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Effect of Cassava (*Manihot esculenta* Crantz) on Hexachlorocyclohexane and/or Malathion Toxicity in Pregnant Rats and their Offspring.

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Dedicated this paper to Late Saraswathy Devi K, Former Professor, Department of Biochemistry, Kariavattom, University of Kerala

ABSTRACT The toxic effect of Malathion (50mg\100gm) Hexachlorocyclohexane (HCH) (6mg/100gm) and HCH+ Malathion (6mg HCH and 50 mg Malathion\100 gm bodyweight) on Cassava diet (65%) during the critical period of organogenesis and theirconsequent effect on the offspring were studied. Smaller size of the fetuses and poor ossification sites showed that cassava diet containing HCN (15.6mg/100gm food) produced maternal and embryo toxicity.The effect was marginal with respect to HCH but Malathion appeared to produce severe effect. The offspring of cassava diet fed rats had lower birth weights and pesticide exposure with the same cassava diet as theirdams on subsequent mating did not conceive at all. Urinary excretion of thiocyanate was elevated and rhodanese activity in the maternal liver and placenta was considerably lower in cassava diet fed pregnant rats and pesticide exposure further magnified the activity. Cassava feeding decreased the hepatic microsomal carboxylesterase and increased the hepatic cytosolic glutathione -S-transferase. Malathion has no effect on the activity of hepatic carboxyl esterase while glutathione -S- transferase was inhibited. HCH appears to deplete the hepatic carboxyl esterase and cytosolic glutathione- S- transferase activity.

Key words: *Manihot esculenta* Crantz; Hexachlorocyclohexane; Malathion; thiocyanate; Rhodanese; Glutathione

There was a high incidence of gynaecological diseases reported among female workers occupationally exposed to pesticides and suffered from menstrual function disorders. Such disorders can be caused by both direct influence of toxic chemicals on ovaries and their indirect effect by increasing the gonadotrophic function of the pituitary gland changing metabolism of estrogens by the liver **(1).** The toxicity of Malathion is attributed to its contamination with impurities which are either originally present or may be developed upon storage and detoxified to a greater extent in mammals **(2)**. The hexacholorocyclohexane (HCH) have lipophilic properties and are highly stable chemically. The population groups most at risk from pesticide exposure are generally low income groups. In India, especially in Kerala Cassava (Manihot esculenta Crantz) is consumed as a staple diet by large section of low income group who are undernourished. Although more than 20 glycosides have been identified in edible plant varieties only four (amygaldin, dhurrin, linamarin and lotaustralin) are of practical toxicological importance**(3)** .Linamarin and lotaustralin are the two cyanogenic glycosides that occur in cassava. Toxic effects of cyanogenic glycosiodes are mediated

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by hydrogen cyanide

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(HCN) released during the hydrolysis of glycoside. Conn et al reported that in cassava HCN is liberated from linamarin and lotaustralin by β-glucosidase, linamarase (4). Cassava consists mainly of starch and contains cyanogenic glycosides. Cassava having a very low protein content and its quality is poor due to the low content of several essential aminoacids lysine, methionine and tryptophan. It has been found that the cyanide concentration of less than 50 mg HCN/Kg fresh peeled tuber is harmless, 50-80 mg/ kg is slightly poisonous, 80-100 mg HCN/kg toxic and above 100 mg HCN/kg fresh peeled tuber is fatal (5) .Traditional processing of cassava is unlikely to remove HCN, although cooking or boiling may drive off the free HCN, a considerable quantity of bound HCN may remain **(6**) . It is believed that consumption of large quantity of tapioca is associated with the development of tropical ataxic neuropathy, endemic goiter cretinism and cardiovascular diseases. The disease is prevalent in Nigeria(7) India, (8) Indonesia, (9) and Brazil (10)where cassava is consumed plenty. Some clinicians in Nigeria suspectcassava as a cause of congenital defects in humans if consumed in excess during pregnancy (11) . Studies by Ayangade etal (12) have shown that plasma and amniotic fluid thiocyanate levels were elevated in cassava eating women. The paucity of information regarding the interactive effects of cassava diet and pesticides has prompted the study.

Collection and processing of Cassava

The Cassava tuber, Malayan variety (M-4) (Manihot esculenta crantz) was collected from the local market . Cassava tubers were washed free of dirt and the rind is peeled off. The tubers were then cut into small chips, sun dried powdered and used directly for the preparation of Basal diets.

The Hydrocyanic acid (HCN) content was estimated (13) .

