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EFFECT OF COFFEE ON SOME BIOCHEMICAL PARAMETERS OF ALBINO RATS

[1] Henrrietta Ogadimma Asuzu-Samuel, [2]Karibo Amakiri Okari

[1] Biomedical Technology Option, School of Science Laboratory Technology, University of Port Harcourt.

[2] Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Medical Sciences, River State University, Nkpolo-Oroworukwo, Port Harcourt.

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Abstract: **The present study was conducted to determine the effect on some biochemical parameters by oral administration of coffee solution. 20 albino rats were used in this study. The animals were placed into 4 groups and were acclimatized for 7 days. Each group was administered a different dose of coffee solution with group 1 been the control group and received no coffee solution, while group 2 was administered with 0.25ml of coffee solution and group 3 was administered with 0.5ml of coffee solution, while group 4 was administered with 0.7ml solution. After 14 days of coffee solution administration, animals were sacrificed and 5ml of blood sample was collected through cardiac puncture into fluoride oxalate bottles. Some biochemical parameters (AST, ALT, ALP Total protein and albumin) as well as some electrolytes (Na+, K+, Cl- and HCO3-) were analyzed using the appropriate reagent and standard procedure. Graphpad software was used for data analysis and significant value was established at P < 0.05. The result showed that coffee solution caused an increase or decrease as follows.0.75ml caused the highest decrease in ALT, 0.5ml caused the highest decrease in ALP, 0.25ml, 0.5ml and 0.75ml all caused an increase in AST, 0.75ml caused the highest decrease in Albumin, 0.75ml caused an increase in total protein, 0.75ml caused an increase in sodium, 0.25ml caused an increase in potassium, 0.25ml caused a decrease in bicarbonate and 0.25ml caused an increase in chloride.**

Keywords: **Coffee, Sodium Ions, Biochemical, Albino Rats and Potassium.**

1. INTRODUCTION

Coffee is a brewed drink prepared from roasted coffee beans, the seeds of berries from certain *Coffea* species. The genus *Coffea* is native to tropical Africa (specifically having its origin in Ethiopia and Sudan) and Madagascar, the Comoros, Mauritius, and Réunion in the Indian Ocean (Maurin, et al., 2007), Coffee plants are now cultivated in over 70 countries, primarily in the equatorial regions of the Americas, Southeast Asia, Indian subcontinent, and Africa. The two most commonly grown are *C. arabica* and *C. robusta*. Once ripe, coffee berries are picked, processed, and dried. Dried coffee seeds (referred to as "beans") are roasted to varying degrees, depending on the desired flavor. Roasted beans are ground and then brewed with near-boiling water to produce the beverage known as coffee. Caffeine which is a major constituent of coffee can have both positive and negative health effects. It can treat and prevent the premature infant breathing disorders bronchopulmonary dysplasia of prematurity and apnea of prematurity. Some people experience sleep disruption or anxiety if they consume caffeine, but others show little disturbance. Evidence of a risk during pregnancy is equivocal; some authorities recommend that pregnant women limit consumption to the equivalent of two cups of coffee per day or less (American College of Obstetricians and Gynecologists, *2010*). The aimed is to determine the effect of coffee on some biochemical parameters of albino rats.Caffeine can produce a mild form of drug dependence – associated

with withdrawal symptoms such as sleepiness, headache, and irritability – when an individual stops using caffeine after repeated daily intake (*Malenka et. al., 2009;* Juliano et. al., 2004). Liver function test are groups of laboratory tests that give information about the state of the liver (Lee Mary, 2009). ALT is commonly measured clinically as a part of a diagnostic evaluation of hepatocellular injury, to determine liver health (Nyblom et. al., 2006). In animals, sodium ions are necessary for the aforementioned functions and for heart activity and certain metabolic functions (Pohl et. al., 2013). Sodium chloride is the principal source of sodium in the diet, and is used as seasoning and preservative, such as for pickling and jerky; most of it comes from processed foods (Geleijnse, et. al., 2004). Potassium is the main intracellular ion for all types of cells, while having a major role in maintenance of fluid and electrolyte balance (*Clausen*, et. al., 2013). The amount of serum chloride is carefully controlled by the kidneys (Morrison et. al., 1990)

2. MATERIALS AND METHODS

Sample Collection

20 albino rats male and female were procured from University of Port Harcourt, Pharmacology Department, Animal House. Their weight was checked with a weighing balance and then, rats where placed into groups.

