CHAPTER IV

RESULTS

Yield percentage of *D. trifoliata* leaves extract

Crude of *D. trifoliata* were extracted from 16 Kg air-dried leaves. The yield percentage is presented in Table 4.1

Table 4.1 The yield percentage of *D. trifoliata* leaves extract.

The extraction part	yield	percent yield (wt. by wt.)	physical appearance
ethanol part	410.10 g	2.56 %	dark green gum
dichloromethane part (water insoluble part)	101.04 g	0.6315 %	dark green gum
water soluble part	210.0 g	1.3125 %	pale brown crystal

Acute Toxicity Test of *Derris trifoliata* leaves extract on juvenile Nile tilapia *Oreochromis niloticus*

Preliminary Toxicity Range-Finding Tests : The mortal fish was not observed in the control group and solvent control group at all. There were not found the death of juvenile Nile tilapia in any test concentrations of the crude from water soluble part, but in the water insoluble part were found the death. The water soluble part was not effected to the juvenile Nile tilapia, the data are presented in table 4.2. Thus the definitive tests were performed only the water insoluble part. The mortality percentage is presented in Table 4.3. In the experiment of water insoluble part, the concentrations of 10, 100 mg/L killed all fish within 24 hours, the mortality percentage of concentration 1, 0.1, 0.01 mg/L were 36.67, 13.33, 13.33 respectively.

Multi-Concentration (Definitive) Toxicity Tests : The mortality percentage of the first experiment is presented in Table 4.4. The mortal fish was not found in control group and solvent control group at all. The mortality percentage of the

Table 4.2 Mortality percentage of juvenile Nile tilapia *O. niloticus* at the concentration range of 0.01-100 mg/L the water extract from *D. trifoliata* leaf over 96 hours.

Concentration	Number of fish in experiment	Number of Replication	Percent mortality											
			24-hour			48-hour			72-hour			96-hour		
			No. of dead	death (%)	Mean death (%)	No. of dead	death (%)	Mean death (%)	No. of dead	death (%)	Mean death (%)	No. of dead	death (%)	Mean death (%)
Control	10	1	0	0		0	0		0	0		0	0	
	10	2	0	0	0	0	0	0	0	0	0	0	0	0
	10	3	0	0		0	0		0	0		0	0	
Solvent Control	10	1	0	0		0	0		0	0		0	0	
	10	2	0	0	0	0	0	0	0	0	0	0	0	0
	10	3	0	0		0	0		0	0		0	0	
0.01 mg/L	10	1	0	0		0	0		0	0		0	0	
	10	2	0	0	0	0	0	0	0	0	0	0	0	0
	10	3	0	0		0	0		0	0		0	0	
0.1 mg/L	10	1	0	0		0	0		0	0		0	0	
	10	2	0	0	0	0	0	0	0	0	0	0	0	0
	10	3	0	0		0	0		0	0		0	0	
1 mg/L	10	1	0	0		0	0		0	0		0	0	
	10	2	0	0	0	0	0	0	0	0	0	0	0	0
	10	3	0	0		0	0		0	0		0	0	
10 mg/L	10	1	0	0		0	0		0	0		0	0	
	10	2	0	0	0	0	0	0	0	0	0	0	0	0
	10	3	0	0		0	0		0	0		0	0	
100 mg/L	10	1	0	0		0	0		0	0		0	0	
	10	2	0	0	0	0	0	0	0	0	0	0	0	0
	10	3	0	0		0	0		0	0		0	0	

Table 4.3 Mortality percentage of juvenile Nile tilapia *O. niloticus* at the concentration range of 0.01-100 mg/L the dichloromethane extract from *D. trifoliata* leaf over 96 hours.

Concentration	Number of fish in experiment	Number of Replication	Percent mortality											
			24-hour			48-hour			72-hour			96-hour		
			No. of dead	death (%)	Mean death (%)	No. of dead	death (%)	Mean death (%)	No. of dead	death (%)	Mean death (%)	No. of dead	death (%)	Mean death (%)
Control	10	1	0	0		0	0		0	0		0	0	
	10	2	0	0	0	0	0	0	0	0	0	0	0	0
	10	3	0	0		0	0		0	0		0	0	
Solvent Control	10	1	0	0		0	0		0	0		0	0	
	10	2	0	0	0	0	0	0	0	0	0	0	0	0
	10	3	0	0		0	0		0	0		0	0	
0.01 mg/L	10	1	1	10		1	10		1	10		1	10	
	10	2	0	0	6.67	0	0	6.67	1	10	13.33	1	10	13.33
	10	3	1	10		1	10		2	20		2	20	
0.1 mg/L	10	1	0	0		1	10		1	10		1	10	
	10	2	2	20	6.67	2	20	10.0	3	30	13.33	3	30	13.33
	10	3	0	0		0	0		0	0		0	0	
1 mg/L	10	1	3	30		3	30		3	30		3	30	
	10	2	3	30	33.33	3	30	33.33	4	40	36.67	4	40	36.67
	10	3	4	40		4	40		4	40		4	40	
10 mg/L	10	1	10	100										
	10	2	10	100	100	-	-	-	-	-	-	-	-	-
	10	3	10	100										
100 mg/L	10	1	10	100										
	10	2	10	100	100	-	-	-	-	-	-	-	-	-
	10	3	10	100										

Table 4.4 Mortality percentage of juvenile Nile tilapia *O. niloticus* at the concentration range of 2.0-5.0 mg/L the dichloromethane extract from *D. trifoliata* leaf over 96 hours.

Concentration	Number of fish in experiment	Number of Replication	Percent mortality											
			24-hour			48-hour			72-hour			96-hour		
			No. of dead	death (%)	Mean death (%)	No. of dead	death (%)	Mean death (%)	No. of dead	death (%)	Mean death (%)	No. of dead	death (%)	Mean death (%)
Control	10	1	0	0		0	0		0	0		0	0	
	10	2	0	0	0	0	0	0	0	0	0	0	0	0
	10	3	0	0		0	0		0	0		0	0	
Solvent Control	10	1	0	0		0	0		0	0		0	0	
	10	2	0	0	0	0	0	0	0	0	0	0	0	0
	10	3	0	0		0	0		0	0		0	0	
2.0 mg/L	10	1	2	20		10	100		10	100		10	100	
	10	2	1	10	13.33	6	60	70.0	7	70	80.0	8	80	83.33
	10	3	1	10		5	50		7	70		7	70	
2.5 mg/L	10	1	3	30		10	100		10	100		10	100	
	10	2	1	10	16.67	8	80	76.67	8	8	93.33	8	8	93.33
	10	3	1	10		5	50		10	100		10	100	
3.0 mg/L	10	1	7	70		10	100							
	10	2	8	80	60	10	100	100	-	-	-	-	-	-
	10	3	4	40		10	100							
3.5 mg/L	10	1	7	70		10	100							
	10	2	10	100	70	10	100	100	-	-	-	-	-	-
	10	3	4	40		10	100							
4.0 mg/L	10	1	10	100		10	100							
	10	2	10	100	90	10	100	100	-	-	-	-	-	-
	10	3	7	70		10	100							
4.5 mg/L	10	1	10	100										
	10	2	10	100	100	-	-	-	-	-	-	-	-	-
	10	3	10	100										
5.0 mg/L	10	1	10	100										
	10	2	10	100	100	-	-	-	-	-	-	-	-	-
	10	3	10	100										

Table 4.5 Mortality percentage of juvenile Nile tilapia *O. niloticus* at the concentration range of 1.0-2.5 mg/L the dichloromethane extract from *D. trifoliata* leaf over 96 hours.

Concentration	Number of fish in experiment	Number of Replication	Percent mortality											
			24-hour			48-hour			72-hour			96-hour		
			No. of dead	death (%)	Mean death (%)	No. of dead	death (%)	Mean death (%)	No. of dead	death (%)	Mean death (%)	No. of dead	death (%)	Mean death (%)
Control	10	1	0	0		0	0		0	0		0	0	
	10	2	0	0	0	0	0	0	0	0	0	0	0	0
	10	3	0	0		0	0		0	0		0	0	
Solvent Control	10	1	0	0		0	0		0	0		0	0	
	10	2	0	0	0	0	0	0	0	0	0	0	0	0
	10	3	0	0		0	0		0	0		0	0	
1.0 mg/L	10	1	0	0		1	10		2	20		2	20	
	10	2	0	0	0	0	0	3.33	0	0	6.67	0	0	6.67
	10	3	0	0		0	0		0	0		0	0	
1.25 mg/L	10	1	0	0		1	10		1	10		1	10	
	10	2	0	0	0	0	0	6.67	0	0	6.67	0	0	6.67
	10	3	0	0		1	10		1	10		1	10	
1.5 mg/L	10	1	0	0		1	10		1	10		2	20	
	10	2	0	0	0	1	10	13.33	2	20	16.67	3	30	23.33
	10	3	0	0		2	20		2	20		2	20	
1.75 mg/L	10	1	1	10		2	20		2	20		2	20	
	10	2	0	0	3.33	5	50	33.33	5	50	33.33	5	50	36.67
	10	3	0	0		3	30		3	30		4	40	
2.0 mg/L	10	1	0	0		0	0		1	10		1	10	
	10	2	0	0	3.33	1	10	23.33	2	20	30.0	4	40	36.67
	10	3	1	10		6	60		6	60		6	60	
2.25 mg/L	10	1	0	0		0	0		0	0		0	0	
	10	2	0	0	3.33	6	60	43.33	8	80	56.67	9	90	60.0
	10	3	1	10		7	70		9	90		9	90	
2.5 mg/L	10	1	0	0		5	50		7	70		8	80	
	10	2	1	10	6.66	9	90	73.33	9	90	80.0	9	90	86.67
	10	3	1	10		8	80		8	80		9	90	

concentration of 4.5, 5 mg/L were 100 % within 24 hours, 3, 3.5, 4 mg/L were 100 % within 48 hours, 2, 2.5 mg/L were 83.33, 93.33 respectively. The mortality percentage of all concentrations in the first experiment were over than 50 %, the data from this experiment can not calculate the LC_{50} so the secound experiment must be decreased the concentration range. The mortality percentage of the secound experiment is presented in Table 4.5. The LC_{50} 96-h. of *D. trifoliata* leaves extract is 1.99 mg/L, NOEC is 1.5 mg/L. The statistical result and the calculation of AF are shown in Appendix C. All fish died in the experiments showed the same series of signs and symptoms; swam at the surface; pectoral fins anteriorly extended; partial loss of equilibrium; light pigmentation; excessive mucus; gill hemorrhage; and reduce respiration.

Table 4.6 The LC_{50} of *D. trifoliata* leaves extract exposure to juvenile Nile tilapia.

Time of exposure	24-hour	48-hour	72-hour	96-hour
LC_{50} (mg/L)	7.66945	2.25345	2.09769	1.98772

Figure 4.1 Probit transformed responses at 96-hour LC_{50}

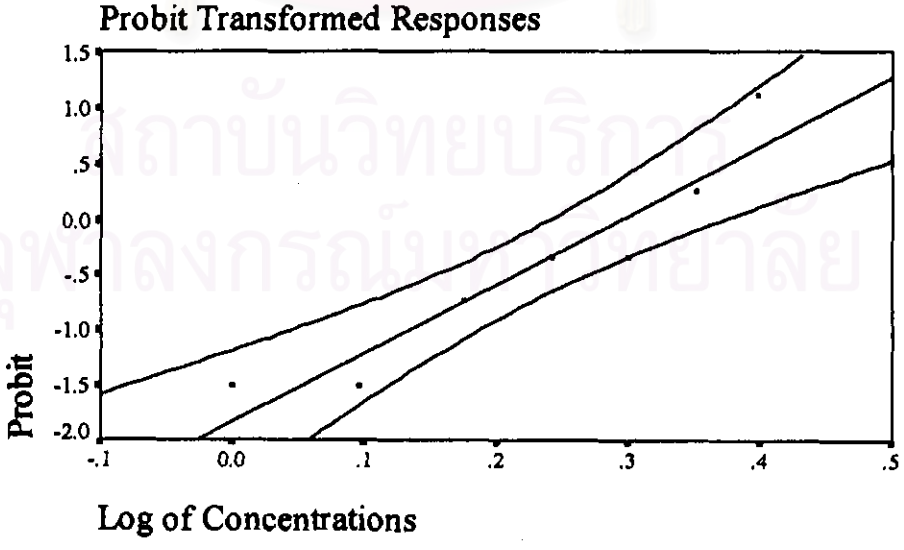
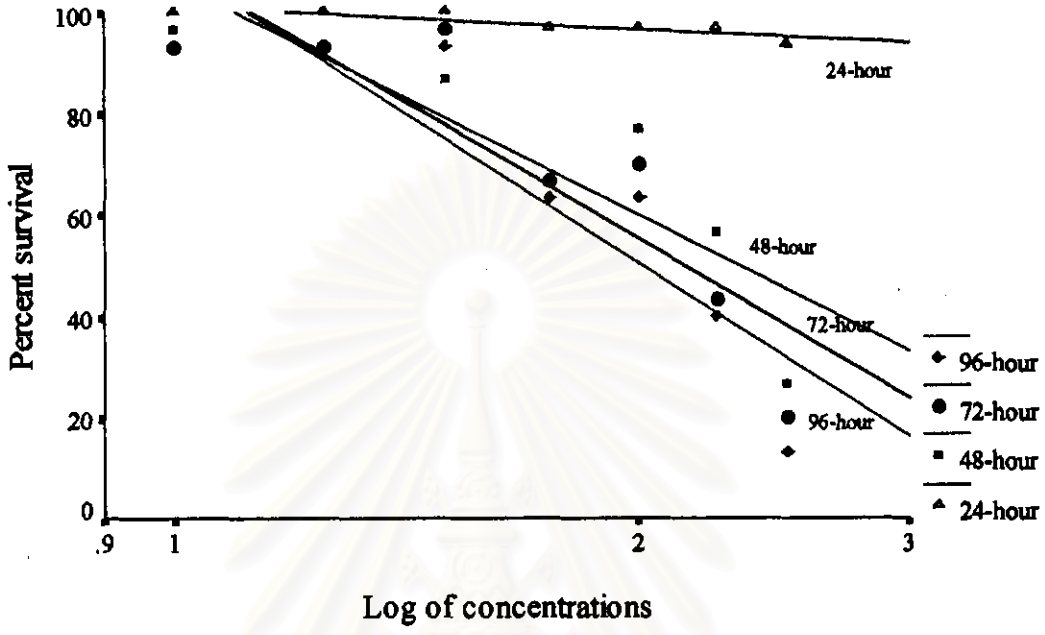


Figure 4.2 Percent survival of *O. niloticus* during acute toxicity



Water Quality : The quality of dilution water during acute toxicity experiments were constant and within a normal requirement of freshwater species. All water quality parameters among treatments were in the same range throughout the tests. It was cleared that the mortality of juvenile Nile tilapia was not affected by the water quality.

Table 4.7 Water quality during the acute toxicity test.

Parameter	Range
Temperature (°C)	26-28
Dissolved oxygen (mg/L)	6-8
pH	7.4-7.8
Hardness (mg/L)	2300-2500

Sub-acute Toxicity Test of *Derris trifoliata* leaves extract on Nile tilapia *Oreochromis niloticus*

Water Quality : All water quality parameters were maintained in the normal range throughout the experiment. The water quality was fading down during the experiment, so in the duration of 72 hours must be renewed the dilution water and test solution of all experiment units. Since all water quality parameters were in the normal range throughout this investigation, it is suggested that the water quality should not be involved the significant differences in growth and histopathological alteration of the liver. The water quality monitoring during 5-month of sub-acute toxicity test is shown in Table 4.8.

Table 4.8 Water quality during the sub-acute toxicity test

Parameter	Range
Temperature (°C)	24-28
Dissolved oxygen (mg/L)	7.2-8
pH	7.3-7.83
Hardness (mg/L)	2300-2500

Gross anatomy of Nile tilapia liver

The liver from control and solvent control group were normal in structure and varies from yellowish brown to dark red in color (Plate1-A&B). Some of the liver from treatment group were observed yellowish brown in color, swelling, large area of subcapsular hemorrhagic, and subcapsular cyst forming (Plate1-C). The lesions of treated liver from the late month of experiment were frequently observed more than the early month. The relative liver weight index from 4th month and 5th month show in Table 4.11

Light Microscopy of Nile tilapia liver

In Nile tilapia liver, the polygonal hepatocytes are arranged between the sinusoids as a mesh-like network form and anastomosing cords. As in other fish, there is no lobulation. Each tube is surrounded by a number of blood capillaries which derive from the portal veins and interpenetrate the entire mass of the liver (Plate 1-A&B). The capillaries merge together to give rise to lager blood-vessels (Harder and Sokolof, 1975). Most of the hepatocytes nuclei are located in the central of cell or located basally in well fed liver. The appearance of hepatocytes were varied between specimens by the degree of vacuolation. In the routine process of tissue preparation, which is removed glycogen and fat that shown the space of vacuole in well fed fish.

Histological alteration of treated liver

In the first month, control and solvent control livers were normal observed in their structure. A few liver from solvent control had some lipid droplets (Plate 2-A&B). Treated livers showed the sign of cellular injury, most of livers were observed slightly hydropic swelling around the sinusoids (Plate 2-C&F). The blood vessels were congested (Plate 2-D). A few case was displayed foci necrosis (Table 4.9), the spot of necrosis area located around sinusoids (Plate 2-D&E). For histochemistry study, the PAS staining of tested livers were not show the difference of glycogen between groups (Plate 3-A-C). Although some of treated livers were pale in color staining less than the livers from both of the control groups. By contrast, treated livers had fat droplets more than control livers (Plate 3-D-F).

