Effect of *Ananas Comosus* (Pineapple) Juice on Hormonal and Biochemical Profile of Male Vertebrates

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Abstract

Background: *Ananas Comosus* (Pineapple) is rich in bromelain (An enzyme that digests protein). *Ananas Comosus* contains aromatase; an enzyme that converts testosterone to oestrogen. Increased oestrogen in male feminizes. Therefore, this research was set up to ascertain the effect of *Ananas Comosus* (Ac) juice on the hormonal, and biochemical profile of male vertebrates.

Methods: Sixteen male albino rats of 14 weeks of age divided into 4 groups of 4 rats per group were used. The rats were kept in 16 standard cages, fed ad libitum and had access to clean water. Treatments were 2 ml/kg body weight of distilled water for control, 250 mg/kg, 500 mg/kg, and 1000 mg/kg body weight of *Ananas Comosus* juice for 56 days. On day 57, a female rat in oestrus was introduced per male to observe the libido of the male. Blood was collected for haematology and serology after which the male rats were sacrificed. Testes were exteriorized and the caudal epididymis lacerated. Spermatozoa concentrations were scoped into bijou bottles containing 1 ml of Phosphate Buffered Saline (PBS). Spermatozoa analysis and histopathology were done using standard methods.

Result: There was significant ($P \leq 0.05$) decrease in serum testosterone in Ac juice treated males while estradiol significantly increased. Libido of Ac juice treated males were significantly ($P \leq 0.05$) reduced, but the histopathology showed evidence of spermatozoa in the seminiferous tubules. Liver profile of Ac treated rats showed significant ($P \leq 0.05$) sera decrease in aspartate aminotransferase and alkaline phosphate.

Discussion: Decrease in serum testosterone leads to a decrease in spermatogenesis which might have led to a decrease in spermatozoa concentration and low libido. However, histopathology of the testes revealed some spermatids and spermatozoa in the seminiferous tubules. This shows that although Ac juice leads to low libido and low spermatogenic activity, it might not lead to infertility. Moreover, significant decrease in aspartate aminotransferase and alkaline phosphate reveals that *Ananas Comosus* juice does not adversely affect the liver.

Conclusion: Pineapple juice (Ac) treatment leads to reduced libido but not contraception. It may be used to treat fatty liver syndrome. Furthermore, Ac may be an ameliorative remedy for colorectal cancer due to its bromelain content.

Keywords: Bromelain • Colorectal-cancer • Contraception • Infertility • Testosterone

Introduction

Contraception, which is a method used to prevent unwanted pregnancy, is an important aspect of human existence. Females have several contraceptive options but males have only two; use of condom which has a high failure rate and vasectomy which is non reversible [1]. This research was carried out to ascertain whether pineapple juice can lead to male contraception.

Hormones synthesized in the gonads of vertebrates are mostly steroid hormones which have their precursor as cholesterol. In its simplest form, the pathway of steroid hormone synthesis is as follows (Figure 1):

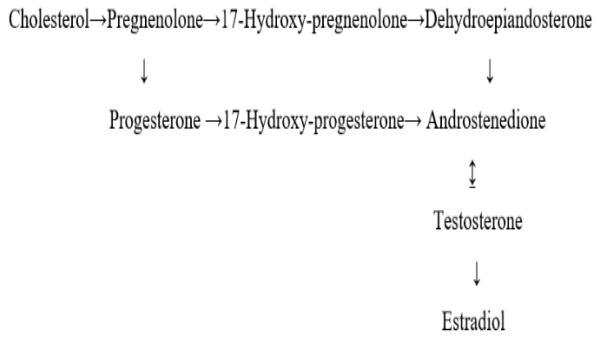

Figure 1. Pathway of steroid hormone synthesis.

Testosterone is the major anabolic hormone in male vertebrates [2]. It maintains the normal reproductive and sexual function of the male vertebrate [3]. Oestrogen receptor-like protein is found in the epididymis of many species including man [4]. Estradiol is the most potent form of oestrogen *in vivo*. It is responsible for modulating libido, erectile function, and spermatogenesis in male vertebrates. Low testosterone and elevated oestrogen increases the incidence of erectile dysfunction in males [5].

Ananas Comosus (Pineapple) fruit is a native of South America. However, important producers of pineapple are Costa Rica, Brazil, Thailand, Philippines, Indonesia, Nigeria, China, India, Mexico, Colombia and others [6]. Pineapple grows best in subtropical and tropical areas were the weather is warm, but cool at night [7].

The consumption of pineapple is wide due to its sweet and acidic flavour. Pineapple is consumed fresh, cooked, juiced, or preserved [8]. It is an excellent source of vitamins, minerals, and nutrients and is rich in antioxidants [9]. Fermentation of pineapple juice is used to make wine. Wine contains antioxidants, phenolic compounds, and flavonoids which prevent cellular damage caused by inflammation and oxidative stress [10]. Moreover, the peel and pineapple by-products from processing can be used as raw materials to prepare natural vinegar by acetic fermentation [11].

Cultivation

There are more than 1000 varieties of pineapple and the cultivation is according to variety [12, 13]. For instance, the queen variety which is used in this research is cultivated in three different ecosystems; freshwater, brackish water, and alluvial soil ecosystem. The research carried out by Rosmaina (2019), revealed that there is no significant difference in yield among the systems of cultivation [7].

Pineapple is an herbaceous perennial plant which grows 1.0 m to 1.5 m (3.3 ft to 4.9 ft) in height. The plant has a short, stocky stem with tough, waxy leaves. During the fruiting process, the plant produces up to 200 flowers. The ovaries develop into berries, which coalesce into a large, compact, multiple fruit. After the first fruit, suckers or side shoots are produced in the leaf axile of the main stem. The suckers may be removed for propagation or left to produce additional fruits on the original plant [14].

Pharmacologically, pineapple contains a protease enzyme (bromelain) which breaks down protein into smaller units making pineapple juice necessary in protein diets such as stakes [15]. Moreover, bromelain found in pineapple is anti-inflammatory and anticancer and can be used to alleviate the effect of routine constipation. It also increases gastric motility [16,17]. According to Seenak (2021), 'Daily consumption of pineapple alleviates hypercholesterolemia-induced cardiac lipid peroxidation and inflammation' [18].

Furthermore, United States Agricultural Data base, revealed that fresh fruit of pineapple has small but substantial quantities of vitamin A, ß - carotene, xanthin, lutein, and ß-cryptoxanthin which collectively play vital roles in antioxidant and vision functions [19].

