EVALUATION OF TRIMESTER EFFECTS OF GRADED DOSES OF PINEAPPLE (*ANANAS COMOSUS*) JUICE TREATMENT IN PREGNANT FEMALES

ABSTRACT

Ethno-medically, pineapple is used as abortifacient. Moreover, pineapple juice was found to have utero tonic effect in vitro just like Oxytocin, but in vivo research, bred at 10 days (second trimester in albino rats) showed no abortifacient effect. Therefore, this research was set up to investigate the trimester effect of the juice using albino rats. A total of 74 albino rats were used. Twenty one rats were used for toxicity testing, 40 female albino rats of 12 weeks of age and 13 males of 14 weeks of age were used for in vivo investigations. The males were kept in 13 cages. The rats were acclimatized for one week. The female rats' estrus were induced using peanut extract (Luchis estrus milk) at a dose of 800mg/kg body weight. Three females picked at random were introduced to each male albino rat for impregnation. The male in cage 6 had very high libido and was given 4 females to impregnate. Twelve female rats each divided into 3 groups of 4 were used to study each trimester effect while the remaining 4 impregnated rats served as control for the three trimester treatment groups. Breeding was confirmed through vaginal cytology. In all the trimesters, the treatments were: 250mg/kg, 500mg/kg and 1000mg/kg body weight (graded doses) of pineapple juice. The control was given 2ml/kg body weight of distilled water. Five days post breeding, graded doses of pineapple juice were given to first trimester treatment groups and control group. Second trimester groups were given graded doses of pineapple juice just as in first trimester at day 10 while third trimester treatment groups were treated just as in first and second trimester at day 20 post conception. Furthermore, 20 days post conception, blood for hematology (in heparinized bottles) and serology (in test tubes) were collected through the ocular vein of the median cantus in first trimester treatment groups and control. Then, the rats in first trimester treatment group and control were sacrificed. The result obtained showed that in the first trimester treatment group, the embryos were resorbed/aborted in female rats treated with 500mg/kg and 1000mg/kg respectively but stunted in rats treated with 250mg/kg. Conclusion: high doses of pineapple juice caused abortion. But low dose caused stunting of the growth of the fetus. This could be why some women experience the stunting of the growth of their fetuses which most often elongates the gestation period.

Key words: Abortion, Gestation, Pineapple juice, Stunted pregnancy, Trimester

Introduction

Pregnancy in vertebrates is usually divided into three trimesters. In primates first three months is known as first trimester, 4-6 months is known as second trimester while 7-9 months is known as third trimester [1]. But in albino rats with 21±2 days duration of pregnancy, first trimester is the first 7 days post conception while second trimester is 8-14 days post conception and the third trimester is 15-21 days post conception [2].

The laboratory rat is widely used in toxicological, nutritional, genetic, behavioral and environmental studies [3]. The small size of rats and the ease of housing and caring for them have made them preferable as pets and research animals [4]. The use of humans and food animals in experiments is restricted for ethical and economic reasons, respectively. Therefore, rats have long been used as models of mammalian health and disease experimentations [5].

The establishment of pregnancy requires the presence of a functional corpus luteum (CL) that is able to produce sufficient progesterone. The female reproductive cycles functions primarily by the interplay between the luteinizing hormone, follicle stimulating hormone, progesterone, estradiol and testosterone. Also the female reproductive organs can be assayed by the serum level of these hormones [6].

Pineapple (*Ananas comosus*) is the leading edible member of the family *Bromeliaceae*, grown in several tropical and subtropical countries including Philippines, Thailand, Indonesia, Malaysia, Kenya, India, and China. It has been used as a medicinal plant in several native cultures [7].

Bromeliaceae embraces about 2,000 species, mostly epiphytic and many strikingly ornamental [8]. Ripe pineapples are cut up in various ways and eaten fresh, as dessert, in salads or cooked in pies, cakes, puddings or as a garnish on ham or made into sauces. It is in fact the most important economic plant of the *Bromeliaceae* family [9].

Pineapple is a vital source of sugars, organic acids, essential minerals, vitamins and fiber for human nutrition. Its fruits are rich in ascorbic acid, flavonoids and carotenoid compounds. The chemical composition of pineapple (sugars, organic acids, minerals, fiber, aromatic compounds, vitamins, amino acids, flavonoids, carotenoids, etc.) depends greatly on the variety [10].

