CHAPTER IV

RESULTS

1. Verification of the Vivid® CYP450 Screening Kits Protocol

The procedure was verified by determination of IC_{50} of the known inhibitors for each individual CYP determined in the study such as α-naphthoflavone, miconazole, sulfaphenazole, imipramine and ketoconazole. The IC_{50} and 95% confidence limits of the inhibitors on the particular CYP were shown in Table 13.

Table 13 Verification of the procedure using the known inhibitors

Enzymes	Inhibitors	IC_{50}		95% Confidence limits
		(μM)	(μg/ml)	(μM)
CYP1A2	α-Naphthoflavone	0.060	0.016	0.019-0.152
CYP2B6	Miconazole	0.805	0.386	0.515-1.211
CYP2C9	Sulfaphenazole	1.076	0.388	0.735-1.747
CYP2C19	Miconazole	0.068	0.033	0.027-0.163
CYP2D6	Miconazole	1.991	0.954	1.709-2.313
CYP2E1	Imipramine	345.930	109.625	272.027-444.996
CYP3A4	Ketoconazole	0.107	0.057	0.075-0.145

2. Inhibitory effects of ECa 233 on human cytochrome P450 enzymes: *in vitro* study

Inhibitory effect of ECa 233 on CYP1A2 activity

Figure 4 displayed the inhibition curve of probit unit of percent inhibition of CYP1A2 activity versus logarithm concentration of ECa 233. ECa 233 inhibited CYP1A2 activity for 35.71% of the control at the concentration of 1,000 μg/ml. The extrapolated IC_{50} value for ECa 233 inhibition of the CYP1A2 activity was 4,356.83 μg/ml.

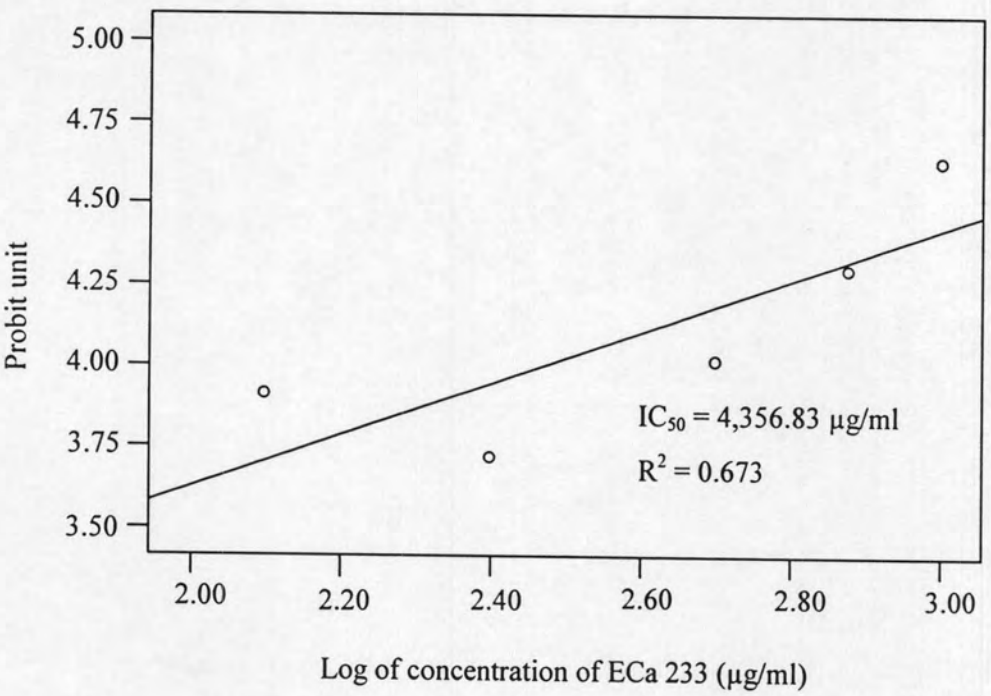

Figure 4 Inhibitory effect of ECa 233 on CYP1A2 activity. Each data point presented the mean of triplicate analysis.

