

Use of pressure cooked Mahuda flowers as an energy yielding component in the diet of pregnant rats Lactating rats and their off-springs.

(Experiment IV)

Objective - 4(a) To explore the effect of feeding pressure cooked Mahuda flowers as the source of carbohydrate, on the nutritional status of pregnant rats and proies, of reproduction.

Objective - 4(b) To investigate the effect of feeding pressure cooked Mahuda flowers as the carbohydrate source, on the lactational performance of the dams in terms of growth rate of pups upto weaning age.

Highlights of the results

During the period of pregnancy and lactation, it would not be safe to consume Mahuda flowers to contribute about 10% (25g Mahuda flowers 100g diet) of the total carbohydrate in the diet but, if flowers are to be eaten due to agricultural, lean months or famines, it is suggested that the dietary preparation containing Mahuda flowers should be avoided during first half period of pregnancy. These results supported the superstition of the tribal women that consumption of Mahuda flowers cause ill effects on reproductive performance.

Introduction

As stated earlier Mahuda flowers form an important food component in tribal diet during the periods of food scarcity in lean months and famine (Health of India 1962). However, the information available indicated that among tribals, mild apprehension existed towards free utilization for all age groups, of Mahuda flowers as a food source (Gopaldas et al 1983c). Surveys conducted to find out utilization and storage practices of Mahuda flowers by the tribals of Chhotaudepur district of Gujarat (Gopaldas et al 1983a,b) revealed that pregnant women were not consuming Mahuda flowers because of the belief that it is a 'hot' food and has abortifacient nature. Likewise, the lactating mothers refrained from eating Mahuda flowers for the fear that the consumption of flowers might cause gastro-intestinal upsets in the infants.

It is believed that food beliefs in any group or community significantly influence the nutritional status of the community, hence become an important factor in any nutritional program. Cultural patterns laid down by parents and associates tend to classify, for their children, food items as being appropriate or inappropriate in certain situations. Such tradition and folk beliefs greatly influence the diet of women during the most important periods of pregnancy and lactation. Devedas and Vanitha (1979) reported that the food fads or beliefs of both the 'wellfed' and 'poorly fed' groups were more or less the same. The authors listed the foods like papaya, brinjal, pineapple, groundnuts and eggs which were avoided during pregnancy for the belief that they have poor digestibility and/or are of "heat," nature. And listed those such as mango, ice cream, cucumber, radish and citrus fruits of

all varieties that were avoided during lactation for fear of intestinal disorders in the infant.

A study conducted by Saxena (1981) on the dietary practices of the Rathwadioli tribe of Gujarat has revealed that spices like salt and chilli, Urad dal, Beans, Rice, Ground nuts, Curd, Papaya, Jowar, Maida and Makh were considered as harmful foods during pregnancy and were believed to damage the fetus. Foods avoided during lactation were Urad dal, Jowar, New Maida, and Makh by the tribal women. The study also revealed that Manuda liquor had a special significance in tribal dietaries during puerperium as it was believed to stop the post-partum bleeding.

Recently, Mittal and Gopaldas (1985) collected information on culturally acceptable mother food in Gujarat. The authors reported that although Methi (fenugreek seeds) formed an important constituent of a special maternal food consumed during pregnancy and lactation in western India, its use during pregnancy was found less common because of the belief that it causes abortion.

Not all the food beliefs could be considered unscientific as adverse effects of consumption of foods that may contain some toxicants, on the pregnancy performance have been reported. Torre et al (1977) had examined the effect of feeding Cassava diet on placental thiocyanate transfer and gestational performance of rats. The pregnant rats were fed for 19 days, on diet containing 57.9% fresh Cassava. The Cassava fed rats exhibited reduction in weight gain and increased levels of thiocyanate in urine, serum and amniotic fluid although fetal thiocyanate content did not alter.

Earlier, Dollabate and Anthon, (1957) suggested that saponin from

The perennial herbaceous plant, *Xanthoxylum microcarpum* which is common, known as the Broom weed, had adversely affected the size, maturity, and strength of the fetuses of cows, however, no teratogenic effects were observed. Later, (Lourno, et al (1972) injected pregnant albino rats with saponin from Broom weed at dosage level of 5, 15 and 25 mg/1g B.wt. from 7th through 21st day of the gestation period. The saponin injected rats exhibited reduction in weight which was observed to be dose dependent. The rats having received the lowest dose (5 mg saponin/1g B.wt.) littered normally in the expected gestation time while those having received 15 mg saponin/1g B.wt. had gestation period beyond term. The rats injected with higher dose of saponin (25 mg/1g B.wt.) showed fetal resorption and some of them died during the gestation period. In addition, the size and weight of the off springs of saponin injected rats were smaller than those of the controls indicating stunting effects of saponins. But no evidence of teratogenicity was found in any of the off spring examined grossly for skeletal and soft tissue malformations.

Placenta is the most vital organ for fetal development. In several ways, it mediates the effects of maternal nutrition on fetal growth. Poor maternal nutrition may impair placental growth thus reducing the surface area of placental villi, or inhibiting the proliferation of villous capillaries. It has been reported that such changes lead to reduced maternal-fetal exchange (Rush et al 1984). The nutrition of embryo and the elimination of its metabolic products are depended upon the state of the placenta. It is necessary for normal fetal growth that the placenta has sufficient size and a normal structure (Groud 1971).

The normal rat placenta as described by Davies and Glazier (1968)

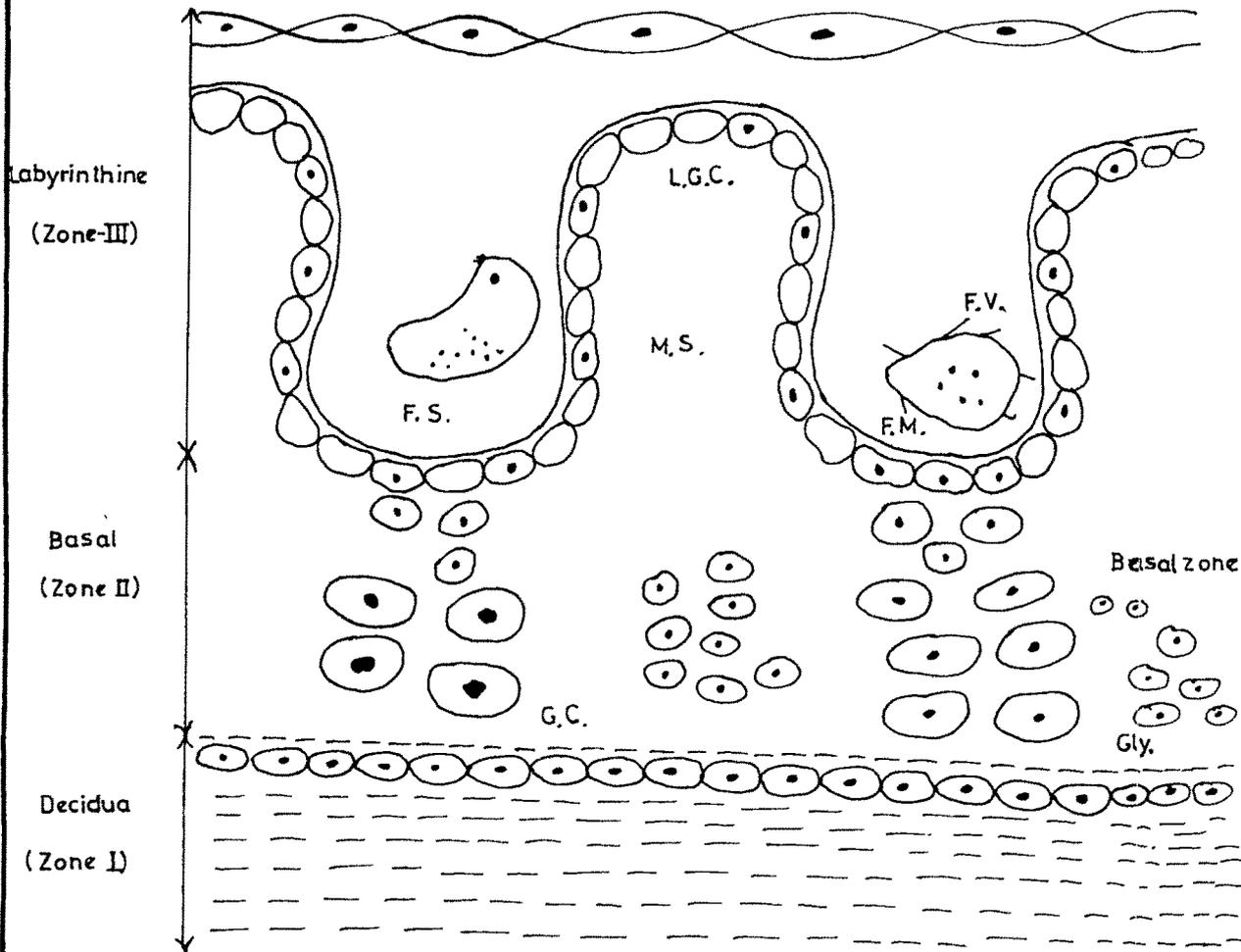
has at least 3 zones (Figure 1.1). The decidua basalis or zone I is composed of cellular and fibrous tissue which imperceptibly continues into the maternal blood at the site of implantation. The basal zone or zone II contains 3 types of cells namely, small giant cells, large giant cells and glycogen cells. The maternal blood passes through the spaces between these cells before entering the labyrinthine zone (zone III) which is comprised of labyrinthine blood vessels of fetus and maternal sinusoids separated from each other by a trophoblast membrane.

The functions of placenta are modified throughout the gestation period. According to Goodman et al (1982) the functions of placenta range from providing a protective barrier that mediates the maternal-fetal exchange of gases, electrolytes, nutrients and wastes, to endocrine functions such as synthesizing gonadotrophins, oestrogens, progesterone and steroids. In addition, the placenta has function of detoxifying the maternal before it reaches to the fetus.

Certain drugs or food toxicants have caused histopathological changes in the rat placenta. Maternal administration of Chlorpromazine hydrochloride (CPZ), a widely used tranquillizing agent led to severe placental histopathological changes in rats irrespective of the dose and stage of administration (Padmanabhan and Singh 1973 and 1980). The authors reported that CPZ injected to mothers caused reduction in weight and volume of the placenta, length of the umbilical cord and volume of the amniotic fluid followed by fetal growth retardation.

Since Mahua flowers are rich source of carbohydrates but its use is restricted during pregnancy and lactation, one experiment IV was designed to explore the impact of feeding Mahua flowers on the

Figure:4-1 Schematic representation of haemotrichorial placenta of rat.



F.M. → fetal mesenchyme

G.C. → giant cell

L.G.C. → large giant cell

F.S. → fetal sinusoids

F.V. → fetal vessel

Gly → glycogen cell

M.S. → maternal sinusoids

source of carbohydrate on the nutritional status of (i) pregnant rats, (ii) lactating rats and (iii) their off-springs. The results of experiment II and III on the use of Mahuda flowers as an energy yielding component in the diet of weaning rats (Chapter 5) suggested that the diet containing 25M20 (25g Mahuda flowers per 100 g diet, cooked for 20 minutes) was nutritionally superior to 25M10 (25g Mahuda flowers per 100 g, cooked for 10 minutes) or 50M10 (50g Mahuda flowers per 100 g diet, cooked for 10 minutes) or 50M20 (50g Mahuda flowers per 100 g diet, cooked for 20 minutes) diets. Therefore, for these studies of experiment IV, 25M20 diet was used.

The two specific objectives of the experiment IV were

- (a) To explore the effect on the nutritional status of pregnant rats and on the products of reproduction, of feeding during first half or second half or entire period of gestation, diet containing Mahuda flowers cooked for 20 minutes (25M20 diet) as the source of carbohydrates. (Objective - 4(a)).
- (b) To investigate the effect on the lactational performance of the dams in terms of growth of pups upto 21 days and on the nutritional status of pups, of feeding 25M20 diet during entire gestation and lactational period or during gestation period only (late or cumulative effects) or during lactation period only (current effect) (Objective - 4(b)).

The effects of feeding 25M20 diet were evaluated against those of feeding sago-bengalgram (SP diet) or Casein (C diet) diet.

To fulfill the above objectives the parameters used or tests performed were .

For objective (a)

- 1) Maternal weight gain
- 2) Food intake
- 3) Litter size (Fetuses/litter)
- 4) Average fetal weight
- 5) Average placental weight
- 6) Serum protein levels and A/G ratio.
- 7) Haemoglobin level of the pregnant rats
- 8) Placental histopathology.

