The Effect of Apple Cider Vinegar on TheLipid Profile And Electrolytes of Wistar Rats

ABSTRACT

Aim: This study was to investigate the effects of apple cider vinegar with "the mother" on lipid profile and electrolytes of Wistar rats.

Materials and Methods: Twelve female albino rats with mean weight of 150±20 were grouped into four groups. The first group was the control. The control was given distilled water and allowed access to normal animal feed *ad libitum* but was not administered apple cider vinegar. The second group was the group to be sacrificed after the first week of experiment. The group was given distilled water, allowed access to normal animal feed *ad libitum* and administered 1ml apple cider vinegar solution twice daily. The third group was the group to be sacrificed after the second week of experiment. The group had same treatment as the second group above. The fourth group was the group to be sacrificed after the third week which was the final week of experiment. The group had same treatment like the second and third groups.

Results: After oral administration of the apple cider vinegar on rats for 7 days up to 21 days, the results revealed that thesignificant reductions in a time dependent manner with the highest reductions obtained on the last week of experiment ($p<0.05$). After 21 days, triglycerides reduced from 3.37 ± 0.14 to 2.73 ± 0.13 mmol/l, total cholesterol from 4.04 ± 0.98 to 3.62 ± 0.33, low density lipoprotein cholesterol from 8.24 ± 1.31 to 7.02 ± 0.30, very low density lipoprotein cholesterol from 1.55 ± 0.07 to 1.42 ± 0.04mmol/l in the blood of rats. It also revealed a significant decrease ($p< 0.05$) in calcium electrolyte concentration from 11.54 ± 0.21 to 7.09 ± 0.20 mmol/l. It also revealed significant decrease ($p<0.05$) in the sodium and elevation in potassium electrolytes concentrations from 153.63 ±0.24 to 120.30 ± 1.31 and 3.61 ± 0.30 to 4.92± 0.46 mmol/l respectively.

Conclusion: The results suggested that the apple cider vinegar reduced triglycerides and cholesterol levels in the blood of Wistar rats. The results also suggested that apple cider vinegar reduced calcium and sodium electrolyte levels in the blood but increased potassium levels in the blood ofWistar rats based on the 1ml administration for 21 days.

Keywords: Apple cider vinegar, Cholesterol, Electrolytes, Lipids, Potassium, Sodium, Triglycerides.

1.0 INTRODUCTION

Vinegar appeared in the British Isles from the French "vinaigre'', a word translated**"**sour taste'' and originated from the Latin vinum *acre*, ''sour wine'' or simply, vinum

acetum,“wine vinegar’’ [1]. More so, the word *acetum* that simply means ‘’vinegar’’ in its basic sense is derived from the verb aceremeaning “to become pungent, go sour’’ and close to the Greek word (akme`), ‘spike’, while the Greek term for vinegar is (o`xos) possessing the same language background of being sharp and pungent.

Indeed, vinegar has been cited since ancient times. According to legend, a Babylonian courtier around 5000BC,was said to have founded wine produced from neglected grape juice and that remarkable discovery led to the advent of vinegar. History also has it that Hippocrates (c. 420BC), the father of modern medicine was said to have used vinegar to treat wounds, and one Chinese physician, Sung Tsein those days advocated the use of vinegar when washing hand to prevent infections during medical procedures [1]

Vinegar is a liquid that has acetic acid of about 5-20% in concentration. It also contains chemicals such as:anthocyanins, flavanols,mineral salts, vitamins, amino acids, non-volatile acids, polyphenolic compounds and water.According to Saladin [2], the reaction between ethanol and oxygen produces acetic acid.Vinegar is a product made from any fermentation of carbohydrate sources like grape, apple, melon, honey, potato, where yeast ferments sugar to alcohol, and the alcohol is then converted to acetic acid by Acetobacter bacteria.

The method for the production of vinegar can either be slow or fast. The slow method is also known as the traditional method or surface culture system. This is where the liquid is exposed to air in the presence of static culture of acetic acid bacteria. The vessels are filled with the juice to some capacity to allow air space, which is kept in contact with the outside air. On the other hand, the fast method also called the submerged culture systemis used to reduce the acidification period. The bacteria form a solid bed on which the vinegar spreads as a result their being immobile on a wood chips. The vinegar moves through the bed of the chips and then it is collected in a vessel at the bottom and forced back into the same fixed bed. This process elevates the acidity level of the vinegar. With this method, quality vinegar can bethe produced faster even in weeks [3].

