

## 4. RESULTS

### 4.1 Effect of fresh *P. speciosa* seeds on paracetamol-induced hepatotoxicity

ALP, AST, and ALT in control rats were  $194.20 \pm 20.05$  IU/L,  $93.70 \pm 6.28$  IU/L, and  $35.50 \pm 1.38$  IU/L, respectively (Table 1), while toxic doses of paracetamol significantly raised the serum enzymes AST and ALT values to  $320.28 \pm 41.23$  IU/L and  $103.91 \pm 12.56$  IU/L, respectively. In rats pretreated with *P. speciosa* seeds at 1 hours before paracetamol administration, the serum levels of AST and ALT were not significantly different from the paracetamol group ( $297.42 \pm 45.69$  IU/L and  $94.33 \pm 11.71$  IU/L, respectively). In rats pretreated with *P. speciosa* seeds for 7 days before paracetamol administration, the enzyme AST ( $431.55 \pm 60.82$  IU/L) was significantly increased ( $P < 0.05$ ) from the paracetamol alone. ALP activities were not significantly different in all groups.

### 4.2 Effect of boiled *P. speciosa* seeds on paracetamol-induced hepatotoxicity

Rats treated with paracetamol alone, *P. speciosa* seeds at 1 hour before paracetamol treatment, and *P. speciosa* seeds for 7 days before paracetamol treatment developed hepatocellular damage as shown by the significant increase in the serum levels of AST, ALT, and ALP compared with the control group (Table 2). The increase in serum AST of rats pretreated with *P. speciosa* seeds for 7 days before paracetamol was significantly higher than the paracetamol treated rats ( $P < 0.05$ ).

Table 1 Effect of fresh *P. speciosa* seeds on paracetamol-induced hepatotoxicity

Group	N	Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
1	10	0.9 % saline 1.5 ml/kg i.p.	93.70 ± 6.28	35.50 ± 1.38	194.20 ± 20.05
2	10	<i>P. speciosa</i> 6 g/kg p.o.	122.60 ± 7.99	41.60 ± 2.21	158.40 ± 9.72
3	9	<i>P. speciosa</i> 6 g/kg p.o. for 7 days	106.44 ± 6.77	39.22 ± 1.38	106.44 ± 6.77
4	11	Paracetamol 500 mg/kg i.p.	320.27 ± 41.23 <sup>*</sup>	103.91 ± 12.56 <sup>*</sup>	194.55 ± 10.12
5	12	<i>P. speciosa</i> 1hour 6 g/kg p.o.+ paracetamol 500 mg/kg i.p.	297.42 ± 45.69 <sup>*</sup>	94.33 ± 11.71 <sup>*</sup>	184.17 ± 8.25
6	11	<i>P. speciosa</i> 7days 6 g/kg p.o.+ paracetamol 500 mg/kg i.p.	431.55 ± 60.82 <sup>*#</sup>	130.00 ± 15.77 <sup>*</sup>	180.64 ± 8.42

Data represent Mean ± SEM of n = 9-12 rats/group determination expressed as IU/L. \*  $p < 0.05$  compared with control group,

#  $p < 0.05$  compared with paracetamol treated rats group

Table 2 Effect of boiled *P. speciosa* seeds on paracetamol-induced hepatotoxicity

Group	Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
1	0.9 % saline 1.5 ml/kg i.p.	125.50 ± 9.18	35.80 ± 2.53	143.70 ± 7.62
2	<i>P. speciosa</i> 6 g/kg p.o.	174.30 ± 38.24	64.30 ± 26.81	162.70 ± 8.28
3	<i>P. speciosa</i> 6 g/kg p.o. for 7 days	125.20 ± 10.53	68.60 ± 25.11	171.00 ± 13.04
4	Paracetamol 500 mg/kg i.p.	389.70 ± 36.70 <sup>*</sup>	110.00 ± 11.43 <sup>*</sup>	184.10 ± 11.22 <sup>*</sup>
5	<i>P. speciosa</i> 1hour 6 g/kg p.o.+ paracetamol 500 mg/kg i.p.	380.10 ± 59.99 <sup>*</sup>	113.60 ± 12.51 <sup>*</sup>	195.50 ± 9.60 <sup>*</sup>
6	<i>P. speciosa</i> 7days 6 g/kg p.o.+ paracetamol 500 mg/kg i.p.	511.80 ± 22.41 <sup>*#</sup>	140.80 ± 6.40 <sup>*#</sup>	180.30 ± 7.55 <sup>*</sup>