Furthermore, in ethno-medicine, *Ananas comosus* (Pineapple) is used for abortifacient purposes and to induce labor when the pregnancy is to term [11]. Also Monji, (2017) in his thesis titled: Investigation of pharmacological properties of *Ananas comosus* on uterine activity, a PhD thesis expressed that ripe and unripe *Ananas comosus* has been used as a traditional medicine in inducing abortion in many countries. He stated that scientific evidence supporting the efficacy of *A. comosus* extracts on uterine activity is clearly lacking. In order to provide the much needed scientific evidence among others, this research was set up to investigate trimester effect of *Ananas comosus* in pregnant albino rats.

Materials and Methods

Ananas comosus (Pineapple) was bought 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 (Fig. 1).

Fig. 1. Queens Pineapple JPG

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 fiber in different compartments. Some quantities of the juice extracted was put in crucible and the remaining stored in a freezer at -4° C until needed.

Establishment of dosage

Five milliliter (ml) each of pineapple juice were put in five crucibles. They were put in hot air oven set at 40°C in order not to denature the substance. The dried yellowish substance obtained were weighed and the average obtained is 700mg in 5ml of pineapple juice. Therefore the concentration was 140mg/ml of pineapple juice.

Acute toxicity study:

This was done using Lorke's up and down method [12]. A total of 21 albino rats were used for the study. The study was carried out in two phases. In the first phase, four groups; A, B, C, D of 3 rats each. Group A was given 2ml/kg of distilled water as control, then, 10mg/kg, 100mg/kg and 1000mg/kg body weight of pineapple juice respectively. All the 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 5000mg/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.

In vivo experimentation

Fifty two albino rats were used for in vivo experimentation; 40 were females of 12 weeks of age and 13 males of 14 weeks of age. Each male was kept in a separate cage. 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 standard cages and fed Topfeed chick marsh *ad libitum* and had access to clean water. They were acclimatized for one week and the estrus of the females induced using peanut extract (Luchis Estrus Milk) at a dose of 800mg/kg body weight [13]. Then three females picked at random were introduced per male for impregnation. The male in cage 6 which has very high libido (Grooms, Sniffs and attempt to mount in split seconds) were given 4 females to impregnate. Breeding was confirmed through vaginal cytology. Twelve female rats were used to study each trimester effect. In all the trimesters, the twelve rats were divided into 3 groups of 4 rats per group while the remaining 4 impregnated rats served as control for the three trimester treatment groups. In each trimester, the first group was given 250mg/kg body weight of pineapple juice, the second group was given 500mg/kg and the third group was given 1000mg/kg of pineapple juice while the control group was given 2ml/kg body weight of distilled water.

Five days post breeding graded doses of pineapple juice were given to first trimester treatment groups while control group was given 2ml/kg of distilled water as stated earlier.

The second trimester treatment groups were treated with graded doses of pineapple juice just as in first trimester at day 10 and allowed to carry their pregnancy to term and allowed normal parturition at days 20-21 [14].

While in the third (last) trimester treatment group, the rats were treated with graded doses of pineapple juice at day 20 post conception.

Blood was collected in the first trimester treatment groups and control group at day 20 through the ocular vein of the median cantus for hematology in heparinized bottles and serology in test tubes, after which the rats were sacrificed through cervical dislocation to avoid pain. Eviscerations were done and ovaries and uteri were exteriorized and isolated. The ovaries were viewed with magnifying glass for the presence of corpora lutea (Yellowish round protrusions at the ovarian walls) which confirms conception and the two fallopian tubes viewed for evidence of embryo resorption and/or abortion. Liver, kidney, spleen and heart of the female albino rats in first trimester treatment and control group were separated and the relative organ weight was done using the formula:

$$\text{Relative organ weight} = \frac{\text{Weight of Organ (g)}}{\text{Live Weight (g)}} \times \frac{100}{1}$$

All the 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 procedure approved stipulated alleviation of pain in laboratory animals.

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 ninety five percent confidence interval ($P \leq 0.05$) and 99% confidence interval ($P \leq 0.01$) were adjudged to showcase positive effect of the treatment.

Result:

Acute toxicity result

After 72 hours and 7 days post administration of the pineapple juice extract at both high and low doses, no death was recorded even at the highest dose of 5000 mg/kg body weight (Table 1).