Inhibitory effect of ECa 233 on CYP2B6 activity

Inhibitory effect of ECa 233 on CYP2B6 activity was assessed at the concentrations between 250 to 2,000 μg/ml. Figure 5 displayed the inhibition curve of probit unit of percent inhibition of CYP2B6 activity versus logarithm concentration of ECa 233. The IC_{50} value for ECa 233 inhibition of CYP2B6 activity was calculated to be 871.14 μg/ml (95% Confidence limits, 686.05-1,163.27 μg/ml).

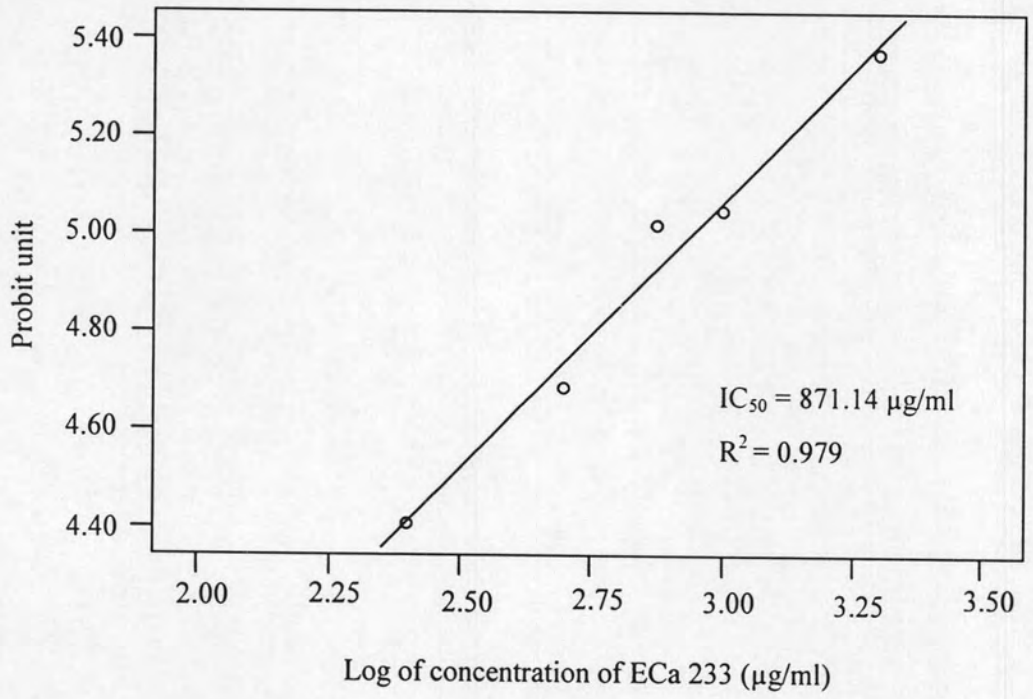

Figure 5 Inhibition effect of ECa 233 on CYP2B6. Each data point presented the mean of triplicate analysis.

Inhibitory effect of ECa 233 on CYP2C9 activity

Inhibitory effect of ECa 233 on CYP2C9 activity was less than 4.11% of the control at concentration up to 750 μg/ml (Table B3). The extrapolated IC_{50} value for ECa 233 inhibition of CYP2C9 activity was approximately 26,090.07 μg/ml.

Inhibitory effect of ECa 233 on CYP2C19 activity

Inhibitory effect of ECa 233 on CYP2C19 activity was assessed at the concentrations between 62.5 to 1,000 μg/ml. Figure 6 displayed the inhibition curve of probit unit of percent inhibition of CYP2C19 activity versus logarithm concentration of ECa 233. The IC_{50} value for ECa 233 inhibition of CYP2C19 activity was calculated to be 365.18 μg/ml, (95% Confidence limits, 321.87-413.90 μg/ml).

