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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 and laboratory 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 medium amber 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 and chutneys. 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 into alcohol.
37 In a second fermentation process, the alcohol is converted into vinegar by acetic acid-forming
38 bacteria (acetobacter). Acetic acid and malic 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 Apple cider vinegar is known to be an acidic solution produced by fermenting apples. The
45 combination of the acidic vinegar and fruit pectin is supposedly the reason behind this solution's
46 fat burning effects. ACV also helps to curb appetite, it is a gentle detoxification agent, it absorbs
47 and blocks fat formation, boosts the immune system, supplies amino acids, minerals and
48 vitamins. It aids in metabolism to help burn and metabolize food efficiently and also boost the
49 immune system, but also has antiseptic and antibiotic action. It is the richest source of amino
50 acids known. ACV has been shown over the years to help arthritis, diabetes, lower cholesterol,

51 increase circulation, tone up skin, help heart problems, chronic headaches, anxiety, and a host of
52 others [1, 2, 3].

53 A hormone is any member of a class of signaling molecules produced by glands in multicellular
54 organisms that are transported by the circulatory system to target distant organs to regulate
55 physiology and behavior. Hormones have diverse chemical structures, mainly of 3 classes:
56 eicosanoids, steroids, and amino acid/protein derivatives (amines, peptides, and proteins). The
57 glands that secrete hormones comprise the endocrine signaling system. The term hormone is
58 sometimes extended to include chemicals produced by cells that affect the same cell (autocrine
59 or intracrine signaling) or nearby cells (paracrine signaling). Hormones are used to communicate
60 between organs and tissues for physiological regulation and behavioral activities, such as
61 digestion, metabolism, respiration, tissue function, sensory perception, sleep, excretion, lactation,
62 stress, growth and development, movement, reproduction, and mood [4, 5, 6].

63 Testosterone is the primary male sex hormone and an anabolic steroid. In male humans,
64 testosterone plays a key role in the development of male reproductive tissues such as the testis
65 and prostate, as well as promoting secondary sexual characteristics such as increased muscle and
66 bone mass, and the growth of body hair [7]. In addition, testosterone is involved in health and
67 well-being, [8] and the prevention of osteoporosis. Insufficient levels of testosterone in men may
68 lead to abnormalities including frailty and bone loss [9]. Since testosterone levels gradually
69 decrease as men age, synthetic testosterone is sometimes prescribed to older men to counteract
70 this deficiency [10]. It is biosynthesized in several steps from cholesterol and is converted in the
71 liver to inactive metabolites. It exerts its action through binding to and activation of the androgen
72 receptor [11]. In humans and most other vertebrates, testosterone is secreted primarily by
73 the testicles of males and, to a lesser extent, the ovaries of females. On average, in adult males,

74 levels of testosterone are about 7–8 times as great as in adult females [12]. As the metabolic
75 consumption of testosterone in males is greater, the daily production is about 20 times greater in
76 men also females are also more sensitive to the hormone [13, 14].

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79 In men, higher levels of testosterone are associated with periods of sexual activity. Men's levels
80 of testosterone, a hormone known to affect men's mating behaviour, changes depending on
81 whether they are exposed to an ovulating or non ovulating woman's body odour. Men who are
82 exposed to scents of ovulating women maintained a stable testosterone level that was higher than
83 the testosterone level of men exposed to non-ovulation cues [15, 16, 17]. Testosterone levels and
84 sexual arousal in men are heavily aware of hormone cycles in females. This may be linked to
85 the ovulatory shift hypothesis [18, 19] where males are adapted to respond to the ovulation
86 cycles of females by sensing when they are most fertile and whereby females look for preferred
87 male mates when they are the most fertile; both actions may be driven by hormones [20].

88 Androgens may modulate the physiology of vaginal tissue and contribute to female genital
89 sexual arousal. Women's level of testosterone is higher when measured pre-intercourse versus
90 pre-cuddling, as well as post-intercourse versus post-cuddling. There is a time lag effect when
91 testosterone is administered, on genital arousal in women. In addition, a continuous increase in
92 vaginal sexual arousal may result in higher genital sensations and sexual appetitive behaviours
93 [21, 22].