Table 1. Result of acute toxicity test in Pineapple juice treated rats

Groups	Dose mg/kg	Number of deaths	Percentage mortality
A	2ml/kg	0/3	0.00
B	10	0/3	0.00
C	100	0/3	0.00
D	1000	0/3	0.00
E	1600	0/3	0.00
F	2900	0/3	0.00
G	5000	0/3	0.00

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

Result of trimester effect on pregnant rats treated with pineapple juice

In the first trimester treatment group, rats treated with 250mg/kg body weight of pineapple juice had stunted embryo (1.5± 815 cm) in the left fallopian tube and (2.0 ± 0.215) in the right fallopian tube against (2.14 ± 0.916) in the left tube and (4.16 ± 0.618) in the right tube of distilled water treated rats. Treatments 500mg/kg and 1000mg/kg of pineapple juice led to abortion in the treated rats (Table 2, Fig. 2 and 3).

However second trimester treatment group of rats went through normal parturition at day 21 with large litter sizes.

In the third trimester treatment groups, at day 20, treatment with 250mg/kg body weight of pineapple juice did not effectively induce parturition but those treated with 500mg/kg went through induced parturition. However, treatment with 1000mg/kg led to parturition at day 20 but the dams lost their lives.

Table 2. Reproductive indices of female albino rats treated with pineapple juice at day 5 post conception

	Parameters					
Treatments	**RCL**	**LCL**	**NFR**	**NFL**	**RHER**	**LHER**
Control (D/water- 2ml/kg)	7.33±0.88	3.00±1.00	5.66±1.33	1.00±1.15	2.33±1.20	2.00±1.52
250 mg/kg	7.33±1.20	4.00±0.57	5.00±2.00	1.33±1.33	2.33±2.02	3.66±1.45
500 mg/kg	6.33±0.33	3.66±0.33	0.00±0.00	0.00±0.00	6.33±1.45	3.66±0.33
1000 mg/kg	8.66±0.66	4.00±1.00	0.00±0.00	0.00±0.00	8.66±0.66	4.00±1.00

The superscript shows aborted embryos in treatments 500mg/kg and 1000mg/kg.

Note: RCL= Right corpus luteum; LCL= Left corpus luteum; NFR= Number of fetuses at the right horn; NFL= Number of fetuses at left horn; RHER= Right horn embryo resorption; LHER= left horn embryo resorption

. Figure 2 and 3 showing stunted growth of fetuses and abortion.

Fig. 2. 250mg/kg treatment ↑ (Stunted embyo), ↓; Ovary

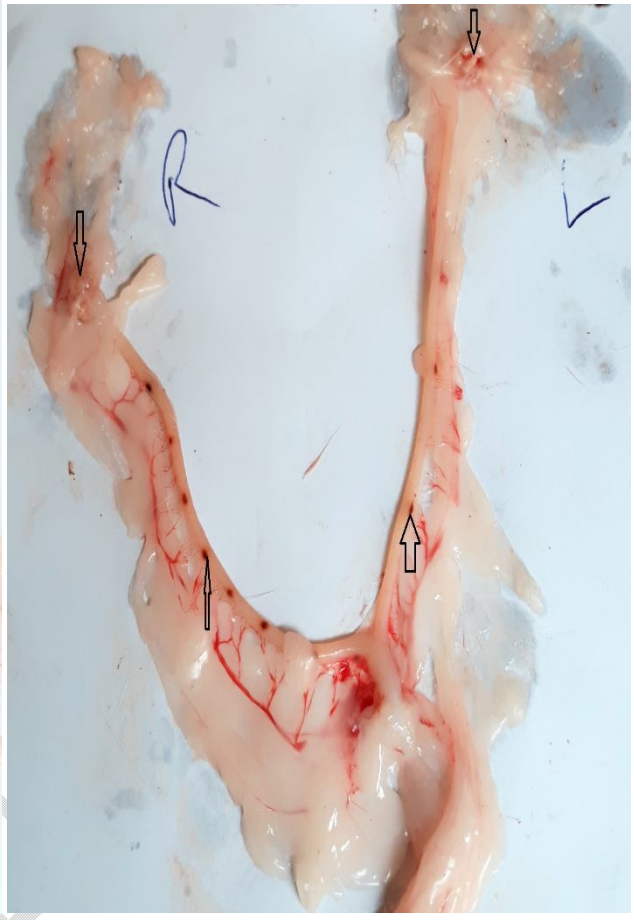

Fig. 3. 500mg/kg ang 1000mg/kg ↑ (Aborted embryo), ↓; Ovary

Result of haematology in female rats treated with pineapple juice in first trimester group

