CHAPTER III

RESULTS

3.1 Chemical constituents and antioxidant activities of *Cleistocalyx nervosum* aqueous extract

Table 3-1 shows the amounts of chemical constituents and antioxidant activities of *C. nervosum* aqueous extract.

The amount of total phenolic compounds of *C. nervosum* extract was 181.16±0.59 GAE in mg per 100 g fresh weight. The amount of total flavonoids of *C. nervosum* extract was 54.86±3.45 CE in mg per 100 g fresh weight. The amount of condensed tannins of C. *nervosum* extract was 1902.72±183.63 CE in mg per 100 g fresh weight. By using a method employing liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS), three anthocyanin components were detected at retention time 17.80, 20.83 and 42.82 min as shown in the total ion chromatogram (TIC) of the aqueous extract of *C. nervosum* (Appendix D). Identification of these anthocyanins based on their UV-Vis and ESI-MS spectra in conjunction with those information presented in the previous reports (Tian, et al., 2005) revealed their structures as cyanidin-3,5-diglucoside, cyanidin-3-glucoside, and cyanidin-5-glucoside, respectively. Among these, cyanidin-3-glucoside was accounted major anthocyanins of *C. nervosum* with its relative content of 9.72 % among the anthocyanins group and overall components in the *C. nervosum* extract.

On DPPH assay, one mg/ml of aqueous extract and 0.1 IU of trolox exhibited 56.43±1.88% inhibition and 92.86±0.47% inhibition, respectively. On deoxyribose assay, % inhibition of 50 mg/ml of aqueous extract and 1 mg/ml of uric acid on non-site specific hydroxyl radical was found to be 28.06±7.35% and 34.14±8.32%, respectively. The % inhibition of 50 mg/ml of aqueous extract and 1 mg/ml of uric acid on site specific hydroxyl radical was found to be 58.84±2.81% and 72.25±2.34%, respectively.

Table 3-1 Chemical constituents and antioxidant activities of *Cleistocalyx nervosum* aqueous extract.

Extract/ Standard	Total phenolics (mg GAE/100 g fresh weight)	Total flavonoids (mg CE/100 g fresh weight)	Condensed tannins (mg CE/100 g fresh weight)	% inhibition		Iron-chelating activity
				DPPH radical	Hydroxyl radical	
Trolox	ND	ND	ND	92.86±0.47	ND	ND
uric acid	ND	ND	ND	ND	34.14±8.32	72.25±2.34
C. nervosum	181.16±0.59	54.86±3.45	1902.72±183.63	56.43±1.88	28.06±7.35	58.84±2.81

Values are means ± SD

ND: Not determined

3.2 Acute toxicity test

After 5000 mg/kg bw of the aqueous extract administration, no dead rat was recorded in the 14 days of observation period in both male and female rats. The animals did not show any changes in the general appearance during the observation period. Body and relative organ weights of treated groups were not significantly different from those of the control group of both genders (Table 3-2). Furthermore, gross examination of internal organs of all rats did not reveal any abnormalities (Table 3-2).

3.3 Subacute toxicity test

After aqueous extract administration, there is no dead rat or change in general behavior or other physiological activities at any point in this study. Body weight and consumptions of diet and water did not show any significant differences in either the control or treated groups of both genders (Table 3-3). The relative internal organ weights (Table 3-3) did not exhibit any gross pathological alteration. However, relative heart weight of male animals given 100 mg/kg of aqueous extract was significantly increased when compared to those of the control group. On the other hand, relative stomach weight of male animals given 100 mg/kg of aqueous extract was significantly decreased when compared to those of a control group. Table 3-4 presents hematological values of male rats in subacute toxicity of aqueous extract from *C. nervosum*. The analyses of female rats were not performed due to blood clotting during collection. There were no significant changes of hemoglobin, hematocrit, platelets, neutrophil, lymphocyte, eosinophil and basophil in male treated groups. The biochemical analysis in Table 3-5 showed no significant difference in any of examined parameters in either the control or treated groups. However, creatinine value of female animals fed with 500 mg/kg bw of aqueous extract was significantly increased when compared to that of the control group. On the other hand, triglyceride level of female animals fed with 100 mg/kg bw of aqueous extract was significantly decreased when compared to that of the control group.