Apple cider vinegar is a type of vinegar produced from cider or apple must. It is made from cider or apple mash in the same way as malt vinegar. It possesses a strong-taste at full strength and a fine quality apple flavour when filtered. But due to the growing notion that the unfiltered organic product is therapeutic, it is sold as an unfiltered unpasteurised product with beneficial bacteria called “the mother’’ which is he unrefined ACV. However, apple cider can also be seen in stores as filtered or finely distilled product [2, 3].

Botanic plant products like apple cider vinegar are said to be therapeutic due to their chemical compositions [4],little wonderthe old Welsh proverb ‘’an apple a day keeps the doctor away’’. As one of the most cultivated and consumed fruits in the world, apples are continuously being praised as a ‘’miracle food’’.Vinegar is a good culinary ingredient [5]. Vinegar isantiglycaemic,it is alsoantibacterial. Interestingly, apple cider vinegar has been identified to be capable of influencing the lipid profiles in Wistar rats and man respectively[6, 7, 8, 9].

The implication of cholesterol in the development of hypercholesterolemia and lipid related diseases have stimulated enormous and growing literature. In simplest terms, it appears there is a statistically significant correlation between high serum cholesterol level and the incidence of lipid related disease. This suggests that it would be desirable to maintain normal level of cholesterol in the blood plasma.

The term lipids are applied to those fatty, oily and waxy substances of animals or vegetable origin that are practically insoluble in water but dissolve freely in non-polar solvent such as chloroform, ether, hexane and benzene [10]. These properties stem from the characteristics of relatively large hydrocarbons portion in lipid molecules that may be branched, or unbranched, cyclic, saturated or unsaturated. In physiological fluids and tissues, most lipids are present in combination with proteins and such lipid-protein complexes are referred to as lipoproteins [11].

Lipoproteins are spherical macro-molecular complexes of lipids with spherical proteins termed apoproteins. Their major function is the transport of lipids of dietary or endogenous origin within the hydrophilic environment of the plasma to tissues which utilise the constituent fatty acid or cholesterol for oxidative metabolism, triglyceride for storage or maintenance of cellular function and membrane integrity [12]. And where the level of the lipoprotein is abnormally high, the organism stands the risk of lipids disease condition known as hyperlipidemia. According to Nelson [13],hyperlipidemia is elevation of fasting total cholesterol concentration.

In physiology, the paired charged particles are referred to as electrolytes.Examples include but not restricted to Sodium ion (Na^+), Potassium ion (K^+) and Calcium ion (Ca^{2+}). These charged particles also referred to as ions are very important for the maintenance of the osmotic gradients between intracellular and extracellular fluid. They regulate fluid balance and blood pressure control. These gradients are important for hydration of the body,

activities of the nerves and muscles and blood pH. Without sufficient amount of these ions, muscle weakness or contraction and expansion issues will emerge.

Electrolyte balance or homeostasis is regulated by hormones. Examples of these hormones are; antidiuretic hormones, aldosterone and parathyroid hormones. Electrolyte balance is very important for normal body functions. When levels of cations such as sodium, potassium or calcium drop too low or rise too high, the health and life of animals are at risk. When the level of the electrolytes for heart and other muscle function istoo low, the organs or tissues stop working [14]. Excess of cations such as calcium or potassium will lead to alkalosis.

Concerned electrolyte issues such as dehydration and overhydration may lead to cardiac and neurological complications in an organism. And until the issue is resolved, it will remain a medical challenge to that organism.Measurement of electrolytes is through a diagnostic procedure- through a blood test or urinalysis.

This aim of this study is to check the effect of apple cider vinegar with "the mother'' on cholesterol, triglyceride and some electrolytes such as sodium, calcium and potassium ions in the blood of Wistar rats.

2.0 MATERIALS AND METHODS

The apple cider vinegar with "the mother'' was bought from a Supermarketin Port Harcourt, Rivers State.

2.1 EXPERIMENTAL ANIMAL

Female albino wistar rats.

2.2 TREATMENT/ DIET

Apple cider vinegar with "the mother'' and normal animal feeds.

2.3 TREATMENT PREPARATION

Sixteen ounce (oz) of concentrated apple cider vinegar was diluted with 480 ml of distilled water. One ml of the diluted solution was used as the treatment.