94 When females have a higher baseline level of testosterone, they have higher increases in sexual
95 arousal levels but smaller increases in testosterone, indicating a ceiling effect on testosterone
96 levels in females. Sexual thoughts also change the level of testosterone but not level of cortisol in

97 the female body, and hormonal contraceptives may affect the variation in testosterone response
98 to sexual thoughts [23].

99 Testosterone may prove to be an effective treatment in female sexual arousal disorders and is
100 available as a dermal patch [24]. Testosterone may be a treatment for postmenopausal women as
101 long as they are effectively estrogenize [24].

102 Estrogen is the primary female sex hormone as well as a medication. It is responsible for the
103 development and regulation of the female reproductive system and secondary sex
104 characteristics. Estrogen may also refer to any substance, natural or synthetic, that mimics the
105 effects of the natural hormone [25]. The estrane steroid estradiol is the most potent and
106 prevalent endogenous estrogen, although several metabolites of estradiol also
107 have estrogenic hormonal activity. Estrogen supplements may be used in some oral
108 contraceptives, in hormone replacement therapy for postmenopausal, hypogonadal, and
109 by transgender women, and estrogen suppressants may be used in the treatment of
110 certain hormone-sensitive cancers like prostate cancer and breast cancer. They are one of three
111 types of sex hormones, the others being androgens/anabolic
112 steroids like testosterone and progestogens like progesterone.

113 Estrogens are synthesized in all vertebrates as well as some insects [26]. Their presence in both
114 vertebrates and insects suggests that estrogenic sex hormones have an ancient evolutionary
115 history. The three major naturally occurring forms of estrogen in women
116 are estrone (E1), estradiol (E2), and estriol (E3). Another type of estrogen called estetrol (E4) is
117 produced only during pregnancy. Quantitatively, estrogens circulate at lower levels than
118 androgens in both men and women (Burger, 2002). While estrogen levels are significantly lower

119 in males compared to females, estrogens nevertheless also have important physiological roles in
120 males [27].

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

130 While estrogens are present in both men and women, they are usually present at significantly
131 higher levels in women of reproductive age. They promote the development of female secondary
132 sexual characteristics, such as breasts, and are also involved in the thickening of
133 the endometrium and other aspects of regulating the menstrual cycle. In males, estrogen
134 regulates certain functions of the reproductive system important to the maturation of sperm and
135 may be necessary for a healthy libido [30]. Furthermore, there are several other structural
136 changes induced by estrogen in addition to other functions.

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

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141 **2. MATERIALS AND METHODS**

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

144

145 **METHOD**

146 **2.1. PREPARATION OF APPLE CIDER VINEGAR “WITH MOTHER”**
147 **TREATMENT**

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

152 **2.2. EXPERIMENTAL DESIGN**

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

157 Group 1(9 rats as control)

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

159 Group2 (test groups)

- 160 • Distilled water, feed and 1ml of apple cider vinegar with mother was administered.

- 161 • 3 animals were sacrificed from each group on day 7, 14, 21 respectively and blood
162 samples was collected.

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166 **2.3.MODE OF SACRIFICE**