Data represent Mean ± SEM of n = 10 rats/group determination expressed as IU/L. \*  $p < 0.05$  compared with control group,

#  $p < 0.05$  compared with paracetamol treated rats group

#### 4.3 Effect of fresh minced *P. speciosa* seeds on the hepatic reduced glutathione contents in rats

The hepatic reduced glutathione levels were determined at 3 and 12 hours after paracetamol administration. Liver glutathione levels at 3 hours after paracetamol injection was significantly depleted in rats receiving paracetamol alone, and those receiving fresh minced *P. speciosa* seeds for 1 hour before paracetamol administration as well as those receiving *P. speciosa* seeds for 7 days before paracetamol treatment. The hepatic glutathione levels were reduced to 57.20, 56.78 and 56.36% of control, respectively. Twelve hours after paracetamol injection, glutathione levels were returned to normal levels in rats receiving paracetamol alone, while those receiving *P. speciosa* seeds at 1 hour before paracetamol injection and *P. speciosa* seeds for 7 days before paracetamol injection, glutathione levels were 104.64 and 114.43% of control, but does not significantly increase (Table 3).

#### 4.4 Effect of boiled *P. speciosa* seeds on the hepatic reduced glutathione contents in rats

At 3 hours after paracetamol injection, glutathione levels were significantly depleted in rats receiving paracetamol alone (45.80% of control), *P. speciosa* seeds at 1 hours before receiving paracetamol (47.06% of control) and in rats receiving *P. speciosa* seeds for 7 days receiving before paracetamol (43.28% of control). After paracetamol injection at 12 hours, the glutathione levels of rats receiving paracetamol alone returned to normal range (83.5% of control), whereas during the same period of time, glutathione level was remain depleted to 65.09% of control in rats treated with *P. speciosa* seeds at 1 hours before receiving paracetamol and 66.04 % of control in rats receiving *P. speciosa* seeds for 7 days before paracetamol, and were returned to normal levels at 24 hours (Table 4).

Table 3 Effect of fresh *P. speciosa* seeds on hepatic reduced glutathione contents in liver after paracetamol pretreatment in rats.

Group	Treatment	Hepatic GSH ( $\mu\text{mole/g liver}$ )			
		3 hours		12 hours	
		$\mu\text{mole/g liver}$	% of control	$\mu\text{mole/g liver}$	% of control
1	0.9 % saline 1.5 ml/kg i.p.	2.36 $\pm$ 0.16	100	1.94 $\pm$ 0.20	100
2	<i>P. specios</i> 6 g/kg p.o.	2.27 $\pm$ 0.09	96.19	1.81 $\pm$ 0.17	93.30
3	<i>P. speciosa</i> 6 g/kg p.o. for 7 days	2.19 $\pm$ 0.07	92.80	2.15 $\pm$ 0.11	110.82
4	Paracetamol 500 mg/kg i.p.	1.35 $\pm$ 0.05*	57.20	1.76 $\pm$ 0.12	90.72
5	<i>P. speciosa</i> 1hr. 6 g/kg p.o.+ paracetamol 500 mg/kg i.p.	1.34 $\pm$ 0.04*	56.78	2.03 $\pm$ 0.20	104.64
6	<i>P. speciosa</i> 7days 6 g/kg p.o.+ paracetamol 500 mg/kg i.p.	1.33 $\pm$ 0.06*	56.36	2.22 $\pm$ 0.08	114.43

Values are Mean  $\pm$  SEM of n = 5 rats/group determination expressed as  $\mu\text{mole/g liver}$ . \*  $p < 0.05$  compared with control group

Table 4 Effect of boiled *P. speciosa* seeds on hepatic reduced glutathione contents in liver after paracetamol pretreatment in rats.