2.4 EXPERIMENTAL DESIGN

The experimental design was made of 12 female albino Wistar rats purchased from the Biochemistry animal house in Choba University of Port Harcourt. The mean weight was 150±20g. The experimental animals were grouped into 4 groups and the method of feed was by gavaging. Each group had its experimental animals of 3 for each week that were allowed access to normal animal feeds*ad libitum*and distilled water.The sacrificing of experimental animals was carried out every 1 week (7days). The experiment lasted for 3 weeks (21 days).The first group was the control. The control was given distilled water and allowed access to normal animal feed *ad libitum* but was not administered apple cider vinegar. The second group was the group to be sacrificed after the first week of experiment. That is, 7 days. The group was given distilled water, allowed access to normal animal feed *ad libitum* and 1ml apple cider vinegar twice daily. The third group was the group to be sacrificed after the second week of experiment. That is, 14 days. The group had same treatment as the second group above. The fourth group was the group to be sacrificed after the third week which was the final week of experiment. The group had same treat as the second and third above but was sacrificed after 21 days (3weeks).

GROUP 1:The group served as the control.The group had access to standard animal feeds *ad libitum*and distilled water but was not administered 1ml apple cider vinegar for the days the experiment lasted before sacrifice.

GROUP 2**:** The group served as the experimental animals to be sacrificed after 1 week. The group had access to normal animal feeds *ad libitum*and distilled water while experiment lasted. The group was also administered 1ml apple cider vinegar morning and evening from day 1 of experiment to day 7 the experiment lasted before sacrifice.

GROUP 3**:** The group served as the experimental animals to be sacrificed after 2 weeks. The group had accessto normalanimal feeds *ad libitum*and distilled waterwhile experiment lasted. The grouphad the same administration as group 2 above. However, the administration lasted for 14 days before sacrifice.

GROUP 4**:** The group served as the experimental animals to be sacrificed after 21 days the experiment lasted. The group also had access to normal animal feeds*ad libitum* and

distilled water andsame administration as groups 2 and 3. However, the administration lasted for 21 days before sacrifice.

2.5 SACRIFICE OF THE EXPERIMENTAL ANIMALS

The administration of the apple cider vinegar was between 10am-11am in morning and 3pm -4pm in the evening. Twenty four hours after the last administration, the animals were anaesthetized under chloroform vapour. Sacrifice was made after the experimental animals have been completely anaesthetized. The experimental animals were dissected and blood was collected through cardiac puncture and stored in sterile lithium heparin bottles for accurate laboratory analysis.

2.6 **ESTIMATION OF LIPID PROFILE PARAMETERS**.

The plasma levels of all the Lipids were determined using Mindray test kits.

PLASMA HDL ESTIMATION

Method

The direct method [15] was used to determine the level of high density lipoprotein – cholesterol in the samples.

Reaction Principle

（1） LDL,VLDL, Chylomicrons ↔ Cholestenone + H_2O_2

$2H_2O_2 \leftrightarrow 2H_2O + O_2$

（2） HDL ↔Cholestenone + H_2O_2

H_2O_2 + HDAOS + 4-aminoantipyrin ↔ Quinonimine

The System monitors the change in absorbance at 600 nm. This change in absorbance is directly proportional to the concentration of cholesterol in the sample and is used by the System to calculate and express the HDL-cholesterol concentration.

Procedure

Two test tubes labelled T1 (reagent blank) and T2 (test sample) were set up. T1 contained 900 µL of reagent (R1) and 12 µL of distilled water, while T2 contained 900 µL of reagent (R1) and 12 µL of test sample. The contents of each tube were mixed and incubated at 37℃ for 5 min. After incubating, 300 µL of the second reagent (R2) was added to both test tubes. The contents of each tube was incubated again for 5 minutes at 37^0C, the absorbance was read immediately.

Calculation

ΔA = [ΔA sample] - [ΔA blank].

Conc. of HDL = [change in absorbance of sample] – [change in absorbance of blank].

The result is expressed in mmol/L.

PLASMA TOTAL CHOLESTEROL ESTIMATION.

Cholesterol oxidase- peroxidase (CHOD-POD) method according to Allain and Roeschlau (Roeschlau*et al.,* 1974) was used to determine the level of total cholesterol in the samples.

