ABSTRACT
Pineapple juice was reported to have nutritional and clinical role in maintaining a relatively constant and healthy body function during condition of reduced immunity and increase prevalence of infection. This study was conducted to determine nutritional effectiveness of pineapple juice on plasma glucose maintenance, body mass index and some hematological parameters in wistar rats. Eighteen wistar rats, divided into three groups were used: A (control), B and C. Rats in A were administered orally with 0.5 ml volume of water, while rats in B and C were administered orally with 0.5 ml and 1.0 ml volume of pineapple juice respectively for 30 days. Blood samples were collected via cardiac puncture using pentobarbionate as anesthesia. Hematological analysis was carried out using Beckman-coulter hematological analyzer and CD4+ Dynal bead technique. Glucose concentration was determined after enzymatic oxidation and BMI calculated by function of rats’ weights and heights. Statistically
analyzed results had significance set at p < 0.05. Rats’ body weight reduced in group A, B and C not significantly when compared with A. Blood glucose levels in both treatment groups as well as CD4+ T cells increased significantly; percentage neutrophil count, percentage lymphocyte count, percentage neutrophil count, percentage lymphocyte count, platelet count and body mass index due to the available nutrient present in the juice, mechanism of action need further clarification.

KEYWORDS: Cluster of differentiation 4 count, Hematocrit, Ananas comosus, BMI, Blood glucose.

INTRODUCTION
Blood glucose level is one of the determinant use to access nutritional status, physiological function, disease states and main source of energy for erythrocytes to activate, achieve and sustain the integrity of their defense system. Hematological indices were used to indicate healthy diet composition(s) and environmental variations in healthy state. It was also used to determine and diagnose stress induced conditions and onset of diseases. Body Mass Index governs healthy state and nutritional status of an individual and fruits were recommended as nutrients and dietary supplements that promote and maintain good health. It was reported that pineapple juice possess anti-degenerative properties and lower the rampant incidence of most non-infectious diseases.

Pineapple (Ananas comosus) fruits were acknowledged for its nutritional and clinical roles in maintaining a relatively constant and healthy body functions. Phytochemicals and micronutrients present in pineapple juice includes chlorogenic acid, coumaric acid, ferulic acid, ellagic acid, manganese, pyridoxine, thiamin, vitamin C, riboflavin, copper as well a proteolytically active bromelain enzyme was reported possessing anti-degenerative properties and lower the rampant incidence of most non-infectious diseases. Fresh or unpasteurized frozen pineapple juice with proteolytically active bromelain enzyme was reported to be safe and decreases inflammation gravity as well as the incidence and multiplicity of inflammation associated with colonic neoplasia. Bromelain also mitigate development of allergic airway disease (AAD),...
suggested its effectiveness in the treatment of asthma and hypersensitivity.\textsuperscript{[11]} Oral treatment with bromelain has a progressive analgesic and anti-inflammatory effects in patients with rheumatoid arthritis.\textsuperscript{[12]} Most medicinal qualities of pineapple were credited to bromelain (EC 3.4.22.32). It has been hypothesized to modulate the immune and hemostatic systems,\textsuperscript{[13]} and to exhibit fibrinolytic, antiedematous, antithrombotic and anti-inflammatory activities in vitro and in vivo.\textsuperscript{[14]} Bromelain was reported to have very low toxicity with an LD\textsubscript{50} (lethal doses) greater than 10 g/kg in mice, rats and rabbits.\textsuperscript{[15]}

Pineapple fruit juice exhibits cardio protective benefit in normolipidemic rat, since pineapple has been reported to relatively improve metabolism and clearance of blood lipoprotein particles.\textsuperscript{[16]} It was reported that pineapple was used traditionally for ailments like sore throat, constipation, jaundice and bronchitis.\textsuperscript{[4]}

