EFFECT OF FEEDING PAPAYA ON THE COURSE OF PREGNANCY IN ALBINO RATS

BY

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

Introduction

I. INTRODUCTION

People have generally known that they must eat to live, but it is unfortunate that many of us know very little about what to eat. Modern sciences has shown that we can add years to our life and life to our years provided we know about and make judicious selection of the right kind of food to eat. There are many kinds of food about which we should know but fruits are by far the most important and need special emphasis in the everyday diet as they supply vitamins, minerals, energy, organic acids, roughage and possess medicinal value as well as (Daulta, 1980).

Fortunately a fruit is not a medicine which may have to be forced down the gullets of unwilling persons. On the contrary, fruits are rich sources of readily available vitamins especially Vitamin A and C and as most of these are eaten in the raw form, the quantitative availability of these vitamin is ensured. However it is paradoxical that the percapita consumption of fruits in India comes to less than 30 g/head/day which is just 1/3 of the minimum quantity prescribed by the nutrition experts, (ICMR, 1981).

Among the fruits, rich in Vitamin C and carotene papaya is an important one and is an inexpensive, indigenous and easily cultivable tropical fruit. Papaya is slowly gaining

importance amongst fruit growers in south India. The main reasons are the nutritive value of the papaya fruit, heavy yield of 150-200 tonnes per hectare during 2 years (Kulasekaran et al, 1983).

Ripe papaya fruit is very refreshing. It is rich in vitamins A, B_1, B_2 and C and is prized for its medicinal properties. Being relatively less expensive and highly nutritious the fruit comes in handy for balancing the diet of the economically weaker sections of population (Purohit, 1981). The fruit contains about 10 per cent sugar, a lot of Vitamin A some Vitamin C and is considered to act as a mild laxative. The fruit is a large, fleshy, hollow berry and usually weighs from 0.5 - 2.0 kg (Samson, 1980). Fresh papaya contains 42 mg vitamin C when immature, to 55 mg when ripe (Arriola, et al 1980). The fruit pulp contains 87-94 per cent water and 2-12 per cent carbohydrate at different stages of maturity. Sucrose, glucose and fructose are the chief forms of sugars present. The colour break stage is characterised by increase in carotene and protein contents (Selvaraj, 1982).

Papaya contains in them a special kind of enzyme-papain which besides being an important aid in the digestion of food also possess considerable therapeutic value. Papain a powerful proteolytic enzyme is obtained from the latex of the full grown

green fruit of carica papaya. Apart from being widely used as a fruit/vegetable, the enzyme obtained from papaya is also used as a food additive to tenderize meat (Watt, 1972). In the preparation of several types of medicines, dental paste, face cream in the tanning industry, textile fibre industry and in breweries also papain is extensively used (Mansha Ram, 1983).

Despite the versatality papaya exhibits, its consumption by population groups in India is selective due to the various kinds of food cults and beliefs that exist. It is largely because of certain misconceptions that consumption of the fruits causes certain disorders (Khader 1984). Papaya is considered by many to be a heat irradiator to the system and hence possessing the capacity to destroy or deform the growing foetus (Swaminathan, 1974, Alanberg, 1973). A belief regarding its powerful abortive properties exists in many parts of India, particularly in southern region (Watt, 1972) where pregnant women are strictly prohibited from eating papaya during pregnancy for fear of inducing abortion and foetopathy (Kvinnsland, 1974).

Nutritional and diet surveys in and around Coimbatore conducted by Devadas and Co-workers (1977) have also indicated that there still exists a strong fallacy against papaya consumption specially during pregnancy, resulting in the prevention of its massive use by adolescent girls and women of child bearing age

specially during pregnancy and lactation. While on one side programmes of agricultural extension insists on growth promotion and many a small farmers have taken up papaya cultivation and papain production and much educational programmes are underway to educate the public about the wide use and benefits of papaya cultivation and consumption, much work needs to be done to make it a culturally acceptable poor man's apple. When massive efforts are being taken to grow special table varieties of this fruit and when there is the added advantage that this fruit can be grown at every door step with least effort or economic input and when it can be a part of various active feeding programmes, conviction on the part of growers and consumers about its great nutritional contribution at low cost or practically no cost and effort is yet to be achieved.

There however is a dearth of information about the scientific knowledge regarding the possible effect of papaya on the development of foetal tissues. In an earlier investigation on albino rats by Chandrasekhar and Ragini (1979) some indications on the possible role of the stage of maturity of the fruit to the harmful effect on foetal tissue growth has been indicated. However, further studies have been suggested before it could be proclaimed that at a particular stage it is harmful or harmless, for foetal implantation, growth and development.

Therefore, further experiments regarding the effect of papaya on the foetal growth and development, becomes a necessity to unravel the scientific truth and to help people understand the nutritional benefits and gain greater acceptance of this fruit. Hence, the objectives of the investigation. In this study, the investigator aims to evaluate the effect of feeding papaya at two different stages of maturity in comparison to papain and seed alone to a group of adult pregnant albino rats and to observe its effect on the course of pregnancy and littering.

II. REVIEW OF LITERATURE

The literature pertinent to this study concerning the effect of feeding papaya on the course of pregnancy in albino rats is reviewed under the following headings:

A. Papaya - Production, Composition, Nutritive value and uses.

B. Papain - Its characteristics and uses.

C. Nutritional taboos in pregnancy.

D. Abortion and prematurity.

A. Papaya - Production, Composition, Nutritive value and uses

Papaya is grown in all states of India and performs well in all regions in the plains (Swaminathan, 1974). The area under papaya cultivation in India is 10,000 hectares (Singh, 1969). In India, the annual production of papaya was about 1.76 lakh tonnes a decade ago and has risen by a lakh tonne in this decade (TNAU, 1984). The world production during 1981 was 19 lakh tonnes, of which Asian countries produced about 7 lakh tonnes and India accounts for 2.70 lakh tonnes (TNAU, 1984).

It is one of the easiest fruit crops to raise both in kitchen gardens or on a field scale from the plains, upto elevations of 110 metres both in arid and rainy areas. It can stand temperatures upto 44°C (110°F) but cannot stand frost. Lack of warm temperature retards maturity and ripening. A dry

climate tends to add to the sweetness of fruits. It yields fruit within a year of souring seeds under Tamil Nadu conditions (Muthuswami and Khader, 1984).

A papaya plantation would yield about 200 kg of papain per hectare in the first year; in the second year it may be 100 kg and in the third year 50 kg. After the third year it will be uneconomical to maintain the plantation as the papain extraction decreases considerably. The edible quality of fruits does not suffer because of the papain extraction (Muthuswami, 1984).

The first crop matures at 12 months of planting and yields 40,000 - 50,000 kg/acre in 3 years (Swaminathan, 1974). Papaya yields a heavy crop of 60 tonnes per hectare/year which few other crops can equal (Muthuswami, 1984).

2. Composition

Fresh fruit contains sucrose, invert sugar, a resin, papain, malic acid, salts of tartaric acid and citric acid, pectin and carotenoids (Chemical abstracts 1949; Indian Academy of Sciences 1949).

The percentage of moisture in papaya is 88.7 and protein 0.6. Papaya is a digestive enzyme. The fruit has a very high digestive value due to the papain - a protein splitting enzyme which it contains (Muthuswami, 1984).

The Vitamin A content per 100 g is 2300 IU and Vitamin C is 70 mg (TNAU reports, 1978). Papaya is a good source of Vitamin A containing 2020 IU in comparison to 150-300 IU in oranges. It also has 0.5 per cent protein. Vitamin C content increases with ripeness (Muthuswami, 1984).

Ripe fruits in the field had increased moisture and fibre content, lower amounts of sugar, acid, beta carotene, cryptoxanthine and papain than fruit raised in a green house. Fruit weight decreased with delay in flowering time so also the moisture, carbohydrate and fibre content. During final stages of ripening there seems a movement of sugar from seed to flesh (Kasinathan et al 1955).

Reports from NIN (1978) states that the black seeds of the ripe fruit contain a toxin carpaine which depressed pulse rate and the nervous system.

The most common method of propogation of papaya is from seeds. The seeds retain vitality for several years if carefully preserved from insect attack, although it would seem that this fact is not true in all papayas and in all places(Cheema et al, 1954). Seeds germinate in two weeks (Samson, 1980). Seeds are multilayered and rich in tannin and can be viably stored at descreated conditions (IARI reports, 1977).

Seeds accounted for about 16 per cent of the weight of fresh papaya. Endosperm represented 43 per cent of dried seed. The seed nitrogen includes free amino acids and amides. Seeds are of two types-those containing carbohydrate and those containing lipids as their main storage material. The fat content on a dry weight basis was 60 per cent in papain endosperm. This is similar to sunflower, castor bean, seasame and coconut and is comparitively higher than that of seeds from other fruits. Ether extraction of papaya seeds yield a slightly green oil in which the major fatty acids were oleic acid 76 per cent and palmitic acid 15 per cent. Seeds also contain minerals and reducing sugar, calcium, sodium, potassium, magnesium, sucrose, β-glucose, fructose and α-glucose. It has been used in poultry seed (Junk, 1981).

Seed protein increased from the 30th day, decreases from the 90th day and again increased to the maximum on the 135th day (4154 mg/g). Thus seeds contain more proteins at the different stages of development (Chithiraichelvan, 1975).

