CHAPTER IV

RESULTS

Results of acute and sub-acute toxicity studies were divided into two parts, 4.1 was results of clinical chemistry parameters and 4.2 was results of histopathological examination as follows:

4.1 Results of clinical chemistry parameters

4.1.1 Experiment I: Effect of PA extract given 24 hours before single oral dose of ethanol (5 g/kg) (acute toxicity study)

1.) Effect of PA extract on levels of serum ALT and AST

The levels of ALT and AST were shown in Table 1. Administration of ethanol (5 g/kg) to rats significantly increased levels of ALT and AST as compared with control rats. Administration of PA extract at doses of 25, 50, 75 mg/kg and SL at a dose of 5 mg/kg 24 hours before ethanol (5 g/kg) showed that PA extract at a dose of 75 mg/kg and SL at a dose of 5 mg/kg significantly decreased levels of ALT and AST and brought back to normal. While PA extract at doses of 25 and 50 mg/kg significantly decreased only levels of AST.

Table 1. Effect of PA given 24 hours before single oral dose (5 g/kg) of ethanol : levels of ALT and AST.

Groups	ALT (U/L)	AST (U/L)
Control (distilled water)	17.24 ± 1.50	44.40 ± 1.93
Ethanol 5 g/kg	26.37 ± 1.24 *	76.88 ± 6.46 *
PA 25 mg/kg	26.47 ± 1.62 *	57.68 ± 3.82 #
PA 50 mg/kg	22.59 ± 1.45	51.87 ± 1.34 #
PA 75 mg/kg	19.10 ± 0.66 #	50.02 ± 2.89 #
SL 5 mg/kg	19.97 ± 0.86 #	52.35 ± 2.36 #

Results are expressed as mean ± SEM, (n=6).

* Significant difference from control group ($p<0.05$).

Significant difference from ethanol group ($p<0.05$).

2.) Effect of PA extract on level of serum triglyceride (STg)

The level of STg was shown in Table 2. Administration of ethanol (5 g/kg) as well as administration of PA extract and SL 24 hours before ethanol showed no difference in STg level as compared with control rats. The values were in the range of 82.75-121.06 mg/dl.

Table 2. Effect of PA given 24 hours before single oral dose (5 g/kg) of ethanol : level of STg.

Groups	STg (mg/dl)
Control (distilled water)	82.75 ± 12.49
Ethanol 5 g/kg	111.55 ± 11.39
PA 25 mg/kg	107.23 ± 14.43
PA 50 mg/kg	113.57 ± 15.31
PA 75 mg/kg	121.06 ± 6.78
SL 5 mg/kg	113.74 ± 10.46

Results are expressed as mean ± SEM, (n=6).

3.) Effect of PA extract on level of hepatic triglyceride (HTg)

The level of HTg was shown in table 3. Administration of ethanol (5 g/kg) as well as administration of PA extract and SL, 24 hours before ethanol showed no difference in HTg level as compared with control rats. The values were in the range of 19.45-33.09 mg/g liver.

Table 3. Effect of PA given 24 hours before single oral dose (5 g/kg) of ethanol : level of HTg.

Groups	HTg (mg/g liver)
Control (distilled water)	22.94 $\pm$ 3.90
Ethanol 5 g/kg	25.17 $\pm$ 5.06
PA 25 mg/kg	20.83 $\pm$ 3.43
PA 50 mg/kg	33.09 $\pm$ 4.68
PA 75 mg/kg	28.84 $\pm$ 1.82
SL 5 mg/kg	19.45 $\pm$ 3.94

Results are expressed as mean $\pm$ SEM, (n=6).

4.) Effect of PA extact on level of hepatic reduced glutathione (GSH)

The level of GSH was shown in Table 4. Administration of ethanol (5 g/kg) as well as administration of PA extract and SL 24 hours before ethanol showed no difference in GSH level as compared with control rats. The values were in the range of 6.04-6.78 μmol/g liver.

Table 4. Effect of PA given 24 hours before single oral dose (5 g/kg) of ethanol : level of GSH.

Groups	GSH (μmol/g liver)
Control (distilled water)	6.78 ± 0.68
Ethanol 5 g/kg	6.47 ± 0.48
PA 25 mg/kg	6.37 ± 0.28
PA 50 mg/kg	6.57 ± 0.31
PA 75 mg/kg	6.23 ± 0.10
SL 5 mg/kg	6.04 ± 0.28

Results are expressed as mean ± SEM, (n=6).

5.) Effect of PA extract on level of hepatic malondialdehyde (MDA)

The level of MDA was shown in Table 5. Administration of ethanol (5 g/kg) as well as administration of PA extract and SL 24 hours before ethanol showed no difference in MDA level as compared with control rats. The values were in the range of 8.60-9.92 nmol/g liver.

Table 5. Effect of PA given 24 hours before single oral dose (5 g/kg) of ethanol : level of MDA.

Groups	MDA (nmol/g liver)
Control (distilled water)	8.60 $\pm$ 0.43
Ethanol 5 g/kg	9.64 $\pm$ 0.24
PA 25 mg/kg	9.10 $\pm$ 0.74
PA 50 mg/kg	9.92 $\pm$ 0.71
PA 75 mg/kg	9.61 $\pm$ 0.75
SL 5 mg/kg	9.51 $\pm$ 0.41

Results are expressed as mean $\pm$ SEM, (n=6).

6.) Effect of PA extract on level of serum TNF-α

The level of TNF-α was shown in Table 6. Administration of ethanol (5 g/kg) as well as administration of PA extract and SL 24 hours before ethanol showed no difference in TNF-α level as compared with control rats. The values were in the range of 44.85-68.69 pg/ml.

Table 6. Effect of PA given 24 hours before single oral dose (5 g/kg) of ethanol : level of TNF-α.

Groups	TNF-α (pg/ml)
Control (distilled water)	44.85 ± 8.84
Ethanol 5 g/kg	68.69 ± 23.46
PA 25 mg/kg	52.93 ± 5.45
PA 50 mg/kg	53.44 ± 10.61
PA 75 mg/kg	49.97 ± 9.84
SL 5 mg/kg	49.98 ± 9.50

Results are expressed as mean ± SEM, (n=6).