Group	Treatment	Hepatic GSH ( $\mu\text{mole/g liver}$ )					
		3 hours		12 hours		24 hours	
		$\mu\text{mole/g liver}$	% of control	$\mu\text{mole/g liver}$	% of control	$\mu\text{mole/g liver}$	% of control
1	0.9 % saline 1.5 ml/kg i.p.	2.38 $\pm$ 0.12	100	2.12 $\pm$ 0.04	100	2.40 $\pm$ 0.11	100
2	<i>P. specios</i> 6 g/kg p.o.	2.22 $\pm$ 0.12	93.27	2.05 $\pm$ 0.05	96.70	2.28 $\pm$ 0.07	95.00
3	<i>P. speciosa</i> 6 g/kg p.o. for 7 days	2.05 $\pm$ 0.06	86.61	2.31 $\pm$ 0.10	108.96	2.39 $\pm$ 0.11	99.58
4	Paracetamol 500 mg/kg i.p.	1.09 $\pm$ 0.07 <sup>*</sup>	45.80	1.77 $\pm$ 0.09	83.50	2.39 $\pm$ 0.09	99.58
5	<i>P. speciosa</i> 1hr. 6 g/kg p.o.+ paracetamol 500 mg/kg i.p.	1.12 $\pm$ 0.10 <sup>*</sup>	47.06	1.38 $\pm$ 0.05 <sup>*,#</sup>	65.09	2.56 $\pm$ 0.05	106.67
6	<i>P. speciosa</i> 7days 6 g/kg p.o.+ paracetamol 500 mg/kg i.p.	1.03 $\pm$ 0.06 <sup>*</sup>	43.28	1.40 $\pm$ 0.07 <sup>*,#</sup>	66.04	2.47 $\pm$ 0.10	102.92

Values are Mean  $\pm$  SEM of n = 5 rats/group determination expressed as  $\mu\text{mole/g liver}$ . \*  $p < 0.05$  compared with control group,

#  $p < 0.05$  compared with paracetamol treated rats group

#### 4.5 Effect of *P. speciosa* seeds on hepatic lipid peroxidation

The production of malondialdehyde was assayed to determine the effect of paracetamol-induced lipid peroxidation. There was no significant difference among all groups in malondialdehyde through the whole periods of the study, i.e. 3, 12 and 24 hours, as shown in Table 5 and 6.

#### 4.6 DPPH radical scavenging activity of fresh and boiled *P. speciosa* seeds

To evaluate the activity of the fresh and boiled *P. speciosa* seeds, the  $EC_{50}$  values or effective concentrations were determined for 50% response. The  $EC_{50}$  values of crude extract of fresh and boiled *P. speciosa* seeds were 862.5  $\mu\text{g/ml}$  and 337.5  $\mu\text{g/ml}$ , respectively, as shown in Figure 11