Pineapple also contains some minerals which are listed per 100 grams fresh fruit weight: Calcium 13 mg, Magnesium 12 mg to 20 mg, Phosphorus 9 mg to 13 mg, Manganese 0.8 mg to 1.6 mg, Iron 0.25 mg to 0,2 mg, Copper 0.08 mg to 0.11 mg and Zinc 0.08 mg to 0.2mg. Pineapple also has high Potassium 125 mg to 178 mg and low Sodium content per 100 g fresh fruit weight [19]. It contains 60%-70% of the daily recommended dose of vitamin C in 100 g fresh weight of pineapple [20].

Physiologically, high potassium and very low sodium is an indication that pineapple consumption leads to reduced blood pressure. This is because potassium ion is normally intracellular and is used to extrapolate cellular membrane resting potential. At the resting potential, every activity of the cell is reduced to the barest minimum [21].

Furthermore, Pavan, asserted that bromelain in pineapple reduces blood pressure and ameliorates asthma effects [16]. But, it is possible that high potassium content or synergistic effect of bromelain and potassium is what reduces the blood pressure. It is also possible that high vitamin C content or synergistic effect of bromelain and Vitamin C reduces broncho-constriction in asthma patients and not necessarily bromelain alone. Moreover, pineapple is found to reduce oxidative stress in high cholesterol fed diet [18]. Cholesterol is a precursor for the synthesis of steroid hormones *in vivo*. Pineapple also has an aromatase enzyme which converts testosterone to estradiol [22-24]. High aromatase in the body of the male leads to mass conversion of testosterone to estradiol cumulating in feminization of the male and male infertility. Consequently, this research was set up to evaluate the effect of pineapple juice on the hormonal and serum biochemistry of male vertebrates. To do this, assessment of the reproductive hormone profile, sexual behaviour, semen analysis, gonado-somatic index, relative organ weight, serum biochemistry, haematology, and histopathology of the testes of the male were considered necessary.

Materials and Methods

One big pineapple (*Ananas Comosus*) was purchased from Ahiaeke market in Umuahia North Local Government area of Abia State, Nigeria, and was sent to Department of Forestry, Michael Okpara University of Agriculture, Umudike for identification and authentication as Queen's Pineapple (Figure 2).

Figure 2. Queens Pineapple JPG (*Ananas Comosus*).

The pineapple was peeled and the back removed. The succulent mesocarp was put in juice extractor (Silvercrest®, IAN: 96115-S/N, Hoyer Handel GMBH, 22761 Hamburg, Germany) and the juice was separated from the fibre. Five millilitre (ml) each of the juice was put in 5 crucibles. The five crucibles were put in hot air oven at 40°C. Dried yellowish substances obtained after drying were weighed. The results obtained shows that the concentration of pineapple in one millilitre of juice is 140 mg. The remaining pineapple juices were stored in a freezer at -4°C until needed.

Acute toxicity study

This was done using Lorke's up and down method [25]. The study was carried out in two phases. In phase 1, four groups; A, B, C, D of 3 rats each were used. Group A was given 2 ml/kg of distilled water as control, then, 10 mg/kg, 100 mg/kg, and 1000 mg/kg body weight of pineapple juice, respectively, in groups B, C and D. All treatments were done orally.

In the second phase, three groups; E, F, G of 3 rats per group also were given 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg body weight of pineapple juice respectively. The rats were observed for 72 hours post administration and for another 7 days to observe any delayed toxicity.

Experimental design

Sixteen male rats of 14 weeks of age divided into 4 groups of 4 rats per group were used for the study. The rats were procured from the Departmental animal house of the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike. The rats were kept in 16 standard cages and fed Topfeed (20% crude protein) chick marsh ad libitum and had access to clean water. They were acclimatized for one week and weighed between 156.86 g to 271.97 g with an average weight of 204.44 g. Treatments were 2 ml/kg body weight of distilled water for control, 250 mg/kg, 500 mg/kg, and 1000 mg/kg body weight of pineapple juice for 56 days. On day 57, a female rat whose oestrus has been induced using 800 mg/kg peanut extract, was introduced per male to observe the libido of the male [26]. Then blood was collected through the ocular vein of the median cantus of the eye for haematology in heparinized bottles and serology in test tubes. Thereafter, the rats were sacrificed after cervical dislocation to eliminate pain.

Concurrently, after evisceration, some of the testes were exteriorized and the caudal epididymis lacerated. Spermatozoa concentration were scoped into bijou bottles containing 1 ml of Phosphate Buffered Saline (PBS) to keep the sperm alive. The contents of the bottles were gently mixed to form caudal epididymis sperm suspension. Spermatozoa analysis were done using standard methods described by El-Sherbiny (1987), Chibundu, 2013 and adopted by Orieke 2019 [27-29].The consistency, cell viability, concentration, and motility of the spermatozoa were evaluated.

Sperm consistency was done through macroscopic observation. Highest score of 4 was allotted to very thick sperm with milky colour while score 1 was allotted to white and watery sperm. The intermediaries include 2 (milky white and slightly thick sperm) and 3 (creamy white and thick sperm).

Sperm motility was done using the method described by El-Sherbiny [27]. A drop of the sperm suspension was smeared on a pre-warmed glass slide and viewed under 40X and 100X magnification of a light microscope. Scoring was done subjectively in percentage.

Assessment of viable spermatozoa was done by putting a drop of the sperm suspension in a glass slide, stained with Eosin-Nigrosin, dried for 30 seconds, fixed with ethanol and viewed under light microscope. Viable sperm do not pick up the stain. A total of 300 sperm were counted and the results were reported using this formula:

$$\text{Percentage viability} = \frac{(\text{Number of live sperm cells} \times 100)}{\text{Total Number of sperm cells}} \quad [27]$$

Sperm concentration was done using a method described by Peter, 2002 [30]. A dilution of 1: 200 was made of one millilitre sperm suspension in phosphate buffered saline. A drop of 10% formalin was added to immobilise the sperm cells. Then a drop of the sperm solution was put on a haemocytometer using a pipette, covered with a cover slips and viewed under 40X magnification in a light microscope.

Sperm concentration per ml=Number of cells counted × DF × 0.04 × 10^6.

Note: DF=Dilution Factor.