7.) Effect of PA extract on level of serum IL-1β

The level of IL-1β was shown in Table 7. Administration of ethanol (5 g/kg) as well as administration of PA extract and SL 24 hours before ethanol showed no difference in IL-1β level as compared with control rats. The values were in the range of 45.33-60.63 pg/ml.

Table 7. Effect of PA given 24 hours before single oral dose (5 g/kg) of ethanol : level of IL-1β.

Groups	IL-1β (pg/ml)
Control (distilled water)	53.96 ± 5.41
Ethanol 5 g/kg	60.63 ± 8.74
PA 25 mg/kg	45.33 ± 6.79
PA 50 mg/kg	55.33 ± 5.57
PA 75 mg/kg	46.90 ± 5.74
SL 5 mg/kg	36.90 ± 8.22

Results are expressed as mean ± SEM, (n=6).

In this acute toxicity study, the best effective dose of PA extract was 75 mg/kg which gave results similar to the effect of SL. Therefore, this dose was chosen for sub-acute toxicity study.

4.1.2 Experiment II: Effect of PA extract given 7 days after administration of ethanol (4 g/kg) for 21 days (sub-acute toxicity study)

1.) Effect of PA extract on levels of serum ALT and AST

The levels of ALT and AST were shown in Table 8. Administration of ethanol (4 g/kg) for 21 days significantly increased levels of ALT and AST as compared with control rats. Treatment with PA extract (75 mg/kg) for 7 days after ethanol caused significant reduction of these values similar to SL treatment. Rats received PA extract alone for 7 days showed no change in values of ALT and AST.

Table 8. Enhancement of rat liver recovery by PA (75 mg/kg) and SL (5 mg/kg) given daily for 7 days after 21 days with ethanol (4 g/kg): levels of ALT and AST.

Groups	ALT (U/L)	AST (U/L)
Control (distilled water)	18.03 $\pm$ 0.49	40.94 $\pm$ 0.89
Ethanol 4 g/kg	27.41 $\pm$ 1.78 *	56.64 $\pm$ 2.74 *
Ethanol + self recovery	19.85 $\pm$ 1.16 #	45.30 $\pm$ 2.85 #
Ethanol + PA 75 mg/kg	19.71 $\pm$ 0.87 #	47.26 $\pm$ 2.33 #
Distilled water + PA 75 mg/kg	17.52 $\pm$ 1.23 #	41.52 $\pm$ 1.51 #
Ethanol + SL 5 mg/kg	18.69 $\pm$ 0.97 #	44.28 $\pm$ 1.83 #

Results are expressed as mean $\pm$ SEM, (n=8).

* Significant difference from control group ($p<0.05$).

Significant difference from ethanol group ($p<0.05$).

2.) Effect of PA extract on level of serum triglyceride (STg)

The level of STg was shown in Table 9. All groups showed no difference in STg level as compared with control rats. The values were in the range of 53.51-120.90 mg/dl.

Table 9. Enhancement of rat liver recovery by PA (75 mg/kg) and SL (5 mg/kg) given daily for 7 days after 21 days with ethanol (4 g/kg): level of STg .

Groups	STg (mg/dl)
Control (distilled water)	57.40 ± 7.85
Ethanol 4 g/kg	120.90 ± 23.29
Ethanol + self recovery	88.50 ± 30.13
Ethanol + PA 75 mg/kg	53.51 ± 8.09
Distilled water + PA 75 mg/kg	60.73 ± 11.71
Ethanol + SL 5 mg/kg	66.46 ± 8.50

Results are expressed as mean ± SEM, (n=8).

3.) Effect of PA extract on level of hepatic triglyceride (HTg)

The level of HTg was shown in Table 10. Administration of ethanol (4 g/kg) for 21 days significantly increased level of HTg as compared with control rats. Treatment with PA extract (75 mg/kg) for 7 days after ethanol caused significant reduction of this value. Rats received PA extract alone for 7 days showed no change in HTg value.

Table 10. Enhancement of rat liver recovery by PA (75 mg/kg) and SL (5 mg/kg) given daily for 7 days after 21 days with ethanol (4 g/kg): level of HTg.

Groups	HTg (mg/g liver)
Control (distilled water)	21.30 ± 3.49
Ethanol 4 g/kg	37.43 ± 4.65 *
Ethanol + self recovery	28.44 ± 2.58
Ethanol + PA 75 mg/kg	22.36 ± 2.31 #
Distilled water + PA 75 mg/kg	20.95 ± 2.90 #
Ethanol + SL 5 mg/kg	29.57 ± 3.47

Results are expressed as mean ± SEM, (n=8).

* Significant difference from control group ($p<0.05$).

Significant difference from ethanol group ($p<0.05$).

4.) Effect of PA extract on level of hepatic reduced glutathione (GSH)

The level of GSH was shown in Table11. Administration of ethanol (4 g/kg) and treatment with PA extract and SL after ethanol showed no difference in GSH level as compared with control rats. The values were in the range of 5.17-5.95 μmol/g liver.

Table 11. Enhancement of rat liver recovery by PA (75 mg/kg) and SL (5 mg/kg) given daily for 7 days after 21 days with ethanol (4 g/kg): level of GSH.

Groups	GSH (μmol/g liver)
Control (distilled water)	5.64 ± 0.27
Ethanol 4 g/kg	5.95 ± 0.25
Ethanol + self recovery	5.25 ± 0.18
Ethanol + PA 75 mg/kg	5.47 ± 0.16
Distilled water + PA 75 mg/kg	5.17 ± 0.14
Ethanol + SL 5 mg/kg	5.37 ± 0.13

Results are expressed as mean ± SEM, (n=8).

5.) Effect of PA extract on level of hepatic Malondialdehyde (MDA)

The level of MDA was shown in Table 12. Administration of ethanol (4 g/kg) for 21 days significantly increased level of MDA as compared with control rats. Treatment with PA extract (75 mg/kg) for 7 days after ethanol showed a significant decrease in the elevated MDA similar to SL treatment. Rats received PA extract alone for 7 days showed no change in MDA formation.

Table 12. Enhancement of rat liver recovery by PA (75 mg/kg) and SL (5 mg/kg) given daily for 7 days after 21 days with ethanol (4 g/kg): level of MDA.

