The Effects of Curcuma Against Paracetamol-induced Liver Damage in Rats

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Abstrak

Pemberian parasetamol sebanyak 2500 mg/kg BB, tunggal dan per oral pada tikus menyebabkan terjadinya nekrosis hati berat. Pemeriksaan kadar SGPT dan SGOT 48 jam setelah pemberian parasetamol menunjukkan kenaikan dari kontrol (5,8 ± 0,9 μ/l dan 17,9 ± 2,3 μ/l) menjadi 407,2 ± 38,7 μ/l dan 536,3 ± 39,3 μ/l. Data biokimiawi ini disokong oleh pemeriksaan ultrastruktur (histopatologi) dimana didapatkan indeks kerusakan 3,0 ± 0,3 dibandingkan 0 pada kontrol. Kurkuma 50 mg/kg BB yang diberikan berulang tidak memberikan efek protektif, tetapi pada dosis tinggi (250 mg/kg BB dan 1000 mg/kg BB) kadar SGPT, SGOT menurun dan gambaran histopatologik membaik dibandingkan dengan kelompok tikus yang hanya diberikan parasetamol saja. Hasil ini disokong lebih lanjut dengan percobaan lain dimana digunakan kurkuminoid yang telah dipurifikasi sebagian. Kurkuma tampaknya dapat mengurangi cedera hati karena parasetamol sama baiknya dengan N- asetilsistein. Mekanisme bagaimana kurkuma dapat mencegah/mengurangi kerusakan hati karena parasetamol belum diketahui. Diduga efek protektif ini dilangsungkan melalui penghambatan sitokrom P450.

Abstract

Paracetamol given as an oral, single dose of 2500 mg/kg BW can produce a severe liver damage in rats as shown by high levels of serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT) and histopathological assessements. Curcuma, if it is given in a high dose regimen (250-1000 mg/kg BW) showed an antihepatotoxic activity. This protective effect is no longer apparent if the dose is lowered (50 mg/kg BW) even though it is given repeatedly. These results were confirmed in a repeated experiment using a partially purified curcuminoid preparation. This study showed that Curcuma appeared to have a similar or better antihepatotoxic action than N-acetylcysteine. The mechanism underlying the antihepatotoxic action of curcuma is not clear yet. Further experiments are needed to clarify this.

Keywords : *Curcuma, Paracetamol-induced liver necrosis, Lipid peroxides, N-acetylcystein, Cytochrome P-450.*

INTRODUCTION

Paracetamol is currently a widely used analgesic-antipyretic in Indonesia and other countries. Although it is considered as safe, it can produce a severe liver damage if used in high dosage. The pathogenesis of toxic liver necrosis caused by paracetamol is not clear yet. According to some authors,[1] the toxic effect of paracetamol is mediated by its metabolites which are formed via cyt P450. Other authors proposed the possible role of free radical production in the metabolism of paracetamol.[2] Normally, the toxic metabolites of paracetamol can be eliminated by conjugation with the available glutathione, which are then metabolized in the intestine or kidney to become its mercapturic derivatives and excreted in the urine. The fact that N-acetylcysteine, a precursor of glutathione has been widely used as an antidote for paracetamol intoxication, provides a support for the above mentioned hypothesis.

Curcuma *(Curcuma xanthorrhiza Roxb.)*, a traditional herb, has been frequently used and said to be

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beneficial in treating liver diseases such as hepatitis or liver cirrhosis. However only few data published[3,4] to have confirmed its beneficial effect. The purpose of the present study is to investigate the effects of curcuma against liver damage induced by paracetamol in experimental animals.

Background

Infectious and degenerative types of liver diseases are endemic especially in Indonesia and other Asian countries. At present, there appears to be practically no specific remedy for viral or toxin-induced liver damage. Several drugs that have been claimed to be beneficial in the treatment of liver diseases have been withdrawn from the market by the Department of Health in our country, because the available data are not convincing. Appropriate medical treatments (drugs and other treatment measures) are therefore in urgent need.

In Indonesia, traditional herbs have been widely used in the treatment of various diseases; one of them is curcuma for the treatment of liver diseases and jaundice. Herbal medicine appears to be promising and probably could play a significant role in the future. Following are some factors which may support the idea:

1) Drug research by the pharmaceutical industries leading to the discovery of new drugs is extremely expensive.
2) Herbs have been used for decades or probably centuries by our people and have been known to be extremely safe. Side effects of herbs have been very rare.
3) There are many examples of established drugs which are currently used that come from herbal medicine investigation such as reserpine, digitalis, quinine, psychoactive drugs etc.

One way of investigating these herbs is to select the most possible effective one in the treatment of a certain disease. We have chosen curcuma as the investigational drug against liver damage induced by paracetamol for the above-mentioned reason in comparison with N-acetylcysteine.

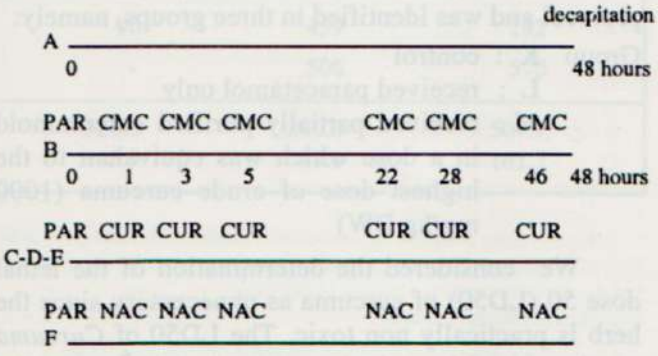
MATERIALS AND METHODS

Materials

Wistar rats of 150-200 g were used in this study, divided into 6 groups, with each group consisting of 10 animals. These animals were adapted and fed ad libitum. Curcuma *(Curcuma xanthorrhiza Roxb.)* and 5% curcuminoid powder were kindly prepared by Professor Sidik, Padjadjaran University, Bandung, while N- acetylcysteine was a generous gift by P.T. Zambon Indonesia. Phosphotungstic acid, sulphuric acid, acetic acid, Tris (hydroxymethyl)-aminomethane, 12150 MIT granutest GOT and 12166 MIT granutest GPT, were purchased from E. Merck, Darmstadst, W. Germany, and Malondialdehyde was from TCI, Japan. All other reagents routinely used were of analytical grade.

