

CHAPTER III

RESULTS

Analysis of chemical components

Results of the nutritive values of Niang beans determined in the present studies are shown in Table 1.

Table 1

The Nutritive values of Niang Beans

Composition (in 100 gm edible food)		Thailand*	Burma**	Present study
Edible portion	(gm)	48.0	-	46.6
Food energy	(Cal)	202.1	-	183.13
Moisture	(gm)	47.1	54.220	51.9
Protein	(gm)	7.9	7.650	9.4
Ether soluble extract	(gm)	0.1	0.390	0.2
Carbohydrate	(gm)	42.4	36.000	36.16
Fiber	(gm)	1.6	-	1.73
Ash	(gm)	0.6	1.400	0.6
Calcium	(mg)	30.0	10.500	62.2
Phosphorus	(mg)	99.3	9.000	151.5
Iron	(mg)	0.4	0.855	0.96
Manganese as MnCl ₂	(mg)	-	82.000	-
Vitamin B ₁	(mg)	0.19	0.421	0.23
Vitamin B ₂	(mg)	0.34	-	0.19
Vitamin C	(mg)	12.8	6.500	-
Folate	(µg)			1.85

* Meevasana, A. (1967) The Bulletin of the Department of Medical Sciences, 9, 1-4, page 1.

** U. Sein Gwan and U. Chit Maung, (1968) J. Sci. & Tech. 1, 221-228.

These figures were in accordance with results reported previously by other authors. The beans contained a high quantity of protein and carbohydrate, vitamins B₁, B₂, C, folic acid and some minerals. Analysis of the amino acids in Niang beans revealed that they contained 19 amino acids as shown in Table 2.

Table 2

The Amino Acids Contents in Niang Beans

Amino Acid	gm/100 gm Edible food	gm/100 gm protein
Tryptophan	0.4423	5.5987
Lysine	0.1748	2.2126
Histidine	0.2882	3.6481
Ammonia	0.2472	3.1291
Arginine	0.2326	2.9443
Aspartic acid	0.2496	3.1594
Threonine	0.1632	2.0658
Serine	0.1266	1.6025
Glutamic acid	0.3483	4.4088
Proline	0.0896	1.1341
Glycine	0.0946	1.1974
Alanine	0.2754	3.4860
Cystine	0.1824	2.3088
Valine	1.1474	1.8658
Methionine	0.0300	0.3797
Isoleucine	0.1422	1.800
Leucine	0.2590	3.2784
Tyrosine	0.1993	2.5227
Phenylalanine	0.2057	2.6037

The comparison of the amino acid contents in Niang beans from the present studies with the previous results obtained by various authors is shown in Table 3.

Table 3

The Amino Acids Contents in Niang Beans As Determined
by Various Author

Amino acid g/100g protein	Thailand*	Burma**	Present study
Tryptophan	1.784	5.3	5.5987
Lysine	3.189	3.3	2.2126
Histidine	2.797	-	3.6481
Ammonia	-	-	3.1291
Arginine	1.924	9.0	2.9443
Aspartic acid	3.936	9.5	3.1594
Threonine	1.962	3.6	2.0658
Serine	2.139	5.8	1.6025
Glutamic acid	4.037	8.8	4.4088
Proline	2.734	15.8	1.1341
Glycine	1.493	7.7	1.1974
Alanine	1.949	8.2	3.4860
Cystine	1.291	-	2.3088
Valine	6.924	4.4	1.8658
Methionine	0.019	-	0.3797
Isoleucine	1.405	-	1.8000
Leucine	2.405	4.2	3.2784
Tyrosine	1.898	-	2.5227
Phenylalanine	1.962	-	2.6037

* Suanpan, S. (1975) 9: 1. page 41. The Bulletin of the Nutrition Division, Department of Health.

** U. Sein Gwan and U. Chit Maung (1966). Proc. Burma Res. Cong. (Med. Sci. Div.). In Press.

The amounts of djenkolic acid extracted from Niang beans with 70% ethanol varied from 1.7 to 10.4%. If the extraction was repeated 2-3 times, the amounts of djenkolic acid increased considerably as shown in Table 4.

