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**EFFECT OF APPLE CIDER VINEGAR (ACV) “WITH MOTHER” ON
PROGESTERONE, TESTOSTERONE, AND ESTROGEN OF WISTAR RATS.ON
ALBINO RAT.**

ABSTRACT

Aim: Apple cider vinegar (ACV) with mother" has been singled out as an especially helpful health remedy.This present research evaluated the effect of ACV ‘with mother’ on hormones(testosterone,estrogen and progesterone) of Wistar rats.

Materials and method: Eighteen rats with average weight range of 120g were grouped into six groups. Three groups served as the control for each week(week 1,2 and 3) while the remaining three groups were treated with 1ml of ACV twice daily. The animals were treated for a total of 21 days. After each week the animals in that group were sacrificed andlaboratory analyses were performed.

Results:After oral administration of the product, the results revealed significant reductions in a time dependent manner with the highest reductions obtained on the last week of experiment. The result obtained for estrogen showed significant reduction($p<0.05$) in week one with test value (7.74 ± 0.19 mlU/ml) as compared to control (8.36 ± 0.01 mlU/ml).For progesterone,there was an increase ($p<0.05$).The highest test value was obtained in week 3, (3.64 ± 0.08 mlU/ml) as compared to control value (3.15 ± 0.01 mlU/ml).However, there was also a reduction in testosterone level.

Conclusion:In conclusion,ACV reduces the levels of estrogen and testosterone but increased progesterone level of Wistar rats.

Key words: Apple cider vinegar, Estrogen, Hormones, Progesterone, Testosterone,

29 **1. INTRODUCTION**

30 Apple cider vinegar (ACV) is useful in preventing metabolic disorders. ACV otherwise known
31 as cider vinegar is a type of vinegar made from cider or apple mustard and it has a pale to
32 mediumamber color. The main component of vinegar is acetic acid. Unpasteurized ACV
33 contains mother of vinegar, which has a cobweb-like appearance and can make the vinegar look
34 slightly congealed. ACV is used in salad dressings, marinades, vinaigrettes, food preservatives,
35 andchutneys. It is made by crushing apples and squeezing out the liquid. Bacteria and yeast are
36 added to the liquid to start the alcoholic fermentation process, and the sugar turned intoalcohol.
37 In a second fermentation process, the alcohol is converted into vinegar by acetic acid-forming
38 bacteria (Acetobacter).Acetic acidandmalic acid give vinegar its sour taste [1, 2. 3]

39 Although styles of cider are extremely diverse and not easy to categorize, depending on the type
40 of apple juices used and the degrees of sweetness, from extra dry to sweet, and alcohol content,
41 cider can be defined as a fermented alcoholic beverage made from apple juice. The modern
42 pharmaceutical industry based on synthetic chemistry severed the historical ties between plants,
43 foods and medicines[1, 2, 3].

44 A hormone is any member of a class of signaling molecules produced by glands in multicellular
45 organisms that are transported by the circulatory system to target distant organs to regulate
46 physiology and behavior. Hormones have diverse chemical structures, mainly of 3 classes:
47 eicosanoids, steroids, and amino acid/protein derivatives (amines, peptides, and proteins). The
48 glands that secrete hormones comprise the endocrine signaling system. The term hormone is
49 sometimes extended to include chemicals produced by cells that affect the same cell (autocrine
50 or intracrine signaling) or nearby cells (paracrine signaling). Hormones are used to communicate
51 between organs and tissues for physiological regulation and behavioral activities, such as

52 digestion, metabolism, respiration, tissue function, sensory perception, sleep, excretion, lactation,
53 stress, growth and development, movement, reproduction, and mood [4, 5, 6].