Preparation of Linamarase from Cassava rind

The enzyme is prepared as acetone dry powder from Cassava rind. Tapioca (Cassava)tubers are washed free of dirt adhering water removed with filter paper. The rind is peeled off. 100 gm rind is crushed in a mixer and extracted with 100 ml phosphate buffer (0.1 M pH6.0) centrifuged and the supernatant collected.The supernatant is brought to 80 % acetone concentration under chilled conditions. The acetone powder is seperated by pouring off the acetone and the brown fluffy material is dissolved in phosphate buffer (0.1 M pH 6.0) in the ratio of 1:100 and used as enzyme, linamarase.

Preparation of Cyanoglucoside extract from food samples

Homogenate of food samples was prepared in orthophosphoric acid (0.1 M)[1g food sample in 10 ml] . Centrifuged and collected the supernatant.

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Determination of Hydrocyanic acid

Neutralised aliquots of the homogenate with 0.2 N NaOH or 0.2 N HCl using phenolphthalein as indicator. To each tube added 0.2 ml of enzyme (linamarase) and incubated at 30◦c to one hour. Then the reaction stopped by the addition of 1 ml of 0.2 N NaOH. Then 1 ml of 0.2 N HCl and 1 ml of chloramines T reagent (1% solution prepared in distilled water) with mixing between each addition. After 10 minutes 3 ml of Barbituric acid - Pyridine reagent (3.0 g barbituric acid is dissolved in 30 ml of pyridine and 6 ml conc.HCl makeupto 100 ml and dissolve by slow stirring(prepared fresh on day of use). Optical density was measured at 570 nm after 30 minutes and before 1 hour. Standard is prepared by using potassium cyanide (dried over conc. H_2SO_4 in a dessicator.)

MATERIALS AND METHODS

Hexachlorocyclohexane and Malathion 98% pure were obtained from Premier Pesticides Co, Pvt Ltd, Kochi, India. Analytical grade chemicals were used and purchased from Sigma Chemicals Co Ltd, USA.

Virgin female Sprague - Dawley albino rats inbred in the animal house of the Department weighing 150-180 gm were used. They were divided into two groups A and B and maintained individually on a control diet (16% casein) and cassava diet (65% cassava) for two weeks and water adlibitum. Composition of the diets is given in (Table 1). There were 35 animals in group A and 150 animals in group B. All the animals were mated with males of proven fertility of same age and strain in the ratio of 4:1 in a mass mating procedure. The day on which the females showed the presence of spermatozoa in their vaginal smear was considered day 1 of pregnancy were used for the experiments.

The pregnant animals were divided into 5 groups of 30 animals each as follows.Group 1 = Control diet + groundnut oil vehicle; group 2= cassava diet + ground nut oil vehicle; group 3 = cassava diet + 6 mg HCH/100 g body weight (LD 25) in ground nut oil from 9-13 of gestation [3 doses] group 4= cassava diet + 50 mg Malathion/100 gm body weight (LD 25) in groundnut oil from 9-13 of gestation [3 doses] group5 = cassava + HCH (6 mg/100g) and Malathion (50mg/100gm) in groundnut oil from 9-13 of gestation [3 doses each] .

The HCH and Malathion were administered on alternate days from day 6 to day 15 of gestation. After giving the 4^{th} dose of Malathion 65% abortion occurred. So the dose was fixed as three for which the abortion rate was minimum. For comparison HCH and combination of HCH + Malathion were given at the same dose level during the critical period of organogenesis viz on the 9, 11, 13 days of gestation.The cassava diet fed rats became inactive and develop ataxia. Signs of toxicity like tremor, ataxia, lacrimation and salivation are exhibited by cassava diet fed dams when exposed to HCH, Malathion and HCH+ Malathion. The signs appeared ½-1 hr after administration

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of 3rd doses and persisted for 3 to 4 hrs during gestation of 1st mating. Offspring of the cassava diet fed rats after mating exhibited ataxia more severe than that occurred

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on during 1st mating. The animals became inactive. Feed intake was measured daily and the animals were weighed periodically.

Table 1. Composition (g /100g) of diets

	Control diet	Cassava diet
Casein	16.0	16.0
CHO[1]	65.0	-
Cassava	-	65.0
Fat[2]	10.0	10.0
Vitamin[3] andMineral mix	6.5	6.5
Cellulose	2.5	2.5

[1] Equal parts of glucose, sucrose dextrin and corn starch.

[2] Groundnut oil.