Papaya seeds in Hawaii are used for oil extraction, its crude protein being 40 per cent and defatted meal 48.9 per cent in it. Two samples of hexane extraction, papaya seeds produced in Somalia were analysed for its physico-chemical characteristics. They have fairly high polyphenol content (2.5 ppm), oxidation stability, aliphatic and terpenic alcohols, 0.4 per cent

phosphatids, 10.3 mg/100 ml carotenoids 3.9 per cent linoleic acid, 7.3 per cent oleic acid, 16 per cent palmitic acid, 5.5 per cent stearic acid (Tangi et al, 1978).

Papain catalase is an enzyme present in papaya puree which is inactivated at acid pH (Brouk et al, 1975).

3. Nutritive value

The nutrient content of the papaya's edible portion (Alice 1956) raw papaya fruit and papaya juice (Bowes, 1956) are given in Table I.

TABLE I

NUTRIENT CONTENT OF EDIBLE PORTION, RAW PAPAYA FRUIT AND PAPAYA JUICE

Nutrients	In 100 grams		
	Edible portion (Alice 1956) (g)	Raw fruit (g) (Bowes, 1956)	Papaya juice (g)
Protein	0.6	0.6	0.4
Fat	0.1	0.1	0
Calories	43 kcals	39 kcals	48 kcals
Carbohydrate	10	10	12.1
Calcium	0.02	0.02	0.01
Phosphorus	0.01	0.01	0.01
Iron	0.0003	0.0003	0.0003
Vitamin A	1800-3000 IU	1750 IU	2000 IU
Thiamine	5-25 IU	30 ug	15 ug
Riboflavin	0.08-0.18 mg	40 ug	10 ug
Vitamin C (mg)	17-55	56	41
Vitamin D	-	-	-
Fibre	-	0.9 g	trace

4. Uses

Papaya is considered to be a wholesome and nutritious food used at all stages of development (Cheema, 1954). The odour of the papaya does not appeal to some (Khader, 1984).

Ripe fruit is used as any other vegetable and the ripe one in confectionaries (TANU reports, 1978). Products such as jams, candies can also be made from the fruits (Khader, 1984).

Papaya is said to cure chronic constipation and piles. It is claimed to set enlarged liver and spleen right. The fruit is a valuable tonic. The fruit is a highly relished table fruit for breakfast and is prepared in a number of ways into delicacies (Cheema, 1954). A slice of papaya rubbed over the skin removes blemishes. Slice of raw fruit are used while cooking for softening meat. Papaya aid in digestion and in fact it is for this specific enzyme action, the fruit is recommended for dyspepsia, ulcers and other intestinal disorders (Muthuswami and Khader 1984).

Carica papaya seeds are indigenous and reported to possess the property of preventing birth when administered orally (Chaudhry, 1966). The seeds also serve as a powerful emmenagogue and helps breakdown the blood of the circulating system and effective in expulsion of the limbrici (Wagner et al, 1977).

Carpaine, the chief alkaloid from papaya possess amoebicidal and bactericidal and pharmacological activity comparable to digitalis and emetin (Burdick, 1971).

Papain finds its use in digestive ailment, meat tenderisation beer clarification, fell mongering of sheep skin, wool preshrinking, chewing gum and degumming silk and rayon (TNAU, 1978).

Leaves or pieces of the fruit are immersed in water which is used to clean coloured clothes and to hold colour fast.

The roots of papaya possess the property of a nerve tonic (Khader, 1984).

B. Papain - its characteristics and uses

Source of papain is the latex of green fruit. It is got in crystalline form as needles or hexagonal plates. Its isoelectric pH is 9. Unstable at pH less than 2.5 and above 12.0. Half life 56 minutes at 75°C. It has a molecular weight of 30,000. Slightly soluble in dilute salt solutions, soluble in 70 per cent alcohol. It contains 15.5 per cent nitrogen, 1.2 per cent Sulphur, 9.0 per cent cystine (Nathan and Sidney, 1955).

Papain is activated by HCN and H_2S. Oxidising agents Iodine, Bromine and dilute H_2O_2 inactivates papaine while HCN, H_2S and cysteine or reduced glutathione restores its activity.

Metals like Zinc, Copper, gold, silver, mercury inhibited its activity. It is active over a wide range of pH. Crystalline papaine is active at pH 3-12 at 30°C (Seemanthini, 1984).

Papain is a plant protease. It shows remarkable stability in concentrated urea solution (Boyer, 1971). Papain is a simple protein containing only amino acids and devoid of carbohydrate (Smith and Kimmel, 1960). Papain is used to describe a proteolytic principle in papaya latex and is applied both to the crude dried latex available commercially as well as to the crystalline proteolytic enzyme (Boyer, 1971). Administration of papain to pregnant rats resulted in significant foetal mortality besides stunted foetal size and haemorrhage scattered over the body and in the viscera (Devi and Singh 1978). Papain has been shown to be an antigen (Osgood, 1945) and it induces the release of histamine. Disruption of rat peritoneal mast cells occurs upon exposure to papain in vitro (Archer, 1965). Its administration to pregnant rats causes rupture of the mast cells and this reduces their total number significantly(Devi and Singh, 1979).

The papain treated foetuses showed a general tendency for haemorrhages which were scattered throughout the body. The liver shows dark patchy areas at haemorrhagic sites, well distinguished from the normal pale areas. The kidney showed petechial

haemorrhage visible in the subscapular areas and their size was markedly enlarged (Singh and Sathi Devi, 1980).

Efficient extraction of β-amylase require treatment with papain and a reducing agent (Boyer, 1971).

Purified papain has been found to be one of the effective enzymes for the removal of Zona Pellucida of mouse egg. It seems to have a specific action on cartilage causing reduction in its normal growth and the disturbed cranio-facial growth in rats has been ascribed to it (Kvinnaslands, 1974). Foetal cell leak was more in induced than in spontaneous abortion cases and increasing with gestational age.

Administration of single oral dose of 375 mg/kg of crude papain on 13th gestation day in rats was well tolerated by the growing foetuses as evidenced by the fact that none of them died and only 12 per cent showed anomalies. A higher dose of 750 mg/kg led to 36 per cent of teratogenicity with no lethality. However with a dosage of 1000 mg/kg the pregnant rats died within 24 hours of administration of enzyme. Though the oral dose of 750 mg/kg repeated on 3 consecutive days during 12-17th gestation day also produced anomalies in 100 per cent of foetuses it was lethal to 25 per cent rats. Lethality in controls was seen in only 2 per cent of rats (Shamer Singh and Sathi Devi 1978).

Papain hydrolyses carboxylic derivatives. It is known that treatment of wheat gluten with crude papain abolishes the toxic effects of the gluten for patients with celiac disease (Messer, 1964).

The overall lethality as found by Singh and Devi (1978) on administration of higher levels of papain was not witnessed though negligible anomalies like skin patches, bluish skin, foetal growth retardation was seen and hemorrhage suspected.

Papain is of great demand abroad for manufacture of several kinds of drugs for digestion, preventing stomach disorders etc., in the breweries for clarifying beer, in the leather and tanning industries for softening leather and in the cosmetics industry for the manufacture of face creams. Even the leaves are in great demand for leather and meat industry (Muthuswami and Abdul Khader, 1984).

Papain enters as a component in various pharmaceutical preparations like glycin papain, liquid papain as iridin and liver tonics. In liquid preparation it has been used to reduce enlarged tonsils, prevention of post-operative adhesions, treatment of sloughing wounds, carbuncles, eschar of burns and for dissolving the membrane of diptheria. It is used as a remedy for haemaptyses, bleeding piles and ulcers of the urinary passages. It has rube-facient properties (Muthuswamy and Khader, 1984).

Papain is applied in psoriasis and other skin effections of the similar characteristic and in the treatment of enlarged spleen and liver. It possess ecbolic properties and often reportedly been used by natives to induce criminal abortions. It is applied externally to prevent suppurations and locally for successive treatment of chronic eczema, more especially of the palms where other remedies do not prove curative.

Papain is also used as a remedy for cases of scorpion sting. It is reported to be an allergen causing sometime severe paroxysmal cough, vasomotor rhinitis and dyspnea. It is a powerful poison when injected intravenously. It is also exceedingly acrid, causing blisters and itching if applied to the skin. It has been widely used for degumming natural silk, especially when the latter is mixed with rayon or wool in fabric weaving. Wool treatment with papain takes a silk-like sheen and becomes soft to touch and also eliminates shrinkage and permits its being washed the same way as cotton. Papain has been employed as a substance for rennet in cheese manufacture (Muthuswami & Abdul Khader 1984).

Irulappan (1980) found the yield of papain to fluctuate from May to October among the different types with the lowest yield in September. At present India produces 50-55 tonnes of papain annually for internal use and export (Veerannan 1984).

C. Nutritional taboos in pregnancy

For the past two and a half decades many researchers have succintly documented that the outcome of pregnancy is directly or indirectly affected by low socio-economic status, poor medical care, anaemia, infections, poor maternal nutrition, parity and age (Arkutu, 1978, Beal 1971, Khanna 1977, Odutun 1976, Thomson 1959). Energy giving food items (Starches) were considered good most often while easily available protective (fruits and vegetables) and body building food items (Proteins) were infrequently mentioned or avoided for cultural, religious or health reasons. Traditional beliefs rather than the most frequently cited reasons of poverty and non-availability of foods are seen as major factors limiting the quality of diet among the pregnant women (ojofeitimi and Tanimowo 1980).