Methods

The experimental procedures were performed as follows:



Group A : control rats

B : liver damaged induced by paracetamol (PAR) which was given orally, 2500 mg/kg BW, in a single dose, with a concentration of 250 mg/ml in 0,5% Carboxymethyl cellulose (CMC). Thereafter a similar amount of CMC 0.5% was given at 1,3,5,22,28, and 46 hours after zero time (PAR administration).

C-D-E : similar to group B, except that curcuma (CUR) replacing 0.5% CMC was given 1,3,5,22,28 and 46 hours after paracetamol
Groups C-D-E received: 50 mg/kg BW, 250 mg/kg and 1000 mg/kg BW of powder curcuma, respectively, at the time points described

F : was treated with oral solution of N-acetyl-cysteine (NAC) in water at a dose of 500 mg/kg BW

Forty-eight hours after paracetamol administration, the experimental animals were anaesthetized with ether and killed by means of decapitation. Blood samples obtained were used to determine the serum

glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and plasma lipid peroxide.

Immediately after decapitation, livers were excised, weighted and immersed in 10% formaldehyde solution for preservation and stained with hematoxyllin-eosin.

SGOT and SGPT were determined by using a commercially available kit from E. Merck.

Plasma lipid peroxides were determined following the method as described by Stringer et al[5].

As results from the first step of experiments involving six groups of animals showed a dose-related protective effect of crude curcuma, we also conducted an additional experiment by using a partially purified curcuminoid (5% of curcuminoid) with similar protocol and was identified in three groups, namely:

Group K : control
L : received paracetamol only
M : received partially purified curcuminoid in a dose which was equivalent to the highest dose of crude curcuma (1000 mg/kg BW)

We considered the determination of the lethal dose 50 (LD50) of curcuma as unnecessary since the herb is practically non toxic. The LD50 of *Curcuma xanthorrhiza* for rats is above 2.5 g/kg BW[6], which far exceeds the crude highest dose we used in this study.

Curcuma is available in tablets of 200 mg in Indonesia with a suggested dosage of 6 tablets daily.

Histopathological assessment by light microscopy was made essentially as described by Mitchell et al[7], in which the severity of liver damage was graded in a scoring system. The severity index of structural liver damage was graded as follows:

Grade 0 : Normal
1 : Ballooning and eosinophilic degeneration of cells in centrilobular regions
2 : Necrotic lesions confined to centrilobular regions
3 : Necrosis extending from central zone to portal triads
4 : Massive necrosis of most of the liver tissue.

The microscopical grading analysis was performed in a blind method by three investigators. Different results of the analyses were discussed and reevaluated until final agreements were obtained.

Statistical Analysis

The Kruskal-Wallis statistic was used for comparison to analyse the multiple groups for SGPT,SGOT and histopathological results, because of the non-normality of the first two data, whereas other data were analyzed by means of one-way Anova method.

RESULTS

Paracetamol (PAR) given orally in a single dose of 2500 mg/kg BW produces a consistent damage of the liver, as shown in group B of experimental animals. SGPT and SGOT which were respectively5.8 $\pm$ 0.9 µ/l and 17.9 $\pm$ 2.3 µ/l in control (group A) increased after PAR administration to 407.2 $\pm$ 38.7 µ/l and 536.3 $\pm$ 39.3 µ/l in group B (Table 1, Fig.1). Multiple comparison by Kruskall-Wallis statistic method showed a statistically significant difference between the various groups (Tables 2,3). In line with these, the histopathological results also showed a severe liver damage (3.0 $\pm$ 0.4) in group B in comparison to the control group (0) (Tables 4,5).

To our surprise, no difference could be seen in plasma lipid peroxides between each group (Fig.2). These data, however, confirmed the results from other authors[8] using isolated hepatocytes as experimental model. As far as the protection of liver damage is concerned, our data strongly suggested a protective effect of curcuma against PAR-induced liver damage, which appeared to be dose-related. Low doses of curcuma (50 mg/kg BW, group C) were not protective. However, higher doses of curcuma, 250 mg/kg BW in group D and 1000 mg/kg BW in group E, clearly showed a beneficial effect of curcuma in ameliorating the bio-chemical and structural derangements of the liver after PAR intoxication.

The antihepatotoxic effects of crude curcuma against PAR cytotoxicity could be a result of drug interaction in the intestinal tract, wherein the absorption of PAR is reduced by the administration of large volumes of curcuma suspension.

In order to exclude this possibility, we performed other experiments using 5% curcuminoid.

In the second study, a dose of partially purified curcuminoid, equivalent to the highest dose of crude curcuma used in the first study, was used to confirm our findings. As shown in Figs.3,4, and Tables 6-10, liver damage induced by PAR (group L) was significantly reduced by repeated administration of partially purified curcuminoid (group M). The histopathological score index was much better in the group with curcuminoid (1,4 $\pm$ 0,4) if compared with the group with PAR only (2,9 $\pm$ 0,4). A similar trend was shown in biochemical parameters, although it was not statistically significant (Fig.3, Tables 6,7,8).

Table 1. Serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) activities in 6 groups of rats

	SGPT (U/L)					
No	Group A	Group B	Group C	Group D	Group E	Group F
1	9	1038	1038	723	125	119
2	3	140	349	260	-	264
3	7	-	231	150	576	103
4	8	703	162	580	148	567
5	2	432	762	-	121	1480
6	8	381	989	-	-	-
7	9	381	1314	343	-	867
8	3	307	723	96	87	401
9	2	135	260	90	439	192
10	7	148	521	-	506	576
Mean	5.8	407.2	634.9	320.3	286.0	507.7
SEM	0.9	38.7	105.5	45.9	77.1	161.7

	SGOT (U/L)					
No	Group A	Group B	Group C	Group D	Group E	Group F
1	10	1476	4870	784	134	198
2	10	157	310	169	-	288
3	27	-	201	104	393	78
4	21	1058	140	953	82	660
5	7	516	1550	-	120	3550
6	24	394	1545	-	-	-
7	22	312	1672	362	-	518
8	14	462	516	62	160	823
9	15	295	413	84	216	267
10	29	157	836	-	666	466
Mean	17.9	536.3	1.205.3	359.7	253.0	760.9
SEM	2.3	39.3	163.8	48.5	85.2	398.3

