



## Effect of Coffee on Some Biological Parameters in the Rabbit

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### ABSTRACT

**Background and Objectives:** There are a number of dietary compounds with purported health benefits such as reducing the risk of heart disease like flavonoids that show bioactivity through their respective antioxidant activity (Walker et al., 2013). Intake of antioxidants and caffeine found in teas, fruits, vegetables, wines and carbonated beverages may be beneficial to body systems at short term and adverse at higher intakes or long term. Caffeine content varies in most of these beverages (Knight et al., 2004). This article investigates the serum concentrations of glucose, total protein, urea, uric acid and creatinine in a cowbell and Nescafe 3in1 sachet coffee fed-rabbits.

**Materials and Methods:** Twenty-five (25) male rabbits housed in rabbit cages allowed free access to food and water after 7days of acclimatization were assigned into five groups, of 5 rabbits. Group 1 served as control, fed only on a basal diet while groups 2 and 3 received 310mg/kg and 470mg/kg Cowbell coffee low and high dose respectively per body weight. Group 4 and 5 received 460mg/kg and 690mg/kg Nescafe low dose and high dose respectively per body weight. All administrations were done orally, daily and standard rabbit chow and water was given *ad libitum*. The treatment lasted for 30days after which the animals were sacrificed and serum obtained for automated analysis.

**Results:** Blood glucose concentration of cowbell high dose group was significantly ( $p < 0.05$ ) higher compared with other experimental groups. Total protein, albumin, globulin content were significantly ( $p < 0.05$ ) higher in Nescafe 3in1 fed animals compared with control. Blood urea nitrogen levels were significantly ( $p < 0.05$ ) higher in the high dose groups of the different brands compared with other experimental groups. Uric acid levels of cowbell high dose and Nescafe treated groups were significantly ( $p < 0.05$ ) lower.

**Conclusion:** Nescafe 3in1 sachet coffee increased total protein, but reduced serum uric acid levels in the rabbit, while cowbell sachet coffee at high dose caused increased glucose concentration and serum urea in the rabbit. Nescafe 3in1 sachet coffee may reduce joint inflammation caused by monosodium urate. These effects are due in part to the caffeine and/or antioxidant levels of the different coffees.

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**Keywords:** Cowbell coffee, Nescafe 3in1, Total Protein, Uric acid, urea, glucose, Rabbit.

#### 1. Introduction:

Coffee, a global beverage prepared and marketed in different formulations is known to contain caffeine, antioxidants among other constituents. Caffeine in coffee naturally found in leaves, seeds, and fruits of different plant species worldwide (Frary et al., 2005; Barone & Roberts, 1996). The common sources of caffeine in diet are coffee-

drinks, tea, chocolate and fizzy drinks. Caffeine concentrations in coffee beverages are quite variable.

McCusker et al. (2003), found that brewed coffee ranged from 72 to 130mg among distributors. Cowbell (Promasidor Nigeria Limited) and Nescafe 3in1 (Nestle, Ghana Plc.) were found to vary in their net weights 20 –



22g, and 32 – 36g per sachet respectively among distributors. Coffee constituents in these different formulations could affect the health of consumers as well as the sweeteners used. Researchers are divided in opinion about the effect of coffee on health.

It has been reported that coffee interferes with the absorption of supplemental iron due to polyphenols (tannins) present which binds to iron in the intestinal lumen, forming insoluble complexes and thereby inhibiting iron absorption (Morck et al., 1983).

Components of coffee such as chlorogenic acid are also thought to interfere with iron absorption (Gutnisky et al., 1992), therefore, coffee consumption may lead to iron deficiency anemia (Munoz et al., 1988) and as well alter cellular or organ function. Caffeine acts on the adenosine A<sub>2A</sub> receptor expressed on the platelet cell (Ledent et al., 1997; Klotz et al., 1997) and under homeostatic conditions the A<sub>2A</sub> receptors sufficiently activate adenosine in the blood. However, caffeine competitively inhibits the action of adenosine, which normally will bind to the adenosine A<sub>2A</sub> receptor, thus altering the platelet function and increasing receptor activity (Paul et al., 1990, Fredholm, 1995).

