CHAPTER IV

RESULTS

1. Precision assay for determination of oxidative stress parameters and PON activities

Precision of the assay for determination of total peroxide (TP), total antioxidant status (TAS) and PON activities were indicated as Optimal Condition Variance (OCV) and Routine Condition Variance (RCV) for the precisions assay as shown in Table 4.

Table 4 Precision of the assay for determination

Test	Precision of the assay			
	Mean ± SD	**OCV**	**Mean ± SD**	**RCV**
Total peroxide (TP) (μmol/L)	19.31 ± 0.77	4.01	19.51 ± 0.47	2.42
Total antioxidant status (TAS) (mmol Trolox equivalent/L)	0.94 ± 0.05	5.53	0.95 ± 0.04	4.55
Paraoxonase1 (PON1) activity				
- ARE (mmol/min/L)	106.54 ± 3.97	3.72	108.16 ± 3.60	3.33
- Paraoxon (μmol/min/L)	171.68 ± 4.33	2.52	172.79 ± 2.56	1.48
Paraoxonase2 (PON2) activity				
- Using control serum (μmol/min/L)	1307.67 ± 59.03	4.51	1330.30 ± 72.25	5.43
- Using vascular rat (μmol/min/L)	625.61 ± 27.32	4.37	620.37 ± 23.92	3.86
Paraoxonase3 (PON3) activity (μmol/min/L)	2255.47 ± 69.63	3.09	2254.51 ± 42.76	1.90

Values are mean ± SD obtained from 20 samples of control serum and 4 samples of vascular rat.

2. Effect of *C. comosa* and simvastatin on lipid parameters

Table 5 showed the effects of *C. comosa* and simvastatin on lipid parameters in rabbits fed with high-cholesterol diet. The plasma lipid levels include; total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C) at the baseline in all groups of rabbits were not significant difference.

Table 5 Lipid parameters at baseline of the 3 treatment groups

Parameters (mg/dl)	**Baseline**		
	Cholesterol	Cholesterol + Simvastatin	Cholesterol + *C. comosa*
TC	53.8 ± 6.4	54.8 ± 5.4	49.3 ± 5.9
TG	76.3 ± 16.2	57.8 ± 8.1	80.0 ± 7.7
HDL-C	41.5 ± 6.0	43.0 ± 5.0	38.5 ± 3.9
LDL-C	14.5 ± 3.5	14.3 ± 2.7	10.5 ± 3.5

Values shown were mean ± SEM obtained from 4 rabbits.
TC=total cholesterol, TG=triglyceride, HDL-C=high density lipoprotein-cholesterol, LDL-C=low density lipoprotein-cholesterol.

Supplement feeding of cholesterol to rabbits successfully raised the level of lipid parameters at 4 months. The levels of TC, HDL-C and LDL-C were highly significant increased at 4 months of treatment in all groups ($p<0.001$) while the level of TG significantly increased at 4 months of treatment in all groups with $p<0.05$ as compared to the levels at baseline (Table 6).

Table 6 Lipid parameters at baseline and 4 months of the 3 treatment groups

Parameters (mg/dl)	**Baseline**			**4 months**		
	Cholesterol	Cholesterol + Simvastatin	Cholesterol + *C. comosa*	Cholesterol	Cholesterol + Simvastatin	Cholesterol + *C. comosa*
TC	53.8 ± 6.4	54.8 ± 5.4	49.3 ± 5.9	2135.3 ± 169.3**	1575.0 ± 146.6**	1642.8 ± 93.4**
TG	76.3 ± 16.2	57.8 ± 8.1	80.0 ± 7.7	139.3 ± 19.3*	88.0 ± 16.4*	216.3 ± 80.9*
HDL-C	41.5 ± 6.0	43.0 ± 5.0	38.5 ± 3.9	386.8 ± 17.8**	384.0 ± 83.7**	317.0 ± 17.0**
LDL-C	14.5 ± 3.5	14.3 ± 2.7	10.5 ± 3.5	2009.3 ± 201.4**	1408.3 ± 103.7**	1463.5 ± 69.0**

Values shown were mean ± SEM obtained from 4 rabbits.