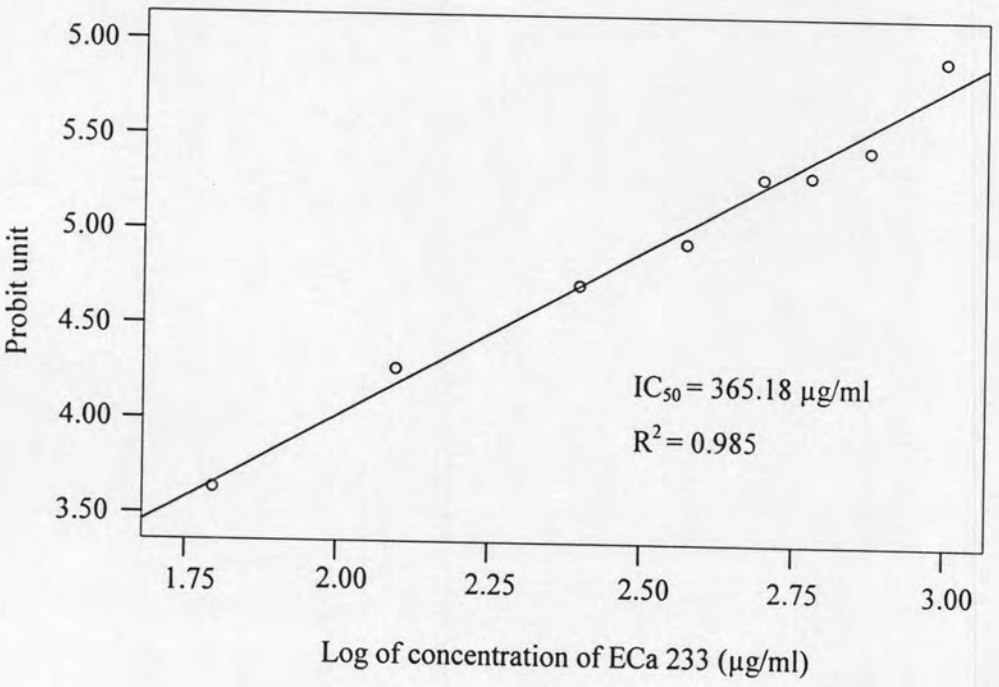

Figure 6 Inhibitory effect of ECa 233 on CYP2C19 activity. Each data point presented the mean of triplicate analysis.

Inhibitory effect of ECa 233 on CYP2D6 activity

Figure 7 displayed the inhibition curve of probit unit of percent inhibition of CYP2D6 activity versus logarithm concentration of ECa 233. ECa 233 inhibited CYP2D6 activity for 36.48 % of the control at concentration of 1,000 μg/ml. The extrapolated IC_{50} value for ECa 233 inhibition of CYP2D6 activity was approximately 1,283.63 μg/ml (95% Confidence limits, 1,104.38-1,643.05 μg/ml).