At 2nd month, livers from both of control groups were normal and almost found cytoplasmic vacuolation of lipids droplets from well feeding (Plate 7-A&B). The treated livers were observed sinusoids dilated and congested (Plate 7-C&D), the areas around blood vessel were seen hydropic degeneration and accumulation of fat droplets (Plate 7-B). Several cases revealed necrotic foci, the most severe case had the hemorrhagic inflammation (Table 4.9 , Plate 7-E&F). The PAS staining showed the sign of glycogen deplete in treated livers. The control livers seem to be the same as

the first month (Plate 8-A-C). The oil red O staining showed that the lipid accumulation of treated livers were more than the control livers (Plate 8-E-F).

At 3rd month, almost livers from both of control groups were observed the accumulation of fat, that showed the cytoplasmic vacuolation (Table 4.9 , Plate 12-A&B). In the treatment group, the sinusoids were congested and dilated (Plate 12-D), numerous of necrotic cell foci were observed and more severely damaged hepatocytes than 2nd month (Table 4.9, Plate 12-C). Several cases were seen hemorhagic inflammation, accumulation of erythrocytes obviously outside the vascular and the white blood cell were also infiltrated to the necrotic area (Table 4.9 , Plate 12-C-F). The glycogen storage in treated livers were less than control livers (Plate 13-A-C). The fat accumulation of treated livers were higher than control liver (Plate 13-D-F).

In the case of 4th month, the control livers liked the liver in 3rd month (Plated 17-A&B). There were severely damage of hepatocytes in treatment liver. The sinusoid were stilled dilated and congested (Plate 17-C&E). Numerous hydropic and fatty degeneration saw in most liver (Table 4.9 , Plate 17-C&F). The erythrocytes and lymphocytes infiltrated (Plate 17-C), large area of necrotic were also found. The fibroplasia (fibrosis) was seen in some cases as a repair response to cell death following (Plate 17-D). In the damage areas were pale stained of PAS less than the connecting area that showed the distinct of glycogen depletion (Plate 18-B&C), the control livers were same as previous months (Plate 18-A). There were a lot of lipid droplets in treated livers (Table 4.9 , Plate 18-D-F).

At 5th month, All the liver from both of control group were observed the cytoplasmic vacuolation that cause of lipid accumulation. The treatment liver were still observed injury cellular like 4th month. But the cases of fibroplasia were frequently found in this month as the regenerative of the liver cell (Plate 22-B,D-F). The glycogen was distinctive depletion on the damage area (Plate 23-A&B), the control livers were same as previous months (Plate 23-A). The accumulation of lipid droplets in treated liver were more than control livers (Plate 23-D-F).

Table 4.9 The presence of cellular injury

The lesion	1st Month	2nd Month	3rd Month	4th Month	5th Month
Hydropic swelling	+	++	+++		
Vacuolation	+	++	+++	+++	+++
Focal Necrosis	+	+	++	+++	+++
Blood congestion	+	+	++	+++	+++
White blood cell infiltration		+	++	+++	+++
Fibroplasia				+	+

+ : mild ; ++ : moderate ; +++ : strong

Ultrastructure of Nile tilapia liver

Control Nile tilapia liver at 1st month old up to 5th month revealed the normal of cytoplasmic organelles, mitochondria exhibited normal configuration, some specimen showed well development of RER cisternea. There were glycogen granule scatter throughout cytoplasm and lipid droplets were found especially the livers from elder month period (Plate 4-A&B, 9-A&B, 14-A&B, 19-A&B).

Ultrastructure Changes of Exposure Nile tilapia liver

At 1st month, most of cell showed normally profile but there were mild degree of cellular injury. The condensation of mitochondria metrix and expansion of intracristae spaces were observed (Plate 5-A). The autophage vacuoles (Plate 6-A), lysosome (Plate 6-B) and membranous-myelin like structure (Plate 5-B) were note in some specimen.

2nd month and 3rd month, the distinctive proliferation and parallel array of cisternea of rough endoplasmic reticulum were observed (Plate 10-A). The contraction were observed in the most of mitochondria (Plate 10-B, 15-A&B), and also found some mitochondria exhibited swelling with flocculent density and rupture of mitochondria cristre (Plate 11-A&B). Numerous of fragmented endoplasmic reticulum were scattering on over the cytoplasm (Plate 10-B, 11-A-B, 15-A). The swelling of mitochondria and fragmented endoplasmic reticulum were starting found on 2nd month that were the additional pathological of cellular injury from 1st month. There were some lipid droplets were found (Plate 16-A).

4th month up to 5th month, the proliferation and fragmentation of rough endoplasmic reticulum were observed (Plate 20-A&B, 21-A). Some hepatocyes displayed the rearrangement of RER to parallel array of cisternea (Plate 21-A&B). Numerous of vesiculated smooth endoplasmic reticulum was notice. Both of the contracted and swelling mitochondria were observed (Plate 20-A&B, 21-A&B, 25-A) and the lipid droplets were also found (Plate 24-A, 25-A). The relatively shift of RER to be SER was very distinctive pathological effect which was starting found on 4th month. Necrosis of hepatocytes were observed especially in 5th month.

Effects of *Derris trifoliata* leaves extract on Growth and Relative Liver Weight Index of Nile tilapia *Oreochromis niloticus*

Statistical testing on Growth : Length and weight of experimental fish *O. niloticus* were used to represent the growth parameters, which were used in statistical testing. An analysis of variance was performed separately for each months, and also separately tested in each group. The data were tested at an $\alpha = 0.05$ for growth differences among experimental groups. Table 4.10 and Table 4.11 show the mean of length and weight for each experimental groups. The bar graph in Figure 4.3 and Figure 4.5 are indicated the difference of length and weight in each month. The line graph in Figure 4.4 and Figure 4.6 are indicated the increasing of length and weight from month to month during five exposure months.

Table 4.10 Mean of length (cm) from growth test

Experimental group	Length (cm) ± SD				
	1st month	2nd month	3 rd month	4 th month	5th month
Control	5.35 (± 0.54)	6.88[a] (± 0.85)	10.68*[b] (± 1.48)	11.18[b] (± 1.15)	15.49*[c] (± 0.76)
Solvent Control	5.34 (± 0.72)	7.19[a] (± 0.87)	11.91*[b] (± 1.03)	12.73*[b] (± 1.18)	15.04*[c] (± 0.88)
Treatment	5.33 (± 0.81)	7.22[a] (± 1.44)	9.18[b] (± 1.00)	10.55[c] (± 1.13)	13.58[d] (± 1.22)

* indicate significant difference in the column at $p \leq 0.05$

The superscript indicate significant difference in the row at $p \leq 0.05$

Table 4.11 Mean of weight (g) from growth test

Experimental group	Weight (g) ± SD				
	1st month	2nd month	3rd month	4th month	5th month
Control	2.63 (± 0.72)	6.06 (± 2.52)	23.91^{*a} (± 9.77)	27.93^{*a} (± 7.75)	68.07^{*b} (± 9.89)
Solvent Control	2.59 (± 0.96)	6.41 (± 2.27)	26.62^{*a} (± 6.78)	37.98^{*b} (± 9.99)	56.6^{*c} (± 11.98)
Treatment	2.62 (± 1.05)	7.35^{a} (± 3.47)	13.40^{b} (± 4.06)	20.29^{c} (± 6.71)	39.39^{d} (± 8.04)

* indicate significant difference in the column at $p \leq 0.05$
The superscript indicate significant difference in the row at $p \leq 0.05$

There was no significant difference in growth among group of the first month and the secound month. Significant reduction in length and weight were starting found in the third month at 0.2 mg/L of *D. trifoliata* leaves extract. The fish length in treatment group is 14.03%, 22.91% lower than the control and solvent control group. And the weight in treatment group is 43.94%, 49.65% lower than the control and solvent control group.

In the forth month, significant difference in length was found only the solvent control group. The length in treatment group is 5.65% lower than the control group which is not statistically significant and 17.14 % lower than the solvent control which is statistically significant. Significant reduction in weight was found in the treatment group. The weight of fish in this group is 27.33%, 46.56% lower than the control and solvent control group.

In the fifth month, Significant reduction in length and weight were found. The fish length in treatment group is 12.34%, 9.69% lower than the control and solvent control group. And the weight in treatment group is 42.13%, 30.4% lower than the control and solvent control group.

The analysis of variance of length in each group variable by month were found the significant difference. In the control group and solvent control group, the length was significantly increase from 1st month to 2nd month, 2nd month to 3rd month, and 4th month to 5th month, but from the 3 rd month to 4th month was not significant. For the treatment group, length was significantly increase from the first month throughout

throughout the fifth month, the significant differences are indicated by the superscript that shown in Table 4.9.

The analysis of variance of weight in each group variable by month were found the significant difference. In the control group, the 1st month to 2nd month and 3rd month to 4th month were not significant, the 2nd month to 3rd month and 4th month to 5th month were significant. In the solvent control group, there was not significant difference in weight from 1st month to 2nd month, the significant of weight increasing were starting found from 2nd month throughout 5th month. For the treatment group, weight was significantly increase from the first month throughout the fifth month. The significant differences are indicated by the superscript that show in Table 4.10.

Figure 4.3 Mean of length of Nile tilapia *O. niloticus* in 5-month test period

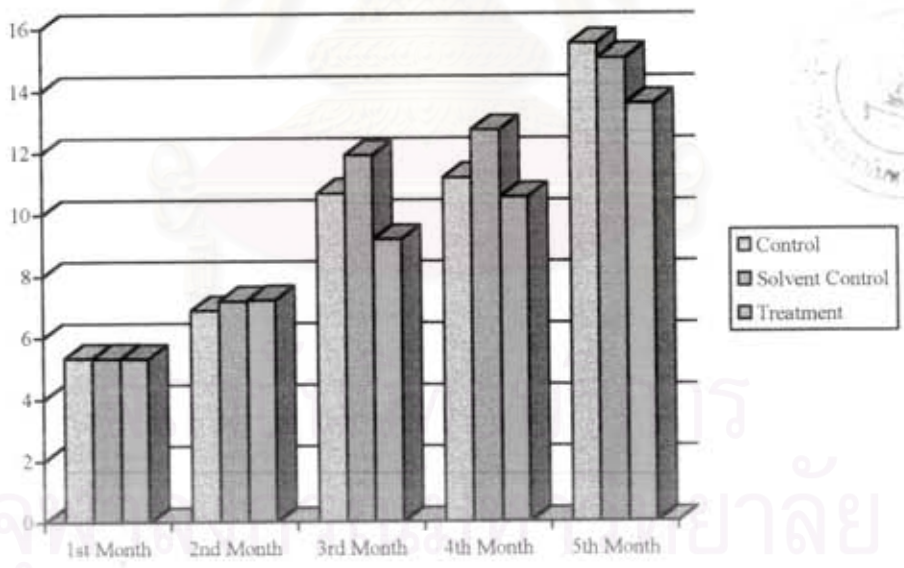


Figure 4.4 Mean of length of Nile tilapia *O. niloticus* in 5-month test period

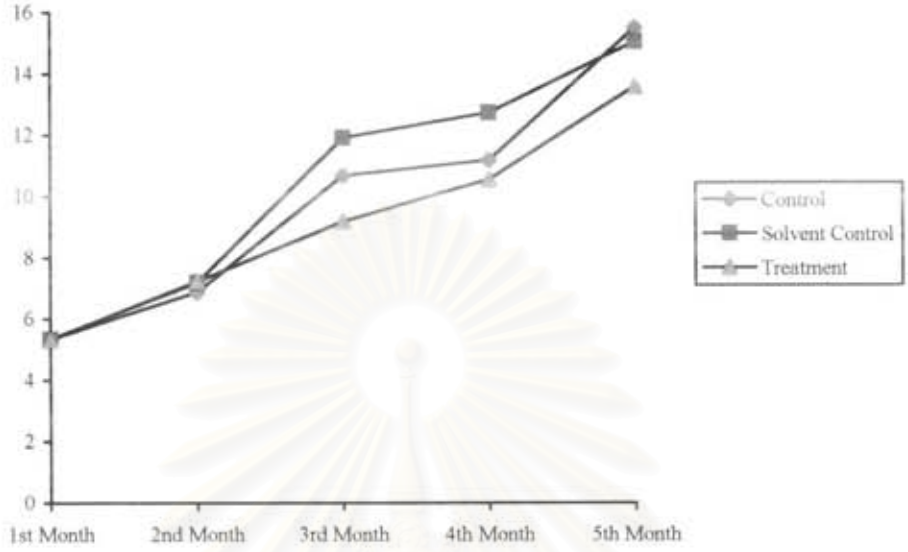


Figure 4.5 Mean of weight of Nile tilapia *O. niloticus* in 5-month test period

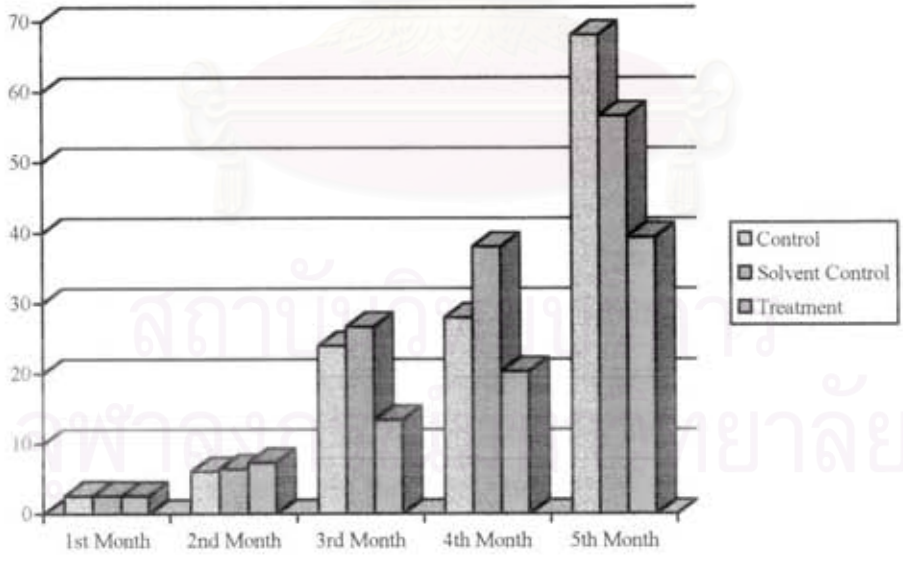
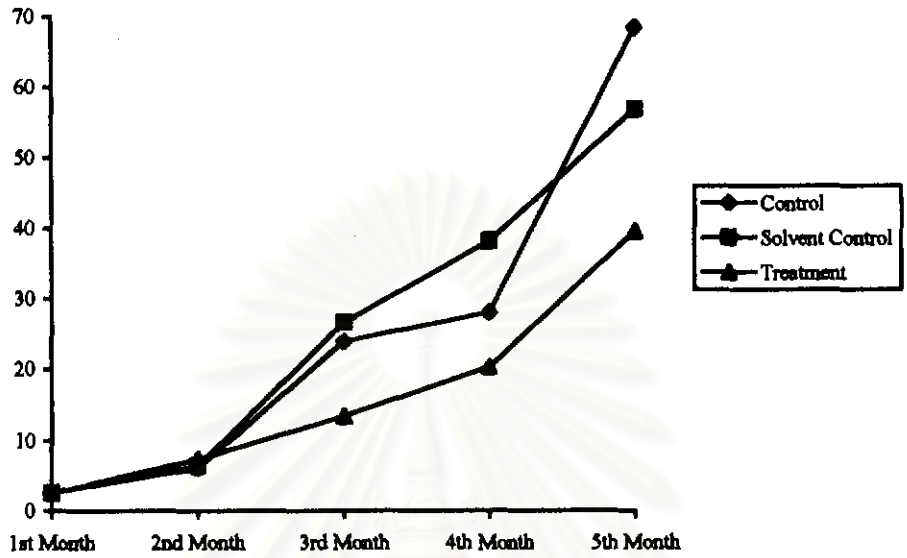


Figure 4.6 Mean of weight of Nile tilapia *O. niloticus* in 5-month test period



Relative Liver Weight Index : The relative liver weight index is calculated by deviding the liver weight by its own body weight of test fish. It can be written in the equation *"Relative weight index = liver weight / body weight"*.

The liver of juvenile fish are very small and hard to dissect it from other organs in abdominal cavity. Therefore, data in which used for calculated the index were only the data from the last two months (4th and 5th month) of the experimental period. The relative liver weight index are shown in Table 4.12. An analysis of variance was separately performed in each month for statistical testing of this parameter. The data were tested at an $\alpha = 0.05$ for the differences among experimental groups. Analysis of variance was also performed to determine the differences between months of the same group.

In the forth month, the index of treatment group (0.031) was higher than the control (0.023) and solvent control group (0.21), and it was significantly difference from both of control groups. There were not found the statistically significant difference in relative weight index from the fifth month of investigation period. The index was increasing from 4th month to 5th month. In the same group, the 4th

treatment index (0.031) was not significantly difference from 5th index (0.033). By contrast, the 4th index of control groups (0.023, 0.021) were significantly difference from 5th month (0.032, 0031).

Table 4.12 Relative liver weight index

Month	Control	Solvent Control	Treatment
4th Month	0.023 ± 0.005	0.021 ± 0.004	0.031* ± 0.008
5th Month	0.032^{a} ± 0.004	0.031^{a} ± 0.007	0.033 ± 0.008

* indicate significant difference in the column at $p \leq 0.05$
The superscript indicate significant difference in the row at $p \leq 0.05$

Figure 4.7 Relative liver weight index (%)

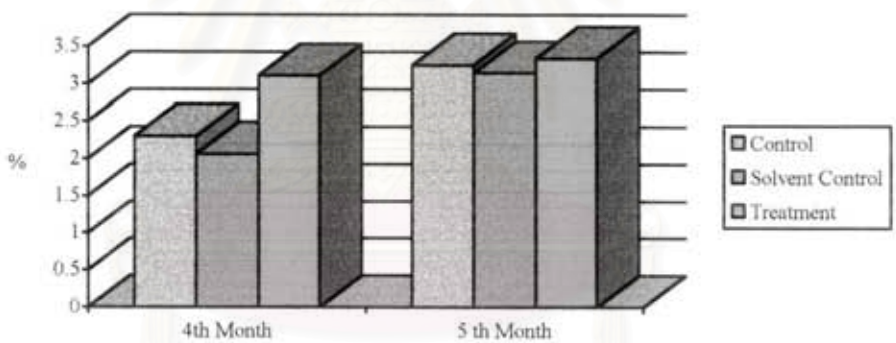


Plate 1

Gross anatomy of Nile tilapia liver

Figure A Control liver : Normal confine in structure, dark red in color.

