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EFFECTS OF APPLE CIDER VINEGAR "WITH MOTHER" ON THE KIDNEY FUNCTION AND HAEMATOLOGICAL PARAMETERS OF WISTAR RATS

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ABSTRACT

Aim: Apple cider vinegar is widely used all over the world and it has been consistently used for its healing properties. In this study, the in vivo effect of apple cider vinegar with the mother on kidney function and haematological parameters of Wistar rat were investigated. **Materials and method:** A total of 18 rats (average weight of 110g) were used for the study. The rats were fed for a period of three weeks with the same concentrations the apple cider vinegar. The rats were grouped into six groups of three rats in each group. Three groups served as the control for each week (day 7, 14 and 21) while the other three groups were administered orally with 1ml of apple cider vinegar "with mother" twice daily for each week. The urea and creatinine levels were determined using spectrophotometric methods, The haemoglobin count, packed cell volume, total white blood cell count, red blood cell count, platelet count, neutrophil and lymphocyte count were also assayed. **Results:** Test results showed that the apple cider vinegar had a slight increase ($p \leq 0.05$) on the urea and creatinine levels in a time dependent manner. The highest increase was observed at the last week of feeding. The results for urea showed that the highest value was obtained in day 21 (11.00 ± 3.82) as compared to control animals (10.99 ± 0.01). The creatinine values also showed slight increase ($P \leq 0.05$) in test animals when compared to control animals. The highest increase was obtained in day 21 (36.10±1.52) as compared to control (34.42±0.02). Test results also showed that the apple cider vinegar had a lowering effect on the haematological parameters studied in a concentration and time dependent manner with differences in concentration and time at 95 % confidence levels ($P < 0.05$). The highest decrease of 11.11 ± 0.18 vs control 12.34 ± 0.24 (g/dl) was obtained for Hb at 21 days duration. PCV, WBC, RBC, platelet, neutrophil and lymphocyte analysis also showed highest decrease at 21 days duration. **Conclusion:** The results showed that using apple cider vinegar in small amounts for a short period of time had little effect. However, it is imperative that anyone intending to take large amounts of bentonite for long periods of time to undergo blood tests from time to time.

KEYWORDS: Apple cider vinegar, Blood, Creatinine, Haematological Parameters, Kidney, Urea.

1.0 INTRODUCTION

Apple cider vinegar is a type of vinegar made from cider or apple and has a pale of medium amber colour. Unpasteurised or organic apple cider vinegar, which was a cobweb like appearance and make the vinegar look slightly congealed. 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 sugars are 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]

True balsamic vinegar, labelled aceto balsamico traditizionale, has been produced for a thousand years in the Emilia-Romagna region of northern Italy. This includes an area starting from the Adriatic Sea and reaching almost to the Gulf of Genoa**.** This type of vinegar is made from the reduced juice of sweet white grapes such as the Trebbiano and Lambrusco varieties and is aged for 12 or more years in a progression of aromatic wooden casks. Sweet and sour, smooth and mellow, and dark purple-brown in colour, balsamic vinegar is full of subtle complex flavors. It is used as an ingredient in salad dressings, sauces and marinades and even as a toping sprinkled on fresh strawberries or ice cream.

A much more readily available quick-processed industrialized version of balsamic vinegar is produced in Modena but even this, by Italian law, has to be aged for at least 3 years to be labeled aceto balsamico. It usually has a 6% acid content.

Traditional balsamic vinegar has been made for over 1000 years in the northern Italian region of **Emilia-Romagna**, in the provinces of Reggio Emilia and Modena from 1300 to 1860 this region was a Duchy ruled by the Este family who were particularly linked to the production and usage of traditional balsamic vinegar. Throughout this time period this precious liquid can be found in notarial deeds recording weddings, inheritances and donations. Records dating back to 1046, show that some of this highly regarded product was even given as a gift to emperor Henry III by the lord of Canossa.

The name balsamic comes from balsam and balm which refer to its attributed medicinal properties since it was used to sooth and heal and to protect against the plague.

Until the 1970's, this traditional vinegar was produced for family use only. It was made in the attic of houses in wooden aging barrels and both the process and the equipment were passed on from generation to generation.[3]

During the 1980's however, the popularity of balsamic soared to the point where demand greatly outpaced the supply of the local families. To meet this worldwide demand, some producers started making this vinegar quickly using non traditional methods and ingredients and it is this inexpensive imitation balsamic vinegar which is readily available in most local supermarkets.

