



Oxytocic Effect of *Ananas comosus* fruit Juice on isolated Pregnant Rats Uteri

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SUMMARY

This work sought to find out whether *Ananas comosus* (pineapple) juice can cause abortion in gravid albino rats. Thirty two pregnant albino rats were used in two separate in vivo experiments. The first set of 16 pregnant rats were divided into 4 groups and groups 2, 3 and 4 were assigned undiluted pineapple juice for 24hrs, 36hrs, 48hrs respectively, while group 1 served as control. The pregnant rats were monitored closely for possible abortions till they littered. The procedure was repeated with the other set of 16 pregnant rats. The effect of the juice was also determined on an isolated pregnant rat uterus. Results obtained in the two separate in vivo trials showed no abortions as the rats had their young ones at term. In the in vitro work, the juice however induced strong uterine contractions similar to that of oxytocin. The results therefore suggest that *Ananas comosus* juice contain some active ingredients with uterine contractile effects which may cause abortion in the same manner as oxytocin but may have been metabolized after oral administration.

Key words: *Ananas comosus*, Abortion, Albino rats, Oxytocin.

INTRODUCTION

Oxytocin is a nine amino acid peptide hormone secreted by the posterior pituitary that elicits milk ejection in female animals and women. In pharmacological doses, oxytocin can be used to induce uterine contraction and maintain lactation (Paul et al; 1998; Gimpl and Fahrenholz; 2001).

Oxytocin and vasopressin are typical neural hormones. The binding protein for oxytocin is designated neurophysin 1 and that for vasopressin is designated neurophysin 11. Both neurophysins are similar in structure. The hormone-neurophysin complex stabilizes the hormone within the neurosecretory granules. Oxytocin is stored

as neurosecretory granules and released from axonal terminals by calcium-dependent exocytosis (Ganong, 1993; Reimers, 2003). In fact oxytocin has been best known for its roles in female reproduction. It is released in large quantities during labor, and after stimulation of the nipples. It is a facilitator for childbirth and breastfeeding. However, recent studies have begun to investigate oxytocin role in various behaviors, including orgasm, social recognition, bonding, and maternal behaviors. oxytocin is believed to be involved in a wide variety of physiological and pathological functions such as sexual activity, penile erection, ejaculation, pregnancy, uterine contraction, milk ejection, maternal behavior, social bonding, stress and probably many more (Lee et al., 2009).

Oxytocin is usually administered intravenously to induce contractions during parturition. It is also available as nasal spray to induce lactation post-partum. Oxytocin infusion near term will produce contractions that decrease the fetal blood supply. It is inactive orally because it is destroyed by gastric and intestinal enzymes (Paul et al., 1998).

Oxytocin is also used to stop bleeding post-partum. For this purpose, it is given intravenously or intramuscularly. It is released into the bloodstream as a hormone in response to stretching of the cervix and uterus during parturition and with stimulation of the nipples during lactation (Huffmeijer et al., 2012).

In estrogen and progesterone primed rodents, injections of prolactin cause the formation of milk droplets and their secretion into the ducts but oxytocin causes contraction of the myoepithelial cells lining the duct walls which results in ejection of milk through the nipple. Membrane receptors for oxytocin are found in both uterine and mammary tissues. These receptors are increased in number by estrogens and decreased by progesterone.

The concomitant rise in estrogen and fall in progesterone occurring immediately before parturition probably explains the onset of lactation in some individuals prior to delivery (Ganong, 1993; Granner, 2000).

The primer for commencement of parturition in humans is secretion of oxytocin by certain cells of the fetus. The oxytocins secreted in turn activate some cells of the placenta to produce and release prostaglandins. Oxytocin and prostaglandins synergize to stimulate the uterine myometria leading to more vigorous and more frequent contractions. At this point, the increasing emotional and physical stresses caused by these contractions activate the mother's hypothalamus which signals for oxytocin release by the posterior pituitary. The elevated levels of oxytocin and prostaglandins trigger the rhythmic expulsive contraction of true labour (Marieb, 1992).

Oxytocin is used to induce parturition and in the treatment of uterine inertia in animal species. However, the mare is the only species susceptible to oxytocin with respect to parturition induction (Thompson, 2004).

Oxytocin can be used to induce maternal behavior in multiparous nonpregnant ewes by a regimen of decreasing progesterone levels, increasing estrogen levels and vaginal-cervical stimulation. This stimulation, which would normally be caused by the passage of the lamb through the birth canal, causes oxytocin release (Haupt, 2004).

However, recent studies seem to incriminate oxytocin in elaborate behavioral activities such as: orgasm, social recognition, bonding and maternal behavior. As a result, oxytocin is sometimes referred to as "Love Hormone" (Marnia, 2011; Broadfoot, 2011).