Groups

The rats where divided into 4 groups according to sex with each group receiving different dosage of coffee solution except for group 1 which is the control group and received feed and water only.

Group 1:The control group

Group 2:0.25ml

Group 3:0.5ml

Group 4:0.75ml

Duration

This study ran for 21 days of which there was 7 days of acclimatization where all rats were acclimatized, during which they were properly fed and their bedding regularly changed.

Coffee solution (Nescafe) was then administered orally to all rats for 14 days, except the control group. After which blood samples were collected and taken to the laboratory for biochemical and analysis.

Calculation of coffee solution for administration per group

Group 1: The Control Group

Group 2: An average man

- Weighing 70kg takes a tea spoon of coffee a day.
- A tea spoon of coffee weighs 2g which in mg is 2000mg
- The weight of an average rat weighs 180g.

:. If 70kg man takes 2000mg then 180g rat will take;

$$\frac{180g \times 2000mg}{70{,}000g} = 5.14mg$$

:. Increasing the dose by 3 gives us 20.56.

Prepare a 20.56mg/ml solution

:. $20.56 \times 20\ animals = 411.2 \times 7$ days of administration = 28.78mg (in every seven days, you prepare a solution)

$$\therefore \frac{20.56 \times 200mg}{1 \times 200ml} = 20.56mg/ml$$

Now weigh out 4.112g of coffee and dissolve in distilled water and make it up to 200ml.

This gives a concentration of 20.56mg/ml which is 0.25ml for group II.

Group 3:

- Twice the dose

4000mg of coffee for a 70kg man.

If 70kg man takes 4000mg then 180g animal will take

$$\frac{180g \times 4000mg}{70{,}000g} = 10.28$$

Preparation of the solution

If 1ml contains 20.56mg, then 10.28mg will be contained in

$$\frac{10.28mg \times 1ml}{20.56} = 0.5ml$$

Therefore each 0.5ml of coffee for group III contains 20.56mg/ml of coffee.

Group 4:

Thrice the dose of coffee for a 70kg man if 70kg man takes 6000mg then 180g animal will take

$$\frac{180g \times 6000mg}{70{,}000} = 15.42mg$$

So, if 1ml contains 20.56mg then 15.42mg will be contained in

$$\frac{15.42 \times 1ml}{20.56mg} = 0.75ml$$

Therefore every 0.75ml of coffee for group iv contains 20.56mg/ml

Table 1: Dosage of administered coffee solution

Group	No	Dosage of coffee
1	5	Feed + water
2	5	0.25ml
3	5	0.5ml
4	5	0.75ml

Procedures for biochemical analysis

Analysis for Sodium (Na)

- Test tube were gotten and labeled as blank, standard, control and sample
- 1ml of Filtrate Reagent was pipette into all test tube
- 50 µl of the blood sample was added to all test tube and distilled water to the blank.
- All tubes where shaken vigorously and mixed for 3 minutes
- All tubes where centrifuged at high speed for ten minutes and supernatant fluids are then test as follows
- 1ml of Acid Reagent was pipette into all tubes

- 50 µl of supernatant was added to respective tubes and mixed
- Zero spectrophotometer with distilled water at 550 nm was carried out
- Absorbance level of all tubes was read and recorded.

Analysis for Potassium (k)

- Test tube were gotten and labeled as blank, standard, control and sample
- 1.0 mL of Potassium Reagent was pipette to all tubes
- 0.01 mL of samples was added to respective tubes. Was mixed and let sit for 3 minutes at room temperature
- After 3 minutes, the wavelength of the spectrophotometer was set at 500 nm, zero spectrophotometer with reagent blank.
- Absorbance level of all tubes was read and recorded

Analysis for Chloride (Cl)

- Test tube were gotten and labeled as blank, standard, control and sample
- 1.5 ml of chloride reagent was pipette into each tube
- 0.01 ml of blood sample was added to respective tubes and mixed
- Incubation was observed for 5 minutes at room temperature
- Spectrophotometer wavelength was set at 480 nm and zero with reagent blank
- Absorbance was read and recorded for all tubes.

Analysis for Bicarbonate (HCO_3^-)

- Prepare CO_2 according to Reagent preparation
- Label tubes appropriately
- Pipette 1.0mL carbon dioxide reagent into each tube
- Incubate all tubes for 3 minutes at 37ºC
- Set spectrophotometer wavelength at 340 nm, temperature to 37ºC
- Pipette 0.005 mL of water, standard and sample to the labelled cuvettes
- Mix gently by invasion and incubate for 5 minutes
- Read and record absorbance of all cuvettes at 340 nm.