Hormone profile of male rats treated with *Ananas Comosus* (Ac) juice

The blood for serology was kept for 24 hours post collection to allow the natural separation of serum through the coagulation of the blood cells. But the one that did not separate well were centrifuged at 2500 rotations per minute and the serum collected with a pipette into clean non-heparinized bottles. The sera were divided into two; a part was used for biochemical analysis while the remaining part was sent to the laboratory in Federal Medical Centre (FMC), Umuahia for analysis of testosterone and estradiol using Elisa kits specific for each hormone. Thereafter, a graph of known quantities of these hormones was used to extrapolate the levels of the individual hormones found in each rat,

Serum biochemistry of male rats treated with *Ananas Comosus* (Ac) juice

Lipid profile (Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C), Triglyceride (TG), and High Density Lipoprotein Cholesterol (HDL-C), analysis of male albino rats treated with *Ananas Comosus* juice were determined spectrophotometric using Randox test kits (Randox Laboratories Ltd, 55 Diamond Road Crumlin County Antrim, BT29 4QY, United Kingdom), and the tests were done according to manufacturer's instruction.

Liver function parameters such as; Total Protein (TP), Aspartate Aminotransferase (AST), and Alanine AminoTransferase (ALT) were also done using Randox test kits instruction manual but Alkaline Phosphatase (ALP) was done using colorimeter optimized standard method for in vitro determination of ALP according to the Deutsche Gesellschaft fur klinische chemie. Furthermore, kidney function of male albino rats treated with *Ananas Comosus* was done using Randox and TECO test kits. Analysis of urea was done using enzymatic kinetic method for the quantitative in vitro determination of urea in serum, creatinine and total bilirubin using colorimetric method. However, sodium, potassium, bicarbonate, and chlorine content of serum were analyzed using TECO test kits, made in USA. The tests were done using colorimeter according to manufacturer's guideline.

Haematology of male rats treated with *Ananas Comosus* (Ac) juice

Haematological parameters which include: Haemoglobin (Hb) concentration, packed cell volume, Red Blood Cell (RBC) and White Blood Cell (WBC) count, differential WBC count, erythrocyte indices which include mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) were analysed using standard methods described by Nwankudu [26].

Histopathology of the brain and testes of male albino rats treated with *Ananas Comosus* juice

Heads and testes were collected from males treated with graded doses of pineapple juice and 2 ml/kg body weight of distilled water as control. The organs were fixed in Bouin's fluid for 48 hours. The skull was cracked and the brain separated. The position of the hypothalamus was identified. Thereafter, the organs were fixed in 70% alcohol and cleared with Xylene. The tissues were embedded in paraffin wax, sectioned with microtome, stained with Hematoxylin and Eosin (H&E), covered with cover slips, and mounted in a Canadaa balsam. Examination of the slides were under light microscope (40X, 100X, 400X and 1000X) magnification. Photomicrographs were taken through capture using a research microscope DN-10, DC 7.5 V, Made in China.

Statistical analysis

The data collected were analyzed using Statistical Package for Social Sciences (SPSS) version 20. Analysis of variance was done using Turkey HSD and values at 95% confidence interval ($P \leq 0.05$) were adjudged to showcase the positive effect of the treatment.

All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) FV-U -IACUC-2020-0262 of University of Nigeria, Nsukka and the procedures approved were in compliance with guide for the care and use of laboratory animals, (eighth edition) which upholds the recognition, and alleviation of pain in laboratory animals according to the Institute for Laboratory Animal Research publication, (2009).
Moreover, we ARRIVE guidelines for *in vivo* experimental animal research and reporting, which stresses that the methods used in research and reporting should be easy to replicate, was observed.

Results

Acute toxicity test results of albino rats treated with *Ananas Comosus* juice

After 72 hours and 7 days post administration of the *Ananas Comosus* juice at both high and low doses, neither death nor distress which included but not restricted to: Hyperactivity, abnormal gait, spasms in rear legs, hyper-reactivity, salivation, sleepiness, lethargy, diarrhea, or death was recorded even at the highest dose of 5000 mg/kg body weight (Table 1).

Table 1 shows 100% safety of *Ananas Comosus* juice consumption using Lorke's up and down method.

Table 1. Result of acute toxicity test in *Ananas Comosus* juice treated rats.

Groups	Dose mg/kg	Number of deaths	Percentage mortality
A	2	0/3	0
B	10	0/3	0
C	100	0/3	0
D	1000	0/3	0
E	1600	0/3	0
F	2900	0/3	0
G	5000	0/3	0

Result of steroid hormone profile of male rats treated with *Ananas Comosus* (Ac) juice

Testosterone profile of male rats treated with *Ananas Comosus* juice for 56 days showed a significant ($P \leq 0.05$) decrease in serum testosterone

in dose-dependent manner when compared to distilled water treated males which served as control. Conversely, serum estradiol in Ac treated rats significantly increased in dose dependent manner (Table 2).

Table 2. Steroid hormone profile of male rats treated with *Ananas Comosus* juice.

Hormones	Treatments			
	2 ml/kg DW	250 mg/kg Ac	500 mg/kg Ac	1000 mg/kg Ac
Testosterone (ng/ml)	7.90 ± 0.62	4.40 ± 0.62*	1.60 ± 0.62*	1.40 ± 0.62*
Estradiol (pg/ml)	66.65 ± 2.86	67.70 ± 2.86	74.95 ± 2.86*	78.95 ± 2.86*

Superscripts indicate significant (P ≤ 0.05) difference in a row.
DW=Distilled water which served as control, Ac=*Ananas Comosus* juice.

Table 2 shows that serum testosterone in Ac juice treated male rats significantly decreased in dose dependent manner while serum estradiol increased significantly also in dose dependent manner. Increased estradiol in blood is a sign of feminization which may lead to decreased libido and infertility in male.

Results of libido in male rats treated with *Ananas Comosus* (Ac) juice

Libido in the rats was determined by physical observation. This was done by observing the male rat's reaction and sexual behaviour or response when a female rat in oestrus was introduced into the cage containing the male. Such sexual behavioural reactions include; general grooming, sniffing, mounting, and thrusting of female rats. Based on these reactions, scores were allocated following the scoring pattern described by (Chibundu, 2013) but with little modification (Table 3a).

Table 3a. Libido grading for experimental rats.

Sexual Behaviour	Score	Grading
Grooms, Sniffs and attempt to mount	5	Very high libido
Grooms, sniffs but no attempt to mount	4	High libido
Sniffs only	3	Moderate
Grooms only	2	Low
Does not pay attention to the female rat	1	Poor libido

Source: Chibundu (2013)

The results obtained showed that male albino rats treated with pineapple juice for 56 days had low libido. Libido waned in dose-dependent manner (Table 3b)

Table 3b. Libido of male rats treated with *Ananas Comosus* (Ac) juice for 56 days.