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

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171 **2.4. Hormone Assay**

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

176

177 **2.5. Statistical Analysis**

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

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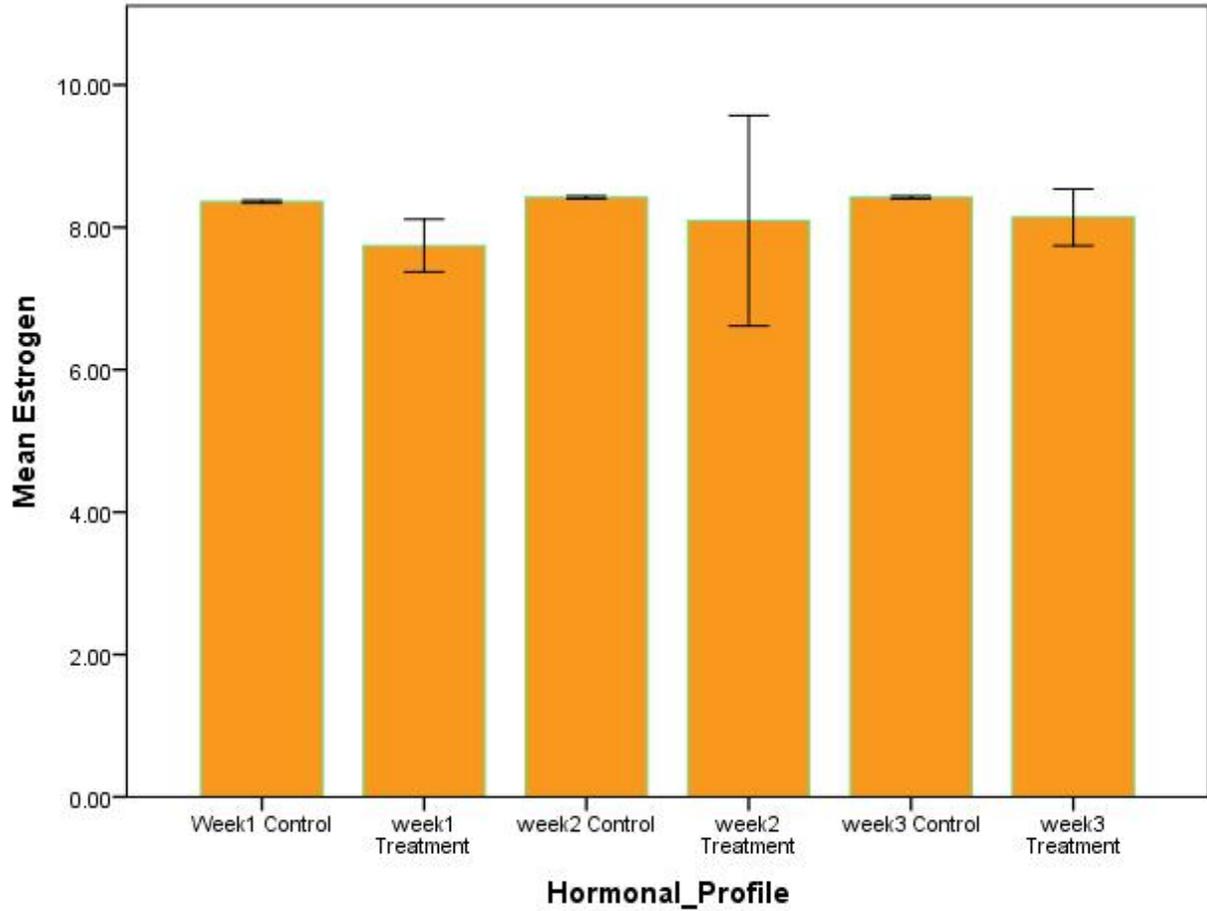
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186 **3. RESULTS**