For objective (b)

- 1) Maternal body weight changes
- 2) Food intake
- 3) Litter size
- 4) Litter weight
- 5) Pup weight on weaning day
- 6) Organ weights of weaned pup (Liver, Heart, Spleen, Kidney, Intestines)
- 7) Haemoglobin level of weaned pup
- 8) Hepatic lipid content of weaned pup
- 9) Serum total protein and its fractions of weaned pups

Materials and Methods

The experiment IV comprised of two separate studies. Study 1 was designed to determine the use of Mahuda flowers as an energy yielding component in the diet of pregnant rats. While study 2 determined, the utility of Mahuda flowers as an energy yielding

component in the diet of lactating dams.

In study 1, 30 adult female rats of the Wistar strain weighing between 190 to 220g were caged overnight with males from the same stock and comparable weight. On the following morning insemination was confirmed by vaginal smear test. The sperm positive day was labelled as day 1 of the pregnancy period. The pregnant females were randomly divided into 2 control and 2 experimental groups of 6 rats each, and were fed control or Mahuda flowers diet ad libitum for 20 days of the gestation period. The composition of the control and Mahuda flower diets (25M20 diet) was the same as presented in Table 3.1 (Chapter 3). The allotment of the diets to the different groups and the symbols used are outlined below

Allotment of the control and Mahuda flowers diets to the pregnant rats and the symbols used.

- Group 1 Casein diet fed throughout the gestation period (C diet/group)
- Group 2 Sago-bengalgram diet fed throughout the gestation period (SB diet/group)
- Group 3 25M20 diet fed throughout the gestation period (M diet/group)
- Group 4 Sago bengalgram diet fed during first half of the gestation period followed by feeding of 25M20 diet during second half of the gestation period (SBM diet/group)
- Group 5 25M20 diet fed during first half of the gestation period followed by SB diet fed during second half of the gestation period (MSE diet/group)

For study 2, one day pregnant rats were randomly divided into 2 control and 2 experimental groups of 6 rats each and were fed ad libitum control or Mahuda flower diet throughout the gestation and lactation period. The composition of the control and Mahuda flower diets were the same as described in table 2.1 (Chapter 2).

The symbols used and diets offered to the different groups of pregnant rats during gestation (P) plus lactation (L) period are presented below

Dietary allotment to the control and Mahuda flower fed pregnant rats during gestation plus lactation period.

- Group 1: Casein diet fed throughout gestation and lactation period (P-LC diet/group)
- Group 2: Sage-bengalgram diet fed throughout gestation and lactation period (P-LS2 diet/group)
- Group 3: 25M20 diet fed throughout gestation and lactation periods (P-L1 diet/group)
- Group 4: Sage-bengalgram diet fed during the gestation period and 25M20 diet during the lactation period (PSB-L1 diet/group)
- Group 5: 25M20 diet fed during the gestation period and sage-bengalgram diet fed during the lactation period (PM-LSB diet/group)

The pregnant rats were housed in individual galvanized iron cages. Food and water were offered ad libitum. Every alternate day the rats were weighed and food bowls were changed. Food consumed was recorded using the methodology described earlier (Chapter 3).

Autopsy procedure

On the 21st day of the gestation period, the pregnant females of each group were killed by ether inhalation. The abdomen was opened by a lower midline incision and the uterine horns were exteriorized. The fetuses and placentas were separated by uterotomy and weighed. The blood was collected from jugular vein, for serum protein estimation. At the same time, 0.02 ml of blood was drawn and immediately expelled into 5 ml of Drabkin's solution for haemoglobin estimation. For histopathological examination, randomly selected two placental tissue of two pregnant female rats from each of the SD, H, NSB, and SBH diets fed rats, were kept in formaldehyde fixative.

The duration of study comprised of gestation and lactation periods. Records were maintained for food intake and maternal weight change during the gestation and lactation periods. The litter size and litter weight were recorded on the day of delivery. Each dam was allowed to nurse 6 pups to study the lactation performance. The pups were weaned at the age of 21 days. Records were maintained on growth rate of the pups until they were weaned. On the 22nd day of lactation period, randomly selected two pups per mother, were lightly ether anaesthetized and the blood was collected from jugular vein for serum protein estimation. The 0.02 ml of blood was measured and immediately expelled into 5 ml of Drabkin's solution for haemoglobin estimation. Midline and crosswise incisions were made in the abdominal and thoracic regions, the liver was quickly removed and immediately placed on ice. The non hepatic tissues were trimmed, the liver was blotted on filter paper, weighed and sampled for total hepatic lipid content. The samples were stored frozen until

analysed. Heart, Liver, Spleen and Intestines were removed and weighed.

Analytical procedures

1) Serum proteins

Serum protein was estimated using Duret method as modified by Varley (1969).

2) Haemoglobin

Haemoglobin was determined by cyanmethaemoglobin method as described by Osler (1976).

3) Hepatic lipids

Hepatic lipids were extracted according to the method of Fulch et al (1957) and estimated gravimetrically.

All the above mentioned methods are described in detail in chapter 5, under the head Materials and Methods.

4) Histopathology of placenta

Methods of Lulling (1957) was used for preparation of slides. The steps involved were as follows :

(a) **Sectioning and fixation :** The placentas were fixed in formaldehyde solution. Fixation is done to prevent putrefaction and autolysis, to preserve and harden the tissue, to solidify colloid material and to facilitate visual differentiation of the structures. Three to five millimetre thick slices were cut from the placental tissue.

(b) **Dehydration :** The tissue slices were dehydrated by immersing them in increasing strengths of ethyl alcohol, 70, 80, 90 and 100%.

- (c) **Cleaning** : The dehydrated tissue slices were blotted on damp filter paper and then cleared with xylene because of its miscibility, in both alcohol and paraffin. The slices were then immersed in 50 to 100 times their volume of xylene for 10 minutes, removed from xylene and blotted on filter paper.
- (d) **Embedding** : After blotting lightly on filter paper, the tissue slices were transferred from the cleaning agent (xylene) to molten paraffin wax maintained at 56°C in an oven. The volume of the wax used was about 25 to 50 times the volume of the tissue. The slices were left in oven at room temperature until they hardened.
- (e) **Microtome sectioning** : Five micron thick sections of the wax embedded tissue slices were prepared with the help of a standard microtome.
- (f) **Staining and mounting** : The sections were stained with haematoxylin and Eosin dyes and were mounted in a commercial (D.P.X.) mountant.
- The prepared slices were examined under microscope to observe morphological changes if any.

3) Serum protein fractions

For serum protein fractions Agar or Agarose gel electrophoresis method was used, as described by, Varle, et al (1980).

Principle of the procedure : The gel electrophoresis is carried out in a buffered medium at a fixed pH so as to charge the particles and hence the rate of migration is stabilised. The Agar gel has the advantage of a low adsorption of proteins with

resulting well defined bands.

Procedure : For preparation of gel, gel slides and reagents see Appendix I. A 5mm slot across the middle of the slide was made and 2 to 3 μ l serum was inserted into this with the help of a fine capillary, pasteur pipette. The slides were inverted and laid acrosses withs of electrophoretic unit. The voltage gradient of 7mA per slide was allowed to run for 60 minutes or until a bromophenol blue marker showed that the albumin band had moved 25 to 30 mm. The slides were removed and placed in cold dilute acid ethanol (ethanol-water-acetic acid, 70:25:5, v/v) for 30 minutes. They were then dehydrated by immersing in acetone-water (90:10, V/V) for 4 hours and were finally, dried in an incubator at 37 $^{\circ}$ C.

The slides were immersed in Amiduschrom 10B solution for 30 minutes. They were then washed by immersing three times in fresh portions of acetic acid (2% w/v in water) for 5 minutes. The slides were rinsed for 10 minutes under water and were kept for drying at 37 $^{\circ}$ C. The slides were made more clearer by placing in xylene and later, on mounted in depex using a 64 x 22 mm cover glass. This separated the beta globulins into beta 1 and beta 2 bands and the gamma globulins moved towards the cathode. The slides were made transparent by impregnating with Ordina oil 17 (shell) which has the same refractive index as of the membrane. The transparent slides were placed for scanning. All the different fractions on slides were scanned using a 100 nm slit. Summation of all the fractions gave the value for total serum protein. Serum was also analysed for total serum protein by biuret method as described in (Varle, 1980).

Statistical Evaluation

Means and standard errors were calculated. The data on all the parameters were subjected to analysis of variance. Where ever F ratio was found significant the group means were tested for significance of differences by using the student's t test. All tests were considered significant at $P = 0.05$ level (Snedecor and Cochran, 1967).

Results and Discussion

The present experiment IV comprised of two separate studies. Study 1 explored the effect of feeding pressure cooked Mahuda flowers as an energy yielding component on the nutritional status of the pregnant rats. While study 2 determined the effect of feeding Mahuda flower containing diet on the lactational performance of dams as evaluated by the growth of off-springs up to weaning age.

Thirty one day pregnant rats were divided into five groups of 6 rats each to be fed for 20 days of the gestation period, either casein diet (C diet/group) or Sago-Bengalgram diet (SB diet/group) or 25%CB diet throughout the gestation period (M diet/group) or SB diet during first half of the gestation followed by M diet during second half of gestation period (SBM diet/group) or M diet during first half of the gestation followed by SB diet during second half of the gestation period (MSB diet/group). Data were collected on food intake and total weight gain. The pregnant rats were autopsied on 21st day of one gestation period to evaluate reproductive performance in terms of litter size, litter weight and placental weight. Bio chemical status of the pregnant rats was judged by estimating total serum proteins and haemoglobin levels. Placental

histopathology, was done to investigate changes if any, in placental morphology in rats fed Mahuda flower diets.

Food intake

The mean values for cumulative food intake are presented in Table 4.1. The F ratio of 6.29 was significant at $P = 0.05$ level indicating that the food intake among various groups significantly differed from each other. The comparisons between the two means indicated that the rats fed SB diet ate significantly more than those fed casein diet. There were no such significant differences in food intake between the rats fed SB or M diets. These results suggest that inclusion of pressure cooled Mahuda flowers into the diet at 25% level did not alter acceptability/palatability of the diet for pregnant rats. Likewise, food intake did not vary among the groups fed Mahuda diet regardless of the stage of pregnancy at which it was fed.

To find out whether the food intake of Mahuda diet fed rats remained consistent throughout the gestation period, the data on food intake was broken into that of first half and second half of the gestation period (Table 4.2). It was noticed that in relation to rats fed SB diet, those fed Mahuda diet in first half of the gestation period (H and MSB groups) tended to eat less food. The gap in food intake between SB and H or MSB groups became narrow in the second half of the gestation period (Figure 4.2A). The difference in cumulative food intake between SB and H diet fed rats was 24.5g in first half and 4.5g in second half of the gestation period. However, such effects were not observed in rats fed H diet in first half of the gestation period (MSB group). The rats fed SB diet in first half of the gestation period (H group) ate as much diet in second half of

Table 4.1 Food intake, total weight and maternal weight gain in various groups during 20 days of gestation period.