*$p<0.05$ significant difference from baseline.

**$p<0.001$ significant difference from baseline.

TC=total cholesterol, TG=triglyceride, HDL-C=high density lipoprotein-cholesterol, LDL-C=low density lipoprotein-cholesterol.

At 4 months of treatment, both TC and LDL-C levels in the cholesterol-fed with *C. comosa* and cholesterol-fed with simvastatin groups were significantly decreased as compared to in the cholesterol-fed control group ($p<0.05$). Remarkably, both TC and LDL-C levels in the cholesterol-fed with simvastatin group were decreased to the same extent as in the cholesterol-fed with *C. comosa*. However, there were no significant differences in the TG and HDL-C levels among the treated groups (Figure 14).

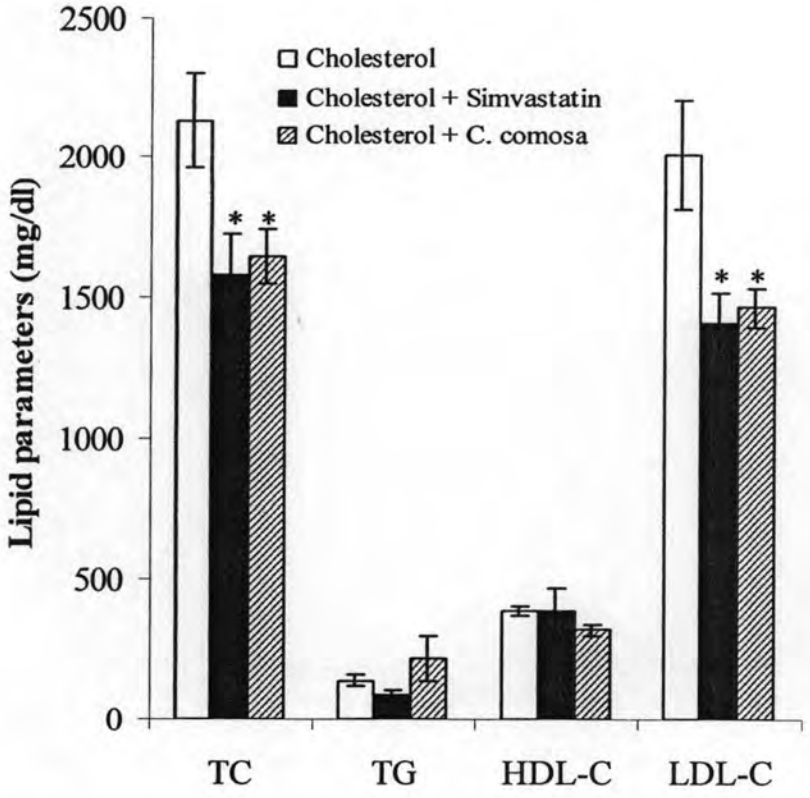

Figure 14 Effects of *C. comosa* and simvastatin on lipid parameters at 4 months. Animals were cholesterol-fed, cholesterol-fed with simvastatin and cholesterol-fed with *C. comosa.* Values are mean ± SEM obtained from 4 rabbits. Significance was determined using One-way ANOVA followed by the Student-Newman-Keuls test in which $p<0.05$ was required for a statistically significant difference.

*$p<0.05$ significant difference from the cholesterol-fed control group.

TC=total cholesterol, TG=triglyceride, HDL-C=high density lipoprotein-cholesterol, LDL-C=low density lipoprotein-cholesterol.

3. Effects of *C. comosa* and simvastatin on oxidative stress parameters

3.1 Effect of *C. comosa* and simvastatin on total peroxide (TP)

At 4 months of treatment, TP levels were significantly increased in both the cholesterol-fed with *C. comosa* (400 mg/kg/day) and the cholesterol-fed with simvastatin (5 mg/day) as compared to the cholesterol-fed control ($p<0.05$). Remarkably, TP levels were lower than that of the cholesterol-fed with *C. comosa* when compared to the cholesterol-fed with simvastatin, but did not reach statistical significant (Figure 15).