Table 3-2 Body and relative organ weights of rats in acute toxicity of the aqueous extract from *Cleistocalyx nervosum*.

Parameters	Female		Male	
	Control	*C. nervosum* (5000 mg/kg bw)	Control	*C. nervosum* (5000 mg/kg bw)
Initial body weight (g)	214±11	219±8	246±23	246±6
Final body weight (g)	240±16	241±7	324±29	330±14
Heart (per 100 g body weight)	0.33±0.01	0.32±0.02	0.29±0.01	0.30±0.02
Lung (per 100 g body weight)	0.43±0.03	0.41±0.04	0.35±0.03	0.38±0.02
Thymus (per 100 g body weight)	0.19±0.02	0.19±0.04	0.21±0.06	0.22±0.04
Liver (per 100 g body weight)	4.00±0.24	3.53±0.13	4.09±0.32	4.10±0.23
Pancreas (per 100 g body weight)	0.46±0.16	0.38±0.12	0.37±0.06	0.35±0.08
Adrenal (per 100 g body weight)	0.03±0.01	0.03±0.01	0.02±0.00	0.02±0.00
Spleen (per 100 g body weight)	0.24±0.02	0.21±0.02	0.20±0.02	0.23±0.02
Kidneys (per 100 g body weight)	0.68±0.04	0.67±0.06	0.65±0.05	0.65±0.05
Stomach (per 100 g body weight)	0.48±0.05	0.49±0.04	0.45±0.03	0.44±0.03
Testis (per 100 g body weight)	-	-	1.14±0.07	1.10±0.10
Ovaries (per 100 g body weight)	0.07±0.01	0.07±0.01	-	-
Utarus (per 100 g body weight)	0.26±0.12	0.21±0.04	-	-

Data are expressed as mean±SD

Table 3-3 Body weight, diet and water intakes and relative organ weights of rats in subacute toxicity of the aqueous extract from *Cleistocalyx nervosum*.

Parameters	Female			Male		
	Control	*C. nervosum* 100 mg/kg bw	*C. nervosum* 500 mg/kg bw	Control	*C. nervosum* 100 mg/kg bw	*C. nervosum* 500 mg/kg bw
Initial body weight (g)	171±9	172±15	172±10	200±15	199±12	199±13
Final body weight (g)	209±11	218±16	213±19	328±23	333±21	321±32
Diet weight (g)	14±2	13±2	15±4	21±4	21±4	23±3
Water weight (ml)	24±7	18±5	20±3	35±7	33±7	29±7
Heart (per 100 g body weight)	0.31±0.02	0.32±0.01	0.33±0.02	0.29±0.02	0.32±0.01*	0.31±0.01
Lung (per 100 g body weight)	0.43±0.02	0.48±0.04	0.43±0.10	0.38±0.06	0.40±0.05	0.39±0.06
Thymus (per 100 g body weight)	0.21±0.02	0.21±0.05	0.19±0.04	0.20±0.04	0.17±0.01	0.19±0.04
Liver (per 100 g body weight)	2.93±0.10	2.94±0.18	2.88±0.10	3.00±0.10	2.96±0.13	3.11±0.20
Adrenal (per 100 g body weight)	0.05±0.01	0.04±0.01	0.05±0.00	0.02±0.01	0.02±0.01	0.02±0.01
Spleen (per 100 g body weight)	0.22±0.04	0.21±0.03	0.21±0.03	0.20±0.04	0.19±0.03	0.18±0.01
Kidneyss (per 100 g body weight)	0.67±0.08	0.64±0.04	0.68±0.06	0.57±0.05	0.56±0.04	0.60±0.03
Stomach (per 100 g body weight)	0.54±0.05	0.54±0.05	0.52±0.04	0.47±0.02	0.42±0.02*	0.48±0.05
Pancreas (per 100 g body weight)	0.36±0.10	0.35±0.13	0.37±0.08	0.30±0.16	0.34±0.08	0.35±0.12
Testis (per 100 g body weight)	-	-	-	1.13±0.08	1.14±0.15	1.15±0.08
Ovaries (per 100 g body weight)	0.07±0.01	0.07±0.00	0.08±0.01	-	-	-
Utarus (per 100 g body weight)	0.26±0.06	0.22±0.05	0.26±0.10	-	-	-