- : Animals died less than 48 h., prior to decapitation

A, control; B, paracetamol-induced liver necrosis (2500 mg/kg BW, single dose, orally); C-D-E, curcuma-treated groupss with a dose of 50 mg/kg BW, 250 mg/kg BW and 1000 mg/kg BW, respectively, after paracetamol; and F, N-acetyl-cysteine treated rats (500 mg/kg BW, orally), after paracetamol. Results are depicted as mean ± SEM

Table 2. Multiple comparison of SGPT activity in rats (Kruskal-Wallis statistical method)

No.	Group A	Group B	Group C	Group D	Group E	Group F
1	9.5	49.5	49.5	44.5	17	15
2	3.5	19	31	26.5	-	28
3	5.5	-	25	22	40.5	14
4	7.5	43	23	42	20.5	39
5	1.5	35	46	-	16	52
6	7.5	32.5	48	-	-	-
7	9.5	32.5	51	30	-	47
8	3.5	29	44.5	13	11	34
9	1.5	18	26.5	12	36	24
10	5.5	20.5	38	-	37	40.5
Total	55.0	279.0	382.5	190.0	178.0	293.5

Group	R	Group A 55.0	Group E 178.0	Group D 190.0	Group B 279.0	Group F 293.5	Group C 382.5
A	55.0	-					
E	178.0	123.0	-				
D	190.0	135.0	12.0	-			
B	279.0	224.0*	101.0	89.0	-		
F	293.5	238.5*	115.5	103.5	14.5	-	
C	382.5	327.5**	204.5	192.5	103.5	89.0	-

R : Sum of rank from each group
* : Significantly different by comparison between the respective group ($p \leq 0.05$)
** : Significantly different by comparison between the respective group ($p \leq 0.01$)
For the explanation of the groups (A-F), please see Table 1.
Results are depicted as ranks

Table 3. Multiple comparison of SGOT activity in rats (Kruskal-Wallis statistical method)

No.	Group A	Group B	Group C	Group D	Group E	Group F
1	2.5	47	52	42	17	23
2	2.5	19.5	29	22	-	27
3	9	-	24	15	32	12
4	6	46	18	45	13	40
5	1	37.5	49	-	16	51
6	8	33	48	-	-	-
7	7	30	50	31	-	39
8	4	35	37.5	11	21	43
9	5	28	34	14	25	26
10	10	19.5	44	-	41	36
Total R	55.0	295.5	385.5	180.0	165.0	297.0

Group	R	Group A 55.0	Group E 165.0	Group D 180.0	Group B 295.5	Group F 297.0	Group C 385.5
A	55.0	-					
E	165.0	110.0	-				
D	180.0	125.0	15.0	-			
B	295.5	240.5*	130.5	115.5	-		
F	297.0	242.0*	132.0	117.0	1.5	-	
C	385.5	330.5**	220.5	205.5	90.0	88.5	-

R : Sum of rank from each group
* : Significantly different by comparison between the respective group ($p \leq 0.05$)
** : Significantly different by comparison between the respective group ($p \leq 0.01$)
For the explanation of the groups (A-F), please see Table 1.
Results are depicted as ranks

Table 4. Histologic index score of the rat liver

No.	Group A	Group B	Group C	Group D	Group E	Group F
1	0	3	3	3	3	1
2	0	3	3	4	-*	2
3	0	-*	2	0	1	2
4	0	4	2	0	2	1
5	0	3	4	-*	1	4
6	0	4	3	-*	-*	-*
7	0	2	4	2	2	3
8	0	2	3	1	1	1
9	0	4	2	3	2	1
10	0	2	3	4	4	2
Mean	0.0	3.0	2.9	2.1	2.0	1.9
SEM	0.0	0.4	0.2	0.6	0.4	0.4

-* : Animals died less than 48 h., prior to decapitation
For explanation of groups (A-F), please see Table 1.
Results are depicted as mean ± SEM

Table 5. Multiple comparison of histologic index score of rats liver (Kruskal-Wallis statistical method)

No.	Group A	Group B	Group C	Group D	Group E	Group F
1	6.5	39.5	29.5	39.5	39.5	16.5
2	6.5	39.5	39.5	50	-	27
3	6.5	-	27	6.5	16.5	27
4	6.5	50	27	6.5	27	16.5
5	6.5	39.5	50	-	6.5	50
6	6.5	50	39.5	-	-	-
7	6.5	27	50	27	27	39.5
8	6.5	50	27	39.5	27	16.5
9	6.5	50	27	39.5	27	16.5
10	6.5	27	39.5	50	50	27
Total R	65.0	349.5	378.5	235.5	220.0	236.5

Group	R	Group A 65.0	Group E 220.0	Group D 235.5	Group B 236.5	Group F 349.5	Group C 378.5
A	65.0	-					
E	220.0	155.0	-				
D	235.5	170.5	15.5	-			
F	236.5	171.5	16.5	1.0	-		
B	349.5	284.5*	129.5	114.0	113.0	-	
C	378.5	313.5*	158.5	143.0	142.0	29.0	

R : Sum of rank from each group
* : Significantly different by comparison between the respective group ($p \leq 0.01$)
For the explanation of the groups (A-F), please see Table 1.
Results are depicted as ranks

Table 6. Serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) activities in rats (3 groups).