Groups	MDA (nmol/g liver)
Control (distilled water)	10.15 ± 0.27
Ethanol 4 g/kg	13.95 ± 0.61 *
Ethanol + self recovery	11.48 ± 0.57 #
Ethanol + PA 75 mg/kg	10.24 ± 0.28 #
Distilled water + PA 75 mg/kg	9.65 ± 0.15 #
Ethanol + SL 5 mg/kg	10.62 ± 0.31 #

Results are expressed as mean $\pm$ SEM, (n=8).

* Significant difference from control group ($p<0.05$).

Significant difference from ethanol group ($p<0.05$).

6.) Effect of PA extract on level of serum TNF-α

The level of TNF-α was shown in Table 13. Administration of ethanol (4 g/kg) for 21 days caused significant increasing level of TNF-α as compared with control rats. Treatment with PA extract (75 mg/kg) for 7 days after ethanol showed a significant decrease in the elevated TNF-α similar to SL treatment. Rats received PA extract alone for 7 days showed no change in TNF-α level.

Table 13. Enhancement of rat liver recovery by PA (75 mg/kg) and SL (5 mg/kg) given daily for 7 days after 21 days with ethanol (4 g/kg): level of TNF-α

Groups	TNF-α (pg /ml)
Control (distilled water)	42.35 ± 6.43
Ethanol 4 g/kg	101.48 ± 26.11 *
Ethanol + self recovery	62.09 ± 9.09
Ethanol + PA 75 mg/kg	48.69 ± 6.84 #
Distilled water + PA 75 mg/kg	37.54 ± 4.62 #
Ethanol + SL 5 mg/kg	41.58 ± 7.50 #

Results are expressed as mean ± SEM (n=8)

* Significant difference from control group ($p<0.05$).

Significant difference from ethanol group ($p<0.05$).

7.) Effect of PA extract on level of serum IL-1β

The level of IL-1β was shown in Table 14. Administration of ethanol (4 g/kg) for 21 days showed a significant increase in IL-1β level as compared with control rats. Treatment with PA extract (75 mg/kg) and SL (5 mg/kg) for 7 days after ethanol and PA extract alone for 7 days showed normal value of IL-1β level.

Table 14. Enhancement of rat liver recovery by PA (75 mg/kg) and SL (5 mg/kg) given daily for 7 days after 21 days with ethanol (4 g/kg): level of IL-1β.

Groups	IL-1β (pg/ml)
Control (distilled water)	36.71 ± 6.75
Ethanol 4 g/kg	74.50 ± 9.68 *
Ethanol + self recovery	46.26 ± 6.77
Ethanol + PA 75 mg/kg	46.12 ± 7.57
Distilled water + PA 75 mg/kg	50.24 ± 4.59
Ethanol + SL 5 mg/kg	42.88 ± 5.66 #

Results are expressed as mean ± SEM (n=8)

* Significant difference from control group ($p<0.05$).

Significant difference from ethanol group ($p<0.05$).

4.2 Results of histopathological examination

The pathological changes of rat liver were evaluated for the damage tissue using the following parameters: the area of liver cell injury, the number of hepatocytic row injury around central vein (centrilobular degeneration), the endothelial cell injury and the formation of fat vacuoles.

The degree of liver injury was graded from 0 to +3 levels as follows:

- Level 0 (normal): normal liver morphology, hepatocytes had the round nucleus centrally and homogenous cytoplasm, the flat endothelial cells around central vein and sinusoid.

- Level +1 (mild degree): some of 1-2 hepatocyte rows around central vein demonstrated hepatic cell degeneration, necrosis (loss of nucleus), less injury of endothelial cells around central vein and less fat vacuoles in hepatocytes.

- Level +2 (moderate degree): hepatocyte rows around central vein had swelling, intracytoplasmic vacuolar degeneration in centrilobular, midzonal and periportal areas, endothelial cells around central vein more injury than level +1 and increasing of fat vacuoles in hepatocytes as compared with level +1.

- Level +3 (severe degree): 3-4 hepatocyte rows around central vein showed hepatocytic degeneration and necrosis (loss of nucleus), degeneration cells including centrilobular, midzonal and periportal areas (diffuse intracytoplasmic vacuolar degeneration), endothelial lining of central vein exhibited more cell injury ,as well as increasing of fat vacuoles in hepatocytes as compared with level +2. Besides, focal necrosis and bile duct proliferation were marked.

4.2.1 Effect of PA extract on histopathological changes of Experiment I (acute toxicity study)

Histopathological grading was shown in Table 15 and histopathological changes of liver were given in Figure 10-15. Normal rats demonstrated normal liver histology with hepatocytes having the round nucleus centrally, homogeneous cytoplasm and arranged in the form of cords at central vein, endothelial cells were flat, no sign of fatty liver and regular distribution of glycogen in hepatocytes. Ethanol (5g/kg) administered rats showed swelling of hepatocytes, increasing areas of sinusoidal spaces, centrilobular degeneration, active kupffer cell. Administration of PA extract(25 mg/kg) 24 hours before ethanol showed centrilobular degeneration

and swelling of hepatocytes. Rats received PA extract (50 mg/kg) showed swelling of hepatocytes. PA extract (75 mg/kg) and SL (5 mg/kg) administered rats showed reversible regeneration of hepatocytes with mitotic figure and normal liver cell morphology.

Table 15. The histopathology of PA extract given 24 hours before single oral dose (5 g/kg) of ethanol (n=6).

Groups	Degree of liver injury
Control (distilled water)	0
Ethanol 5 g/kg	+1
PA 25 mg/kg	+1
PA 50 mg/kg	0
PA 75 mg/kg	0
SL 5 mg/kg	0

Results are expressed as grading:

0 = normal, +1 = mild, +2 = moderate and +3 = severe.

