

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

ABSTRACT

Aim: This study is 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 g 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 1 ml 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 the 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, 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 ± 0.04 mmol/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 of Wistar 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,

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

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

49 Vinegar is a liquid that has acetic acid of about 5-20% in concentration. It also contains
50 chemicals such as: anthocyanins, **flavanols**, flavonols, mineral salts, vitamins, amino acids,
51 non-volatile acids, polyphenolic compounds and water.

52 According to Saladin [2], the reaction between ethanol and oxygen produces acetic acid.

53 Vinegar is a product made from any fermentation of carbohydrate sources like grape, apple,
54 melon, honey, potato, where yeast ferments sugar to alcohol, and the alcohol is then
55 converted to acetic acid by acetobacter bacteria.

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

66 Apple cider vinegar is a type of vinegar produced from cider or apple must. It is made from
67 cider or apple mash in the same way as malt vinegar. It possesses a strong-taste at full
68 strength and a fine quality apple flavour when filtered. But due to the growing notion that
69 the unfiltered organic product is therapeutic, it is sold as an unfiltered unpasteurised

70 product with the bacteria called “the mother”. However, apple cider can also be seen in
71 stores as filtered or finely distilled product.

72 Botanic plant products like apple cider vinegar are said to be therapeutic due to their
73 chemical compositions [4], little wonder the old Welsh proverb “an apple a day keeps the
74 doctor away”. As one of the most cultivated and consumed fruits in the world, apples are
75 continuously being praised as a “miracle food”. Vinegar is a good culinary ingredient [5].
76 Vinegar is antiglycaemic, it is also antibacterial. Interestingly, apple cider vinegar has been
77 identified to be capable of influencing the lipid profiles in Wistar rats and man respectively
78 [6, 7, 8, 9].

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

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

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

99 In physiology, the paired charged particles are referred to as electrolytes. Examples include
100 but not restricted to Sodium ion (Na^+), Potassium ion (K^+) and Calcium ion (Ca^{2+}). These
101 charged particles also referred to as ions are very important for the maintenance of the

102 osmotic gradients between intracellular and extracellular fluid. They regulate fluid balance
103 and blood pressure control. These gradients are important for hydration of the body,
104 activities of the nerves and muscles and blood pH. Without sufficient amount of these ions,
105 muscle weakness or contraction and expansion issues will emerge.

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

113 Concerned electrolyte issues such as dehydration and over hydration may lead to cardiac
114 and neurological complications in an organism. And until the issue is resolved, it will
115 remain a medical challenge to that organism.

116 Measurement of electrolytes is through a diagnostic procedure- through a blood test or
117 urinalysis.

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

121

122 **2.0 MATERIALS AND METHODS**

123 The apple cider vinegar with “the mother” was bought from a Supermarket in Port
124 Harcourt, Rivers State.

125 **1.1. EXPERIMENTAL ANIMAL**

126 Female albino wistar rats.

127 **1.2.TREATMENT/DIET**

128 Apple cider vinegar with “the mother” and normal animal feeds.

129 **1.3.TREATMENT PREPARATION**

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

132 **1.4 EXPERIMENTAL DESIGN**

133 The experimental design was made of 12 female albino Wistar rats purchased from the
134 Biochemistry animal house in Choba University of Port Harcourt. The mean weight was
135 150 ± 20 g. The experimental animals were grouped into 4 groups and the method of feed
136 was by gavaging. Each group had its experimental animals of 3 for each week that were
137 allowed access to normal animal feeds *ad libitum* and distilled water. The sacrificing of
138 experimental animals was carried out every 1 week (7 days). The experiment lasted for
139 3 weeks (21 days). The first group was the control. The control was given distilled water
140 and allowed access to normal animal feed *ad libitum* but was not administered apple cider
141 vinegar. The second group was the group to be sacrificed after the first week of experiment.
142 That is, 7 days. The group was given distilled water, allowed access to normal animal feed
143 *ad libitum* and 1 ml apple cider vinegar twice daily. The third group was the group to be
144 sacrificed after the second week of experiment. That is, 14 days. The group had same
145 treatment as the second group above. The fourth group was the group to be sacrificed after
146 the third week which was the final week of experiment. The group had same treat as the
147 second and third above but was sacrificed after 21 days (3 weeks).

148

149

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

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

157 GROUP 3: The group served as the experimental animals to be sacrificed after 2 weeks.
158 The group had access to normal animal feeds *ad libitum* and distilled water while
159 experiment lasted. The group had the same administration as group 2 above. However,
160 the administration lasted for 14 days before sacrifice.

161 GROUP 4: The group served as the experimental animals to be sacrificed after 21 days
162 the experiment lasted. The group also had access to normal animal feeds *ad libitum* and
163 distilled water and same administration as groups 2 and 3. However, the administration
164 lasted for 21 days before sacrifice.

165 1.4. SACRIFICE OF THE EXPERIMENTAL ANIMALS

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

172

173 1.5.ESTIMATION OF LIPID PROFILE PARAMETERS.

