTAT). Differences among the groups were considered significant at $P<0.05$.

3. Results

Table (1): Effect of instant coffee and/ or non-dairy creamer on the activity of GSH and level of MAD in the heart tissues of male albino rats.

Parameters \ Groups	G1	G2	G3	G4
GSH (mg/g tissue)	**2.76 ±0 .17^{a}**	**1.53 ± 0.7^{b}**	**1.67 ±0.87 ^{b}**	**0.62±0.45 ^{c}**
MAD (mmol/g tissue)	**33.81 ± 1.2 ^{c}**	**40.3± 3.67 ^{b}**	**36.05 ± 6.75 ^{c}**	**45.29 ± 5.35 ^{a}**

Values are shown as means ± SD of N=6.

Different small letters in the same row indicate significant difference at $P< 0.05$

Table1 shows the activity of GSH and level of MAD in the heart tissues of rats supplied with instant coffee and/ or non-dairy creamer. Heart tissues GSH levels were significantly decreased in all treated groups compared to the control group. The MAD levels in the heart tissues were significantly increased in G2 and G4, but G3 showed non significant increase compared to the control group.

Table 2 shows the mean activities of serum LDH, CPK and Troponin levels of the tested groups. There was a significant increase in the level of LDH in G2 compared to the control group. The levels of CPK showed significant increases in all treated groups, compared to the control group. All treatments did not affect the levels of Troponin.

Table3 shows the mean values of TC, TG, LDL-c, HDL-c, VLDL-c and LDL-c/HDL-c ratio for all tested groups. All treated groups showed significant increases in serum TG, LDL-c and VLDL-c but, VLDL-c in G4 was not significant, compared to control the group. Significant reductions were observed in levels of HDL-c in G3 and G4 compared

to the control group. Atherogenic index showed significant increase in G3 and G4 with non-significant increase in G2 compared to the control group.

Table (2): Effect of instant coffee and/ or non-dairy creamer on the activities of serum LDH and CPK and levels of Troponin.

Groups / Parameters	G1	G2	G3	G4
LDH(U/L)	4915.7±282 [b]	10550±854 [a]	5652.4±459 [b]	5422.3± 191 [b]
CPK(mg/dl)	4783.3±125.7[c]	6970.3± 41.6[b]	88139±136.58[a]	7480.3± 605 [a]
Troponin (complex)(U/L)	0.13±0.02 [a] (-ve)	0.09± 0.02 [a] (-ve)	0.13± 0.04 [a] (-ve)	0.122± 0.03 [a] (-ve)

Values are shown as means ± SD of N=6.
Different small letters in the same row indicate significant difference at $P< 0.05$.

Table (3): Effect of instant coffee and/ or non-dairy creamer on serum lipid profile in male albino rats.

Groups / Parameters	G1	G2	G3	G4
TC (mg/dl)	71.18 ± 5.02 [a]	78.4±12.68 [a]	75.9±12.7 [a]	70.58±1.4 [a]
TG (mg/dl)	47.6 ±9.4 [c]	75 ±5.3 [a]	61.8±3.1 [b]	56.9±5.5 [b]
LDL (mg/dl)	11.82±0.59 [d]	17.24±1.6 [c]	42.68± 7.31[a]	31.56±4.81 [b]
HDL(mg/dl)	49.19±5.22[a]	46.08±14.45 [a]	20.91±7.24 [b]	27.63±4.63[b]
VLDLc(mg/dl)	10.16 ± 1.03 [c]	15.13±1.2 [a]	12.28±0.71 [b]	11.37±1.11[bc]
LDL/HDLratio	0.24± 0.01 [c]	0.41±0.01 [c]	2.23±0.74 [b]	1.19±0.55 [b]

Values are shown as means ± SD of N=6.
Different small letters in the same row indicate significant difference at $P< 0.05$.

Table 4 shows the levels of serum glucose and insulin hormone in rats supplied with instant coffee and/ or non-dairy creamer. There was significant increase in glucose levels in G2 and G3, but significant decrease in G4, compared to the control group. All the tested groups showed significant reduction in the levels of insulin hormone, compared to the control group.

Table 5 shows the mean values of T_3 and T_4 of the tested groups. There were significant decrease in levels of T_3 and T_4 in G3, compared to the control group.

Table (4): Effect of instant coffee and/ or non-dairy creamer on serum insulin and glucose in male albino rats.