4.2.2 Effect of PA extract on histopathological changes of Experiment II (sub-acute toxicity study)

Histopathological grading was shown in Table 16 and histopathological changes of liver were given in Figure 16-29. The liver samples from ethanol administered rats for 21 days showed swelling of hepatocytes, increasing area of sinusoidal spaces, lobular necrosis, active kupffer cell, bile duct proliferation in portal tract, fatty liver, hyaline globule and loss of glycogen in hepatocytes including fibrosis. On treatment with PA extract for 7 days after ethanol, the liver showed less disarrangement and degeneration of hepatocytes, reversible regeneration of hepatocytes with mitotic figure similar to SL treatmant. SL administered rats showed reversible regeneration of hepatocytes with the cell division and most of liver cell appeared normal. Rats treated with ethanol and then self recovery showed irregular patterns and variety sizes of hepatocytes, focal

necrosis and reversing of the cell to normal was slower than rats received PA and SL. Administration of PA extract alone demonstrated mitotic figure and normal liver cell morphology.

Table 16. The histopathology of PA extract given 7 days after administration of ethanol (4 g/kg) for 21 days (n=8).

Groups	Degree of liver injury
Control (distilled water)	0
Ethanol 4 g/kg	+3
Ethanol + self recovery	+2
Ethanol + PA 75 mg/kg	+1
Distilled water + PA 75 mg/kg	0
Ethanol + SL 5 mg/kg	0

Results are expressed as grading:

0 = normal, +1 = mild, +2 = moderate and +3 = severe.

Experiment I (acute toxicity study)

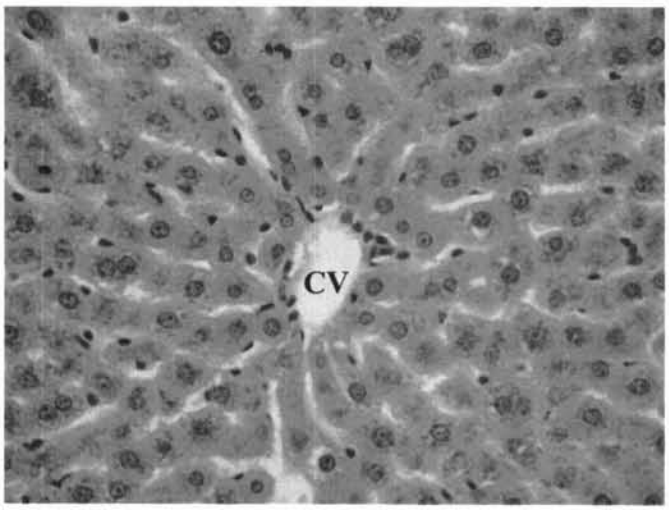

Figure 10. Light micrograph of normal rat liver (received distilled water). Liver section showing hepatocytes had the round nucleus centrally, homogeneous cytoplasm and arranged in the form of hepatocytic cords from central vein, endothelial cells around central vein and sinusoids were flat. (grading: 0)(H&Ex400), CV = central vein.

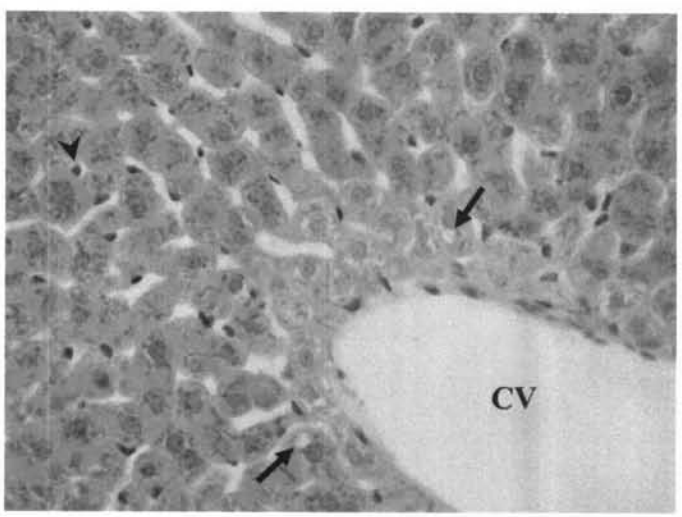

Figure 11. Light micrograph of rat liver after administration of ethanol (5 g/kg). Liver section showing periacinar intracytoplasmic vacuolar degeneration (arrows), active kupffer cells (arrow head), swelling of hepatocytes and increasing area of sinusoidal spaces.
(grading: +1)(H&Ex400), CV = central vein.

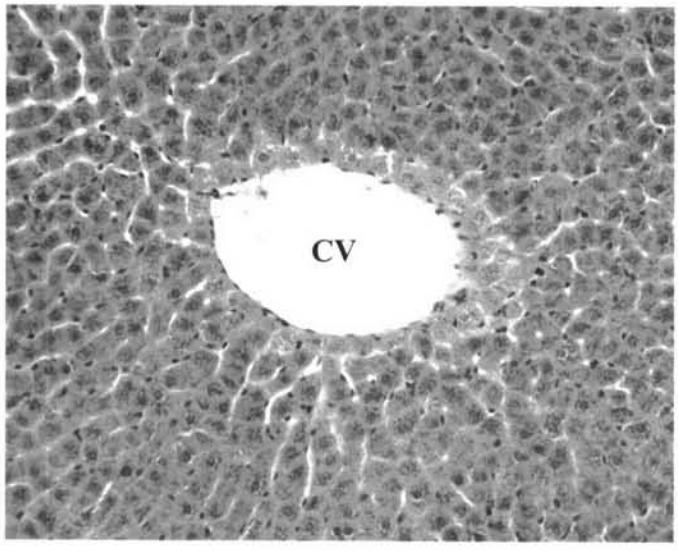

Figure 12. Light micrograph of rat liver after administration of PA 25 mg/kg before ethanol (5 g/kg).
Liver section showing periacinar intracytoplasmic vacuolar degeneration, swelling of hepatocytes. (grading: +1)(H&Ex200), CV = central vein.