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

175

176 PLASMA HDL ESTIMATION

177 Method

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

180 Reaction Principle

181 (1) LDL,VLDL, Chylomicrons \leftrightarrow Cholestenone + H_2O_2

182 $2H_2O_2 \leftrightarrow 2H_2O + O_2$

183 (2) HDL \leftrightarrow Cholestenone + H_2O_2

184 $H_2O_2 + HDAOS + 4\text{-aminoantipyrin} \leftrightarrow$ Quinonimine

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

188 Procedure

189 Two test tubes labelled T1 (reagent blank) and T2 (test sample) were set up. T1 contained
190 900 μ L of reagent (R1) and 12 μ L of distilled water, while T2 contained 900 μ L of reagent
191 (R1) and 12 μ L of test sample. The contents of each tube were mixed and incubated at 37°C
192 for 5 min. After incubating, 300 μ L of the second reagent (R2) was added to both test tubes.

193 The contents of each tube was incubated again for 5 minutes at 37°C, the absorbance was
194 read immediately.

195

196 Calculation

197 $\Delta A = [\Delta A \text{ sample}] - [\Delta A \text{ blank}]$.

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

199 The result is expressed in mmol/L.

200 PLASMA TOTAL CHOLESTEROL ESTIMATION.

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

203 Reaction Principle

204 Cholesterol ester + H₂O ↔ Cholesterol + Fatty acid

205 Cholesterol + O₂ ↔ Δ⁴-Cholestenone + H₂O₂

206 2 H₂O₂ + 4-Aminoantipyrine + Phenol ↔ Quinoneimine + 4H₂O

207 By the catalysis of cholesterol esterase and cholesterol oxidase, Cholesterol ester is
208 catalyzed to yield H₂O₂, which oxidizes 4- aminoantipyrine with phenol to form a colored
209 dye of quinoneimine. The absorbance increase is directly proportional to the concentration
210 of cholesterol.

211 Procedure

212 Two test tubes **labelled** T1 (reagent blank) and T2 (test sample) were set up. T1 contained
213 1000 μL of reagent (R1) and 10 μL of distilled water, while T2 contained 1000 μL of
214 reagent (R1) and 10 μL of test sample. The contents of each tube were mixed thoroughly at
215 37°C. The absorbance was read 10 min. later.

216 Calculation

217 $\Delta A = [\Delta A \text{ sample}] - [\Delta A \text{ blank}]$

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

220 The result is expressed in mmol/L.

221 PLASMA TRIGLYCERIDES (TG) ESTIMATION.

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

224 Reaction Principle

225 Triglycerides + 3H₂O ↔ Glycerol + fatty acid

226 Glycerol + ATP ↔ Glycerol-3-phosphate + ADP

227 Glycerol-3-phosphate + O₂ ↔ Dihydroxyacetone Phosphate + H₂O₂
 228 H₂O₂ + 4-Aminoantipyrine + 4-Chlorophenol ↔ Quinoneimine + HCl + H₂O
 229 Through a sequence of enzymatic catalysis steps by lipase, glycerol kinase and
 230 Dihydroxyacetone phosphate dehydrogenase, triglycerides is catalyzed to yield H₂O₂, which
 231 oxidize 4-aminoantipyrine to yield a colored dye of quinoneimine. The absorbance increase
 232 is directly proportional to the concentration of triglycerides.

233 Procedure

234 Two test tubes **labelled** T1 (reagent blank) and T2 (test sample) were set up. T1 contained
 235 1000 µL of reagent (R1) and 10 µL of distilled water, while T2 contained 1000 µL of
 236 reagent (R1) and 10 µL of test sample. The contents of each tube were mixed thoroughly at
 237 37°C. The absorbance was read at a wavelength of 546 nm 10 min. later.

238 Calculation

239 $\Delta A = [\Delta A \text{ sample}] - [\Delta A \text{ blank}]$

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

241 The result is expressed in mmol/L.

242

243 **1.6. ELECTROLYTE TEST**

244 Sodium levels were determined by colorimetric test.

245 Magnesium-uranyl acetate method. The Principle of this method is that after the
 246 precipitation of sodium magnesiumuranyl acetate, in the supernatant form with uranyl ions
 247 in solution with thioglycolic acid a yellow-brown coloured complex is formed. The optical
 248 density difference between the reagent blank (without precipitation of sodium) and the result
 249 of the analysis is proportional to the sodium concentration [17]. Reagent A kit contained
 250 uranylacetate (19 mM) and magnesium acetate (140 mM) while reagent B kit contained
 251 ammonium thioglycolate (550 mM), ammonia (550 mM) and the standard aqueous solution
 252 of sodium equivalent 150 mmol. 2.00 ml of reagent A was mixed with 0.02 ml of the sample.
 253 For the standard, 2.00 ml of reagent A and 0.02 ml of the standard were mixed. The
 254 mixtures were let to stand for 5 minutes, they were then shaken thoroughly for 30 seconds.
 255 The mixtures were allowed to stand for 30 minutes. They were centrifuged at 2,000 rpm for
 256 5 minutes. The supernatant was then separated. 0.05ml of the clear supernatant was mixed
 257 with 2.00 ml of reagent B. For the blank, 0.05 ml of reagent A and 2.00 ml of reagent B
 258 were mixed, while the standard tube contained 0.05 ml of supernatant and 2.00 ml of