In Ethiopia, a pregnant woman must avoid roasted meat as it is believed to induce abortion. Eggs are thought to cause baldness or sterility and hence not consumed by pregnant women. In India, consumption of papaya fruit by pregnant women is believed to lead to abortion and consumption of garlic by lactating women to increase milk production (Swaminathan, 1974).

Nigerian studies show that they believe Pumpkin leaf to cause slit on new born. Mango and Guava disliked for its odour, wild rabbits and antelope forbidden by family and causes animalistic

behaviour in new babies, pork disliked on religious grounds, snakes causes threatened abortion and the child "may look like snakes" and cowpea seed causes upset stomach. Plantain, fish, eggs, liver, bread, rice, yam flour were considered good in pregnancy (Ojofeitimi and Tanimono 1980).

Consumption of meat from the underside of the animal by young married women will prevent child bearing, a belief in West Bengal. There is also a belief that the baby will not be normal if the pregnant women does not eat clay or starch, otherwise known as PICA (Swaminathan, 1974).

The pregnant women of Coimbatore were particular to avoid certain foods like pumpkin, ashgourd, jack fruit, cabbage, fresh fish, banana and blue berries as pumpkin and ashgourd were believed to be 'cold' foods. Jackfruits, cabbage and banana, fresh fish were believed to be gas producing and blue berries as not good for the health of the child. Papaya was believed to be a 'hot' food (Devadas et al, 1975).

Food cults and traditional beliefs in food habits are still prevalent with a large majority of our population who are illiterate and ignorant, regarding the nutritive value of food. In India especially in rural area consumption of the papaya fruit by pregnant women is believed to lead to abortion. It is thought of to be a heat irradiator to the system and hence destroy the growing foetus (Swaminathan, 1974).

D. Abortion and Prematurity

An abortion is the loss of the embryo of fetus during the first 20 weeks regardless of cause in humans (Anderson, 1976). Abortion is defined as the expulsion of foetus at a stage when it has not attained sufficient development to live external to its parent (Arthur, 1964). Ecobolic action of herbivorous plant, endocrine disturbances, serological defects, lethal genes and drugs all cause abortion (Ghosh 1971).

Abortion is a very important and common clinical problem. Antibodies formed in the mother due to heterospecific pregnancy cross the placental barrier and results in destruction of foetal cells, thus causing death of the fetus (Pande, 1973).

Foetal death is not an essential prelude to abortion. Foetal death, and expulsion later results in abrivelled, dried contorbed and parchment like mass with amnion reabsorbed in the cattle (Arthur, 1964).

Abnormalities in uterus contractions noted during abnormal pregnancy include disorder of shape, frequency and resting tone of contractions with a skew. Systole goes on and on. In abortion at advanced pregnancy it is similar to parturition with dilated and softer cervix, uterus is congested with distened blood vessels. Decomposition occurs according to the period of expulsion of the

perished factors (Low et al, 1973). Premature rupture of membranes is defined as the spontaneous rupture of membranes before the onset of labour (Perviz Heera, 1971).

Placental bed spiral arteries was studied in 68 pregnancy complicated by foetal growth retardation. The mothers had hypertension and uroplacental changes of the spiral arteries and acute atherosis was associated (Corlet et al, 1974).

The placenta showed labyrinthine layer with ruptured endothelial wall and clumping RBC amidst disintegrating endothelium, congesting and producing redematous foetus-degenerated chorionic villi and cytolysis whereas in normal pregnancy the maternal and foetal sinusoids are well differentiated with well defined endotheluim (Singh and Devi, 1978).

The incidence of complications after 5018 therapeutic abortions were investigated. Endometritis was frequent with laceration of the ascending branch of the uterine activity (Low et al 1973).

Vaginal cytology is an inexpensive method for determining the progesterone status in threatened and habitual abortion (Pierce 1954, Ghosh 1971). The presence of trophoblast and decidual tissues in the smear indicated inevitable abortion. Superficial cells more than 10 per cent except during labour

and para basal cells more than 4 per cent in the absence of infection and labour is considered abnormal. Superficial cells more than 10 per cent indicated progesterone deficiency (Garg 1976).

Oviductal fluid at ovulation was reported to have high levels of sodium, low chloride and high potassium with low protein content. Estrogen index and probability of intra-uterine growth retardation are related (Low et al 1973). It is clear that the commonest single cause of foetal loss is intra-uterine growth retardation whilst low birth weight is the commonest factor associated with neonatal death (McIlwaine 1979).

The babies born to diabetic mothers are prone to die in neonatal period and the probable causes being prematurity, respiratory distress syndrome, infections, congenital malformations and hypoglycaemia (Dhirawani 1973).

Zaajman et al (1982) have suggested that infection perhaps the most commonly quoted cause of spontaneous premature rupture, may be the consequence of rupture than its cause. Bourne (1962) demonstrated degeneration of amniotic epithelial cells in term membranes, whether this also occurs in premature ruptured cells is not known.

Ovarian hormones influence spontaneous contractivity of uterus but their individual roles are yet controversial (CSAPO 1969). Hyper function of islets in pregnancy is due to the constant stimulation of the endocrine pancreas (Van Assche 1973).

Immuno reactive plasminogen was identified immuno histologically within the amniotic epithelium of spontaneously ruptured membranes and their tissues showed microscopic evidence of degenerative changes. Moreover, much less or no plasminogen was found in artificially ruptured membranes and did not show degenerative changes of same degree (Jenkins 1983).

Crude papain a proteotylic enzyme from papaya administered to 100 pregnant rats from 8-17 days of gestation and foetus collected on the 20th day shows marked foetal mortality in repeated doses, more severly intraperitoneally. Milky juice of papain applied on uteri caused abortion (Devi and Singh 1977).

Ateiology of abortion and premature births are widely variable. It may be the result of pelvic lesions systemic diseases and more frequently the result of endocrine disturbances, emotional imbalance, heterogenous blood groupings, serological defects, Rh incompatibility or inherited lethal or sublethal genes. Hypermotility of uterus and cervical incompetence are not the least important causes in late abortions and premature births of foetuses (Nagen Ghosh 1971).

Spontaneous and recurrent abortions may be due to immunological factors (Bresnihan et al, 1971; Taylor and Faulk, 1981). Based on serial diamine oxidase determination in a small number of subjects several investigators have suggested that low plasma diamine oxidase levels are associated with still births or spontaneous abortions (Beanen et al, 1975, Southeren et al, 1968).

Experimental Procedure

III. EXPERIMENTAL PROCEDURE

The procedure involved in the conduct of this study concerning the effect of feeding papaya on the course of pregnancy in albino rats is discussed under the following headings:

A. Determination of the experimental variables
B. Selection and grouping of the albino rats
C. Preparation of diets
D. Conduct of the study
E. Criteria for evaluation

A. Determination of the Experimental Variables

Reports of the cultivation of papaya and tapping of the papaya fruit for papain extraction indicates that the fruit is first tapped at 70 days maturation, a stage at which it becomes edible (TNAU, 1978). It also indicates that tapping is done subsequently at 90 and 110 days of maturity to get maximum extraction of papain and full maturation of fruit occurs at 133 days after the fruit set.

In an earlier investigation on albino rats, Chandrasekhar and Ragini (1979) observed that papaya fed at 70 and 90 days of maturity had no effect on foetal growth. They also observed that papain fed as such at lower level of proteolytic activity (0.51 per cent) had no effect on foetal growth and in all the

above variation the growth and development of the foetus and the histopathology of the foetal tissues were similar to the normal control rats fed the basal diet. They however, observed that papaya of 110 days maturity, papaya plus seed, seeds alone and papain at higher levels of activity had some detrimental effect on foetal growth and development, and there was a positive indication that papaya fruit at full maturity, a stage at which it is normally consumed does not seem to have any detrimental effect on the course of pregnancy and foetal growth in albino rats. They also suggested that to draw more valid conclusions, similar studies be repeated.

Based on the above experiences, it was decided that in the present investigation, papaya of 110 days and 135 days (fully riped) of maturity will be fed to albino rats. In order to further investigate and establish without any doubt the effect of feeding papaya at these stages of maturity on the course of pregnancy only these two stages, the one which showed a detrimental effect and the other which had no detrimental effect but is the stage at which it is normally consumed were selected to further substantiate the results of the earlier investigation. Since papain extraction has become a very lucrative industry today, much of the papaya that comes to the table is tapped papaya and hence tapped ripe papaya was also thought of interest to be fed

in this investigation to corraborate the earlier findings. Again since seed alone and papain extracts at higher dosage seemed to have some detrimental effect during the earlier observations, it was further thought of interest to study the effect of these two variables and hence chosen as the other two variables to be experimented in this study. Thus in all five experimental variables namely, 110 days old papaya, untapped ripe papaya (135 days of maturity), tapped ripe papaya, seeds alone and papain at 0.68 per cent level were chosen to be investigated in this study along with the basal diet as the control unsupplemented group.

The Tamil Nadu Agricultural University Research station Horticulture Department has developed the new CO_2 variety of papaya and they are being grown and tapped continuously. With the full co-operation of the Horticulture Department of Tamil Nadu Agriculture University, the investigator was able to procure a continuous supply of papaya at different stages of maturity and papain.