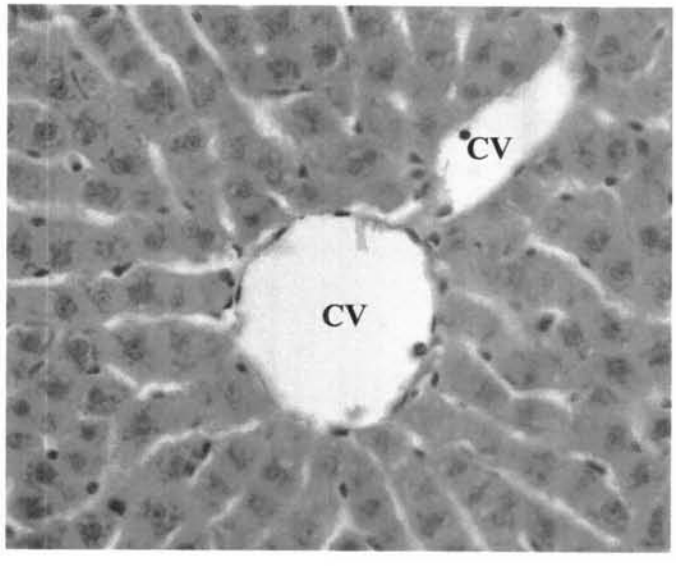

Figure 13. Light micrograph of rat liver after administration of PA 50 mg/kg before ethanol (5 g/kg).
Liver section showing slight swelling of hepatocytes.
(grading: 0)(H&Ex400), CV = central vein.

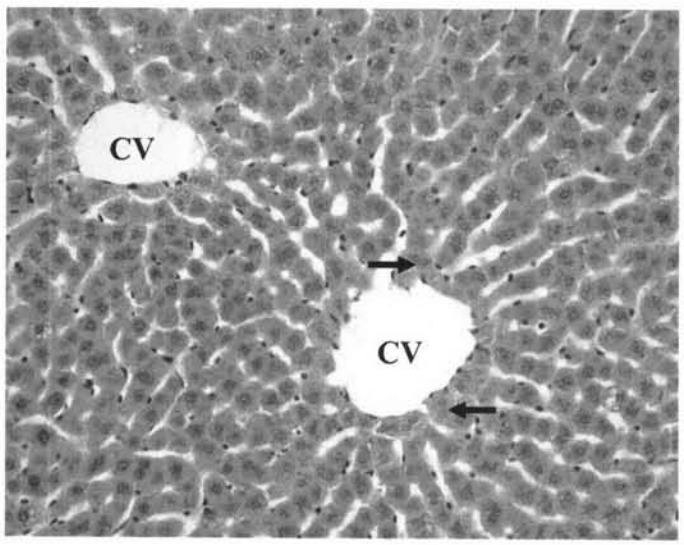

Figure 14. Light micrograph of rat liver after administration of PA 75 mg/kg before ethanol (5 g/kg).
Liver section showing reversible regeneration with mitotic figure of liver cells (arrows) and morphology was similar to normal liver.
(grading: 0)(H&Ex200), CV = central vein.

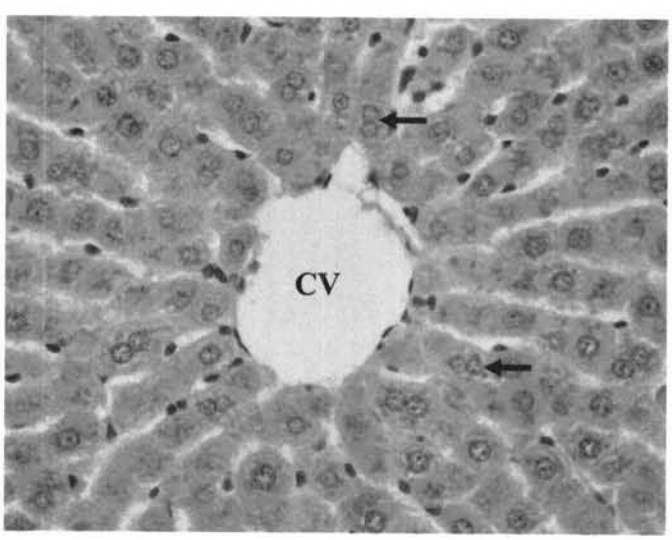

Figure 15. Light micrograph of rat liver after administration of SL 5 mg/kg before ethanol (5 g/kg).
Liver section showing reversible regeneration with mitotic figure of liver cells (arrows) and morphology was similar to normal liver.
(grading: 0)(H&Ex400), CV = central vein.

Experiment I (sub-acute toxicity study)

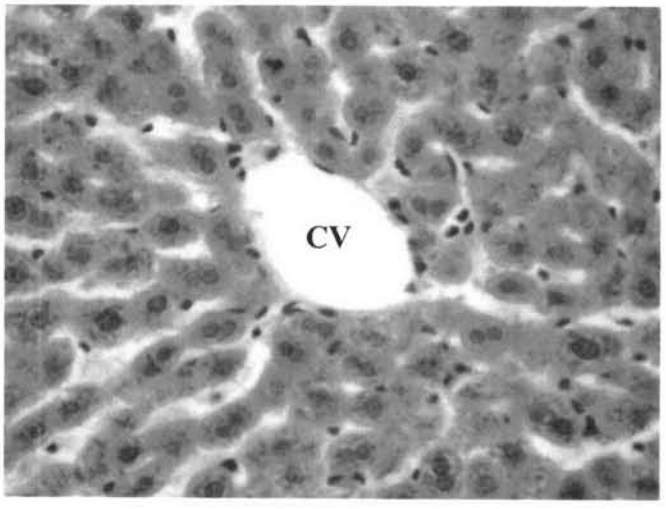

Figure 16. Light micrograph of normal rat liver (received distilled water) for 21days
Liver section showing hepatocytes had the round nucleus centrally, homogeneous cytoplasm and arranged in the form of hepatocytic cords from central vein, endothelial cells around central vein and sinusoids were flat. (grading: 0)(H&Ex400), CV = central vein.