Urea is a waste product formed from the breakdown of proteins. Urea is usually passed out in the urine. A level of urea (uraemia) indicates that the kidney may not be working properly.

Creatinine is usually a more accurate markers of kidney function than urea. The effect of muscle mass needs to be taken into account. A person with a lot of muscle and little fat on their body is likely to have a higher creatinine than a person who has a lot of fat and little muscle.

Haematology refers to the study of the numbers and morphology of the cellular elements of the blood the red cells (erythrocytes), white cells (leucocytes), and the platelets thrombocytes) and the use of these results in the diagnosis and monitoring of disease**.**[4] Haemtological studies are useful in the diagnosis of many diseases as well as investigation of the extent of damage to blood.[5] Haematological studies are of ecological and physiological interest in helping to understand the relationship of blood characteristics to the environment and so could be useful in the selection of animals that are genetically resistant to certain diseases and environmental conditions.[6,7] Haematological parameters are good indicators of the physiological status of animals**.**[8] Haematological parameters are those parameters that are related to the blood and blood forming organs**.** Blood act as a pathological reflector of the status of exposed animals to toxicant and other conditions.[9] As reported by Isaac et al., (2013),[10] animals with good blood composition are likely to show good performance. Laboratory tests on the blood are vital tools that help detect any deviation from normal in the animal or human body. The examination of blood gives the opportunity to investigate the presence of several metabolites and other constituents in the body of animals and it plays a vital role in the physiological, nutrition and pathological status of an organism.[11] Examination of blood for their constituents can provide important information for the diagnosis and prognosis of diseases in animals. Blood constituents change in relation to the physiological conditions of health**.** These changes are of value in assessing response of animals to various physiological situations.[8,12] Changes in haematological parameters are often used to determine various status of the body and to determine stresses due to environmental, nutritional and/or pathological factors.[13]

2.0 MATERIALS AND METHOD

Eighteen (18) albino rats of both sexes weighing between 100g and 120g were used for the experiments. They were purchased from the Department of Biochemistry animal house in Choba campus at the University of Port Harcourt. The animals were left to acclimatize to the environment during which they were fed with normal feed (Top feeds' grower's mash) and clean water. Three (3) animals were kept in each cage as a group and labelled. Three (3) cages were labelled control with specific days that is, one (1) cage had seven (7) days, another fourteen (14) days and the last twenty-one (21) days. Another three (3) set of cage were labelled, this time, those that would be administered with the apple cider vinegar "with mother". E.g a cage was labelled group 1 "seven (7) days", group two (2) "14 days" and group three (3) "21 days".

2.1 Dillution of Apple Cider Vinegar "With Mother"

Two (2) table spoon (30ml) of the Apple cider vinegar 'with mother' was measured with volumetric flask. Exactly 240ml of distilled water was measured with a volumetric cylinder. The 30ml of apple cider vinegar "with mother" was poured into the 240ml of distilled water. The solution was mixed properly.

2.2 Administration of Apple Cider Vinegar "With Mother"

Each animal from each cage (except the ones in control cages) was administered with 1ml of the diluted Apple cider vinegar "with mother" which was measured with a syringe. The administration was done morning and evening; the cages labelled for seven (7) days, the animal were sacrificed on the 8^{th} day without any administration on that 8^{th} day.

2.3 Mode of Sacrifice

A desicator was made ready by pouring 5ml of chloroform into the cotton wool placed at its base, each rat were placed on the gauze horizontally over the cotton wool and the lid of the dessicator was shut tightly. The rats remained in the desicator for about a minute to

inhale the chloroform. The effect of inhalation of the chloroform causes weakness and suffocation which led to the passing out of the rat. With a glove hand, a sharp blade was used to slice the body cavity of the animal and the syringe was used to suck out its blood. Each blood sucked out were poured into a heparin bottle for chemistry test and also EDTA bottle for haematological test (before then both bottles were labelled) and taken to the lab for analysis. This process was done for each sacrificial day.

Table 1: Diagramatic Illustration of The Experiment.