Data are expressed as mean±SD

* Significant difference from control, $p < 0.05$.

Table 3-4 Hematological values of male rats in subacute toxicity of the aqueous extract from *Cleistocalyx nervosum*.

Hematological parameters	Normal value	Control	*C. nervosum* extract	
			100 mg/kg bw	500 mg/kg bw
White blood cell (10^3 /µl)	5.7±2.62	3.80	5.63±1.45	4.83±0.39
Hemoglobin (g/l)	16.1±1.44	15.60	15.43±2.05	15.18±0.30
Hematocrit (%)	49.9±4.40	41.60	41.40±5.65	46.63±1.04
Platelets (10^3 /µl)	824.5±198.48	719.00	493.25±290.78	852.00±15.71
Neutrophil (%)	6.1±8.86	57.00	60.43±13.77	39.98±26.00
Lymphocyte (%)	92.8±9.58	40.80	38.08±13.39	55.60±22.24
Monocyte (%)	0.1±0.12	1.70	0.55±0.45	0.48±0.33
Eosinophil (%)	0.9±1.54	0.90	0.83±1.08	1.53±1.57
Basophil (%)	0.0±0.00	0.00	0.13±0.15	0.00±0.00

Data are expressed as mean±SD

Table 3-5 Blood chemistry values of rats in subacute toxicity of the aqueous extract from *Cleistocalyx nervosum*.

Blood chemical parameters	Female			Male		
	Control	*C. nervosum* 100 mg/kg, bw	*C. nervosum* 500 mg/kg, bw	Control	*C. nervosum* 100 mg/kg, bw	*C. nervosum* 500 mg/kg, bw
Glucose (mg/dl)	148.40±31.23	150.20±49.69	151.67±28.75	145.25±15.59	143.20±14.46	128.20±11.37
BUN [a] (mg/dl)	21.40±4.98	22.80±2.28	21.33±4.51	18.50±2.65	22.60±5.27	17.20±2.39
Creatinine (mg/dl)	0.40±0.00	0.42±0.04	0.47±0.06*	0.40±0.00	0.52±0.16	0.40±0.00
Uric acid (mg/dl)	2.94±0.61	2.88±0.22	2.83±1.19	2.00±0.83	1.76±0.62	1.48±0.29
Cholesterol (mg/dl)	61.20±6.61	62.20±13.5	56.33±3.21	70.25±8.02	67.20±14.15	57.00±8.94
Triglyceride (mg/dl)	52.80±18.42	33.40±3.44*	41.00±1.00	84.50±15.78	100.60±50.44	85.60±12.22
Total protein (g/dl)	6.44±0.18	6.38±0.29	6.47±0.25	6.28±0.39	6.06±0.25	6.06±0.23
Albumin (g/dl)	3.38±0.04	3.34±0.21	3.40±0.10	3.20±0.20	3.16±0.13	3.14±0.09
Direct Bilirubin (mg/dl)	0.04±0.01	0.04±0.01	0.03±0.01	0.03±0.01	0.03±0.00	0.03±0.00
SGOT [b] (U/l)	143.40±48.17	150.80±67.52	120.33±25.11	115.75±32.75	111.40±11.67	97.20±10.64
SGPT [c] (U/l)	27.60±5.32	34.40±21.27	23.33±3.06	30.00±11.43	30.60±4.16	27.80±3.70
ALP [d] (U/l)	55.00±15.38	62.80±18.17	63.00±19.16	109.25±14.80	118.00±19.36	102.20±12.64
Total calcium (mg/l)	10.54±0.34	10.70±0.29	10.30±0.62	10.05±0.89	9.72±0.40	9.80±0.45
Sodium (mmol/l)	145.20±1.30	144.40±1.82	144.67±1.15	148.50±2.38	147.00±1.58	147.00±1.00
Potassium (mmol/l)	5.66±0.51	5.76±1.00	5.10±0.82	4.80±0.72	4.90±1.07	4.16±0.72
Chloride (mmol/l)	110.60±0.89	109.60±1.14	111.33±0.58	107.00±0.82	107.20±0.84	106.20±0.84