Groups	Food intake ¹ (g)	Total weight gain (g)	Maternal weight gain ² (g)
		MEAN ± SE	
C	214.3 ±11.11	70.3 ±2.62	24.4 ±2.31
SE	328.5 ±19.24	98.0 ±6.17	34.5 ±3.22
M	299.4 ±16.64	77.3 ±5.27	48.2 ±6.05
SBM	319.1 ±15.86	70.2 ±4.05	50.5 ±4.46
MbD	377.1 ±22.14	79.2 ±6.94	51.1 ±4.27
F-ratio	5.39*	4.16*	5.10*

¹Values of Mahuda /lower diet have been adjusted for the moisture content of the diet

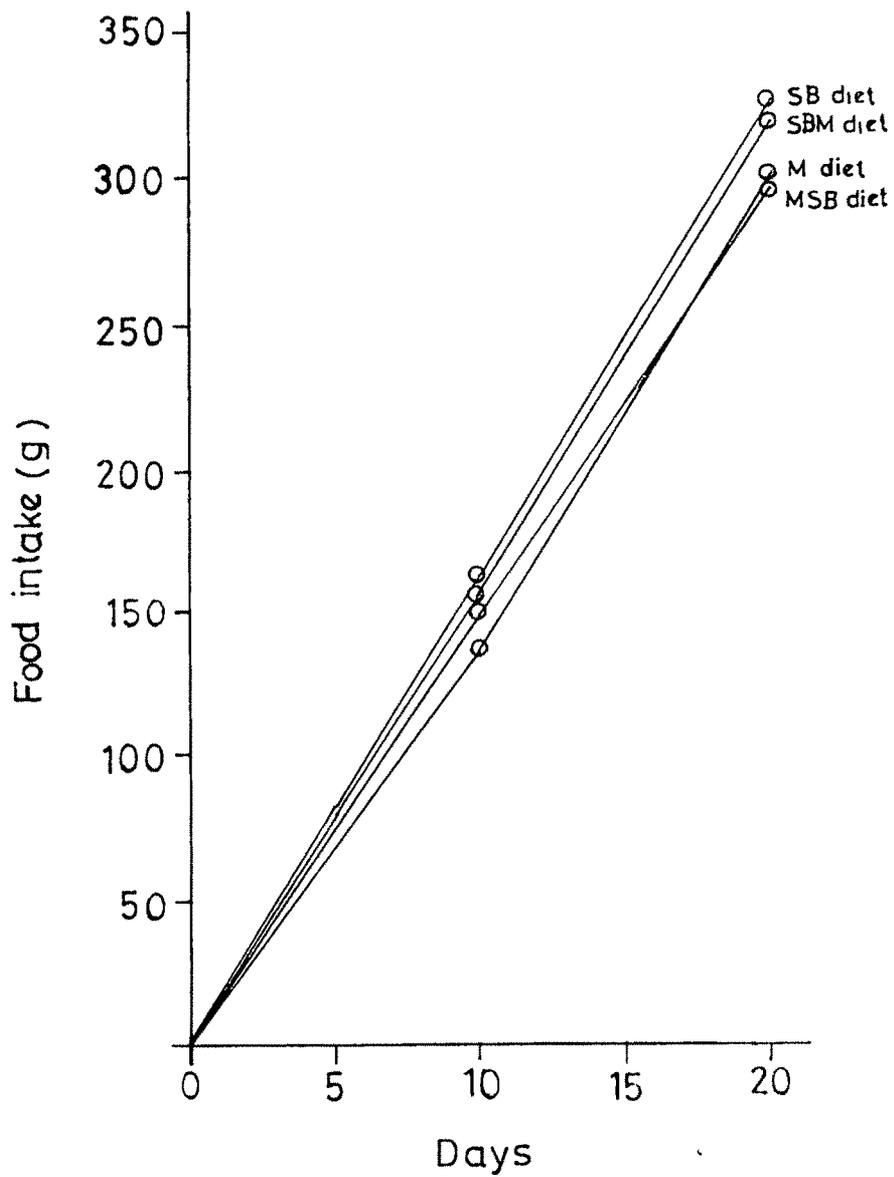
- ²Maternal = Total weight - (fetal weight + placental weight)
weight gain gain

- *Significance at P = 0.05 level

Table 4.2 Food intake and total weight gain of females fed various diets for first 10 days and latter 10 days of gestation period.

Groups	Food intake (g)		Total weight gain (g)	
	1-10 days	11-20 days	1-10 days	11-20 days
	MEAN ± SE			
SE	142.50 ±10.67	159.00 ± 7.71	26.20 ±4.18	41.90 ±5.63
M	136.20 ± 8.50	163.20 ± 7.48	24.60 ±4.14	57.16 ±4.09
SPM	157.20 ± 9.27	161.90 ±13.27	25.20 ±2.31	55.00 ±4.37
MEP	148.70 ±16.00	148.00 ±11.75	29.20 ± 3.00	50.00 ±6.16

Figure 4-2-A Food intake of pregnant rats fed various diets for first 10 days and latter 10 days of gestation period.



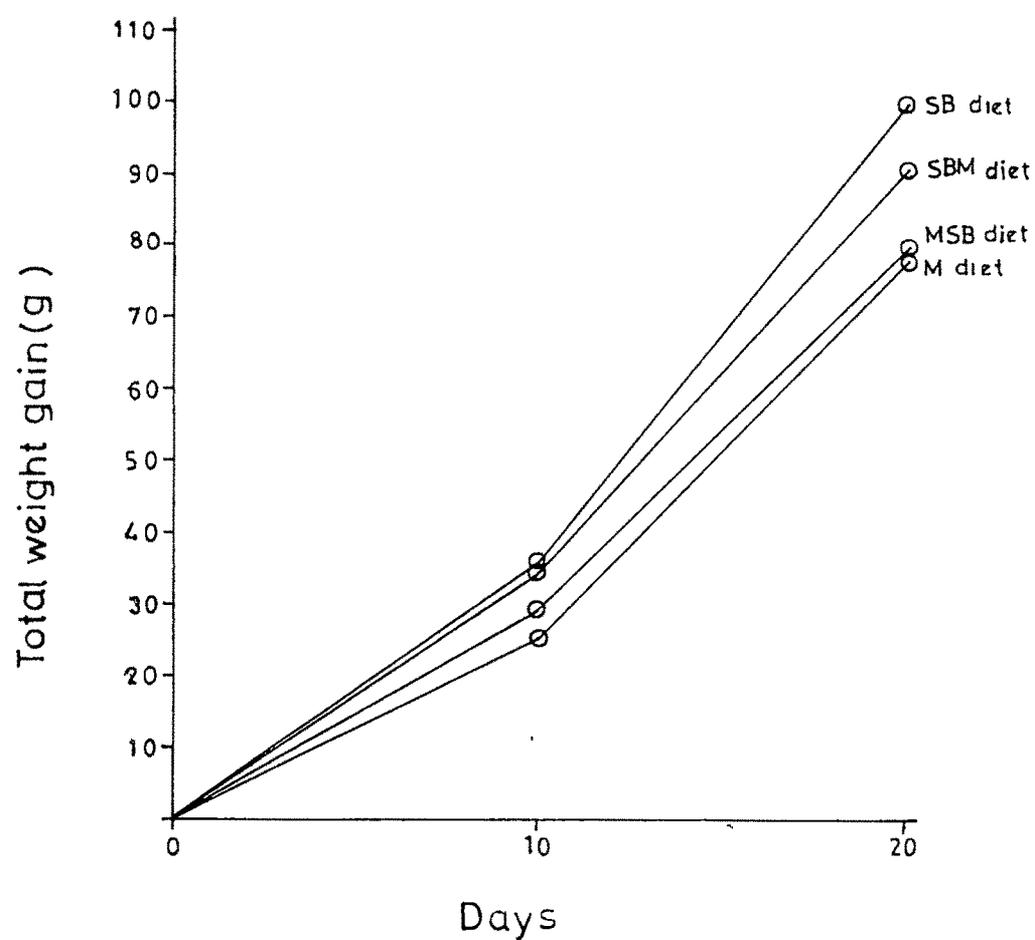
The gestation period of those fed SB diet throughout the gestation period. These results point to the fact that perhaps, the rats took sometime to adapt to Mahuda diet. Similar findings were observed when weaning rate were fed Mahuda diet for 18 days (Chapter 3 Table 3.7).

Body weight gain

The pattern of total weight gain of pregnant rats fed various diets was the mirror image of their food intake (Table 4.1). The significant F ratio of 4.16 at $P = 0.05$ level, suggested that the various dietary treatments had exerted significant effects on total weight gain of the pregnant rats (Table 4.1). The contrast of mean values between the two groups indicated that the rats fed SB diet gained significantly more than those fed C or H diet (whether the latter diet was fed during first half (MSB group) or during the entire gestation period (H group)). Among the Mahuda fed groups, the rats fed Mahuda diet during second half of the gestation period (SM group) gained significantly more weight than those fed the same diet during first half of the gestation period (MSB group) or throughout the gestation period (M group) (93.2 vs 79.2 or 77.8g). These data indicated that adverse effects of feeding Mahuda flower containing diet on total weight gain of the pregnant rats was more pronounced when Mahuda flower diet was fed at early stage of pregnancy, which is considered a nutritionally sensitive period of embryogenesis.

The tabulation of the data on weight gain, separately for first and second half of the gestation period (Table 4.2) revealed that the rats fed H diet in first half of the pregnancy period gained less weight than those fed SB diet during the same period. Figure 4.2 I displays that the gap in total weight gain was wider between rats

Figure:4-2-B Total body weight gain of pregnant rats fed various diets for first 10 days and latter 10 days of gestation period.



fed M diet during first half of the gestation period (MSB group) or during entire gestation period (M group) as compared to those fed SBM and SB diets. The lower weight gain in MSB or M diet fed rats as compared to those fed SB diet, was perhaps, due to the lower food intake during first half of the gestation period. The wider gap in weight gain between MSB/M groups and SBM/SB groups as compared to the gap in food intake between these groups indicated that perhaps Mahuda flower containing diet was not being utilized efficiently by pregnant rats for weight gain.

To examine whether the decreases observed in total weight gain of pregnant rats fed Mahuda diet were specifically, due to decreased maternal weight gain, the latter was calculated by subtracting total fetus plus placental weight from the total weight gain of the pregnant rats (Table 4.1). The calculated F value of 6.10 significant at $P = 0.05$ level revealed that the maternal weight gain was significantly altered in response to various dietary treatments. Among various groups, the maternal weight gain was significantly lower in rats fed Mahuda diet irrespective of whether the diet was fed throughout the gestation period or at initial or latter 10 days of the gestation period, as compared with those fed SB diet (18.1 or 51.1 or 50.3 vs 64.9g). No such differences in maternal weight gain were observed among the Mahuda fed groups indicating that the pregnant rats maintained their weight gain regardless of the stage of pregnancy, at which Mahuda diet was fed. Recent evidences indicate that during periods of malnutrition the mother protects herself against severe depletion of body nutrient stores, and does not supplement fetal requirements or prevent fetal growth retardation (Miles et al 1975). In the present study, I of experiment IV, females fed Mahuda diet during any stage of pregnancy,

maintained their maternal weight gain at the cost of their products of reproduction (to be discussed later).

Litter size

The mean values for litter size are enumerated in Table 4.1. The F ratio of 5.51 was found significant at $P = 0.05$ level, which suggested that feeding of various diets had exerted significant effect on the number of implantation sites in pregnant mothers. The fetus size did not significantly differ in rats fed C or SE diets although the number of fetuses of SE diet fed rats was smaller by one fetus to C diet fed rats (9.2 vs 10.2 fetuses). Similar magnitude of decrease in fetus size was observed between H or SB diet fed rats (8.2 vs 9.2). Within the nonpurified diets, those fed H diet during the first half NSB group or throughout the gestation period (H group) had smaller number of fetuses than those fed SE diet in first half of gestation period (7.5 or 8.2 vs 10.5). The variations in fetus size could account for the difference observed in total body weight gain of pregnant mothers between H and SE, and between NSB and SE groups. It is possible that carbohydrates of *Helius florens* was not fully available to the body when it was needed the most during first half of the gestation period. Taylor et al (1983) have highlighted the need for carbohydrate during pregnancy, specifically, in the period of embryogenesis which is a nutritionally sensitive period following implantation and prior to placentalation of fetus in the mother. In the present study, the data on litter size indicated impairment in embryogenesis particularly when rats were fed H diet in first half of the gestation period. It may be that carbohydrate from the *Helius florens* was not efficiently absorbed/utilized by the mother which resulted in lack of energy,

Table 4.3 Average litter size, fetal and placental weights of various diet fed pregnant groups.

Groups	Litter size (fetuses/litter)	Average litter weight (g/fetus)	Average placental weight (g/placental)
		ML/N \pm SE	
C	10.20 ± 0.60	1.29 ± 0.31	0.79 ± 0.07
EB	9.20 ± 1.01	1.17 ± 0.20	0.46 ± 0.06
M	9.20 ± 0.49	1.24 ± 0.24	0.44 ± 0.01
SBM	10.50 ± 0.42	1.38 ± 0.10	0.41 ± 0.03
MSE	7.50 ± 1.30	1.14 ± 0.12	0.45 ± 0.04
F-ratio	5.54 [*]	2.77	1.07

*Significance at $P = 0.05$ level

supply during the sensitive period of embryogenesis and thereby it caused an impairment in implantation sites. It can perhaps, also be due to anti-fertility effect of toxicant present in Mahoe flowers particularly, when ingested in early part of the gestation period. Many plants or food stuffs containing toxic constituent are known to affect fertility index in pregnancy, if consumed in large amounts. Mare et al (1980) screened ethereal extract of Melon seeds to observe its fertility effect in rats. The results indicated that single dose of 20mg of extract/kg body weight given orally from day 1 to day 10 of pregnancy produced about 20% impairment in implantation activity. The authors observed a significant decrease in implantation sites in methic extract fed rats as compared to the control group (7.2% vs 5.2 sites).

