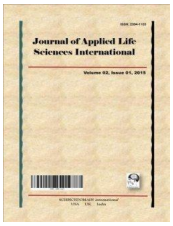

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Effect of Apple Cider Vinegar (ACV) "With Mother" on Progesterone, Testosterone and Estrogen of Wistar Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author ONF designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Author EO managed the literature searches. Both authors read and approved the final manuscript.

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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 Methods: Eighteen rats with average weight range of 120 g 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 1 ml 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 mIU/ml) as compared to control (8.36±0.01 mIU/ml).For progesterone, there was an increase ($p<0.05$). The highest test value was obtained in week 3, (3.64±0.08 mIU/ml) as compared to control value (3.15±0.01 mIU/ml). However, there was also a reduction in testosterone level.

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

Keywords: Apple cider vinegar; estrogen; hormones; progesterone; testosterone.

1. INTRODUCTION

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

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

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

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

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

are the most fertile; both actions may be driven by hormones [20,21,22].

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

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

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

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

2. MATERIALS AND METHODS

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

2.1 Preparation of Apple Cider Vinegar "With Mother" Treatment

Two table spoons (30 ml) of the Apple cider vinegar 'with mother' was measured with volumetric flask. Then 240 ml of distilled water was measured with a volumetric flask. The 30 ml of apple cider vinegar "with mother" was poured into the 240 ml of distilled water. The solution was mixed properly.

2.2 Experimental Design

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

Group 1(9 rats as control)

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

Group2 (test groups)

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

2.3 Mode of Sacrifice

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

2.4 Hormone Assay

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

3. METHOD USED FOR PROGESTERONE TEST

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

3.1 Test Procedures

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

3.2 Method Used For Testosterone Test

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

3.3 Test Procedure for Testosterone

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

3.4 Method Used for Estrogen Test

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

3.5 Test Proceedure

The sample was centrifuged for 15 minutes at 1000×g, at 4°c within 30 minutes of collection and was assayed. All reagents and samples were prepared and the number of wells to be used was determined and the remaining wells and the desiccant were put back into the pouch and sealed back and stored at 4°c. A blank was set without any solution. The sample (50 µl) was added per well. HRP-conjugate (50 µl) was added to each well (not to the blank well) and 50µlwas added to each well and was mixed properly and incubated for one hour at 37°c. Each well was aspirated and washed and the process was repeated twice for a total of three washes. Washing was done by filling each well with wash buffer (200 µl) using an autowasher and allow to stand for 10 seconds. After the last wash, the remaining wash buffer was aspirated or decanted and the plate was inverted and blotted against clean papers towels. Substrate A (50 µl) and 50 µl of substrate B were added to each well and was mixed properly and incubated for 15 minutes at 37°c. Stop solution (50 µl) was added to each well, and was mixed properly. The optical density of each well was determined within 10 minutes using a microplate reader set to 450 nm and was read.

3.6 Statistical Analysis

Data analysis was performed using the Statistical package for the Social Sciences software (SPSS, version 11.0). Data is displayed in mean ± SD. 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 is p=0.05.

4. RESULTS

The results obtained in this study are as presented in the graph below.

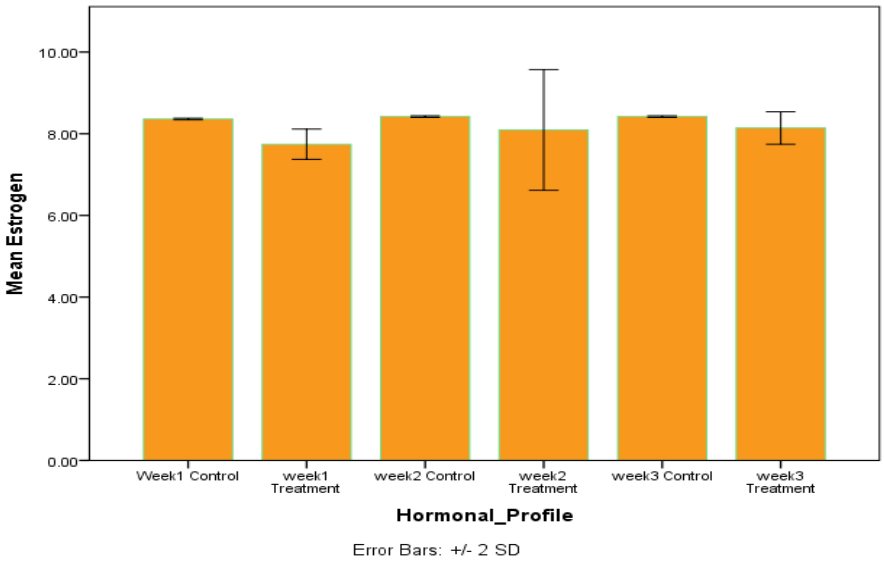

Fig. 1. Graph showing mean estrogen concentration (mIU/ml) of Wistar rats treated with apple cider vinegar