Analysis for Aspartate Aminotransferase (AST)

Measurement against Reagent Blank

- Reagents where prepared and mixed with the blood sample
- The mixture was incubated at 37°C for 30 minutes and then pipette into a cuvette
- The absorbance of the sample and standard was read and recorded.

Measurement against Sample Blank

- Reagents where prepared and mixed with the blood sample
- The mixture was incubated at 25°C for 20 minutes and then pipette into a cuvette
- The absorbance of the sample and standard was read and recorded.

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Analysis for Alanine Aminotransferase (ALT)

Measurement against Reagent Blank

- Reagents where prepared and mixed with the blood sample
- The mixture was incubated at 37°C for 30 minutes and then pipette into a cuvette
- The absorbance of the sample and standard was read and recorded.

Measurement against Sample Blank

- Reagents where prepared and mixed with the blood sample
- The mixture was incubated at 25°C for 20 minutes and then pipette into a cuvette
- The absorbance of the sample and standard was read and recorded.

Analysis for Akaline Phosphatase (ALK PHOS)

- Reagents where prepared and mixed with the blood sample
- The mixture was incubated at 37°C for 5 minutes and then pipette into a cuvette
- The absorbance of the sample and standard was read and recorded.

Analysis for Total Protein

- Reagents where prepared and mixed with the blood sample
- The mixture was incubated at 25°C for 30 minutes and then pipette into a cuvette
- The absorbance of the sample and standard was read and recorded.

Analysis for Albumin

- Reagents where prepared and mixed with the blood sample
- The mixture was incubated at 37°C for 10 minutes and then pipette into a cuvette
- The absorbance of the sample and standard was read and recorded.

3. RESULTS AND DISCUSSION

Table 2: Effect of coffee solution on Liver function parameters

Group	AST (IU/L)	ALT (IU/L)	Alk-Phos (IU/L)	Total protein (g/l)	Albumin (g/l)
A (Control)	60.2 ± 22.55	30.6 ± 10.13	55.4 ± 24.08	56 ± 19.14	23.8 ± 3.56
B (0.25ml)	86.4 ± 5.81	25.4 ± 7.79	34.2 ± 14.67	68.2 ± 19.99	22 ± 1.58
C (0.5ml)	78.6 ± 14.51	24.2 ± 1.78	26.8 ± 3.83 *	83.4 ± 10.69	21.8 ± 1.30
D (0.75ml)	83.8 ± 7.12	22.6 ± 4.56	32 ± 4.47	73 ± 13.83	22.6 ± 1.81

Each value represents a mean ± S.D; *= P < 0.05

Table 3: Effect of coffee solution on Electrolytes parameters

Group	Sodium (mmol/l)	Potassium (mmol/l)	Bicarbonate (mmol/l)	Chloride (mmol/l)
A (Control)	59 ± 19.72	5.82 ± 2.26	25 ± 4.63	54.4 ± 9.18
B (0.25ml)	51.8 ± 7.82	7.66 ± 1.07	20.4 ± 9.50	56.2 ± 12.07
C (0.5ml)	50.8 ± 5.45	4.1 ± 1.04	23 ± 7.34	50.8 ± 11.09
D (0.75ml)	64.8 ± 11.38	6.38 ± 2.79	24.4 ± 3.91	53.8 ± 10.44

Each value represents a mean ± S.D; *= P < 0.05

From the mean values obtained, it can be observed that all dosage levels of coffee solution caused a general increase in AST concentration.

Also, from the mean values of the result obtained, it can be deduced that all dosage levels of coffee solution caused a general decrease in ALT with the 0.75ml causing the highest decrease.

Normal levels of AST and ALT may slightly vary depending on the individual laboratory's reference values (Siamak et al, 2019). Typically, the range for normal AST is reported between 10 to 40 units per liter and ALT between 7 to 56 units per liter (Siamak et al, 2019). Mild elevations are generally considered to be 2-3 times higher than the normal range.

Elevated levels of liver enzymes in general signify some form of liver (or hepatic) damage or injury. These levels may be elevated acutely (short term) indicating sudden injury to the liver, or they may be elevated chronically (long term) suggesting ongoing liver injury. In addition to the duration, the level of abnormal elevation of the aminotransferases is also significant. In some conditions the elevation could be mild, consistent with a mild injury or inflammation of the liver. They can also be severely elevated, possibly up to 10 to 20 times the normal values, suggesting more significant damage to the liver. It can also be deduced that all dosage levels also caused a general decrease in Alkaline Phosphatase (ALP) with the 0.5ml dose causing the most decrease.