Groups	Title	Administration
Control 1	Seven (7) days	The animals in this group were given normal feed and water for seven (7) days before sacrifice.
Control 2	Fourteen (14) days	The animals in this group were given normal feed and water for fourteen (14) days before sacrifice.
Control 3	Twenty-one (21) days	The animals in this group were given normal feed and water for twenty-one (21) days before sacrifice.
Group 1	Seven (7) days	The animals in this group were administered with 1ml of Apple cider vinegar "with mother" for seven days (7) before sacrifice.
Group 2	Fourteen (14) days	The animals in this group were administered with 1ml of Apple cider vinegar "with mother" for fourteen (14) days before sacrifice.
Group 3	Twenty-one (21) days	The animals in this group were administered with 1ml of Apple cider vinegar "with mother" for twenty-one (21) days before sacrifice.

2.4 Urea Determination

Urea levels were determined by enzymatic colorimetric endpoint method. The principle of this method is that urea is hydrolysed by urease into ammonia and carbon dioxide. The ammonia generated reacts with alkaline hypochlorite and sodium salicylate in the presence of sodium nitroprusside as coupling agent to yield a blue cromophore. The intensity of the colour formed is proportional to the concentration of urea in the sample.[14,15]

The reagent kit contained reagent 1: (urease >500U/ml), stabilizers. Reagent 2/; (buffered chromogen), phosphate buffer (20mmol/l pH 6.9), EDTA (2 mmol/l), sodium salycilate (60 mmol/l), sodium nitroprusside (3.4mmo/l). Reagent 3: Alkaline hypochlorite, sodium hypochlorite (10 mmol/l), NaOH (150mmol/l), urea standard, urea (8.3 mmol/l). The working reagent was prepared by mixing 1ml of reagent 1 with 24 ml of reagent 2.

1.00ml of the working reagent was mixed with 10µl of the sample. The standard tube contained 1.00ml of the working reagent and 10µl of the standard. The blank tube had 1.00ml of working reagent. The mixture was incubated for 5 minutes at 37^{o}C and absorbance of sample read against the reagent blank at 600nm with Spectronic-20 spectrophotometer.

Calculations:

Concentration of Sample = $\frac{\Delta A_{sample}}{\Delta A_{standard}} \times C_{standard}$ = mg/dL urea x 0.1665 = mmol/l

Normal values: 2.5 – 6.6mmol/l.

2.5 Creatinine Determination

Creatinine levels were determined by colorimetric method (with deproteinization)

The principle of this method is that creatinine in alkaline solution reacts with picrate to form a coloured complex.[16]

The Reagent kit contained solution 1: Standard (177umol/l), solution 2: Picric acid (35 mmol/l), solution 3: Sodium hydroxide (1.6 mol/l), TA 651 Trichloroacetic acid (TCA) (1.2mol/l). The Working reagent was prepared by mixing 10ml of solution 2 and 10ml of solution 3.

The sample was first deproteinized by mixing 1.0ml of Trichloroacetic acid (TCA) and 1.0ml of sample. The mixture was vigorously stirred with a glass rod to evenly disperse the precipitate. The mixture was then centrifuged at 2500 rpm for 10 minutes, the supernatant was then separated and used for the assay as listed below:

1.00ml of the working reagent was mixed with 1.00ml of the supernatant. The standard tube contained 1.00ml of the working reagent, 0.5ml of TCA and 0.5 ml of solution 1. The blank tube had 1.00ml of working reagent, 0.5ml of TCA and 0.5ml of distilled water. The mixture was let to stand for 20 minutes at 25 ^{o}C and the absorbance of the sample and standard were read against the blank at 520nm with Spectronic -20 spectrophotometer.

Calculations:

$\frac{\Delta A_{sample}}{\Delta A_{standard}} \times 177$ = µmol/l

Normal values: 44 – 80 µmol/l

2.6 Haemoglobin Determination

Blood (0.20ml) was measured and dispensed into 4ml Drabkins solution. The mixture was allowed to stay at room temperature for 4 – 5 minutes, absorbance were

measured using spectrophotometer and read at 540nm. The haemoglobin values were read using the calibrate graph.

Packed Cell Volume
The capillary tube was filled with ¾ well mixed EDTA blood. The unfilled end was sealed with a sealant and place in a microhaematocrit centrifuge for 5 minutes. After centrifuging, the PCV was read using a microhaematocrit reader.