Oxytocin also functions as facilitator in building trust which is necessary in developing emotional relationships, a process also referred to as social bonding. Social bonding is essential to species

survival since it favors reproduction, protection against predators and environmental changes, and furthers brain development. Exclusion from the group results in individual physical and mental disorders and leads ultimately to death, both in animal models and in primitive human tribes (Rerlpath *et al.*, 2005; Kosfeld *et al.*, 2005; Kavaliers *et al.*, 2006)

Furthermore, oxytocin and its receptors have been found to be involved in a plethora of social and affective, physiological and pathophysiological behaviors, ranging from attachment security, mating, paternal behavior and motherhood to autism and obsessive compulsive disorder (Carmichael *et al.*, 1987; Leckman *et al.*, 1994; Hollander *et al.*, 2007; Yamasue *et al.*, 2009).

Vasopressin appears to have a similar effect in males. Plasma concentrations of oxytocin have been reported to be higher amongst people who claim to be falling in love. Oxytocin injected into the cerebrospinal fluid causes spontaneous penile erections in rats (Thackare *et al.*, 2006; Manning *et al.*; 2008; Gupta *et al.*; 2008 and Succus *et al.*, 2008).

Oxytocin increases sexual receptivity and can counteract impotence. As a result partners cuddle up. Studies have shown increases in plasma oxytocin at orgasm in both men and women (Navneet and Sanjay, 2011).

It was discovered that *Ananas comosus* juice is capable of inducing powerful uterine contractions on isolated non pregnant rats (Nwankudu *et al.*, 2014). The detailed mechanism through which *Ananas comosus* juice induced the observed uterine contractions remained unclear. Hence this research was designed to find out whether *Ananas comosus* truly cause abortion in vivo. Further investigation revealed that the activity is oxytocin-like.

MATERIALS AND METHODS

Preparation of pineapple juice

Freshly harvested Victoria Queen Pineapple (*Ananas comosus*) was purchased from Ahiaeke fruit market in Umuahia North Local Government Area of Abia state. The fruits were washed thoroughly with clean water, cut into two and the juice was extracted using a manual juicer. The resulting juice was put in a sterile bottle and stored in the refrigerator until needed.

Animals

Thirty six matured albino rats comprising of thirty two females (180 to 200g) and four males (230 to 250g) were used for the study. The rats were procured from the animal production unit of the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike and were kept in standard clean aluminum cages and fed *ad libitum* with vital feed and clean drinking water all through the study period. The animals were acclimatized for two weeks before the commencement of experimentation and the experiments were conducted in compliance with NIH guidelines for care and use of laboratory animals (Pub. No. 85-23, Revised 1985). The study was conducted in the Physiology Laboratory of the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike.

In vivo effect of *Ananas comosus* juice on Pregnant rats uteri

The female rats were divided into four groups of four rats each, placed in separate cages and had estrus synchronization using Diethylstilbestrol dissolved in paraffin oil and administered at the dose of 1mg/kg body weight. A male was then introduced into each cage for mating. On the 7th day vaginal smear of each of the female rats was made on a clean glass slide by carefully inserting a cotton-tipped swab moistened

with normal saline into the vaginal cavity of the rats and rolled gently against the wall before withdrawal. The smear was stained with Giemsa and observed under microscope to check for presence of protein coagulates. After confirmation of pregnancy, the pregnant rats were grouped in the following order:

Group 1: Normal saline *ad libitum* (control)

Group 2: Undiluted Pineapple juice *ad libitum* for 24 hrs

Group 3: Undiluted Pineapple juice *ad libitum* for 36 hrs

Group 4: Undiluted pineapple juice *ad libitum* for 48hrs

The animals were then observed daily till they littered. The procedures were repeated after one month to establish the result obtained in this first experiment.

***In vitro* effect of *Ananas comosus* juice on isolated rat uteri**

The method described by Uchendu, (1999) was adopted: Briefly matured pregnant female rats were sacrificed by stunning and decapitation. The lower abdomen was opened and the two uterine horns were harvested and transferred into De jalon solution that continuously bubbled with air and maintained at 37⁰C (pH 7.4). The De jalon solution was constituted such that each liter contained: NaCl (9g), KCl (0.42g), CaCl₂ (0.06g), NaHCO₃ (0.5g), and Glucose (0.5g). Each uterine horn was suspended vertically in a 35ml organ bath by means of ligatures attached at one end to a tissue holder and at the other end to an isometric

force displacement transducer connected to a digital physiological recorder (Medicaid Physiopac) and computer screen for displaying isometric contractions. Resting tension in the muscle strip was readjusted, just sufficient to remove the slack. The preparation was allowed to equilibrate within 30 minutes of mounting. After recording regular rhythmic contractions, dose-response relationships were established for Pineapple juice and other standard drugs used. For all administrations, a minimum time of 1 minute was allowed for individual tissue responses before being washed 3 times with De jalon solution. The test substances were administered as final bath concentration (FBC).