B. Selection and Grouping of the Albino Rats

Albino rats of Wistar strain from the laboratory stock colony was taken for conducting the experiment. Female rats of breeding age (120-150 days old) free from obvious disease and

ill-health were chosen. Forty eight rats were divided into 6 groups of 8 rats each for the six diets studied. They were housed in individual cages and diets and water fed adlibitum.

C. Preparation of Diets

Since papaya is not eaten alone and never forms the only food eaten under normal circumstances in a practical situation, the investigator aimed to stimulate as realistic a situation as possible in the formulation of diets. This resulted in the investigator selecting a basal diet and substituting it with papaya, papain and seeds. Considering the ICMR 1981 recommended allowance for a moderate adult woman, the percentage of cereals and pulses were worked out which formed the basic ingredient in the basal diet. Calories and proteins in the diet were adequate and skimmed milk at 30 per cent level, was added to ensure, the quality of protein. Mineral mixture at 4 per cent level and vitamin mixture at 2 per cent level were added to make the basal diet adequate with regard to minerals and vitamins (Devadas et al, 1977). The composition of the mineral and vitamin mixture is given in Appendix I. The composition of the basal diet is given in Table II.

TABLE II

COMPOSITION OF BASAL DIET

Food items	Amount in g/100g
Rice flour	45
Bengal gram flour	10
Skimmed Milk	30
Vitamin Mixture	2
Mineral Mixture	4
Groundnut oil	9

The ingredients of the diet were purchased in one lot, basal diet for the entire feeding period mixed and stored under refrigeration. Previous experience has shown that the adult rats can tolerate an 80 per cent replacement of papaya in the basal diet. On this basis the five experimental diets along with the control diet without any substitution were formulated. The composition of the six diets thus formulated is given in Table III.

TABLE III

EXPERIMENTAL DIET COMPOSITION

S.No.	Stage of Papaya/ diet content	Amount of papaya/ papain in %	Amount of basal diet in %
1.	110 days papaya	80	20
2.	Untapped ripe	80	20
3.	Tapped ripe	80	20
4.	Papain	0.68	100
5.	Seeds	8.5	86
6.	Control	-	100

In formulating the quality of seeds to be added, the enzyme activity as determined in the previous study was taken as the basis and the enzyme activity to match 80 per cent substitution of the 110 days old papaya was chosen as the criteria and the amount of seeds determined.

D. Conduct of the Study

The female albino rats in the preestrous phase, weighed and grouped for the six diets were transferred to cages containing male rats and were left such, till on vaginal examination a span positive was noted which was designated as 0 day of pregnancy

(Devi & Singh 1977, Adkat 1975). Then the females were separated, housed in individual cages and the five experimental diets and the control basal diet were fed to the six groups of eight each and their daily food intake and weight gain per week recorded. The study was carried out for a period of 18-20 days depending upon when the rats littered and later until litters were separated from mother rat.

E. Criteria for Evaluation

1. Weight record and food intake

The amount of food consumed by each rat was recorded everyday and their weight changes noted weekly. Though, the weight gained was different in different groups, there was appreciable increment in weight, recorded at intervals of 7th and around 17-21st day of gestation depending when the different groups delivered.

2. Isolated uterine muscle contractions

One pregnant rat from each group was sacrificed every week. Their uterus was detached and their contractions were recorded on Kymograph using the Dale's organ bath. The composition of Dale's fluid is tabulated in Appendix II.

3. Evaluation of foetus

Naked eye evaluation of the size of the foetus for and number of implants also served as the indices for evaluating normal growth and development.

4. Evaluation of litters

Naked eye examination of the physical appearance of the colour of the skin, transparency of the viscera, time of appearance of hair, head to body proportionality, weight of the rats at birth and at 21 days were noted. Litter size, male to f female ratio, Litter mortality and consumption of litters by mother rats were also recorded.

Results And Discussion

IV. RESULTS AND DISCUSSION

The observations made by feeding papaya at two different stages of maturity, in the tapped and untapped form and seeds alone in comparison to feeding pure forms of enzyme papain and a control group on the course of pregnancy in albino rats is presented and discussed under the following headings:

A. Trends in the weight records of the rats

B. Pattern of food intake

C. Isolated uterine muscle contractions.

D. Foetus evaluation

E. Evaluation of litters

A. Trends in the Weight Records of the Rats

The mean initial weight, mean weight of each group during the first week of gestation and weight before delivery that is around the 17th day of gestation were the three periods at which weight records were maintained. Mean weights of each group during the above three periods along with the total gain in weight and the average days of gestation is presented in Table IV. The individual weights of each rat during the different periods of gestation is appended (Appendix III).

TABLE IV

MEAN WEIGHTS OF RATS DURING DIFFERENT STAGES ON GESTATION

	110 days papaya	Tapped ripe papaya	Untapped ripe papaya	Papain	Seed	Control
	I	II	III	IV	V	VI
Weight at the start (g)	145.34± 10.603	179.25± 33.827	138.94± 6.399	139.72± 7.629	148.13± 13.804	182.7± 23.64
Weight gain at the I week of gestation (g)	163.44± 11.579	193.71± 38.934	155.82± 5.54	154.18± 6.614	167.93± 10.524	209.78± 19.824
Weight before delivery (g)	186.6± 19.957	217.8± 45.130	188.14± 18.949	176.32± 18.697	203.2± 21.916	237.04± 16.647
Gain in weight (g)	41.25± 15.882	36.71± 15.879	52.57± 1.066	35.17± 22.535	47.33± 20.259	54.56± 12.576
Average days of gestation	15–17	15–18	18–19	16–18	15–16	21–23

The control group recorded the highest weight gain in comparison to all the other experimental groups and the weight gain was steady during the course of gestation. Next highest weight gain was recorded by the group of rats fed untapped ripe papaya. In terms of weight gain, the other experimental groups may be ranked as groups fed seeds alone, groups fed 110 days old papaya, groups fed tapped ripe papaya and groups fed papain extract in the descending order of weight gain. Though there were difference in weight gains, statistical analysis (Appendix IV) of the weight gain between different groups indicated that there were no significant difference.

It is however, evident that the highest and steady growth pattern was recorded by the control group. From among the experimental diets, untapped ripe papaya fed group ranked to be the best in its steady pattern of growth and weight gain. At this juncture it is interesting to note that although the other experimental groups like 110 days old papaya and seeds alone also resulted in higher weight gains, the number of days of gestation and the carrying of the pregnancy to full term is considerably impaired. It may be noticed from Table IV that while full term gestation of 21-23 days typical of albino rats was exhibited only by control group, the untapped ripe papaya group came very closer to full term gestation. In spite of weight gain, both the 110 days old group and the seed group

delivered around 16 days of gestation that is almost one week ahead of full term. This is indicative of some abnormality in the proper implanation of the foetus and in this case could well be considered as premature delivery. This indicates the possible role of papaya at 110 days old maturity and papaya seed in incapacitating the pregnant animals to carry its normal course of gestation to full term and associated arrest in the growth and development of the foetus. These results needs further exploration but is indicative of the possible harmful effect these variables can play on the course of pregnancy. Earlier observations of Chandrasekhar and Ragini (1979) were quite in tune with this findings, in that control group was the best and untapped ripe papaya gave the best result from among the experimental groups. The 110 days old and the seeds also did not promote weight gain in the earlier study thus preventing even the implanatation right from the early stages of gestation. Hence it is recommended that more studies be done with these groups at different levels of substitution to actually find the harmful dosage and if possible the modality of operation.

B. Pattern of Food Intake

In all the experimental diets changes in food intake was linearly related to changes in papaya or papain intake. The mean total food intake and papaya intake of the rats fed different experimental diet is given in Table V. The individual papaya and food intake is given in Appendix V.

TABLE V

FOOD AND PAPAYA INTAKE OF RATS FED DIFFERENT DIETS

Group	110 days I	Tapped II	Untapped III	Papain IV (680mg)	Seed V	Control VI
Mean food intake (g)	76.29± 0.8625	77.79± 0.4878	78.88± 0.4192	68.98± 0.5681	61.39± 0.8088	80.44± 0.3894
Mean (g) papaya intake	61.04± 0.6901	62.24± 0.3890	63.11± 0.3339	781.73± 6.3582 (mg)	8.94± 0.1296	-

The animals fed the control diet had the highest food intake and consequently resulted in the highest weight gains. Among experimental diets untapped papaya resulted in the highest food intake followed by tapped papaya, 110 days old papaya, papain and seeds respectively in the descending order of intake. Intake of papain also followed the same order.

In general all the experimental groups and the control group recorded a food intake which showed a significant difference ($p < 0.01$) when compared statistically (Appendix VI). Although food intake normally corresponds to body weight gain during pregnancy gain in weight represents the weight gains of various components of material organism and the foetus (Hytten and Leitch 1964). Similarly though weight gains have been good for seed intake, the actual food intake seems to be rather low in this case. This however points out that the animals were selective in food intake and ate and tolerated foods of better quality.

C. Isolated Uterine Muscle Contractions

Uterine contractions in pregnancy is an index to the maintenance and growth of foetus (Harris, 1969). Experimental rats in this investigation one per group was sacrificed on the 7th day and 14th day of gestation to find the effect of the experimental diet on their uterine activity and course of pregnancy. Using the Dale's apparatus the uterine contractions were recorded and the data obtained is summarised in Table VI.