54 Testosterone is the primary male sex hormone and an anabolic steroid. In male humans,
55 testosterone plays a key role in the development of male reproductive tissues such as the testis
56 and prostate, as well as promoting secondary sexual characteristics such as
57 increased muscle and bone mass, and the growth of body hair [7]. In addition, testosterone is
58 involved in health and well-being, [8] and the prevention of osteoporosis. Insufficient levels of
59 testosterone in men may lead to abnormalities including frailty and bone loss
60 [9]. Since testosterone levels gradually decrease as men age, synthetic testosterone is sometimes
61 prescribed to older men to counteract this deficiency [10]. It is biosynthesized in several steps
62 from cholesterol and is converted in the liver to inactive metabolites. It exerts its action through
63 binding to and activation of the androgen receptor [11]. In humans and most other vertebrates,
64 testosterone is secreted primarily by the testicles of males and, to a lesser extent,
65 the ovaries of females. On average, in adult males, levels of testosterone are about 7–8 times as
66 great as in adult females [12]. As the metabolic consumption of testosterone in males is greater,
67 the daily production is about 20 times greater in men also females are also more sensitive to the
68 hormone [13, 14].

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71 In men, higher levels of testosterone are associated with periods of sexual activity. Men's levels
72 of testosterone, a hormone known to affect men's mating behaviour, changes depending on
73 whether they are exposed to an ovulating or non ovulating woman's body odour. Men who are
74 exposed to scents of ovulating women maintained a stable testosterone level that was higher than

75 the testosterone level of men exposed to non-ovulation cues [15, 16, 17]. Testosterone levels and
76 sexual arousal in men are heavily aware of hormone cycles in females. This may be linked to
77 the ovulatory shift hypothesis [18, 19] where males are adapted to respond to the ovulation
78 cycles of females by sensing when they are most fertile and whereby females look for preferred
79 male mates when they are the most fertile; both actions may be driven by hormones [20,21, 22].

80 Testosterone may prove to be an effective treatment in female sexual arousal disorders and is
81 available as a dermal patch [23]. Testosterone may be a treatment for postmenopausal women as
82 long as they are effectively estrogenized [24, 25,26].

83 The actions of estrogen are mediated by the estrogen receptor (ER), a dimeric nuclear protein
84 that binds to DNA and controls gene expression. Like other steroid hormones, estrogen enters
85 passively into the cell where it binds to and activates the estrogen receptor. The estrogen:ER
86 complex binds to specific DNA sequences called a hormone response element to activate the
87 transcription of target genes (in a study using an estrogen-dependent breast cancer cell line as
88 model, 89 such genes were identified [27, 28]. Since estrogen enters all cells, its actions are
89 dependent on the presence of the ER in the cell. The ER is expressed in specific tissues including
90 the ovary, uterus and breast. The metabolic effects of estrogen in postmenopausal women has
91 been linked to the genetic polymorphism of the ER [29].

92 While estrogens are present in both men and women, they are usually present at significantly
93 higher levels in women of reproductive age. They promote the development of female secondary
94 sexual characteristics, such as breasts, and are also involved in the thickening of
95 the endometrium and other aspects of regulating the menstrual cycle. In males, estrogen
96 regulates certain functions of the reproductive system important to the maturation of sperm and

97 may be necessary for a healthy libido[30]. Furthermore, there are several other structural changes
98 induced by estrogen in addition to other functions.

99 From literature, there is not much information on the effects of ACV on hormones. Hence, the
100 aim of this present research is to evaluate the effect of ACV ‘with mother’ on hormones
101 (testosterone, estrogen and progesterone) of Wistar rats.

102

103 **2. MATERIALS AND METHODS**

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

106

107 **METHOD**

108 **2.1.PREPARATION OF APPLE CIDER VINEGAR “WITH MOTHER”** 109 **TREATMENT**

110 Two table spoons (30ml) of the Apple cider vinegar ‘with mother’ was measured with
111 volumetric flask. Then 240ml of distilled water was measured with a volumetric flask. The 30ml
112 of apple cider vinegar “with mother” was poured into the 240ml of distilled water. The solution
113 was mixed properly.

114 **2.2.EXPERIMENTAL DESIGN**

115 Eighteen female rats of average weight (120g) were purchased from the Department of
116 Biochemistry animal farm in Choba campus at the University of Port-Harcourt and was
117 acclimatized for 14 days prior to treatment. On acclimatization the rats were divided into 2
118 groups.

119 Group 1(9 rats as control)

- 120 • Distilled water and feed(top growers and marsh) was fed to them for 21 days.

121 Group2 (test groups)

- 122 • Distilled water, feed and 1ml of apple cider vinegar with mother was administered.
- 123 • 3 animals were sacrificed from each group on day 7,14,21 respectively and blood
- 124 samples was collected.