This increased receptor activity subsequently results in an increase in cyclic adenosine monophosphate (cAMP) which acts directly to inhibit platelet aggregation (Paul et al., 1990) and may result in altered cellular functions.

Coffee consumption has been found to impair glucose tolerance and to decrease insulin sensitivity in a number of controlled clinical trials (Greer et al., 2001). Several formulations of coffee compounds are on sale in most remote parts of an urban settlement with the poor public enlightenment of their safety, weak regulation, their effects or action on the body systems poorly understood. The aim of this study is to investigate the biological effect of cowbell and Nescafe 3in1sachet coffees on glucose, total protein, urea, uric acid and creatinine in the rabbit.

## 2. Materials and Methods:

### 2.1. Purchase and Preparation of Beverage

Cowbell coffee (Promasidor Nigeria Limited) and Nescafe 3in1 (Nestle Ghana PLC) were purchased from Market Store in Okuku, Yala Local Government Area of Cross River State. The beverages were prepared in boiling water that was cooled before administration.

### 2.2. Experimental Animals and Maintenance

Twenty-five (25) rabbits (400g -600g) were housed in rabbit cages, kept at 28 °C, 58 % humidity and a 12 hour light/dark cycle and were allowed free access to food and water. After 7days of acclimatization, the animals were assigned into five groups, of 5 rabbits. Group 1 served as control, fed only on a basal diet while animals in group 2 and 3 received 310mg/kg and 470mg/kg Cowbell coffee (low and high dose respectively) per body weight. Animals in group 4 and 5 received 460mg/kg and 690mg/kg

Nescafe 3in1 low dose and high dose respectively per body weight. All administrations were done orally, daily and standard rabbit chow and water was given *ad libitum*. The treatment lasted for 30days.

### 2.3. Collection and Analysis Blood Sample

Blood samples were collected by cardiac puncture into centrifuge tubes and allowed to stand for one hour after which they were spun for ten minutes at 3000rpm. Other portions of blood were put in oxalate sample bottles for the determination of blood glucose. The serum derived was used for analysis of total protein, albumin, globulin, blood urea, uric acid, creatinine using Clinical Chemistry Analyzer (Erba Chem 7).

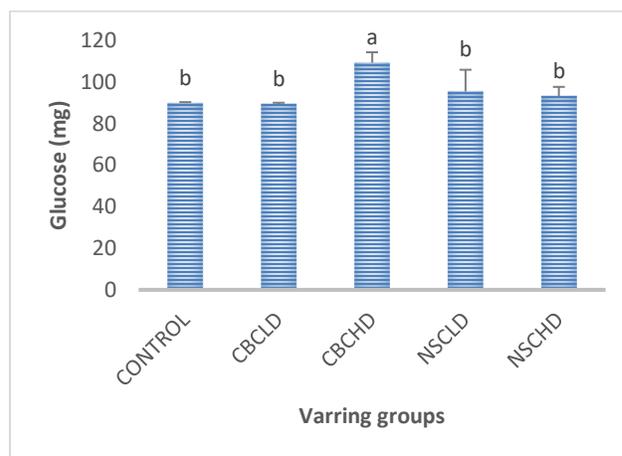
### 2.4. Statistical Analysis

Statistical analysis was carried out using SPSS. One way Analysis of variance (ANOVA) was used to compare means. Data were expressed as the mean  $\pm$  Standard deviation (SD) and a *p* – significance level < 0.05 was accepted as statistical significance.

## 3. Results:

### 3.1. Glucose level of Experimental groups of rabbits

The blood glucose concentration of the group administered cowbell coffee (high dose) was significantly (*p*<0.05) higher compared to control group and groups administered with Nescafe 3in1. Cowbell coffee low dose and Nescafe coffee 3in1treated groups were not statistically significant (Figure 1).