Reaction Principle

Cholesterol ester + H_2O ↔ Cholesterol + Fatty acid

Cholesterol + O_2 ↔ Δ4-Cholestenone + H_2O_2

$2H_2O_2$ + 4-Aminoantipyrine + Phenol ↔ Quinoneimine + $4H_2O$

By the catalysis of cholesterrol esterase and cholesterol oxidase, Cholesterol ester is catalyzed to yield H_2O_2, which oxidizes 4- aminoantipyrine with phenol to form a colored dye of quinoneimine. The absorbance increase is directly proportional to the concentration of cholesterol.

Procedure

Two test tubes labelled T1 (reagent blank) and T2 (test sample) were set up. T1 contained 1000 µL of reagent (R1) and 10 µL of distilled water, while T2 contained 1000 µL of reagent (R1) and 10 µL of test sample. The contents of each tube were mixed thoroughly at 37℃. The absorbance was read 10 min. later.

Calculation

ΔA = [ΔA sample] - [ΔA blank]

Conc. of cholesterol = [change in absorbance of sample] – [change in absorbance of blank].

The result is expressed in mmol/L.

PLASMA TRIGLYCERIDES (TG) ESTIMATION.

Glycerokinase Peroxidase- Peroxidase method according to Tietz colorimetric method [16] was used to determine the level of Triglyceride in the samples.

Reaction Principle

Triglycerides + $3H_2O$ ↔ Glycerol + fatty acid

Glycerol + ATP ↔ Glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O_2 ↔ Dihydroxyacetone Phosphate + H_2O_2

H_2O_2 + 4-Aminoantipyrine + 4-Chlorophenol ↔ Quinoneimine + HCl + H_2O

Through a sequence of enzymatic catalysis steps by lipase, glycerol kinase and Dihydroxyacetone phosphate dehydrogenase, triglycerides is catalyzed to yield H_2O_2, which oxidize 4-aminoantipyrinel to yield a colored dye of quinoneimine. The absorbance increase is directly proportional to the concentration of triglycerides.

Procedure

Two test tubes labelled T1 (reagent blank) and T2 (test sample) were set up. T1 contained 1000 µL of reagent (R1) and 10 µL of distilled water, while T2 contained 1000 µL of reagent (R1) and 10 µL of test sample. The contents of each tube were mixed thoroughly at 37℃. The absorbance was read at a wavelength of 546 nm10 min. later.

Calculation

ΔA = [ΔA sample]- [ΔA blank]

Conc. of triglyceride = [change in absorbance of sample] – [change in absorbance of blank].

The result is expressed in mmol/L.

2.7 ELECTROLYTE TEST

Sodium levels were determined by colorimetric test.

Magnesium-uranyl acetate method. The Principle of this method is that after the precipitation of sodium magnesiumuranyl acetate, in the supernatant form with uranyl ions in solution with thioglycolic acid a yellow-brown coloured complex is formed. The optical density difference between the reagent blank (without precipitation of sodium) and the result of the analysis is proportional to the sodium concentration [17]. Reagent A kit contained uranylacetate (19mM) and magnesium acetate (140mM) while reagent B kit contained ammonium thioglycolate (550mM), ammonia (550mM) and the standard aqueous solution of sodium equivalent150mmol. The reagent A (2.00ml) was mixed with 0.02 ml of the sample. For the standard, 2.00 ml of reagent A and 0.02 ml of the standard were mixed. The mixtures were let to stand for 5 minutes, they were then shaken thoroughly for 30 seconds. The mixtures were allowed to stand for 30 minutes. They were centrifuged at 2,000rpm for 5 minutes. The supernatant was then separated. The clear supernatant (0.05ml) was mixed with 2.00ml of reagent B. For the blank, 0.05 ml of reagent A and 2.00 ml of reagent B were mixed, while the standard tube contained 0.05 ml of supernatant and 2.00ml of reagent B.

The absorbance of the mixtures was read after 10 minutes at 405nm with spectronic – 20 spectrophotometer.

Calculations: $\frac{\text{Blank O.D – Sample O.D}}{\text{Blank O.D – Standard O.D}} \times 150 = \text{mmol/L}$

Calculations Normal values 135-150 mmol/l.

Potassium levels were determined by colorimetric endpoint method.