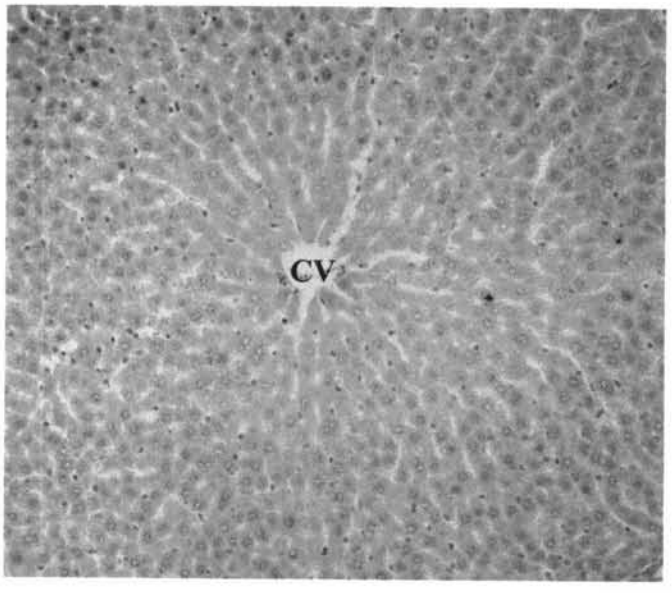

Figure 17. Light micrograph of rat liver after administration of ethanol (4 g/kg/day) for 21 days.
Liver section showing periacinar and midzonal hepatocytic degeneration and necrosis (lobular necrosis), swelling of hepatocytes, active kupffer cells and increasing area of sinusoidal spaces. (grading: +3)(H&Ex100), CV = central vein.

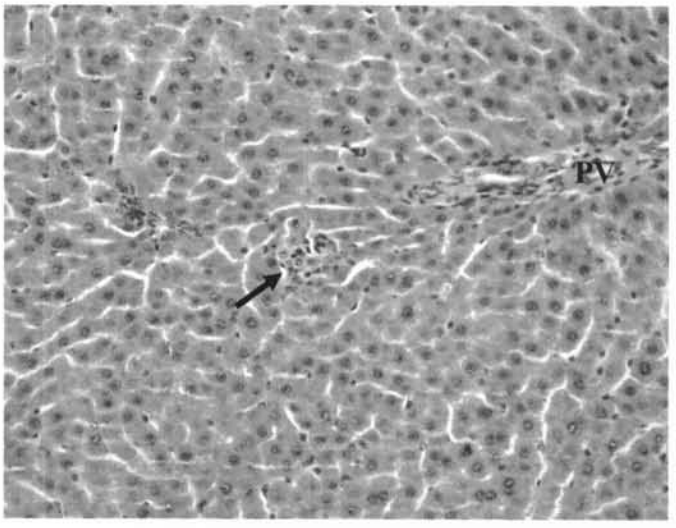

Figure 18. Light micrograph of rat liver after administration of ethanol (4 g/kg/day) for 21 days

Liver section showing focal fibrosis in midzonal area (arrow), swelling of hepatocytes. (grading+3)(H&Ex200), PV = portal vein.

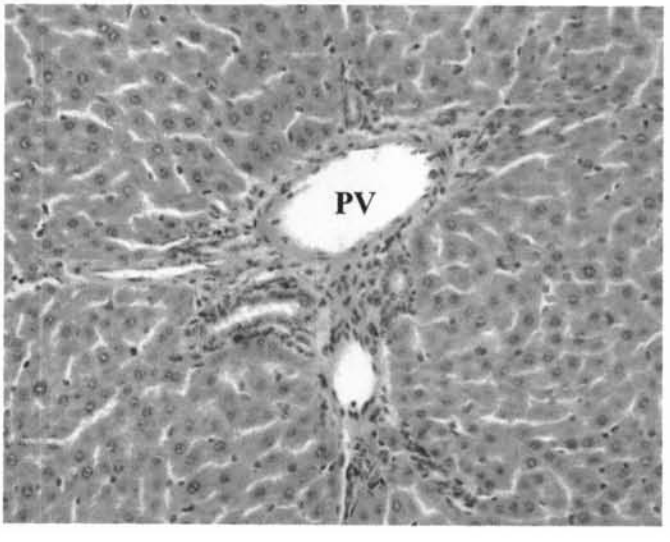

Figure 19. Light micrograph of rat liver after administration of ethanol (4 g/kg/day) for 21 days

Liver section showing bile duct proliferation in portal tract and swelling of hepatocytes. (grading: +3)(H&Ex200), PV = portal vein.

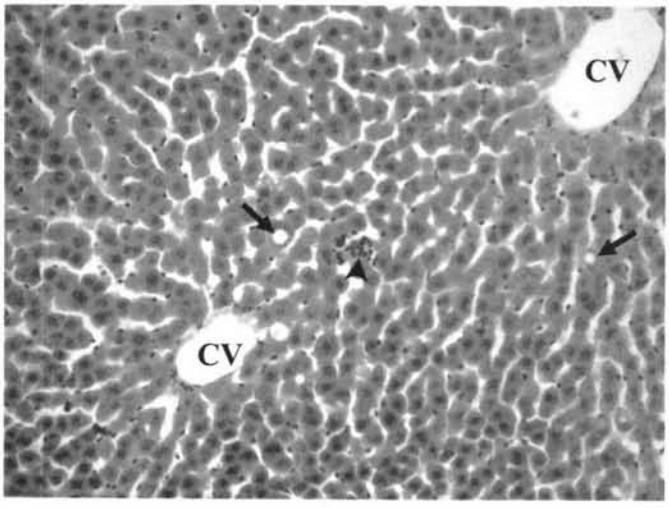

Figure 20. Light micrograph of rat liver after administration of ethanol (4 g/kg/day) for 21 days and then self recovery.
Liver section showing midzonal intracytoplasmic vacuolar degeneration (arrows), focal necrosis (arrow head), disarrangement of hepatocytes and reversible regeneration of hepatocytes with the cell division.
(grading: +2)(H&Ex200), CV = central vein.

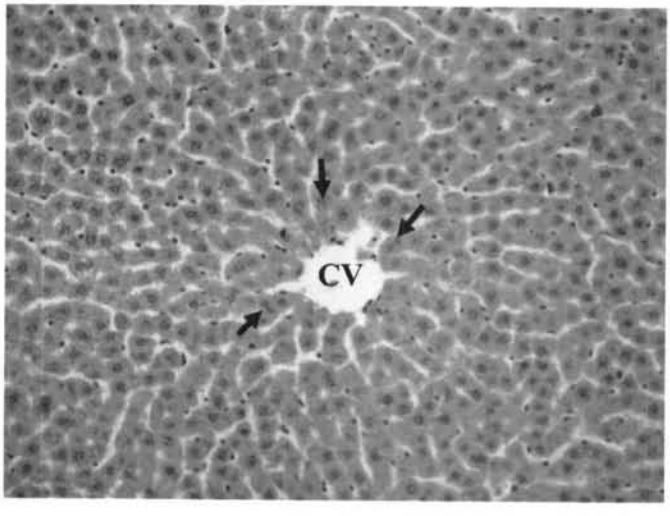

Figure 21. Light micrograph of rat liver given PA 75 mg/kg treatment for 7 days after ethanol (4 g/kg/day) administration for 21 days.
Liver section showing less disarrangement and degeneration of hepatocytes, reversible regeneration and mitotic figure of liver cells (arrows) (grading: +1)(H&Ex200), CV = central vein.