Earlier, Flournoy, et al (1977) reported alterations in fetus size in response to administration of Broad wood saponin at various levels. The rats injected with 20mg saponin/kg body weight, showed complete resorption of fetuses while such effects were not observed when the dose injected was reduced to 15 or 5mg/kg body weight. According to the authors, the complete resorption of fetuses observed in 20mg saponin injected rats might have been due to hypoxia and insufficient nutrient supply to the mothers.

Litter Weight

Table 4.7 presents mean litter weight per fetus, of rats fed control or Mahoe diets. The F ratio (2.77) was not found significant at $P = 0.05$ level which indicated that various dietary treatments produced no variations on weight/litter. The average total weight of the C and SB diets fed rats was found comparable (7.17 vs 7.17g/fetus). Likewise, no influences were observed in fetal weight

between the rats fed SB or M diet (3.17 Vs 3.24g/fetus). Also no significant differences were observed in fetal weight of rats fed SB and MSB diets (3.16 Vs 3.21g/fetus).

Placental weight

Table 4.7 also exhibits the mean values for placental weight of rats fed various diets. The non significant F ratio is suggestive of the fact that dietary variations exerted no effects on placental weights. The average placental weights ranged between 0.78 to 0.86g/placenta.

Since litter size was smaller in MSB and M groups as compared to that of SB and MSM groups it seemed that litter weight and placental weight were maintained at the cost of litter size. This may be body's protective effect on fetal growth. These data support the speculation made earlier that the carbohydrate of Mahade flowers may not be of full available type thus when mahade flower containing diets were fed during early pregnancy period it resulted in energy deficiency, consequently, produced impairment in implantation sites as indicated by smaller litter size. It may also be that Mahade flowers contain some toxic factors which exerted adverse effects on the embryogenesis.

Serum proteins

The values for serum protein concentration of rats fed various diets for 20 days of the gestation period are displayed in Table 4.4. The serum of rats fed SB diet tended to contain less proteins than that of M diet fed rats (7.72 Vs 7.71g/dl). The decrease in serum total protein in SB diet fed rats was due to decrease in globulin levels as the albumin levels were comparable between both the groups.

Table 4.4 Serum protein, A/G ratio and haemoglobin concentration of various groups.

Groups	Serum total proteins (g/dl)	Albumin (g/dl)	Globulins (g/dl)	A/G ratio	Haemoglobin level (g/dl)
			MEAN ± SE		
C	7.71 ±0.18	5.55 ±0.14	4.15 ±0.29	0.80 ±0.09	12.74 ±0.32
SP	7.22 ±0.21	5.52 ±0.15	3.70 ±0.15	0.96 ±0.05	12.04 ±0.45
M	6.91 ±0.13	5.00 ±0.22	3.91 ±0.11	0.78 ±0.07	12.57 ±0.50
SBH	7.26 ±0.10	5.50 ±0.15	3.76 ±0.24	0.99 ±0.11	12.75 ±0.24
MSB	6.97 ±0.25	5.01 ±0.16	3.98 ±0.20	0.78 ±0.08	12.87 ±0.38

Consequently, the A/G ratio of the SB diet fed rats tended to be higher than that of C diet fed rats (0.73 vs 0.59g/dl). No differences in serum total protein levels were observed between Bahuda flowers and SB diet fed rats (Table 4.4). Also, the serum proteins levels did not vary among any of the groups fed Bahuda diets. However, the serum albumin levels of H and MSB groups were lower than those of C group while the globulin levels of these groups did not vary from each other. Consequently, the A/G ratios of rats fed H diet either throughout or during first half of the gestation period, were lower than their counterparts fed SB diet.

Haemoglobin levels

Table 4.4 also includes the mean values for haemoglobin levels of rats fed various diets. The haemoglobin levels of C or SB diet fed rats were found comparable (11.74 vs 12.04 g/dl). Likewise, no difference was observed in haemoglobin levels between the rats fed SB or H diets (12.04 vs 12.57 g/dl). Also, haemoglobin values did not vary among H or SBM or MSB diet fed rats. The data of present study indicated that Bahuda flowers cooked for 20 minutes when incorporated into the diet at 25% level exerted no adverse effect on the haematological status of the pregnant rats.

Histopathology of placenta

The rat placenta has three distinct zones as in other rodents. These are decidua basalis, basal or spongy zone and labyrinthine zone. In the present study, the cytoarchitectural of the placenta of rats fed SB diet revealed normal structure. But, the placenta of rats fed Bahuda diet either throughout the period of gestation (H group) or during the first or second half of the gestation period (MSB or SBM

groups) showed certain abnormalities. Of the three Mahuda diet fed groups, maximal histologically observable lesions were manifested in the M group followed by MSB and SEM groups.

The decidua basalis in the placenta of SB diet fed rats was lined peripherally, by well formed smooth muscle capsula underlain by dermal layer composed of cellular and fibrous elements. Between the capsular envelope (composed of smooth muscles and connective tissue) and the basal zone, a thick layer of giant cells was present (Figures 4.3a,b). The cells in this zone were well formed and compactly packed with few sinusoidal spaces (Figure 4.3c). The nuclei exhibited intense basophilic with rich chromatin material and prominent nucleoli. The cells ranged in size from large to extra large.

The spongy, or the basal zone was constituted of a mixture of large and small cells, compactly packed and rich in small sinusoids containing blood elements (Figures 4.4d,e). The labyrinthine zone was rich in foetal blood vessels and large numerous maternal sinusoids (Figures 4.4d,f,g).

In the placenta of the Mahuda diet fed rats throughout the gestation period, histologically, observable lesions were noted in almost all the three zones. The decidua basalis was marked by reduction in the smooth muscle envelope with increased dermal substance and less fibrous elements. Many cells in this area were found to be hypertrophied. At many places some distended spaces could also be observed (Figure 4.4a). The giant cell zone in general, gave a disrupted appearance with many distended spaces and loosely packed cells (Figure 4.4a,b). The nuclei were less basophilic and cytoplasm more acidophilic indicating reduced functional competence of the

Figure 4.3 Photomicrograph of the placenta of the rats fed a,b,c, SE diet throughout the gestation period.

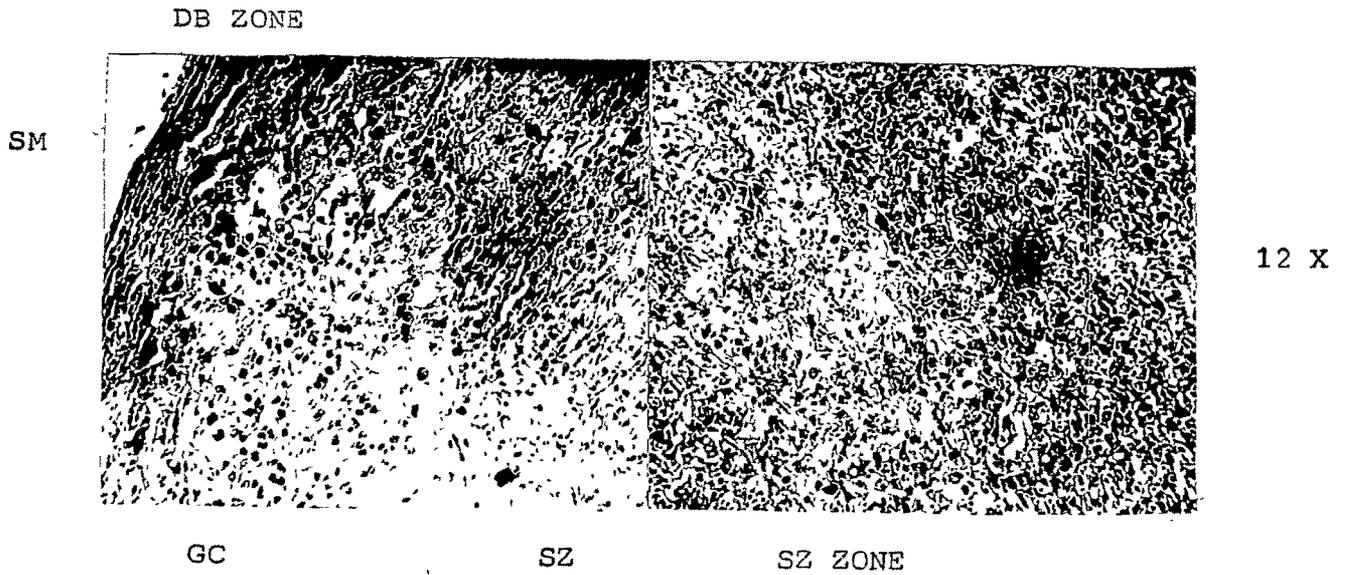


Fig. 4.3a

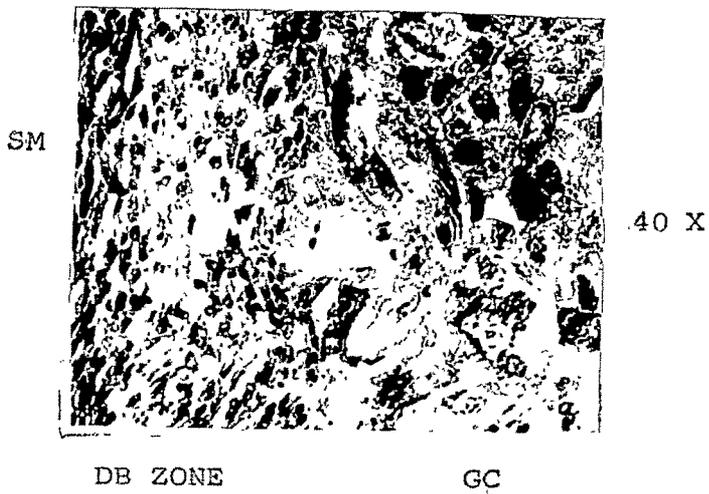


Fig. 4.3b

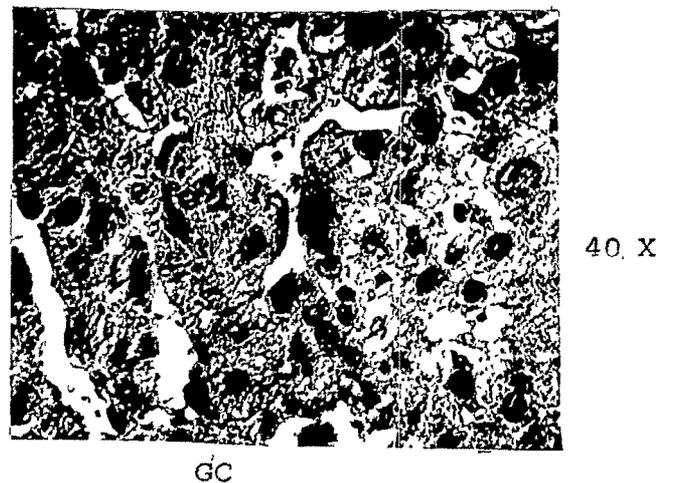


Fig. 4.3c

DB = Decidua basalis, S M = Smooth muscle, G C = Giant cells,
 SZ = Spongy zone, L Z = Labyrinthine zone

Figure 4.3 Photomicrograph of the placenta of the rats fed SB diet showing SZ & LZ zones.
d,e,f,g.