The ALP level in healthy adults should be 20–140 units per liter (U/L) (Siamak et al, 2019). Children tend to have significantly higher levels of ALP than adults because their bones are still growing. All dosage levels caused a general increase in total protein levels with the 0.25ml causing the least increase and 0.5ml causing the most increase. Also, all dosage levels caused a general decrease in Albumin, this decrease is slight with the 0.5ml dose having the most decrease and 0.75ml dose having the least decrease.

The normal range for total protein is typically 60-80g/L. (It is also sometimes reported as "6.0-8.0g/dl") (Siamak et al, 2019), but this may vary depending on the method of analysis.

Concentrations below the reference range usually reflect low albumin concentration, for instance in liver disease or acute infection. Rarely, low total protein may be a sign of immunodeficiency.

Concentrations above the reference range are found in paraproteinaemia, Hodgkin's lymphoma, leukaemia or any condition causing an increase in immunoglobulins. Total protein is also commonly elevated in dehydration and C677T gene mutation (Siamak et al, 2019).

The effect on sodium concentration with 0.25ml and 0.5ml causing a decrease in sodium concentration, while 0.75ml caused an increase in sodium concentration.

Also, an effect on potassium concentration, with the 0.25ml and 0.75ml causing an increase, while the 0.25ml causing the most increase. While the 0.5ml dose caused a decrease in potassium concentration.

Normal serum sodium levels are between approximately 135 and 145 mEq/liter (135 - 145 mmol/L). A serum sodium level of less than 135 mEq/L qualifies as hyponatremia, which is considered severe when the serum sodium level is below 125 mEq/L (Melissa C. S. 2019). And serum sodium level above 145 mEq/L qualifies as hypernatremia which can lead to hypertension (Melissa C. S. 2019).

While excess potassium in the bloodstream can result from diseases of the kidneys or adrenal glands as well as from certain medications, this is a condition known as hyperkalemia. Hyperkalemia can also be the result of potassium moving out of its usual location within cells into the bloodstream.

The normal potassium level in the blood is 3.5-5.0 milliEquivalents per liter (mEq/L) (Melissa C. S. 2019).

Potassium levels between 5.1 mEq/L to 6.0 mEq/L are considered to be mild hyperkalemia (Melissa C. S. 2019).

Potassium levels of 6.1 mEq/L to 7.0 mEq/L are moderate hyperkalemia, and levels above 7 mEq/L reflect severe hyperkalemia (Melissa C. S. 2019).

Potassium levels below 3.5 mEq/L is considered to be hypokalemia (Melissa C. S. 2019).

All dosage level caused a general decrease in bicarbonate concentration with the 0.25ml dose causing the most decrease and 0.75ml dose causing the least decrease.

Bicarbonate (HCO^-_3) is a vital component of the pH buffering system of the human body (maintaining acid–base homeostasis). 70%–75% of CO_2 in the body is converted into carbonic acid (H_2CO_3), which is the conjugate acid of HCO^-_3 and can quickly turn into it. With carbonic acid as the central intermediate species, bicarbonate – in

conjunction with water, hydrogen ions, and carbon dioxide forms this buffering system, which is maintained at the volatile equilibrium (Melissa C. S. 2019) required to provide prompt resistance to pH changes in both the acidic and basic directions. This is especially important for protecting tissues of the central nervous system, where pH changes too far outside of the normal range in either direction could prove disastrous by causing acidosis or alkalosis.

Effect on chloride concentration with the 0.25ml causing an increase in chloride concentration, while the 0.5ml and 0.75ml dose caused a decrease in chloride concentration with the 0.5ml causing the most decrease.

Chloride is one of the most important electrolytes in the blood. It helps keep the amount of fluid inside and outside of your cells in balance. It also helps maintain proper blood volume, blood pressure, and pH of your body fluids.

The normal blood reference range of chloride for adults in most labs is 96 to 106 milliequivalents (mEq) per liter.

4. CONCLUSION

In conclusion, it can be established that based on the result obtained from this study, coffee solution has effects on the various biochemical parameters which have been looked into. And these effects can be beneficial or detrimental.

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DISCLOSURE OF CONFLICT OF INTEREST

There was no conflict of interest between the authors.

STATEMENT OF ETHICAL APPROVAL

Ethical approval was sought and received from the Head of Department and Committee.

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