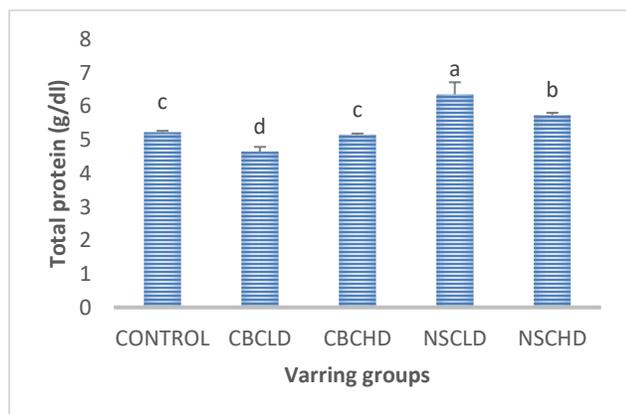
**Figure 1.** Effect of Cowbell and Nescafe coffee Administration on Serum Glucose concentration in test and control rabbit.

Values are expressed as mean  $\pm$  SD (n=5). The different superscript indicates statistically significant differences between groups at *p*< 0.05. CBLD (Cowbell coffee Low dose), CBHD (Cowbell coffee High dose), NSLD (Nescafe Low dose), NSHD (Nescafe High dose)



### 3.2. Total Protein of Experimental groups of rabbits

The Total protein levels of Nescafe3in1 treated groups were significantly ( $p < 0.05$ ) higher compared to control and other groups while total protein levels of Cowbell coffee (low dose) was significantly ( $p < 0.05$ ) lower compared with control group and other experimental groups (Figure 2).

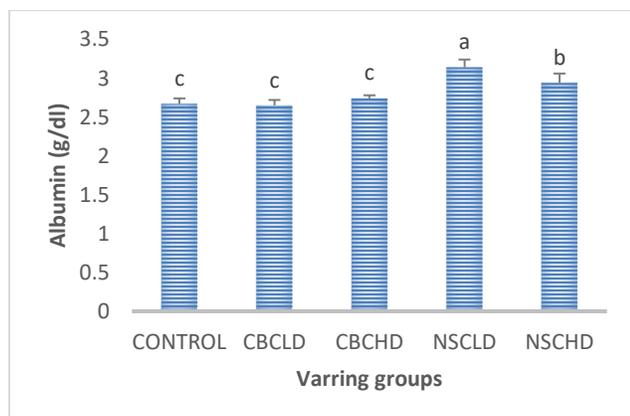


**Figure 2.** Effect of Cowbell and Nescafe coffee Administration on Serum Total Protein concentration in test and control rabbits.

Values are expressed as mean  $\pm$  SD ( $n=5$ ). The different superscript indicates statistically significant differences between groups at  $p < 0.05$ . CBLD (Cowbell coffee Low dose), CBHD (Cowbell coffee High dose), NSLD (Nescafe Low dose), NSHD (Nescafe High dose)

### 3.3. Albumin levels of Experimental groups of rabbits

The Albumin levels of Nescafe 3in1 treated groups were significantly ( $p < 0.05$ ) higher compared with control group and other Cowbell coffee administered groups. There was no significant change in albumin levels of the groups treated with cowbell coffee (Figure 3).