12 X

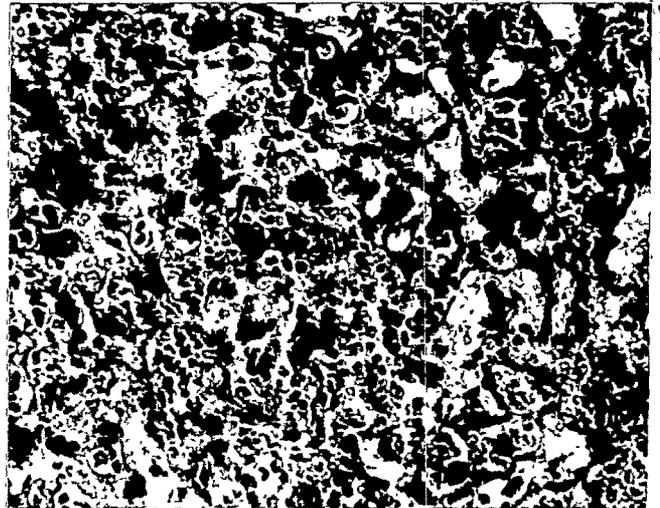


SZ ZONE

LZ ZONE

Fig. 4.3d

40 X



SZ ZONE

Fig. 4.3e

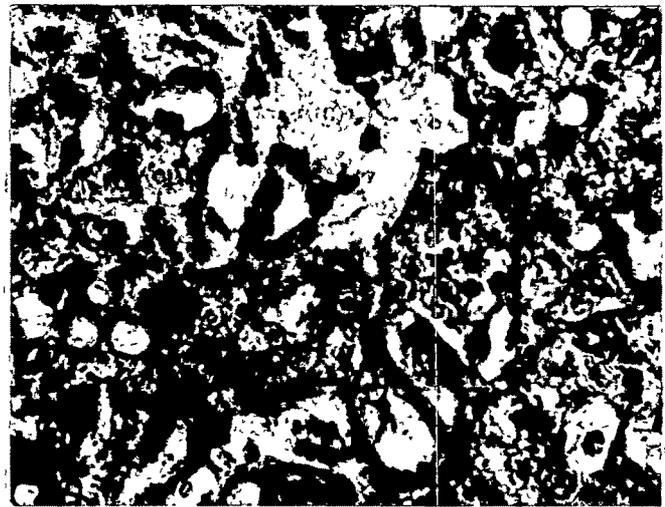
40 X



LZ ZONE

Fig. 4.3f

60 X



LZ ZONE

Fig. 4.3g

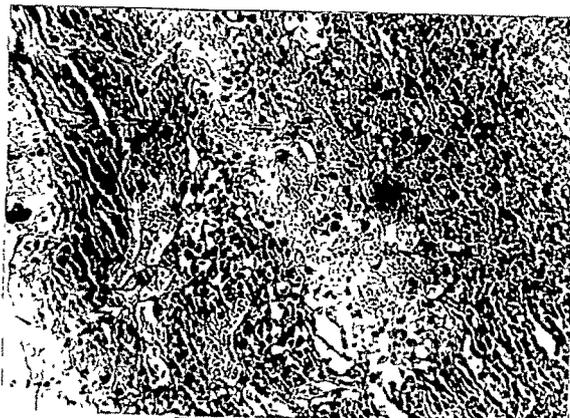
SZ = Spongy zone, LZ = Labyrinthine zone

Figure 4.4 Photomicrograph of the placenta of the rats fed
a,b,c,d, M diet throughout the gestation period.

12 X

40 X

DS



GC DB ZONE

SZ ZONE

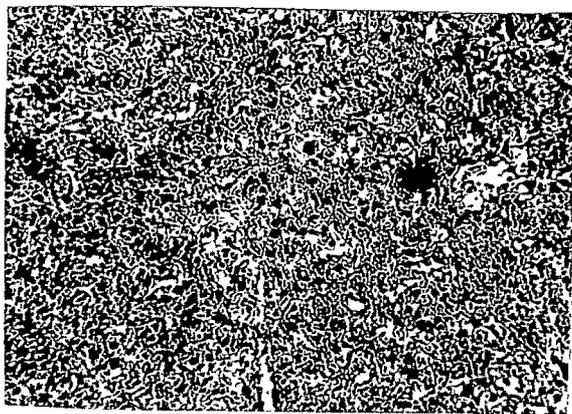
GC

Fig. 4.4a

Fig. 4.4b

12 X

12 X



SZ ZONE

SZ ZONE

LZ ZONE

Fig. 4.4c

Fig. 4.4d

DS = Dermal substances, DB = Decidua basalis, GC = Giant cells,

SZ = Spongy zone, LZ = Labyrinthine zone

cells.

The spongy zone was also marked by loss of compactness and cytolysis. Also, haemorrhagic changes were present which resulted into the merging of smaller sinusoids to form larger sinusoids. These set of changes tended to give the spongy zone a false appearance of labyrinthine zone (Figures 4.4a,c,d,e,f,g).

The structural integrity of the labyrinthine zone was greatly affected which was marked by intense cytolysis, merging of sinusoids and formation of large space filled cisternae. The continuity of the membrane partitions was grossly disrupted and even the sinusoidal linings were ruptured at many places (Figures 4.4d,h).

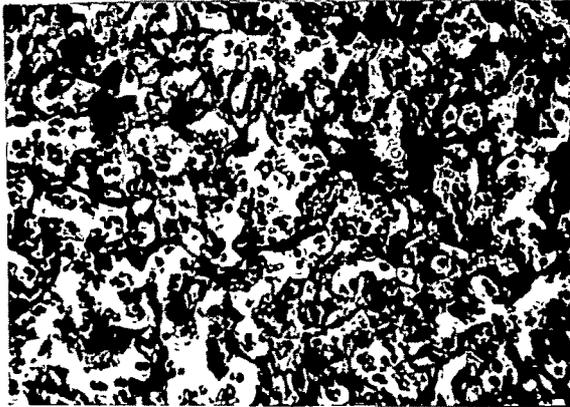
As compared to the placental histology of M group, the histology of the placenta of MSR diet fed group of rats revealed lesser degree of lesions and the smooth muscle lining of the decidua basalis was better developed. Also, other features in this zone such as decidual exuberance and giant cells were better organised but cells were less compactly packed (Figures 4.5a,b,c). The better organization of the giant cell zone was apparently due to the less extensive cytolysis although, nuclear damage was still noticeable. The nuclei was less basophilic and the cytoplasm was found to be vacuolated. Prominent dilated spaces were clearly evident.

The spongy zone in contrast to the decidua basalis zone, decided damage almost as severe as was observed in the placenta of M group of rats. The nuclei in this zone were also less basophilic and cytolysis as well as presence of large sinusoids were still the characteristic feature (Figures 4.5d,e). The rupture of the membranous partitions and discontinuities in the labyrinthine zone

Figure 4.4
e,f,g,h.

Photomicrograph of the placenta of the
rats fed M diet showing SZ & LZ zones.

40 X



SZ ZONE

Fig. 4.4e

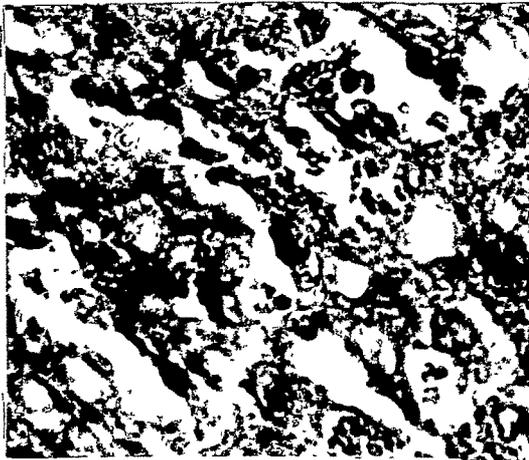
40 X



SZ ZONE

Fig. 4.4f

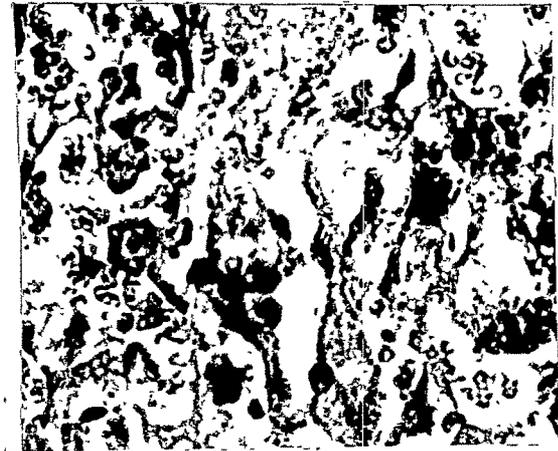
60 X



SZ ZONE

Fig. 4.4g

60 X



LZ ZONE

Fig. 4.4h

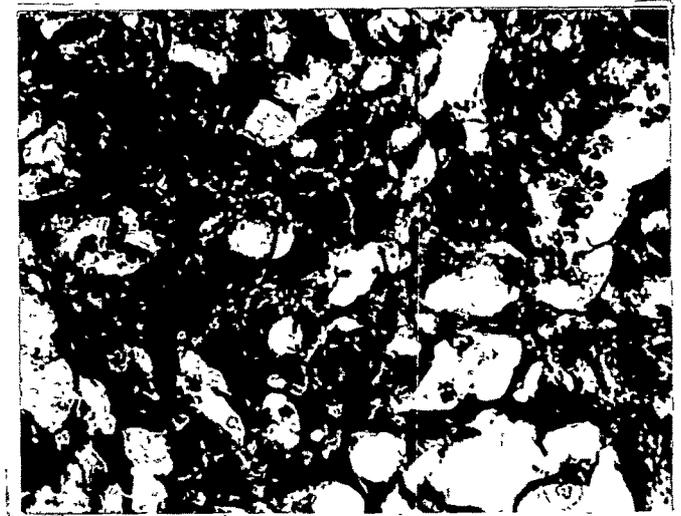
SZ = Spongy zone, LZ = Labyrinthine zone

Figure 4.5
a,b,c,d,

Photomicrograph of the placenta of the
rats fed MSB diet during the gestation
period of 20 days.

12 X

40 X



DB ZONE GC SZ ZONE

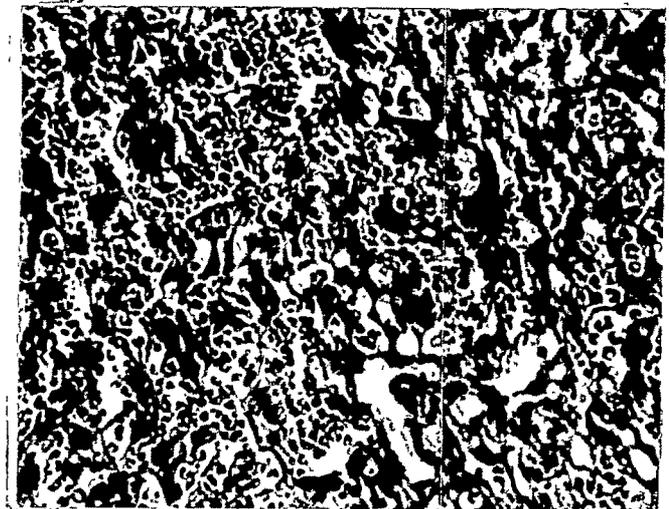
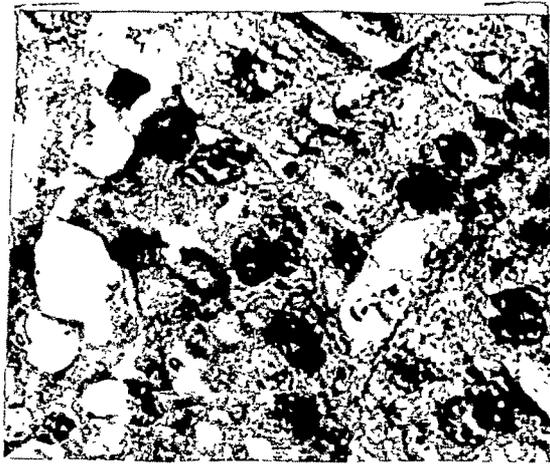
GC

Fig. 4.5a

Fig. 4.5b

60 X

12 X



GC

SZ ZONE

Fig. 4.5c

Fig. 4.5d

SM = Smooth muscle, DB = Decidua basalis, GC = Giant cells, SZ = Spongy zone

were frequent and uniform. Although, they were found to be less rampant and less severe as compared to the rat placenta of M group. Nevertheless, the damage was obvious, the sinusoids were larger and the cytolysis of the cells were marked (Figures 4.5 f,g).

The placenta of the group of rats which were fed SB diet during first half of the gestation period followed by M diet during second half of the gestation period (SBM group), depicted least degree of lesions and in general, the structure was more comparable with that of the placenta of rats fed SB diet. The smooth muscle layer in decidua basalis was well developed and compactly held with the giant cell zone by connective tissue elements. The giant cells showed more compactness (Figure 4.6a). Some of the vacuolar spaces were also evident but were discontinuous and scattered. The cytolysis was less obvious and nuclear damage was found almost negligible. The nuclei was basophilic in nature as was observed in the placenta of SB diet fed rats.

Both the spongy zone as well as the labyrinthine zone showed a nearly normal appearance and compared with those of the placenta of rats fed SB diet. The cells in both the zones were well formed and cytological lesions were negligible. Also, the spongy zone like the placenta of SB diet fed group, was marked by small sinusoids (Figures 4.6b,c,d).