The Principle of this method is that the amount of potassium is determined by using sodium tetraphenylboron (2.1mmol/l) in a specifically prepared mixture to produce a potassium concentration in the range of 2 – 7 mEq/L. 1.0ml of reagent was mixed with 0.1ml of sample except for the controls, which had no samples. The blank tube contained 1.0ml of reagent while the standard tube contained 1.0ml of reagent and 0.1ml of standard. The mixtures were incubated at 25oC for 3mins. The absorbance was read against reagent blank at 500nm with Spectronic -20 spectrophotometer.

Calculations: $\frac{\Delta A \text{ unknown}}{\Delta A \text{ standard}} \times C \text{ standard} = \text{potassium concentration mEq/L}$

CALCIUM

Collection of blood sample was carried out after cardiac puncture.The blood was collected by a sterile syringe into a sterile lithium heparin bottle.Spinning of the blood sample was by a centrifuge in order to separate the plasma from the blood cells.Selected three clean dry test tubes were labelled as blank (B), standard(S) and test (T).Buffer reagent (L1) 0.5ml was measured into B, into S and T, respectively. Colour reagent (L2) 0.5ml was measured into B, S, and T, respectively.Distilled water 0.02ml was measured into B only.For Calcium

standard, the measurement was only 0.02ml into S.Into Sample, the measurement was 0.02ml into T only.The tubes were well mixed and incubated at room temperature for 5 minutes.Measurement of the absorbance at 570nm for the standard and test sample against the blank was within 60 minutes.

CALCULATION

$$= \frac{\text{Absorbance of the test x 10}}{\text{Absorbance of standard}}$$

2.8 STATISTICAL ANALYSIS

Data analysis was performed using the Statistical package for the Social Sciences software (SPSS, version 11.0). The statistical method of one way analysis of variance (ANOVA) was used to compare the mean values obtained among different groups. Differences were considered significant whenever the p-value was P=0.05.

3.0 RESULTS

The data were expressed as the Mean ± SD and represent the average values for the animals in the same group. Each analysis was repeated three times and the average was used to compare between the groups. These data were subjected to statistical analysis using ANOVA in order to display their significance p>0.05.

Table 1: Effect of 7 days oral administration of apple cider vinegar with "the mother" on Sodium, Potassium and Calcium electrolytes of Wistar rats.

Sample	Na^+ (mmol/l)	K^+ (mmol/l)	Ca^{2+}(mmol/l)
Control/ Group1	153.67 ± 0.23	3.63 ± 0.31	11.58 ± 0.22

Group2	149.32 ± 1.30[a]	4.00± 1.60[a]	8.89± 1.30[a]
Group3	139.69± 1.33[a]	4.99± 0.41[a]	8.69± 0.06[a]
Group4	131.30 ± 1.30[a]	5.01± 0.46[a]	8.59± 0.21[a]

307 The values are reported as Mean ± Standard deviation **(**M± SD), N= 3.

308 [a]Statistically significant at 95% confidence level, (P < 0.05).

309 **Table 2:** Effect of 14 days oral administration of apple cider vinegar with "the mother" on
310 Sodium, Potassium and Calcium electrolytes ofWistar rats.

Sample	Na^+ (mmol/l)	K^+ (mmol/l)	Ca^{2+}(mmol/l)
Control/ Group1	153.65 ± 0.24	3.62± 0.30	11.55± 0.21
Group2	143.22 ± 1.40[b]	3.99± 1.60[b]	7.89± 1.30[c]
Group3	135.67 ± 1.23[b]	4.48± 0.41[b]	7.79± 0.07[b]
Group4	121.30 ± 1.30[b]	4.96± 0.46[b]	7.59± 0.20[b]

311 The values are reported as Mean ± Standard deviation **(**M± SD), N= 3.

312 [b]Statistically significant at 95% confidence level, (P < 0.05).

313 **Table 3:** Effect of 21 days oral administration of apple cider vinegar with "the mother" on
314 Sodium, Potassium and Calcium electrolytes of Wistar rats.

Sample	Na^+ (mmol/l)	K^+ (mmol/l)	Ca^{2+}(mmol/l)
Control/ Group1	153.63 ± 0.24	3.61 ± 0.30	11.54 ± 0.21
Group2	133.22 ± 1.41[c]	3.97 ± 1.60[c]	7.79± 1.31[c]

Group3	125.67 ± 1.24[c]	4.46 ± 0.41[c]	7.19± 0.08[c]
Group4	120.30 ± 1.31[c]	4.92± 0.46[c]	7.09± 0.20[c]

315 The values are reported as Mean ± Standard deviation **(**M± SD), N= 3.

316 [c]Statistically significant at 95% confidence level, (P < 0.05).