Total White Blood Cells
Measured 0.38ml of diluting fluid was dispensed into a small container and 0.20ml of well mixed EDTA blood was added and mixed and remixed in the counting chamber using Pasteur pipette. The chamber was left undisturbed for 20 minutes to allow time for the white blood cells to settle. They were then counted.

$$\text{WBC count (per/liter)} = \frac{N \times DF \times 10}{A \times D}$$

Where N = no of cell counted
DF = dilution factor
A = Area counted
0.1 = depth of chamber

Platelet Count
Measured 0.38ml of diluting fluid was dispensed into a small container and 0.20ml of well mixed EDTA blood was added and mixed and remixed in the counting chamber. The chamber was left undisturbed for 20 minutes to allow time for the white blood cells to settle.

They were then counted.

$$\text{Platelet count} = \frac{N \times 20 \times 10}{0.2 \times 0.1}$$

Red Blood Cell Count
Measured 4.00ml of formal citrate fluid was dispensed into a small container and 0.20ml of well mixed EDTA blood was added and mixed and remixed in the counting chamber using Pasteur pipette. The chamber was left undisturbed for 20 minutes to allow time for the white blood cells to settle. They were then counted.

$$\text{RBC count (per liter)} = \frac{N \times DF \times 10^9}{A \times D}$$

Where N = no of cell counted
DF = dilution factor
A = Area counted
0.1 = depth of chamber

Neutrophil and Lymphocyte: A drop of blood was placed on the end of a clean dry slide. A clean smooth edge of the spreader was used to spread the blood to make film of about 40-50mm in length. The film was air dried and fixed in absolute methanol. The slide was covered with undiluted leishman stain and allowed to stand for 2 minutes. Buffered water of pH 6.8 was used to mix the stain and it was allowed to stand for 8 minutes. It was washed with tap water and put back in the rack for the smear to dry and examined using oil immersion objective lens.

2.7 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.

3.0 RESULTS

Table 2: Kidney functions test (creatinine and urea) was analysed to determine their concentration in various samples.

SAMPLE	Urea (mmol /l)	Creatinine (µmol/l)
Control 1	10.17 ± 0.01^{a}	34.16 ± 0.09^{a}
Group 1	10.88 ± 3.78^{b}	36.02 ± 2.34^{b}
Control 2	10.19 ± 0.01^{a}	34.15 ± 0.02^{a}
Group 2	10.92 ± 0.25^{c}	36.08 ± 2.14^{b}
Control 3	10.99 ± 0.01^{a}	34.42 ± 0.02^{a}
Group 3	11.00 ± 3.82^{b}	36.10 ± 1.52^{b}

Haematological parameters (PCV, Hb, RBC, WBC, Platelet, MCV, MCHC, MCH) were also analysed to determine their concentration in the various samples. The results obtained were shown below;

Table 2: The haematological profile of PCV= Packed Cell Volume (%), Hb = haemoglobin (g/dl), WBC = White blood cell count, RBC = Red Blood Cell MCH = Mean cell haemoglobin, MCHC = mean cell haemoglobin concentration, MCV= mean cell volume. Values represents Mean ± standard error of mean (SEM). Means in the same column with same alphabet are significantly p≤0.05 while mean in same column with different alphabets are not significantly different p≤0.05.

Sample	PCV (%)	Hb (g/dl)	WBC (x10^9/l)	RBC (x10^7/l)	Platelet (x10^9/l)	MCH (X10^18p)	MCV (x10^18fl)	MCHC (g/l)
Control 1	41.33±0.67[a]	12.67±0.20[a]	4.63± 0.00[a]	1.64± 0.01[a]	61.67±0.89[a]	7.83± 0.02[a]	2.54±0.1[a]	0.31±0.00[a]
Group 1	37.33±6.36[b]	11.24±1.84[b]	2.77± 0.62[b]	2.45± 0.26[b]	150.0±24.01[a]	4.58± 0.26[a]	1.60±0.1[b]	0.31±0.00[b]
Control 2	40.00±0.00[c]	12.26±0.10[c]	4.70± 0.12[c]	1.73± 0.01[c]	146.0±1.15[b]	7.03± 0.02[b]	2.21±0.01[c]	0.31±0.00[c]
Group 2	39.32±0.64[d]	11.16±0.10[d]	2.0± 0.60[d]	1.94± 0.22[d]	139.3±17.84[a]	43.46± 0.84[c]	2.10±0.27[d]	0.31±0.00[d]
Control 3	30.58±0.67[e]	12.34± 0.24[e]	4.77± 0.33[,e]	1.42± 0.01[e]	162.0±1.15[a]	7.42± 0.01[d]	2.07±0.01[e]	0.31±0.00[e]
Group 3	36.70±0.05[f]	11.11±0.18[f]	2.26± 0.23[a,b,c]	1.19± 3.11[f]	124.5±45.50[c]	42.97±3.04[a]	2.22±0.98[a,b]	0.31±0.00[f]