Table 4

Amount of Djenkolic Acid in Niang Beans After Extraction
with 70% Ethanol

No. of experiment	Wt. of Niang (gm)	Final volume (ml)	Djenkolic acid		
			mg/ml	mg/gm Niang (Exclude Precipitate)	% of Extraction
1	500	80	-	-	-
2	250	65	0.87	0.23	1.66
3	400	1475	0.38	1.43	10.36
4	800	80	2.50	0.25	1.81
5	600	150	4.32	1.08	7.82
6	600	180	2.20	0.66	4.78
7	1,100	300	2.76	0.75	5.43
8	1,300	350	2.39	0.64	4.63
9	2,100	300	4.82	0.69	5.00

The state of djenkolic acid in Niang beans was determined in the present study. About 69% of djenkolic acid was extracted in the filtrate and only 7% of the amount was found to be in a bound form to proteins. The result illustrated in Table 5 demonstrated that most of djenkolic acid in Niang beans was in a free form.

Table 5

The Free and Bound Forms of Djenkolic Acid in
35 gm Niang Beans

No.	Filtrate		Precipitate		Resuspension in HCl		% of D.A. in Niang
	mg	%	mg	%	mg	% of activity in filtrate	
1	97.50	63.93	55.00	36.07	10.9	11.17	0.44
2	348.25	70.52	145.60	29.48	4.6	1.32	1.41
3	445.05	74.79	150.00	25.21	16.25	3.65	1.70
4	310.25	66.68	155.00	33.32	32.25	10.39	1.33
Mean	300.26	68.98	126.40	31.02	15.00	6.63	0.97

The amount of djenkolic acid in Niang beans could be reduced considerably after boiling with different solvents. Results in Table 6-8 showed that the djenkolic acid content

left in Niang beans were 30%, 32% and 32% after boiling with water, 5% NaHCO_3 and 5% HCl for 10 minutes respectively. If the boiling time in the water and 5% NaHCO_3 was prolonged to 20 minutes the djenkolic acid content in Niang beans were reduced to 8% and 13% respectively as shown in Table 9.

Table 6

The Djenkolic Acid Content (mg) in 10 gm Niang Beans After Boiling with Water for 10 Minutes

No	Distilled water			
	Niang	%	H_2O	%
1	112.0	35.30	205.32	64.70
2	83.0	35.91	148.10	64.09
3	44.0	24.34	136.70	75.70
4	37.0	28.30	93.60	71.70
5	50.0	34.60	94.70	65.40
6	13.59	22.62	46.50	77.38
Mean	56.60	30.17	120.82	69.83



Table 7

The Djenkolic Acid Content (mg) in 10 gm Niang Beans After Boiling with 5% NaHCO₃ for 10 minutes

No	5% NaHCO ₃			
	Niang	%	NaHCO ₃	%
1	73.95	48.03	80.0	51.97
2	93.5	50.62	91.2	49.38
3	39.0	24.9	117.7	75.1
4	29.0	20.9	109.4	79.1
5	30.0	28.5	75.2	71.5
6	13.6	21.0	51.2	79.0
Mean	46.51	32.33	87.45	67.67

Table 8

The Djerkolic Acid Content (mg) in 10 gm Niang Beans After
Boiling with 5% HCl for 10 Minutes

No	5% HCl			
	Niang	%	HCl	%
1	215.6	38.00	352.0	62.0
2	60.0	36.21	105.7	63.79
3	38.5	24.9	116.1	75.1
4	37.5	30.7	84.6	69.3
5	40.5	36.8	69.6	63.2
6	36.4	24.3	113.5	75.7
Mean	71.45	31.82	140.25	68.18

Table 9

The Djenkolic Acid Content (mg) in 10 gm Niang Beans After Boiling
in Solvents for 10 and 20 Minutes