Statistical Analysis

Results were expressed as mean \pm standard error of mean (SEM) and Percentages. Statistical analysis was done using one way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Protein coagulates were observed in vaginal smears and indicated that pregnancy had occurred in the experimental rats (Plate 1).

***In vivo* activity of fresh Pineapple juice on pregnant rats' uteri**

No abortions were observed all through the period of pregnancy following administration of Pineapple juice as all the pregnant rats appeared physically healthy and successfully littered at the end of pregnancy. There was no difference between

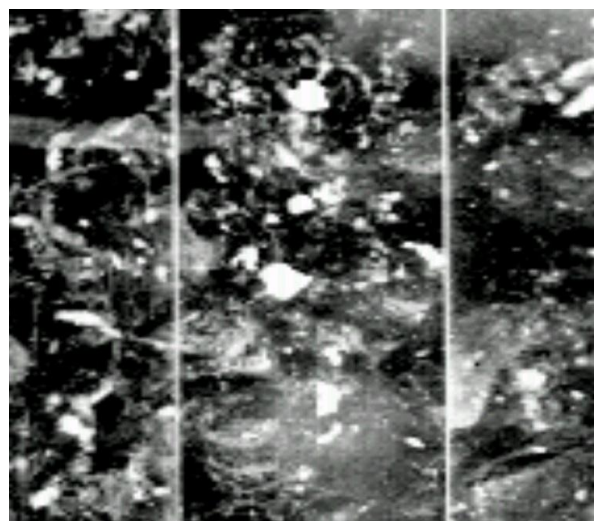


Plate 1: Protein coagulates observed on slides on which vaginal smears of mated rats were made (positive test)

Table I: *In vivo* activity of fresh Pineapple juice on pregnant rats' uteri

Group	Pregnancy Test	Treatment	Number that Littered	Percentage (%) that littered
1 (control)	++++	Normal Saline	4	100
2	++++	Undiluted Pineapple juice per os for 24hrs	4	100
3	++++	Undiluted Pineapple juice per os for 36 hrs	4	100
4	++++	Undiluted Pineapple juice per os for 48 hrs	4	100

++++ = positive pregnancy assessment

Table II: Repeat *in vivo* activity of fresh Pineapple juice on pregnant rats' uteri

Group	Pregnancy Test	Treatment	Number that littered	Percentage (%) that Littered
1 (control)	++++	Normal Saline	4	100
2	++++	Undiluted Pineapple juice for 24 hrs	4	100
3	++++	Undiluted pineapple juice for 36 hrs	4	100
4	+++-	Undiluted pineapple juice for 48 hrs	3	75

++++ = positive pregnancy assessment

27.35± 1.80 respectively. The contractions induced by Pineapple juice compared favourably with that of the standard agent oxytocin.

the control group and all test groups (Table I).

The repeat experiment after one month produced results that were not significantly different ($P < 0.05$) from that of the first experiment, as in all groups 100% of the pregnant animals again littered except group 4 which had 75% (Table II).

***In vitro* effect of Pineapple Juice on isolated pregnant rats' uteri**

All doses of pineapple juice administered significantly induced contractions of the isolated rat uteri ($p < 0.05$) with 0.05, 0.1, 0.2, 0.3 and 0.4 raised the amplitudes of contractions from 5mm to 18.20± 1.31, 23.40± 2.00, 24.80± 2.52, 26.10± 1.81,

In Table I, all the pregnant rats in group 1 (control) treated with normal saline throughout the experiment littered. In the second group undiluted pineapple juice was

given for 24hrs, and at the end of the experiment, all littered. In the third group undiluted pineapple juice was given to the pregnant rats for 36hrs and they all littered as well. In the fourth group, the pregnant rats were given undiluted pineapple juice for 48hrs and they all littered at the end of the experiment.

In the repeated in vivo experiment Table II, all the animals in group one (control) to group three littered, but in group four only three out of four littered, this was because one of the animals in group four was not pregnant.

In the in vitro experiment, *Ananas comosus* juice elicited a dose dependent multiple contractions of the pregnant rat's uterus.