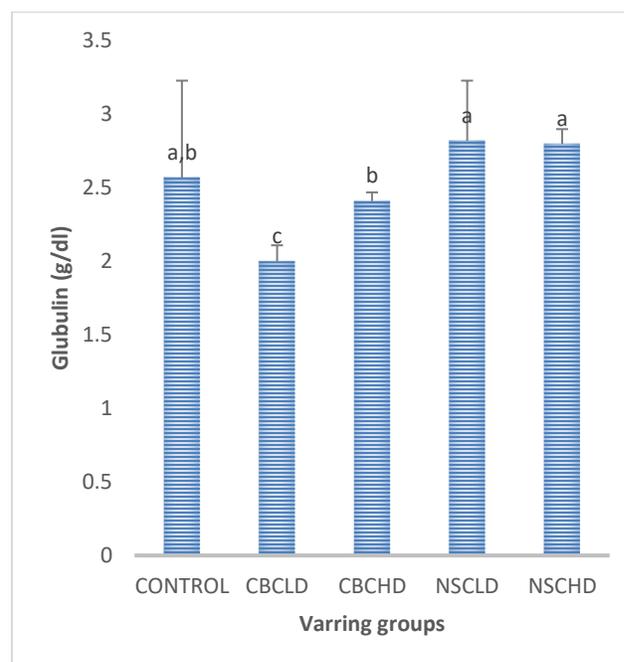


**Figure 3.** Effect of Cowbell and Nescafe coffee Administration on Serum Albumin concentration in test and control rabbits

Values are expressed as mean  $\pm$  SD ( $n=5$ ). The different superscript indicates statistically significant differences between groups at  $p < 0.05$ . CBLD (Cowbell coffee Low dose), CBHD (Cowbell coffee High dose), NSLD (Nescafe Low dose), NSHD (Nescafe High dose)

### 3.4. Globulin levels of Experimental group of rabbits

Cowbell coffee treated groups had low globulin levels compared with control group and other groups (Figure 4). There was no statistically significant difference ( $p > 0.05$ ) observed in the globulin levels of control and groups administered Nescafe 3in1.

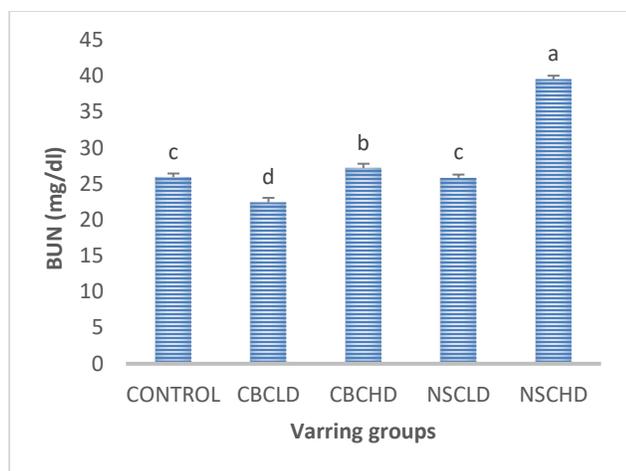


**Figure 4.** Effect of Cowbell and Nescafe coffee Administration on Serum Globulin concentration in test and control rabbits.

Values are expressed as mean  $\pm$  SD ( $n=5$ ). The different superscript indicates statistically significant differences between groups at  $p < 0.05$ . CBLD (Cowbell coffee Low dose), CBHD (Cowbell coffee High dose), NSLD (Nescafe Low dose), NSHD (Nescafe High dose).

### 3.5. Blood Urea levels of Experimental groups of rabbits

The blood urea nitrogen (BUN) of the high doses of Nescafe coffee 3in1 and Cowbell was observed to be significantly higher ( $p < 0.05$ ) compared with control and other experimental groups. The cowbell coffee low dose group had significantly reduced BUN compared to control and other groups. There was a significant difference between Nescafe coffee 3in1 low dose group and the control (Figure 5).

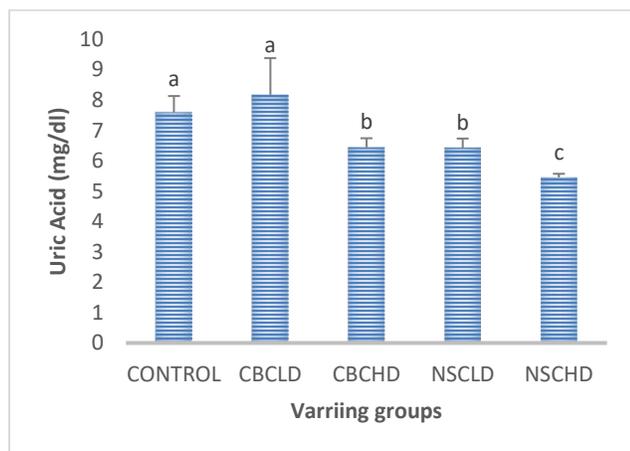


**Figure 5.** Effect of Cowbell and Nescafe coffee Administration on Serum BUN in test and control rabbits

Values are expressed as mean  $\pm$  SD, (n=5). The different superscript indicates statistically significant differences between groups at ( $p < 0.05$ ). CBLD (Cowbell coffee Low dose), CBHD (Cowbell coffee High dose), NSLD (Nescafe Low dose), NSHD (Nescafe High dose)