Data are expressed as mean±SD

[a] Blood urea nitrogen [b] Aspartate transaminase [c] Alanine transaminase [d] Alkaline phosphatase

* Significant difference from control, $p < 0.05$

3.4 The effect of *C. nervosum* extract on oxidative stress and antioxidant system

The effect of *C. nervosum* extract on oxidative stress and antioxidant system was summarized in Table 3-6. The MDA formation resulting in lipid peroxidation was measured in terms of the TBARs formation. The level of hepatic MDA was significantly increased in the rats fed with 100 mg/kg bw of *C. nervosum* extract when compared to that of a control group. However, high dose of *C. nervosum* extract (500 mg/kg bw) significantly increased heme oxygenase activity. The aqueous extract did not modulate total glutathione level, glutathione peroxidase, glutathione reductase and catalase activities.

Table 3-6 Effect of aqueous extract from *Cleistocalyx nervosum* on antioxidant status in male rat liver.

Parameters	Control	Aqueous extract of *C. nervosum*	
		100 mg/kg bw	500 mg/kg bw
Hepatic MDA (pmol/mg protein)	12.36±4.03	20.60±5.37*	17.49±2.70
Total hepatic glutathione (nmol/mg protein)	17.61±3.72	15.94±5.92	18.28±2.42
Glutathione reductase activity (pmol/min/mg protein)	27.23±1.69	26.92±2.69	27.65±3.42
Glutathione peroxidase activity (pmol/min/mg protein)	25.22±2.62	27.11±4.94	28.38±8.50
Catalase activity (pmol/min/mg protein)	77.05±6.89	82.98±12.72	68.63±12.66
Heme oxygenase activity (nmol/min/mg protein)	19.1±1.87	22.8±3.80	28.9±5.31*

Data are expressed as mean±SD

* Significant difference from control, $p < 0.05$.

3.5 The preliminary study for protocol of carcinogens induced oxidative stress in early stages of rat hepatocarcinogenesis.

Table 3-7 showed effect of DEN administration on various biological parameters in chemicals induced hepatocarcinogenesis in rat. Final body weight and diet intake of rats given PB 500 ppm after 2 or 3 times DEN injection (Groups 2 and 3) did not significantly decrease as compared to those of the control group (Group 1), however, fluid intake was significantly increased in groups 2 and 3 as compared to those of group 1. In groups 2 and 3, there were significant increased in relative liver weight when compared to that of group 1, however, relative spleen and kidneys weights of groups 2 and 3 showed no significantly differences when compared to those of group 1.

The level of serum and hepatic MDA in DEN injected rats significantly increased when compared to those of a control group.

Both double and triple DEN injections induced number of GST-P positive foci in rat liver. GST-P positive foci were not found in the liver of the control group.

Table 3-7 Effect of DEN administration on various biological parameters in chemicals induced hepatocarcinogenesis in rat.