The result of the haematology revealed that hemoglobin concentration of the blood was lower in all the treatment groups but erythrocyte indices were not significantly different from control. Also white blood cells (WBC) or leukocytes were low in all the treatments with 250mg/kg treatment being significantly ($P \leq 0.05$) lower than control. Differential leukocyte count revealed that lymphocytes were significantly ($P \leq 0.05$) lower in 250mg/kg treatment than control, but neutrophils were significantly higher in 250mg/kg treatment than control (Tables 3a, 3b and 3c).

Table 3a. Haematology of female albino rats treated with pineapple juice in first trimester group

Haematological Parameters	Treatments			
	250mg/kg	500mg/kg	1000mg/kg	Control (2ml/kg)
Haemoglobin (Hb) (g/dl)	16.47 ± 0.50	16.40 ± 0.72	17.07 ± 0.50	19.00 ± 2.11
Packed cell volume (PCV) (%)	35.67 ± 2.52	35.67 ± 2.52	37.33 ± 1.53	41.33 ± 5.51
Red blood cell (RBC) ($\times 10^6 mm^3$)	5.68 ± 0.40	5.70 ± 0.51	5.93 ± 0.27	6.44 ± 0.83
White blood cell (WBC) ($\times 10^3 mm^3$)	7.30 ± 0.89	8.27 ± 0.73	9.07 ± 0.84	10.87 ± 1.86

The superscript shows significant ($P \leq 0.05$) difference in a row. Table 3a shows that rats treated with 250mg/kg body weight of pineapple juice showed significantly lower white blood cell count. White blood cells fight infectious agents that enter the body. Lowered WBC is an indication of the pregnant rat's lowered ability to fight infection.

Table 3b. Erythrocyte indices in Pineapple juice treated female albino rats in first trimester group

Erythrocyte Indices	Treatments			
	250mg/kg	500mg/kg	1000mg/kg	Control (2ml/kg)
Mean Corpuscular Volume (MCV) fl	62.76 ± 1.13	62.68 ± 1.25	63.00 ± 0.35	64.13 ± 0.79
Mean Corpuscular Haemoglobin (MCH) pg	29.03 ± 1.14	28.87 ± 1.45	28.81 ± 0.47	29.53 ± 0.52
Mean corpuscular Hb conc (MCHC) g/dl	46.26 ± 1.97	46.71 ± 2.75	45.73 ± 0.53	46.06 ± 1.20

$P \leq 0.05$. MCV = PCV x 10/ RBC, MCH = Hb (mg/dl) x 10/RBC and MCHC= Hb/PCV×100/1

Variation in MCV and MCH is used to assess anemia in treated rats. Table 3b shows that the erythrocyte indices of pineapple juice treated rats were not significantly different from control. This shows that pineapple juice does not cause anemia.

Table 3c. Differential Leukocytes of female albino rats treated with pineapple juice in first trimester group

Differential Leukocyte Count	Treatments			
	250mg/kg	500mg/kg	1000mg/kg	Control (2ml/kg)
Lymphocytes	*57.67 ± 2.52	60.67 ± 1.53	62.00 ± 1.73	64.33 ± 3.06
Neutrophils	*34.33 ± 2.52	33.00 ± 2.65	30.00 ± 2.65	26.67 ± 3.06
Monocytes	5.00 ± 1.00	5.00 ± 0.00	5.67 ± 1.53	6.67 ± 0.58
Eosinophils	3.00 ± 1.00	1.33 ± 1.15	2.33 ± 1.15	2.33 ± 0.58

The superscripts show significant ($P \leq 0.05$) difference in a row. Lymphocytes is used to access the immune status of the animal, and neutrophilia is an indication of bacteremia. Table 3c shows that rats treaded with 250mg/kg body weight of pineapple juice had significantly lowered lymphocytes when compared to control which shows that they had lowered immunity. Moreover, the same treatment group had neutrophilia, which shows that they had bacterial infections but could not effectively eradicate the infectious agents due to lowered immunity.