### 3.6. Uric Acid levels of Experimental groups of rabbits

The Uric acid (UA) levels of cowbell coffee high dose and Nescafe coffee 3in1 treated groups were significantly ( $p < 0.05$ ) lower compared with control, but there was no significant difference between the cowbell low dose low group and the control group (Figure 6).

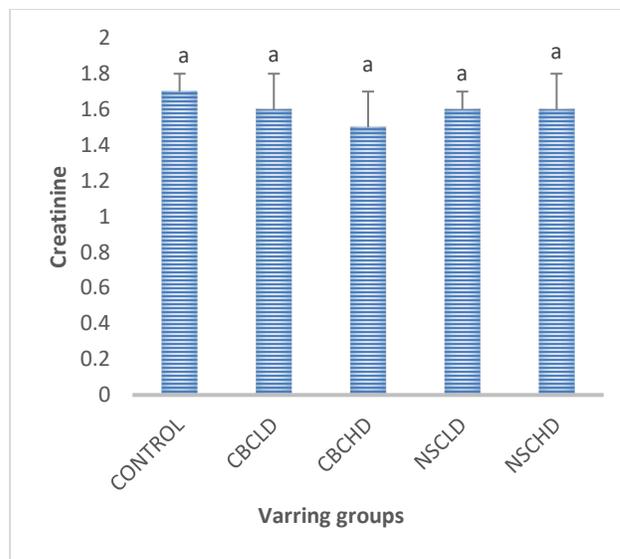


**Figure 6.** Effect of Cowbell and Nescafe coffee Administration on Serum Uric acid concentration in test and control rabbits.

Values are expressed as mean  $\pm$  SD (n=5). The different superscript indicates statistically significant differences between groups at  $p < 0.05$ . CBLD (Cowbell coffee Low dose), CBHD (Cowbell coffee High dose), NSLD (Nescafe Low dose), NSHD (Nescafe High dose).

### 3.7. Creatinine levels of Experimental group of rabbits

There was no statistically significant difference ( $p > 0.05$ ) in the creatinine levels of control group and groups administered cowbell coffee and Nescafe (low and high doses) (Figure 7).



**Figure 7.** Effect of Cowbell and Nescafe coffee Administration on Serum Creatinine concentration of rabbit in test and control rabbits.

Values are expressed as mean  $\pm$  SD (n=5). The different superscript indicates statistically significant differences between groups at  $p < 0.05$ . CBLD (Cowbell coffee Low dose), CBHD (Cowbell coffee High dose), NSLD (Nescafe Low dose), NSHD (Nescafe High dose).

## 4. Discussion:

In this study, we investigated the effects of Cowbell and Nescafe 3in1 Coffee sachet consumption on glucose, total protein, urea, uric acid and creatinine in the rabbit. The cowbell coffee usage at High Dose caused a significant increase in serum glucose level while the Nescafe coffee 3in1 showed no significant change in serum glucose levels. Increased serum levels of glucose and raised serum protein after caffeine administration to rat has been observed (Stawiarska-Pieta et al., 2006; Grucka-Mamczar et al., 2007). Constant intake of coffee (caffeine) impaired glucose tolerance as increase concentration in the blood decreases tissue sensitivity to insulin. This is said to result from an increased adrenaline concentration and inhibition of glucose uptake by muscles dependent on adenosine receptor (Keijzers et al., 2002).

Insulin deficiency caused by caffeine, result in increased lipolysis, intensified oxidation of fatty acids, induction of gluconeogenesis, and inhibition of glycolysis which additionally perpetuates and intensify insulin resistance (Grucka-Mamczar et al., 2007; van Dam, 2008).