Groups / Parameters	G1	G2	G3	G4
Glucose (mg/dl)	130 ± 2.7 [c]	201.3 ± 20.8 [a]	176.3 ± 10.4[b]	105.7 ± 7.2 [d]
Insulin (Uu/ml)	78.4 ± 2.9 [a]	62.7 ±2.6 [b]	40 ± 4.2 [c]	63.4 ±10.9 [b]

Values are shown as means ± SD of N=6.
Different small letters in the same row indicate significant difference at $P< 0.05$.

Table (5): Effect of instant coffee and/ or non-dairy creamer on serum levels of T3and T4 in male albino rats.

Groups / Parameters	G1	G2	G3	G4
T3 (ng/dl)	146.4 ±8.9 [a]	137.1 ± 4.8 [ab]	129.3 ±10.7 [b]	146.4±8.7 [a]
T4 (ug/dl)	9.3 ± 0.76 [a]	8.7± 0.62 [ab]	8.3 ± 0.64 [b]	9.6 ± 0.59 [a]

Values are shown as means ± SD of N=6.
Different small letters in the same row indicate significant difference at $P< 0.05$.

4. Discussion

In the current study, an oxidative stress might be expressed through the significant decreases of heart tissue's GSH after consumption of instant coffee and /or non-dairy creamer with the significant increases of MAD levels after each of instant coffee and instant coffee plus non-dairy creamer consumption. **Schafer and his colleagues (2001**) recommended that oxidative stress is associated with increased production of oxidizing species or a significant decrease in the effectiveness of antioxidant defenses such as GSH. The antioxidant defense GSH, a cysteine-containing tripeptide, is the most abundant non-protein thiol in mammalian cells and plays an important role in the scavenging of ROS and free radicals (**Hashimoto *et al.*, 2008**). **Oyama *et al.* (2002)** stated that the decrease in cellular glutathione content increases cell vulnerability to oxidative stress. The significant decrease of liver GSH after non-dairy creamer consumption, in the present study, is in agreement with **Ojieh *et al.* (2009)**. The authors showed that hydrogenated palm oil significantly decrease liver content of GSH. Malondialdhyde is one of several end products formed via the decomposition of certain primary and secondary lipid peroxidation of product. It is commonly known as a marker of oxidative stress and an increase in free radicals causes over production of MAD (**Gawet *et al.*, 2004**). Consequently, the significant increase of MAD, in the present study might be indicative to an increase of free radicals that in turn might affect cell membranes and functions. **Kuper *et al.* (2000**) stated that powerful oxidants attack poly unsaturated fatty acids in cell membrane phospholipids result in damage of cell membranes and function. The findings of the present study are in parallel with other authors. **Vistisn *et al.* (1992)** and **Diazani *et al.* (1991)** observed that caffeine favor the production of free radicals and leads to a subsequent increase of lipid peroxidation. **Al Moutaery *et al.* (2003)** showed that, in rats that had been given experimental head trauma, a high intraperitoneal dose (100-150 mg/kg) of caffeine increased MAD in the cortex. In addition, administration of caffeine 150 mg/kg orally increased MDA levels in the livers of rats that had been treated with allyl alcohol (**Karas *et al.*, 2001**). Moreover, rats fed on hydrogenated palm oil showed significant increased liver MAD (**Ojieh *et al.*, 2009**).

In contrast, **Pasaoglu *et al.*(2011)** reported that caffeine reduces levels of MAD in heart tissues of rats. This discrepancy could be attributed to dose of caffeine, rout of treatment and duration.

Consumption of instant coffee plus non-dairy creamer reflected synergistic effect on the levels of heart GSH and MAD. Rats consumed coffee with sugar and non-dairy creamer in the study of **Renouf *et al.*(2010)** showed lower blood levels of antioxidants.

Lactate dehydrogenase and CPK are enzymes present in cells of the body with high concentrations in heart. The present study showed significant elevations in the levels of serum LDH and CPK in rats consumed instant coffee. In addition to significant increase of serum CPK levels in rats consumed each of non-dairy creamer and instant coffee plus non-dairy creamer. Instant coffee proved to be more effective on levels of serum LDH than non-dairy creamer. The elevations of LDH and CPK might be indicative to heart injury. **Tietz (2006)** stated that an increase of LDH and CPK are seen in myocardial infarction and myocarditis. Also, **Kasap *et al.* (2007)** confirmed that these two enzymes leak out into serum during myocardial injury due to disintegration of contractile elements and sarcoplasmic reticulum. Many studies have examined the association between coffee consumption and risk of cardiovascular disease however, the results remain controversial.