317 **Table 4:** Effect of 7 days oral administration of apple cider vinegar with "the mother" on
318 Triglycerides and Total cholesterol levels of albino Wistar rats

319

Samples	Triglyceride (mmol/l)	Total Cholesterol (mmol/l)
Control/ Group 1	3.39 ±0.15	4.06 ± 0.89
Group 2	3.00 ± 0.46[a]	4.05 ± 1.08[a]
Group 3	2.89 ± 0.18[a]	3.95 ± 1.67[a]
Group 4	2.86± 0.15	3.83 ± 0.35[a]

320 The values are reported as Mean ± Standard deviation (M±SD), N= 3.

321 [a]Statistically significant at 95% confidence level, (P < 0.05).

322

323 **Table 5:** Effect of 14 days oral administration of apple cider vinegar with "the mother" on
324 Triglycerides and Total cholesterol levels of albino Wistar rats.

325

Samples	Triglyceride (mmol/l)	Total Cholesterol (mmol/l)
Control/ Group 1	3.38 ±0.14	4.05 ± 0.99
Group 2	2.97 ± 0.56[b]	4.04 ± 1.09[b]

Group 3	2.88 ± 0.19[b]	3.75 ± 1.57[b]
Group 4	2.83 ± 0.14[b]	3.73 ± 0.34[b]

The values are reported as Mean ± Standard deviation (M±SD), N= 3.

[b]Statistically significant at 95% confidence level, (P < 0.05).

Table 6: Effect of 21 days oral administration of apple cider vinegar with "the mother" on Triglycerides and Total cholesterol levels of albino Wistar rats.

Samples	Triglyceride (mmol/l)	Total Cholesterol (mmol/l)
Control/ Group 1	3.37 ±0.14	4.04 ± 0.98
Group 2	2.87 ± 0.57[c]	4.02 ± 1.08[c]
Group 3	2.78 ± 0.18[c]	3.65 ± 1.56[c]
Group 4	2.73± 0.13[c]	3.62 ±0.33[c]

The values are reported as Mean ± Standard deviation (M±SD), N= 3.

[c]Statistically significant at 95% confidence level, (P < 0.05).

Table 7: Effect of 7 days oral administration of apple cider vinegar with "the mother" on High density lipoprotein (HDL), very low density lipoprotein (VLDL) and low density lipoprotein (LDL).

Sample	HDL (mmol/l)	VLDL (mmol/l)	LDL (mmol/l)
Control/Group 1	2.67 ± 0.46	1.59 ± 0.08	8.29 ± 1.33

Group 2	2.35 ± 0.33[a]	1.50 ± 0.12[a]	7.99 ± 1.20[a]
Group 3	2.45 ± 0.25[a]	1.45 ± 0.09[a]	7.73 ± 1.40[a]
Group 4	2.55 ± 0.31[a]	1.38 ± 0.04[a]	7.54 ± 0.20[a]

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339 The values are reported as Mean ± Standard deviation (M±SD), N= 3.

340 [a]Statistically significant at 95% confidence level, (P < 0.05).

341 **Table 8**: Effect of 14 days oral administration of apple cider vinegar with "the mother" on
342 High density lipoprotein (HDL), very low density lipoprotein (VLDL) and low density
343 lipoprotein (LDL).

Sample	HDL (mmol/l)	VLDL (mmol/l)	LDL (mmol/l)
Control/Group 1	2.66 ± 0.45	1.57 ± 0.06	8.26 ± 1.32
Group 2	2.15 ± 0.31[b]	1.49 ± 0.15[b]	7.90 ± 1.30[b]
Group 3	2.25 ± 0.15[b]	1.48 ± 0.07[b]	7.69 ± 1.50[b]
Group 4	2.35 ± 0.21[b]	1.43 ± 0.05[b]	7.32 ± 0.40[b]

344

345 The values are reported as Mean ± Standard deviation (M±SD), N= 3.

346 [b]Statistically significant at 95% confidence level, (P < 0.05).