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

261 Calculations: $\frac{\text{Blank O.D} - \text{Sample O.D}}{\text{Blank O.D} - \text{Standard O.D}} \times 150 = \text{mmol/L}$
262
263

264 Calculations Normal values 135-150 mmol/l.

265

266 Potassium levels were determined by colorimetric endpoint method.

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

274
275 Calculations: $\frac{\Delta A \text{ unknown}}{\Delta A \text{ standard}} \times C \text{ standard} = \text{potassium concentration mEq/L}$
276
277

278 **CALCIUM**

279 Collection of blood sample was carried out after cardiac puncture.

280 The blood was collected by a sterile syringe into a sterile lithium heparin bottle.

281 Spinning of the blood sample was by a centrifuge in order to separate the plasma
282 from the blood cells.

283 Selected three clean dry test tubes were labelled as blank (B), standard(S) and
284 test (T).

285 Buffer reagent (L1) 0.5 ml was measured into B, into S and T, respectively.

286 Colour reagent (L2) 0.5 ml was measured into B, S, and T, respectively.

287 Distilled water 0.02 ml was measured into B only.

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

293 **CALCULATION**

$$294 \quad = \frac{\text{Absorbance of the test} \times 10}{295 \quad \text{Absorbance of standard}}$$

296 **2.8 STATISTICAL ANALYSIS**

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

301

302

303

304 **3.0 RESULTS**

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

309

310 **Table 1:** Effect of 7 days oral administration of apple cider vinegar with “the mother” on
 311 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

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

313 ^aStatistically significant at 95% confidence level, (P < 0.05).

314 **Table 2:** Effect of 14 days oral administration of apple cider vinegar with “the mother” on
 315 Sodium, Potassium and Calcium electrolytes of Wistar 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

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

317 ^bStatistically significant at 95% confidence level, (P < 0.05).

Table 3: Effect of 21 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	153.63 ± 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

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

^cStatistically significant at 95% confidence level, (P < 0.05).

Table 4: Effect of 7 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	3.39 ±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

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

^aStatistically significant at 95% confidence level, (P < 0.05).

328 **Table 5:** Effect of 14 days oral administration of apple cider vinegar with “the mother” on
 329 Triglycerides and Total cholesterol levels of albino Wistar rats.

330

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

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

332 ^bStatistically significant at 95% confidence level, (P < 0.05).

333

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

336

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

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

338 ^statistically significant at 95% confidence level, (P < 0.05).

339

340 **Table 7:** Effect of 7 days oral administration of apple cider vinegar with “the mother” on
 341 High density lipoprotein (HDL), very low density lipoprotein (VLDL) and low density
 342 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

343

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

345 ^aStatistically significant at 95% confidence level, (P < 0.05).

346 **Table 8:** Effect of 14 days oral administration of apple cider vinegar with “the mother” on
 347 High density lipoprotein (HDL), very low density lipoprotein (VLDL) and low density
 348 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

349

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

351 ^bStatistically significant at 95% confidence level, (P < 0.05).

352

Table 9: Effect of 21 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	2.65 ± 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.

^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 6.2 mmol/l for many years are at the risk of having a heart attack compared with people whose plasma cholesterol level is below 5.2 mmol/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, total cholesterol from 4.04 ± 0.98 to 3.62 ± 0.33 mmol/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 ± 0.20 mmol/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, chylomicrons made 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 triacylglycerol-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 work, 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

407 controlled conditions [8, 21]. Furthermore, Budak, *et al.*, [22] showed with their experiment
408 the effect of apple cider vinegar produced from different techniques on blood lipids in high-
409 cholesterol fed rats. This research mirrored the work of Beheshti, *et al.*, [9] who awakened
410 the consciousness of researchers regarding the influence of apple cider vinegar on blood
411 lipids in human beings. The results from the study showed that there were reductions in
412 harmful lipids, that is, total cholesterol, LDL, triglyceride in blood samples of the
413 hyperlipidemic individuals.

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

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

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

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

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434 This study revealed that apple cider vinegar decreased the levels of calcium electrolyte,
435 perhaps this is why it erodes tooth enamel when consumed since apple cider vinegar
436 contains acetic acid and has low pH. From this study it could be deduced that prolonged
437 intake of apple cider vinegar could reduce bone density. This results concurred with the

work of Murray, *et al.*, [14] because the rats's motility was affected as the days went by. This research work also pitch tent with the literature of Stryer [28], which stated that the physiologic regulator of muscle contraction is calcium ion. It is evident that movement of muscle will be blocked if calcium ion is absent. The binding of calcium ion to the troponin(TN)- tropomyosin(TM)- actin complex triggers a shift in the position of TM, which then produces an allosteric transition in 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 the TN-1 sub-unit. Then, a signal is sent to TM that triggers the muscle contraction process.

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449 **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 the cases 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.

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462 **Competing Interests**

Authors have declared that no competing interests exist.

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6.0 ETHICAL APPROVAL:

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

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