Figure B Solvent Control liver : Normal confine in structure, dark red in color.

Figure C Treated liver is showing the injury, yellowish brown in color, swelling, large area of subcapsular hemorrhagic, and subcapsular cyst forming.

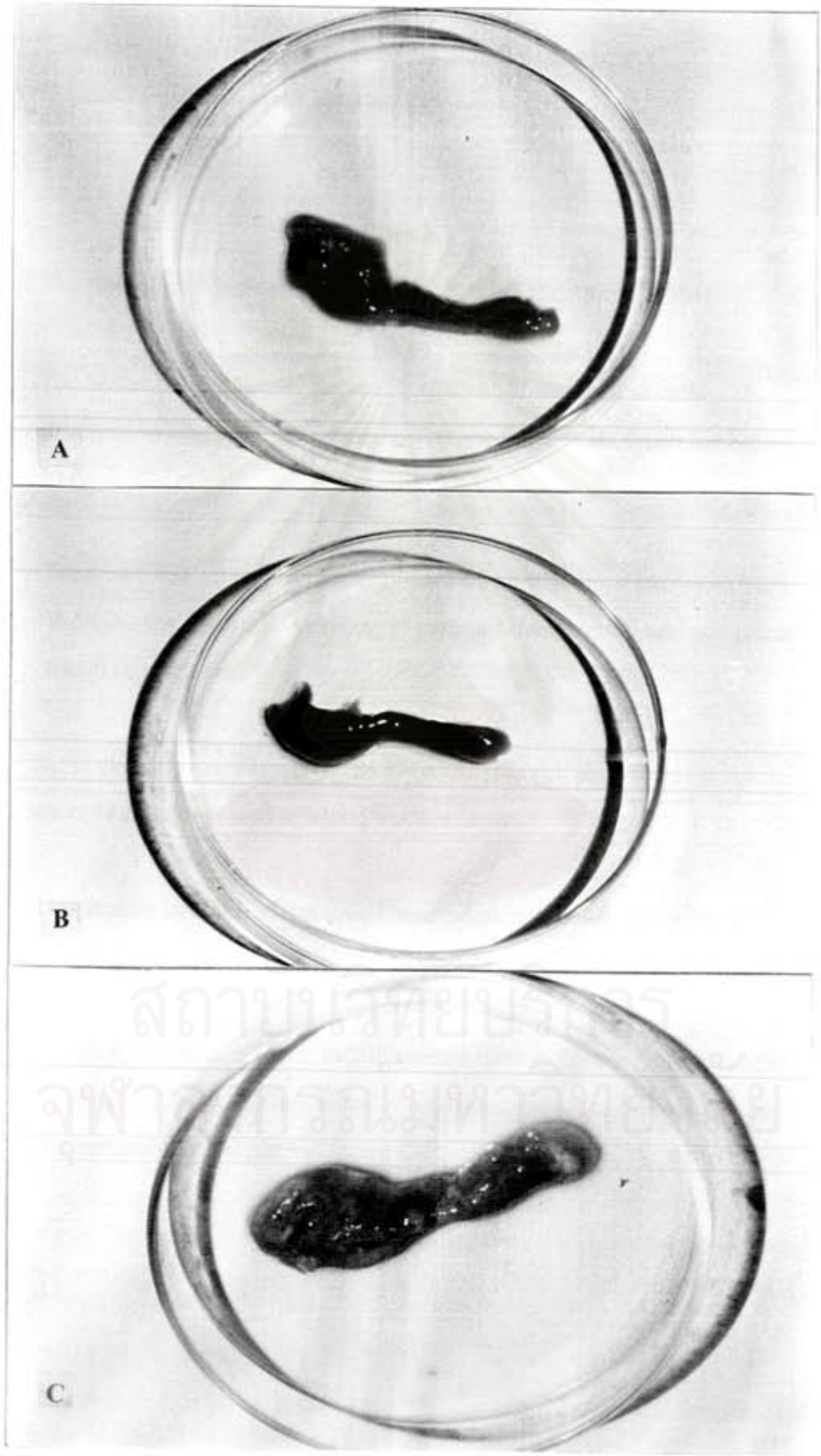


Plate 2

Photomicrograph of the liver from 1st month of experimnt

(H&E staining)

Figure A The photomicroraph of control liver showing the normal structure

Figure B The photomicrograph of solvent control liver showing normal structure with some lipid droplets. The bar scale is 1000 μm for figure A, B.

Figure C Treated liver after 1 month exposure of *D. trifoliata* leaves extract showing the hydropic swelling (*) around the sinusoid and congested blood in sinusoid.

Figure D Same condition as Figure D, the photomicrograph shows the necrosis area (*) and congested of blood vessels (arrow).

Figure E The photomicroraph of 1st month treated liver is showing the focal necrosis areas (*) and congested of blood vessels (arrow).

Figure F The photomicroraph of 1st month treated liver is showing the hydropic sweeling (*) around sinusoid and dilatation of congested blood vessel. The bar scale is 250 μm for figure C-F.

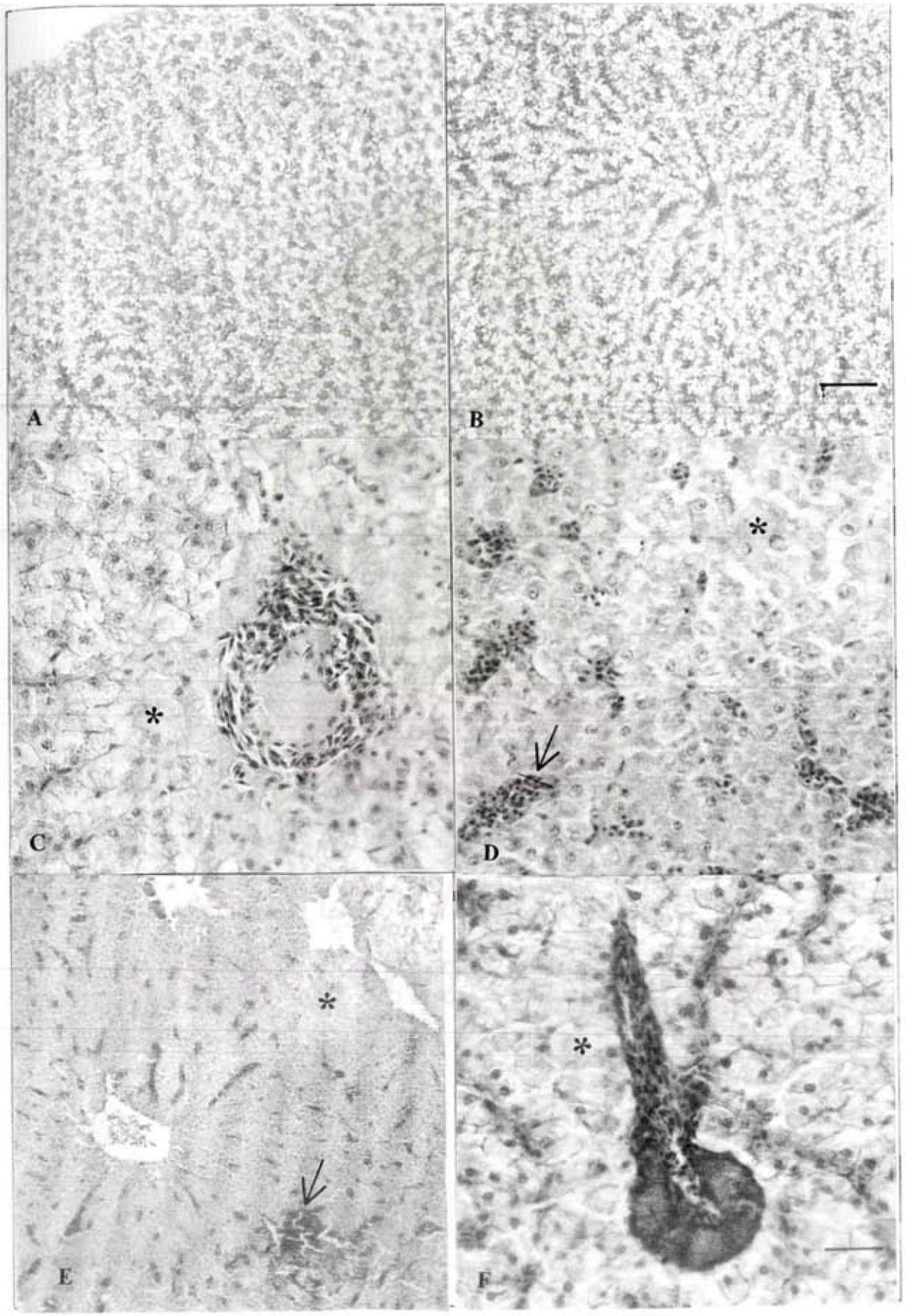

A
B
C
D
E
F

Plate 3

Photomicrograph of the liver from 1st month of experiment
(PAS and Oil red O staining)

Figure A 1st month of control liver staining with PAS, shows the normal deposition of glycogen in liver tissue.

Figure B 1st month of solvent control liver staining with PAS, shows the normal deposition of glycogen in liver tissue.

Figure C 1st month of treated liver staining with PAS, shows less deposition of glycogen than both of control groups.

Figure D 1st month of control liver staining with Oil red O, shows lipid deposition in hepatocytes.

Figure E 1st month of solvent control liver staining with Oil red O, shows lipid deposition in hepatocytes.

Figure F 1st month of treated liver staining with Oil red O, shows the density of lipid droplets deposition in hepatocytes (arrow). The bar scale is 250 μm for all figures.

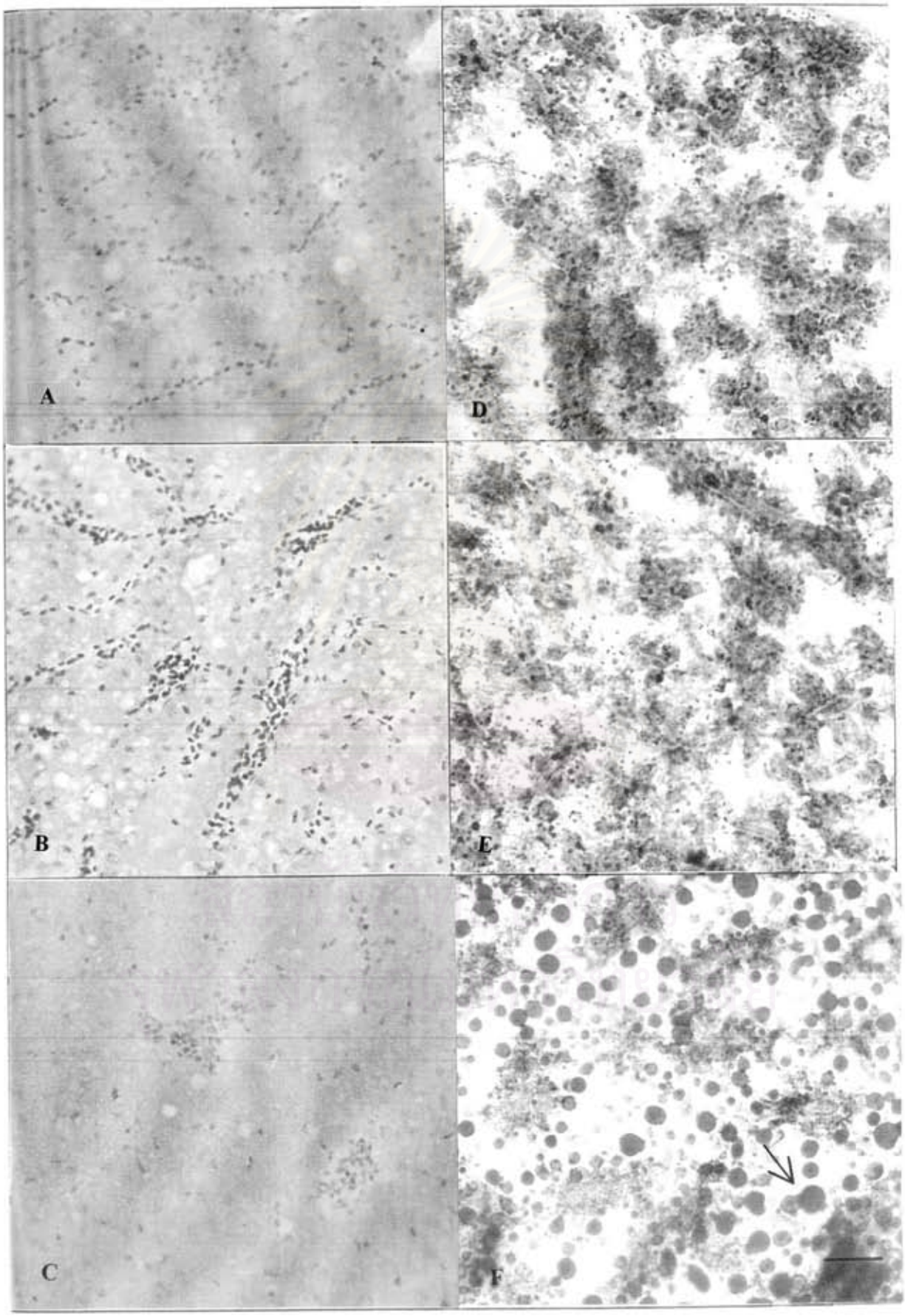

A
D
B
E
C
F

Plate 4

Electronmicrograph of the control liver from 1st month of experiment

Figure A Electron micrograph of 1st control liver, the hepatocyte is normal in its structure and cytoplasmic organalle.

Figure B Electron micrograph of 1st solvent control liver, the hepatocyte is normal in its structure and cytoplasmic organalle. There are some lipid (L) deposit in the hepatocyte. The bar scale is 1 μm.

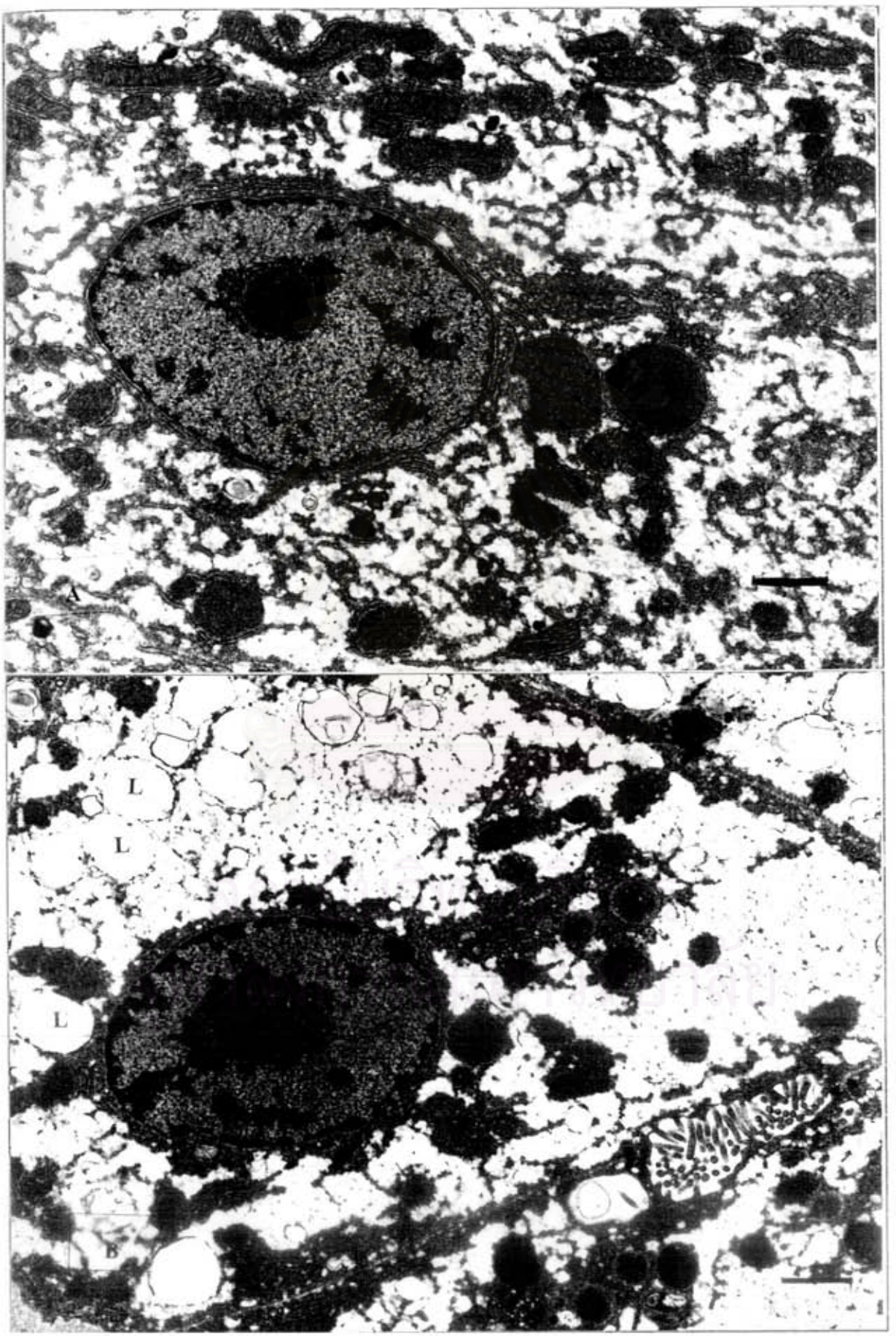

A
L
L
L
B

Plate 5

Electronmicrograph of the treated liver from 1st month of experiment

Figure A Electron micrograph of 1st month treated liver, the mitochrondrias are showing condensation of mitochrondrial metrix and expansion of intracristae spaces and RER dilation (arrow).