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128 **2.3.MODE OF SACRIFICE**

129 Blood samples were collected from the rats via cardiac puncture technique under chloroform
130 anaesthesia and transferred to a well labelled plain lithium heparin bottles and was taken to the
131 laboratory for analyses.

132

133 **2.4.Hormone Assay**

134 The levels of hormones were measured in serum by ELISA testosterone, progesterone and
135 estrogen standard kits (Biocheck, Inc. Foster City CA, USA). The procedure described in the
136 hormone assay kits was used according to the principle highlighted by Tietz [31]for testosterone
137 and progesterone and estrogen.

138 **METHOD USED FOR PROGESTERONE TEST**

139 Progesterone test EIA is based on the principle of competitive binding between
140 progesterone in the test specimen and progesterone-HRP conjugate for a constant
141 amount of rabbit anti- progesterone.

142

143 **TEST PROCEDURES**

144 The desired number of coated wells in the holder was secured.Standards, specimen
145 and control (25µl) were dispensed into appropriate wells.Progesterone-HRP
146 conjugate reagent (100µl) was dispensed into each well.Rabbit anti-progesterone
147 reagent (50µl) was dispensed into each well and was mixed thoroughly for

148 30seconds.They were incubated for 90minutes at room temperature (18-25⁰c). The
149 microwells were rinsed and flicked 5 times with distilled water.TMB reagent
150 (100µl) of was dispensed into each well and gently mixed for 20minutes and
151 incubated at the same temperature and time as previous.The reaction was stopped
152 by adding 100µl of stop solution to each well.It was gently mixed to ensure that all
153 the blue colour changes to yellow colour completely.Absorbance was read at
154 450nm with a microtiter well reader within 15minutes

155

156 **METHODS USED FOR TESTOSTERONE TEST**

157 Testosterone test EIA is based on the principle of competitive binding between
158 testosterone in the test specimen and testosterone-HRP conjugate for a constant
159 amount of rabbit anti- testosterone.

160 **TEST PROCEDURE FOR TESTOSTERONE**

161 The desired number of coated wells in the holder was secured. Standards,
162 specimen and control (25µl) were dispensed into appropriate wells. Testosterone-
163 HRP conjugate reagent 100µl of was dispensed into each well. Rabbit anti-
164 testosterone reagent 50µl of was dispensed into each well, and was mixed
165 thoroughly for 30seconds. They were incubated for 90minutes at room temperature
166 (18-25⁰c). The microwells were rinsed and flicked 5 times with distilled water.
167 TMB reagent (100µl) of was dispensed into each well and gently mixed for
168 20minutes and incubated at the same temperature and time as previous. The
169 reaction was stopped by adding 100µl of stop solution to each well. It was gently
170 mixed to ensure that all the blue colour changes to yellow colour completely.
171 Absorbance was read at 450nm with a microtiter well reader within 15minutes.

172

173 **METHOD USED FOR ESTROGEN TEST**

174 This assay employs the competitive inhibition enzyme immunoassay technique.
175 The microtitre plate provided in the kits has been precoated with goat anti-rabbit
176 antibody.

177 **TEST PROCEEDURE**

178 The sample was centrifuged for 15 minutes at 1000×g, at 4⁰c within 30 minutes of
179 collection and was assayed. All reagents and samples were prepared and the
180 number of wells to be used were determined and the remaining wells and the
181 desiccant were put back into the pouch and sealed back and stored at 4⁰c. A blank
182 was set without any solution. The sample (50µl) was added per well. HRP-
183 conjugate (50µl) was added to each well (not to the blank well) and 50µl was added
184 to each well and was mixed properly and incubated for one hour at 37⁰c. Each well
185 was aspirated and washed and the process was repeated twice for a total of three
186 washes. Washing was done by filling each well with wash buffer (200µl) using an
187 autowasher and allow to stand for 10 seconds. After the last wash, the remaining
188 wash buffer was aspirated or decanted and the plate was inverted and blotted
189 against clean papers towels. Substrate A (50µl) and 50µl of substrate B were added
190 to each well and was mixed properly and incubated for 15minutes at 37⁰c. Stop
191 solution (50µl) was added to each well, and was mixed properly. The optical
192 density of each well was determined within 10 minutes using a microplate reader
193 set to 450nm and was read.