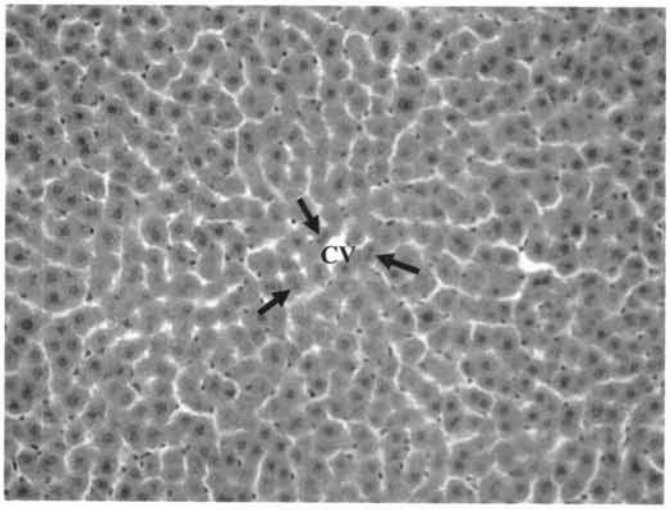

Figure 22. Light micrograph of rat liver after administration of PA 75 mg/kg alone for 7 days.
Liver section showing mitotic figure of hepatocytes (arrows) and normal liver cell morphology.
(grading: 0)(H&Ex200), CV = central vein.

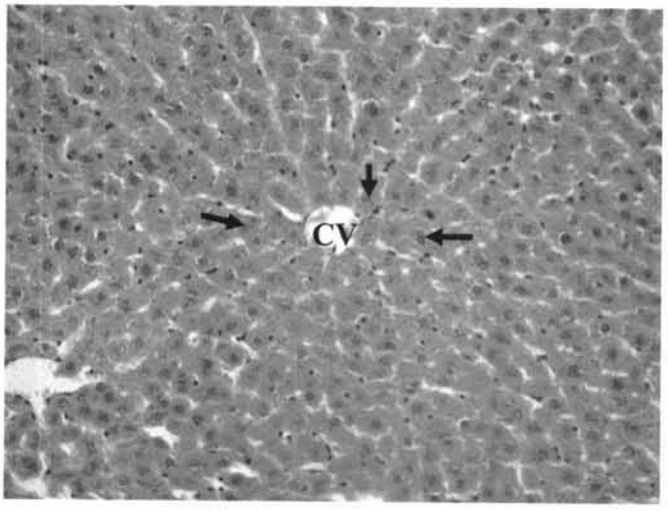

Figure 23. Light micrograph of rat liver given SL 5 mg/kg treatment for 7 days after ethanol (4 g/kg/day) administration for 21 days.
Liver section showing reversible regeneration, mitotic figure of liver cells (arrows) and almost normal liver morphology.
(grading: 0)(H&Ex200), CV = central vein.

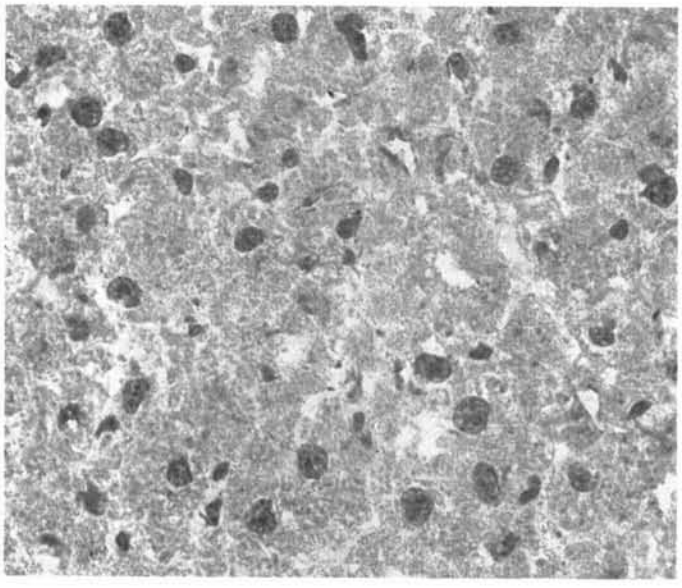

Figure 24. Light micrograph of normal rat liver (received distilled water)
Liver section showing homogeneous cytoplasm and no sign of intracytoplasmic bright-red fat vacuolar of hepatocytes (Oil red Ox400).

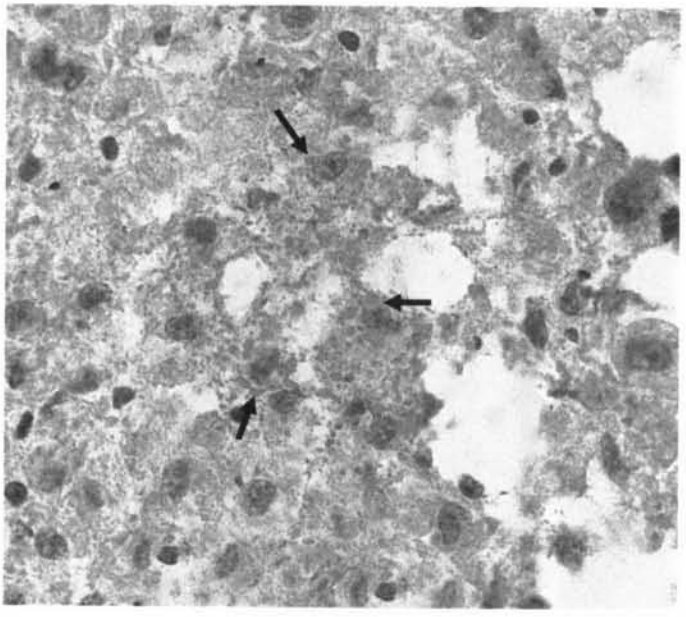

Figure 25. Light micrograph of rat liver after administration of ethanol (4 g/kg/day) for 21days.
Liver section showing intracytoplasmic bright-red fat vacuolar of hepatocytes (arrows)(Oil red Ox400).