Previous studies showed that intake of fruit form important part of diets that are healthy and it has reduced the risk of diseases such as cancer, coronary heart disease, stroke, formation of cataract, hypertension, dyslipidaemia, diabetics, high blood pressure among others.\textsuperscript{[17, 18, 19, 20, 21, 22]} Likewise Oyesola et al 2015\textsuperscript{[23]} in one of their studies also showed that pineapple juice had some gastric protective effects because it was found to reduce some toxic gastric enzymes and cholesterol level. It also has positive hematological and biochemical significances because it potentiated increased erythropoietin activities (increase Hemoglobin content, total white blood cell count and red blood cell count). This is not so in the case of its effect on the levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine amino transferase (ALT) in the liver because, excess consumption significantly will express level of alkaline phosphatase, aspartate aminotransferase and alanine amino transferase in the experimental rat. Elevation in ALP, AST and ALT are usually secondary to tissue damage because such damage results in the leakage of these enzymes from their intracellular stores into plasma. Significant increases in the transaminases commonly accompany such liver diseases as toxic hepatitis, acute liver necrosis and hepatic cirrhosis.\textsuperscript{[23]}

Therefore this study was undertaken as an additional study to examine the nutritional influence that pineapple juice administration in Wistar rats will express on BMI, blood glucose, percentage lymphocyte, percentage neutrophils, platelet count, hematocrit and CD4 count.

Animal Grouping and Pineapple Juice Administration
Eighteen (18) female Wistar rats weighed between 120-150 g were used for this study. The rats were housed under standard environmental conditions and were allowed free access to food and water. They were bred in plastic and wire gauze cages in the animal holding of Department of Physiology, Igbinedion University, Okada, Edo state, Nigeria. The rats were allowed to acclimatize for a period of two weeks and were maintained on standard rat pellet (Capsfeed, Ibadan, Nigeria). The animals were randomly assigned into three groups (A, B and C) with six rats in each group. Group A (control group) was administered 0.5 ml volume of distilled water. Group B was administered with 0.5 ml volume of pineapple juice. Group C was administered with 1.0 ml volume of pineapple juice.

The pineapple juice was administered with an oral cannula for 30 days. Body mass indices (BMI), glucose concentrations were determined and hematological parameter analysis was conducted.

Blood sample collection
Blood samples were collected after 30 days, through cardiac puncture under anesthesia (sodium pentobarbital) at a dose of 50 mg/kg body weight of rats, into EDTA (ethylenediaminetetraacetate) bottle as described by Hoff 2000\textsuperscript{[24]} and reported by Oruganti and Gaidhani.\textsuperscript{[25]} They were immediately transferred to the laboratory for the estimation of different variables.

Hematological Analysis
Percentage lymphocyte, percentage neutrophils, platelet count and hematocrit were estimated using an automated Beckman-coulter hematological analyzer.

CD4\textsuperscript{+} Count
CD4\textsuperscript{+} was determined using the CD4\textsuperscript{+} Dynal bead technique which uses the principle of immune-magnetic cell isolation using Dynal bead at T4-T8 Quant kit. This process enables rapid quantification of T4 and T8 lymphocytes directly from samples in EDTA and anticoagulated blood. This is based on the isolation technique of depletion of monocytes, isolation of CD4\textsuperscript{+} T cells and counting of CD4\textsuperscript{+} T cells.\textsuperscript{[26]}

Glucose Concentration
Glucose concentration was determined after enzymatic oxidation in the presence of glucose oxidase, the hydrogen peroxide formed reacted under catalysis of peroxidase with phenol and 4-amino phenazone forming a red violet quinoneimine dye as indicator.\textsuperscript{[27]}

Determination of Body Mass Indices (BMI)
Ohaus Scout digital weighing balance was used to determine the weights of the rats before and on the last day of administration before blood collection. Lengths of...
the rats were determined with the use of tread and a ruler. Rat’s lengths were measured from the head of the rats to their tails end. BMI was determined for individual rats using below formula. The lengths of the rats were taken as their heights.

\[
\text{BMI} = \frac{\text{weight (kg)}}{\text{height (m)}}^2
\]

**Statistical Analysis**

All the values are expressed as mean ± standard error of mean (SEM). Data were analysed by one-way analysis of variance (ANOVA) followed by Student’s Newman-Keuls post-hoc test using the Graph Pad software. \( P \) value of < 0.05 was declared as statistically significant.