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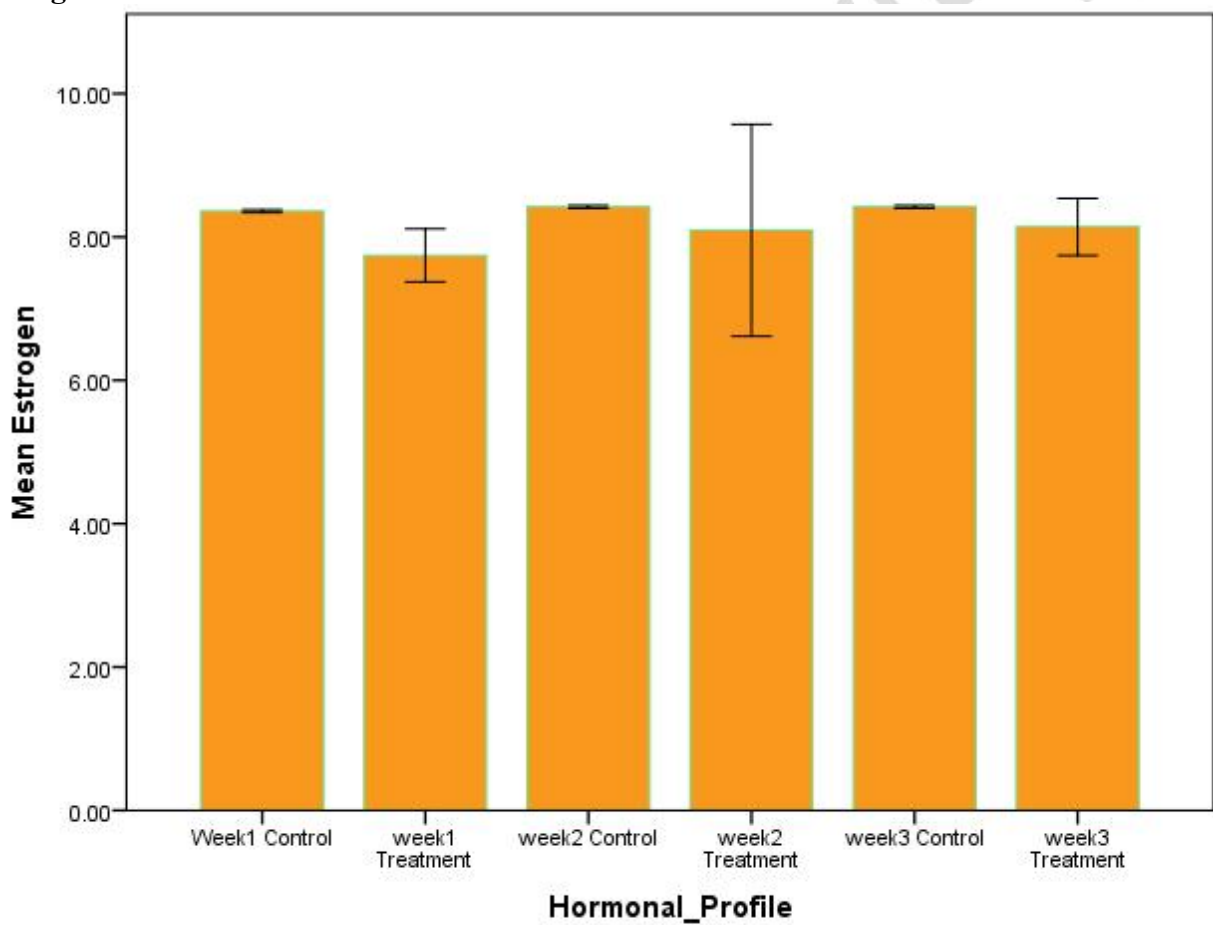
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196 **2.5.Statistical Analysis**

197 Data analysis was performed using the Statistical package for the Social Sciences software
198 (SPSS, version 11.0). Data is displayed in mean \pm SD. The statistical method of one way analysis
199 of variance (ANOVA) was used to compare the mean values obtained among different groups.
200 Differences were considered significant whenever the p-value is $p=0.05$.