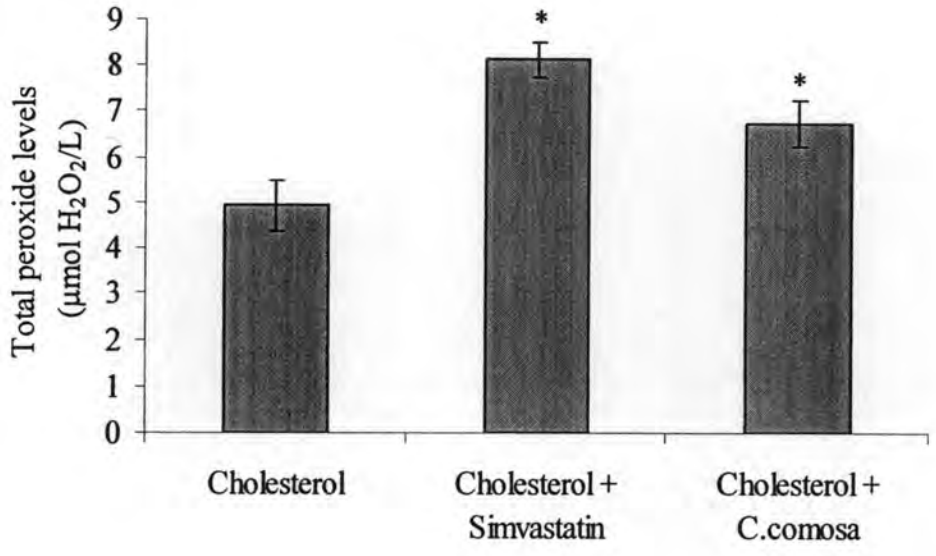

Figure 15 Effect of *C. comosa* and simvastatin on lipid peroxide (TP) levels. Rabbits were administered orally with cholesterol-fed, cholesterol-fed with simvastatin (5 mg/day) and cholesterol-fed with *C. comosa* (400 mg/kg/day) for 4 months. The individual bar graph represented mean of TP levels with a standard error of the mean (n =4). Significance was determined using One-way ANOVA follow by the Student-Newman-Keuls test in which $p<0.05$ was required for a statistically significant difference.

*$p<0.05$ significant difference from cholesterol-fed control group.

The experiment was performed in triplicate for 4 rabbits in each group.

3.2 Effect of *C. comosa* and simvastatin on total antioxidant status (TAS)

At 4 months of treatment, cholesterol-fed with simvastatin (5 mg/day), caused significantly decreased of TAS levels as compared to the cholesterol-fed control and cholesterol-fed with *C. comosa* groups ($p<0.05$). However, TAS level of cholesterol-fed with *C. comosa* group was not statistically different as compared to those of the cholesterol-fed control (Figure 16).