The non-significant levels of glucose in the Nescafe 3in1 sachet coffee groups suggest its beneficial effect in glucose homeostasis. Several authors have reported on the hypoglycemic effect of coffee (Nicasio et al., 2005; Rezaq & Fathy, 2010; Jang *et al.*, 2012).

Total protein levels in Nescafe 3in1 coffee treated groups were significantly higher which is consistent with Birkner et al., (2006a, 2006b). These authors observed that an increase in levels of protein after coffee consumption result from intensified renal filtration and diuresis. In that circumstance, there is increased urea excretion by the kidney and according to Stawiarska-Pieta et al., (2006) and Grucka-Mamczar et al., (2007) higher concentration of protein abound in serum. Raised protein content after coffee administration was observed by Delfiol et al., (2012) in horses. On the other hand, the cowbell coffee groups caused a significant decrease in protein levels. The concentration of these macro nutrient markers is affected by protein malnutrition associated with end-stage liver and kidney disease as well as severe infection and most significantly by stress injury (Bishop et al., 2013). Reduced protein levels may reflect low hepatic production or loss of transfer of albumin between the extravascular and vascular component. Decreased albumin, a determinant of chronic protein deficiency under the condition of non-calorie intake may lead to Kwashiorkor or marasmus (Bishop et al., 2013).

The blood urea levels were significantly increased in the high dose groups of the two brands of coffees but were significantly reduced in the low dose of Cowbell coffee. Many research workers have shown that coffee increased serum urea (Tofovic et al., 2002; Portolés et al., 1985). Some studies indicate that caffeine in coffee through inhibition of A2A adenosine receptor accelerate the development of interstitial inflammation, for example, activation of adenosine receptor to inhibit poly morphonuclear cells infiltration and protect the kidney from ischemic reperfusion. Others report no association between coffee or caffeine and serum urea (Cheul Do et al., 1997). Blood urea was significantly reduced in the low dose group of the Cowbell coffee. Birkner et al., (2006a) reported slightly reduced serum urea. Reduction in serum urea may indicate decreased renal blood flow, dehydration, acute or chronic glomerulonephritis (Bishop et al., 2013).

The study showed that the different brands of Coffee decreased uric acid levels in the high dose groups. This is in agreement with the findings of Kiyohara et al., (1999).

They reported that coffee consumption was negatively associated with serum uric acid. Also, Choi and Curhan (2007), found that serum uric acid levels decreased significantly with increasing coffee intake and that the mean serum levels of individuals consuming larger quantities of coffee daily were lower than those individuals consuming lesser milligrams of coffee. Wu et al. (2005), reported that coffee decreased serum uric acid levels. They maintained as evidence that, there was a positive

correlation between serum insulin resistance and elevated uric acid that is the higher insulin resistance, the higher uric acid levels (Choi & Curhan, 2007; Fam, 2002).

Also, the result showed that there was no significant change in creatinine levels following treatment with Cowbell coffee, however, there was a significant reduction among the Nescafe 3in1 groups. Plasma creatinine in blood stream remains reasonably stable, although the protein content of diet does influence it. Reduction in creatinine levels in the Nescafe 3in1 coffee groups with increased serum concentration suggests renal fitness in the rabbit, although the mechanism was not studied.

## 5. Conclusion:

In conclusion consumption of Cowbell coffee and Nescafe 3in1 coffee appear to have some favorable biological effects on glucose and markers of renal function. Increased serum glucose levels in the cowbell high dose group suggest that chronic consumption might precipitate diabetes. On the other hand, Nescafe 3in1 coffee had no effect on glucose. Both brands of coffee increased blood urea at high doses.