TABLE VI

ISOLATED UTERINE CONTRACTION OF THE EXPERIMENTAL VERSUS CONTROL RATS

	7th day			14th day		
	Frequency of contraction	Contraction time (sec)	Relaxation time (sec)	Frequency of contraction	Contraction time (sec)	Relaxation time (sec)
110 days papaya fed	Less frequent	12.90	25.6	Frequent	Irregular	Irregular
Tapped papaya fed	less frequent	Wavy	Irregular	Frequent	Wavy	Wavy
Untapped papaya fed	less frequent	12.90	2.48	Frequent	56.00	7.15
Papain fed	less frequent	52.00	25.80	Frequent	Wavy	Wavy
Seed fed	less frequent	Irregular	Irregular	Frequent	Irregular	Irregular
Control	less frequent	15.50	19.50	less frequent	15.50	19.50

On the 7th day of gestation, it was observed that for all the rats sacrificed in each one of the experimental and control group, the frequency of contraction was rather low. The experimental rats fed tapped papaya and seeds had an irregular or wavy contraction and realaxation on the 7th day of gestation. However during this period of gestation, the control group showed regular pattern of uterine contraction with a contraction and a relaxation phase which lasted for a period of 15.5 seconds and 19.5 seconds respectively and the amplitude was good. In the untapped papaya group also the contraction was irregular with a contraction and relaxation phase of 12.9 and 2.48 seconds respectively. The uterine contraction of 110 days old papaya fed group was for 12.9 seconds and relaxation phase lasted for 25.6 second which was almost double the contraction time. On the other hand in the papain fed group the contraction time was rather high (52 seconds) and the relaxation time was almost half (25.8 seconds).

On the 14th day of gestation, the picture of uterine contraction was quite different. Both tapped papaya and seed fed group exhibited irregular to wavy contractions which was pronounced and frequent in the case of seeds fed group. This higher frequency even during the 14th day of gestation probably is an indication of early delivery and on an average this group had a gestation period of only 15-16 days, the least among the groups investigated.

During the 14th day of gestation, the uterine contraction of the control group continued to be less frequent and exhibited the same kind of contraction and relaxation phases as on the 7th day. This again is indicative of the possibility of continuing the course of pregnancy to full term. It may be pointed out that the average gestation period observed in this group was 21-22 days, the normal pattern in albino rats. When looking at the uterine contractions of the untapped papaya fed group it is observed that on the 14th day of gestation, the frequency was increased but it still had a regular contraction and relaxation. This again can be correlated to the number of days of gestation in that on an average, animals of this group delivered around 19th day of gestation, approximating the full term. The 110 days old papaya fed as well as the papain fed groups also had frequent and irregular contractions again indicative of the possibility of not carrying the course of pregnancy to full term and it was observed that in both these groups the rats delivered around 15th and 18th day of gestation.

Since in this investigation all the experimental animals delivered before both the 21st day of gestation, it was not possible to record the uterine contraction on the 21st day and obtain regular graphical representation of uterine contraction. The fact that they were irregular and wavy even during 7th and 14th day did not enable the investigator to do full justice of recording.

However, a comparison of the uterine contractions of the experimental rats with that of the control rat indicates that in all the experimental animals, the course of pregnancy was in some way or other affected and the fact that many of them displayed irregular strong contractions possibly indicates the impending or already dead foetus ready to be expelled. Similar observations were made by Chandrasekhar and Ragini (1979) wherein 110 days old papaya, seed and papain gave such results. In the present investigation also the control rats showed similar trends as observed by the above investigators. The possible effect of uterine contractions as observed in this investigation and the period of gestation as observed here correspond to each other and is indicative of the possible harm these experimental diets could cause on the normal course of pregnancy. It may however be pointed out that untapped ripe papaya was the one which was nearer to the control group an observation similar to the earlier study (Chandrasekhar and Ragini 1979).

D. Foetus Evaluation

In order to record uterine contraction one rat from each experimental and control group was sacrificed on the 7th and 14th day and on the 21st day of gestation for the control group alone. After recording the uterine contraction, uterine membrane was cut open to evaluate foetal implantation. The number of foetus for each group as observed is given in Table VII.

TABLE VII

FOETUS EVALUATION

Groups	Gestation period (days)		
	7th	14th	21st
110 days	7	6	-
Tapped	7	6	-
Untapped	8	6	-
Papain	1	3	-
Seed	4	2	-
Control	8	8	7

It is evident that control group had a steady implantation and the foetus were healthy and the number normal. Looking at the experimental group, the untapped papaya fed group had implantations similar to the control group and the litter size somewhat similar. Both the 110 days old papaya fed group, tapped papaya fed group also showed implantations, but the foetus was not as healthy as in the control group. It may also be recalled that while the untapped group delivered on the 19th day on an average in the case of 110 days and tapped papaya fed group, the average period of gestation was around 15-17 days. These observations are indicative of the fact that while papaya of this maturity facilitates implantation there is some factor which hinders full term gestation. Both papain fed group and seed fed group showed

very poor implantation and foetal size. This observation is in tune with the observation on weight gain and days of gestation.

E. Evaluation of litters

1. Morphological features

Morphological examination of the litters from the different experimental groups showed that on parturition the litters from untapped papaya fed group were healthier, deep pink in colour and showed healthy movement. They were nearly full term and the appearance was almost similar as in the control group which also exhibited healthy appearance, movements and deep pink colouration. The ones on tapped and 110 days old papaya were smaller in appearance, did not show movement and were very pale in colouration. This may be partly due to the fact that the gestation was not complete and the litters were not full term. In the case of papain and seed fed groups, the litters were abnormally small, very pale in appearance and the movements were limited. This again could be attributed to incomplete gestation period and these observations are intune with earlier observations of Chandrasekhar and Ragini (1979).

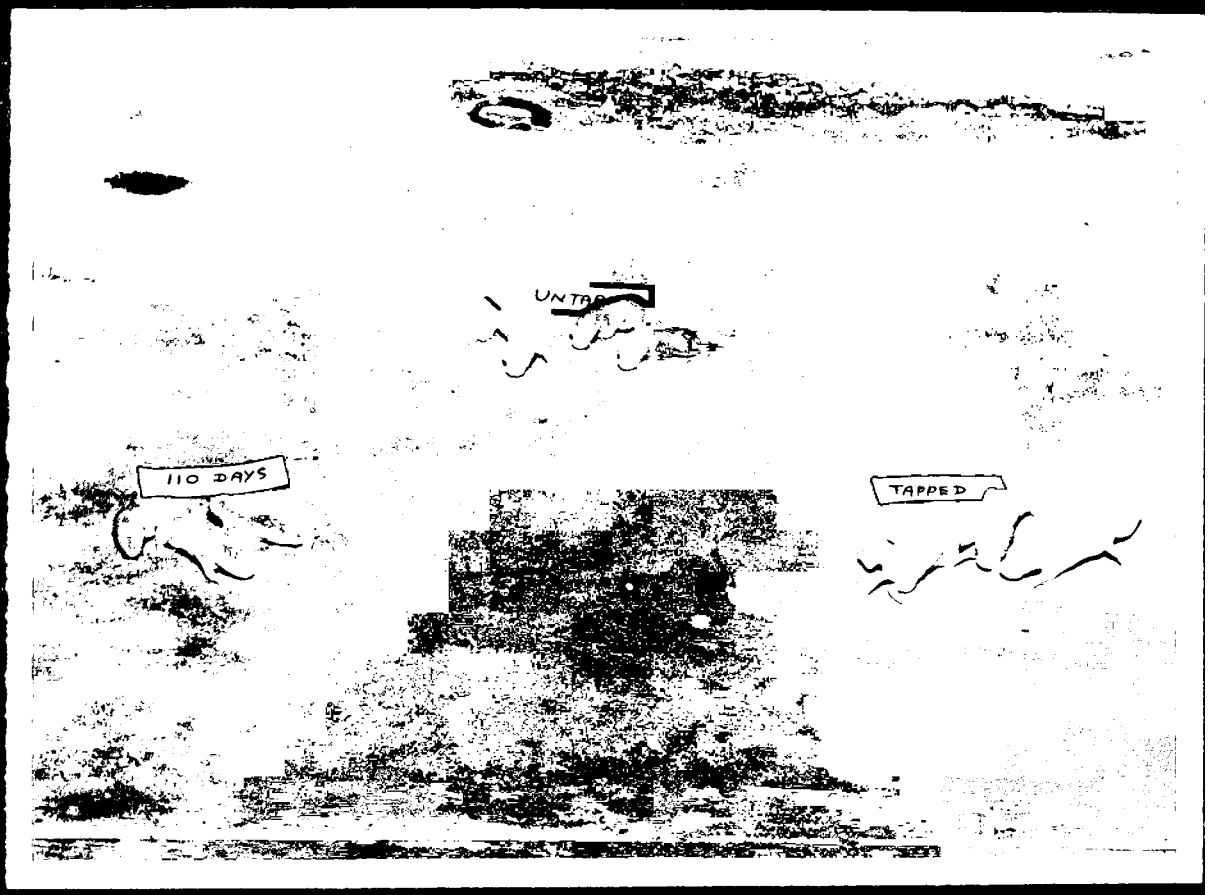
Transparency of the viscera were noted to a very great extent in all experimental groups, papain fed and seed fed having the maximum transparency with superficially white and bluish patches on the skin which was rather pronounced in these groups.

The litters were carefully observed for a period of 30 days and the summary of morphological characteristics observed is given in Table VIII. Figures 1 to 5 shows the litters, experimental, dead and alive, control and the characteristic tail morphology.