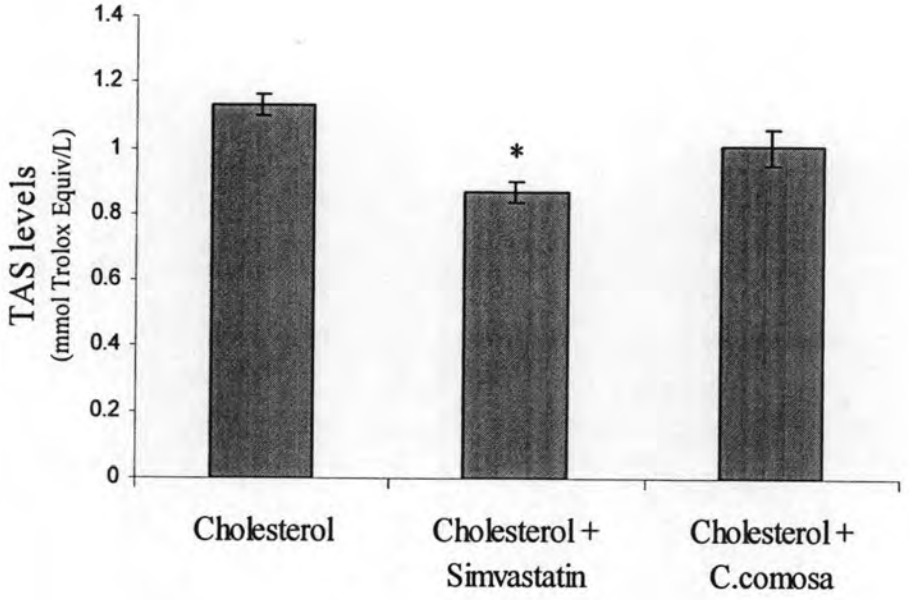

Figure 16 Effect of *C. comosa* and simvastatin on total antioxidant status (TAS) levels. Rabbits were administered orally with cholesterol-fed, cholesterol-fed with simvastatin (5 mg/day) and cholesterol-fed with *C. comosa* (400 mg/kg/day) for 4 months. The individual bar graph represented mean of TAS levels with a standard error of the mean (n =4). Significance was determined using One-way ANOVA follow by the Student-Newman-Keuls test in which $p<0.05$ was required for a statistically significant difference.

*$p<0.05$ significant difference from cholesterol-fed control and cholesterol-fed with *C. comosa* groups.

The experiment was performed in triplicate for 4 rabbits in each group.

3.3 Effect of *C. comosa* and simvastatin on oxidative stress index (OSI)

At 4 months of treatment, OSI of cholesterol-fed with *C. comosa* and cholesterol-fed with simvastatin were significant higher than the cholesterol-fed control. OSI of cholesterol-fed with *C. comosa* was significant lower than that of the cholesterol-fed with simvastatin. (Figure 17)

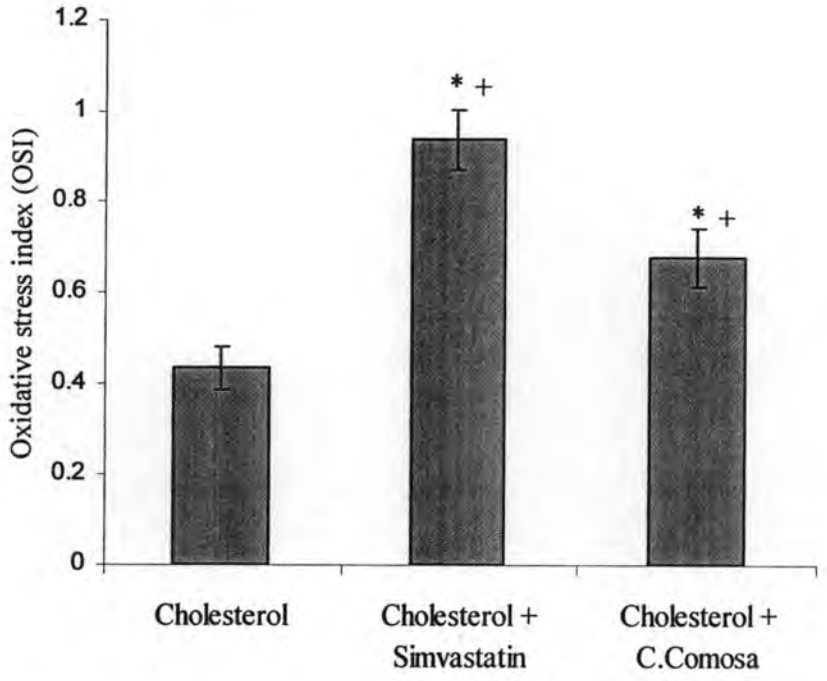

Figure 17 Effect of *C. comosa* and simvastatin on oxidative stress index (OSI). Rabbits were administered orally with cholesterol-fed, cholesterol-fed with simvastatin (5 mg/day) and cholesterol-fed with *C. comosa* (400 mg/kg/day) for 4 months. The individual bar graph represented mean of OSI with a standard error of the mean (n =4). Significance was determined using One-way ANOVA follow by the Student-Newman-Keuls test in which $p<0.05$ was required for a statistically significant difference.