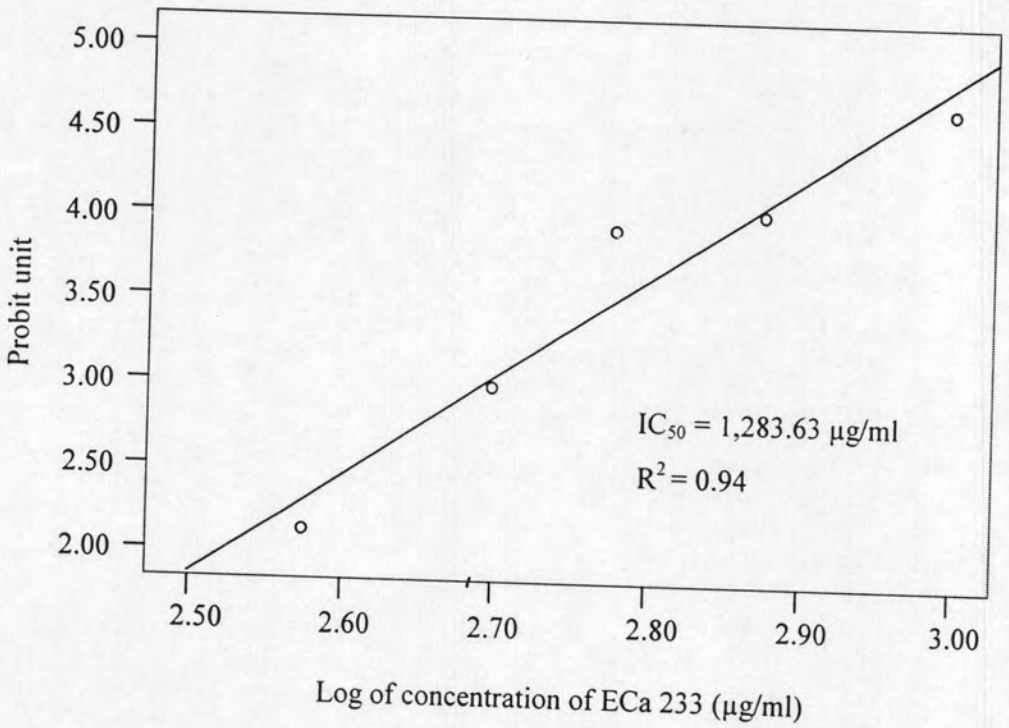

Figure 7 Inhibitory effect of ECa 233 on CYP2D6 activity. Each data point presented the mean of triplicate analysis.

Inhibitory effect of ECa 233 on CYP2E1 activity

ECa 233 was not found to exhibit concentration-dependent inhibition on CYP2E1 activity at all concentrations used in the study (Table B6). Assessment of the inhibitory effect of ECa 233 on this CYP was limited to the solubility of ECa 233.

Inhibitory effect of ECa 233 on CYP3A4 activity

Inhibitory effect of ECa 233 on CYP3A4 activity was assessed at the concentrations between 62.5 to 750 μg/ml. Figure 8 displayed the inhibition curve of probit unit of percent inhibition of CYP3A4 activity versus logarithm concentration of ECa 233. The IC_{50} value for ECa 233 inhibition of CYP3A4 activity was calculated to be 210.98 μg/ml (95% Confidence limits, 169.43-251.09 μg/ml).

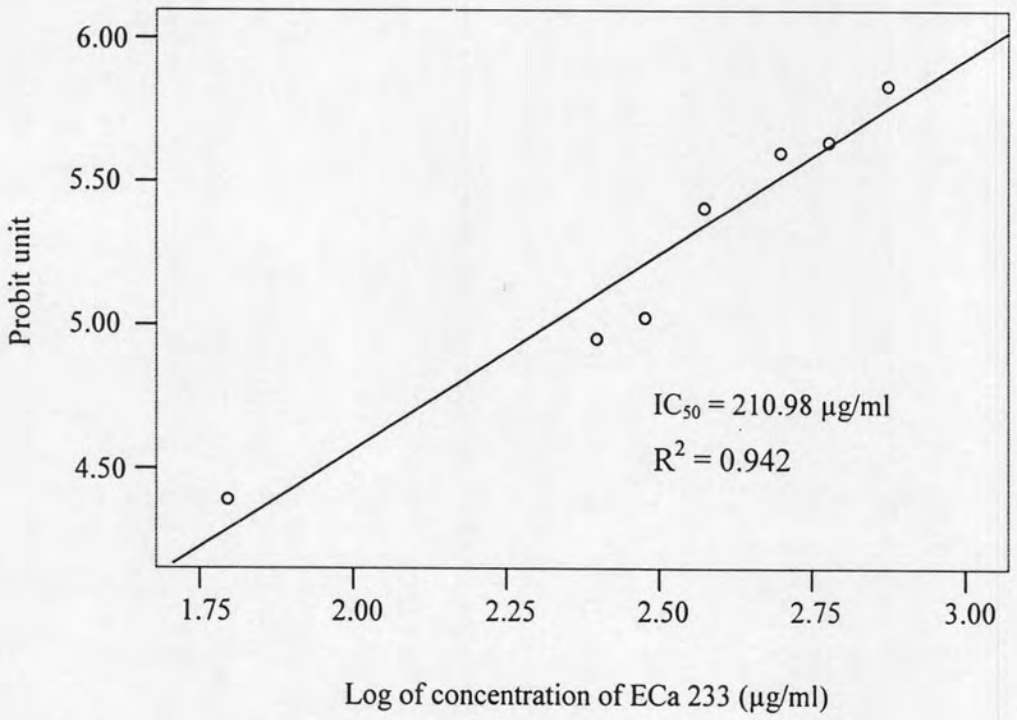

Figure 8 Inhibitory effect of ECa 233 on CYP3A4 activity. Each data point presented the mean of triplicate analysis.