187 **Estrogen Result**



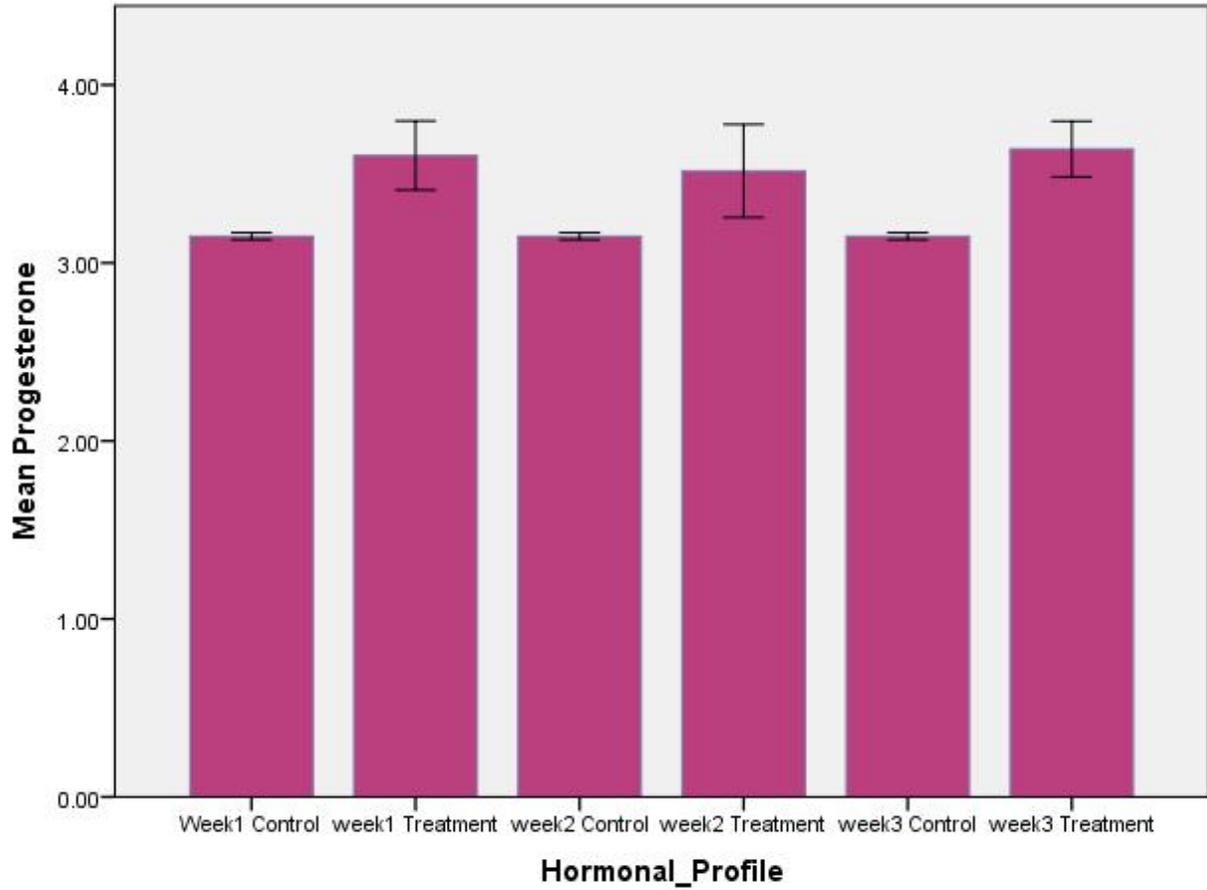
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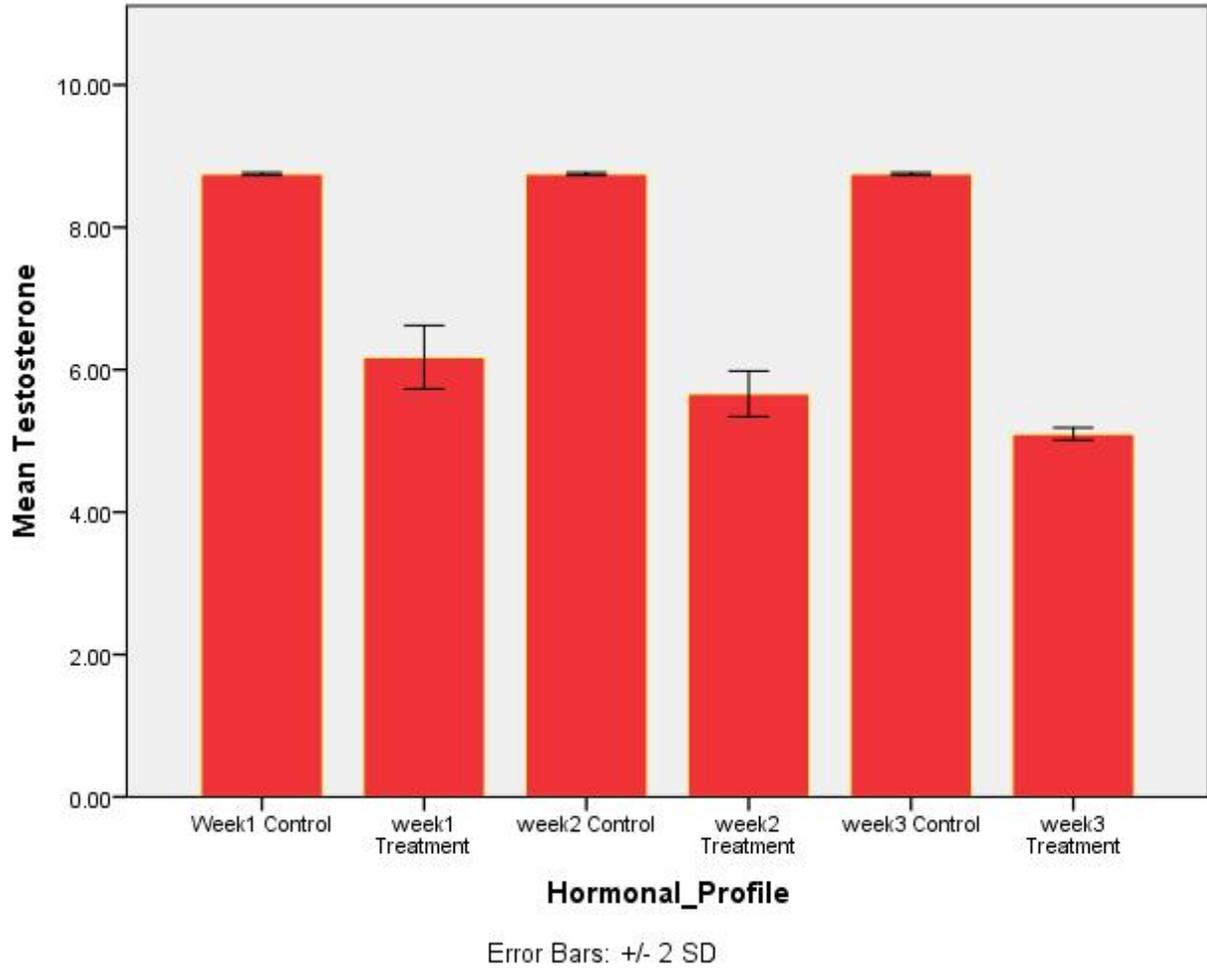
Fig. 3.1 Graph showing mean estrogen concentration (mIU/ml) of Wistar rats treated with apple cider vinegar.