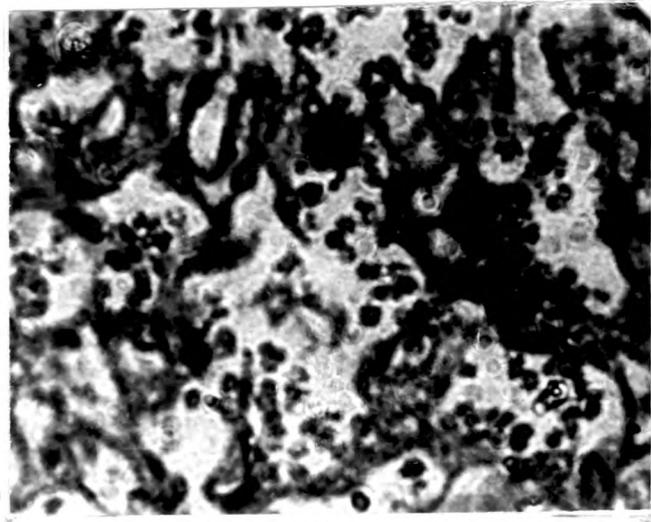
Another interesting observation noted was that the blood elements in the sinusoids of the placenta of M and MSB diet fed rats appeared shrunken and distorted, while in SB and SBM groups they appeared normal.

The placenta is the most vital organ for fetal development, and any

Figure 4.5
e,f,g.

Photomicrograph of the placenta of the rats
fed MSB diet showing SZ & LZ zones.

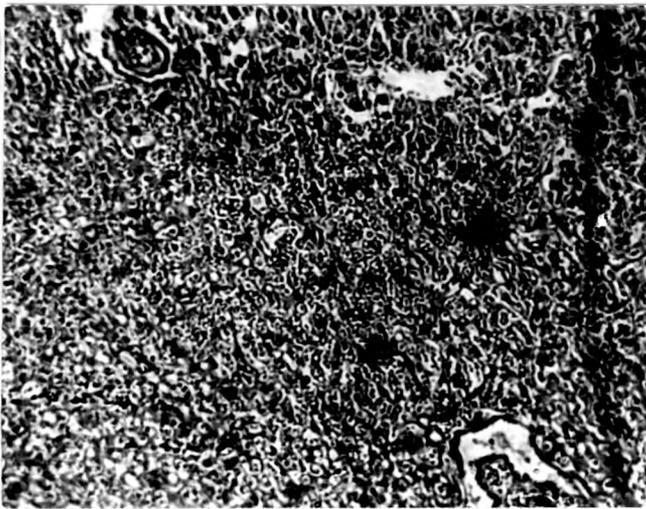
60 X



SZ ZONE

Fig. 4.5e

12 X

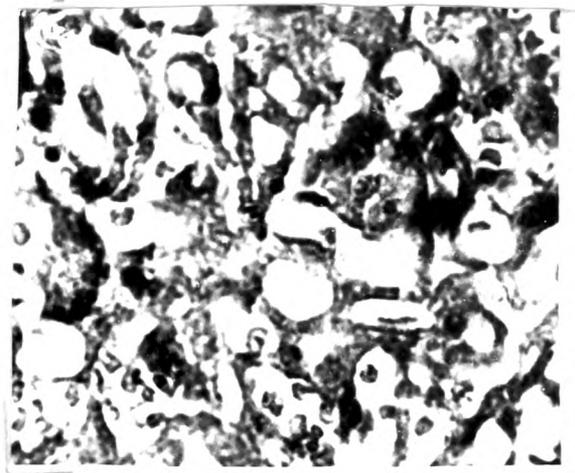


SZ ZONE

LZ ZONE

Fig. 4.5f

60 X



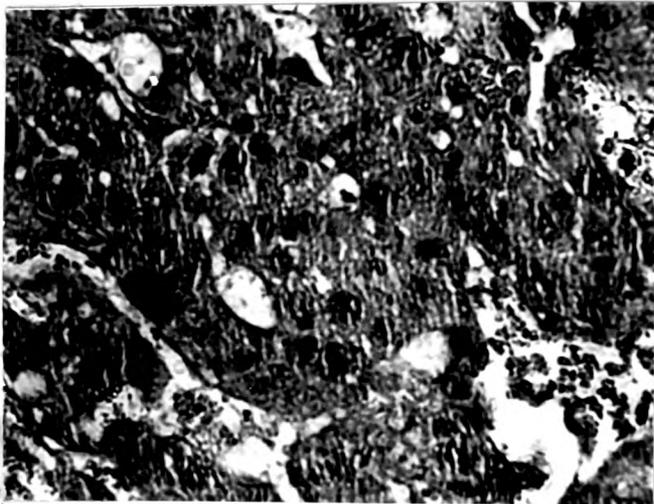
LZ ZONE

Fig. 4.5g

SZ = Spongy zone, LZ = Labyrinthine zone

Figure 4.6 Photomicrograph of the placenta of the rats fed SBM diet showing GC, SZ zone & LZ zone. a,b,c,d.

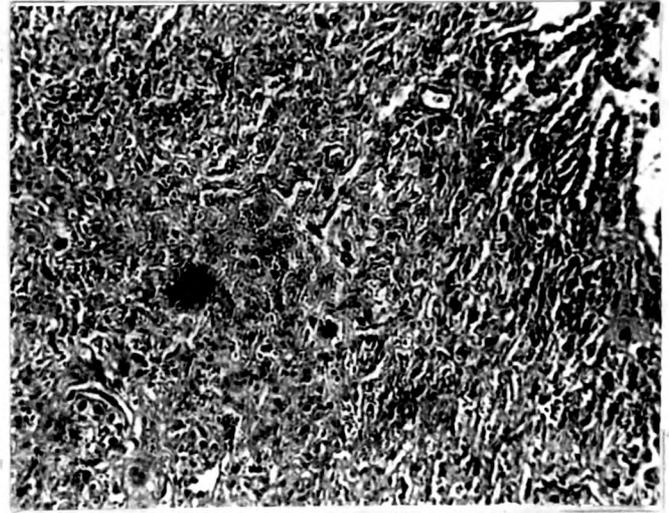
40 X



GC

Fig. 4.6a

12 X

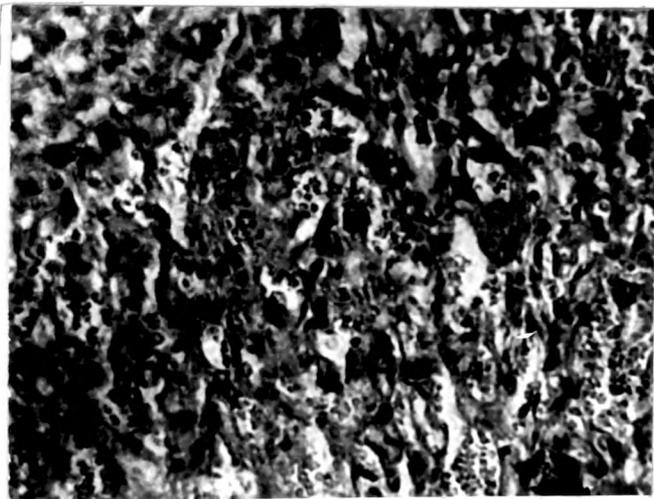


SZ ZONE

LZ ZONE

Fig. 4.6b

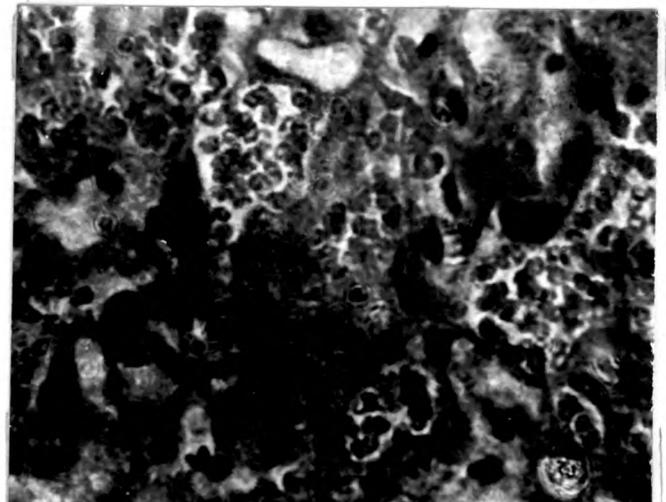
40 X



SZ ZONE

Fig. 4.6c

60 X



LZ ZONE

Fig. 4.6d

GC = Giant cells, SZ = Spongy zone, LZ = Labyrinthine zone

injury inflicted on this organ can be reflected directly, or indirectly on the fetus (Benirschke 1975). Number of factors which are of teratogenic nature have been reported to cause pathological changes in the placenta such as papain (Devj and Singh 1978), Chlorpromazine (Singh and Padmanabhan 1980), prednisolone (Blackburn et al 1965), Yolk sac antiserum (Brent et al 1971), trypan blue (Beek et al 1967) and hyperthermia (Arora et al 1979). More recently, Singh et al (1985) have observed pathological lesions in the placenta of rats subjected to experimental ischaemic hypoxia. The authors noted distended spaces, infraction and homogeneous necrotic material in the decidua basalis zone which were attributed to vascular congestion resulting from uterine vessel clamping leading to possible loss of local function.

In the study I of experiment IV histologically observable lesions were seen in the structural features of all the three zones of the placenta of the M, MSB and SAM groups. The reduced smooth muscle cells, less fibrous tissue, necrotic giant cells and appearance of loose space filled areas in the decidua might be indicative of probable failure in local function. Also, loss of structural integrity of cells in both the spongy and labyrinthine zones as well as formation of larger sinusoids could be suggestive of reduced functional efficiency of the placenta. The rat placenta which is haemotrichorial in structure (Enders 1965) has a trichorial membrane separating the foetal blood from the maternal sinusoids. However, the formation of larger sinusoids due to rampant cellular damage and rupturing of the membranous barriers in the placenta of rats fed M and MSB groups might have produced some degree of mixing of foetal and maternal blood. The observation of the distorted and shrunken blood cells in the sinusoids of these groups of rats could be the

possible reason of affecting the functional ability of the ¹³³ blood flowing through the placenta which forms an essential and vital link between the mother and the fetus.

These histopathological findings suggest that feeding of Mahuda flower diet exerted deleterious effects on placental structure. However, these placental changes were more marked in rats fed Mahuda diet either during the entire period of gestation or during the first half of the gestation period than of those fed Mahuda diet during the second half of the gestation period. However, ingestion of Mahuda diet did not lead to complete functional impairment of the placenta as no adverse effects were observed on fetal weights of the rats fed Mahuda diets at any stage of the gestation period.

The salient features of feeding Mahuda flowers diet during the gestation period (study 1) were :

- (i) no appreciable variations in food intake in pregnant rats fed SB or M or SBM or MSB diet;
- (ii) smaller body weight gain in M and MSB versus SB and SBM groups;
- (iii) smaller litter size of M and MSB groups versus SB and SBM groups;
- (iv) no significant adverse effects on litter and placental weights of rats fed Mahuda diets;
- (v) moderate decreases in serum albumins and consequently decreases in A/G ratio in M and MSB groups;
- (vi) no adverse effects on the haematological status of rats fed Mahuda diets;
- (vii) histopathologically abnormal changes in rat placenta of M and MSB groups.

Based on these results, the author feels inclined to state that during pregnancy, it would not be safe to consume Mahuda flowers to provide about 10% (25g flowers/100g diet) of the total dietary carbohydrate in the diet. But if the flowers are to be consumed because of food scarcity, such diets should be avoided during first half of the gestation period.

In stud. 2, thirty, one day pregnant rats were divided into five groups of 6 rats each to be fed for throughout the gestation and lactation periods, either casein diet (P-LC diet/group) or sago-bengalgram diet (P-LSB diet/group) or 25M20 diet (P-L M diet/group) or SB diet during the gestation period followed by M diet during the lactation period (PSR-LM diet/group) or M diet during the gestation period followed by SB diet during the lactation period (PM-LSB diet/group). Data were collected on food intake and maternal weight changes throughout the gestation and lactation periods. On the day of delivery, the number of pups, their mean weight and deformity, if any, were recorded. Each dam nursed 6 pups for 21 days. Records were maintained on growth rate of the pups. The pups were sacrificed on 22nd day of the lactation period. The lactational performance of the dams was evaluated in terms of growth rate of the pups. The bio-chemical status of the pups was evaluated by, estimating haemoglobin levels, hepatic lipid content and concentration of serum total protein and its fractions.

Food intake

The mean values for food intake during gestation and lactation periods are outlined in Table 4.5. The non significant F ratio indicated that various dietary treatments exerted no effects on food intake. The food intake of pregnant rats fed on diet containing

Table 4.5 Food consumption during gestation and lactation periods in dams fed control and Mahuda flowers diets.

Groups	Food intake ¹ (g)	
	During gestation (g)	During lactation (g)
	MEAN \pm SE	
P-LC	315.20 \pm 17.75	339.00 \pm 21.70
P-LSB	256.20 \pm 44.31	295.50 \pm 54.21
P-LM	315.00 \pm 11.54	328.20 \pm 18.18
PGB-LM	277.00 \pm 20.26	379.50 \pm 30.43
PM-LSB	208.20 \pm 8.98	408.00 \pm 9.00
F-ratio	1.05	0.31

* Values of Mahuda flower diet have been adjusted for the moisture content of the diet.