[3] The vitamin mixture contained (mg /100 g diet)

Choline chloride-200, inositol-20, α-tocopherol-12, niacin-5.0. calcium pantothenate - 4.0, Thiamine-0.80, pyridoxine hydrochloride-0.6, folic acid-0.40 Menadione-0.30 and the following units indicated per 100 gm diet.Vitamin acetate- 1000 IU, Vitamin-D-150 IU, Biotin-20 µg and vitamin B 12 - 2 µg.

The salt mixture (g/Kg) KH_2PO_4-310. $CaCO_3$- 210. $Ca_3(PO_4)_2$ – 149. KCl- 120, NaCl-105. $MgSO_4$ (anhydrous) -90.0. $FePO_4$ - $4H_2O$-14.7, NaF-0.57. $CuSO_4.5H_2O$- 0.39 $MnSO_4$ (anhydrous)- 0.20, $K_2SO_4Al_2(SO_4)_3.24H_20$ – 0.09 and KI-0.05. In addition the diet was supplemented with15 mg $ZnCl_2$ and 0.15 mg $CoCl_2.6H_2O$ per kg diet.

Effect of HCH and /or Malathion on the fetuses of cassava diet fed pregnant rats

Twentyfour hour urine was collected individually on 15, 17, 19 days of gestation for thiocyanate estimnation. On the 20th day of gestation 10 pregnant females were anaesthetized with phenobarbitone. The abdominal wall was incised longitudinally and both the uterine horns were carefully exposed. The amniotic fluid sac pierced with a hypodermic needle and the amniotic fluid drawn off. Fetal, placental, amniotic fluid weights and umbilical cord length (UCL) were measured. Crown rump length (CRL) and tail length (TL) were recorded. Three fetuses chosen at random from each litter were fixed in neutral

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formalin and another 4 fetuses litter in rectified sprit and processed for Alizarin red-S-staining by Staples technique(14). Fetuses and skeletal preparations were observed for malformations under a dissecting microscope.

Effect of HCH and /or Malathion on the fertility and reproductive data of cassava diet fed rats 1st and 2nd mating

The remaining animals from 1A (group1-17, group 2- 14, group 3 – 11, group 4 – 10, group 5 – 9) were allowed to deliver. All the animals were maintained on the respective diets as described before in ad libitum. All litters were examined after parturition and daily thereafter. Litter size at parturition and the number of live and dead pups at parturition were recorded. Additional parameters noted over 4 days of postpartum included the number of live pups , and litter weights. Pups were sexed at gross necropsy. Reproductive indices such as fertility (% of bred females that were pregnant) gestation survival (% of pups born alive) and pups viability (% of live pups born that survival to days 1 or 4 postpartum were calculated. Body weight changes of pups were recorded periodically.

On 21 day of lactation 6 dams and 6 pups at random from each of the group were sacrificed by cervical dislocation after 12 hour fasting. The organs liver, brain from dams and pups were removed, blotted , weighed and stored in ice-cold containers at-4º c for analysis.

The remaining female offspring from experiment 1 B were maintained on the respective diets as described earlier group 1- control diet, groups 2, 3, 4 and 5 on cassava diet for 3 months. Growth rate of all rats of all groups were periodically examined. Allowed to mate with males of proven fertility of same age and strain from departmental colony in a ratio of 4:1 in a mass mating procedure. There were 20 females in group 1, 20 females in group 2, 15 females in group 3, 13 females ingroup 4, 10 females in group 5. The females from group 3, 4, and 5 were not pregnant. Pregnant females of group 1 and 2 were maintained on the 16% casein and cassava diet and allowed to deliver. Reproductive and fertility data were examined.

Effect of HCH and /or Malathion on the thiocyanate content, Rhodanese activity of cassava diet fed rats 1st and 2nd mating

Serum, urine, amniotic fluid, placenta and liver were used for the the following analysis. The thiocyanate content was determined by the method of Bowler (15) Rhodanese [Thiosulphate, Cyanide sulphur transferase, E.C. 2.8.1.1] was assayed by Sorbo´s method (16) . Tissue homogenates were prepared in double distilled water containing 0.0125 M sodium thiosulphate and 0.025% bovine albumin (SIGMA) and the chilled homogenates were centrifuged at 3000 rpm for 10 minutes, at 4 ° c. The supernatants were used as the enzyme source. The protein content in the tissue homogenates was estimated after trichloroacetic acid precipitation following the method of Lowry et al (17) using bovine serum albumin
as standard.