		10 Minutes				20 Minutes			
	Niang	%	H ₂ O	%	Niang	%	H ₂ O	%	
H ₂ O	12.57	13.55	76.82	82.85	10.50	11.32	82.22	88.68	
	17.92	20.29	70.38	79.71	3.46	3.91	84.00	96.09	
Mean	-	16.92	-	31.28	-	7.62	-	92.39	
NaHCO ₃	5.08	21.00	15.84	65.48	3.29	13.60	20.90	86.40	
	10.77	19.29	34.08	61.05	7.16	12.82	48.68	87.18	
	7.50	28.52	18.8	71.48	1.25	4.75	25.05	95.25	
	28.00	26.08	62.40	58.13	23.63	22.01	83.72	77.99	
	21.88	23.05	68.34	71.99	12.13	12.78	82.80	87.22	
Mean	-	23.59	-	65.63	-	13.19	-	86.81	

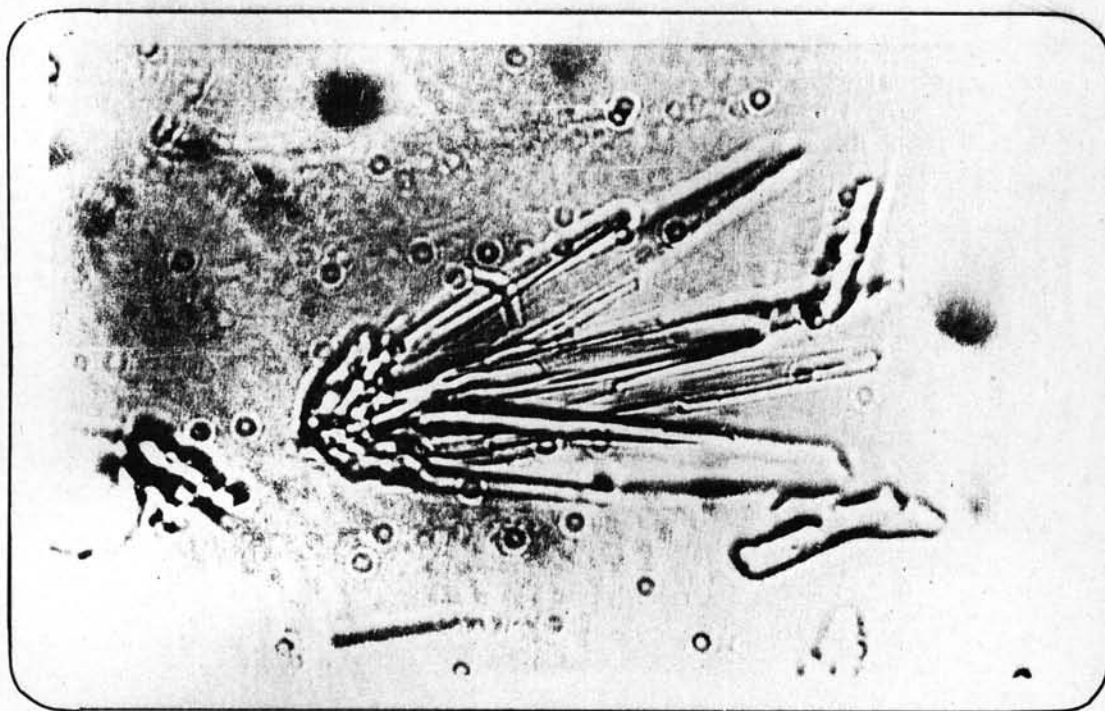


Fig.5 Crystals of djenkolic acid prepared from a commercial L-djenkolic acid

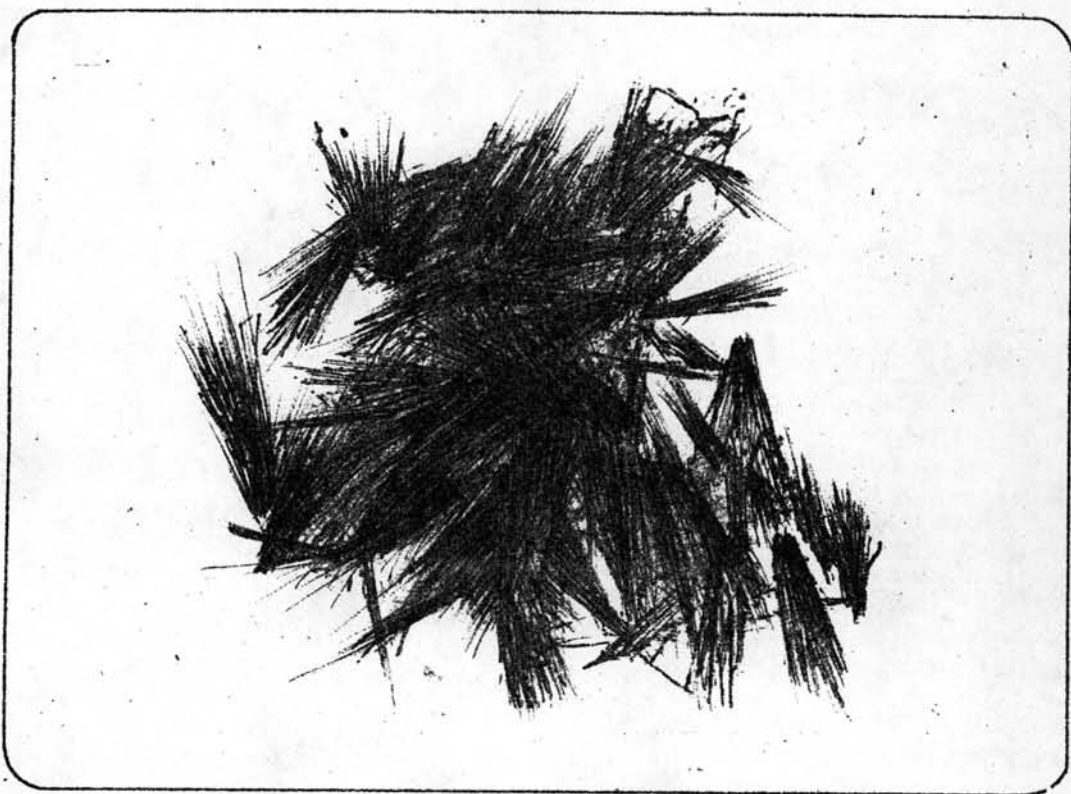


Fig. 6 Crystals of djenkolic acid prepared from a commercial L-djenkolic acid

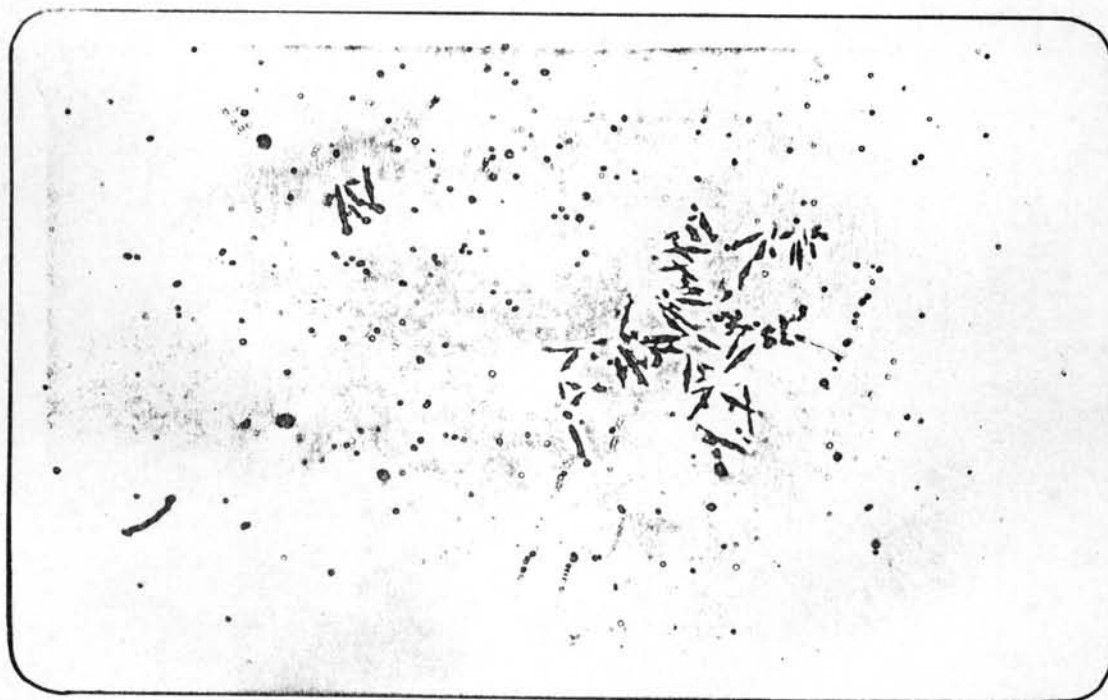


Fig. 7 Crystals from the urine of a mouse during feeding with the extract of Niang beans.