Parameters	Control	Double injection of DEN	Triple injection of DEN
Number of rat	2	3	3
Initial body weight (g)	195±7	195±10	195±18
Final body weight (g)	383±39	353±25	337±38
Food intake (g/rat/day)	26±1	21±0	22±2
Fluid intake (ml/rat/day)	44±8	36±6*	29±7*
Liver weight (per 100 g body weight)	3.38±0.38	4.26±0.14*	4.56±0.17*
Spleen weight (per 100 g body weight)	0.58±0.00	0.51±0.05	0.56±0.06
Kidneys weight (per 100 g body weight)	0.24±0.03	0.19±0.01	0.23±0.02
Hepatic MDA (pmol/mg protein)	4.26±0.87	13.5±6.19*	14.7±1.80*
Serum MDA (nmol/mg protein)	4.94±0.00	5.72±0.15*	5.68±0.16*
GST-P positive foci (foci/cm^2)	0.00±0.00	8.47±5.76	36.0±6.59*

Data are expressed as mean±SD * Significant difference from control, $p < 0.05$.
DEN: Diethylnitrosamine 100 mg/kg bw
PB: Phenobarbital 500 ppm in drinking water

3.6 Effect of *Cleistocalyx nervosum* extract on oxidative stress induced early stage of hepatocarcinogenesis.

3.6.1 Protocol I

The treated rats in this protocol were injected with DEN for 3 times and followed by the administration of PB in drinking water. There was no significant difference in the final body weight, diet and fluid intakes among the rat in this study (Table 3-8). Relative liver weight of DEN and PB treated rats significantly increased when compared to that of a control group. The treatment of aqueous extract of *C. nervosum* and silymarin tended to reduce liver weight of DEN and PB treated rats. The relative weights of spleen and kidneys and serum AST and ALT levels were no significant difference when compared to those of a control group.

The numbers of GST-P positive foci in the rats given DEN and PB (Group 2) was significantly increased as compared with the values obtained from rats treated with 0.9% NSS (Group 1). The treatment of aqueous extract of *C. nervosum* (Groups 3 and 4) tended to decrease in the number of GST-P positive foci when compared to those of group 2. However, the treatment of silymarin (Group 5) showed significantly increase in the number of GST-P positive foci when compared to that of group 2.

The levels of MDA in serum and liver of rats treated with DEN and PB (Group 2) were increased when compared to those of a control groups. The treatment of aqueous extract of *C. nervosum* (Group 3) significantly reduced level of MDA in serum. However, the treatment of aqueous extract of *C. nervosum* (Group 4) and silymarin (Group 5) showed no significant difference in level of MDA in serum when compared to those of group 2. Treatment of aqueous extract of *C. nervosum* (Groups 3) tended to increase level of MDA in liver when compared to those of group 2. However, treatment of aqueous extract of *C. nervosum* (Group 4) and silymarin (Group 5) tended to decrease level of MDA in liver when compared to those of group 2. The level of total hepatic GSH of rats treated with DEN and PB (Group 2) tended

Table 3-8 Effect of *Cleistocalyx nervosum* extract on various biological parameters in protocol I

Treatment	Test compound;ig	n	body weight(g)		Intake		Relative organ weight (per 100 g body weight)			AST activity (U/l)	ALT activity (U/l)
			Initial	Final	Diet (g/rat/d)	Fluid (ml/rat/d)	Liver	Spleen	Kidneys		
1 NSS+Tap water	Vehicle (wk1-8)	5	96±7	304±52	17±3	37±8	3.28±0.52	0.23±0.05	0.63±0.12	174±66	37±7
2 DEN+PB	Vehicle (wk1-8)	9	96±6	341±21	18±5	31±6	5.09±0.32*	0.25±0.04	0.60±0.03	226±84	61±20
3 DEN+PB	Aqueous extract 500 mg/kg bw (wk1-8)	9	98±6	340±23	19±5	33±6	4.88±0.45	0.22±0.05	0.58±0.06	177±62	83±19
4 DEN+PB	Aqueous extract 500 mg/kg bw (wk5-8)	8	97±5	332±22	19±6	35±7	4.61±0.33	0.20±0.02	0.60±0.08	170±22	52±14
5 DEN+PB	Silymarin 100 mg/kg bw (wk5-8)	6	99±2	337±17	19±5	31±9	4.65±0.20	0.22±0.03	0.58±0.03	187±40	61±16