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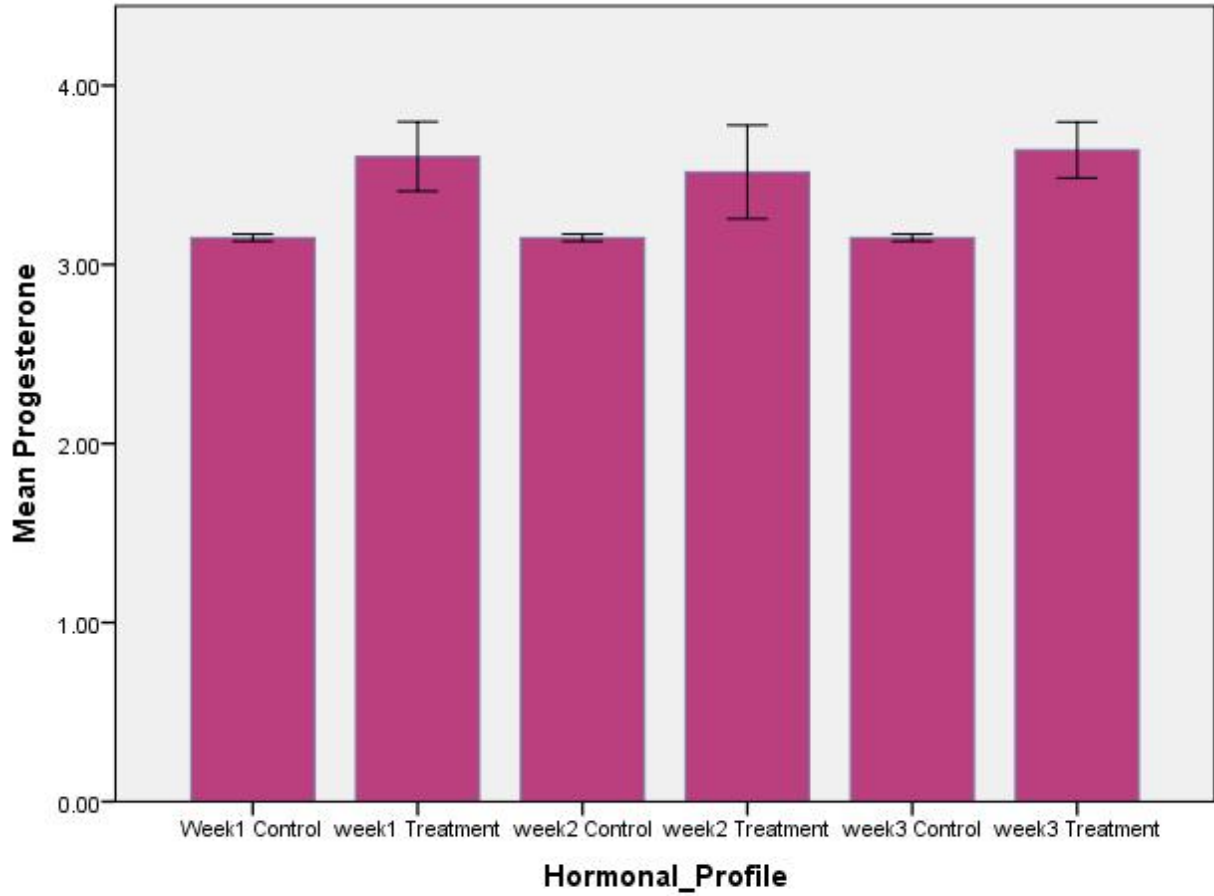
3. RESULTS