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Table 11

The Amount of Djenkolic Acid (% of Dose Given)
in Urine of Mice

No	Days after feeding									Mean
	1	2	3	4	5	6	7	8	9	
8	27.14	27.84	4.29							19.75
11	18.80	13.60	8.20							13.52
17	4.80	12.60	5.0	17.60	23.67	25.52	8.12	25.64	30.39	17.03
20	9.40	33.60	4.0	49.40						24.10
21	18.00	19.8	50.0	49.40	54.29					38.29
22	24.0									24.0
25	16.82									16.82
27	7.54									7.54
28	7.89									7.89

2. Monkeys The changes of the volume of urine in rhesus monkey before, during and after feeding with Niang extract are shown in Table 12. All 5 monkeys showed decreased volumes of urine during the period of feeding. These volumes returned to the original values after feeding with Niang extract. Urine samples became darker with a typical smell of sulfur. The pH of the urine ranged from 8.0-9.6 and the

Table 12

Changes of the Volume of Urine in Rhesus Monkeys before, during and after Feeding with Niang Extracts.

The volumes are expressed as the mean values in ml/day.

No	Before		During		After	
	No of days	Volume	No of days	Volume	No of days	Volume
R-1	5	75	26	61	6	77
R-2	5	77	23	62	6	88
R-3	5	82	5	54	13	71
R-5	4	72	2	43	2	109
R-7	5	87	4	52	8	66

specific gravity varied from 1.006 - 1.026. Microscopic examination of these samples showed the amorphous particles and mucus. A few epithelial cells and the albumin appeared in the urine throughout the period of experiment. Occasionally cast and white blood cells were seen. Red blood cells appeared only during the first few days. The monkey did not show any obvious signs and symptoms of toxicity. The amounts of djenkolic acid in the urine were 18-22 mg/day or 49-58% of the dose given per day as illustrated in Tables 13 and 14 respectively.

3. Rats The gross appearance of rat's urine after feeding with Niang extracts was similar to that of mice. The reaction of the urine was alkaline and no significant increase in crystals, epithelial cells, red blood cells, white blood cells and casts were seen during the feeding period. The amounts of djenkolic acid in rat's urine after feeding Niang extract varied from 0.90 to 4.29 mg per day or from 3.13 to 29.95% of dose given per day as shown in Table 15 and 16 respectively.

Table 13
 The Amount of Djenkolic Acid (mg/day) in Urine of Monkeys

No	Days after feeding																									X ± SD
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
R-1	17.6	32.5	11.6	24.7	24.7	24.7	24.0	24.0	40.7	11.2	26.3	6.8	32.4	15.5	3.8	36.9	2.6	15.8	12.5	27.8	4.41	16.7	8.2	9.2	40	18.30
R-2	-	14.0	14.0	42.0	42.0	42.0	33.0	33.0	34.4	20.5	50.9	7.2	36.0	23.4	17.2	13.2	20.8	16.2	11.6	22.0	4.41	26.6	13.6	0.9	0.82	22.48

Table 14

The Amount of Djenkolic Acid (% of Dose Given) in Urine of Monkeys

No	Days after feeding																									X ± SD
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
R-1	40.7	75.2	26.8	57.2	57.2	57.2	55.5	55.5	94.2	25.9	60.9	20.6	77.3	37.4	9.2	89.1	8.9	38.2	52.1	100	18.4	69.6	34.3	34.2	16.7	48.50
R-2	-	32.4	32.4	97.2	97.2	97.2	76.4	76.4	79.6	47.4	100	21.8	86.9	56.5	41.5	31.9	50.2	39.1	48.2	91.7	18.4	100	56.7	3.7	3.4	57.76