347

348 **Table 9**: Effect of 21 days oral administration of apple cider vinegar with "the mother" on
349 High density lipoprotein (HDL), very low density lipoprotein (VLDL) and low density
350 lipoprotein (LDL).

Sample	HDL (mmol/l)	VLDL (mmol/l)	LDL (mmol/l)

Control/Group 1	2.65 ± 0.45	1.55 ± 0.07	8.24 ± 1.31
Group 2	2.05 ± 0.30[c]	1.48 ± 0.14[c]	7.80 ± 1.31[c]
Group 3	2.15 ± 0.14[c]	1.46 ± 0.08[c]	7.29 ± 1.60[c]
Group 4	2.25 ± 0.20[c]	1.42 ± 0.04[c]	7.02 ± 0.30[c]

The values are reported as Mean ± Standard deviation (M±SD), N= 3.

[c]Statistically significant at 95% confidence level, (P < 0.05).

4.0 DISCUSSION

The research work has shown that the administration of 1ml apple cider vinegar can alter lipid profile level as well as electrolytes such as sodium, potassium and calcium in the blood of an albino Wistar rat.There is considerable discussion about elevated cholesterol and its link to cardiovascular diseases because there is a direct relationship between elevated levels of cholesterol in the plasma and incidence of heart disease. Experts generally agreed that people with levels of total cholesterol in plasma above 6.2mmol/l for many years are at the risk of having a heart attack compared with people whose plasma cholesterol level is below 5.2mmol/l. It is also generally recommended that adults endeavour to achieve levels of both free cholesterol and cholesteryl ester in plasma of 5.2mmol/l or less [18]

Consumption of high cholesterol diet can increase the chances of an organism developing the metabolic disorder [8, 19].

This research work has deduced the effect of apple cider vinegar with “the mother” on the lipid profile and electrolytes such as sodium, potassium and calcium of albino Wistar rats for 21 days. After oral administration of the apple cider vinegar on rats for 7 days up to 21 days, the results revealed significant reductions in a time dependent manner with the highest reductions obtained on the last week of experiment ($p<0.05$). After 21 days, triglycerides reduced from 3.37 ± 0.14 to 2.73 ± 0.13 mmol/l, totalcholesterol from 4.04 ± 0.98 to 3.62 ±0.33mmol/l, low density lipoprotein cholesterol from 8.24 ± 1.31 to 7.02 ± 0.30 mmol/l, very low density lipoprotein cholesterol from 1.55 ± 0.07 to 1.42 ± 0.04 in the blood of rats.

Also there was an increase in high density lipoprotein cholesterol.This study further revealed a significant decrease ($p< 0.05$) in calcium electrolyte concentration from 11.54 ± 0.21 to 7.09± 0.20mmol/l.The work also showed significant decrease ($p> 0.05$) in the sodium electrolyte concentrations from 153.63 ± 0.23 to 120.30 ± 1.31.However, plasma potassium showed significant increase of 3.61 ± 0.30 to 4.92 ± 0.46 mmol/l.

The results suggested that the apple cider vinegar must have influenced lipase enzyme resulting in the increase in HDL (good cholesterol) level which functions in the return of cholesterol to the liver, where it is metabolized and secreted but reduced LDL (bad cholesterol) level which is carried as cholesteryl ester in the blood plasma of albino wistar rats [18, 19].

Metabolically, chylomicronsmade in the intestine and secreted into the lymphatic system serve as the means of transport of triacylglycerol and cholesteryl ester in the presence of the cholesterol acyltransferase(ACAT)) from the intestine to other tissues in the body. VLDL functions in a similar way for the transport of lipid to other tissues but it is secreted from the liver directly into the blood. These two triacyglycerol-rich particles are initially degraded by the lipoprotein lipase. Lipoprotein lipase catalyzes the hydrolysis of triacylglycerols. This enzyme is specifically activated by apoprotein C-II, which is associated with chylomicrons and VLDL. As a result, this lipase supplies the heart, muscle, adipose, and other tissues with fatty acids, derived from these lipoproteins in the plasma. As the lipoproteins are depleted of triacylglycerol, the particles become smaller. The surface molecules (apoproteins) are transferred to HDL. In rats, 'remnants'that results from chylomicrons and VLDL catabolism are taken up by the liver for metabolism and possibly secretion.