Treatments	2 ml/kg (DW)	250 mg/kg (Ac)	500 mg/kg (Ac)	1000 mg/kg (Ac)
Score	5	3	2	1
Reaction time	5 seconds	10 seconds	20 seconds	>20 seconds
Grade	Very high libido	Moderate libido	Poor libido	Very poor libido

Table 3b shows libido pattern observed in Ac juice treated male rats and control males. The scoring is reflected in column. The scoring system is according to Chibundu, 2013. However, reaction time was considered necessary and added. Also reaction time above 20 seconds was depicted as very poor libido. DW=Distilled water as control.

Result of spermatozoa analysis of male rats treated with *Ananas Comosus* (Ac) juice

The results of spermatozoa analysis of male albino rats treated with *Ananas Comosus* juice for 56 days indicated that Ac treated male rats had significantly (P ≤ 0.05) lower spermatozoa consistency of 3 while controls had 4. Motilities of the spermatozoa were significantly reduced in dose-dependent manner than controls. Sperm concentration (sperm count) was significantly lower in all Ac treatment groups at 95% confidence interval in dose-dependent manner. Normal sperm ratio was also significantly (P ≤ 0.05) lower in all the Ac treatment groups in dose-dependent manner while the ratios of abnormal sperms were significantly higher in all the Ac treatment groups in dose-dependent manner (Table 4).

Table 4. Spermatozoa analysis of male rats treated with *Ananas Comosus* (Ac) juice.

Treatment Group	Spermatozoa Consistency	Sperm Motility	Live Sperm Proportion	Sperm Count (×106/CE)	Normal Spermatozoa	Abnormal Spermatozoa
DW. Control	4.00 ± 0.00	74.66 ± 0.88	81.73 ± 1.79	113.33 ± 1.76	94.17 ± 1.73	5.82 ± 1.73
Ac. 250 mg/kg	3.33 ± 0.33^{b}	62.00 ± 1.15^{b}	71.08 ± 0.58^{b}	88.33 ± 1.85^{b}	84.28 ± 1.44^{b}	15.71 ± 1.44^{a}
Ac. 500 mg/kg	3.00 ± 0.00^{b}	59.66 ± 1.76^{bc}	68.42 ± 1.29^{bc}	82.66 ± 1.76^{bc}	83.16 ± 1.31^{b}	16.84 ± 1.31^{a}
Ac.1000 mg/kg	3.00 ± 0.00^{b}	53.00 ± 3.78^{c}	57.33 ± 1.45^{c}	70.66 ± 1.76^{c}	76.54 ± 4.09^{b}	23.45 ± 4.09^{a}

Superscripts indicate significant (P ≤ 0.05) difference in columns. Sperm consistency score; 4=very thick and 3=slightly thick.

InTable 4, superscript 'a' indicate increase, 'b' shows decrease, 'c' shows further decrease. DW=Distilled water. Sperm analysis is used to show fecundity of the male.This shows that there was decrease in fecundity of the male treated with pineapple juice as the dose increased which shows that high dose and/or prolonged *Ananas Comosus* (pineapple) juice treatment might lead to infertility in male.

Gonadosomatic index of male albino rats treated with *Ananas Comosus* (Ac) juice

The result of the gonadosomatic index presented showed that the size of the testes relative to the animal live-weights were significantly (P ≤ 0.05) decreased in all the Ac juice treated groups compared to the control group. The male rats in the control group recorded higher mean relative testicular weight of 1.34 ± 0.05% compared to a mean of 0.95 ± 0.00%, 1.00 ± 0.01% and 0.80 ± 0.00% in 250 mg/kg, 500 mg/kg and 1000 mg/kg treatments respectively. Moreover, the relative epididymis weights were significantly (P ≤ 0.05) reduced in Ac treatment groups (Table 5). Table 5 Shows that males treated with 1000mg/kg Ac significantly increased in weight. This could be due to anabolic effect of oestrogen whose profile in 1000 mg/kg treated rats significantly increased as showcased in Table 2. Relative testicular and epididymis weight were greatly reduced in Ac treated groups when compare to control.

Table 5. Gonadosomatic index of male albino rats treated with *Ananas Comosus* (Ac) juice.

Treatments	Live weight	Relative testicular weight	Relative epididymis weight
DW 2ml/kg (control)	228.66 ± 14.51	1.34 ± 0.05	
250 mg/kg Ac	257.00 ± 2.30	0.95 ± 0.00*	**0.10 ± 0.01***
500 mg/kg Ac	212.00 ± 7.54	1.00 ± 0.01*	0.10 ± 0.02*
1000 mg/kg Ac	333.33 ± 19.78*	0.80 ± 0.00*	0.11 ± 0.02*

Superscripts indicate significant (P ≤ 0.05) difference in a column.
DW=Distilled water

Relative organ weight of male rats treated with *Ananas Comosus* (Ac) juice

Male rats treated with 1000 mg/kg Ac significantly (P ≤ 0.05) weighed the heaviest from 271.97, which was the highest weight of the rats at the commencement of the experiment to 333.33 g ± 14.51g. Others weighed within the range of weight (156.86-271.97) which was the range at the commencement of the experiment. However, there were notable mean growth changes in all treatment groups. The results of relative organ weight showed that Ac juice has no significant effect on the size of the spleen, lungs, and heart relative to the animal body weight, while the size of the liver and kidney relative to the body weight were significantly (P ≤ 0.05) decreased with the highest dose of 1000 mg/kg body weight of Ac treatment compared to 2 ml/kg DW which served as control (Table 6).

Table 6. Relative organ weight of male rats treated with *Ananas Comosus* (Ac) juice.

Treatments	Live weight	Liver	Kidney	Spleen	Lungs	Heart
2ml/kg DW	228.66 ± 14.51	2.59 ± 0.11	0.72 ± 0.03	0.31 ± 0.02	0.68 ± 0.05	0.46 ± 0.03
250 mg/kg Ac	257.00 ± 2.30	2.57 ± 0.01	0.67 ± 0.06	0.26 ± 0.01	0.55 ± 0.00	0.32 ± 0.00
500 mg/kg Ac	212.00 ± 7.54	2.66 ± 0.03	0.52 ± 0.00	0.21 ± 0.07	0.60 ± 0.25	0.36 ± 0.07
1000 mg/kg Ac	333.33 ± 19.78	2.03 ± 0.03	0.39 ± 0.11	0.32 ± 0.08	0.42 ± 0.10	0.27 ± 0.12
Superscripts indicate significant (P ≤ 0.05) difference in a column						

Table 6 shows that the male rats treated with 1000 mg/kg Ac weighed the heaviest, yet their liver and kidney weighed the least which indicates that Ac at very high dose causes reduction in weight of liver and kidney in-vivo. DW=Distilled water (control).