Data are expressed as mean±SD

* Significant difference from control, $p < 0.05$.

DEN: Diethylnitrosamine 100 mg/kg bw, 3 time injection

PB: Phenobarbital 500 ppm in drinking water

to decrease when compared to that of a control groups. The treatment of aqueous extract of *C. nervosum* (Group 3) showed no significant difference in total hepatic GSH when compared to that of group 2. However, the treatment of aqueous extract of *C. nervosum* (Group 4) and silymarin (Group 5) were decreased in total hepatic GSH when compared to those of group 2. The GR and CAT activities were significantly increased in group 2 as compared to those of group 1. However, the GPx and HO activities tended to decrease when compared to those of group 2. The treatment of aqueous extract of *C. nervosum* (Group 4) significantly decreased in GR activity when compared to that of group 2. However, the treatment of aqueous extract of *C. nervosum* (Group 3) and silymarin (Group 5) showed no significant difference when compared to those of group 2. Treatment of aqueous extract of *C. nervosum* (Groups 3 and 4) and silymarin (Group 5) did not affect on the activities of GPx, CAT and HO.

Table 3-9 Effect of *Cleistocalyx nervosum* extract on Glutathione-*S*-transferase P positive foci in protocol I

Treatment	Test compound; ig	n	Number of GST-P positive foci (foci/cm^2)	Size of GST-P positive foci			
				21-30 cell/focus	31-50 cell/focus	51-100 cell/focus	>100 cell/focus
1 NSS+Tap water	Vehicle (wk1-8)	5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2 DEN+PB	Vehicle (wk1-8)	9	23.2±7.54*	7.32±1.17	8.50±3.25	4.84±2.40	2.51±1.53
3 DEN+PB	Aqueous extract 500 mg/kg bw (wk1-8)	9	16.8±8.69	4.46±1.68	7.23±4.55	4.27±3.25	0.82±1.27
4 DEN+PB	Aqueous extract 500 mg/kg bw (wk5-8)	8	22.5±4.68	7.52±2.25	7.54±1.90	6.28±2.71	1.17±1.23
5 DEN+PB	Silymarin 100 mg/kg bw (wk5-8)	6	38.6±3.20**	11.72±0.35	12.80±3.11	8.85±2.43	5.23±2.51

Data are expressed as mean±SD

* Significant difference from control, $p < 0.05$.

** Significantly different from positive control, $p < 0.05$.

DEN: Diethylnitrosamine 100 mg/kg bw, 3 time injection

PB: Phenobarbital 500 ppm in drinking water

Table 3-10 Effect of aqueous extract from *Cleistocalyx nervosum* on oxidative stress and antioxidant status in protocol I