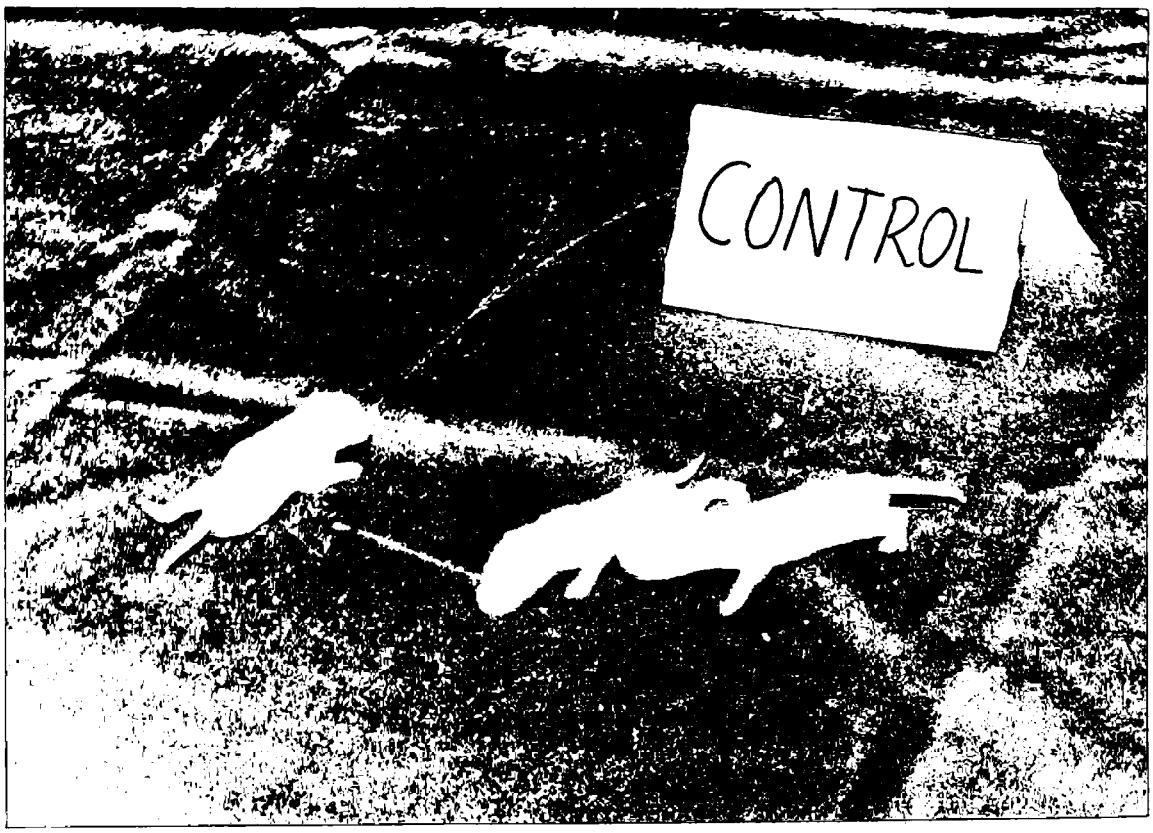
TABLE VIII

SUMMARY OF MORPHOLOGICAL CHARACTERISTICS OF THE LITTERS

Group	Appearance at birth	Viscera at birth	Hair appearance (days)	Opening of eyes and activity (day)	Full growth (day)
110 days papaya fed	Smaller pale coloured	Transparent	9th	22nd	27th
Tapped papaya fed	Smaller, pale pink colour	Transparent	7th	22nd	26th
Untapped papaya fed	Healthy, deep pink colour, active	Transparent	7th	22nd	26th
Papain fed	Very small, very pale movements limited	Transparent	9th	21st	26th
Seed fed	Very small, very pale, limited movements	Transparent	10th	22nd	27th
Control	Healthy, deep pink, active	Not transparent	7th	20th	25th

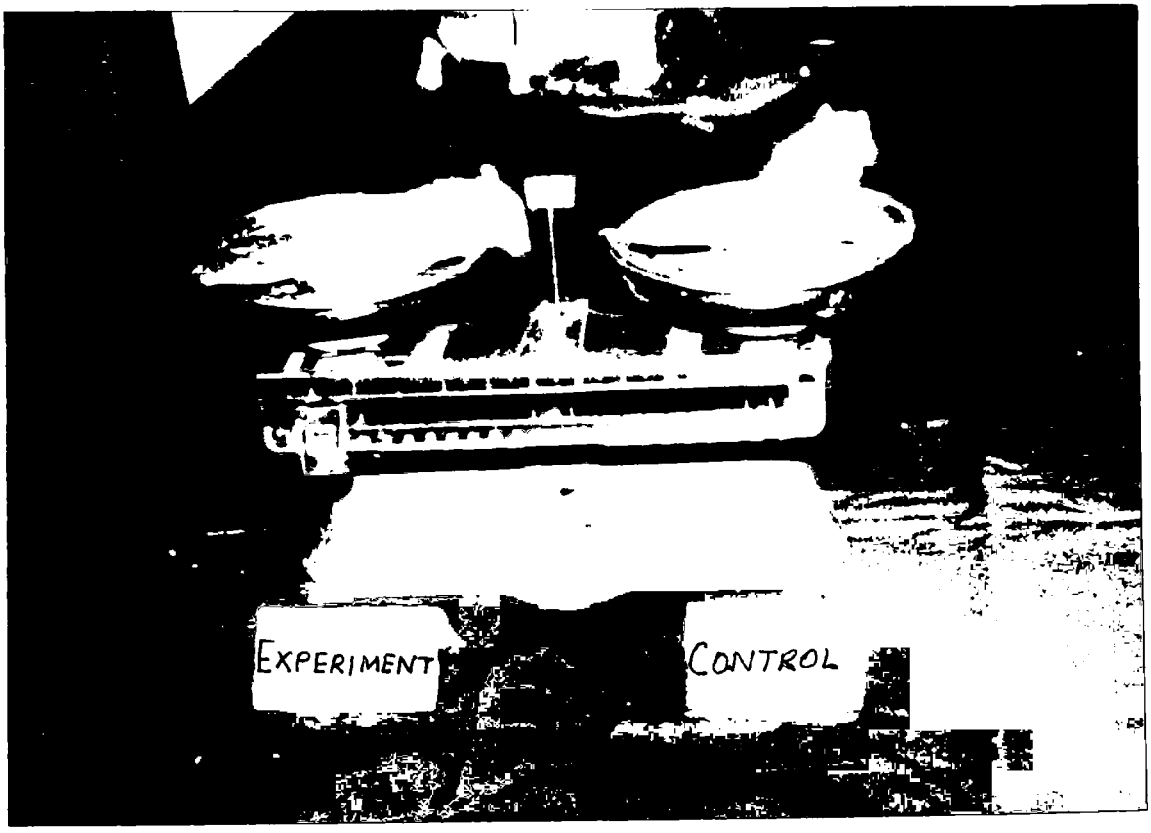


MORPHOLOGY OF LITTERS OF RATS FED DIFFERENT DIETS



MORPHOLOGY OF LITTERS OF RATS FED DIFFERENT DIETS

ABNORMALITY IN THE TAIL NOTICED IN THE EXPERIMENTAL GROUP LITTERS



DEAD LITTERS OF RATS FED DIFFERENT EXPERIMENTAL DIETS

In general opening of the eyes and hair growth was similar in all the groups except that the control group opened the eyes on 20th day and became active in movement. Hair appearance was evident even on the 7th day in the control group as well as in the tapped and untapped ripe papaya fed groups. But it was delayed to the 9th day both in the 110 days old papaya fed group and papain fed group and to the 10th day in the seed fed group. It may also be noted that though appearance of hair was evident the hair was rather sparse, short and coarse in the case of 110 days old, papain fed and seed fed groups. Though the tapped and untapped groups had better hair growth it was not comparable to the smooth, silky and lushy hair growth of control litters.

Head to body proportionality appeared normal but tail appeared to be effected in all the experimental groups. The tails appeared cut and there were bruises specially in the 110 days old, papain fed and seed fed groups. The length of the tail in almost all the experimental groups were ⅔ that of the control group. This observation was evident even three days after littering and failed to show any improvement over the 30 days period of observation. All the rats in the experimental group towards the end of 30 days had noticeably small tails with a chopped off appearance which was rather peculiar and characteristic of all the groups. Similar trends have been reported by earlier workers also (Chandrasekhar and Ragini, 1979).

2. Litter size

Table IX gives the average litter size of the different groups studied.

TABLE IX

AVERAGE LITTER SIZE

Group	Average
110 days	7.0
Tapped	8.0
Untapped	8.0
Papain	5.0
Seed	5.0
Control	8.0

On an average the litter size of control group and untapped and tapped papaya fed group were eight, whereas in the 110 days old papaya fed group it was 7 and strikingly small (5) in the case of papain and seeds fed group. These results are indicative of the anti-implantational effect of both papain and seed and these observations are intune with earlier observations of Chandrasekhar and Ragini (1979).

3. Litter weight and mortality

Table X gives information on litter weight and mortality in groups fed different diets.