SGPT (U/L)			
No.	Group K	Group L	Group M
1	10	881	653
2	7	84	632
3	14	2063	87
4	8	540	49
5	13	750	169
6	4	575	100
7	6	232	117
8	9	1613	67
9	9	270	307
10	11	240	135
Mean	9.1	724.8	231.6
SEM	0.9	193.8	68.5

SGPT (U/L)			
No.	Group K	Group L	Group M
1	7	546	456
2	11	46	396
3	8	2836	84
4	11	417	63
5	8	695	177
6	9	553	230
7	9	326	80
8	16	692	94
9	9	139	141
10	6	160	88
Mean	9.4	641.0	180.9
SEM	0.8	241.4	41.9

K, control; L, paracetamol-induced liver necrosis (2500 mg/kg BW, single dose, orally); M, partially purified curcuminoid treated rats.
Results are depicted as mean ± SEM

Table 7. Multiple comparison of SGPT activity in rats (Kruskal-Wallis statistical method)

No.	Group K	Group L	Group M
1	7	28	26
2	3	13	25
3	10	30	14
4	4	23	11
5	9	27	18
6	1	24	15
7	2	19	16
8	5.5	29	12
9	5.5	21	22
10	8	20	17
Total R	55.0	234.0	176.0

Group	R	Group-K 55.0	Group-L 176.0	Group-M 234.0
K	55.0	-		
M	176.0	121.0*	-	
L	234.0	179.0*	58.0	-

R : Sum of rank from each group
* : Significantly different by comparison between the respective group ($p \leq 0.01$)
For the explanation of the groups (K,L,M), please see Table 6.
Results are depicted as ranks

Table 8. Multiple comparison of SGOT activity in rats (Kruskal-Wallis statistical method)

No.	Group K	Group L	Group M
1	2	26	25
2	8.5	11	23
3	3.5	30	14
4	8.5	24	12
5	9	29	20
6	6	27	21
7	6	22	13
8	10	28	16
9	6	17	18
10	1	19	15
Total R	55.0	233.0	177.0

Group	R	Group-K 55.0	Group-L 177.0	Group-M 233.0
K	55.0	-		
M	177.0	122.0*	-	
L	233.0	178.0*	56.0	-

R : Sum of rank from each group
* : Significantly different by comparison between the respective group ($p \leq 0.01$)
For the explanation of the groups (K,L,M), please see Table 6.
Results are depicted as ranks

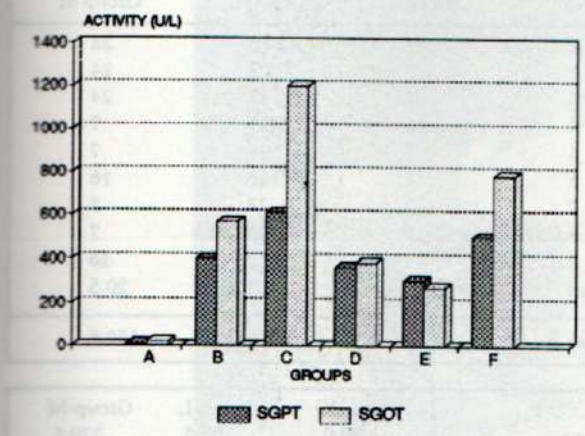


Figure 1. SGPT and SGOT in rats

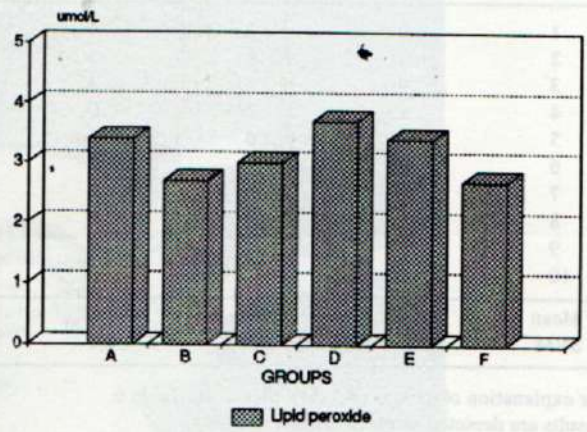


Figure 2. Plasma lipid peroxide in rats

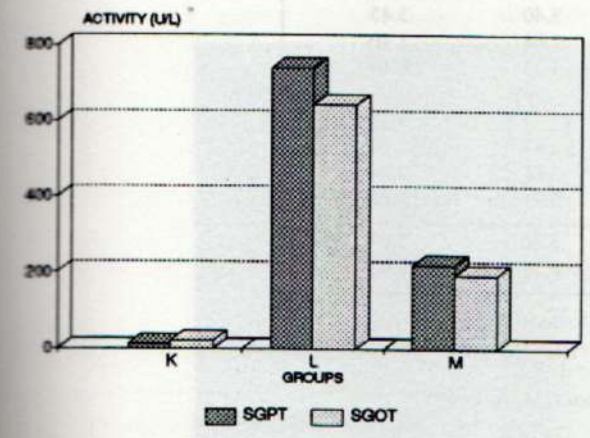


Figure 3. SGPT and SGOT in rats

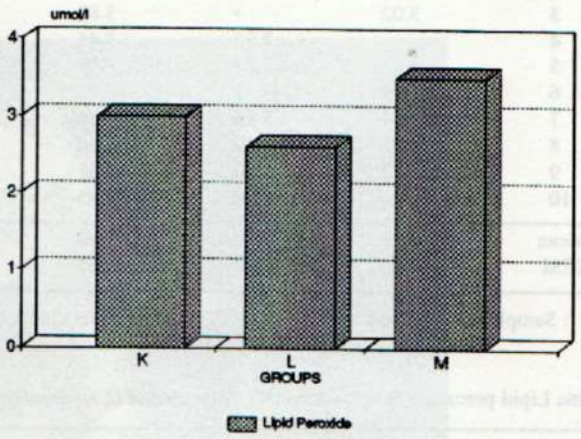


Figure 4. Plasma lipid peroxide in rats

Table 9. Histologic index score of the rat liver.*

No.	Group K	Group L	Group M
1	0	2	4
2	0	4	3
3	0	4	3
4	1	3	0
5	0	4	0
6	0	1	1
7	0	4	0
8	0	3	0
9	0	2	1
10	0	2	2
Mean	0.1	2.9	1.4
SEM	0.1	0.4	0.4

* For explanation of groups (K,L,M), please see Table 6.
Results are depicted as mean SEM.