3. Effects of ECa 233 on phase II drug metabolizing enzymes

ECa 233 was orally administered to rats daily for 90 consecutive days, then their liver microsomes and cytosols were determined for phase II drug metabolizing enzymes. ECa 233 did not show significant effect on the activity of UDPGT at all doses (10, 100 and 1,000 mg/kg/day) given to male (Figure 9) and female rats (Figure 10). A significant decrease in the SULT activity was observed in male rats treated with ECa 233 at all doses used in this study as compared to the control group (Figure 11). However, the activity of SULT was not significantly affected by ECa 233 in female rats as compared to the control group (Figure 12). ECa 233 given at all dosage regimens used in this study did not modulate the activity of GST in male rats (Figure 13) and female rats (Figure 14). Regarding the effect on NQOR activity, all doses of ECa 233 did not affect the activity of this enzyme in both male (Figure 15) and female rats (Figure 16).

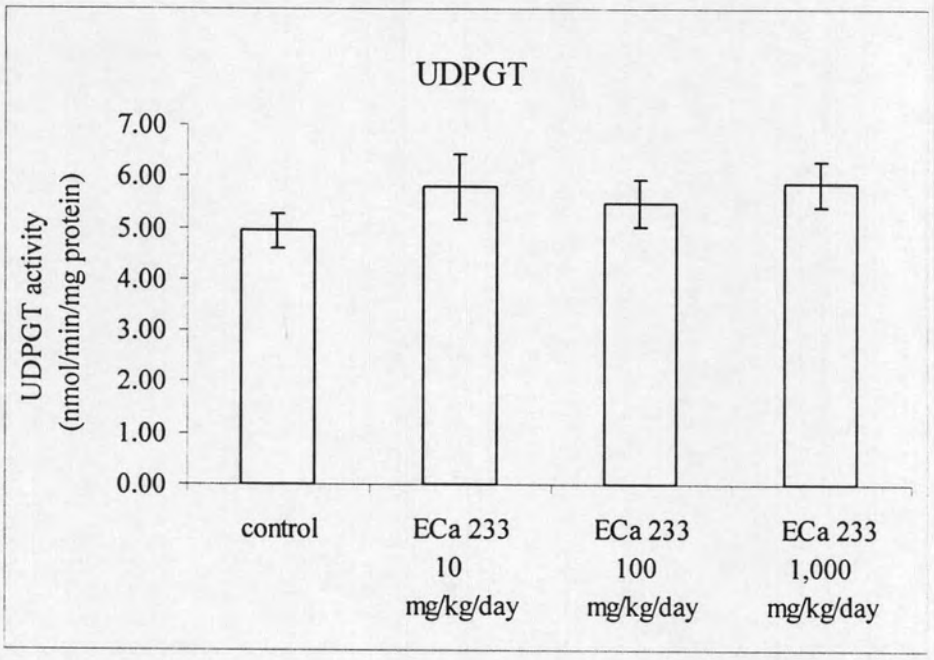

Figure 9 Effects of ECa 233 on liver microsomal UDPGT activity in male rats. Rats were treated orally with water (1 ml/kg/day) in the control group, ECa 233 (10, 100 and 1,000 mg/kg/day) for 90 consecutive days. Data were presented as mean ± SEM of 10 rats/group. For statistical analysis, one-way ANOVA and Student-Newman-Keuls test were carried out, $p < 0.05$ was considered statistically significant.