Result of serum biochemistry of male rats treated with *Ananas Comosus* juice

Serum biochemical profile of male albino rats treated with *Ananas Comosus* (Ac) juice showed significant (P ≤ 0.05) increase in Total Cholesterol (TC), Triglyceride (TG), and Low Density Lipoprotein (LDL) in treatments 250 mg/kg and 500 mg/kg body weight Ac when compared to Distilled water (DW) treatment which served as control, but all were within the normal ranges for each parameter. Treatment with low dose of Ac (250 mg/kg) led to very high secretion of Cholesterol and triglycerides, medium dose (500 mg/kg) led to mild increase while high dose (1000 mg/kg) led to no significant increase in TC and TG (Table 7a).

Table 7a. Lipid profile of male rats treated with *Ananas Comosus* juice

Treatments	TC Normal : 200 mg/dl	HDL-C. normal : 45 (mg/dl)	TG. Normal:< 150 (mg/dl)	LDL-C Normal : 50-100 (mg/dl)	VLDL. Normal : 2-30 (mg/dl)
250 mg/kg Ac	118.66 ± 4.30a	43.50 ± 4.20	91.60 ± 1,30a	56.77 ± 0.35[a]	18.33 ± 0.25
500 mg/kg Ac	106.23 ± 3.30b	39.7 ± 2.35	79.60 ± 3.20b	50.60 ± 0.35[b]	15.93 ± 0.65
1000 mg/kg Ac	99.73 ± 4.55	35.80 ± 6.30	72.50 ± 2.70	46.10 ± 6.96	14.50 ± 0.50
2 ml/kg DW	92.23 ± 3.91	37.9 ± 2.20	69.50 ± 0.60	40.10 ± 2.97	13.90 ± 0.10
Superscript indicates significant (P≤ 0.05) difference in a column					

Strength of significance is in ascending order; a is stronger than b. Cholesterol measurement is used in the diagnosis and treatment of lipid lipoprotein metabolism disorders. Lipids serve as hormones or hormone precursors. Cholesterol is precursor for steroid hormone synthesis and was enhanced though not above expected value of 200 mg/dl by Ac treatment. This explains why spermatogenesis still took place despite low libido observed in treated males.

Liver function of male albino rats treated with Ac showed significant (P ≤ 0.05) decrease in serum availability of Aspartate Aminotransferase (AST) in all the treatment groups at the same strength when compare to control (DW). Moreover, there was significant decrease in serum availability of Alkaline Phosphatase (ALP) (35.47 ± 2.11) and serum total protein (TP) (4.87 ± 0.12) in treatment 250 mg/kg, but significant (P ≤ 0.05) increase in serum ALP (101.27 ± 3.33) and TP (4.17 ± 0.25) in treatment 1000 mg/kg body weight of Ac when compared to control ALP (68.50 ± 1.65) and TP (6.60 ± 0.20), but all within the normal serum ranges of ALP (Normal: 60 (iu/l) -170 (iu/l) at 25°C) and TP (Normal: 6.4(g/dl)-8.3 (g/dl)). Furthermore, there was significant (P ≤ 0.05) decrease in serum availability of Alanine Aminotransferase (ALT) enzyme in all the treatment groups, and, there was significant decrease in serum globulin in treatments 250 mg/kg and 500 mg/kg body weight of Ac but, no significant difference in serum total bilirubin due to Ac treatments (Table 7b).

Table 7b. Liver function of male rats treated with *Ananas Comosus* (Ac) juice

Treatments	AST. Normal: 18×10 (iu/l)	ALP Normal: 60-170 (iu/l) at 25°C	ALT. normal: 22×10 (iu/l)	TP. Normal: 6.4-8.3 (g/dl)	ALB. Normal: 3.8-4.4 (g/dl)	Globulin. Normal: 2.0-3.5 (g/dl)	T. Bilirubin. Normal: 1.0 (g/dl)
250 mg/kg Ac	[a]88.97 ± 2.63	[a]35.47 ± 2.11	[a]70.40 ± 2.95	[a]4.87 ± 0.12	[b]2.77 ± 0.15	[a]2.10 ± 0.10	0.30 ± 0.00
500 mg/kg Ac	[a]122.53 ± 3.86	73.70 ± 2.71	[a]46.47 ± 3.91	[c]6.00 ± 0.20	3.23 ± 0.21	[b]2.70 ± 0.40	0.20 ± 0.00
1000 mg/kg Ac	[a]146.43 ± 4.00	[a]101.27 ± 3.33	[b]105.53 ± 2.05	[b]7.27 ± 0.25	[a]4.17 ± 0.25	3.10 ± 0.00	0.30 ± 0.00
2 ml/kg DW	169.70 ± 1.71	68.50 ± 1.65	120.40 ± 1.97	6.60 ± 0.20	3.33 ± 0.25	3.23 ± 0.57	0.30 ± 0.00
Superscript indicate significant (P≤ 0.05) difference in a column							

Strength of significance is in ascending order; a is stronger than b. Aspartate Amino Transferase (AST) is an enzyme that catalyses amino acids and alpha-oxoacids by transfer of amino group. Elevated AST signal myocardial infaction, hepatic disease, muscular dystrophy and organ damage. However, *Anannas Comosus* treatment significantly reduced AST at the same strength across the treatments when compared to control which indicates that *Ananas Comosus* does not cause liver damage. Moreover, ALT was significantly lower in all the treatment groups which further showed that *Ananas Comosus* did not cause any liver damage. Increased level of ALT in blood is an indication of liver damage.

Finally, kidney function of male albino rats treated with *Ananas Comosus* (Ac) juice showed significant (P ≤ 0.05) increase in serum urea in treatment 250 mg/kg, but significant decrease in serum urea in treatment 500 mg/kg body weight of Ac when compare to control. Furthermore, serum creatinine, serum potassium and serum bicarbonate significantly varied in dose dependent manner in reverse proportion between 250 mg/kg and 500 mg/kg Ac treatment when compared to control. However, there was no significant difference among Ac treatment groups or between Ac treated and control in serum sodium level, but serum chloride level varied significantly (P ≤ 0.05) between 250 mg/kg body weight Ac treatment and control (Table 7c).

Table 7c. Kidney function in *Ananas Comosus* (Ac) treated male rats.