Table 10. Multiple comparison of histologic index score of rat liver (Kruskal-Wallis statistical method)

No.	Group K	Group L	Group M
1	7	20.5	28
2	7	28	24
3	7	28	24
4	16	20.5	7
5	7	28	7
6	7	16	16
7	7	28	7
8	7	24	7
9	7	16	16
10	7	20.5	20.5
Total R	79.0	229.5	156.5

Group	R	Group-K 79.0	Group-L 156.5	Group-M 229.5
K	79.0	-		
M	156.5	77.5	-	
L	229.5	150.5*	73.0	-

R : Sum of rank from each group
* : Significantly different by comparison between the respective group ($p \leq 0.01$)
For the explanation of the groups (K,L,M), please see Table 6.
Results are depicted as ranks

Table 11. Plasma lipid peroxides from normal (A), paracetamol (B), curcuma (C,D,E) and N-acetylcysteine treated rats (F) (μ/mol/l)

No	Group A	Group B	Group C	Group D	Group E	Group F
1	2.05	2.30	2.83	3.67	1.40	2.87
2	3.50	2.97	3.02	3.32	+	3.59
3	3.02	+	3.82	4.23	3.40	3.45
4	-	2.23	3.45	4.25	2.98	1.40
5	4.28	2.67	2.99	+	4.33	0.14
6	2.99	3.11	2.92	+	+	+
7	3.04	2.16	2.76	4.37	+	3.38
8	4.01	2.16	2.60	4.82	3.87	2.69
9	3.73	2.90	2.51	1.33	3.85	2.64
10	-	3.38	2.85	+	3.71	3.84
Mean	3.33	2.65	2.98	3.71	3.36	2.67
SEM	0.50	0.15	0.05	0.78	0.71	0.50

- : Sample not obtained
+ : Animals died less than 48 h. prior to decapitation

Plasma Lipid peroxides from normal (K), paracetamol (L), partially purified curcuminoid (M) (μ mol/l)

No	Group K	Group L	Group M
1	3.01	2.43	3.31
2	2.72	2.21	5.44
3	3.75	2.13	4.14
4	3.68	2.06	3.38
5	3.10	2.13	2.57
6	2.43	2.06	3.10
7	2.72	2.32	3.10
8	3.24	2.21	3.97
9	2.87	3.97	2.57
10	2.35	4.26	2.43
Mean	2.99	2.58	3.49
SEM	0.10	0.29	0.19