Figure B Electron micrograph of 1st month treated liver, the hepatocyte is showing membranous-myelin like strucrure (*). The bar scale is 1 μm.

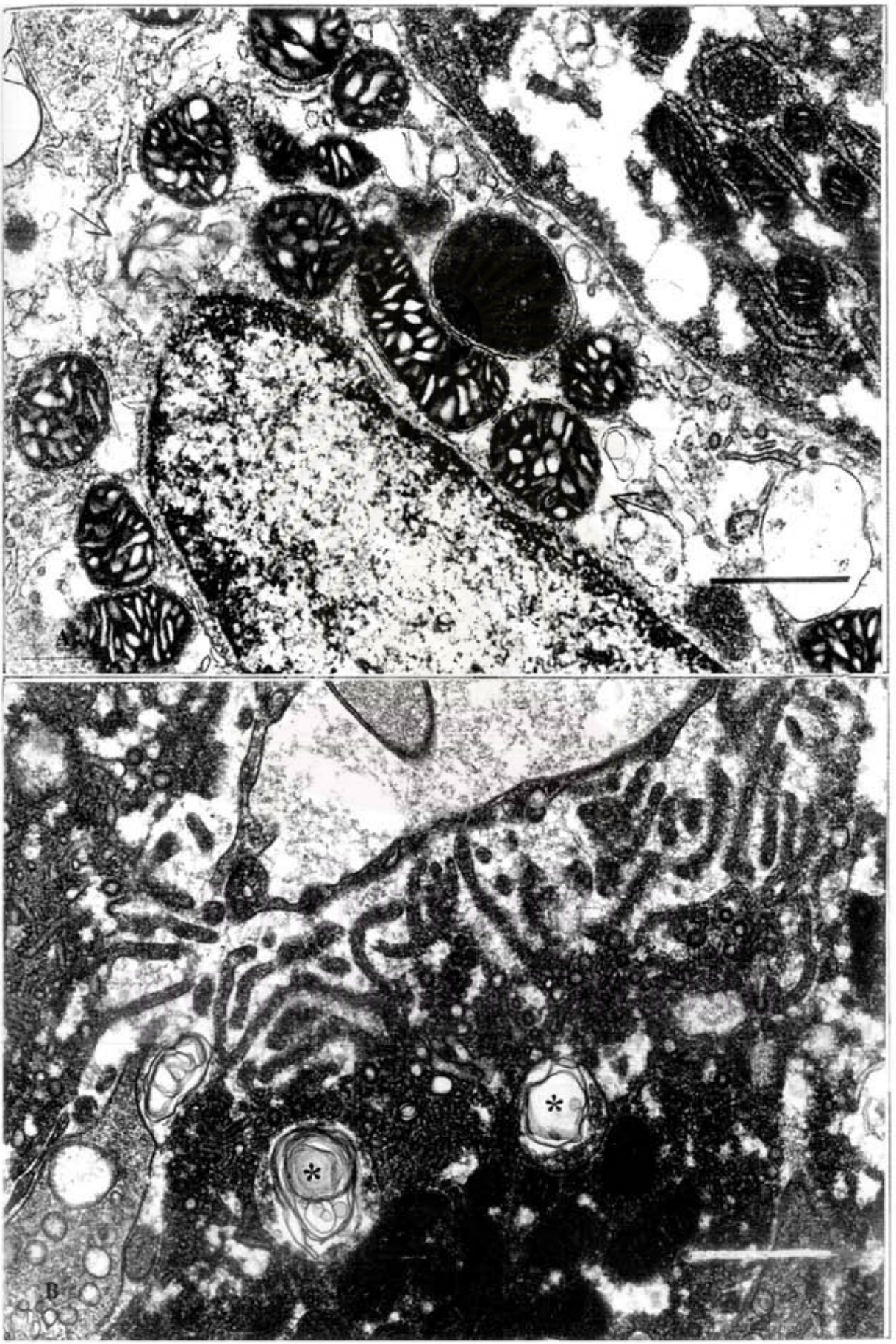

A
B
*
*

Plate 6

Electronmicrograph of the treated liver from 1st Month of experiment

Figure A Electron micrograph of 1st month treated liver, shows the autophagic vacuole and abnormal nucleus.

Figure B Electron micrograph of 1st month treated liver, shows the autophagic vacuole and secoundary lysosome (arrow). The bar scale is 1 μm.

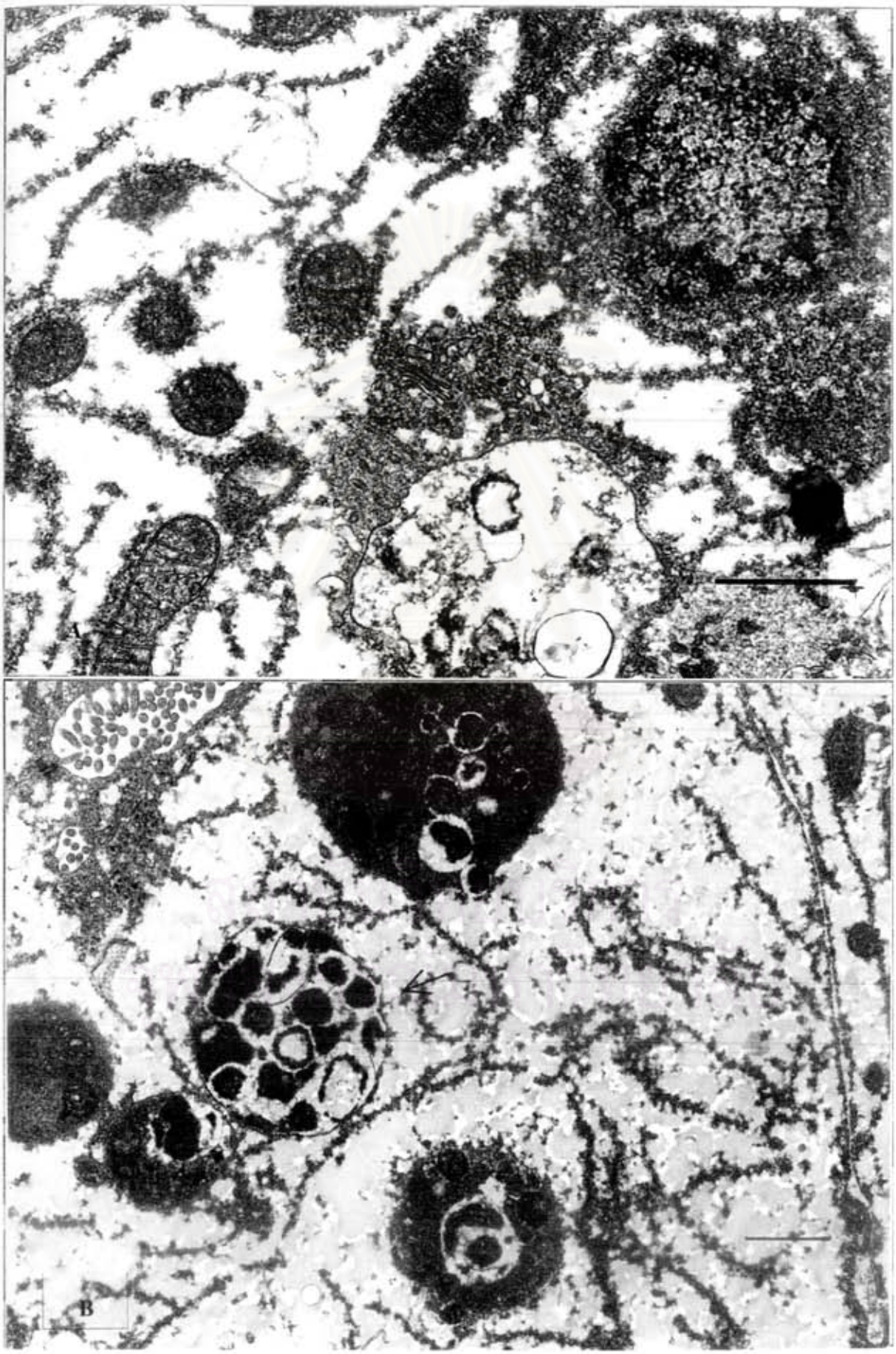

A
B

Plate 7

Photomicrograph of the liver from 2nd month of experiment
(H&E staining)

Figure A Photomicrograph of 2nd month control liver, shows normal in their structure and have some lipid droplets (L).

Figure B Photomicrograph of 2nd month solvent control liver, shows normal in their structure and have some lipid droplets (L).

Figure C Photomicrograph of 2nd month treated liver, shows the hydropic swelling hepatocytes (*) around sinusoid (S).

Figure D Photomicrograph of 2nd month treated liver, shows the congested blood vessels (arrow) and diffuseed necrotic cell (*).

Figure E Photomicrograph of 2nd month treated liver, shows the hemorhagic inflamation, infiltration of erythrocytes and lymphocytes (*) in the necrosis area.

Figure F Photomicrograph of 2nd month treated liver, shows the dilatation of sinusoids (*), erythrocytes infiltrate into the parenchymal area (arrow). The bar sacle is 250 μm for all figures.

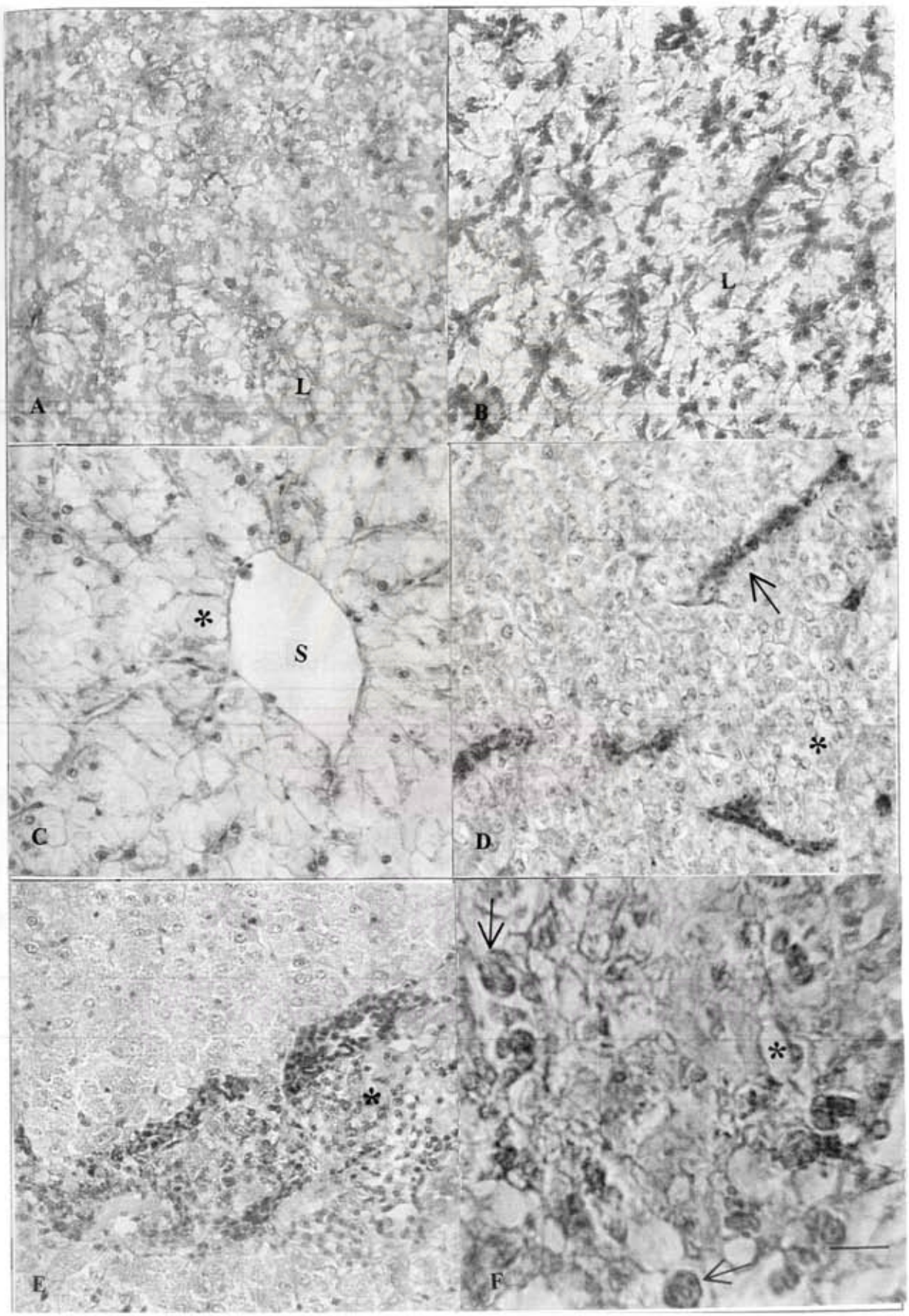

A
L
B
L
C
*
S
D
*
E
*
F
*

Plate 8

Photomicrograph of the liver from 2nd month of experiment
(PAS and Oil red O staining)

Figure A 2nd month of control liver staining with PAS, shows the normal deposition of glycogen in liver tissue.

Figure B 2nd month of solvent control liver staining with PAS, shows the normal deposition of glycogen in liver tissue.

Figure C 2nd month of treated liver staining with PAS, shows less deposition of glycogen (*) than both of control groups.

Figure D 2nd month of control liver staining with Oil red O, shows lipid deposition in fish liver.

Figure E 2nd month of solvent control liver staining with Oil red O, shows lipid deposition in fish liver (arrow).

Figure F 2nd month of treated liver staining with Oil red O, shows the density of lipid droplets deposition in fish liver (arrow). The bar sacle is 250 μm for all figures.

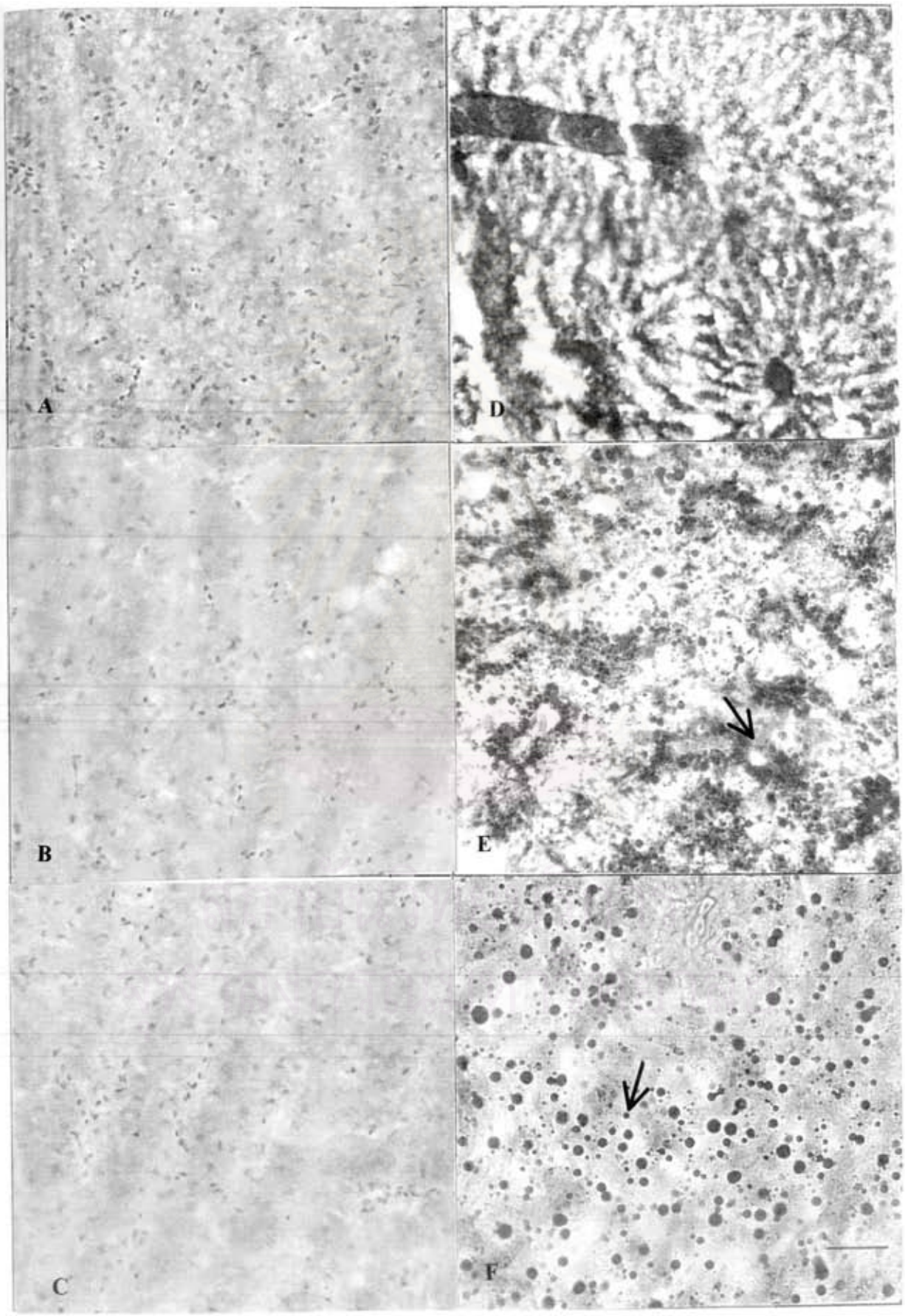

A
D
B
E
C
F

Plate 9

Electronmicrograph of the control liver from 2nd month of experiment

Figure A Electron micrograph of 2nd month control liver, shows the arrangement of RER connecting with nucleus. The bar scale is 0.5 μm.