Table 15

The Amount of Djenkolic Acid (mg/day) in Urine of Rat

No	Days after feeding																- X	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
1	0.95	0.34	0.96	6.62	-	-	-	-	-	-	-	-	-	-	-	-	-	4.29
2	1.34	2.04	2.60	10.46	11.44	4.0	2.36	4.03	0.40	-	-	-	-	-	-	-	-	2.25
3	0.75	1.30	4.38	2.20	7.36	1.68	0.07	0.27	-	-	-	-	-	-	-	-	-	3.47
4	0.95	3.88	5.87	9.21	3.20	0.63	0.36	9.20	3.92	1.47	1.60	7.10	1.70	3.89	0.11	2.43	-	2.23
5	3.51	0.96	2.24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.18
6	3.48	1.13	1.44	0.24	1.56	2.40	0.36	0.02	0.07	-	-	-	-	-	-	-	-	1.26
7	0.18	0.97	1.74	1.92	0.72	1.82	1.48	-	-	-	-	-	-	-	-	-	-	0.90
8	1.61	0.20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.51
9	0.59	3.35	1.88	5.20	6.57	-	-	-	-	-	-	-	-	-	-	-	-	-



Table 16

The Amount of Djenkolic Acid (% of Dose Given) in Urine of Rats

No	Days after feeding											Mean	
	1	2	3	4	5	6	7	8	9	10	11		
1	6.62	2.37	6.69	46.16									15.57
2	9.34	14.22	18.13	72.93	79.78	27.89	16.46	28.10	2.79				29.95
3	5.23	9.06	30.54	15.34	51.32	11.71	0.49	1.88					15.69
4	6.62	27.06	40.93	64.22	22.31	4.39	2.51	64.15	27.33	5.09	5.54		24.55
5	24.48	6.69	15.62										15.59
6	24.27	7.88	4.98	0.83	5.40	6.64	1.25	0.07	0.24				5.72
7	2.80	3.36	6.02	6.64	2.44	6.30	5.12	5.12					4.67
8	5.57	0.69											3.13
9	2.04	11.59	6.50	18.00	22.73								12.17

Histological examination

Mice The histological examination of kidneys were studied in 10 mice.

M-17 (10 days after feeding with Niang extract). Kidneys showed moderate necrosis and cloudy swelling of tubular cells, with an edema of glomerular cells (Fig. 44).

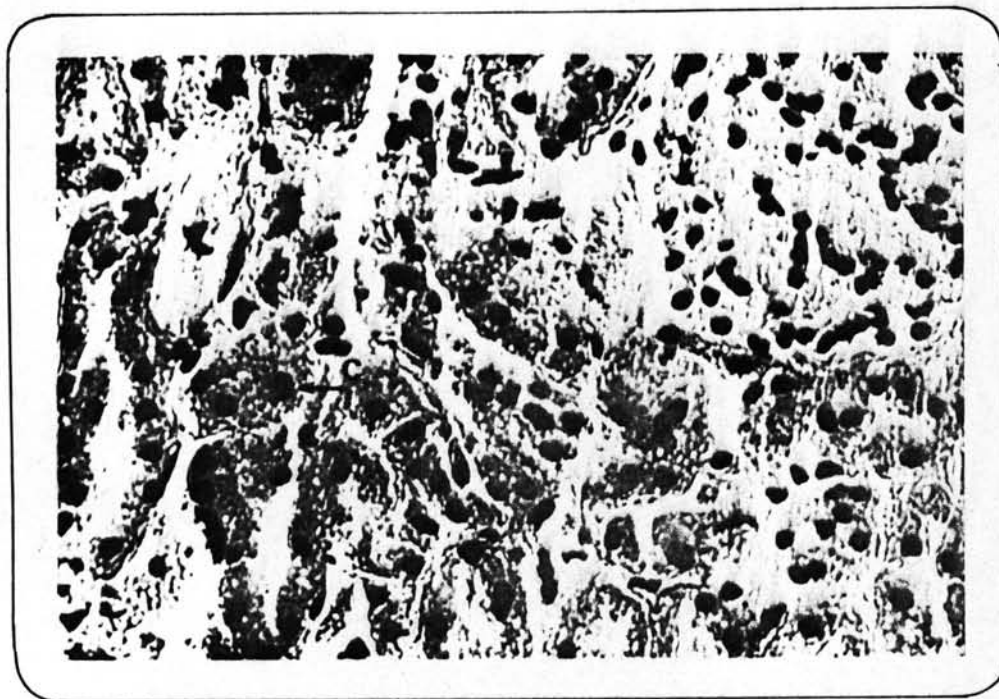
M-20 (4 days after feeding with Niang extract). Kidneys showed a hydropic degeneration and cloudy swelling of tubular cells.