Treatments	Urea Normal: 10-50mg/dl	Creatinine Normal: 0.6-1.1mg/dl	Sodium. Normal: 135-155 (meq/l)	Potassium. Normal: 3.4-5.3 (meq/l)	Bicarbonate. Normal: 23-33 (meq/l)	Chlorine. Normal: 98-140 (meq/l)
250mg/kg Ac	45.87± 2.37[a]	0.77±0.06[a]	135.50± 0.89	3.73±0.06[a]	33.63±0.85[a]	98.27± 0.86*
500mg/kg Ac	22.20± 1.28[b]	0.63±0.06[a]	134.23± 0.35	3.63±0.06[b]	27.93±2.57[b]	86.37± 3.80
1000mg/kg Ac	27.53 ± 3.05	0.30±0.00	133.57± 0.59	3.53±0.06	24.13±2.30	82.83± 4.04
2ml/kg DW	33.53± 3.81	0.30±0.00	134.80± 0.70	3.40±0.10	22.60±0.10	87.47± 4.58
Superscript indicates significant (P ≤ 0.05) difference in a column						

Creatinine is used to measure kidney function. The rate of creatinine production is constant. The research showed a significant increase in serum creatinine when compare to control in 250 mg/kg and 500 mg/kg Ac treatment which suggests that Ac treatment might reduce kidney function due to loss in kidney mass but the increased serum availability of creatinine is within the expected or normal range of creatinine in blood. Therefore, Ac treatment does not affect kidney function.

Result of haematology of male rats treated with *Ananas Comosus* (Ac) juice

Blood Haemoglobin (Hb) concentration, Packed Cell Volume (PCV), Red Blood Cell (RBC), and White Blood Cell (WBC) counts in Ac juice treatment were not significantly (P ≤ 0.05) different from Distilled Water (DW) treatment which served as control. Moreover, erythrocyte index; Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) in Ac treatments were not significantly different from control. Further differentiation of the white blood cell count revealed that there were no significant (P ≤ 0.05) differences between control treatment and all the Ac treatments in terms of lymphocytes, neutrophils, monocytes and eosinophils (Tables 8a and 8b).

Table 8a. Haematology of male rats treated with *Ananas Comosus* (Ac) juice

Treatments	Blood parameters						
	Hb (g/dl)	PCV (%)	RBC ($\times10^6$ m^3)	WBC ($\times10^3$ m^3)	MCV	MCH	MCHC
2 ml/kg DW	16.00 ± 0.72	37.67 ± 3.48	6.05 ± 0.58	9.93 ± 1.04	62.34 ± 0.49	26.61 ± 1.44	42.65 ± 2.09
250 mg/kg Ac	17.47 ± 0.72	44.00 ± 3.48	6.97 ± 0.58	6.73 ± 1.04	63.13 ± 0.49	25.10 ± 1.44	39.75 ± 2.09
500 mg/kg Ac	16.67 ± 0.72	40.33 ± 3.48	6.35 ± 0.58	8.02 ± 1.04	63.48 ± 0.49	26.37 ± 1.44	41.52 ± 2.09
1000 mg/kg Ac	16.27 ± 0.72	38.00 ± 3.48	6.06 ± 0.58	6.88 ± 1.04	62.70 ± 0.49	27.04 ± 1.44	43.12 ± 2.09

There was no significant difference between *Ananas Comosus* treated groups and Distilled Water (DW) treated group which served as control in terms of the blood parameters and the erythrocyte indices of the blood which shows that Ac treatment at any dose does not affect the blood.

Table 8b. Differential white blood cell count in Ac treated male rats.

Treatments	Differential WBC			
	Lymphocytes	Neutrophils	Monocytes	Eosinophil
2 ml/kg DW	58.33 ± 1.53	36.67 ± 1.93	4.33 ± 0.91	0.67 ± 0.94
250 mg/kg Ac	62.33 ± 1.53	31.67 ± 1.93	4.67 ± 0.91	1.33 ± 0.94
500 mg/kg Ac	62.00 ± 1.53	33.33 ± 1.93	3.33 ± 0.91	1.33 ± 0.94
1000 mg/kg Ac	59.67 ± 1.53	32.33 ± 1.93	5.00 ± 0.91	2.67 ± 0.94

Table 8b shows that there was no significant difference in the columns showing lymphocytes, neutrophil, monocytes and eosinophil in all the *Ananas Comosus* treatments when compare to Distilled water (DW) which served as control treatment. This indicates that pineapple juice is well tolerated by the body and does not affect immune status of the male.

Results of histopathology of testes and hypothalamus of male rats treated with Ac juice:

There were fewer spermatogonia in the *Ananas Comosus* (Ac) juice treated rats. Some round spermatids were atrophied. But there were elongated spermatids and spermatozoa in the lumen of the seminiferous tubules ready for evacuation into the epididymis when compared to the control which had similar spermatids and spermatozoa ready for evacuation into the epididymis. On the other hand, the hypothalamus of Ac juice treated rats showed necrotic and normal acidophil, but there were more necrotic basophils than normal ones. But the control had even spread of acidophil and basophil (Figures 3A, 3B)

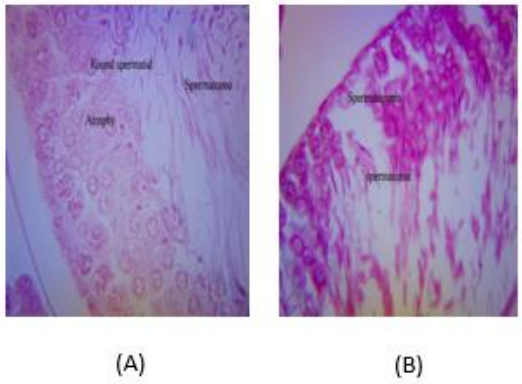

Figure 3. Testis × 1000 (control) and Testis × 1000 Ac treated rats. (**A**) (control) shows many spermatozoa ready, while (**B**) shows few spermatozoa ready for evacuation into the epididymis.

However, there were atrophy among round spermatids and spermatocytes in Ac treated male rats. The aggregation of spermatozoa in the lumen of the seminiferous tubules and spermatozoa could be because the rats were teased with female in oestrus before they were sacrificed. Ac= *Ananas Comosus* juice (Figures 4A and 4B).

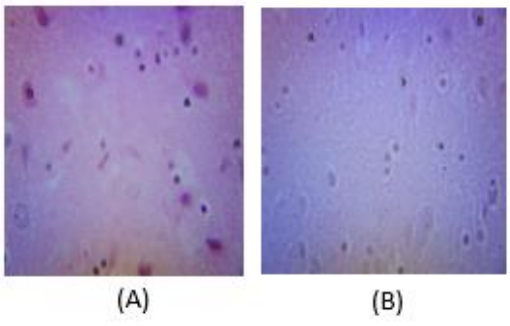

Figure 4. Hypothalamus ×1000 (control) and Hypothalamus × 1000 Ac treated rats **(A)** shows almost even spread of acidophil secreting prolactin and basophil secreting gonadotropin releasing hormone. Almost even spread of A and B is an indication of normal. (**B**) showed normal

acidophil and necrotic acidophil. Also there, very few basophil and many necrotic basophil.