TABLE X

LITTER WEIGHT AND MORTALITY

Group	Weight (g)	Mortality %
110 days	7.1	49
Tapped	7.2	46
Untapped	8.0	40
Papain	6.7	75
Seed	6.0	60
Control	8.3	-

The litter weight was around 8.3 g in control group and 8.0 g in the untapped papaya fed group. The litter weights of tapped and 110 days old papaya fed group were 7.2 g and 7.1g respectively as against 6.7 g and 6.0 g in the papain fed and seed fed groups. This information on the average litter weight at birth correlates well with litter size and foetus evaluation and the morphological observations on the litters. Figure 6 represents diagramatically the litter size and weight in rats on different diets.

LITTER WEIGHT AND SIZE OF RATS FED DIFFERENT DIETS

SCALE :-

x axis - 1cm - 1 rat

y axis - 1cm - 1g

KEY

I - 110 DAYS PAPAYA

II - TAPPED PAPAYA

III - UNTAPPED PAPAYA

IV - PAPAIN

V - SEED

VI - CONTROL

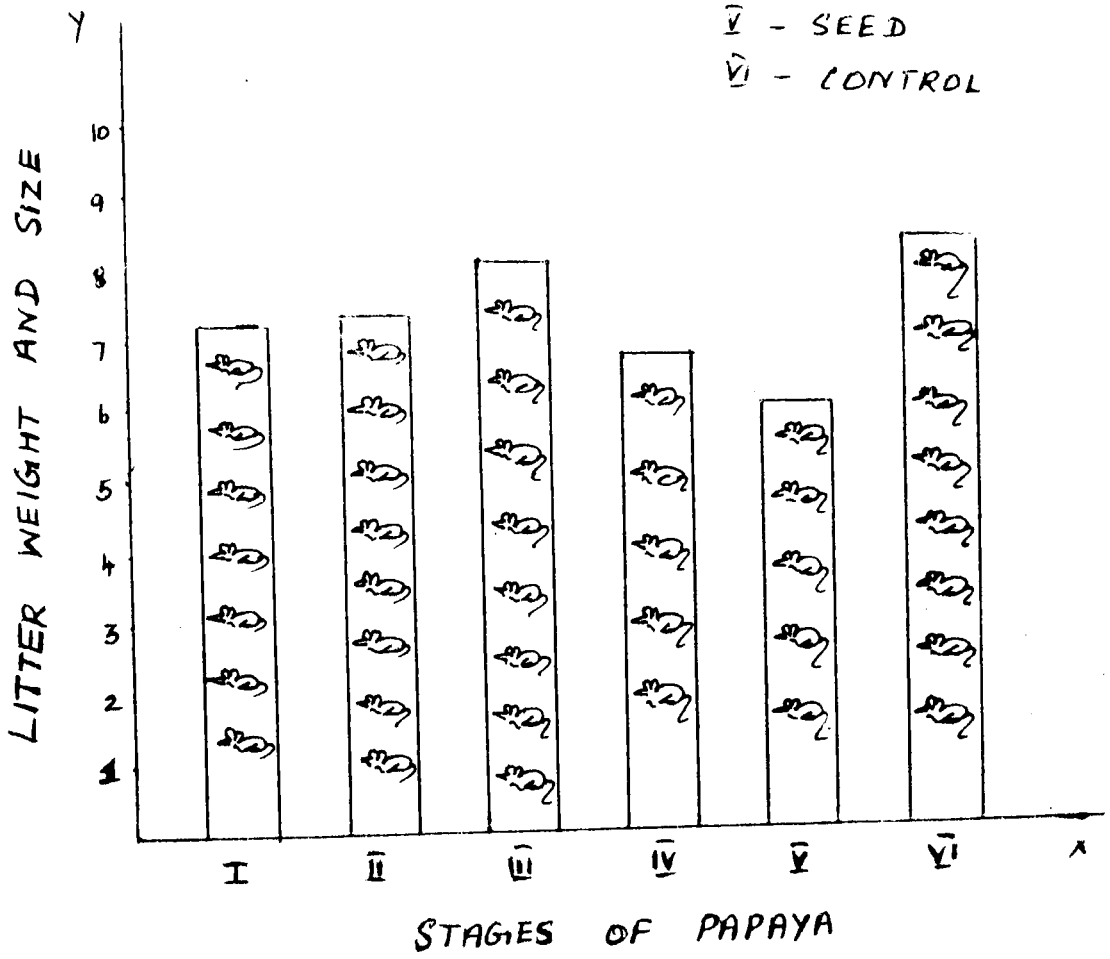


FIGURE . 6

Looking at the percentage mortality in the different experimental group, the highest per cent mortality was noted in papain fed group (75 per cent) with seed fed group coming next in the descending order (60 per cent). The least mortality (40 per cent) was observed in the untapped papaya fed group and the mortality rate in the tapped papaya fed and 110 days old papaya fed groups were 46 and 49 per cent respectively. There was no mortality in the control group. These observations again indicates the possible lethal effects of seeds and papain with least harmful effect of untapped papaya.group. This observation was also very similar to earlier observations by Chandrasekhar and Ragini (1979). Infomation of the weight of litters on separation from the mother rats was maintained and gain in weight computed from average litter weight taken at birth. This data is presented in Table XI.

TABLE XI

LITTER WEIGHT ON SEPARATION

Group	Weight on separation (g)	Gain in weight (g)
110 days	12.19	19.29
Tapped	13.24	20.44
Untapped	20.00	28.00
Papain	3.85	10.55
Seed	6.54	12.54
Control	25.62	33.92

The gain in weight was maximum in the control group (33.92 g). The least weight gain was observed in the papain fed group having a weight gain of ~~12.54~~ 10.55 g. The untapped papaya fed group had a weight gain of 28.00 g while the weight gain of tapped group was 20.44 g and that of 110 days old group 19.2 g. This information of weight gain of litters follows the same pattern of initial weight and percentage mortality indicating that both papain and seed has some lethal quality.

4. Consumption of litters by mother rats

Abnormal consumption of litters by mother rats was another characteristic feature observed in this investigation also and was similar to the observations of the earlier investigations (Chandrasekhar and Ragini, 1979). The data as observed is presented in Table XII.

TABLE XII

CONSUMPTION OF LITTERS BY MOTHER RATS

Group	Number consumed by mother rats	% of litters consumed
110 days	5 rats	21.7
Tapped	2	25.0
Untapped	3	20.0
Papain	5	21.7
Seed	6	37.5
Control	-	-

On an average the per cent consumption was highest in the seed fed group (37.5 per cent) and the least in untapped papaya fed group while no such phenomena was exhibited in the control group.

These observations again provokes us to think that there is some abnormal activity which induces the mother rat to consume their young ones and the trend observed is very similar to the pattern exhibited in other characteristics as well.

5. Male to Female Ratio

The male to female ratio of the litters from the various groups studied is given in Table XIII.

TABLE XIII

MALE TO FEMALE RATIO

Group	Male	Female
110 days	3	2.75
Tapped	1	2.75
Untapped	2	3
Papain	1	3
Seed	2	3.5
Control	2	2

Observations on male to female ratio is similar to earlier observations in that the ratio seems to be even in the control group whereas variations was observed in the other groups.

From the foregoing discussions it may be well observed that the implantation of foetuses, time of delivery, course of gestation, foetal death, premature delivery, consumption of litters by mother rats, morphological characteristics of the litters, their growth pattern are all grossly different in the experimental groups when compared to the control group. Greatest difference in the above characteristics were observed in papain fed group and seeds fed group followed by 110 days old papaya fed group. The untapped papaya fed group seems to be very near to normal. These observations in general were quite similar to earlier observations although the overall lethality as observed by Singh and Devi (1978) on high administration of papain was not witnessed in this study and no littering on seed group as observed by Chandrasekhar and Ragini (1979) was also not observed in this investigation. However, both these groups exhibited abnormalities in the course of pregnancy short gestational period, early delivery high litter mortality, low litter size and weight.

These observations though were of a lesser degree in the 110 days although this group also did not permit normal course of pregnancy to its full term. The tapped papaya fed group was

better in certain characteristics like litter size, but the untapped papaya fed group was closer to the control group in carrying through the course of pregnancy closer to full term and resulting in better litter characteristics.

Thus judging from the various parameters studied in this investigation the untapped ripe fruit, the form in which papaya is normally consumed by human beings proved to be the best in carrying through the course of pregnancy as evidenced by significant weight gains, food intakes, normal uterine contraction, littering morphologically healthy status of litters, production of viable litters and better growth pattern of litters. While papain at higher concentration and seeds are positively detrimental to the course of pregnancy in rats as observed in this investigation, effect of tapped papaya needs further investigation. The 110 days old papaya as in the earlier investigation displayed negative deviations in aspects of weight gain, food intake, uterine contractions, term of gestation and litter morphology thus making us speculate the possibility of considering papaya at this stage of maturity to be less acceptable, anti implantational foetal growth retarding and blasto cyto toxic. This might have been the cause for having some negative effect on the course of pregnancy. Farther studies on experimental animals and possible studies with adolescent girls on the course of menstruation may throw further light on this aspect.

Summary And Conclusion

V. SUMMARY AND CONCLUSION

The aim of this investigation was to evaluate the effect of feeding papaya at different stages of maturity to a group of adult pregnant albino rats and to observe its effect on the course of pregnancy and littering. In order to maintain uniformity and to avoid variations in the variety of papaya fed CO_2 variety from the Tamil Nadu Agricultural University Research station was procured everyday from trees marked ahead of the experiment, at 110 days raw papaya, tapped and untapped ripe papaya and the wet latex of the immature fruit.