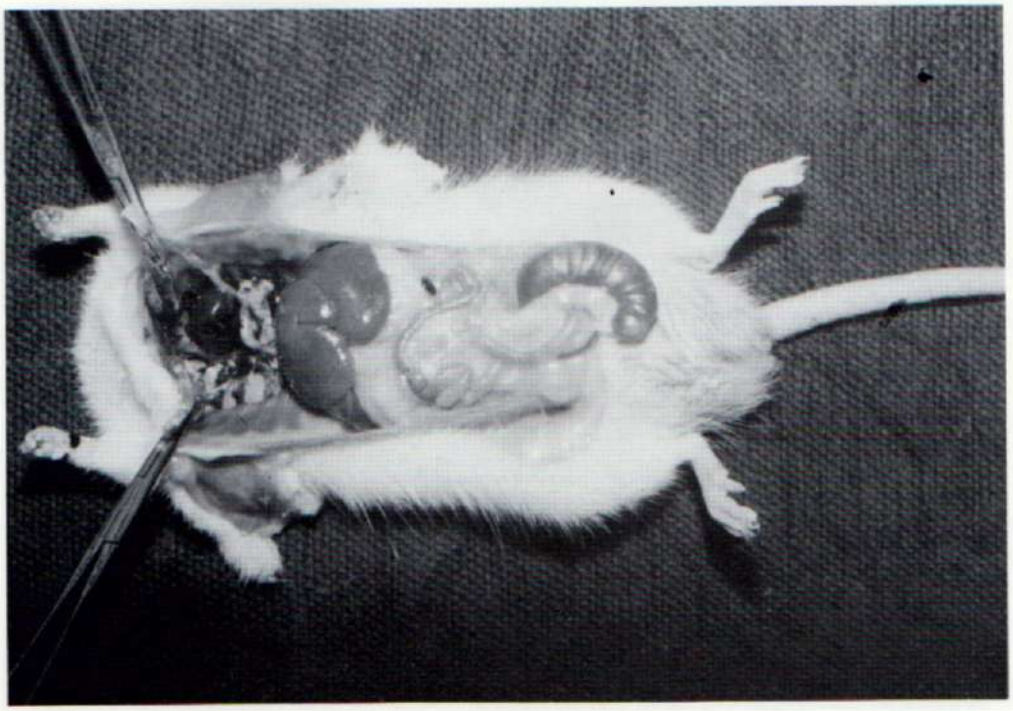


Figure 5. Macroscopical pictures of the normal rat liver

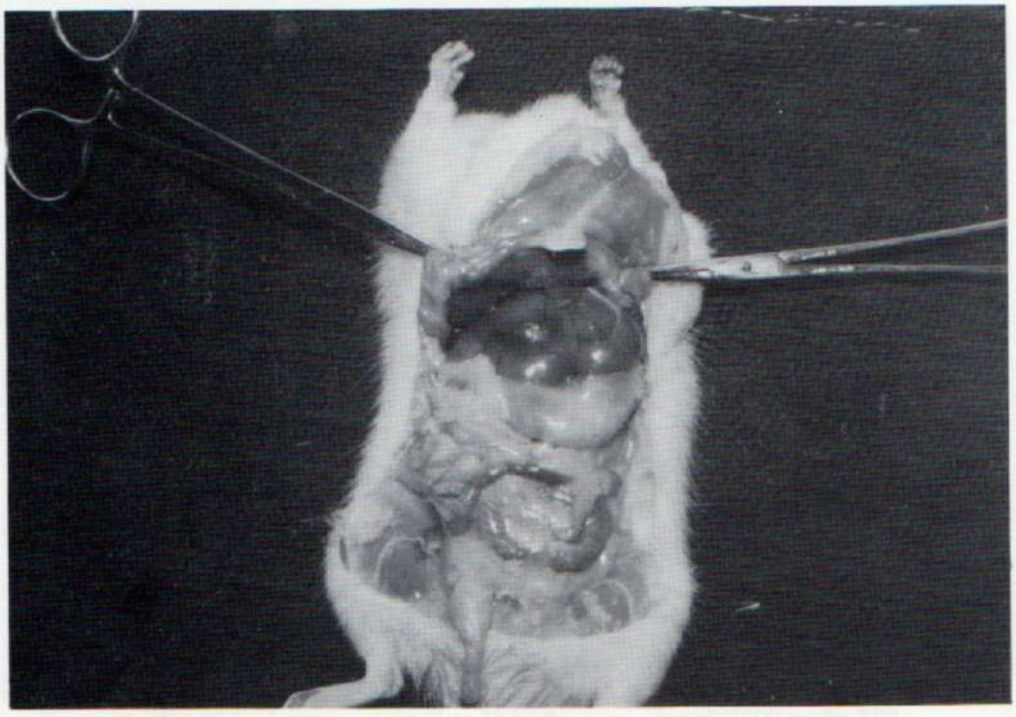


Figure 6. Macroscopical picture of the rat liver. 48 h after paracetamol (2500 mg/kg BW) showing a marked hemorrhagic lesion in the upper right lobe

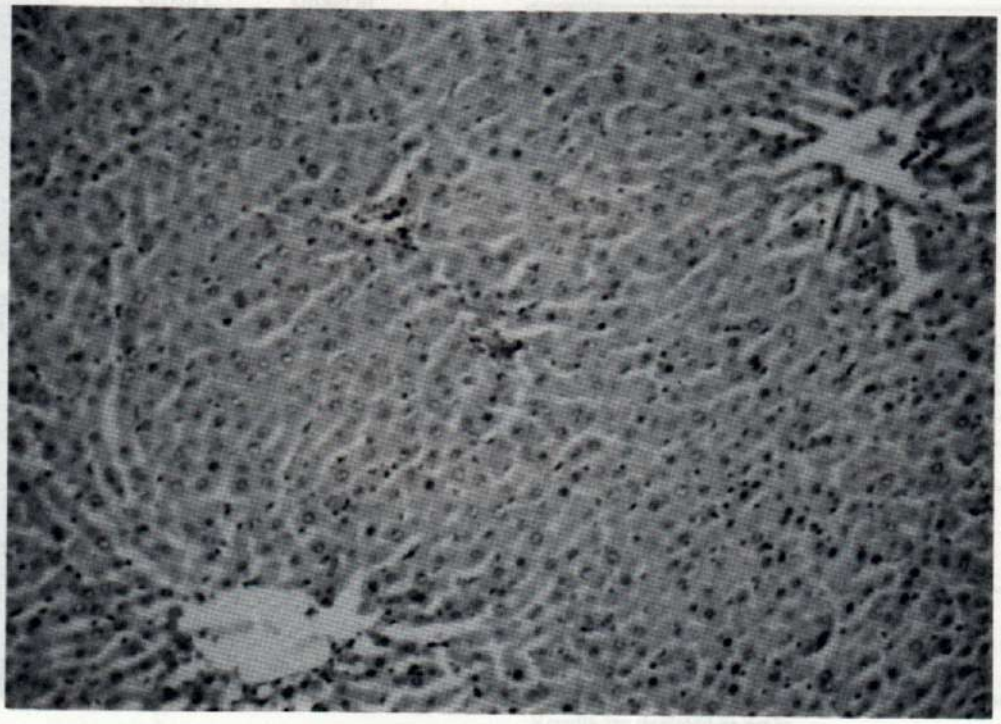


Fig.7. Histological picture of normal rat liver

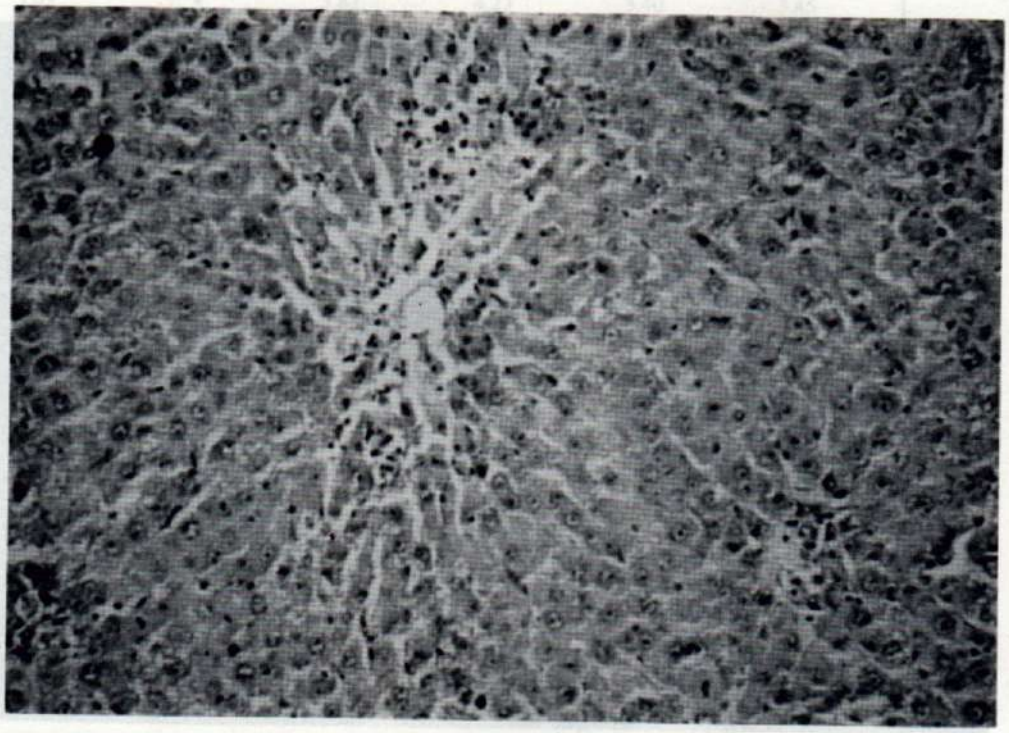


Figure 8. Histological picture of the liver 48 h after paracetamol (2500 mg/kg BW). Structural damage grade 1.