Estrogen Result



Error Bars: ± 2 SD

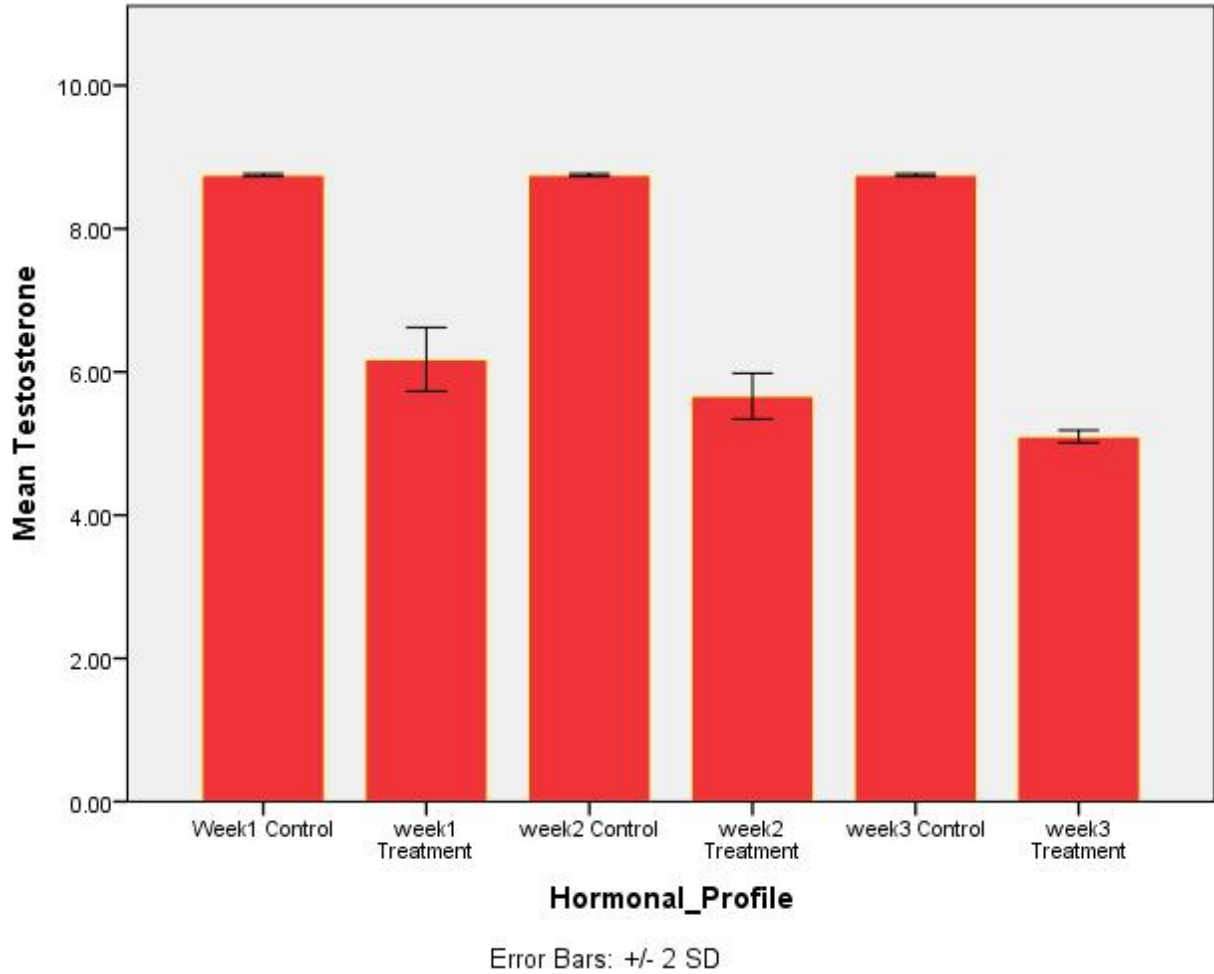
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208 **Fig. 3.1 Graph showing mean estrogen concentration(mlU/ml) of Wistar rats treated with**
209 **apple cider vinegar.**



Error Bars: +/- 2 SD

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Fig. 3.2 Graph showing mean progesterone concentration (mlU/ml)of Wistar rats treated with apple cider vinegar



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Fig. 3.3 Graph showing mean testosterone concentration (mIU/ml)of Wistar rats treated with apple cider vinegar.

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4. DISCUSSION

222 This research work showed the effect of apple cider vinegar with “the mother” on the
223 progesterone, testosterone and estrogen of Wistar rats for 21 days. After oral administration of
224 the product, the results revealed significant reductions in a time dependent manner with the
225 highest reductions obtained on the last week of experiment.