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Effect of HCH and /or Malathion on acetylcholinesterase, carboxylesterase, glutathione –s- transferase of cassava diet fed rats and their 21 day old pups.

The tissues were homogenized in 1:4 0.25 M sucrose /0.01M Tris – HCl buffer (pH 7.5) and centrifuged. An aliquot of the 700 x g supernatant was saved for the measurement of catalase and acetylcholinesterase activity. The remaining supernatant was centrifuged for 60 minutes at 100,000 x g to obtain the soluble fraction. Liver homogenate (12.5 w/v) was prepared in icecold 1.15% KCl in 0.01 M phosphate buffer pH 7.4 with a Potter- Elvehjem homogenizer. The crude homogenate centrifuged at 10,000 x g for 10 minutes, for obtaining the mitochondrial supernatant. Microsomes were prepared by spinning in aliquot of each pool of the post mitochondrial supernatant for 1 hour at 105,000 xg. The acetylcholinesterase activity [E.C. 3.1.1.7] was assayed following the method of Ellman et al (18). The Carboxyl activity [E.C. 3.1.1.1] was assayed according to the method of Spenny (19). The glutathione – s- transferase [E.C.2.5.1.18] was determined by the method of Bell et al (20).

Statistical Analysis

Analysis of covariance of bodyweights between groups with normal diet and cassava with initial body weight as a covariate. One way analysis of variance (ANOVA) and t- test for difference between mean levels of the various parameters depending upon the nature of data. Analysis of variance under a 2^2- factorial experiment. The use of CD (critical difference) to evaluate possible differences among treatment combinations.

RESULTS

Effect of HCH and /or Malathion on the fetuses of cassava diet fed pregnant rats

The body weight and the daily food intake of the pregnant rats fed on cassava dietdiffered from the control. (Table 2 A, 2 B) .Feeding of cassava diet during gestation reduced the body weight gain . The pregnant rats fed on cassava diet combined withHCH, Malathion and HCH + Malathion , it is seen that administration of Malathion showed a significant decrease in weight gain during gestation when compared with cassava diet pregnant rats.The interaction is not significant.

Table 2.A. Effect of Cassava	and pesticide exposure in	pregnant rats
	Food intake/day/rat (mgs)	Average cyanide intake/day/rat (gms)
16% casein	15.5±0.314	
Cassava	13.4±0.190	2.01
Cassava+HCH	11.8±0.120[ab]	1.77
Cassava+Malathion	11.5±0.105 [a]	1.72

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Cassava+HCH+Malathion	12.0±0.11 [b]	1.80
Critical difference*	0.40	

Values shown are mean ± SE from six rats.
Groups with common superscript are not significantly different at $P < 0.05$
* Critical difference for all treatment combinations.

In cassava diet fed dams the number of implantation sites and number of live fetuses per litter were low as compared to the control. It is further low in HCH, Malathion and HCH+Malathion exposed cassava diet fed dams

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Table 2 B. Effect of Cassava and pesticide exposure in pregnant rats

Values shown are mean ± SE from six rats.
Groups with common superscript are not significantly different at P < 0.05 Critical difference for all treatment combinations.

Table 3. A Placental and fetal changes -1 st mating

	16% casein	Cassava	Cassava+HCH	Cassava+ Malathion	Cassava HCH+Malathion	Critical difference
Fetal weight (g)	5.513±0.112	4.710±0.071	4.040±0.040[a]	4.210±0.073[a]	3.750±0.042	0.174
Crown rump length(cm)	4.45±0.095	4.21±0.080	3.57±0.037[a]	3.49±0.040[a]	3.04±0.023	0.146
Tail length(mm)	2.21±0.045	1.69±0.030	1.52±0.022	1.35±0.015	1.26±0.515	0.053
Umbilical cord length(cm)	3.40±0.07	3.00±0.051	2.10±0.018	2.58±0.037	2.40±0.024	0.100
Placental Weight (mgs)	860±17.38	761±13.20	606±8.20[a]	656±9.18	594±5.56[a]	27.0
Amniotic fluid weight (mgs)	676±13.7	620±10.5	558±8.0	495±4.3	463±4.0	21.87

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Values shown are mean ± SE from six rats.
Groups with common superscript are not significantly different at P < 0.05
* Critical difference for all treatment combinations.

There was a significant reduction in the fetal weight, tail length , umbilical cord length, placental weight and amniotic fluid weight in fetuses from cassava diet fed dams on the first and second mating when compared with the respective control groups. (Table 3, A, 3B).These parameters were further decreased when cassava diet fed exposed to pesticides.