Treatment	Test compound; ig	n	Serum MDA (nmol/mg protein)	Hepatic MDA (nmol/mg protein)	Total hepatic GSH (nmol/mg protein)	GR activity (pmole/min/ mg protein)	GPx activity (pmole/min /mg protein)	CAT activity (pmole/min /mg protein)	HO activity (nmole/min /mg protein)
1 NSS+Tap water	Vehicle (wk1-8)	5	8.29±0.63	67.1±30.2	19.1±2.38	11.7±1.34	24.6±4.13	57.7±18.9	14.9±1.52
2 DEN+PB	Vehicle (wk1-8)	9	14.1±0.75*	531.4±431.2*	17.0±5.04	23.0±4.77*	19.0±5.08*	74.3±14.1*	13.5±1.57
3 DEN+PB	Aqueous extract 500 mg/kg bw (wk1-8)	9	11.2±1.17**	869.1±547.9	17.0±6.53	23.9±4.29	18.7±2.15	82.2±15.3	14.8±1.83
4 DEN+PB	Aqueous extract 500 mg/kg bw (wk5-8)	8	13.1±0.35	436.4±291.9	11.2±5.13**	18.4±1.84**	18.4±3.02	67.9±9.77	14.4±2.25
5 DEN+PB	Silymarin 100 mg/kg bw (wk5-8)	6	14.5±0.80	492.4±406.4	11.6±2.60	19.6±1.84	20.2±5.87	72.0±6.68	12.9±1.33

Data are expressed as mean±SD

* Significant difference from control, $p < 0.05$.

** Significantly different from positive control, $p < 0.05$.

DEN: Diethylnitrosamine 100 mg/kg bw, 3 time injection

PB: Phenobarbital 500 ppm in drinking water

3.6.2 Protocol II

The treated rats in this protocol were injected by DEN for 2 times and followed by the administration of PB in drinking water. There was no significant difference in the final body weight, diet intake among the groups of rats in this study (Table 3-11). However, fluid intake of DEN and PB treated rats was significantly decreased when compared to that of a control group. Relative liver weight of DEN and PB treated rats significantly increased when compared to that of control group. The treatment of aqueous extract of 1000 mg/kg bw of *C. nervosum* tended to reduce liver weight of DEN and PB treated rats. However, the treatment of 500 mg/kg bw of *C. nervosum* tended to increase liver weight of DEN and PB treated rats. The relative weights of spleen and kidneys and serum AST and ALT levels were no significant difference when compared to those of control group.

The numbers of GST-P positive foci in the rats given DEN and PB (Group 2) was significantly increased as compared with those values of rats treated with 0.9% NSS (Group 1). The treatment of aqueous extract of 1000 mg/kg bw of *C. nervosum* tended to reduce liver weight of DEN and PB treated rats (Table 3-12).

The levels of MDA in serum and liver of rats treated with DEN and PB (Group 2) were increased when compared to those of control group. The treatment of aqueous extract of *C. nervosum* reduced level of MDA in serum when compared to that of group 2. The levels of total GSH in serum and liver of rats treated with DEN and PB (Group 2) were decreased when compared to those of control group. The treatment of 1000 mg/kg bw of aqueous extract of *C. nervosum* tended to increase total GSH in serum and liver when compared to those of group 2. The GR activity of rats treated with DEN and PB was significantly increased when compared to that of a control group. The rats given aqueous extract (Groups 3 and 4) showed increased GR activity when compared to that of group 2. The GPx and CAT activities of rats treated with DEN and PB were significantly decreased when compared to those of a control group. The rats given 500 and 1000 mg/kg bw of aqueous extract (Groups 3 and 4) showed significantly increased CAT activity when compared to that of group 2. The rats given 1000 mg/kg bw of aqueous extract (Group 4) showed significantly

Table 3-11 Effect of *Cleistocalyx nervosum* extract on various biological parameters in protocol II

Treatment	Test compound; ig	n	body weight (g)		Intake		Relative organ weight (per 100 g body weight)			AST activity (U/l)	ALT activity (U/l)
			Initial	Final	Diet (g/rat/d)	Fluid (ml/rat/d)	Liver	Spleen	Kidneys		
1 NSS+Tap water	Vehicle (wk1-8)	4	101±8	356±9	19±2	27±5	3.20±0.37	0.20±0.04	0.60±0.01	89±14	34±5
2 DEN+PB	Vehicle (wk1-8)	7	99±2	383±35	21±3	37±10*	4.00±0.22*	0.21±0.02	0.59±0.02	88±6	48±14
3 DEN+PB	Aqueous extract 500 mg/kg bw (wk1-8)	7	100±3	375±20	21±3	34±10	4.15±0.21	0.20±0.02	0.61±0.04	85±5	47±9
4 DEN+PB	Aqueous extract 1000 mg/kg bw (wk1-8)	5	102±4	370±15	22±5	33±7	3.91±0.18	0.20±0.02	0.60±0.05	101±26	71±44

Data are expressed as mean±SD

* Significant difference from control, $p < 0.05$.