Result of endocrine (hormone) profile of female rats treated with pineapple juice in the first trimester group

Endocrine profile of female rats treated with pineapple juice showed significant difference ($P \leq 0.05$) in estradiol secretion between those treated with 250mg/kg and 1000mg/kg. But, prolactin secretion showed similarity between 500mg/kg and 1000mg/kg treatments which is significantly lower than control at 99% confidence interval. Also prolactin secretion in 250mg/kg treatment is significantly ($P \leq 0.05$) lower than control. Furthermore, there is significant difference in progesterone secretion among all the treatments when compared to control at 95% confidence interval (Table 4).

Table 4. Hormonal Profile of female rats treated with pineapple juice in first trimester group

Hormonal Profile	Treatments			
	250mg/kg	500mg/kg	1000mg/kg	Control (2ml/kg)
Estradiol (pg/ml)	56.00 ± 7.20	77.00 ± 8.40	89.00 ± 12.30	69.00 ± 9.60
Prolactin (ng/ml)	16.00 ± 2.70^{b}	4.00 ± 0.80^{a}	4.40 ± 0.80^{a}	22.00 ± 3.30
Progesterone (nmol/ml)	49.60 ± 3.80^{b}	36.87 ± 1.75^{a}	52.60 ± 2.80^{c}	78.00 ± 9.10

The superscripts show significant ($P \leq 0.05$) difference in a row. Prolactin is used to maintain corpus luteum and corpus luteum secretes progesterone which maintains pregnancy. Table 4 shows that prolactin in blood was reduced significantly in 500 mg/kg and 1000mg/kg body weight of pineapple juice at 99% confidence interval which shows that the pregnancy has been aborted

Result of relative organ weight of females in first trimester treatment group

The result showed that the weight of the liver relative to the body weight was significantly ($P \leq 0.05$) decreased across treatments (500 and 1000 mg/kg) in dose dependent manner when compared to control. However, mean relative value of liver in the 250 mg/kg treatment was not statistically different from the value in the control rats. The weights of the kidney, lungs, and heart relative to the animal body weights, although were not significantly affected by the test juice, but their mean values were numerically higher than the mean value of the control rats except spleen mean value of 500mg/kg and 1000mg/kg treatments which were lower than control (Table 5)

Table 5. Relative organ weight of female albino rats treated with pineapple juice in first trimester treatment group

	Parameters					
Dose	Live weight	Liver%	Kidney%	Spleen%	Lungs%	Heart%
Control (D/water- 2ml/kg)	193.66±29.66	4.05±0.12	0.45± 0.20	0.34± 0.02	0.56±0.04	0.33± 0.01
250 mg/kg	202.33 ± 6.96	3.61±0.34	0.61± 0.04	0.42± 0.17	0.65±0.07	0.40± 0.08
500 mg/kg	248.33 ± 9.06	3.06 ± 0.18^{b}	0.60± 0.05	0.29± 0.01	0.72±0.09	0.34± 0.01
1000 mg/kg	220.00 ± 5.50	2.92 ± 0.22^{a}	0.60 ± 0.03	0.32± 0.03	0.82±0.07	0.40± 0.02

Superscripts show significant ($P \leq 0.05$) difference in a column. Table 5 shows that the weight of the liver in pineapple juice treated rats significantly reduced when compared to control in descending dose dependent manner with 1000 mg/kg pineapple juice treatment showing the least weight.

Discussion

Acute toxicity testing result showed that pineapple juice is safe in high and low doses in both male and female animal species however the result of trimester effect showed that low doses can lead to complications. For instance low dose of 250mg/kg body weight treatment with pineapple juice was observed to lead to significantly ($P \leq 0.05$) lowered white blood cell which was further differentiated to be lowered lymphocytes which means lowered immunity. This agrees with Chiu et al., 2019 [15]; lymphocytes are one of the main immune cells.

Also differential white blood cell count revealed that low dose of 250mg/kg led to neutrophilia which is an indication of bacteraemia. It is possible that low dose of pineapple juice led to proliferation of bacteria which induced increased release of neutrophils to the blood as the body's defensive mechanism. This agrees with Branzk et al., 2014 [16] who stipulated that Neutrophils phagocytise, degranulate, and release nuclear material in the form of neutrophil extracellular traps against bacterial infections.