In conjunction with this research wok, Fushimi, *et al*., [20] demonstrated profoundly how dietary acetic acid which is a key constituent of apple cider vinegar can reduce serum cholesterol and triacylglycerol in rats fed a cholesterol-rich diet. This work agrees with Shishehbor, *et al.,* [8]who revealed how the lipid profile levels could be attenuated by using apple cider vinegar on some normal and diabetic male wistar rats kept in cages under controlled conditions[8, 21]. Furthermore, Budak,*et al*., [22]showed with their experiment the effect of apple cider vinegar produced from different techniques on blood lipids in high-cholesterol fed rats. This research mirrored the work of Beheshti, *et al*., [9]who awakened the consciousness of researchers regarding the influence of apple cider vinegar on blood lipids in human beings. The results from the study showed that there were reductions in

harmful lipids, that is, total cholesterol, LDL, triglyceride in blood samples of the hyperlipidemic individuals.

Laszlo and Balazs, [1]illustrated with their model experiment on mice how apple cider vinegar can affect the plasma lipids. The concentration of plasma and liver triglyceride remained the same in all groups no matter the treatment.

This work also agreed with the study of Ajaykumar,*et al*., [23]who demonstrated how apple cider vinegar can be used for its antihyperlipidemic properties. Hyperlipidemia is elevation of fasting total cholesterol concentration which may or may not be associated with triglyceride concentration [23].This work also corresponded with the work of Allegier [24**]** who noted in their research work the effect of apple cider vinegar intake on lipid profile in albino wistar rats and also concurred with the work of Naziroglu*et al*., [25].

Halima, *et al*., [26]showed the antihyperglycemic, hyperlipidemic and modulatory effects of apple cider vinegar on digestive enzymes in experimental diabetic rats. These results were tied to the fact that apple cider vinegar inhibited key enzymes of lipid metabolism and absorption which resulted to a significant reduction in serum total cholesterol, low-density lipoprotein cholesterol and triglyceride rates, but an elevation in the level of high- density lipoprotein cholesterol.

This study is in agreement with the work of Bourderbala, *et al*., [27] whodemonstrated how apple vinegar can have a significant impact on the lipid profile of rats subjected to high-fat diet.

This study revealed that apple cider vinegar decreased the levels of calcium electrolyte, perhaps this is why it erodes tooth enamel when consumed since apple cider vinegar contains acetic acid and has low pH. From this study it could be deduced that prolonged intake of apple cider vinegar could reduce bone density. This results concurred with the work ofMurray, *et al*., [14] because the rats's motility was affected as the days went by. This research work also pitch tent with the literature ofStryer [28], which stated that the physiologic regulator of muscle contraction is calcium ion. And that it is evident that movement of muscle will be blocked if calcium ion is absent. The binding of calciumion to the troponin(TN)- tropomyosin(TM)- actin complex triggers a shift in theposition of TM,

which then produces an allosteric transitionin actin. The allosteric transition in the actin facilitates the release of Pi from myosin, which strengthens the interaction between actin and myosin. Eventually, changes occur within the TN complex, which overcomes the inhibitory effect of theTN-1 sub-unit. Then, a signal is sent to TM that triggers the muscle contraction process.

5.0 CONCLUSION

The findings showed that the use of natural plant products such as apple cider vinegar as diet supplement can drastically reduce bad cholesterol (LDL) level considered to be a threat while increasing good cholesterol (HDL) in the blood of albino Wistar rats. It will also increase the absorption of sodium and potassium electrolytes in the blood of albino Wistar rats so as to aid metabolism. However, it reduces the absorption of calcium electrolyte necessary for bone formation and muscle contraction in the blood of albino Wistar rats.

It is recommended that in view of the rapid rise in thecases of elevated cholesterol level in the plasma and electrolyte shortage which alters metabolism, illustrations of its risk factors as well as multiple health campaign and awareness particularly regarding the promotion of widespread use of natural plant products such as apple cider vinegar as diet supplements are advised to diminish the prevalence and complications of these health challenges.

Competing Interests

Authors have declared that no competing interests exist.

6.0 ETHICAL APPROVAL:

This research work was carried out with the approval of the University of Port Harcourt research ethics committee.

Author Contribution: Okoye Ngozi Franca designed the study, performed the statistical analysis, wrote the first draft of the manuscript. Porolo Sinenye Barikpoa managed the literature searches. Both authors read and approved the final manuscript

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