DEN: Diethylnitrosamine 100 mg/kg bw, 2 time injection PB: Phenobarbital 500 ppm in drinking water

Table 3-12 Effect of *Cleistocalyx nervosum* extract on Glutathione-*S*-transferase P positive foci in protocol II

Treatment	Test compound; ig	n	Number of GST-P positive foci (foci/cm^2)	Size of GST-P positive foci		
				21-30 cell/focus	31-50 cell/focus	51-100 cell/focus
1 NSS+Tap water	Vehicle (wk1-8)	4	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2 DEN+PB	Vehicle (wk1-8)	7	5.21±2.83*	3.00±1.69	1.55±1.19	0.67±0.39
3 DEN+PB	Aqueous extract 500 mg/kg bw (wk1-8)	7	5.42±3.39	3.29±2.11	1.53±1.07	0.60±0.66
4 DEN+PB	Aqueous extract 1000 mg/kg bw (wk1-8)	5	2.20±2.01	1.33±0.91	0.59±0.90	0.28±0.41

Data are expressed as mean±SD

* Significant difference from control, $p < 0.05$.

** Significant difference from positive control, $p < 0.05$.

DEN: Diethylnitrosamine 100 mg/kg bw, 2 time injection

PB: Phenobarbital 500 ppm in drinking water

increased GPx activity when compared to that of group 2. However, the rats given 500 mg/kg bw of aqueous extract (Group 3) tended to increase GPx activity when compared to that of group 2. The HO activity of rats treated with DEN and PB showed no significant difference when compared to that of control group. The rats given aqueous extract (Groups 3 and 4) showed no significant difference in HO activity when compared to that of group 2 (Table 3-13).

Table 3-13 Effect of aqueous extract from *Cleistocalyx nervosum* on oxidative stress and antioxidant status in protocol II

Treatment	Test compound; ig	n	Serum MDA (nmol/mg protein)	Hepatic MDA (nmol/mg protein)	Total serum GSH (nmol/mg protein)	Total hepatic GSH (nmol/mg protein)	GR activity (pmole/min/ mg protein)	GPx activity (pmole/min/ mg protein)	CAT activity (pmole/min/ mg protein)	HO activity (nmole/min/ mg protein)
1 NSS+Tap water	Vehicle (wk1-8)	4	12.7±1.0	66±36	6.09±2.65	6.91±3.82	15.7±3.48	29.8±9.27	99.2±27.7	13.0±1.85
2 DEN+PB	Vehicle (wk1-8)	7	13.4±1.1	200±74*	5.91±2.48	2.55±1.71*	25.2±4.32*	24.0±4.21	91.8±14.9	13.0±2.26
3 DEN+PB	Aqueous extract 500 mg/kg bw (wk1-8)	7	13.8±1.0	127±92	5.63±2.89	2.04±0.48	29.1±1.94	28.4±4.15	112.9±11.4**	17.2±2.38
4 DEN+PB	Aqueous extract 1000 mg/kg bw (wk1-8)	5	11.3±1.0**	109±54	6.11±2.75	3.63±1.85	26.5±3.85	37.2±3.27**	117.0±16.5**	16.0±1.93

Data are expressed as mean±SD

* Significant difference from control, $p < 0.05$.

** Significant difference from positive control, $p < 0.05$.

DEN: Diethylnitrosamine 100 mg/kg bw, 2 time injection

PB: Phenobarbital 500 ppm in drinking water