Figure B Electron micrograph of 2nd month solvent control liver, shows the normal configuration of mitochrondria and RER. The bar scale is 1μm.

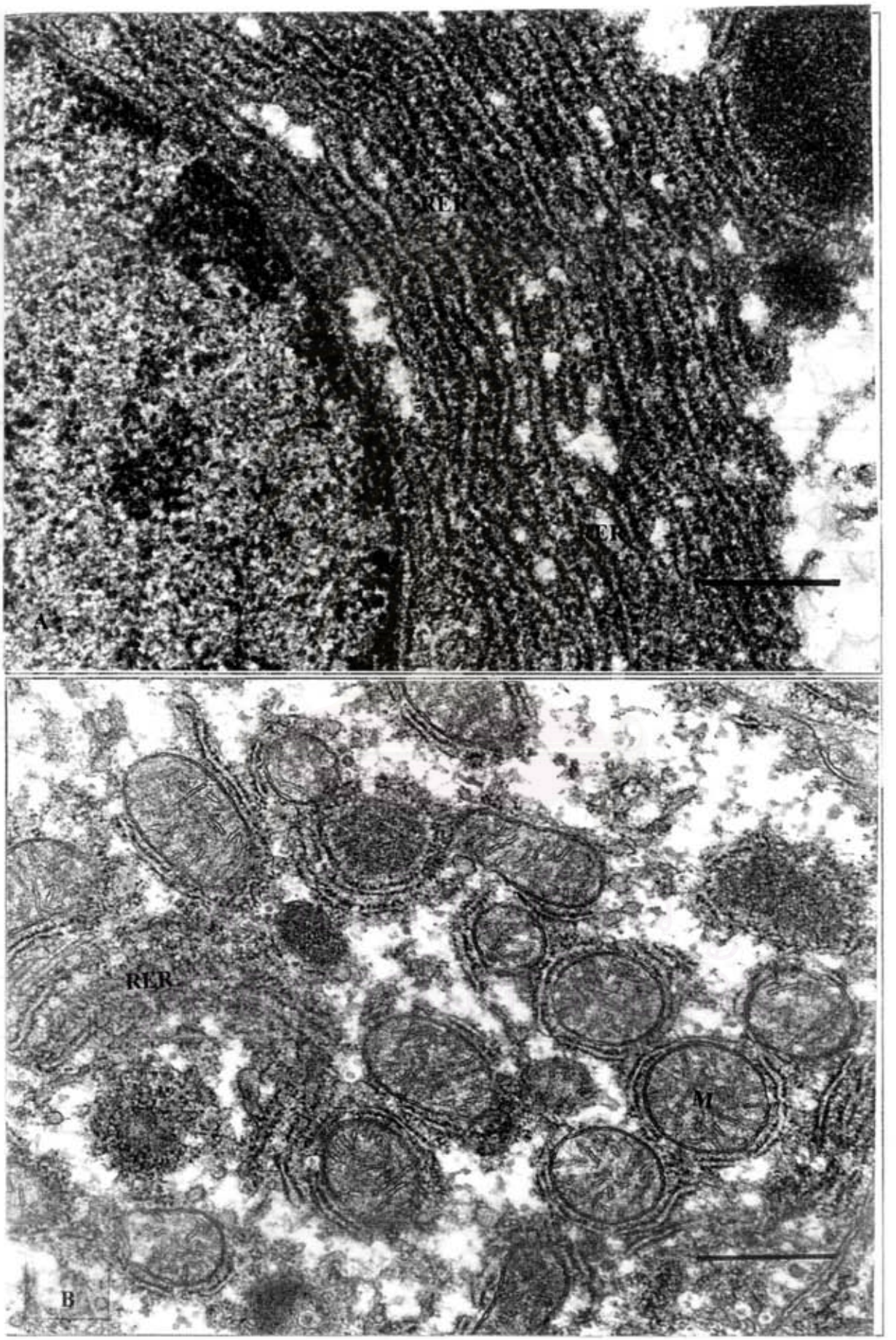

RER
RER
A
RER
M
B

Plate 10

Electronmicrograph of the treated liver from 2nd month of experiment

Figure A Electron micrograph of 2nd month treated liver, shows the distinctive proliferation and paralell aray of cicternea of RER (arrow), and some lipid droplets (L).

Figure B Electron micrograph of 2nd month treated liver, shows the contraction of mitochrondria, dilatation (*) and fragmentation (arrow) of RER and some lipid droplets (L). The bar scale is 1 μm.

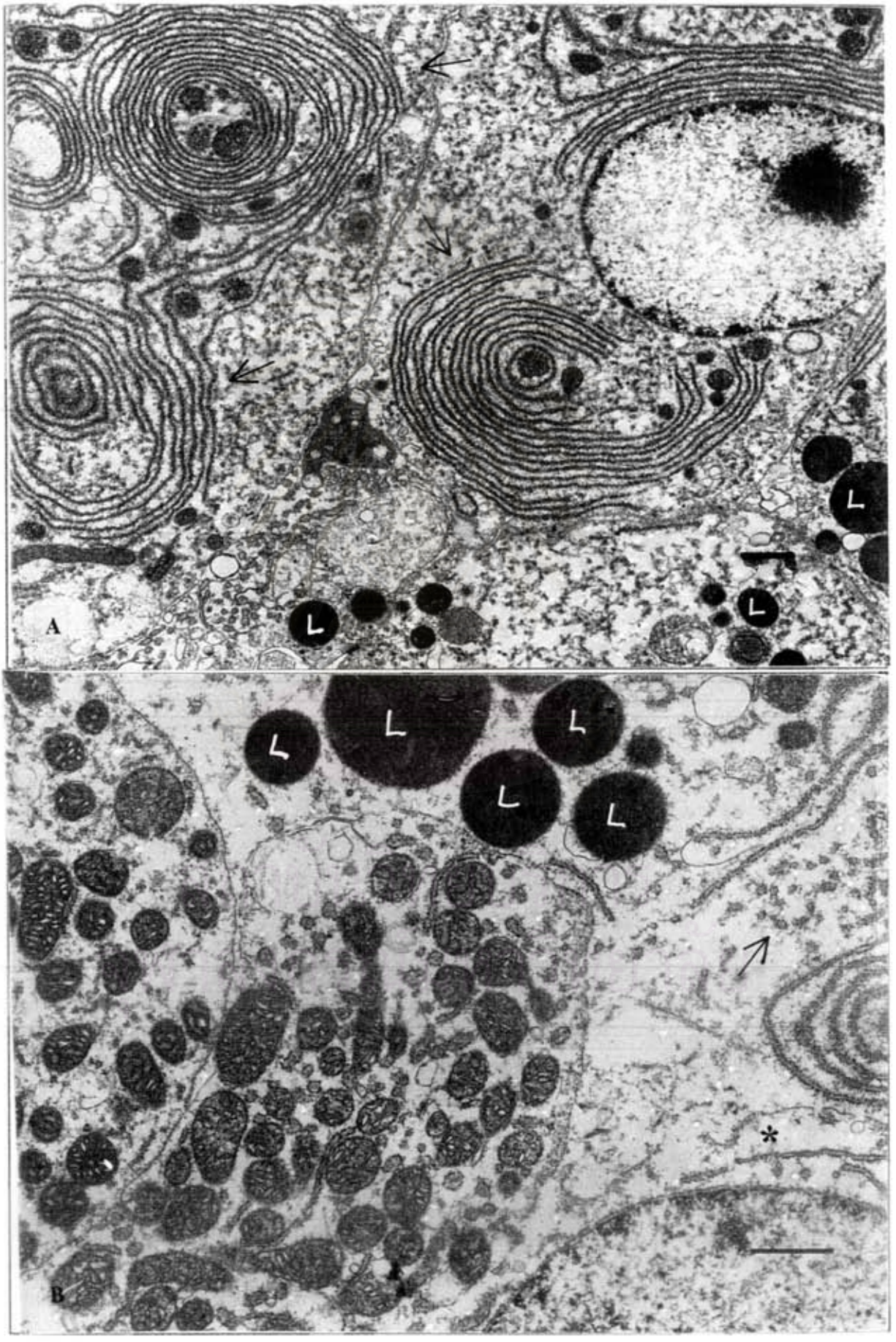

A
B
*

Plate 11

Electronmicrograph of the treated liver from 2nd month of experiment

Figure A Electron micrograph of 2nd month treated liver, shows the mitochrondria exhibit swelling with flocculent density (arrow), fragmentation of RER and some lipid droplets (L).

Figure B Electron micrograph of 2nd month treated liver, shows the swelling and dilation of mitochrondria, rupture of mitochrondria membrane (*), dilatation and fragmentation of RER (arrow). The bar scale is 1 μm.

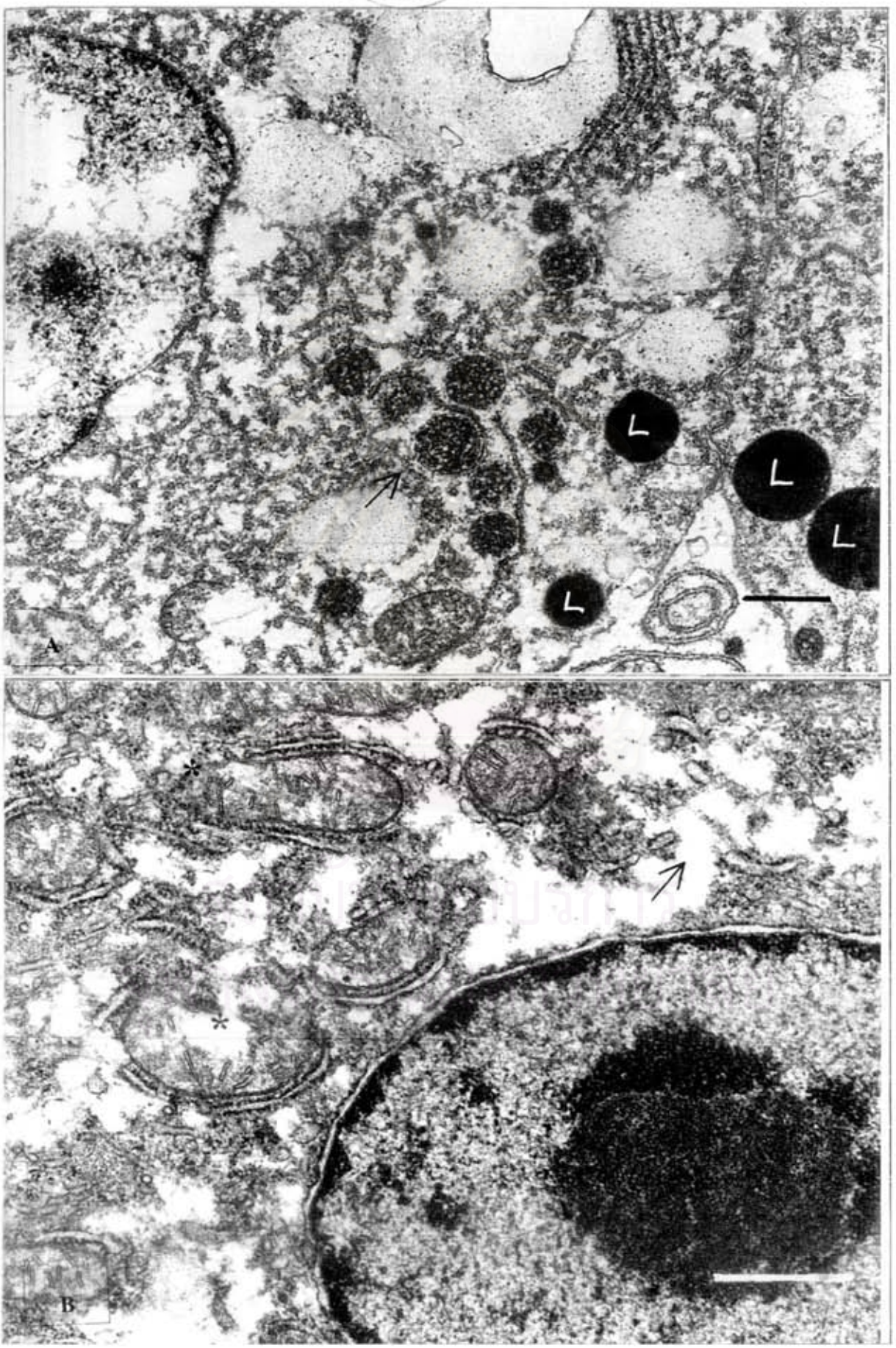

L
L
L
L
A
*
*
B

Plate 12

Photomicrograph of the liver from 3rd month of experiment
(H&E staining)

Figure A Photomicrograph of 2rd month control liver, shows normal in their structure.

Figure B Photomicrograph of 3rd month solvent control liver, shows normal in their structure and have the accumulation of fat, that shows the concentric cytoplasmic vacuolation (L). The bar sacle is 250 μm for figure A, B

Figure C Photomicrograph of 3rd month treated liver, shows the large area of focal necrotic (*), and hemorhagic inflamation (arrow). The bar scale is 2000 μm.

Figure D Photomicrograph of 3rd month treated liver, shows the hemorhage area (arrow head) along the necrotic area. The accumulation of erythrocytes and invastion of macrophage (*). The bar scale is 1000 μm.

Figure E Photomicrograph of 3rd month treated liver, shows the hemorhagic inflamation area and necrotic cell (*) around blood vessel. The deformed erythrocytes and invastion of macrophage (arrow).

Figure F Photomicrograph of 3rd month treated liver, shows the hemorhagic inflamation area and vacuolation of fatty degenerate cell (L) around lood vessel. The deformed erythrocytes and invastion of macrophage (arrow). The bar scale is 250 μm.

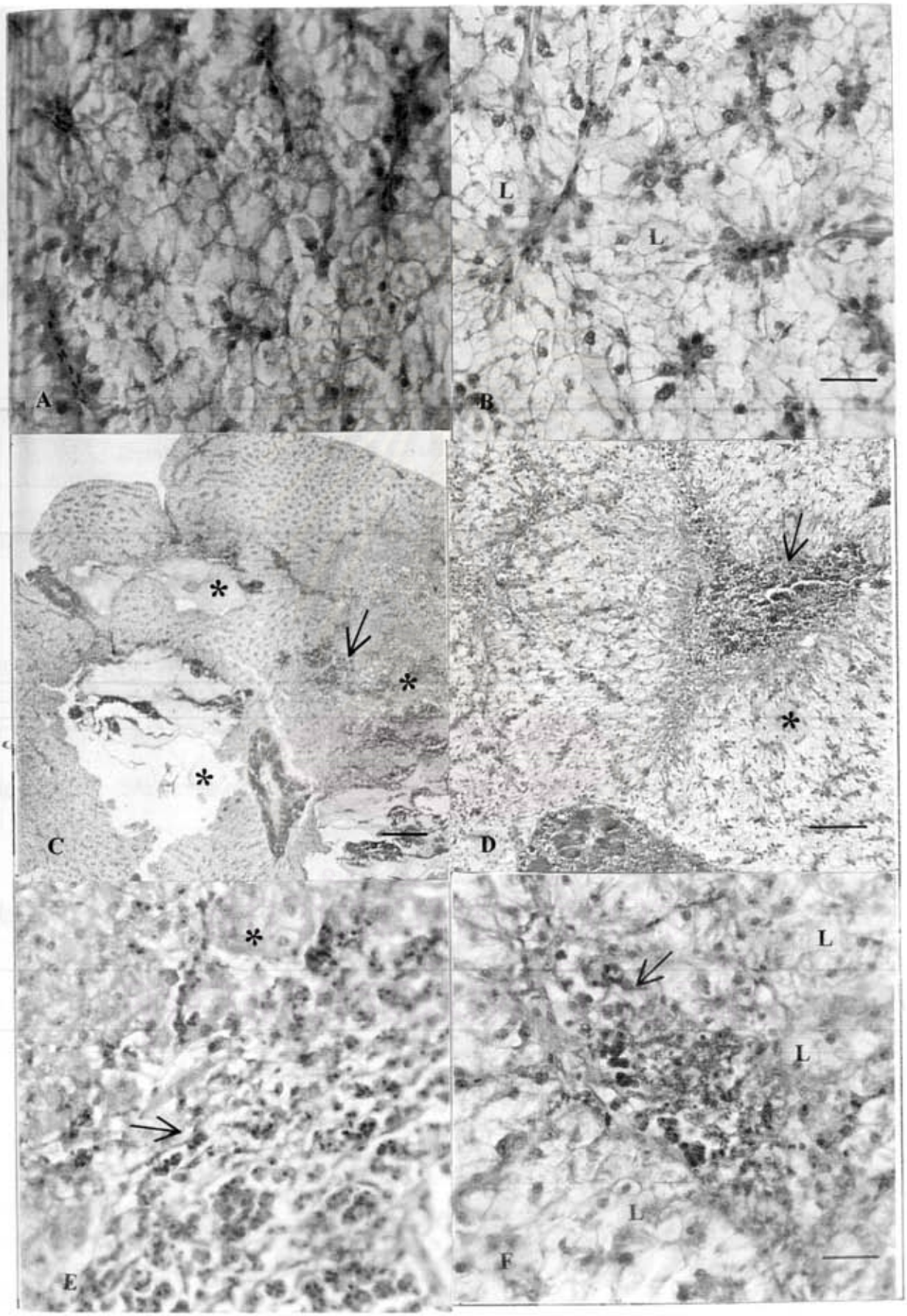

A
B
L
L
C
D
E
F
L
L
L

Plate 13

Photomicrograph of the liver from 3rd month of experiment
(PAS and Oil red O staining)

Figure A 3rd month of control liver staining with PAS, shows the normal deposition of glycogen in liver tissue.