M-21 (5 days after feeding with Niang extract). Kidneys slight focal necrosis and cloudy swelling of tubular cells. Liver cells showed mild fatty metamorphosis.

M-25 (3 days after feeding with Niang extract). Kidneys showed progressive necrosis of tubular cells with pyknosis, karyorrhexis and karyolysis. Some glomeruli also showed pyknotic nucleus of endothelial and mesengial cells (Fig. 45).

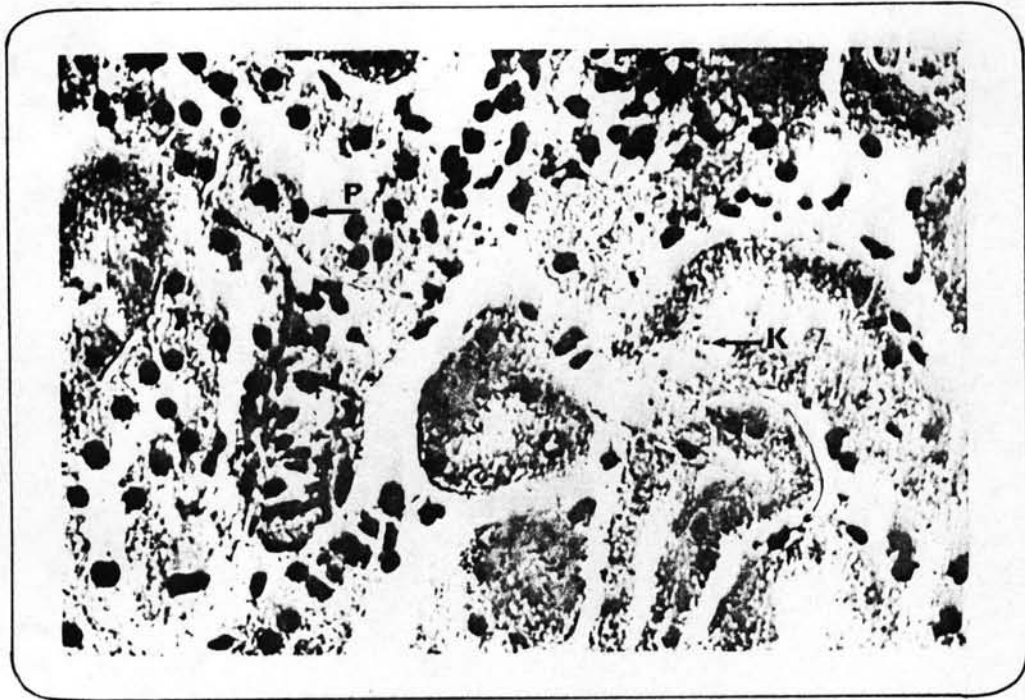
Rats Kidney: Degeneration of kidney especially tubular cells, cloudy swelling. Occasionally the tubules were surrounded with a clear - like area, haemorrhage and constricted tubules.

Liver: Mild degree of fatty metamorphosis with congestion was observed in the liver cells.



C = cloudy swelling

Fig. 8 Histological section of kidney of mouse No. 17, 10 days after feeding with Niang extract, kidneys showed moderate necrosis and cloudy swelling of tubular cells with an edema of glomerular cells.



P = pyknotic nucleus
K = karyorrhexis

Fig.9 Histological section of kidney of mouse No. 25, 3 days after feeding with Niang extract, kidney showed progressive necrosis of tubular cells with pyknosis, karyorrhexis. Some glomeruli also showed pyknotic nucleus of endothelial and mesengial cells.