**RESULT**

Table 1 shows the observed changes in body mass index during pineapple administration in Wistar Rats. Body mass index of the control group A (28.4 ± 1.63) was statically compared with the control group B (15.2 ± 2.07) and group C (27.9 ± 4.48). The data revealed that group B reduced significantly and group C showed a reduction that was not significant at \( p < 0.05 \) when compared with the control group A. This gave an indication that the effect that the that pineapple juice had on the rats may be dependent on the volume of pineapple juice administered because there may be further increase in body mass index if administered volume was increase.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Initial BMI Kg/m²</th>
<th>Final BMI Kg/m²</th>
<th>% BMI Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Animals received distilled water only</td>
<td>0.95 ± 0.04</td>
<td>1.22 ± 0.04</td>
<td>28.4 ± 1.63</td>
</tr>
<tr>
<td>B</td>
<td>Animals received 0.5ml pineapple juice</td>
<td>0.99 ± 0.02</td>
<td>1.14 ± 0.03</td>
<td>15.2 ± 2.07*</td>
</tr>
<tr>
<td>C</td>
<td>Animals received 1.0ml pineapple juice</td>
<td>0.86 ± 0.06</td>
<td>1.10 ± 0.02</td>
<td>27.9 ± 4.48</td>
</tr>
</tbody>
</table>

* Significantly different from control at \( P < 0.05 \)

Table 2 showed the effect of administered pineapple juice on CD4 count, percentage lymphocyte, percentage neutrophil, platelet and hematocrit in female wistar rat. Data from the hematological parameter were statistically analyzed group A the control were compared with group B and group C. The result from showed a significant increase in both group B (7.33 ± 0.88) and group C (7.00 ± 1.15) when compared with group A (5.00 ± 1.15) the control group. But it was noted that the difference in the CD4 value of group A and group B was as low as 0.33 cell/µL. This suggested that that consumption of pineapple juice may be a booster for the production of CD4 cells. The result from percentage lymphocyte count showed a reduction that was not significant in group B (82.6 ± 3.57) and in group C (84.0 ± 2.77) showed an increase that was not significant when compared with group A (84.0 ± 2.77) rats. Importantly we noted a slight difference of 0.9 between group C and group A. The result from the percentage neutrophil count that were compared showed increase that was not significant in group B (17.42 ± 3.57), and a reduction that was also not significant in group C (14.6 ± 1.94) when compared with group A (15.7 ± 2.89) rats. It was observed from the percentage neutrophil count that the reduction in group C as compared to group B is lower than group A rats. The various pattern of increases and decreases observed in the percentage lymphocyte and neutrophil count suggested that the juice may have ability to potentiate or initiate production of lymphocyte and neutrophil. The result from platelet count when compared showed a decrease which is not significant in group B (397.8 ± 53.8) and an increase which is not significant in group C (554.7 ± 130.6) when compared with group A (397.8 ± 53.8). It was noted that the increase recorded in group C is more than both group A and C.

**CD4Count- Cluster of Differentiation 4 Count, LYM- Lymphocytes, NEUT- Neutrophils, PLT- Platelets and HCT- Hematocrit Volume**

Obtained results were increase that were significant in group B (29.0 ± 1.18) and C (29.3 ± 1.12).

**Table 2: Hematological changes during Pineapple juice administration in females Wistar rats.**

<table>
<thead>
<tr>
<th>Blood Parameters</th>
<th>0.5ml of Distilled water administered (Group A)</th>
<th>Volume of Pineapple Juice Administered (Group B)</th>
<th>Volume of Pineapple Juice Administered (Group C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4Count (Cell/µL)</td>
<td>5.00 ± 1.15</td>
<td>7.33 ± 0.88</td>
<td>7.00 ± 1.15</td>
</tr>
<tr>
<td>LYM %</td>
<td>84.0 ± 2.77</td>
<td>82.6 ± 3.57</td>
<td>84.9 ± 2.27</td>
</tr>
<tr>
<td>NEUT %</td>
<td>15.7 ± 2.89</td>
<td>17.42 ± 3.57</td>
<td>14.6 ± 1.94</td>
</tr>
<tr>
<td>PLT (x10³/µL)</td>
<td>397.8 ± 53.8</td>
<td>342.7 ± 79.0</td>
<td>554.7 ± 130.6</td>
</tr>
<tr>
<td>HCT %</td>
<td>46.7 ± 1.47</td>
<td>44.6 ± 1.88</td>
<td>50.0 ± 5.29</td>
</tr>
</tbody>
</table>