Table 3. B Placental and fetal changes – 2ND mating

Groups	Fetal weight (g)	Crown rump length (cm)	Tail length (mm)	umbilical cord length (cm)	lacental weight (mgs)	amniotic fluid weight (mgs)
16%Casein	5.51±0.11	4.6±0.091	2.30±0.042	3.2±0.062	862±17.35	684±13.9
Cassava	4.30±0.063	4.2±0.072	1.73±0.044	2.8±0.055	783±13.57	616±9.4

Values shown are mean ± SE from six rats.

Alizarin stained preparations revealed the absence of sternebrae, rib defects and poor ossification of skull bones in about a number of fetuses from cassava diet fed dams. (Table 4). Most of the defects occurred in sternebrae, ribs and bones of thoracic limb. Significantly poor ossification of parietals, intraparietal, pectoral girdle bones, pelvic girdle bones was observed in fetuses from cassava diet fed Malathion + HCH exposed dams.

Table: 4 Effect of HCH and\or Malathion on skeletal anomalies

	16% casein	Cassava	Cassava+ HCH	Cassava+ Malathion	CassavaHCH+ Malathion
Fetuses Examined	60	52	50	48	45
Fetuses with Anomaly	9	14	16	19	20
Litters with Anomalous fetuses	6	7	7	8	8
Average % of Anamolous fetuses[a]	20±0.36	33.0± 0.589	37±0.534	37±0.534	50±1.32
Types % b					
Incomplete sternebrae	10.7	23	28	31	34

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Missing sternebrae	5.5	23	29	32	38
Missing metatarsals	9.6	24	26	32	34

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Rudimentary rib Incomplete ossification	12	24	28	30	32
Parietals	1.0	25	26	28	28
Occipitals	1.3	21	23	24	25
Intraparietal	1.0	20	21	23	23
Pectoral girdle bones	1.8	20	24	25	27
Pelvic girdle bones	2.0	21	23	24	26
siiR	1.1	23	26	27	29

a- Values represent mean of 100 x (no. of anomalous fetus in litter/total number of fetuses in litter ±SE)

b- 100 x (no.of anomalous fetuses in a group/total number of fetuses in a group)

Effect of HCH and /or Malathion on the fertility and reproductive data of cassava diet fed rats 1st and 2nd mating

The fertility was low in the cassava diet fed dams as compared with the control(Table 5). Ingestion of pesticides exhibited a significantly lower fertility index.Treatment with HCH, Malathion and HCH+ Malathion to cassava diet fed dams reduced the live fetuses and the reduction was greater in Malathion and HCH + Malathion exposed groups. There was a smaller number of pups per litter in the cassava diet fed dams as compared to the control. Cassava diet fed dams exposed to pesticides showed a more or less equal or a comparable increase in the number of pups per litter as compared to cassava diet alone fed rats.The sex ratio of the pups atbirth varied within and amongst the groups. The survival rate of the pups was unaffected in the cassava diet fed rats during the period of lactation. The survival rateof the female pups of the prenatal HCH treated dams was decreased during lactation period. Prenatal exposure to Malathion and HCH +Malathion caused a decreased survival rate of both male and female pups during lactation.

Table:5 Fertility and reproductive data of the 1st mated dams – survival andgrowth of their young ones during first 21 days of post partum.

	16% casein	Cassava	Cassava +HCH	Cassava+ Malathion	CassavaHCH+ Malathion
No. of mated females	30	30	30	30	30
No.pregnant at term	27	24	21	20	19
No.for reproductive examination	17	14	11	10	9
Fertility index	90	80	70	65	63

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No of live pups	144	102	76	65	54

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Sex ratio (live males:livefemales)	1.54	1.24	0.77	0.59	0.75
Viability index at day 4	99	98	94	92	90
Lactation index at day 21	98	95	90	87	85

Fertility index (%) - (No. of pregnant females/no. of mated females) x 100

Viability index at day 4 - (No. of pups at day 4/ total number of pups at day 1 x 100)

Lactation index - (No. of live pups at day 21 / no. of pups at day 4 x 100)

There was a significant decrease in the bodyweight of pups on Cassava diet fed groups when compared with the control lactation period. (Table 6) The pups from the pregnant rats maintained on cassava diet exposed to HCH, Malathion and HCH + Malathion gained less weight over the total period of lactation than did those fed on cassava diet only. There was some statistically different decrease in bodyweight in the groups fed cassava diets compared with the controls.