Necrosis of the acidophil and basophil is an indication of reduced reproductive capacity. Ac=*Ananas Comosus* juice.

Discussion

Ananas Comosus (Pineapple) juice is safe even at the highest dose of 5000 mg/kg. However, the steroid hormone profile of *Ananas Comosus* (Ac) juice treated male rats revealed that Ac significantly ($P \leq 0.05$) reduced serum testosterone in dose-dependent manner but significantly increased serum estradiol in males also in dose-dependent manner. This could be as a result of aromatase found in pineapple and other fruits [24].

Aromatase converts testosterone to estradiol in a non-reversible reaction. Reduced amount of testosterone in the seminiferous tubules could have led to decreased spermatogenic activity which led to a significant decrease in spermatozoa concentration cumulating in low libido observed in Ac treated rats. Furthermore, reduced spermatogenic activity was also buttressed by the fact that the gonads and epididymis relative to body weight were significantly reduced in size. This agrees with Ramalan (2021) [31], who positioned that there is a positive relationship between increased testicular weight and increased fertility.

Evaluation of relative organ weight revealed that treatment with pineapple juice in very high doses for 56 days led to a significant ($P \leq 0.05$) decrease in the weight of the liver. Liver is involved in the digestion of carbohydrate and protein. Excess carbohydrate is stored as glycogen in the liver and excess protein is stored as part of the liver mass. Pineapple contains very little protein which might have been completely utilized by the rat and the demand made to the liver to release the already stored protein to supplement for protein insufficiency. This might have led to a decrease in liver mass relative to body weight. However, Cattley and Cullen, 2013, asserted that a decrease in liver weight reflects a loss in function mass associated with atrophy or hepatocellular injury [32]. However, biochemical analysis of liver function in *Ananas Comosus* treated male rats revealed that liver function were not affected by Ac treatment since Aspartate Aminotransferase (AST) were significantly reduced due to Ac treatment Elevated AST signals liver disease. Moreover, alanine aminotransferase was significantly ($P \leq 0.05$) lower in all the Ac treatment groups which further showed that *Ananas Comosus* did not cause any liver damage. According to Claus 2021 [33], increased ALT in blood is an indicator for non-alcoholic fatty liver disease (macrosteatosis). But Ac reduced serum ALT which indicates that Ac may be of therapeutic importance in the treatment of fatty liver disease,

Furthermore, pineapple juice contains very little quantity of protein about 0.54 g per 100 g fresh weight. In addition, pineapple juice contains rich quantities of bromelain; an enzyme that breaks down protein into smaller units. As the rats were not fed an extra protein diet, it is possible that bromelain quick digested the little protein available in the feed creating protein insufficiency, which led to the decrease in kidney mass in the male rats treated with very high doses of pineapple juice. This agrees with Fotheringham (2021) who affirmed that a decrease in protein in the diet leads to a decrease in relative kidney weight and function [34]. This assertion is substantiated by the fact that Ac treatment led to increase in serum creatinine when compare to control in low and medium dose of Ac treatment. Creatinine is used to measure kidney function. The rate of creatinine production is constant; therefore, an elevation of plasma creatinine is an indication of kidney impairment due to loss in kidney mass. However, loss in kidney mass was only observed in 1000 mg/kg body weight of Ac treatment which has similar creatinine value to control (Tables 6 and 7c). Moreover, serum creatinine level was within normal range among Ac treatment groups and control. Therefore, Ac treatment does not lead to kidney malfunction in male.

Furthermore, *Ananas Comosus* juice treatment did not affect blood parameters or erythrocyte indices of treated male rats. Moreover, differential white blood cell count revealed that Ac juice treatment led to a mean increase in lymphocyte secretion but they were not significantly different from control.

Histopathology of the testes showed fewer spermatogonia, spermatocytes, and spermatids in the seminiferous tubule of Ac treated male rats. Some of the cells of the seminiferous tubule were atrophied. However, spermatogenic activities were not halted since elongated spermatids and spermatozoa were observed in the lumen of the seminiferous tubules ready for evacuation into the epididymis. The same was observed in distilled water (control) treated male rats. Mass movement of spermatids and spermatozoa towards the epididymis could be because the male rats were teased with the female in oestrus just before they were sacrificed.

Hypothalamus of *Ananas Comosus* juice treated male rats showed few acidophil and very few basophil nuclei. Moreover, there was more necrotic basophil than normal. This shows reduced reproductive capacity. Basophil secret gonadotropin releasing hormone and Thyrotropin releasing hormone. Gonadotropin Releasing Hormone (GnRH) passes through the portal system to the anterior pituitary to induce the secretion of two important peptide hormones necessary for reproduction; Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) which passes through the general circulation to stimulate the synthesis of steroid hormones from the gonads. When there is a reduction in GnRH, the anterior pituitary will then be minimally stimulated to secrete FSH and LH, which leads to reduced synthesis of testosterone in the gonads leading to decreased spermatogenesis cumulating in reduced libido which may result to infertility. However Ac treatment does not lead to infertility, rather it led to reduced libido [35].

Conclusion

Ananas Comosus juice treatment in high and low doses led to significantly decreased testosterone synthesis, reduction in libido, reduction in spermatozoa concentration, and reduction in relative size of the testes and epididymis. All these are signs of infertility, but the histology of the testes showed spermatid and spermatozoa in the lumen of the seminiferous tubules, which is an indication of spermatogenic activity. Therefore, treatment with *Ananas Comosus* juice may not lead to contraception rather it is suggested that increased use of *Ananas Comosus* juice should be compensated with increased protein content of diet to avoid loss in kidney mass which may lead to reduced kidney function. Predominantly protein diets lead to fermentation in the colon and rectum which leads to an increased risk of colorectal neoplasm. But the predominant protein diet in conjunction with pineapple juice which is rich in protease enzymes leads to quick digestion of protein and absorption is done without involvement of colon and rectum. Therefore, *Ananas Comosus* (pineapple) or its products can be an ameliorative solution for colorectal cancer.

Finally, Ac treatment led to increased bicarbonate in blood. Bicarbonate is used by the body to buffer differences in acidity *in vivo*. *Ananas Comosus* treatment led to significant increase in bicarbonate especially between 250 mg/kg body weight of Ac treatment and control. This shows that *Ananas Comosus* juice has the capacity to ameliorate acidosis or alkalosis *in vivo*.