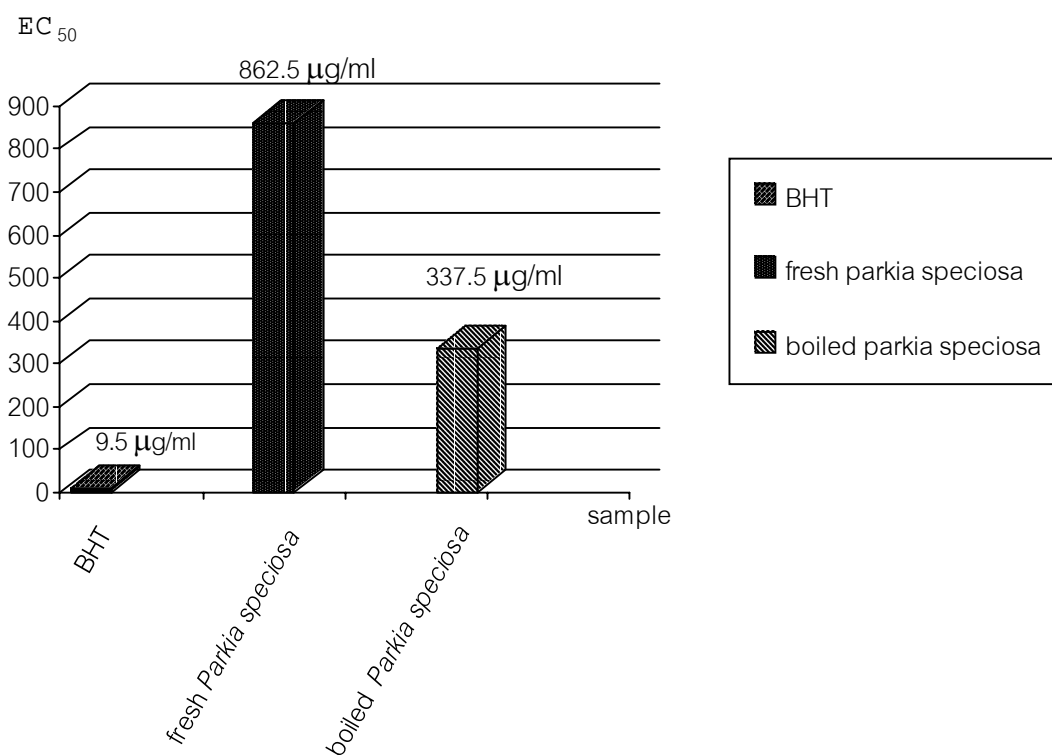


Figure 11  $EC_{50}$  values of crude extract of *P. speciosa* by DPPH assay.

Standard BHT (1), fresh *P. speciosa* (2) and boiled *Parkia speciosa* (3).

Table 5 Effect of fresh *P. speciosa* seeds on hepatic lipid peroxidation in rats at 3 hours and 12 hours after paracetamol administration.

Group	Treatment	Malondialdehyde (nmole/g liver)	
		3 hours	12 hours
1	0.9 % saline 1.5 ml/kg i.p.	21.47 ± 0.78	20.10 ± 0.89
2	<i>P. specios</i> 6 g/kg p.o.	22.79 ± 1.23	20.21 ± 0.31
3	<i>P. speciosa</i> 6 g/kg p.o. for 7 days	23.86 ± 0.59	22.08 ± 1.41
4	Paracetamol 500 mg/kg i.p.	21.12 ± 1.56	21.02 ± 1.34
5	<i>P. speciosa</i> 1hr. 6 g/kg p.o.+ paracetamol 500 mg/kg i.p.	21.27 ± 0.68	23.25 ± 1.13
6	<i>P. speciosa</i> 7days 6 g/kg p.o.+ paracetamol 500 mg/kg i.p.	21.37 ± 0.43	24.12 ± 0.94

Values are Mean ± SEM of n = 5 rats/group determination expressed as nmole/g liver. \*  $p < 0.05$  compared with control group



Table 6 Effect of boiled *P. speciosa* seeds on hepatic lipid peroxidation in rats at 3 , 12 and 24 hours after paracetamol administration.