Furthermore low dose of 250mg/kg bodyweight of pineapple juice led to stunting of the embryo growth which may be the answer to some women who are told that their embryo stopped growing due to unknown cause. This agrees with Kep et al., 2022 [17] who inferred that indicators contributing to maternal and child factors that affect the stunting have not been properly analyzed.

However, higher doses of 500mg/kg and 1000mg/kg body weight of pineapple juice treatment led to abortion which did not significantly affect the haematological parameters of the animals treated.

The result of the hormonal profiling revealed that in the first trimester treatment group, 500mg/kg and 1000mg/kg treatments led to increased estradiol secretion which was not significantly different from control. This may be because the pregnancy of the control rats were to term and increased estradiol secretion is part of parturition events. But there was significantly ($P \leq 0.05$) lowered secretion of estradiol in 250mg/kg treatment when compared to 1000mg/kg treatment. Estradiol is a primer for uterine contraction which lead to expulsion of fetus but in this case embryo. Increased secretion of estradiol could have led to the abortion observed in 500mg/kg and 1000mg/kg treatments. This agrees with Wu *et al.,* 2004 [18] who said that estradiol can in the absence of increased plasma cortisol stimulate uterine prostaglandin production and induce labor. Estradiol secretion is lower in 250mg/kg treatment than in 1000mg/kg treatment which explains why the treated females retained their fetuses though stunted.

Prolactin: There was lowered secretion of prolactin in all the treatments. However, 500mg/kg and 1000mg/kg showed the greatest lowering at 99% confidence interval. Prolactin is used to maintain corpus luteum and corpus luteum secretes progesterone which maintains pregnancy [19]. Lowered secretion of prolactin is an indication that the corpus luteum was not maintained which led to expulsion of the embryo. However in 250mg/kg treatment, prolactin secretion though significantly different from control, was not grossly lowered when compared to 500mg/kg and 1000mg/kg. This also explains why the embryo, though stunted were not aborted.

Furthermore, progesterone secretion in the first trimester treatment group was significantly reduced in all the treatments. Significant reduction in progesterone when compared to control

showed that the pregnancy was not maintained. Therefore abortion occurred especially in 500mg/kg and 1000mg/kg treatments.

However, the result of the relative organ weight showed that 500mg/kg and 1000mg/kg treatments caused a significant reduction in the weight of the liver. This suggest that the juice had significant adverse effect on the liver tissues and no significant effect on the other organs (Kidney, spleen, lungs and heart) evaluated. Reduction in liver mass could be adverse in metabolism of carbohydrates and protein. This agrees with Han et al., (2016) [20] who stated that liver has a major role in the control of glucose homeostasis by controlling various pathways of glucose metabolism; glycogenesis, glycogenolysis, glycolysis and gluconeogenesis. Alamri, (2018) [21] also confirmed protein metabolism in the liver by declaring; in metabolism of protein, urea cycle occurs in the liver to remove toxic ammonia accumulated as a by-product of protein metabolism. Therefore, treatment with pineapple juice should be controlled or ameliorative mechanism put in place to avoid undue interference with liver function due to the treatment. However, more work should be done to confirm the activity of pineapple juice in the liver of female vertebrates.

In second trimester treatment group, the rats were allowed to go through normal parturition events. Some kidded at day 21 while others kidded at day 22 which agrees with Ochiogu et al., 2006 [11]; length of pregnancy for albino rats is 21±2 days.

Finally in the third (last) trimester treatment group, 250mg/kg treatment did not lead to induced parturition at day 20 post conception but 500mg/kg treatment led to induced parturition. The dams kidded at day 20. However 1000mg/kg treatment led to induced parturition at day 20. The dams kidded but they lost their lives. This confirms the assertion by Nwankudu et al., 2015 [22]; Pineapple juice is oxytocin-like in activity.

1.1. Conclusion

Pineapple (*Ananas comosus*) juice treatment does not lead to anemia. Therefore, pineapple juice is a safe abortifacient at higher doses. Pregnant women should avoid consumption of pineapple during first trimester of pregnancy to avoid miscarriage. Low doses are as dangerous as high doses because stunted embryo or fetus is a source of stress to the expectant mother. Pineapple juice could be used to induce parturition at a moderate dose. However more research should be done to accurately establish the dose ranges needed to induce parturition.

Ethic approval: All the experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC); FV-U -IACUC-2020-0262 of University of Nigeria, Nsukka

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