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## References:

1. Rezaq, A. A., & Fathy, N. M. (2010). Effect of regular drinking of boiled, filtered or Turkish coffee and its impact on some biochemical parameters relevant to atherogenicity and the functions of the kidney and liver in a rat model. *European J. of Biol. Sci.*, 2(3), 46-54.
2. Birkner, E. Grucka-Mamczar, E. Zwirska-Korczala, K. Zalejska-Fiola, J. Stawiarska-Pieta, B. Kasperczyk, S. Kasperczyk, A. (2006a). Influence of sodium fluoride and caffeine on kidney function and free radical processes in that organ in adult rat. *Boil Trace Elem Res* 109: 35-47
3. Birkner, E., Grucka-Mamczar, E., Zalejska-Fiolka, J., Chlubek, D., Kasperczyk, S., Stawiarska-Pieta, B., & Błazarczyk, U. (2006b). Influence of sodium fluoride and caffeine on the concentration of fluoride ions, glucose, and urea in blood serum and activity of protein metabolism enzymes in rat liver. *Biological trace element research*, 112(2), 169-174.
4. Bishop, M. L., Fody, E. P., & Schoeff, L. E. (Eds.). (2013). *Clinical chemistry: principles, techniques, and correlations*. Lippincott Williams & Wilkins.
5. Barone, J. J., & Roberts, H. R. (1996). Caffeine consumption. *Food and Chemical Toxicology*, 34(1), 119-129.