Error Bars: +/- 2 SD

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



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

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4. DISCUSSION

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This research work showed the effect of apple cider vinegar with “the mother” on the

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progesterone, testosterone and estrogen of Wistar rats for 21 days. After oral administration of

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the product, the results revealed significant reductions in a time dependent manner with the

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highest reductions obtained on the last week of experiment.

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

212 From Fig 3.2, the study also showed that at week 1 mean progesterone concentration (mIU/ml) of
213 control rats (3.15 ± 0.01) was significantly ($P < 0.05$) lower than treatment rats (3.60 ± 0.10). At
214 week 2, mean progesterone concentration of control rats (3.15 ± 0.01) was significantly ($P < 0.05$)
215 lower than treatment rats (3.15 ± 0.13). At week 3 mean progesterone concentration of control rats
216 (3.15 ± 0.01) was significantly ($P < 0.05$) lower than treatment rats (3.64 ± 0.08). The study further
217 showed reduction in the testosterone results as shown in Fig 3.3.

218
219 Progesterone is an endogenous steroid and progestogen sex hormone involved in the menstrual
220 cycle, pregnancy, and embryogenesis of humans and other species. It belongs to a group of
221 steroid hormones called the progestogens and is the major progestogen in the body. It has been
222 discovered that progesterone can be taken as a medication mainly used for hormone replacement
223 therapy for menopause, hypogonadism and transgender [17, 32]. In this present study, the effect
224 of apple cider” with mother” was able to reduce the level of progesterone in Wistar albino rats
225 during the 21 days of treatment.

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

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

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

239 Previous studies has shown that apple cider vinegar” with mother” has been effective in the
240 reduction of excess sugar level. Its likely good for both type1 and type 2 diabetes, especially
241 lowering postprandial glucose and also supports weight loss [2, 33, 34]. Apple cider vinegar”
242 with mother has also been useful in reducing harmful lipid in the blood. Apple cider vinegar
243 helps the body to convert the proteins found in foods into usable amino acids. Amino acids are
244 the building blocks for many different bodily processes, including the creation of the hormones.
245 So, in drinking a shot of apple cider vinegar one is actually giving the body what it needs to
246 make hormones – addressing any imbalances between estrogen, progesterone and testosterone.
247 Apple cider vinegar balances the blood sugar, preventing blood sugar soars and crashes and
248 supporting healthy, consistent ovulation. Apple cider vinegar balances acid/alkaline levels in the
249 body, allowing good bacteria to flourish in the micro biome. Apple cider vinegar also supports
250 weight loss by contributing good bacteria to the gut. (2, 3, 33, 34, 35).

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253 **5. CONCLUSION**

254 In conclusion, apple cider vinegar reduces the levels of estrogen and testosterone but
255 progesterone was increased over the period of the weeks when administered to Wistar rats.

256 **Competing Interests**

257 Authors have declared that no competing interests exist.

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260 **6. ETHICAL APPROVAL:**

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

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