4.0 DISCUSSION

The result of the effect of apple cider vinegar on plasma urea and creatinine levels are presented on Table 2. Analysis of plasma urea showed slight increase (p≤0.05) in test animals when compared to the controls. The highest value was obtained in day 21 (11.00 ± 3.82) as compared to control animals (10.99 ± 0.01). The creatinine values also showed slight increase (P≤0.05) in test animals when compared to control animals. The highest increase was obtained in day 21 (36.10±1.52) as compared to control (34.42±0.02).

Plasma level of urea and creatinine are primarily used to determine the efficiency of the kidney. Urea and creatinine accumulate in the plasma when renal excretion is reduced. Cause of increased blood urea levels include high protein diet, intestinal haemorage, dehydration, severe haemorage, shock etc. Urea level could be decreased due to the following: liver failure, low protein diet, anabolic steroids etc.[17] The normal reference range for blood urea is 2.9-8.2mmol/l, while blood creatinine is 50-110µmol/l .In this present study, apple cider vinegar "with mother" was able to increase the levels of urea and creatinine (p≤0.05), (day 7,14 and 21).

There are numerous health benefits of regular consumption of ACV. ACV can be used for weight loss which can prevent obesity, reduce inflammation throughout the body, lowering the incidence of high blood pressure and high cholesterol and reduces the risk of heart disease, heart attack stroke and fight diabetes in patients with insulin resistance. ACV can help detoxify the body by providing support to the liver and kidneys.[18]

The Mother is what gives unfiltered raw apple cider its distinctive, cloudy appearance and is the most beneficial part of ACV. It is full of antioxidants, important chemicals that help to promote new cell growth and can help prevent cancer, it's full of probiotics which are important for the immune system by promoting healthy bacteria in the gut. Scientific research into the benefit of the mother is fairly limited. Side effects of ACV include if undiluted can irritation of the throat and stomach, excess consumption can lead to depleted nutrient level in the body particularly potassium.

A full blood count (FBC) or complete blood count (CBC) is a very common clinical procedure and often the "starting point" for most medical investigation. The cells that circulate in the blood stream are generally divided into three types: white blood cells (leukocytes), red blood cells (erythrocytes) and platelets (thrombocytes). References ranges of Hb :12.0 –18.0g/l, WBC: 3.5 –12.0 x10^9 /L Plt:130 –400 x10^9/l and PCV: 37 – 54%.Abnormally high or low counts may indicate the presence of many forms of diseases, and hence blood counts are among the most commonly performed blood test in medicine as they can provide an overview of a patient's general health status.[19]

Packed cell volume/ haemoglobin and mean corpuscular haemoglobin are major indices for evaluating circulatory erythrocytes and are significant in the diagnosis of anaemia and also serve as useful indices of the bone marrow capacity to produce red blood cells as in mammals.[19,20] Furthermore,[20] posited that high PCV reading indicate either an increased in the number of RBC or reduction in circulating plasma volume. Mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration indicate blood level conditions. A low level is an indication of anaemia while high level is an indication of polycythemia.[21] Also in this present study, the effect of apple cider vinegar "with mother" was able to increase the level of RBC, platelets, PCV, Hb, WBC, MCH, MCV, MCH and reduces the level over the period of some days except RBC and platelets. Also on multiple comparism, each of these

days (sample days) their values are not still significantly different ($p \leq 0.05$) except for WBC (day 7&21) which had significant difference ($p \leq 0.05$).

There was a significant decrease the in weight for the control group compared to the test groups. This suggests that apple cider vinegar can be used for weight management. Current findings displayed on ACV, had no significant changes in the number of WBC, RBC count and related indices such as MCHC and Hb while MCH and MCV were reduced and platelets were increased.[22]

5.0 CONCLUSION

High urea and creatinine levels in our blood streams tells us that our kidney tissues is damaged or damaging and may indicate the presence of many form of diseases, the apple cider vinegar "with mother" when administered to the Wistar albino rats had no significant difference on kidney functions (creatinine & urea) when compared to the control animals used for the research study.

The apple cider vinegar "with mother" when administered to the Wistar albino rats had no significant difference on haematological parameters when compared to the control animals used for the research study.

Competing Interests
Authors have declared that no competing interests exist.

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

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