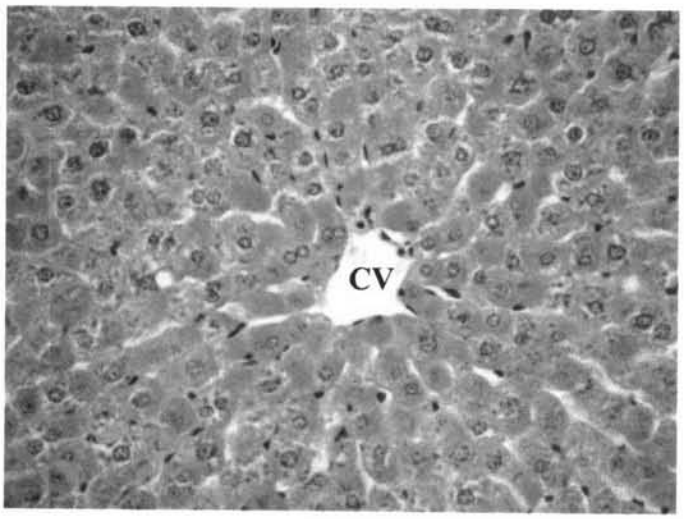

Figure 26. Light micrograph of normal rat liver (received distilled water)
Liver section showing regular distribution of intracytoplasmic pink-red glycogen accumulation. (PASx400), CV = central vein.

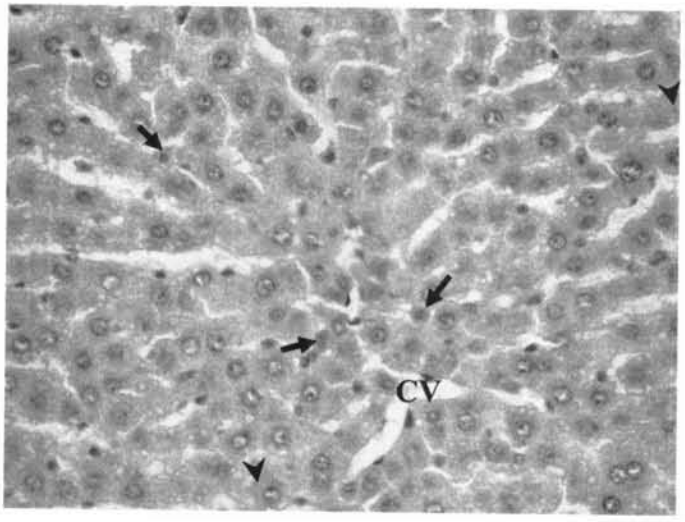

Figure 27. Light micrograph of rat liver after administration of ethanol (4 g/kg/day) for 21days.
Liver section showing irregular distribution of intracytoplasmic pink-red glycogen accumulation,glycogen globule (hyaline globule)(head arrows) and active kupffer cell (arrows) (PASx400), CV = central vein.

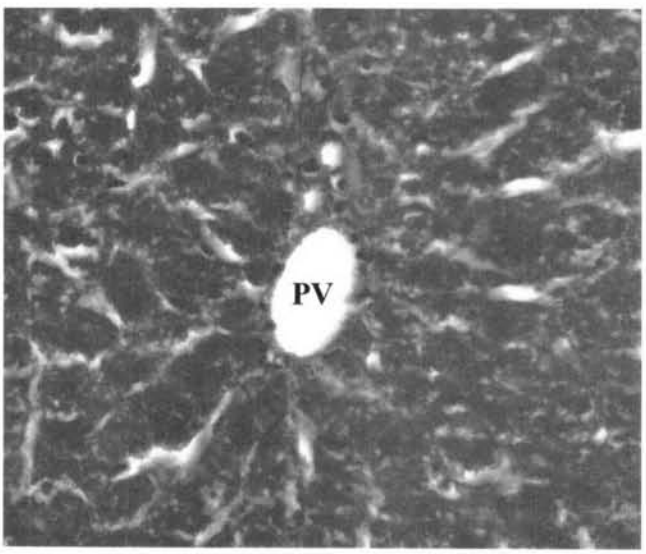

Figure 28. Light micrograph of normal rat liver (received distilled water)
Liver section showing blue color of thin connective tissue in normal portal tract (Masson's Trichrome x400), PV = portal vein.

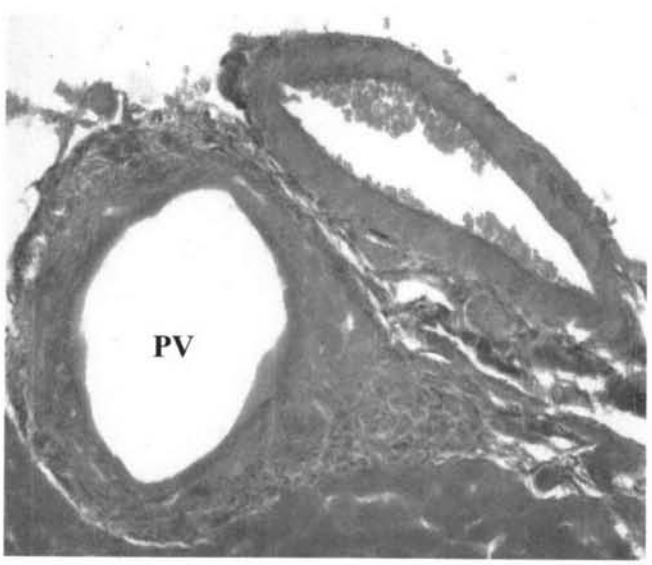

Figure 29. Light micrograph of rat liver after administration of ethanol (4 g/kg/day) for 21days
Liver section showing blue color of thick connective tissue forming fibrosis in portal tract. (Masson's Trichrome x400), PV = portal vein.