*$p<0.05$ significant difference from the cholesterol-fed control group.

$^{+}p<0.05$ significant difference between the cholesterol-fed with *C. comosa* and cholesterol-fed with simvastatin group.

The experiment was performed in triplicate for 4 rabbits in each group.

4. Effects of *C. comosa* and simvastatin on PON1 and PON3 activities

PON1 activity toward paraoxon and ARE as well as PON3 activity were not significantly affected in the cholesterol-fed with *C. comosa* and the cholesterol-fed with simvastatin as compared to the cholesterol-fed control (Figure 18).

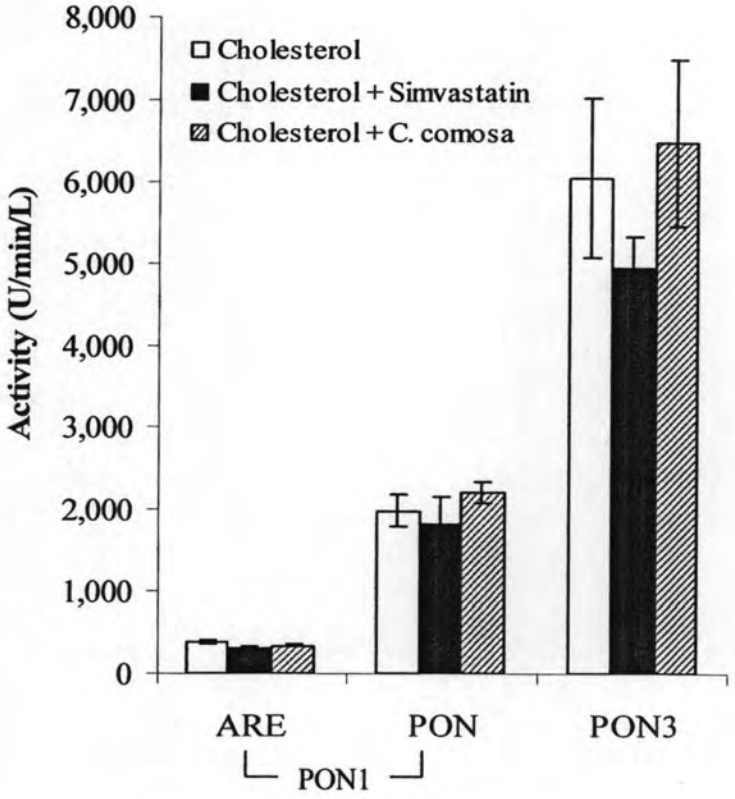

Figure 18 Effects of *C. comosa* and simvastatin on PON1 (using phenyl acetate (ARE) and paraoxon (PON) as substrates) and PON3 activities at 4 months. Animals were cholesterol-fed, cholesterol-fed with simvastatin and cholesterol-fed with *C. comosa.* Values are mean ± SEM obtained from 4 rabbits. The experiment was performed in triplicate for 4 rabbits in each group.

5. Effect of *C. comosa* and simvastatin on PON2 activity

5.1 Total protein assay

Linearity of total protein assay was determined using bovine serum albumin (BSA) as a standard. The coefficient of determination was shown by R^2 of 0.9963 (Figure 19). Then, protein concentrations of samples were determined using this standard curve.