Figure B 3rd month of treated liver staining with PAS, shows the depleatation of glycogen deposition in liver tissue (*).

Figure C 3rd month of treated liver staining with PAS, shows the depleatation of glycogen deposition in liver tissue (*).

Figure D 3rd month of control liver staining with Oil red O, shows lipid deposition in fish liver.

Figure E 3rd month of solvent control liver staining with Oil red O, shows a very dense of lipid droplets deposition in fish liver (arrow).

Figure F 3rd month of solvent control liver staining with Oil red O, shows a very dense of lipid droplets deposition in fish liver (arrow). The bar sacle is 250 μm for all figures.

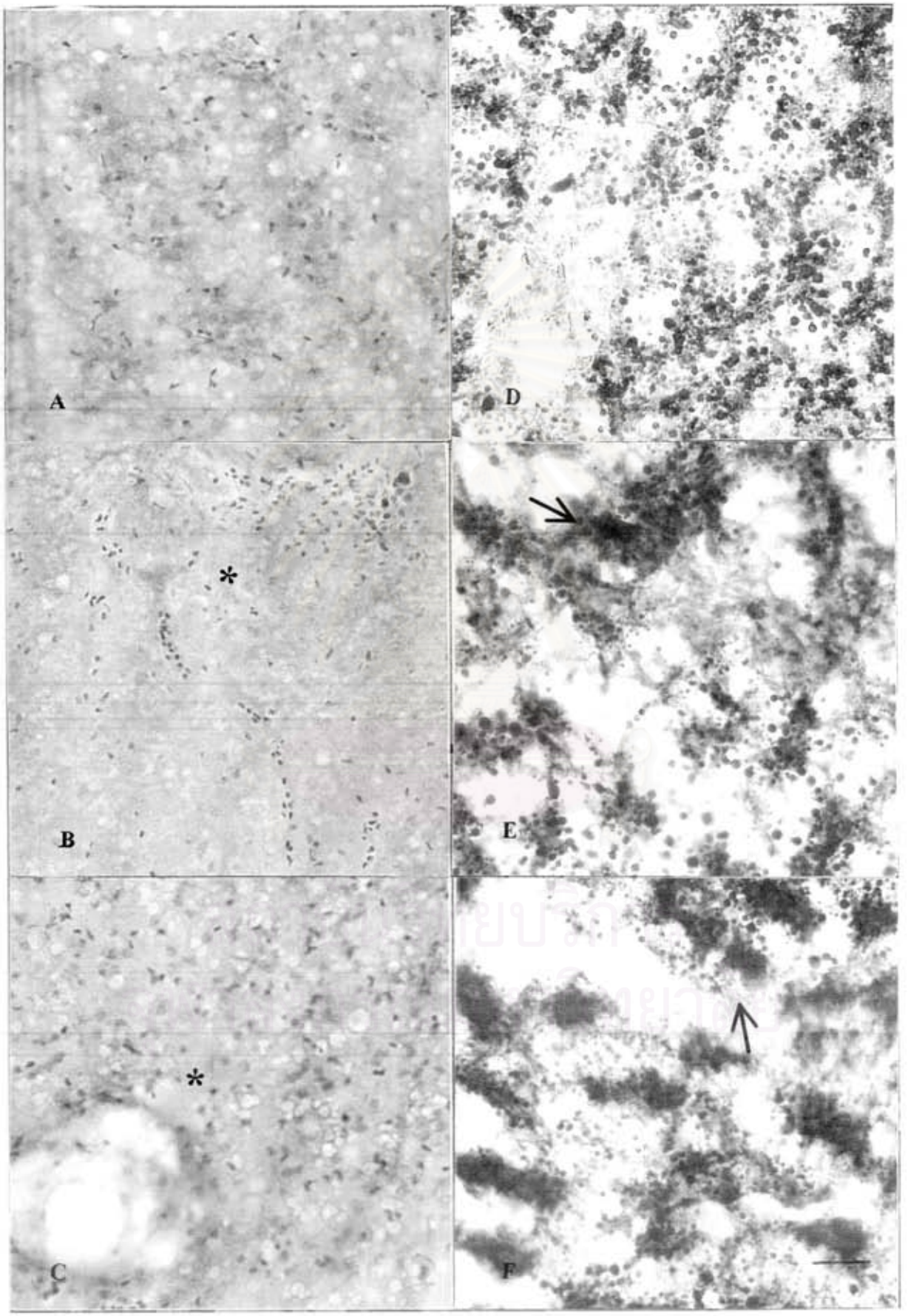

A
B
C
D
E
F

Plate 14

Electronmicrograph of the control liver from 3rd month of experiment

Figure A Electron micrograph of 3rd month control liver, shows the normal mitochondria and nucleus and arrangement of RER, glycogen granule scatter throughout cytoplasm, lipid droplet (L).

Figure B Electron micrograph of 3rd month control liver, shows the normal configuration of mitochrondria, proliferation and arrangement of RER, glycogen granule scatter throughout cytoplasm and lipid droplet (L). The bar scale is 1 μm.

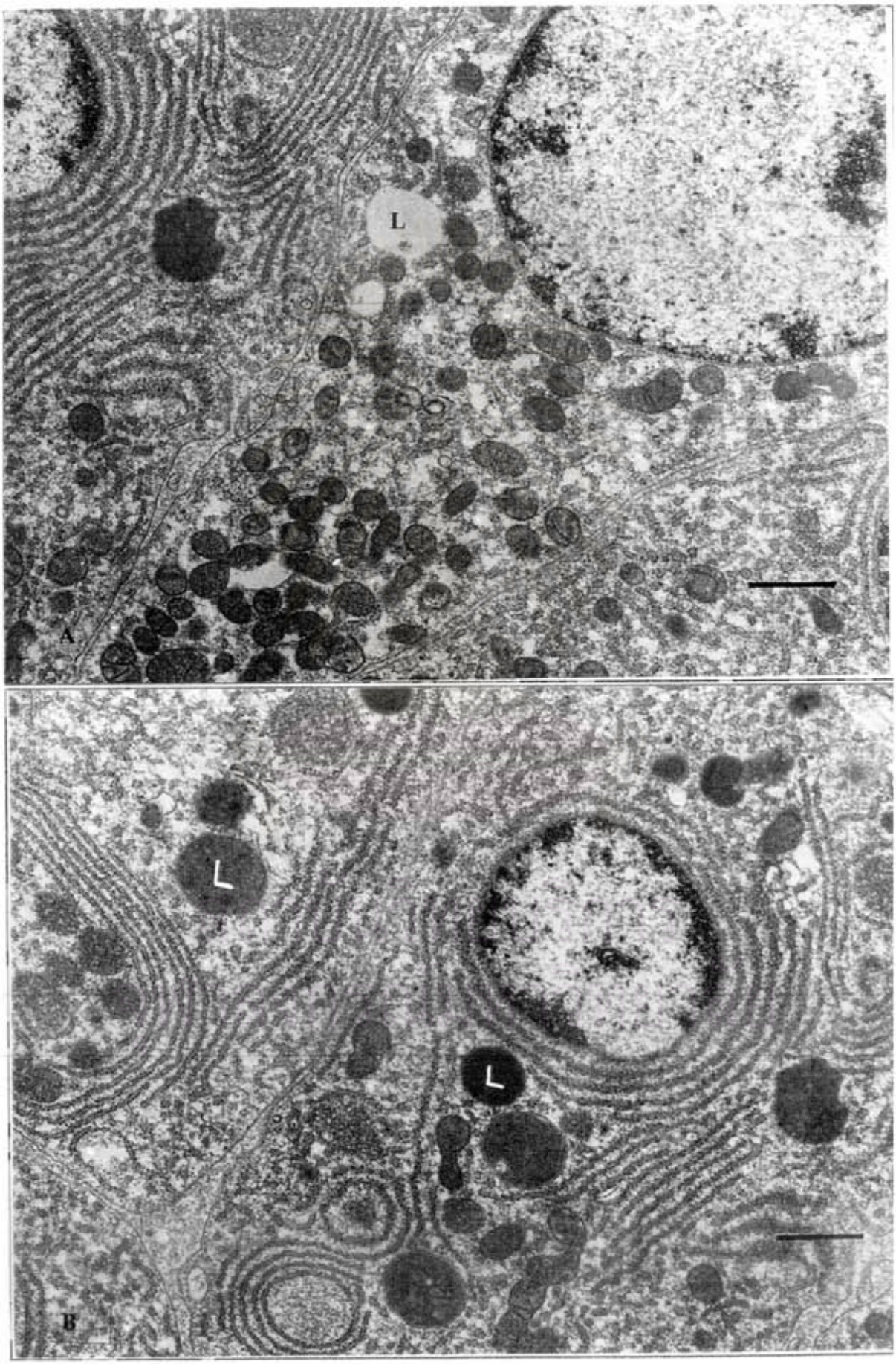

L
A
L
L
B

Plate 15

Electronmicrograph of the treated liver from 3rd month of experiment

Figure A Electron micrograph of 3rd month treated liver, shows the contraction of mitochrondrias matrix, fragmentation of RER (arrow) and lipid droplets (L).

Figure B Electron micrograph of 3rd month treated liver, showsthe distinctive proliferation and paralell aray of cicternea of RER (*), fragment RER, contraction of mitochrondrias matrix and lipid droplets. The bar scale is 1 μm.

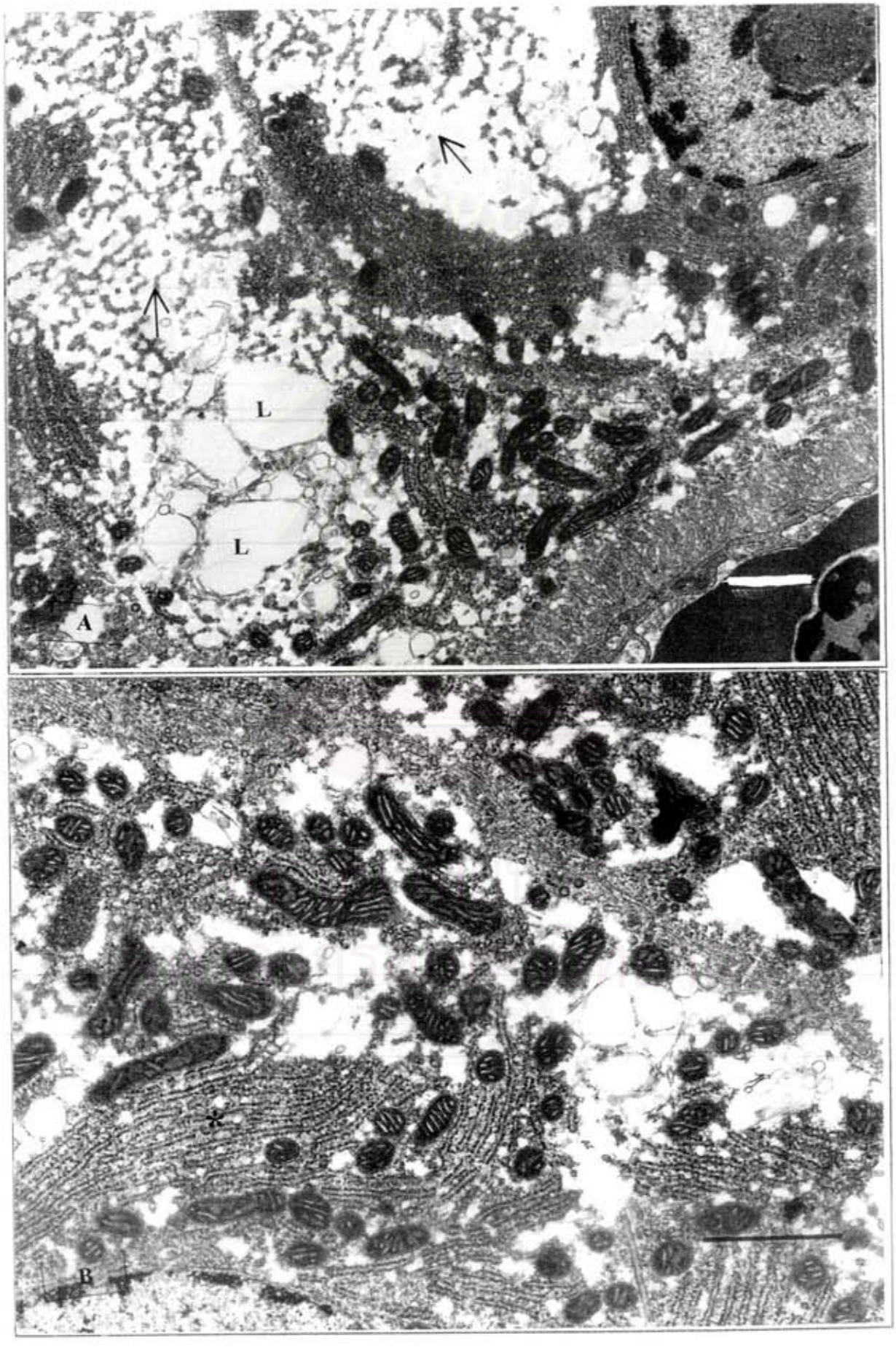

L
L
A
*
B

Plate 16

Electronmicrograph of the treated liver from 3rd month of experiment

Figure A Electron micrograph of 3rd month treated liver, shows the contraction of mitochrondrias matrix and fragmentation of RER (arrow).

Figure B Electron micrograph of 3rd month treated liver, shows the proliferation and paralell aray of cicternea of RER (*), fragmentation of RER (arrow) and swelling mitochondria. The bar scale is 1 μm.

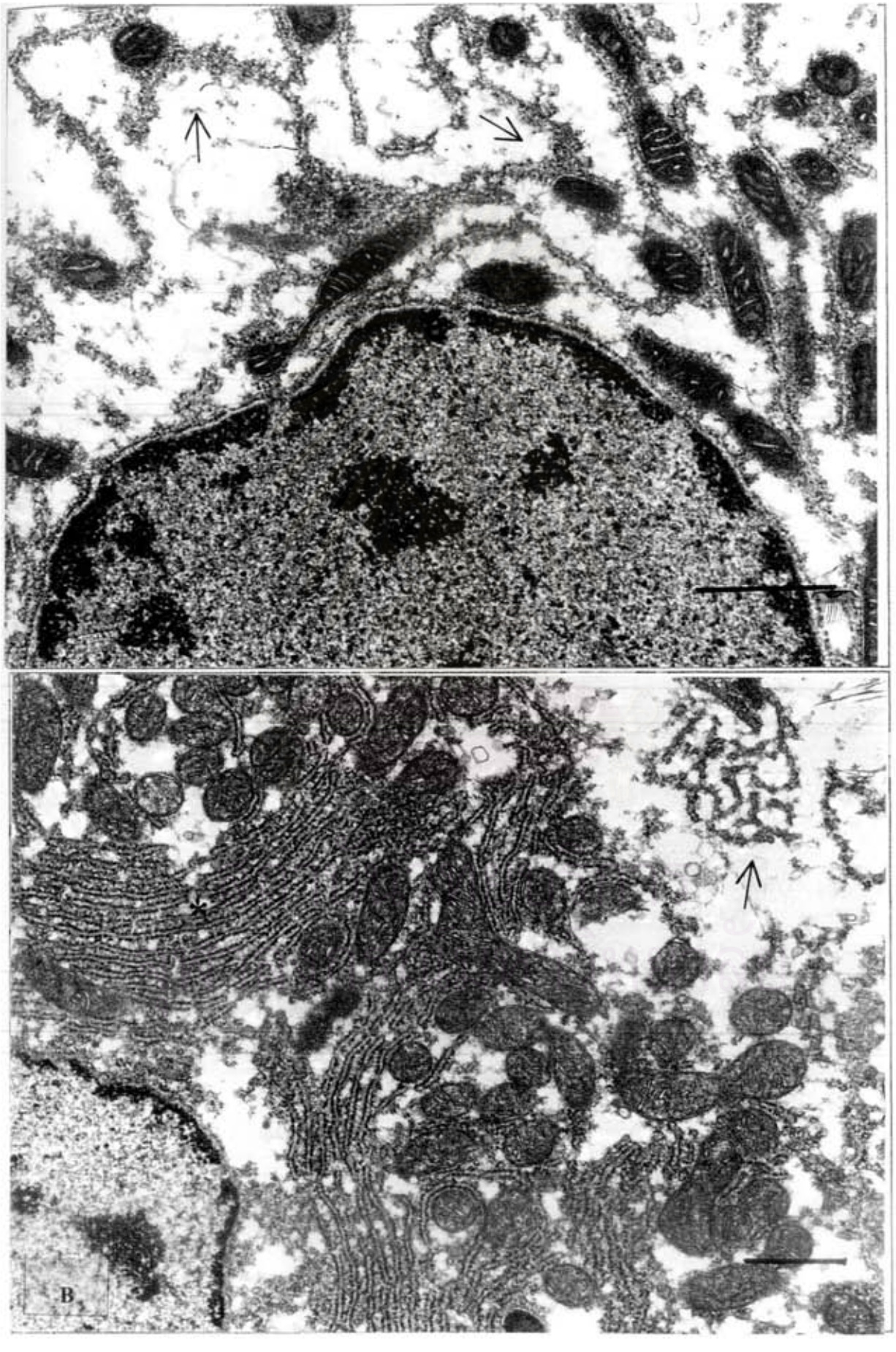

B

Plate 17

Photomicrograph of the liver from 4th month of experiment

(H&E staining)

Figure A Photomicrograph of 4th month control liver, shows normal in their structure with some lipid droplets.

Figure B Photomicrograph of 4th month solvent control liver, shows normal in their structure.