Mahuda flowers did not differ from those fed on sago-bengalgram diet during the gestation period. These results are in confirmation with those of study 1 where no differences were observed in food intake between M and SB groups. Also, food intake during the lactation period did not vary among any of the groups fed either casein or sago-bengalgram or Mahuda flower diets (Table 4.5). Considering the values for daily food intake (Table 4.6) it was observed that the rats ate 11 to 39% more food during the lactational over that of the gestational periods. The increases in food intake were 19% and 16% in P-LC and P-LSB groups respectively.

The rats fed Mahuda flowers containing diet during the gestational period (P-LM and PM-LSB groups) exhibited larger increases in food intake regardless of the type of diet fed, during the lactational period. These data indicate that irrespective of the type of diet fed, lactating rats required more food than the pregnant rats. This is understandable because nutrient requirement is higher during the latter period to meet the nutrient stress imposed by pregnancy, and to produce adequate amount of good quality, milk for infants growth (Vijayalaxmi and Lakshmi 1982). However, when the rats were switched over from Mahuda diet fed during the gestational period onto sago-bengalgram diet during the lactational period, they tended to eat more food than those continued to be fed on the same diet (PM-LSB Vs P-LM groups). If the speculation made earlier holds true that the carbohydrate of Mahuda flowers may be less available to the body, then the M diet fed groups could be considered as undernourished or semi starved. It could be that the rats of PM-LSB group remained semi starved during the gestation period because of unavailability of the Mahuda flower carbohydrate and when they were offered SB diet during the lactation period they just ate more

Table 4.6 Daily food intake during gestation and lactation periods in dams fed various diets.

Groups	intake during gestation period (g)	intake during lactation period (g)	increase in food intake (%)
	MEAN \pm SE		
F-LC	14.80 ± 1.70	17.60 ± 1.12	19
P-LSB	16.20 ± 2.04	18.80 ± 1.58	16
F-LM	14.40 ± 0.67	16.00 ± 0.66	25
PSP-LM	17.10 ± 0.92	19.00 ± 0.50	11
PM-LSB	14.00 ± 0.41	17.40 ± 0.42	26

perhaps in an attempt to fulfill their nutritional demands. Many studies conducted in rats, to explore the effects of chronic under nutrition during pregnancy on food intake during lactation, have revealed that when the diet was fed ad libitum during the lactation period, the food intake increased and was almost found closer to the control values (Cowley Grieco 1966, Zamenhof et al 1971 and 1972, Zamenhof and Marthens 1977).

Body weight changes

The pattern of weight gain of pregnant rats fed various diets during gestation period are presented in Table 4.7. The F ratio of 4.62 significant at $P = 0.05$ level suggested that the various diets fed to pregnant rats during the gestation period had caused variations in weight gain. The group mean comparisons revealed that rats fed SB diet (P-LSB or PSB-LM groups) gained significantly more than those fed C or M diets (P-LC or P-LM or PM-LSB groups) during the gestation period. These data indicated that adverse effects of feeding Mahuda diet on gestational weight gain was more pronounced when it was fed during the gestation period. The weight gain of PSB-LM group as compared to that of the P-LM group supported this view as the rats fed SB diet during gestation and M diet during lactation period, gained more weight than those fed M diet during gestation and lactation periods (101.3 Vs 76.2g). The pattern in weight gain was similar to that observed in study 1 (Table 4.1). The daily weight gain during gestation period in rats fed SB or M diets in study 1 was 4.9 or 3.9 g and in study 2 it was 4.7 or 3.5 g respectively.

Table 4.7 Total weight gain during gestation, weight at parturition and weight at 21st day of lactation in dams fed various diets.

Groups	Initial weight on zero day of pregnancy (g)	Total weight gain in gestation period ¹ (g)	Weight at parturition (g)	Weight on 21 st day of lactation period (g)
	MEAN ± SE			
P-LC	219.20 ± 2.27	81.50 ± 6.07	294.40 ± 16.34	289.20 ± 11.21
P-LSF	204.00 ± 4.87	102.10 ± 11.28	255.70 ± 12.78	212.10 ± 10.08
P-LM	198.30 ± 6.15	76.70 ± 5.18	214.10 ± 13.21	203.80 ± 8.70
PSF-LM	179.60 ± 5.18	101.10 ± 6.54	240.50 ± 12.05	209.20 ± 9.51
PM-LSF	203.50 ± 4.91	73.00 ± 6.80	227.20 ± 14.15	208.20 ± 8.87
F-ratio	1.97	4.62*	2.71	1.13

¹Total weight gain = Weight on 21st day of gestation period - Initial weight on zero day of gestation period.

*Significance at P = 0.05 level

Table 4.7 also includes the mean values for weights taken at parturition. The non-significant F ratio suggested that weight at parturition did not significantly differ in rats subjected to various dietary treatments. However the mean weight at parturition of rats fed M diet during the gestation period (P-LM or PM-LSB groups) tended to be lower than that of SB diet (P-LSB group) fed group (234.1 or 237.2 Vs 255.7g). Since the mean initial weight (Table 4.7) between the three groups did not significantly differ from each other, the non significant differences in weight at parturition did suggest that Mahuda diet was not being efficiently utilized for maternal weight gain during gestation period.

At end of the 21 day lactation period, no significant differences were observed in body weights among any of the groups (Table 4.7). But all the dams fed various diets lost weight during the period of lactation. The difference between parturition weight and lactation weight in C diet fed dams (P-LC group) was found comparable with that of those fed SB diet (P-LSB group). But the differences in weight of the dams on Mahuda diet fed at any stage. (P-LM or PM-LSB or PSM-LM groups) were lower than that of those fed SB diet (27.3 or 29.0 or 31.3 Vs 43.5g). These findings were attributed to the relatively lower weight gain of these rats during the gestation period resulting in less amount of the stored fat and consequently, less utilization of the stored fat for the increased demands of energy required for lactational activities. However, no such differences were observed between dams fed M diet during gestation (PM-LSB group) or during lactation (PSM-LM group) periods. Earlier, Delgado et al (1977) have shown a linear trend in weight loss in lactating mothers during the period of lactation. The authors opined that as the energy demand of lactating women remain unmet the women

utilize stored fat to subsidize their increased demand of energy, required for lactation.

The data of present study 2 of experiment IV on body weight changes revealed that mother fed on Mahuda flower diet during the gestation period (P-LM or PM-LSB groups) could not gain as much weight as those fed on SB diet (P-LSB or P'SB-LM groups), although their dietary intake remained more or less comparable throughout the gestation period. It probably, was due to the fact that the carbohydrate of Mahuda flowers was not fully available to the body to meet its increased requirement during the period of embryogenesis which is a nutritionally sensitive period of pregnancy.

Litter size

The mean values for litter size are exhibited in Table 4.6. The F ratio of 2.87 was significant at $P = 0.05$ level, which suggested that feeding of various diets had exerted significant effect on the number of pups delivered by the pregnant rats. The average number of pups delivered by rats fed casein (P-LL group) or sago-bengalgram diet during the gestation period (P-LSB or P'SB-LM groups), did not differ from each other. But rats fed M diet during the gestation period (P-LM and PM-LSB groups) gave birth to less number of pups. The average number of pups/dam was 8.3 in Mahuda diet fed rats as against 10.7 to 10.8 in sago-bengalgram diet fed rats. The smaller pup size perhaps had caused the variations observed in total weight gain between Mahuda diet (P-LM or PM-LSB groups) and sago-bengalgram diet (P-LSB or P'SB-LM groups) fed groups. These results are in accordance with those observed in study I (Table 4.3) where the smaller size of fetus observed in Mahuda fed groups (M or MSB groups) was attributed to non availability of the Mahuda

Table 4.8 Average litter outcome, birth weight and weight of pup at 21 day of lactation period in various diets fed groups.

Groups	No. of pups delivered (pups/litter)	Birth weight (g/pup)	weight of 21 day old pup (g/pup)
		MEAN \pm SE	
F-LC	10.00 ± 0.96	5.44 ± 0.25	23.60 ± 0.71
F-LSE	10.10 ± 1.22	5.10 ± 0.11	27.70 ± 1.77
F-LM	8.20 ± 0.55	5.20 ± 0.15	20.90 ± 0.62
FSE-LM	10.50 ± 1.74	5.70 ± 0.19	24.20 ± 1.25
FM-LSE	9.20 ± 0.79	5.14 ± 0.28	22.00 ± 0.72
F-ratio	2.87*	1.69	5.13*

*Significance at P = 0.05 level

carbohydrate and/or to presence of any toxic/antinutritional factor in Mahuda flowers.

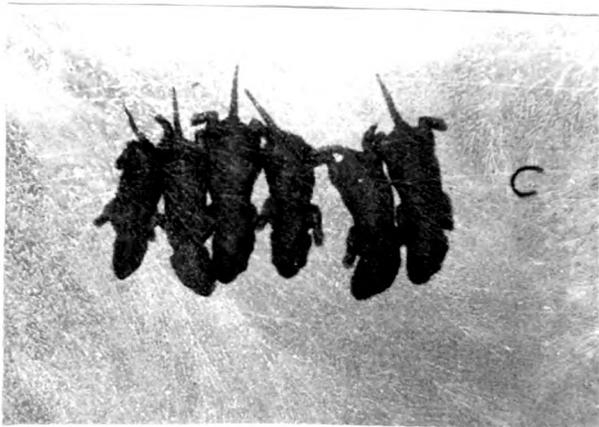
Litter weight at birth

Table 4.8 presents mean litter weight per pup, of rats fed control or Mahuda diets. The F ratio (1.69) was not found significant at $P = 0.05$ level which indicated that various diets given to pregnant females produced no variations in birth weight of pups. The mean weight per pup of P-LC and P-LSB groups did not differ from each other (5.4 Vs 5.3g/pup). Likewise, no differences were observed (Figure 4.7) in weight per pup, of rats fed P-LSB or P-LM diets (5.3 Vs 5.2g/pup). Also, no significant differences were observed in weight per pup whether the pregnant rats were fed M or SB diets during entire the gestation period although pups born to mothers fed SB diet during the gestation period tended to be heavier than those fed M diet (5.7 Vs 5.1g/pup).

Weight of weaned pups

Table 4.8 also exhibits the mean values for weight of 21 day old pups. The F ratio of 5.13 significant at $P = 0.05$ level is suggestive of the fact that the various diets fed to lactating dams exerted significant effects on the growth rate of pups until they were weaned. The average weight of pups of dams fed SB diet (P-LSB group) was significantly lower than that of dams fed on P-LC diet (27.7 Vs 22.6g/pup). This was understandable because C diet supplied protein of high biological value. The mean weight of 21 day old pups nursed by dams fed on M diet throughout the gestation and lactation periods was significantly lower than that of the pups nursed by dams fed on SB diet (20.8 Vs 22.7g/pup). As a matter of

Figure 4.7 Photographs of pups (zero day old)
from the dams fed various diets.



Pups of dams fed C diet



Pups of dams fed SB diet



Pups of dams fed M diet



Pups of dams fed SBM diet



Pups of dams fed MSB diet

fact the weight of pups nursed by dams fed on Mahuda diet whether during the gestation or lactation periods, was significantly lower than the mean weight of those of non Mahuda diet fed dams. It was also noticed that the pups of the dams fed on M diet during the gestation period, tended to grow at a slower rate than those of the dams fed on SU diet during the same period regardless of the type of diet fed during the lactation period. Since birth weight of the pups did not vary among all the five groups and since the growth rate upto 21 days of age varied, it appeared that feeding of M diet to the dams adversely influenced either milk secretion and/or its composition. It may also be that so called toxic factor present in Mahuda flowers reached pups through milk and in turn, impaired their growth rate.

Man, studies have been conducted to investigate the effect of maternal diet on the lactational performance in women (Lechtig 1930; Delgado et al 1977). Delgado et al (1977) are of the opinion that infant growth is dependent not only on the breast milk quantity and quality but also on maternal nutritional status during and after pregnancy. Earlier, Venkatchalam (1962) and Bailey (1965) had shown that severely malnourished mothers produced inadequate amounts of milk of poor quality. Recently, Bhatia et al (1983) have related fetal growth with the maternal calorie and protein intake during pregnancy and demonstrated that mothers with better body size were able to meet demands of growing fetus even if their diet remained nutritionally inadequate. More recently, Butte et al (1984) have found a significant correlation between milk production and maternal energy intake. They have opined that inadequate energy intake for a longer duration might result in diminishing milk production.