6. Cheul Do J, C. P. N., Jun Jang, S., Hyun Cho, K., Hwa Park, I., Kwon Son, K., & Woong Kim, S. (1997). Changes in the blood chemistry components in the serum of the rat after oral administration of caffeine. *Korean J. Vet. Serv*, 20, 297-306.
7. Choi, H.K. and Curhan, G (2007). Coffee, tea and caffeine consumption and uric acid levels. The third National Health and Nutritional Examination Survey. (Arthritis and Rheumatism) *Arthritis Care Res* 57(5) pp 816-821
8. van Dam, R. M. (2008). Coffee consumption and risk of type 2 diabetes, cardiovascular diseases, and cancer. *Applied physiology, nutrition, and metabolism*, 33(6), 1269-1283.
9. Delfiol, D. J. Z., Oliveira-Filho, J. P., Casalecchi, F. L., Kievitsbosch, T., Hussni, C. A., Riet-Correa, F., ... & Borges, A. S. (2012). Equine poisoning by coffee husk (*Coffea arabica* L.). *BMC Veterinary research*, 8(1), 4.
10. Fam, A. G. (2002). Gout, diet, and the insulin resistance syndrome. *The Journal of Rheumatology*, 29(7), 1350-1355.
11. Frary, C. D., Johnson, R. K., & Wang, M. Q. (2005). Food sources and intakes of caffeine in the diets of persons in the United States. *Journal of the American Dietetic Association*, 105(1), 110-113.
12. Fredholm, B.B. (1995). Astra Award lecture. Adenosin, Adenosin receptors and the action of caffeine. *Pharmacology and Toxicology*, 76: 93 -101.
13. Greer, F., Hudson, R., Ross, R., & Graham, T. (2001). Caffeine ingestion decreases glucose disposal during a hyperinsulinemic-euglycemic clamp in sedentary humans. *Diabetes*, 50(10), 2349-2354.
14. Grucka-Mamczar, E., Birkner, E., Zalejska-Fiolka, J., Machoy, Z., Kasperczyk, S., & Blaszczyk, I. (2007). Influence of extended exposure to sodium fluoride and caffeine on the activity of carbohydrate metabolism enzymes in rat blood serum and liver. *Fluoride*, 40(1), 62-66.
15. Gutnisky, A., Rizzo, N., Castro, M. E., & Garbossa, G. (1992). The inhibitory action of chlorogenic acid on the intestinal iron absorption in rats. *Acta physiological, pharmacological et therapeutic Latinoamericana: organo de la Asociacion Latinoamericana de Ciencias Fisiologicas y [de] la Asociacion Latinoamericana de Farmacologia*, 42(3), 139-146.
16. Jang,, E. S., Jeong, S. H., Hwang, S. H, Kim, H. Y, Ahn, S. Y & Lee, J. (2012). Effect of Coffee, Smoking and Alcohol on liver function tests, a comprehensive cross-sectional study. *Gastroenterol* 12:145-4.
17. Nicasio, P., Aguilar-Santamaria, L., Aranda, E., Ortiz, S., & González, M. (2005). Hypoglycemic effect and chlorogenic acid content in two *Cecropia* species. *Phytotherapy Research*, 19(8), 661-664.
18. Keijzers, G. B., De Galan, B. E., Tack, C. J., & Smits, P. (2002). Caffeine can decrease insulin sensitivity in humans. *Diabetes care*, 25(2), 364-369.
19. Kiyohara, C., Kono, S., Honjo, S., Todoroki, I., Sakurai, Y., Nishiwaki, M., ... & Nakagawa, K. (1999). The inverse association between coffee drinking and serum uric acid concentrations in middle-aged Japanese males. *British Journal of Nutrition*, 82(2), 125-130.
20. Klotz, K. N., Hessling, J., Hegler, J., Owman, C., Kull, B., Fredholm, B. B., & Lohse, M. J. (1997). Comparative pharmacology of human adenosine receptor subtypes—characterization of stably transfected receptors in CHO cells. *Naunyn-Schmiedeberg's archives of pharmacology*, 357(1), 1-9.
21. Knight, C. A., Knight, I., Mitchell, D. C., & Zepp, J. E. (2004). Beverage caffeine intake in US consumers and subpopulations of interest: estimates from the Share of Intake Panel survey. *Food and Chemical Toxicology*, 42(12), 1923-1930.
22. Ledent, C., Vaugeois, J. M., Schiffmann, S. N., & Pedrazzini, T. (1997). Aggressiveness, hypoalgesia and high blood pressure in mice lacking the adenosine A2a receptor. *Nature*, 388(6643), 674.
23. McCusker, R. R., Goldberger, B. A., & Cone, E. J. (2003). Caffeine content of specialty coffees. *Journal of analytical toxicology*, 27(7), 520-522.
24. Morck, T. A., Lynch, S. R., & Cook, J. D. (1983). Inhibition of food iron absorption by coffee. *The American journal of clinical nutrition*, 37(3), 416-420.
25. Munoz, L. M., Lönnerdal, B., Keen, C. L., & Dewey, K. G. (1988). Coffee consumption as a factor in iron deficiency anemia among pregnant women and their infants in Costa Rica. *The American journal of clinical nutrition*, 48(3), 645-651.
26. Paul, S. U. B. I. R., Feoktistov, I., Hollister, A. S., Robertson, D., & Biaggioni, I. (1990). Adenosine inhibits the rise in intracellular calcium and platelet aggregation produced by thrombin: evidence that both effects are coupled to adenylate cyclase. *Molecular pharmacology*, 37(6), 870-875.
27. Portolés, M., Miciana, M. D., Jordá, A., & Grisolia, S. (1985). Caffeine-induced changes in the composition of the free amino acid pool of the cerebral cortex. *Neurochemical research*, 10(7), 887-895.
28. Stawiarska-Pieta, B., Grucka-Mamczar, E., Stojko, R., Birkner, E., & Zalejska-Fiolka, J. (2006). The effect of sodium fluoride and caffeine on concentrations of calcium, phosphorus, and magnesium in rat serum. In *Annales Academiae Medicae Stetinensis* (Vol. 52, pp. 97-101).
29. Tofovic, S. P., Kost, C. K., Jackson, E. K., & Bastacky, S. I. (2002). Long-term caffeine consumption exacerbates renal failure in obese, diabetic, ZSF1 (fa fa cp) rats. *Kidney international*, 61(4), 1433-1444.

30. Walker, B. R., Colledge, N. R., Ralston, & S. H, Penman, D. I. (2013). *Davidson's Principles and Practice of Medicine E-Book*. Elsevier Health Sciences..

31. Wu, T., Willett, W. C., Hankinson, S. E., & Giovannucci, E. (2005). Caffeinated coffee, decaffeinated coffee, and caffeine in relation to plasma C-peptide levels, a marker of insulin secretion, in US women. *Diabetes Care*, 28(6), 1390-1396.

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