Figure C Photomicrograph of 4th month treated liver, shows the fatty degenerate (L), infilltration of erythrocytes and lymphocytes in blood vessel (arrow) and necrotic area (*).

Figure D Photomicrograph of 4th month treated liver, shows the fibroplasia (fibrosis) a repair response to cell death (*).

Figure E Photomicrograph of 4th month treated liver, shows the vauolation and fatty degeneration. The bar sacle is 1000 μm for figure A, E.

Figure F Photomicrograph of 4th month treated liver, shows the fatty degenerate (L) and infilltration of erythrocytes and lymphocytes (arrow). The bar sacle is 250 μm for figure B-D, F.

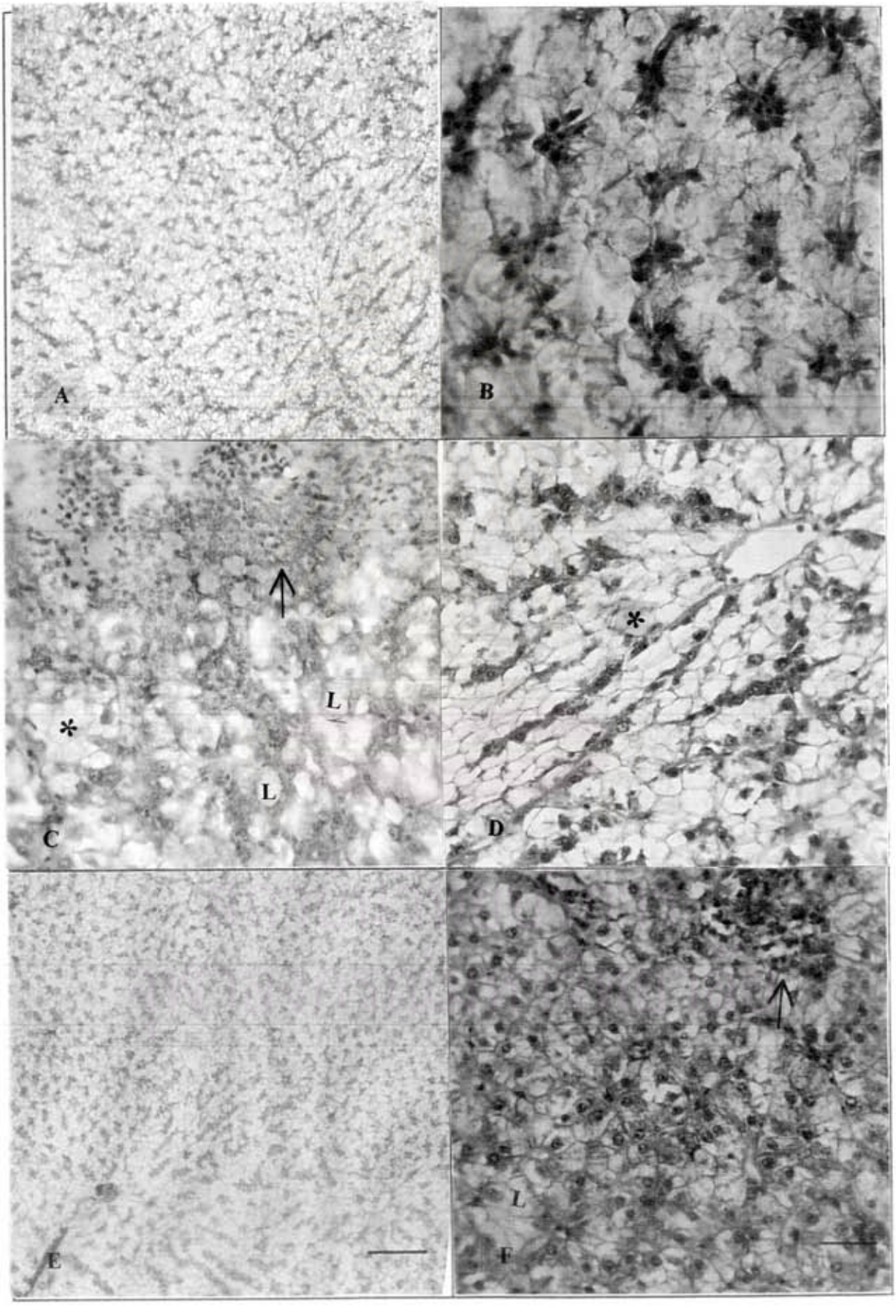

A
B
C
L
L
*
D
*
E
L
F

Plate 18

Photomicrograph of the liver from 4th month of experiment
(PAS and Oil red O staining)

Figure A 4th month of control liver staining with PAS, shows the normal deposition of glycogen in liver tissue.

Figure B 4th month of solvent treated liver staining with PAS, shows the depleatation of glycogen deposition of in liver tissue (*).

Figure C 4th month of solvent treated liver staining with PAS, shows the depleatation of glycogen deposition of in liver tissue (*).

Figure D 4th month of control liver staining with Oil red O, shows lipid deposition in fish liver.

Figure E 4th month of solvent treated liver staining with Oil red O, the lipid droplets in fish liver is very density (arrow).

Figure F 4th month of solvent treated liver staining with Oil red O, the lipid droplets in fish liver is very density (arrow). The bar sacle is 250 μm for all figures.

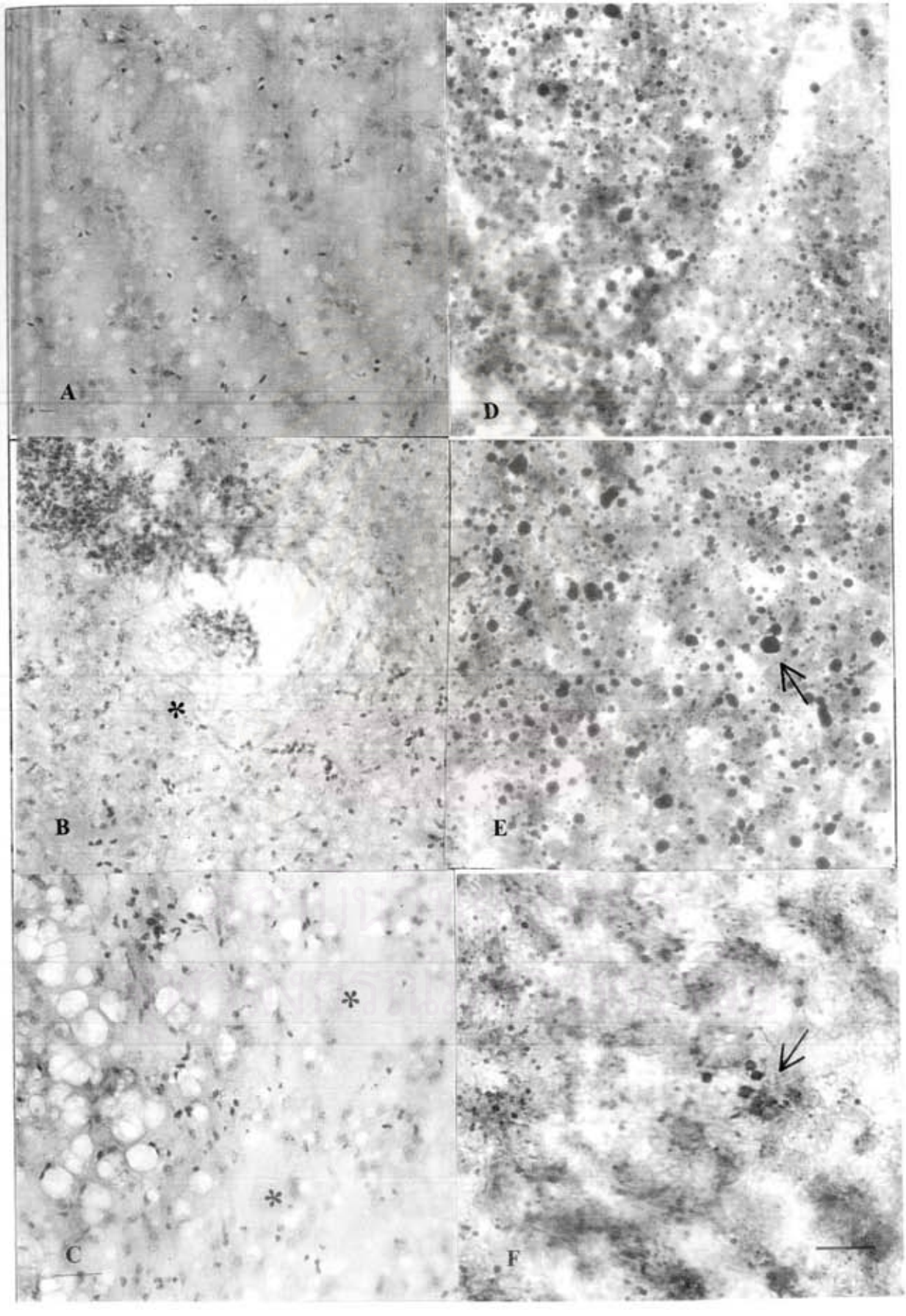

A
D
B
E
C
F

Plate 19

Electronmicrograph of the control liver from 4th month of experiment

Figure A Electron micrograph of 4th month control liver, shows the parallel arrangement of RER close to nucleus (arrow) and some lipid droplets (L).

Figure B Electron micrograph of 4th month control liver, shows normal structure with parallel arrangement of RER and mitochondria. The bar scale is 1 μm.

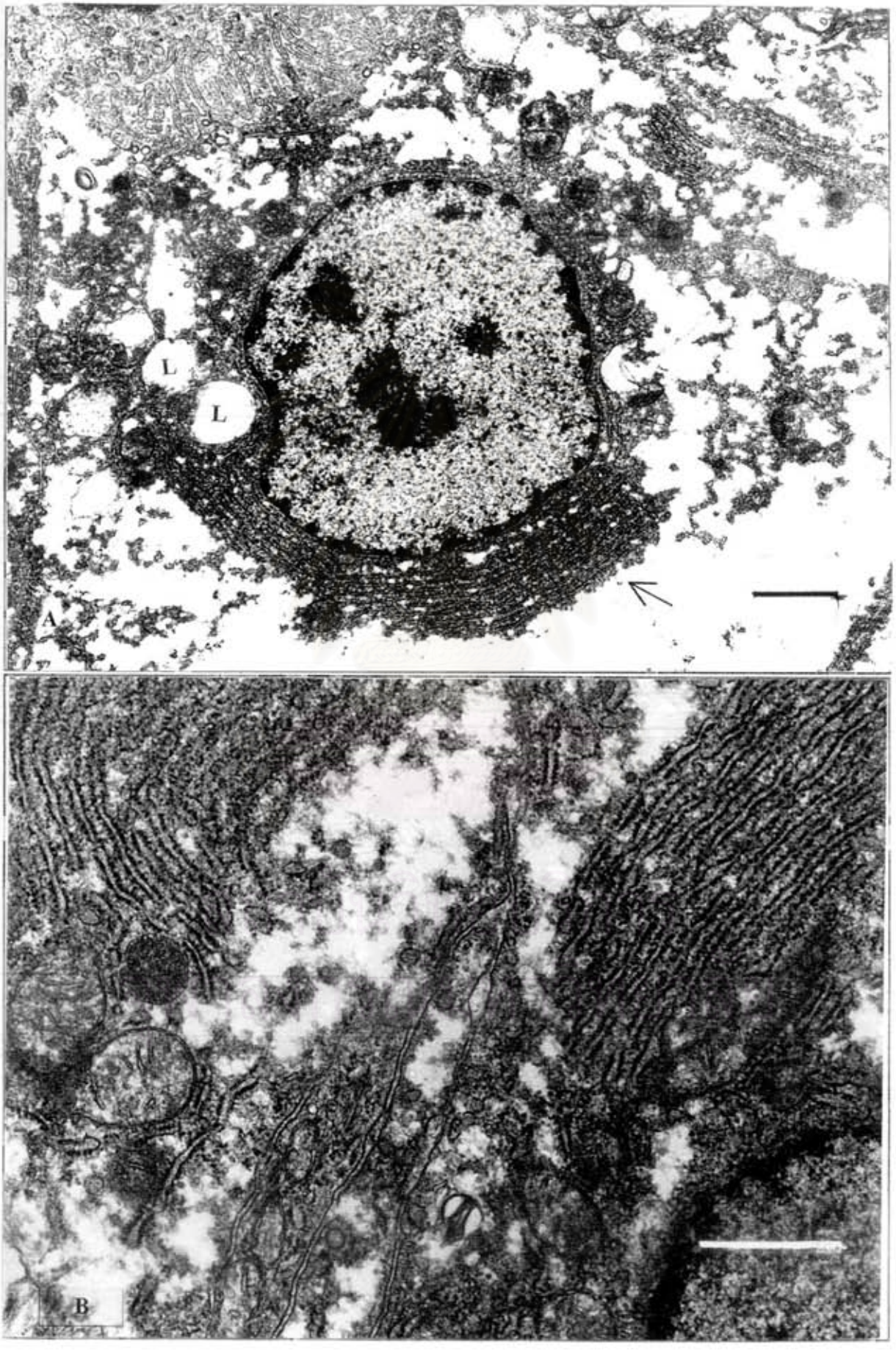

L
L
A
B

Plate 20

Electronmicrograph of the treated liver from 4th month of experiment

Figure A Electron micrograph of 4th month treated liver, shows dilatation (arrow) and fragmentation of RER and shif to be SER, SER and free ribosome are scatter all over the cytoplasm (*).

Figure B Electron micrograph of 4th month treated liver, shows the contraction of mitochrondria, dilatation and fragmentation of RER (*) and shif to be SER, and lipid droplets (L). The bar scale is 1 μm.

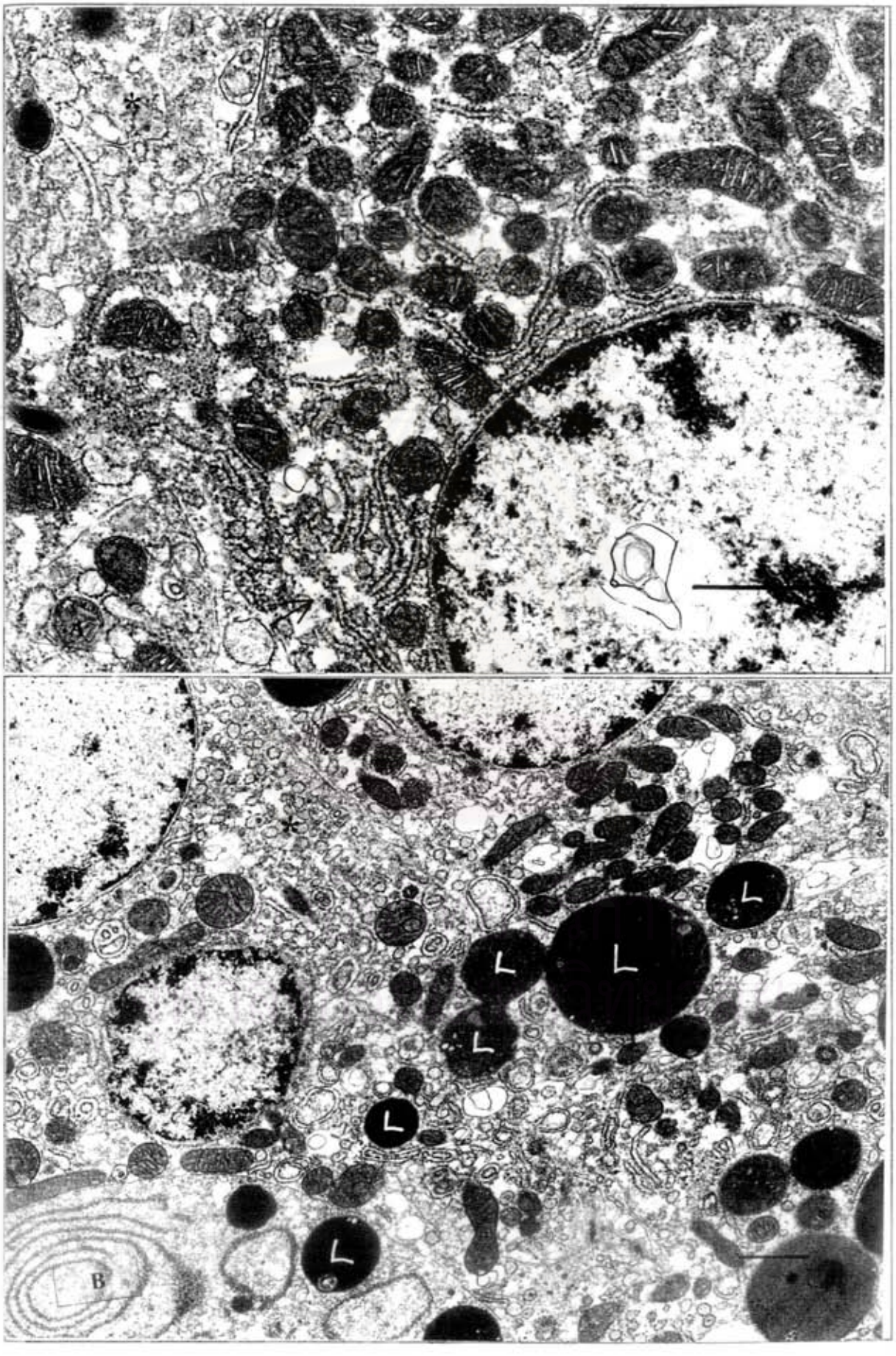

L
L
L
L
L
L
B

Plate 21

Electronmicrograph of the treated liver from 4th month of experiment

Figure A Electron micrograph of 4th month treated liver, shows the concentric membranous lamella (*) fragmentation of RER (arrow), SER are scatter all over the cytoplasm, contracted mitochrondria and some lipid droplets (L).