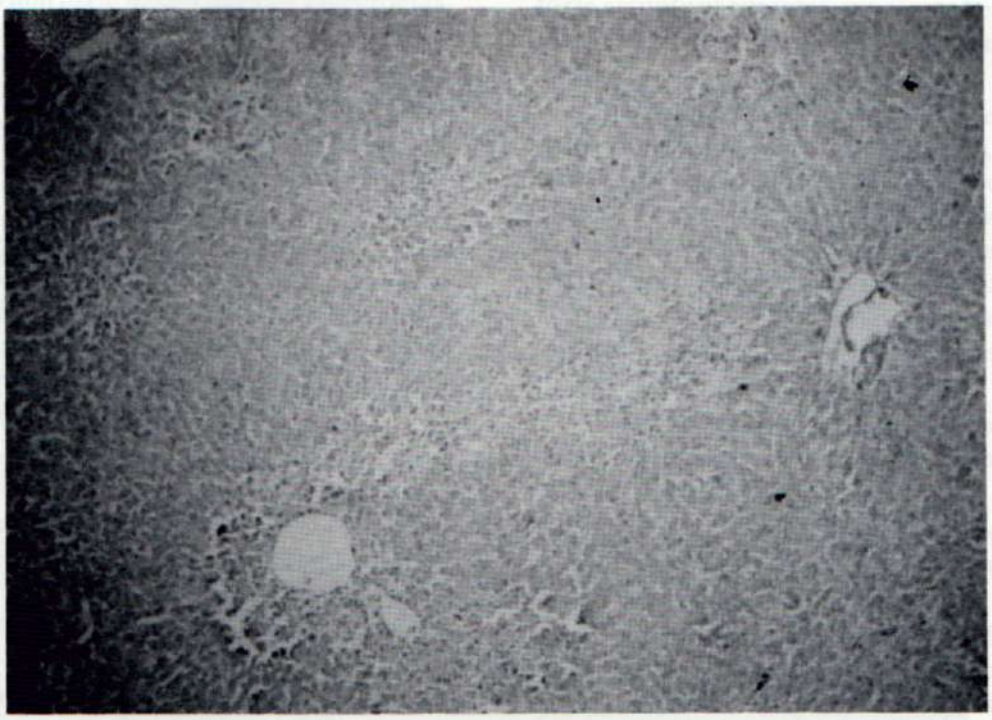


Figure 9. Histological picture of the liver 48 h after paracetamol (2500 mg/kg BW). Structural damage grade 2.

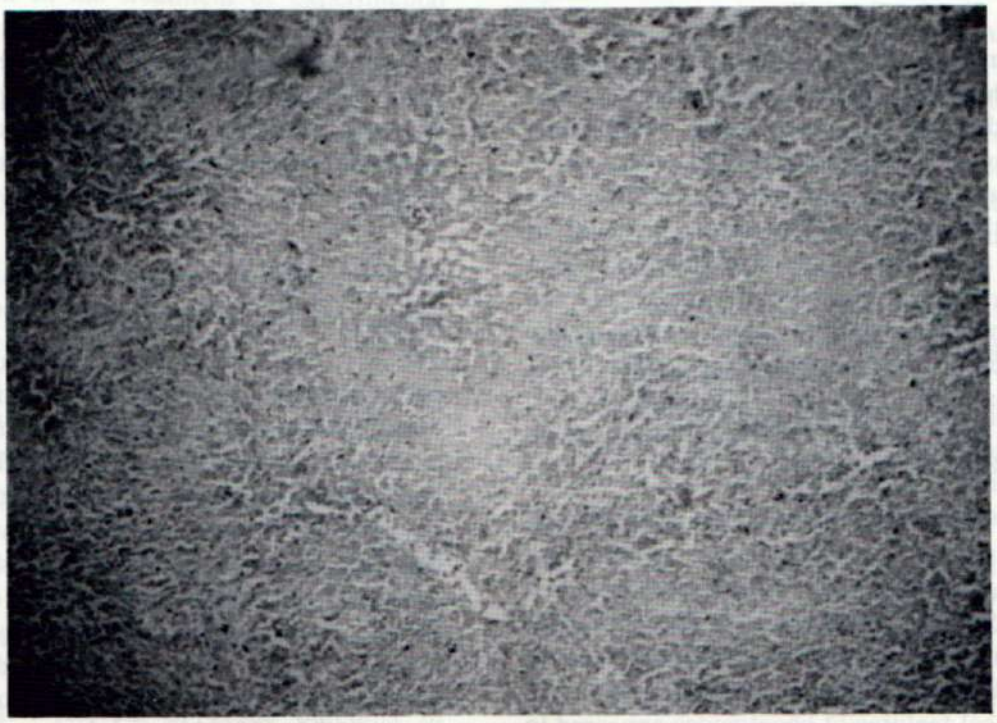


Figure 10. Histological picture of the liver 48 h after paracetamol (2500 mg/kg BW). Structural damage grade 3.

Figure 11. Histological picture of the liver 48 h after paracetamol (2500 mg/kg BW). Structural damage grade 4.

In addition to the above-mentioned parameters to assess the antihepatotoxic action of curcuma, we also collected the data on wet liver weight. Although it could only show macroscopical changes of the pathological condition, measurement of liver weight may give an additional information on the presence of edema or fluid accumulation in the affected liver. Our data, however, showed that curcuma treatments and NAC caused an increase in liver weight; control (group A), 5.04 ± 0.06 g; paracetamol only (group B), 5.87 ± 0.16 g; curcuma-treated (groups C,D,E), 5.90 ± 0.12 g, 6.78 ± 1.32 g, 6.54 ±1.21 g, and NAC-treated group (F), 6.86 ± 0.88 g, respectively.

A similar result was obtained during the second study; control (group K), 3.32 ± 0.08 g; paracetamol only (group L), 4.63 ± 0.14 g and curcuminoid-treated group (M), 5.25 ± 0.10 g, respectively. The increase in liver weight was presumably because of fluid or inflammatory cells accumulation in interstial tissue since the microscopical features of hepatocytes appeared to be normal. This is a new finding and needs further clarification.

In the present study, the number of rats that had died before decapitation was 1 (group B), 3 (group D), 3 (group E), and 1 (group F), respectively; death occurred mostly during the night, beyond our ability to monitor.