Group	Treatment	Malondialdehyde (nmole/g liver)		
		3 hours	12 hours	24 hours
1	0.9 % saline 1.5 ml/kg i.p.	19.91 ± 0.30	22.61 ± 1.15	17.21 ± 0.63
2	<i>P. speciosa</i> 6 g/kg p.o.	19.42 ± 0.45	23.53 ± 1.36	17.43 ± 0.93
3	<i>P. speciosa</i> 6 g/kg p.o. for 7 days	18.98 ± 0.72	26.11 ± 1.13	17.55 ± 0.28
4	Paracetamol 500 mg/kg i.p.	19.42 ± 0.83	26.57 ± 0.97	16.78 ± 0.29
5	<i>P. speciosa</i> 1hr. 6 g/kg p.o.+ paracetamol 500 mg/kg i.p.	18.72 ± 0.72	24.52 ± 0.87	17.32 ± 0.48
6	<i>P. speciosa</i> 7days 6 g/kg p.o.+ paracetamol 500 mg/kg i.p.	19.91 ± 0.97	21.82 ± 1.15	17.16 ± 0.75

Values are Mean ± SEM of n = 5 rats/group determination expressed as nmole/g liver. \*  $p < 0.05$  compared with control group

#### 4.7 Histological Study

No histopathological change was found in all treated groups.

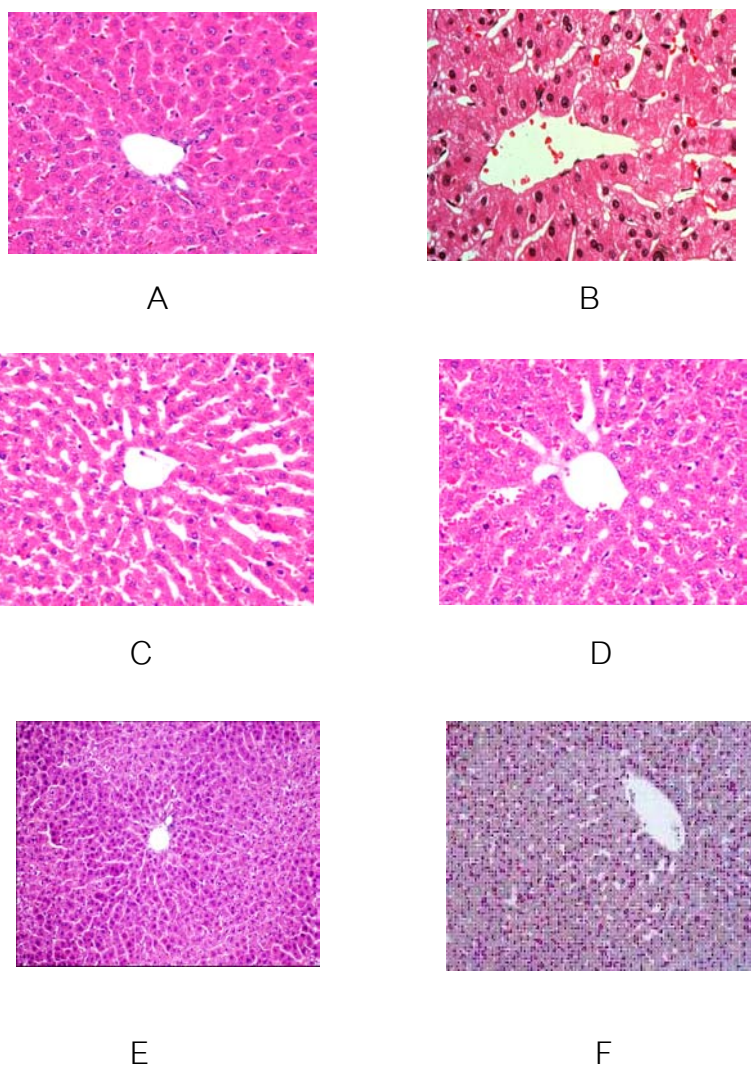


Figure 12 Representative H&E-stained liver section of rats. (A) control : the liver was histologically normal with no change in the lobular architecture, (B) 12 hours after paracetamol injection, (C) fresh *P. speciosa* alone either 1 hour or 7 day, (D) fresh *P. speciosa* alone either 1 hour or 7 day pretreated before paracetamol injection, (E) boiled *P. speciosa* alone either 1 hour or 7 day, (F) boiled *P. speciosa* alone either 1 hour or 7 day pretreated before paracetamol injection