Figure B Electron micrograph of 4th month treated liver, shows the contraction of mitochrondria, fragmentation of RER and lipid droplets. The bar scale is 1 μm.

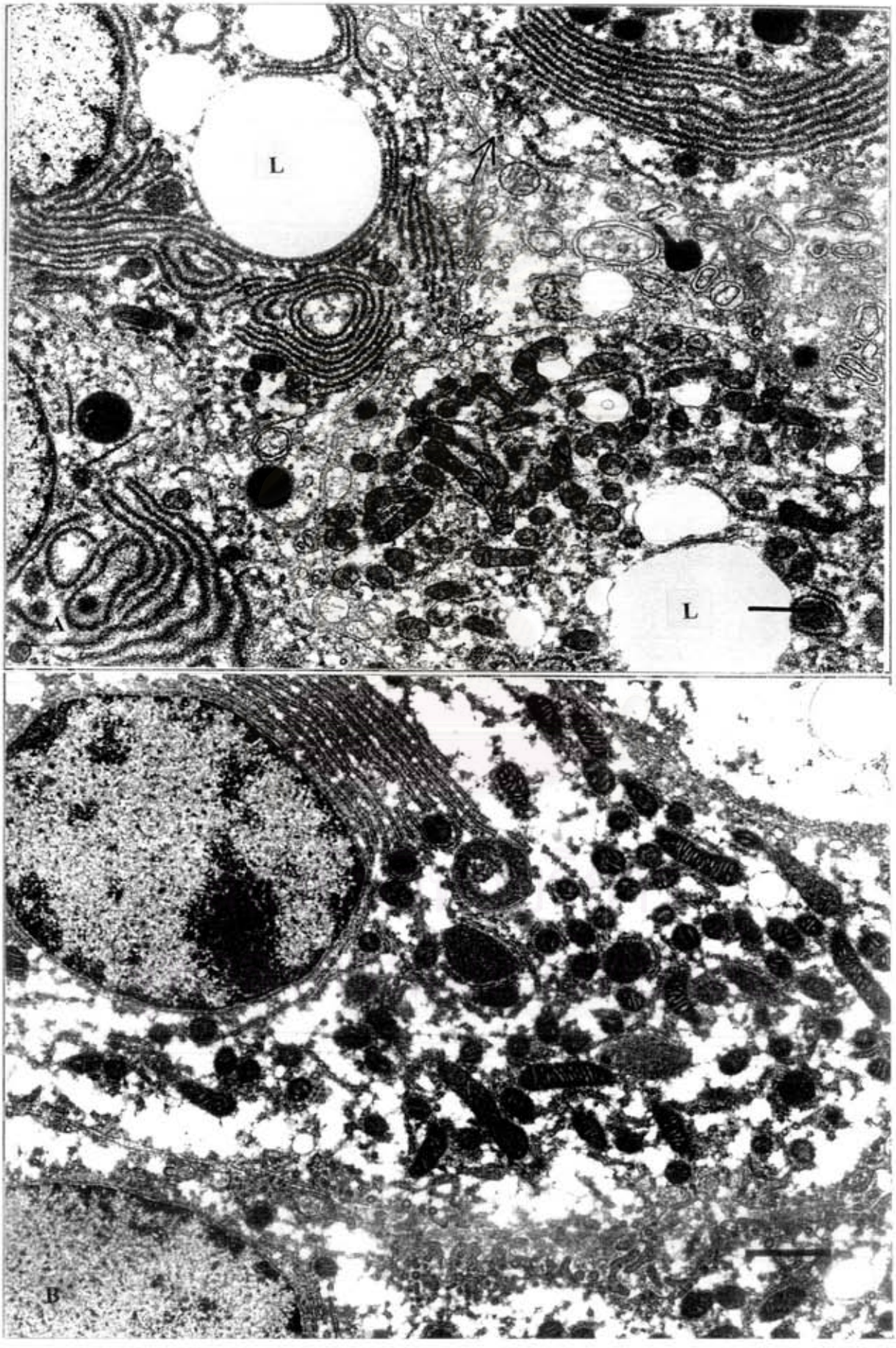

L
L
A
B

Plate 22

Photomicrograph of the liver from 5th month of experiment
(H&E staining)

Figure A Photomicrograph of 5th month control liver, shows normal in their structure and have some lipid droplets (L).

Figure B Photomicrograph of 5th month treated liver, shows the fibroplasia degeneration area (*).

Figure C Photomicrograph of 5th month treated liver, shows the most severe case of large necrotic area. The bar sacle is 1000 μm for figure B, C.

Figure D Photomicrograph of 5th month treated liver, shows the fibroplasia degeneration area (*) and death erythrocyte (arrow).

Figure E Photomicrograph of 5th month treated liver, shows the fibroplasia degeneration area (*).

Figure F Photomicrograph of 5th month treated liver, shows the fibroplasia degeneration area (*). The bar sacle is 250 μm for figure A, D-F.

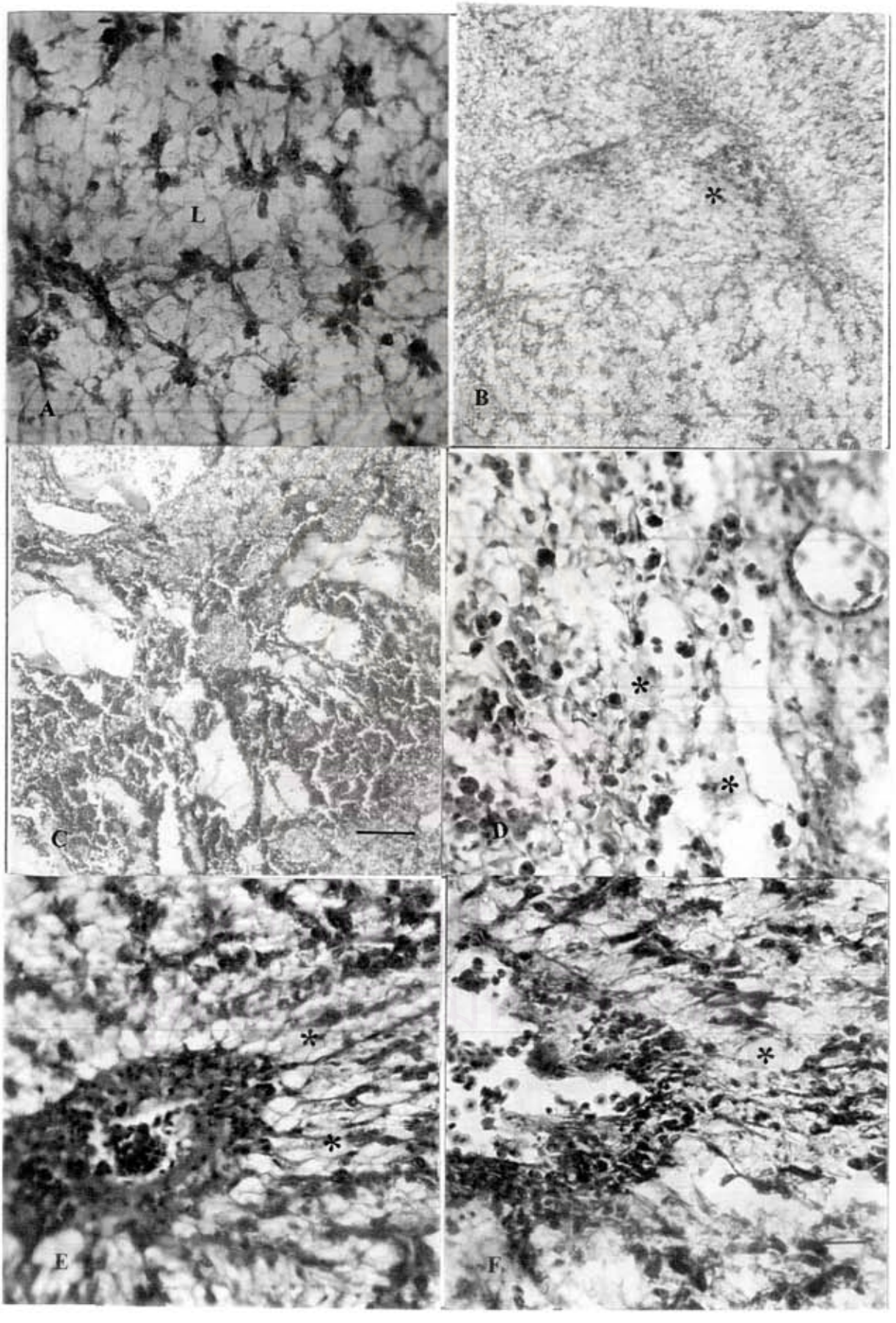

L
A
B
C
D
E
F

Plate 23

Photomicrograph of the liver from 5th month of experiment
(PAS and Oil red O staining)

Figure A 5th month of control liver staining with PAS, shows the normal deposition of glycogen in liver tissue

Figure B 5th month of solvent treated liver staining with PAS, shows the large damage area with depleate of glycogen deposition (*).

Figure C 5th month of solvent treated liver staining with PAS, shows the depleatation of glycogen deposition (*) around blood vessel (Bv).

Figure D 4th month of control liver staining with Oil red O, shows lipid deposition in fish liver.

Figure E 4th month of solvent treated liver staining with Oil red O, the lipid droplets in fish liver is very density (arrow).

Figure F 4th month of solvent treated liver staining with Oil red O, the lipid droplets in fish liver is very density (arrow). The bar sacle is 250 μm for all figures.

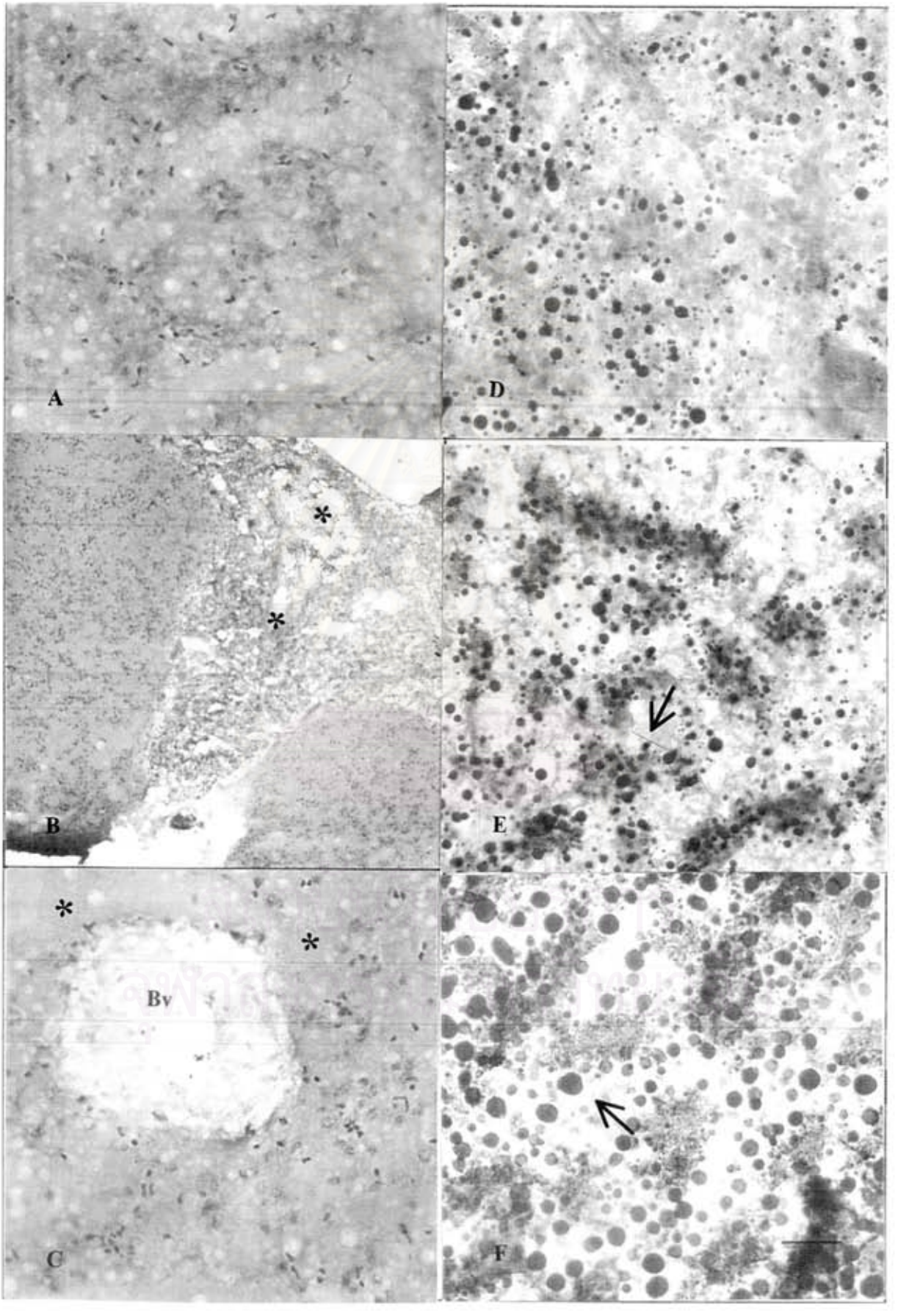

A
D
B
E
Bv
C
F

Plate 24

Electronmicrograph of the treated liver from 5th month of experiment

Figure A Electron micrograph of 5th month treated liver, shows the proliferation and fragmentation of RER (arrow), the contracted mitochrondria and myeline like structure (*) are also found, and some lipid droplets (L).

Figure B Electron micrograph of 5th month treated liver, shows the fragmentation of RER, the contracted and sweeling mitochrondria (arrow) myeline like structure are also found, and some lipid droplets (L), lysosome and secoundary lysosome. The bar scale is 1 μm.

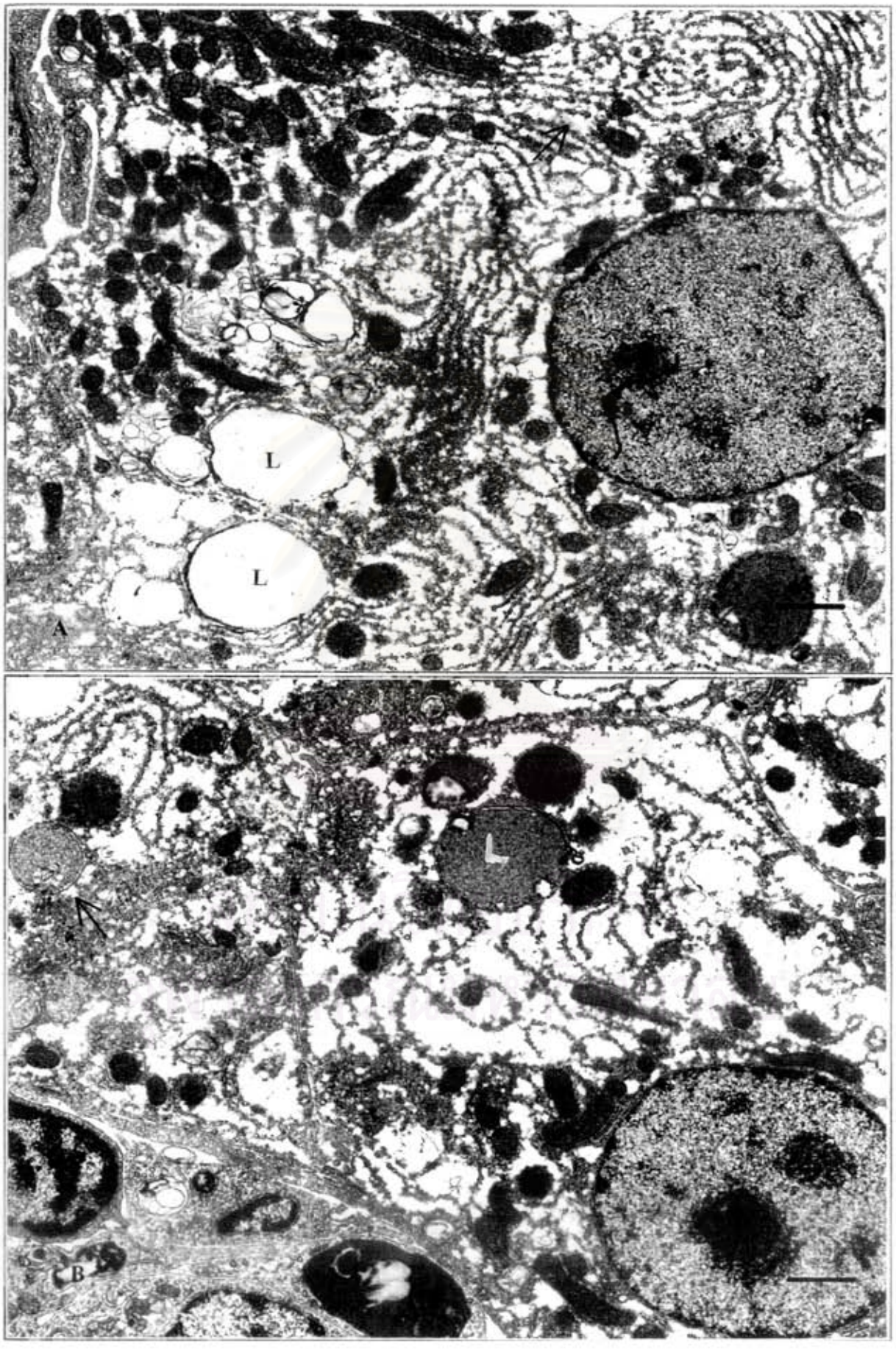

L
L
A
L
B
C

Plate 25

Electronmicrograph of the control liver from 5th month of experiment

Figure A Electron micrograph of 5th month treated liver, shows the contracted and swelling of mitochrondria, the hepatocytes lost in the cytoplasmic organalle. The bar scale is 1 μm.