Table:6 Body weight of pups on lactation (gms)

Groups	Day 1	Day 14	Day 21	Weight gain
16% Casein	6.4±0.316	18.9±0.950	38.5±1.43	32.10±1.21
Cassava	5.4±0.233	12.5±0.510	18.0 ±0.76	12.6±0.53[a]
Cassava + HCH	4.80±0.170	10.5±0.350	14.0 ±0.76	9.2±0.33[ab]
Cassava + Malathion	4.60±0.127	9.0±0.254	11.0 ±0.310	6.4±0.32[ac]
Cassava+ HCH+ Malathion	4.26±0.140	8.0±0.170	10.0 ±0.210	5.74±0.24[bc]
Critical difference*				6.72

Values shown are mean ± SE from six rats.
Groups with common superscript are not significantly different at P < 0.05 Critical difference for all treatment combinations.

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The value for mean bodyweight of dams treated with pesticides was significantly lower than cassava diets alone fed groups on 3 months after lactation.When mated the offspring from the first mating with males of proven fertility of same age and strain showed that the number of successful mating as expressed by viability and fertility indices was low in cassava diet fed groups.(Table7)

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In Cassava diet fed groups the number of pups and survival rate of the pups were affected during the lactation. The mean bodyweight of the pups was also reduced significantly during lactation. Failure to maintain pregnancy was exhibited by cassava + HCH admintered group during 2nd mating. On the other hand , the offspring from Malathion and HCH+ Malathion exposed cassava diet fed groups did not conceive atall.

Table 7. Fertility and reproductive data of 2 nd mated dams- Survival and growthof their youngones during first 21 days of postpartum.

	16% casein	Cassava	Cassava + HCH	Cassava+ Malathion	Cassava HCH+ Malathion
No. of matedfemales	20	20	15	13	10
No.pregnant at term	18	13	-	-	-
Fertility index	90	65	-	-	-
Total no of pups born alive	83	47	-	-	-
Sex ratio (live males:live females)	1.52	0.86	-	-	-
Viability index at day 4	98	94	-	-	-
Lactation index at day 21	97	91	-	-	-
Mean pup weight at					
Day 1	6.0±0.120	5.0±0.070			
Day 14	20.0±0.404	12.0±0.173			
Day 21	35.0±0.708	15.0±0.217			

Effect of HCH and /or Malathion on the thiocyanate content, Rhodanese activity of cassava diet fed rats 1st and 2nd mating

The thiocyanate concentration in the serum, urine and amniotic fluid increased on feeding of

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concentration. There was a decreased Rhodanese activity in the liver and placenta ofcassava diet fed rats during first and second mating and pesticides further decreased the activity. (Table 8, &9)

Table:8 Activity of thiocyanate and Rhodanese on 20th day of gestation- 1 st mating

	Thiocyanate (mg thiocyanate/min/gProtein)			Rhodanese	
	Serum mg/100ml	Urine mg excreted/100g feed intake	Amniotic fluidmg/100ml	Liver	Placenta
16% Casein	1.45±0.012	2.32±0.020	0.54±0.0014	26.5±0. 54	0.473±0.0.008
Cassava	1.56±0.014	2.49±0.021	0.57 ±0.0050	23.5±0.27	0.422±0.004
Cassava+HCH	1.69±0.015	2.69±0.026	0.62 ±0.0050	21.9 ±0.0.16[a]	0.395±0.003[a]
Cassava+ Malathion	1.81±0.037 [a]	2.87±0.056[a]	0.64 ±0.007 [a]	21.7±0.152[a]	0.392±0.003[a]
Cassava+HCH+ Malathion	1.79±0.034 [a]	2.85±0.0.05 [a]	0.65 ±0.010 [a]	21.4±0.17[a]	0.389±0.002[a]
Critical difference*	0.083	0.125	0.012	0.58	0.012

Values shown are mean ± SE from six rats.
Groups with common superscript are not significantly different at P < 0.05 Critical difference for all treatment combinations.

Table:9 Activity of thiocyanate and Rhodanese on 20th day of gestation- 2 nd mating

	Thiocyanate (mg thiocyanate/min/gProtein)			Rhodanese	
	Serum(mg/100ml feed intake)	Urine(mg excreted/100g)	Amniotic fluid(mg/100ml)	Liver	Placenta
16% Casein	1.53±0.014	2.5±0.05	0.55±0.11	27.0±0.653	0.48±0.01
Cassava	1.71±0.020	2.7±0.026	0.62±0.01	22.8±0.260	0.29±0.002

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Values shown are mean ± SE from six rats.