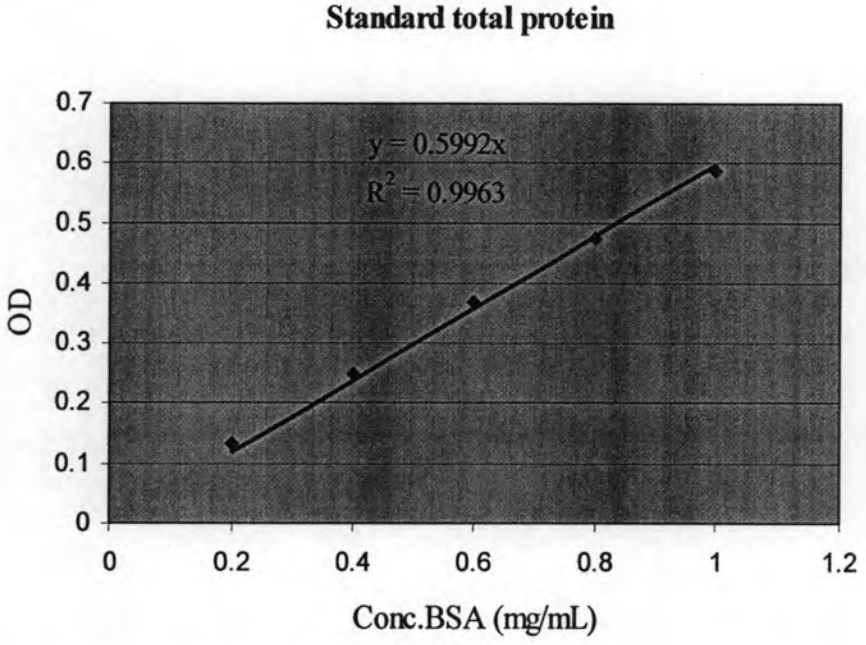

Figure 19 Linearity of the assay for determination of protein concentration using Bradford method (Bradford, 1976).

5.2 Effect of *C. comosa* and simvastatin on PON2 activity

Administration of cholesterol-fed, cholesterol-fed with simvastatin (5 mg/day) and cholesterol-fed with *C. comosa* (400 mg/kg/day) to rabbits for 4 months, demonstrated that both cholesterol-fed with *C. comosa* and cholesterol-fed with simvastatin did not statistically affected PON2 activity as compared to the cholesterol-fed control group (Figure 20).

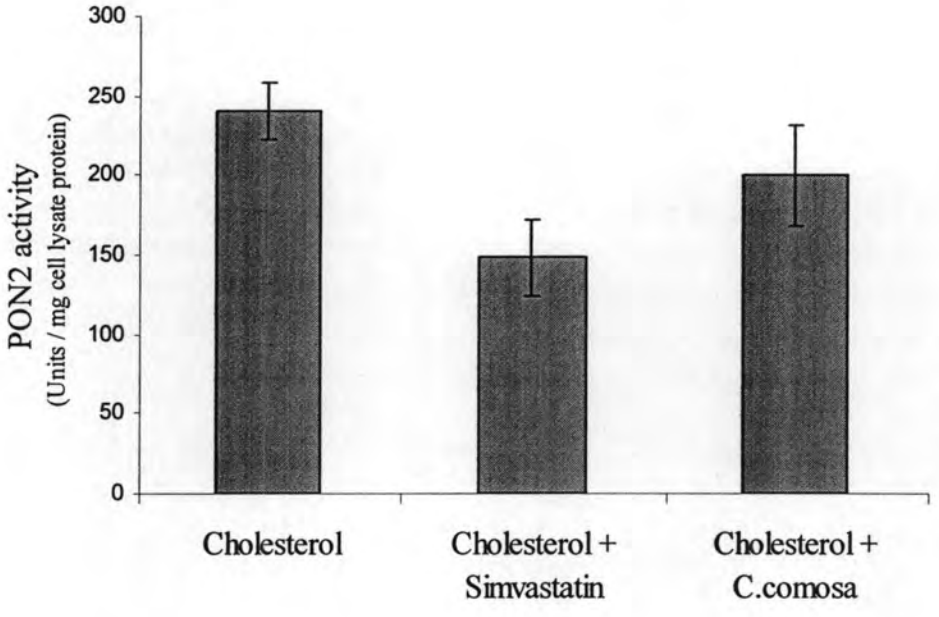

Figure 20 Effect of *C. comosa* and simvastatin on PON2 activity using dihydrocoumarin (DHC) as a substrate. Animals were cholesterol-fed, cholesterol-fed with simvastatin and cholesterol-fed with *C. comosa.* Values are mean ± SEM obtained from 4 rabbits. The experiment was performed in triplicate for 4 rabbits in each group.