226 From Fig 3.1, the study showed that at week one, mean estrogen concentration (mIU/ml) of
227 control rats (8.36 ± 0.01) was significantly ($P < 0.05$) higher than treatment rats (7.74 ± 0.19). At
228 week 2 there was no significant ($p < 0.05$) difference between control rats (8.42 ± 0.01) and
229 treatment rats (8.09 ± 0.74). Also at week 3, there was no significant ($p < 0.05$) difference between
230 control rats (8.42 ± 0.01) and treatment rats (8.14 ± 0.20).

231 From Fig 3.2, the study also showed that at week 1 mean progesterone concentration (mIU/ml) of
232 control rats (3.15 ± 0.01) was significantly ($P < 0.05$) lower than treatment rats (3.60 ± 0.10). At
233 week 2, mean progesterone concentration of control rats (3.15 ± 0.01) was significantly ($P < 0.05$)
234 lower than treatment rats (3.15 ± 0.13). At week 3 mean progesterone concentration of control rats
235 (3.15 ± 0.01) was significantly ($P < 0.05$) lower than treatment rats (3.64 ± 0.08). The study further
236 showed reduction in the testosterone results as shown in Fig 3.3.

237
238 Progesterone is an endogenous steroid and progestogen sex hormone involved in the menstrual
239 cycle, pregnancy, and embryogenesis of humans and other species. It belongs to a group of
240 steroid hormones called the progestogens and is the major progestogen in the body. It has been
241 discovered that progesterone can be taken as a medication mainly used for hormone replacement
242 therapy for menopause, hypogonadism and transgender [17, 32]. In this present study, the effect of
243 apple cider vinegar with mother tincture was able to reduce the level of progesterone in Wistar albino rats
244 during the 21 days of treatment.

245 Testosterone is the primary male sex hormone and an anabolic steroid. In male humans,
246 testosterone plays a key role in the development of male reproductive tissues such as the testis
247 and prostate, as well as promoting secondary sexual characteristics such as increased muscle and
248 bone mass, and the growth of body hair [8]. In addition, testosterone is involved in health and

249 well-being and the prevention of osteoporosis [8, 9]. Insufficient levels of testosterone in men
250 may lead to abnormalities including frailty and bone loss. Based from the present study carried
251 out it was discovered that apple cider vinegar” with mother” reduces the testosterone level of the
252 Wistar rats for the 21 days of treatment.

253 Estrogen is the primary female sex hormone as well as a medication. It is responsible for the
254 development and regulation of the female reproductive system and secondary sex
255 characteristics. Estrogen supplements may be used in some oral contraceptives, and also in
256 hormone replacement. From the study carried out, it showed that the level of estrogen reduced
257 during the twenty one days of treatment.

258 Previous studies has shown that apple cider vinegar” with mother” has been effective in the
259 reduction of excess sugar level. Its likely good for both type 1 and type 2 diabetes, especially
260 lowering postprandial glucose and also supports weight loss [2, 33, 34]. Apple cider vinegar” with
261 mother has also been useful in reducing harmful lipid in the blood. Apple cider vinegar helps the
262 body to convert the proteins found in foods into usable amino acids. Amino acids are the
263 building blocks for many different bodily processes, including the creation of the hormones. So,
264 in drinking a shot of apple cider vinegar one is actually giving the body what it needs to make
265 hormones – addressing any imbalances between estrogen, progesterone and testosterone. Apple
266 cider vinegar balances the blood sugar, preventing blood sugar soars and crashes and supporting
267 healthy, consistent ovulation. Apple cider vinegar balances acid/alkaline levels in the body,
268 allowing good bacteria to flourish in the micro biome. Apple cider vinegar also supports weight
269 loss by contributing good bacteria to the gut. (2, 3, 33, 34, 35).

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272 **5. CONCLUSION**

273 In conclusion, this study showed that apple cider vinegar reduced the levels of estrogen and
274 testosterone but increased progesterone level. This results from this study suggests that apple
275 cider vinegar when ingested did not compromise the reproductive system. Furthermore it may
276 be used to boost fertility.

277 **Competing Interests**

278 Authors have declared that no competing interests exist.

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281 **6. ETHICAL APPROVAL:**

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

284 Author Contribution: Okoye Ngozi Franca designed the study, performed the
285 statistical analysis, wrote the first draft of the manuscript. Ekpudjuren
286 Oghenevware managed the literature searches. Both authors read and approved
287 the final manuscript.

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