Figure 1 showed the result of the blood glucose during pineapple juice administration in wistar rats group A (22.4 ± 0.6) was compared with group B and C, the
DISCUSSION
The reduction in body mass index recorded in group B during administration of 0.5ml of pineapple juice was consistent with known finding about fruit juice consumption.[28] Relatively high water content, low energy density and relatively high dietary fibers present in fruit was implicated for fruit juice ability to reduce body weight, because they increase post – prandial satiety and decrease subsequent hunger in short term study but in long term study it may lead to decrease in energy intake subsequently reduction in body weight.[29]

However when the animal was administered with 1.0ml of the pineapple juice the body mass index increase in group C when compared with group B and this is not consistent with that of group B result because group A differ from group B by value of 0.5. But when we refer to the increased blood glucose level recorded in both group B and group C we may suggest that the sugar content of the juice may be among the factors that is responsible for the increase. Likewise pineapple juice may tend to increase food consumption of the rats with increased volume, although this was not measured in this study.

100 grams of pineapple juice was reported by Nutritiondata.com[30] to contain water (86.5g), energy (48kcal), fat (0.43g), protein (0.39g), carbohydrate (12.39g), fiber (1.2g), potassium (113mg), phosphorus (7mg), Iron (0.37mg), sodium (1mg), Magnesium (14mg), Calcium (7mg), Zinc (0.08mg), Selenium (0.6mcg), Vitamin C (15.4mg), Vitamin A (23UI), Vitamin B, (0.092mg), Vitamin B2 (0.036mg), Vitamin E (0.10mg), Niacin (0.42mg) and folic acid (11mcg). Some of these nutrients were reported to be effective for body building. Vitamin C which is a powerful antioxidant support formation of collagen in bones, blood vessels cartilage and muscles. Vitamin B1 was reported to be effective promoting cells of the body to covert carbohydrate to energy, vitamin B2 was known to promote metabolism of carbohydrate, protein and fat, it also enable the body to get energy from food. Several essential elements found in pineapple juice were reported to promote activation of certain enzymes and absorption of Iron, folic acid was known to promote to promote cell proliferation and production of red blood cells.[30]

The recorded increase in blood glucose in both group B and C, increase platelet count in group C, increase percentage neutrophil count in group B, increase percentage lymphocyte count in group C and as well as CD4 count in both group can be associated with the nutrient content of pineapple juice as earlier mentioned.

CONCLUSION
It is concluded from this study that administration of pineapple juice in rats can potentiate increase blood glucose, CD4 count, percentage neutrophil count, and percentage lymphocyte count and body mass index due to the available nutrient present in the juice. The suggested mechanism may be through metabolism of food substances and or increase efficiency of absorption and transportation of nutrient. This mechanism need further clarification.

REFERENCES
10 Hale LP, Chichlowski M, Trinh CT, Greer PK. Dietary supplementation with fresh pineapple juice decreases inflammation and colonic neoplasia in IL-10-deficient mice with colitis. Inflamm Bowel Dis., 2010; 16(12): 2012-2021.
22 Chobanian AV, Bakris GL, Black HR. The seventh report of the joint national committee on prevention, detection, evaluation and treatment of high blood pressure. JAMA., 2003; 289: 2560-2572.
23 Oyeshola TO, Oyeshola OA, Odukoya SOA, Adelodun ST. Assessment of some Biomarkers in Liver, Kidney and Stomach Following Pineapple Juice Administration IJPE., 2015; 4: 27–35.
27 Barham D and Trinder P. An improved color reagent for the determination of blood glucose by the oxadase system. Analyst., 1972; 97(151): 142-145.
30 Nutritiondata.com Nutrient data for pineapple, raw, all varieties per 100g serving Nutritiondata.com, 2011; USDA SR – 21 retrieved 2011 – 03 – 01.