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Effect of HCH and /or Malathion on acetylcholinesterase, carboxylesterase, glutathione –s- transferase of cassava diet fed rats and their 21 day old pups.

The cassva diet fed pregnant rats exhibited a significant decrease in brain acety lcholine esterase activity on 20 th day of gestation and 21 st day of lactation. Pups from the above treated rats also showed a similar inhibition. (Table 10) Administration of HCH, Malathion and HCH+ Malathion caused a further inhibition ofbrain acetylcholinesterase activity . The effect was more significant in Malathion treated groups.

Table:10 Activity of acetylcholinesterase in dams and pups (nmoles of thiocholineiodide formed/mg protein)

	0th day of gestation	21 st day of lactation	
	Dams	Dams	Pups
16% Casein	84.6±0.709	85.3±0.713	78.6±0.647
Cassava	78.4±0.921	76.43±0.712	72.4±0.510
Cassava+HCH	68.7 ±0.567	72.00±0.643	67.1±0.550
Cassava+Malathion	58.5±0.337	63.00±0.363[a]	59.6±0.547
Cassava+HCH+Malathion	53.6±0.516	61.40±0.710[a]	57.8±0.655
Critical difference*	1.83	1.84	1.67

Values shown are mean ± SE from six rats.
Groups with common superscript are not significantly different at $P < 0.05$
*Critical difference for all treatment combinations.

Cassava diet caused a significant decrease in the activity of hepatic microsomal carboxylesterase activity of dams and pups when compared with the control. (Table 11) Prenatal exposure of cassava diet fed rats to HCH (6mg/100gm body weight) and HCH+Malathion also exhibited decreased activity in dams and pups. On the other hand prenatal exposure of cassava diet fed rats to Malathion (50 mg/100gm body weight) has no or little effect on hepatic microsomal carboxyl esterase activity of dams and pups when compared with cassava diet fed control.

The hepatic cytosolic glutathione – s- transferase activity was observed to be increased significantly in cassava diet fed dams and their 21 day old pups.(Table 11)

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Administration of HCH, Malathion and HCH+ Malathion from the 9 -13 days gestation (3 doses) caused marked decrease in the activity . Similar changes were seen in the 21 day old pups of the treated dams. Table:11 Activity of hepatic microsomal carboxyl esterase and glutathione-s-transferase on 21 st day of lactation .

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Table: 11 Activity of Microsomal carboxy esterase and Glutathione –s-transferase on 21 st day of Lactation.

	Microsomal carboxyl esterase (n mol salicyclic acid produced/min/per min per mg protein)		Glutathione – s-transferase (n mol NO2 mg protein)	
Dams		Pups	Dams	Pups
16% casein	11.2±0.095	10.34±0.090	3.93±0.028	3.57±0.026
Cassava	9.40±0.073	9.20±0.094	5.09±0.044	4.43 ±0.038
Cassava+HCH	7.18 ±0.033	7.05±0.036	1.56±0.014	1.68±0.017
Cassava+Malathion	9.16±0.085	8.97±0.047	1.73±0.017	1.84±0.021
Cassava+HCH+Malathion	6.22±0.026	6.5±0.030	1.39±0.013	1.45±0.015
Critical difference	0.178	0.171	0.076	0.076

Values shown are mean ± SE from six rats.
All the groups are significantly different at $P < 0.05$
*Critical difference for all treatment combinations.

DISCUSSION

The investigation revealed that administration of 65% cassava diet to pregnant rats caused the development of ataxia and the animal became inactive.The average weight gained during gestation by cassava diet 23 % which was lower than the weight gained (34%) in control dams. The reduction in the maternal weight gain may be due to the decreased fetal size and placental weight. Exposure of cassava diet fed pregnant rats in addition toHCH, Malathion independently or in combination would impair the normal physiological environment for normal and proper placental development resulting in the decreased placental weight which inturn influences the mobilisation and translocation of nutrients to

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the developing fetus thereby causing fetal growth retardation. The less weight gain of the dams and the fetus showed thatcassava diet produced feto toxicity. This is in agreement with Olusi (21) and Frakes(22) . Devi et al reported that HCH and Malathion are embryotoxic when maintained ona low protein diet (23) No reports are available on the combined effect of pesticides and cassava diet on pregnancy. Treatment during 9-13 day of gestation with HCH, Malathion and HCH plus Malathion significantly decreased the live fetuses and