In the study of **Machado *et al.* (2009)**, caffeine (one of the main biological active compounds of coffee) supplementation has no influence upon serum LDH and CPK. However, **Li *et al.* (2011)** postulated that caffeine administration reduces LDH and CPK and inhibits activation and attenuate expression of pro-inflammatory mediators such as inducible nitric acid oxide synthetase, IL-6 and TNF-a.

The present data recorded that rats consumed instant coffee beverage revealed significant increase in the serum levels of TG, LDL-C, and VLDL-c, while induce significant decrease in HDL-C. The elevations of lipid fractions could be attributed to the lipid component of coffee, cafestol and kahweol, which classified as diterpenes. **Nawrot *et al.*(2003)** suggested that caffeine in not coffee that is responsible for the hypercholesterolemic but to the two diterpenoid alcohols (cafestol and kahweol). The obtained results are in agreement with many other authors. **Rezq and Fathy (2010)** revealed that administration of boiled and Turkish coffee induce significant increase in atherogenic index represented as increase in total lipids, TG, TC, LDL-c and VLDL-c. Also, **Abd El-Fatta (2008)** showed significant elevation of serum total lipids, TC, TG, LDL-c, while a significant decrease of HDL-c in rats fed on diet supplemented with low or high dose of coffee. **Glass and Witztum (2001)** recorded that increased plasma cholesterol, particularly LDL-c, is one of the most important risk factor for coronary vascular disease. Low density lipoprotein-c particles are taken up by macrophage cells after oxidized or

modified and deposited in the arterial intimae leading to formation of atheroma.

The significant increases of serum levels of TG, LDL-C, VLDL-C and LDL-c/ HDL-c ratio in rats consumed non-dairy creamer, could be attributed to the fact that the non-dairy creamer contains hydrogenated palm kernel oil, the major source of trans fats, and corn syrup. **Ibegbulem and Chikezie (2012)** proved that palm kernel oil is one atherogenic factor because it contains more short chain saturated **fatty acid**s and few antioxidant phytochemicals. Increased the serum LDL-c and LDL-c/ HDL-c ratio are indicative to promote the synthesis of cholesterol rather than phospholipids, whereas, HDL-c contains phospholipids more than cholesterol (**Nelson and Cox, 2000; Glew, 2006**).

The significant elevation in fasting serum glucose and decrease in fasting insulin in rats consumed instant coffee may be attributed to caffeine and /or chlorogenic acid, two components in coffee. Several authors reported that caffeine interferes with metabolism of glucose. **Pizzio *et al.* (1998)** found that caffeine consumption raises blood sugar. Also, consumption of caffeine during and after meal raises blood sugar levels in type 2 diabetics (**Lane *et al.*, 2004**). Moreover, caffeine in coffee elevates the stress hormones epinephrine and norepinephrine (**Lane, 1994**). Norepinephrine (noradrenaline) strongly inhibits release of insulin leading to increase blood glucose level during stress (**Lane,1994**) and increased epinephrine level decreases insulin sensitivity (**Keijzers *et al.*, 2002**).

However, **Bidel *et al.* (2006)** reported that coffee consumption is significantly and inversely associated with impaired fasting glucose, impaired glucose regulation and hyper insulinemia. Moreover, **Sadeek *et al.*(2010)** showed that chlorogenic acid has an antagonistic effect on glucose level suggesting a novel function of coffee on lowering the risk factors of diabetes and delaying the progress of diabetes complications as well. In addition, **Afify *et al.*(2009)** proved that caffeine consumption reduced fasting glucose and insulin levels.

Coffee plus non-dairy creamer (G4) demonstrated a synergistic action on lowering fasting blood glucose than the rest tested groups.

The significant decrease of T_3 and T_4 hormone level in rats consumed non-dairy creamer might be attributed to the presence of hydrogenated palm kernel oil. **Doerge and Hebron (2002)** stated that hydrogenated or partially hydrogenated vegetable oil damage cell tissue and negatively affect thyroid as well as health in general, also the long chain fatty acids deposit in cells more often as rancid and oxidizing fats results in impair conversion of T4 to T3.

Conclusion

In the current study, instant coffee augmented the oxidative stress and it was the most effective on increasing the level of LDH. Non-dairy creamer augmented the risk of myocardial integrity and it was the most effectiveness on the levels of LDL, HDL and LDL/HDL. Instant coffee plus non-dairy creamer reflected synergistic effect on increasing the oxidative stress and decreasing the glucose level. These results suggest that instant coffee and non-dairy creamer consumption has been shown to have adverse effects on various biological markers of heart suggestive of increased cardiovascular disease risk.

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