These effect was significantly different ($p < 0.05$) from the basal contractions, with 0.40ml of juice eliciting the highest increase in amplitude of 447% (Table III, Figure I)

The effect of *Ananas comosus* juice on an isolated pregnant rat uterus compared with that of a standard utero-tonic agent Oxytocin, showed that Oxytocin was giving slightly higher effect at low volumes and *Ananas comosus* juice producing higher effects at higher doses (Figure I).

Ananas comosus juice when administered to the isolated pregnant rat uteri induced multiple uterine contractions in a manner similar to that of Oxytocin. This result suggests that *Ananas comosus* juice may contain bioactive principles capable of

Table III: In vitro effect of Pineapple Juice on isolated pregnant rats' uteri

Volume administered (ml)	Basal contraction (mm)	Amplitude of contraction (mm)	Amplitude of contraction with pineapple juice (mm)	Percentage rise in Amplitude of contraction
0.05	5.00	18.20± 1.31	264	
0.10	5.00	23.40± 2.00	368	
0.20	5.00	24.80± 2.52	396	
0.30	5.00	26.10± 1.81	422	
0.40	5.00	27.35± 1.80	447	

P < 0.05 for test versus basal values

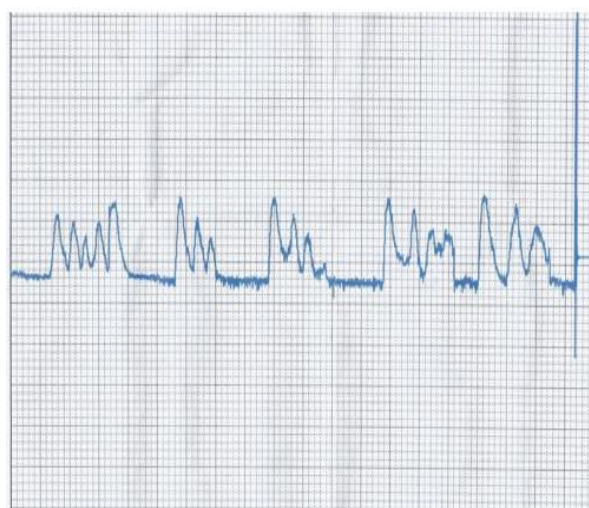


Figure I: Contractile effects of *Ananas comosus* juice on isolated pregnant rat uterus

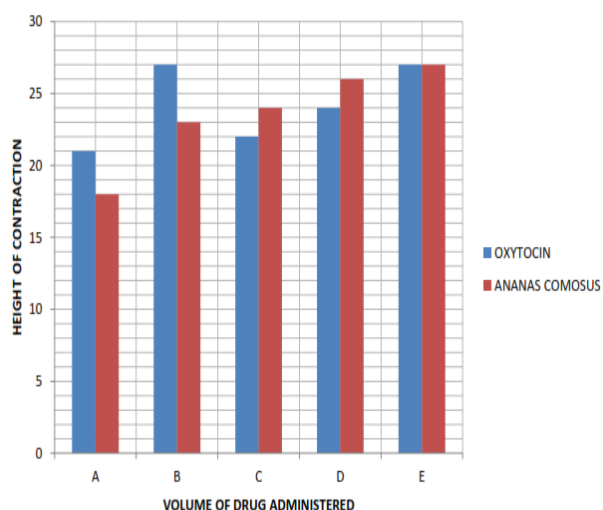


Figure II: Comparative effects of oxytocin and pineapple juice on an isolated pregnant rat uterus

From A-E represents volumes administered as 0.05, 0.1, 0.2, 0.3 and 0.4ml respectively

inducing uterine contractions and as such could be used to facilitate labor or as an abortifacient. Possible mechanisms through which the juice achieved this contractile effect may be combined effect of bromelain (an enzyme present in the juice) and other components of the juice. Their combined action seems to be effected by binding to histaminergic (H₂) receptors present in the rat uterus (Xiao *et al.*; 1999), promoting calcium flux in the smooth muscles (Alexandria and Soloff; 1979).

In the *in vivo* work, the contractile effect of *Ananas comosus* juice was not observed as all pregnant rats given oral *Ananas comosus* juice littered at full term with no abortions induced. This result suggest that the active component(s) of the juice that has the uterine contractile effect may have been affected and transformed by digestive enzymes, thereby causing the juice to lose its utero tonic property when taken orally.

CONCLUSION

Ananas comosus juice induced multiple contractions of the pregnant rat uteri following invitro administrations but did not induce abortion when administered to pregnant rats. This suggests that *Ananas comosus* juice contains active principles which could be isolated and processed into pure utero-tonic agents for use by routes other than the oral. Hence, the consumption of pineapple remains relatively safe in pregnancy.

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