In the present stud., it could be that energ. supplied by carbohydrate of Mahuda flowers was not fully available to the lactating dam which in turn, adversely, affected milk yield and/or its composition. Consequently, the growth rate of the pups of the dams fed on M diet was impaired.

Organ weight of pups

Table 4.9 exhibits mean values for weight of fresh organs expressed as percent body weight, of pups of the dams fed control and Mahuda flowers diets for entire gestation and/or lactation periods. Except for liver weight, the F ratios for the weight of intestine (4.61), heart (2.91), spleen (7.35), and kidney (6.08) were found significant at $P = 0.05$ level indicating that weight of these organs was significantly altered in response to various dietary treatments (Table 4.9).

The comparisons between the two means revealed that the mean weight of pup intestines of Mahuda diet fed dams, was significantly higher than that of the pups of dams fed casein diet. But no such significant differences were observed in intestinal weights between the pups of the dams fed sago-bengalgram diet or any of the Mahuda diets. Except for the dams fed on casein or on Mahuda diet during the entire gestation and lactation periods, no significant differences were observed in mean weight of pup heart among the various groups. The mean values for spleen weight of the pups of dams fed Mahuda diet were smaller than those of the dams fed SB or C diets. On the other hand, the pup kidneys of the dams fed M diet were heavier than those of the pups of C or SB diets fed dams.

It may be worth recalling here (Chapter 3) that similar effects on

Table 4.9 Organ weight as percent body weight, of pups of the dams fed control and Mahuda flower diets

Groups	Organs				
	Liver	Intestine	Heart	Spleen	Kidney
	MEAN \pm SE				
P-LC	4.11	4.30	0.15	0.41	0.91
	± 0.11	± 0.08	± 0.008	± 0.05	± 0.04
P-LSB	4.36	4.29	0.37	0.26	0.71
	± 0.22	± 0.10	± 0.02	± 0.05	± 0.02
P-LM	3.97	4.05	0.17	0.25	0.95
	± 0.15	± 0.28	± 0.01	± 0.01	± 0.04
PSE-LM	4.01	4.72	0.36	0.29	0.90
	± 0.21	± 0.24	± 0.02	± 0.02	± 0.02
PM-LSP	3.57	4.01	0.36	0.28	0.71
	± 0.13	± 0.22	± 0.12	± 0.02	± 0.03
F-ratio	2.22	4.31*	2.91*	7.15*	1.03*

*Significance at $P = 0.05$ level

the organ weights were observed in weanling rats fed diet containing Mahuda flowers for 20 days. The higher weight of various organs in relation to bod. weight, in response to feeding diet containing sa! meal containing 7% tannins, has been reported earlier (Bandhi et al 1975). In the present study since pups of P-LM or PM-LSB group of dams gained significantly less than those of the P-LSB group of dams, it is possible that the higher organ weight expressed as percent body weight, were indeed, due to their lower body weight gain during the gestation period. On the other hand, it may also be that feeding of Mahuda diet to the dams during gestation or lactation periods had caused alterations in the structure or cellularity of the organs of weaned pups as has been demonstrated in rats, by Kochnowski and Sherman (1962).

Hepatic lipids

Table 4.10 presents the mean values for hepatic lipid content of pups of the dams fed control or Mahuda flower diet. The F ratio of 1.98 was not significant at $P = 0.05$ level which suggested that the hepatic lipid content was not altered in response to various dietary treatments. The values for hepatic lipid of P-LC or P-LSB groups did not vary from each other (3.11 Vs 3.14g/100g wet tissue). Likewise, no significant difference in values for hepatic lipids was observed between pups of the dams fed P-LM or PM-LSB and P-LSB or PM-LM diets (2.97 or 3.08 Vs 3.14 or 3.06g/100g wet tissue). Similar observations were made in weanling rats fed 25M20 diet for 28 days (Chapter 3).

Haemoglobin levels

Table 4.10 also displays the mean concentrations for haemoglobin

Table 4.10 Hepatic lipids and haemoglobin levels of pups of the dams fed various diets.

Groups	Hepatic lipids (g/100g wet tissue)	Haemoglobin levels (g/dl)
	MEAN \pm SE	
P-LC	7.11 ± 0.11	12.22 ± 0.14
P-LSB	5.14 ± 0.05	12.64 ± 0.14
P-LM	2.97 ± 0.07	11.86 ± 0.15
PDI-LM	1.86 ± 0.11	11.60 ± 0.14
PM-LSB	7.02 ± 0.10	12.06 ± 0.15
P-ratio	1.98	2.01

levels of the pups of the dams fed on various diets during gestation and lactation periods. The haemoglobin levels of the pups of the dams fed P-LC or P-LSB diet were comparable. Likewise, no difference was observed in haemoglobin levels between the pups of the dams fed P-LSB or P-LM diets (12.64 Vs 11.86g/dl). Also, haemoglobin values did not vary among pups of the dams fed P-LM or P-SB-LM or PM-LSB diet (11.25 or 11.60 or 12.06g/dl). In line with the results of the study 1, the data of the study 2 indicated that Mahuda flowers cooled for 20 minutes and incorporated into the diet of nursing mothers at 25% level exerted no adverse effects on the haematological status of the pups.

Serum total protein and serum protein fractions

Table 4.11 indicates that inclusion of Mahuda flowers into the diet at 25% level as carbohydrate source and fed to dams during the lactation period exerted no marked ill effects on the total serum proteins, of weaned pups. The serum total protein in pups of the P-LSB and P-LC groups did not differ from each other but that of the P-LM group tended to be lower. Within the pups of Mahuda fed groups, no appreciable differences were observed in serum total protein levels. The level of albumin however, tended to be lower in the pup serum of the dams fed Mahuda diet particularly during the gestation (PM-LSB group) or during entire gestation and lactation periods (P-LM group). In contrast, the serum globulin levels in pups of these groups (P-LM and PM-LSB groups) tended to be higher than the non Mahuda fed groups (P-LC and P-LSB groups). Consequently, the A/G ratio of the pup serum of P-LM and PM-LSB groups was lower than that of the pups of other groups.

The determination of various globulin fractions using gel

Table 4.11

Mean values for serum total protein, A/G ratio and serum protein fractions in weaned pups of control and Mahuda diet fed dams.

Groups	Serum total protein (g/dl)	Albumins (g/dl)	Globulins (g/dl)	A/G ratio	MEAN ± SE			
					Serum Protein	Fractions	(g/dl)	
					Alpha1	Alpha2	Beta	Gamma
P-LL	6.21 ±0.11	3.33 ±0.15	1.98 ±0.21	1.01 ±0.02	1.10 ±0.10	0.51 ±0.03	0.42 ±0.02	0.46 ±0.08
P-LSV	6.97 ±0.19	3.77 ±0.18	2.64 ±0.20	0.91 ±0.17	1.91 ±0.20	0.55 ±0.02	0.54 ±0.07	0.54 ±0.05
P-LM	6.77 ±0.22	3.04 ±0.23	3.72 ±0.23	0.82 ±0.06	1.92 ±0.18	0.76 ±0.08	0.61 ±0.01	0.51 ±0.07
PSE-LM	7.01 ±0.30	3.40 ±0.15	3.61 ±0.21	0.94 ±0.18	1.98 ±0.16	0.57 ±0.09	0.58 ±0.10	0.40 ±0.12
PT-LSE	5.71 ±0.25	3.11 ±0.16	2.79 ±0.19	0.92 ±0.12	1.71 ±0.12	0.77 ±0.11	0.57 ±0.05	0.55 ±0.02

electrophoresis method (Figure 4.9 a,b,c,d,e) revealed that increases in globulin levels in the pup serum of P-LM and PM-LSB groups were mainly due to increases in beta and gamma fractions of the globulin.

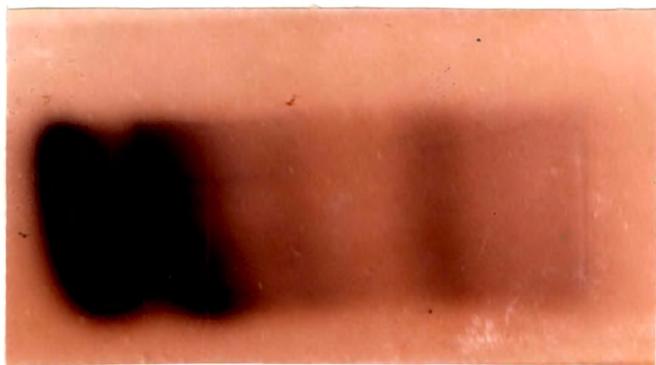
Similar trends were observed in total serum proteins and A/G ratio in weanling and pregnant rats (Chapter 3 and 4 (study 1)) fed on Mahuda flowers diet (25M20 diet).

The salient features of incorporating Mahuda flowers (cooled for 20 minutes) into the diet at 25% level and fed to rats during gestation and/or 21 days of lactation period (stud. 2) were :

- (i) no effect of Mahuda flower diet on food intake of pregnant or lactating rats;
- (ii) lower body weight at parturition in dams fed Mahuda diet as compared to that of those fed SF or C diet;
- (iii) smaller litter size in rats fed Mahuda diet during gestation period (P-LM and PM-LSB groups) versus those fed M diet during lactation period;
- (iv) no effect on birth weight of pups;
- (v) stunted growth of pups nursed by dams fed on Mahuda diet at any stage, during gestation or lactation period (P-LM or PM-LSB or PSB-LM groups);
- (vi) hypertrophy of kidneys, heart and intestines in pups of dams fed Mahuda diet (P-LM, PM-LSB groups);
- (vii) no alterations in hepatic lipid contents of pups nursed by dams fed on Mahuda diet (P-LM, PM-LSB, PSB-LM groups);
- (viii) no adverse effect on the haematological status of the pups of dams received Mahuda diet at any stage during gestation or lactation;

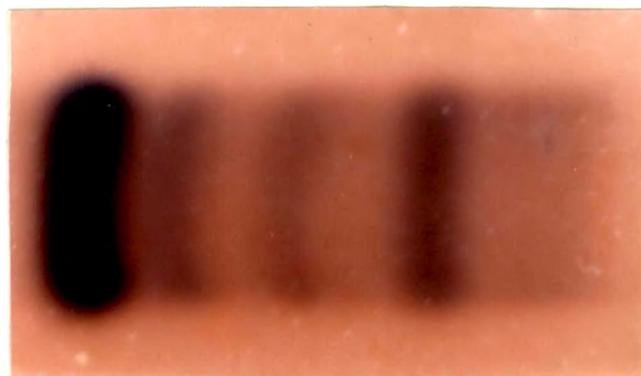
Figure 4.8
a,b,c,d,e.

Electrophoretic pattern of serum protein
fractions in groups of weaned pups of
dams fed various diets.



A α1 α2 B r

Fig.4.8a P - LC diet fed dams pups



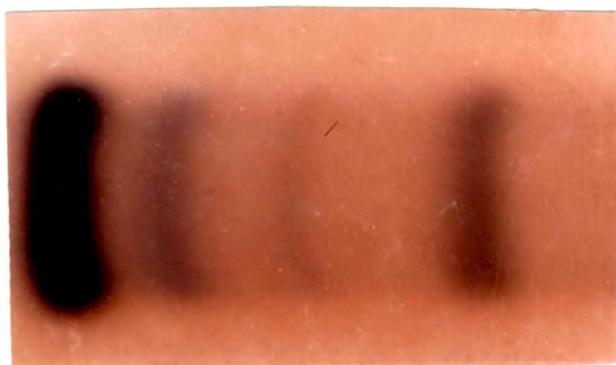
A α1 α2 B r

Fig. 4.8b P - L SB diet fed dams pups



A α1 α2 B r

Fig.4.8c P - L M diet fed dams pups



A α1 α2 B r

Fig.4.8d PM - LSB diet fed dams pups



A α1 α2 B r

Fig.4.8e PSB - L M diet fed dams pups

A = Albumins, α1 = Alpha 1, α2 = Alpha 2, B= Beta, r = Gamma

- (1.) no striking alteration in serum total protein of the pups nursed by dams fed on Mahuda diet (P-LM, PM-LSB, P5B-LM groups), however moderate increase in beta and gamma fractions of globulins in Mahuda fed groups (P-LM or PM-LSB groups).

The findings of study 2 suggested that consumption of Mahuda flowers by the dams to provide about 30% (25g Mahuda flowers/100g diet) of the total dietary carbohydrate during entire gestation and lactation periods (P-LM group) or only during gestation period (PM-LSB group) or only during lactation period (P5B-LM group) exerted adverse effect on the growth of pups.