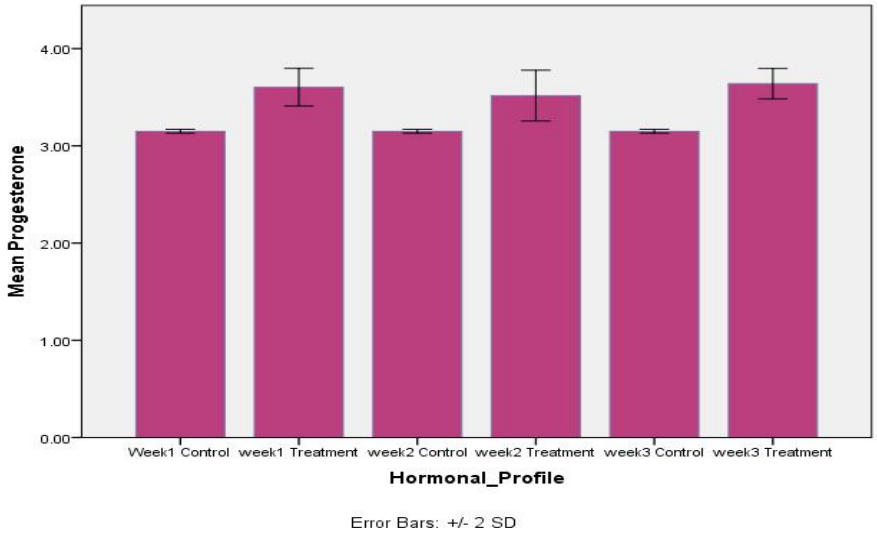

Fig. 2. Graph showing mean progesterone concentration (mIU/ml)of Wistar rats treated with apple cider vinegar

5. DISCUSSION

This research work showed the effect of apple cider vinegar with "the mother" on the progesterone, testosterone and estrogen of Wistar rats for 21 days. 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.

From Fig. 1, the study showed that at week one, mean estrogen concentration (mIU/ml) of control rats (8.36±0.01) was significantly ($P<0.05$) higher than treatment rats (7.74±0.19). At week 2 there was no significant ($p<0.05$) difference between control rats (8.42±0.01) and treatment rats (8.09±0.74). Also at week 3, there was no significant ($p<0.05$) difference between control rats (8.42±0.01) and treatment rats (8.14±0.20).

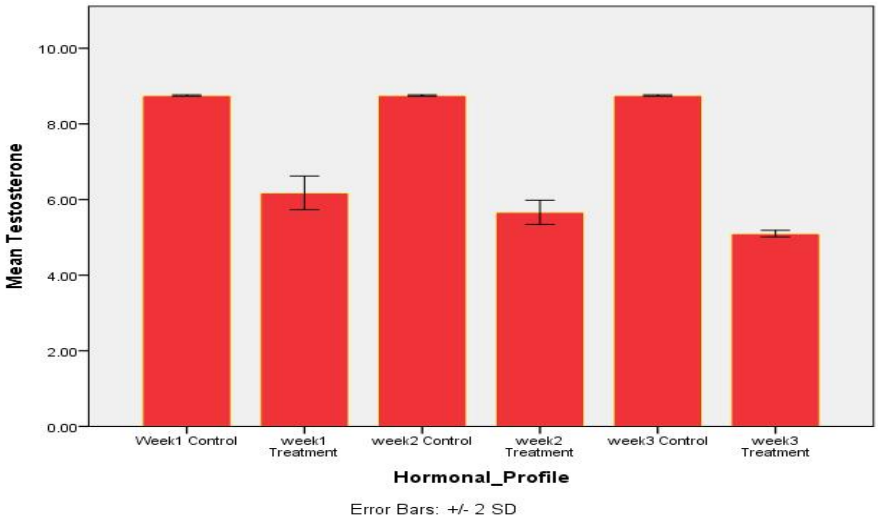

Fig. 3. Graph showing mean testosterone concentration (mIU/ml)of Wistar rats treated with apple cider vinegar

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

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

Testosterone is the primary male sex hormone and an anabolic steroid. In male humans, testosterone plays a key role in the development of male reproductive tissues such as the testis and prostate, as well as promoting secondary sexual characteristics such as increased muscle and bone mass, and the growth of body hair [8]. In addition, testosterone is involved in health and well-being and the prevention of osteoporosis [8,9]. Insufficient levels of testosterone in men may lead to abnormalities including frailty and bone loss. Based from the present study carried out it was discovered that apple cider vinegar" with mother" reduces the testosterone level of the Wistar rats for the 21 days of treatment.

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

Previous studies have shown that apple cider vinegar" with mother" has been effective in the reduction of excess sugar level. Its likely good for both type1 and type 2 diabetes, especially lowering postprandial glucose and also supports weight loss [2,33,34]. Apple cider vinegar "with mother" has also been useful in reducing harmful lipid in the blood. Apple cider vinegar helps the body to convert the proteins found in foods into usable amino acids. Amino acids are the building blocks for many different bodily processes, including the creation of the hormones. So, in drinking a shot of apple cider vinegar one is actually giving the body what it needs to make hormones – addressing any imbalances between estrogen, progesterone and testosterone. Apple

cider vinegar balances the blood sugar, preventing blood sugar soars and crashes and supporting healthy, consistent ovulation. Apple cider vinegar balances acid/alkaline levels in the body, allowing good bacteria to flourish in the micro biome. Apple cider vinegar also supports weight loss by contributing good bacteria to the gut [2,3,33,34,35].

6. CONCLUSION

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

ETHICAL APPROVAL

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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