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increased resorption sites per dam as 25%, 28%. and 37% respectively. The more severe reduction in the maternal weight gain and placental weight in pesticide exposed cassava diet fed dams suggests potentiation of maternal toxicity. The effect was more pronounced in Malathion exposed cassava diet fed group. Interaction however is not significant. The development of maternal toxicity in turn lower the number of fetuses, which seem to involve both implantation failures and resorption that may be the result of embryo lethality. The absence of sternebrae, poor ossification of parietals, occipitals and reduced ribs exhibited by the fetuses from cassava diet fed dams shows signs of embryo toxic response. The offspring of cassava diet fed rats had significantly lower birth weight and never attained the same adult weights as those of the control.The offspring of cassava diet fed HCH, Malathion and HCH+Malathion exposed dams exhibited lower birth weights, growth retardation and survived for more than 3 months but never became pregnant.

The average hydrocyanic acid content in the experimental diet was 15.6 mg /100 gm food and the average daily intake per rat per group was in between1.8- 2.01 mg (Table 2A).

The major pathway of cyanide detoxification in man and animals is through the cyanide thiocyanate sulphur transferase (Rhodanese) enzyme pathway. The detoxification of cyanide as well as pesticides (HCH and Malathion) is influenced by the level of sulphur containing aminoacids. The sulphur aminoacid especially cystein is utilized in cyanide detoxification. Manor and Gomez (24) confirm that cyanide detoxification in rats can condition a methionine deficiency in an otherwise balanced diet. Eventhough the diet containing 16% casein that may not be sufficient to supply the sulphur containing aminoacids necessary for the detoxification of HCH, Malathion and cyanide.The higher thiocyanate concentration witnessed in pregnant rats fed cassava diet alone and incombination with pesticides indicated that inspite of the detoxification of cyanide to thiocyanate and its subsequent excretion in the urine , considerable thiocyanate is retained in the bloodstream and the retention is greater in Malathion exposed dams. The retention of thiocyanate in the bloodstream causes the presence of thiocyanate in the amniotic fluid.

Amniotic fluid may play an important role in the fetal nutrition (25). The development of maternal toxicity by accumulation of toxic materials, thiocyanate, the persistent HCH, Malathion and its active metabolite malaoxon leads to the transfer of these chemicals through placental barrier to the fetus, which may be the reason for the developments of fetal toxicity and retarded growth in pesticide exposed cassava diet fed groups. Kamalu reported (26) that growing dogs fed on a rice diet, the cassava diet(gari) and rice plus hydrocyanic acid (equivalent to that present in gari) for 14 weeks indicate that dogs that consumed the gari diet had high blood thiocyanate content and rice with cyanide suffered hypothyroidism and goiter.

A number of studies have been made to establish the correlation between the toxicityof pesticides with the inhibition of AChE (27,28). The symptoms of ataxia and inactiveness of the cassava diet fed rats observed might be due to the inhibition of the

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acetylcholinesterase activity.

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The important pathways of Malathion detoxification are hydrolytic cleavage of esterbond by carboxylesterases and glutathione-s- transferase mediated dealkylation.Nadur et al (29) reported that in rats, exposure to Malathion (30 mg/kg bodyweight orally for 21 days) increased the carboxlyesterases and glutathione – s- transferase activities associated with 25% decrease in acetylcholinesterase. Hydrolysis of carboxylesterases was reported to have decreased with decreased dietary protein (30). The low activity of glutathione-s-transferse, carboxylesterase, Rhodanese and high cyanide (thiocyante) clearly showed that the detoxification of Malathion, ,HCH, and cyanide are depressed when cassava diet fed pregnant rats were exposed to pesticides.The presence of cyanide in the body appears to influence the detoxification of Malathion by inhibiting the glutathione - S- transferase thus inhibit the rapid degradation of Malathion to less toxic metabolite resulting in the development of toxicity. The study revealed that exposure of cassava diet fed rats to Malathion has not further influenced the activity of hepatic carboxylesterase. So the detoxification of Malathion through carboxylesterase may be limited in the presence of cyanide in thebody. The same pattern of changes were was showed by the 21 day old pups.

CONCLUSION

Co-exposure to cassava diet and pesticides results in maternal and developmental toxicity. The pattern of changes in the mother and their 21 day old pups was the same as that observed during gestation, but to a lesser degree. So ingestion of pesticides is less tolerated during the combined stress of cassava diet containing cyanogenic glycosides, and pregnancy. The more severe changes were observed in Malathion exposed dams than in HCH exposed rats.

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