DISCUSSION

Injury to the liver can take the form of hepatocellular necrosis, cholestatic disease, or a disease of mixed patterns. Generally speaking, drug-induced liver damage has clinicopathological features similar to that caused by biological agents.[9] Based on this fact, we have selected paracetamol as the experimental model in inducing the liver damage. Paracetamol (in high doses) might also be a relevant investigated toxin as it is a widely use analgetic antipyretic drug in daily life. When taken as a suicidal drug, it may cause fulminant hepatic failure. Paracetamol has perhaps become the best understood toxic agent because much research has been conducted on its model of toxicity. The precise molecular mechanisms underlying the hepatotoxic effects of paracetamol are still open for discussion.

In its metabolism, a major fraction of paracetamol is converted into non toxic metabolites by glucuronidation or sulfation; only a small amount is metabolized by cytochrome P450 into a toxic metabolite which is thought to be the electrophilic N-acetyl-p-benzoquinoneimine (NAPQI)[1]. The reactive NAPQI will covalently bind to thiol groups of cellular proteins and will eventually lead to cell necrosis.

According to other authors[8], however, the toxicity of paracetamol is probably due to oxidative stress which is caused by the reactive NAPQI, resulting in lipid peroxidation and depletion of protein thiols. These alternative mechanisms appear to occur after depletion of glutathione (GSH).

Glutathione is a physiological substance that is used in the detoxication of oxygen radicals and in the protection against oxidation of protein thiol groups which are critical in maintaining of a number of cellular functions.[10] Depletion of cytosolic glutathione by NAPQI will result in the loss of protection against chemically-induced oxidative cellular injury. Similarly, a loss of protein thiols will also result in cytotoxicity.

This knowledge has supported a rational use of glutathione precursors in the treatment of paracetamol intoxication such as cysteine, cysteamine and NAC[11]; the latter being the most established antidote, as others are relatively toxic. Another approach which has also been investigated in the treatment of experimental paracetamol intoxication is by means of inhibiting of cytochrome P450 activity by certain drugs such as cimetidine,[7] PTU,[12] etc.

Although it may theoretically sound reasonable, this approach can not be recommended for clinical use, since the efficacy is not clear and because of harmful effects they may produce.

In this preliminary investigation, the potency of curcuma could therefore be assessed using NAC as the standard antidote.

The present study clearly shows a dose-related protective effect of curcuma against liver damage caused by paracetamol.

A dose of 50 mg/kg BW of curcuma appears to be insufficient and a marked protective effect was obtained when we increased the dose to 250 mg/kg BW and, 1000 mg/kg BW (Tables 1,2,3, Fig.1). A similar protective effect was also observed when we used 5 % curcuminoid (Tables 9, 10).

The protective effects of crude curcuma in ameliorating liver damage by PAR were shown by biochemical (SGOT, SGPT) and histopathological parameters. Our results suggest that the antihepatotoxic activity of curcuma appears to be similar or better than NAC in the protection of liver damage induced by PAR.

We could not find any difference in plasma lipid peroxide content between control, PAR and curcuma-treated paracetamol intoxication groups. Conflicting results on lipid peroxide contents have been reported elsewhere. According to some authors[8], there appears to be a dissociation between lipid peroxide content and liver damage induced by PAR. Our results suggest that free radical production might not be involved in PAR-induced liver damage.

Nevertheless, the protective effect of curcuma is confirmed when we use 5% curcuminoid in other groups of rats (Tables 9,10).

The mechanism underlying the protective effects of curcuma against liver damage induced by PAR is not clear yet.

Theoretically, curcuma may mediate its antidote effect by its ability to inhibit the cyt. P450 activity. This possible mechanism of action has been shown by several investigators[13] who found that xanthorrhizol, a fraction of curcuma, prolonged the phenobarbital sleeping time, an effect that could result from the inhibition of cyt. P450. This herb may also behave as a radical scavenger as has been shown in the CCl4-induced liver necrosis using cultures of hepatocytes.[3,14,15]

Another possibility is that curcuma may have interfered with paracetamol absorption in the intestinal tract due to its bulky volume used in this experiment and that the interval between the administration of paracetamol and curcuma is only 1 hour. We can not exclude this possibility yet, although the repeated experiment in which a partially purified curcuminoid was used gave similar result.

A confirmation study in using a highly purified curcuminoid or other fraction is suggested in order to obtain convincing results.

Nevertheless, to our contention the experiments from other authors revealed that various substances that have been isolated from curcuma are also protective to some extent against liver damage induced by CCl4.[3]

In conclusion, this preliminary study on the effect of curcuma against experimental liver damage induced by xenobiotic showed promising results.

These results may encourage us to investigate in a more comprehensive manner to elucidate the mechanism of the hepatoprotective action of curcuma and its possible of clinical applications.

CONCLUSION

Oral administration of a single, high dose of paracetamol (2500 mg/kg BW) produces a severe liver damage in rats. Serum glutamic pyruvic transaminase and serum glutamic oxaloacetic transaminase measured 48 hours after paracetamol administrations were 407.2 ± 38.7 μ/l and 536.3 ± 39.3 μ/l, respectively, indicating extensive liver cell damage as compared to control values of 5.8 ± 0.9 μ/l and 17.9 ± 2.3 μ/l.

These biochemical data were supported by the presence of a severe structural damage as judged by histopathological analysis which were arbitrarily designated to have a score index of 3.0 ± 0.3 compared to grade 0 in normal rats.

Curcuma as low as 50 mg/kg BW given in repeated doses does not show a protective effect, whereas at higher doses (250 mg/kg BW and 1000 mg/kg BW) the antihepatotoxic action of the herb becomes obvious as revealed by a significantly lower SGPT and SGOT levels and a less severe histopathologic features in curcuma-treated rats as compared with the group receiving paracetamol only. The results are confirmed in repeated experiment using a partially purified curcuminoid preparation. Curcuma appears to have a similar or better antihepatotoxic action than N-acetylcysteine as reflected by several parameters we have used. The mechanism underlying the antihepatotoxic action of curcuma is not clear yet. According to other investigators, the antihepatotoxic action of curcuma might be mediated by the inhibition of cyt. P450.

Further experiments are needed to elucidate this hypothesis.

Aknowledgements

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