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Research approval

The research was approved by institutional animal care; FV-U - IACUC-2020-0262 of University of Nigeria, Nsukka and the reporting of the research was thorough and conforms to we ARRIVE guidelines for *in vivo* experimental animal reporting (PLOS BIO. B (6), E1000412 2010).

Availability of data and materials

Data is available upon request from the corresponding author.

Conflict of interest

All authors certify that they have no affiliation with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or material discussed in this manuscript.

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Author contribution

ONN designed the research, supervised the research, analysed part of the data, collated and wrote the manuscript, and is the corresponding author, while DCI saw to the day-to-day experimentation, took care of the animals, collected the data and analysed part of the data.

References

1. Abbe, Carmen R., et al. "Focus: Sex & Reproduction: Male Contraception." *The Yale Journal of Biology and Medicine* 93.4 (2020): 603..
2. Acconcia, F., and Maria M.. "Steroid hormones: synthesis, secretion, and transport." *Principles of endocrinology and hormone action* (2018): 43-72..
3. Kaufman, J., et al. Aging and male reproductive system. *Endocrine Reviews*. 40.4(2019). 906-972.
4. Hess, Rex A. "Estrogen in the adult male reproductive tract: a review." *Reprod Biol Endocrinol.* (2003): 1-14.
5. Schulster, M., et al.. "The role of estradiol in male reproductive function." *Asian j androl.* (2016): 435.
6. Bartholomew, Duane P., et al.. The pineapple: botany, production and uses. *CABI publishing*, (2003).
7. Almaktsur, M. A., and Elfianis, R. "Morphology and fruit quality characters of pineapple (*Ananas Comosus* L. Merr) cv. Queen on three sites planting: freshwater peat, brackish peat and alluvial soil." *IOP Conf. Ser.: Earth Environ. Sci.* 391.1. (2019). IOP Publ.
8. Pandhare, G. R., et al. "Effect of natural preservatives on pineapple juice." *J Pharmacogn Phytochem.* (2018): 746-750.
9. Li, T., et al. "Major polyphenolics in pineapple peels and their antioxidant interactions." *Int j food prop.* (2014): 1805-1817.
10. Boondaeng, A., et al. "Fermentation condition and quality evaluation of pineapple fruit wine." *Fermentation* (2022): 11.
11. Clutton, A. The vinegar cupboard: Recipes and history of an everyday ingredient. *Bloomsbur*y (2019): 57.
12. d'Eeckenbrugge, G. Coppens, and Freddy Leal. "Morphology, anatomy and taxonomy." *pineapple: bot. prod. uses,* Wallingford UK: CABI Publ, (2003). 13-32.
13. d'Eeckenbrugge, Geo Coppens, et al. "Ananas." *Wild crop relat.: genom. breed. resour.: trop. subtrop. fruits* (2011): 21-41.
14. Bartholomew, D.P., et al. "The Pineapple: Botany, Production, and Uses." CABI, ISBN: 9780851999791, 2022, 23.
15. Zatul Iffah Mohd, A., et al. "Bromelain: an overview of industrial application and purification strategies." *Appl. microbiol. biotechnol*. (2014): 7283-7297.
16. Rajendra, P., et al. "Properties and therapeutic application of bromelain: a review." *Biotechnol. res. int.* (2012).
17. Zhou, Z., et al. "Fruit bromelain ameliorates rat constipation induced by loperamide." RSC advances 7.72 (2017): 45252-45259.
18. Seenak, P., et al. "Pineapple consumption reduced cardiac oxidative stress and inflammation in high cholesterol diet-fed rats." *Nutr. Metab.* 18.1 (2021): 1-10.
19. de Ancos, Begoña, Concepción Sánchez-Moreno, and Gustavo Adolfo González-Aguilar. "Pineapple composition and nutrition." Handb. *pineapple technol.: Prod. postharvest sci. process. nutr.* (2017): 221-239.
20. Ivanova, N. N., et al. "Apple juice nutritional profile." *Voprosy pitaniia* 86.4 (2017): 125-136.
21. Moini, Jahangir, et al. "Epidemiology of Brain and Spinal Tumors." *Academic Press,* (2021).
22. Zemlicka, L., et al. "Analysis of natural aroma and flavor of MD2 pineapple variety (*Ananas Comosus* [L.] Merr.)." *Acta Chimica Slovaca* 6.1 (2013): 123-128.
23. Zheng, L.Y., et al. "Aroma volatile compounds from two fresh pineapple varieties in China." *Int J Mol Sci.* (2012): 7383-7392.
24. Blakemore, J., and Fredrick N. "Aromatase: contributions to physiology and disease in women and men." *Physiology* 9 (2016).
25. Lorke, D. "A new approach to practical acute toxicity testing." *Archives of toxicology* 54 (1983): 275-287.
26. Nwankudu, O. N., et al. "Phytochemical, hematologic and histopathologic evaluation of male albino rats treated with peanut (Arachis hypogaea) extract." *World j Innov res.* (2020): 62-72.
27. El-Sherbiny AM. "Seasonal variation in seminal characteristics of rabbits". *Fac. of Agric.,* Ain-Shams University (1987).
28. Chibundu UC. "Response of pre-pubertal bucks to administration of estradiol B." *Proj. Rep. Fed. Univ. Technol.* Owerri (2013), 30
29. Orieke, Daniel, et al. "Semen quality, hormone profile and histological changes in male albino rats treated with Corchorus olitorius leaf extract." *Avicenna J Phytomed.* (2019): 551.
30. Peter IR. Artificial Insemination Technology 1. *Natl. Anim. Prod. Res. Inst.* Ahmadu Bello Univ. Zaria (2002), 16-17.
31. Ramalan, Mansur A., et al. "Effects of hydromethanol hypocotyls extract of Borassus aethiopum on sperm and gonadal indices of male Wistar rats." *J Med Plants Res.* (2021): 515-521.
32. Cattley, Russell C., and John M. Cullen. "Liver and gall bladder." *Haschek Rousseaux's handb. toxicol. pathol.,* Acad. Press, (2013). 1509-1566.
33. Claus, M., et al. "Factors associated with elevated alanine aminotransferase in employees of a German chemical company: results of a large cross-sectional study." *BMC gastroenterology* 21 (2021): 1-13.
34. Fotheringham, Amelia K., et al. "Kidney disease risk factors do not explain impacts of low dietary protein on kidney function and structure." *Iscience* 24.11 (2021): 103308.
35. Yang, J., and Jun, Y. "The association of diet, gut microbiota and colorectal cancer: what we eat may imply what we get." *Protein cell* 9.5 (2018): 474-487.

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