Five experimental diets were planned using the raw and ripe papaya, papain and seeds with a group of comparable rats which were given the stock diet, serving as the control. The planned diets were given to groups of adult female albino rats, each from the day of pregnancy till the time it delivered, litters and the weanling rats separated from it. The rats were evaluated with indices of weight gain, food and papaya intake, uterine contraction during the gestational period of the pregnant papaya fed group in contrast to its non-papaya fed counterpart and foetus and litter status.

<u>The findings revealed that</u>

1. The mean body weight gains ranged from 35.17 to 54.54 g in all groups during pregnancy. Rats fed the control diet recorded the highest weight gain followed by rats fed the

untapped ripe papaya. The least weight gain was exhibited by rats fed papain. There was no significant difference between the experimental groups and control group, when analysed statistically.

2. The mean food intake of rats fed different diets ranged from 61.89 g to 80.44 g, while the mean papaya intake ranged from 61.04 g to 63.11 g. The mean food intake between groups when analysed statistically was significant between all the experimental groups and the control group.

3. Isolated uterine muscle contractions were recorded on the 7th and 14th days of gestation from the experimental group and compared with the control group. The control group and all experimental rats showed low frequency contractions on the 7th day. The contraction relaxation phase was irregular and wavy whereas the control showed a regular pattern of uterine contraction and relaxation phase. On the 14th day of gestation, the contractions were irregular but strong, probably ready to expell the foetus in the case of tapped papaya fed group, seed fed group, 110 days papaya fed group and papain group. The untapped papaya fed group showed regular contraction and relaxation with increased frequency which was nearer to control group observations.

4. The gestation period was 21-23 days, the normal period for albino rats in the case of control rats as against the lower gestational periods observed in the experimental groups.

From among the experimental groups untapped ripe papaya came nearer to full term gestation (19 days) while all others had a gestational period of 15-17 days only.

5. Foetus evaluation on the 7th and 14th day of gestation in all the experimental groups and control group revealed that untapped papaya fed group had implantations similar to the control group. The implantations in the 110 days papaya fed group and tapped papaya fed group showed unhealthy foetus in comparison to the control group; whereas papain fed and seed fed groups showed very poor implantations and foetal size.

6. Comparison of yield of litters between the experimental groups and the control groups revealed that untapped papaya fed group neared the control group in litter size, litter weight on birth and weight on separation from the mother rats. The litter mortality was highest in the papain fed group and limited in the untapped papaya fed group. The abnormal consumption of litters by mother rats was highest in the seed fed group.

7. Morphological features of the litters of the rats in the experimental groups were nearer to the control litters, but for its sparseness in hair, lightness in weight and abnormalities noted in their tails.

The results indicate that judged from the parameters of weight gain, food intake, isolated uterine muscle contractions, evaluation of foetus and litters, groups fed untapped papaya and control diet were superior to all other experimental diets. They recorded the maximum weight gain in contrast to the lowest weight gain exhibited by papain fed group. Groups fed control diet recorded the maximum food intake followed by the untapped papaya fed group. The isolated uterine muscle contractions showed that the contractions of the untapped papaya fed group was similar to that of the control group with regular contractions and relaxations; and the foetal size and implantation was comparitively better in the untapped papaya fed group than other experimental groups. The litter size, weight, morphology and pattern of growth resembled the control in the untapped papaya fed group and low littering quality was observed in the papain fed and seed fed groups.

From the foregoing discussions, it may well be concluded that although papain and seed fed groups exhibited detrimental effects, there is a positive indication that papaya at its full maturity (riped, untapped) a stage and state at which it is normally consumed does not seem to have any detrimental effects on the course of pregnancy and foetal growth in albino rats.

To draw more valid conclusions it is however suggested that similar studies be repeated on albino rats and higher animals

like monkeys atleast with papaya of 110 days old maturity and riped fruit.

Promises for further research in this field are wide. Papaya in different amounts, with different food combinations in different forms, at different time intervals in the period of gestation, through different modes of administration and repetition is essential to draw further conclusions.

Longitudinal studies, stretching through generations need also to be done, to further establish their effect on the morphology of the reproductive organs and of the external morphology of the animal. It would also be of interest to study via isolating the factors responsible for the harmful effect. Varietal difference in papaya can also be experimented upon and longterm feeding of papaya alternating it with no papaya diet can also be taken up. It is also suggested that impact of feeding papaya be done on the course of menstruation in young adolescent girls to further establish any possibility of harmful effect on menstrual cycle and such continued work may throw light on the actual possible role, if any of papaya and thus gives core for further research.

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Appendices

APPENDIX I

Composition of Mineral Mixture (g/100 g)

Potassium dihydrogen phosphate	38.9
Calcium carbonate	38.14
Cobalt chloride	0.0025
Cupric sulphate	0.0477
Ferrous sulphate	2.7
Magnesium sulphate	5.73
Manganese sulphate	0.401
Potassium iodide	0.079
Sodium chloride	13.93
Zinc sulphate	0.0548

Composition of Vitamin Mixture (in 1 g)

Vitamin A	1000 IU
Vitamin D	100 IU
Vitamin E	10 IU
Vitamin K	0.5 mg
Thiamine	0.5 mg
Riboflavin	1.0 mg
Pyridoxine	1.4 mg
Pantothenic acid	4.0 mg
Niacin	4.0 mg
Choline	200 mg
Inositol	25 mg
PABA	10 mg
Vitamin B_{12}	2 μg
Biotin	0.02 mg
Folic acid	0.2 mg

Add sufficient corn starch to make upto 1 gm.

APPENDIX II

COMPOSITION OF DALE'S FLUID

Components	Concentration in g %
Sodium chloride	0.9
Potassium chloride	0.042
Calcium chloride	0.024
Magnesium chloride	0.0005
Sodium bicarbonate	0.05
Dextrose	0.05

APPENDIX - III

WEIGHT GAIN OF RATS FED DIFFERENT DIETS

Diet/Wt. Gain (g)	Rat number							
	1	2	3	4	5	6	7	8
I. 110 days Papaya fed	19.1 *	44.7	41.1	55.2	64.8	23.6	44.3	15.1
II. Tapped Papaya fed	15.8 *	31.6	31.8	5.8 *	12.7	58.6	29.9	55.7
III. Untapped papaya fed	52.1	8.2 *	8.1 *	11.2 *	25.7 *	52.9	54.1	51.2
IV. Papain fed	69.2	24.1	12.6	21.7	10.1	64.9	14.1 *	43.6
V. Seed fed	75.1	12.4 *	24.8	41.2	75.2	32.1	4.2 *	35.6
VI. Control	20.3 *	20.7 *	16.4 *	49.6	54.2	50.1	40.7	78.1

* For calculating the means, these weights was not considered because these rats were either sacrificed for recording uterine contractions in the first or second week or they did not litter.

APPENDIX IV

STATISTICAL ANALYSIS OF GAIN IN WEIGHT

Diet	't'	Diet	't'
I VS II	0.20 NS	III VS IV	1.38 NS
I VS III	1.29 NS	III VS V	0.68 NS
I VS IV	0.24 NS	III VS VI	0.40 NS
I VS V	0.17 NS	IV VS V	0.34 NS
I VS VI	0.43 NS	IV VS VI	0.55 NS
II VS III	0.63 NS	V VS VI	0.25 NS
II VS IV	0.06 NS		
II VS V	0.42 NS		
II VS VI	0.74 NS		

NS - Not significant

APPENDIX V

TOTAL FOOD AND PAPAYA INTAKE OF RATS FED DIFFERENT DIETS

(g)

		Rat Number							
		1	2	3	4	5	6	7	8
I	110 days								
	Food	75.60	76.92	75.44	77.81	75.34	75.57	76.84	76.86
	Papaya	60.48	61.53	60.35	62.25	60.27	60.46	61.47	61.49
II	Tapped								
	Food	78.61	77.45	77.52	77.65	77.92	78.55	77.45	77.23
	Papaya	62.88	61.96	62.02	62.12	62.34	62.84	61.96	61.78
III	Untapped								
	Food	78.11	78.65	79.21	79.22	78.76	78.52	79.16	79.44
	Papaya	62.49	62.92	63.36	63.38	63.01	62.82	63.33	63.55
IV	Papain								
	Food	68.40	68.65	69.75	69.09	69.62	68.43	68.32	69.60
	Papain (mg)	775.2	778.0	790.5	783.02	789.0	775.5	774.3	788.80
V	Seed								
	Food	61.07	63.42	61.42	63.00	61.45	61.42	61.32	62.06
	Seed	8.76	9.18	8.89	9.12	8.89	8.89	8.88	8.89
VI	Control								
	Food	79.90	80.62	80.00	80.88	80.75	80.88	80.54	79.99
	Papaya	-	-	-	-	-	-	-	-

APPENDIX VI

STATISTICAL ANALYSIS OF FOOD INTAKE

Diet	't'	Diet	't'
I VS II	4.00 **	III VS IV	37.09 **
I VS III	7.14 **	III VS V	49.34 **
I VS IV	18.72 **	III VS VI	7.21 **
I VS V	32.21 **	IV VS V	18.97 **
I VS VI	11.60 **	IV VS VI	44.02 **
II VS III	4.48 **	V VS VI	54.67 **
II VS IV	31.12 **		
II VS V	44.53 **		
II VS VI	11.23 **		

** significant at one per cent level