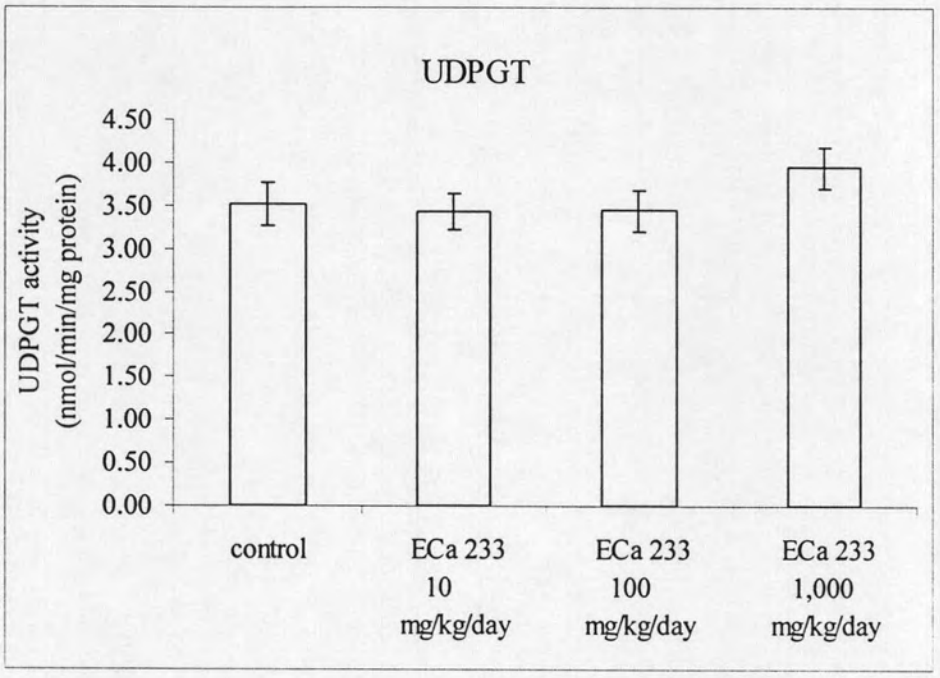

Figure 10 Effects of ECa 233 on liver microsomal UDPGT activity in female rats. Rats were treated orally with water (1 ml/kg/day) in the control group, ECa 233 (10, 100 and 1,000 mg/kg/day) for 90 consecutive days. Data were presented as mean ± SEM of 10 rats/group. For statistical analysis, one-way ANOVA and Student-Newman-Keuls test were carried out, $p < 0.05$ was considered statistically significant.

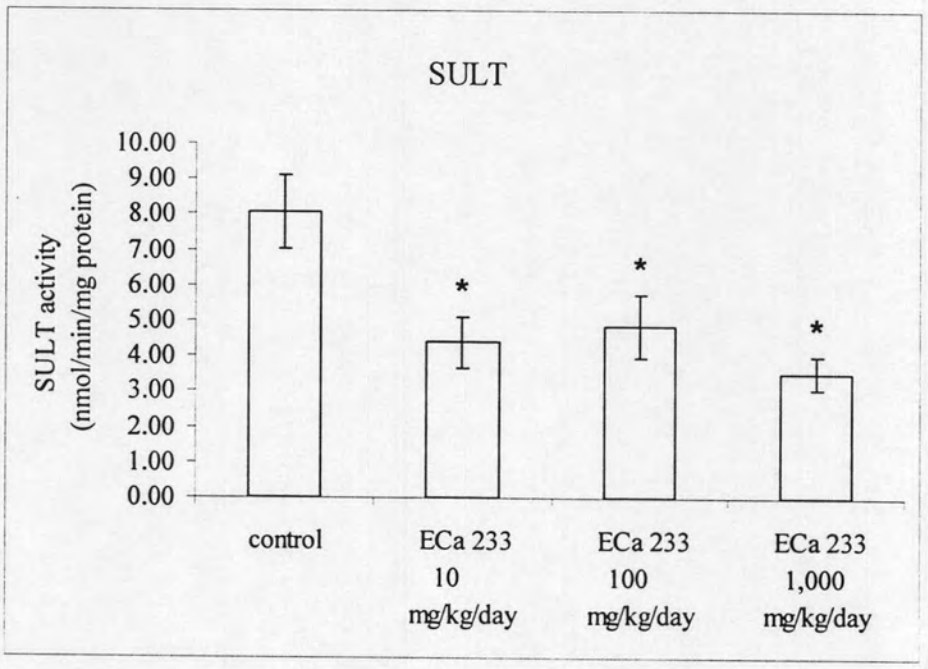

Figure 11 Effects of ECa 233 on liver cytosolic SULT activity in male rats. Rats were treated orally with water (1 ml/kg/day) in the control group, ECa 233 (10, 100 and 1,000 mg/kg/day) for 90 consecutive days. Data were presented as mean ± SEM of 10 rats/group. For statistical analysis, one-way ANOVA and Student-Newman-Keuls test were carried out, $p < 0.05$ was considered statistically significant. * $p < 0.05$, ECa 233 treated group vs control group.

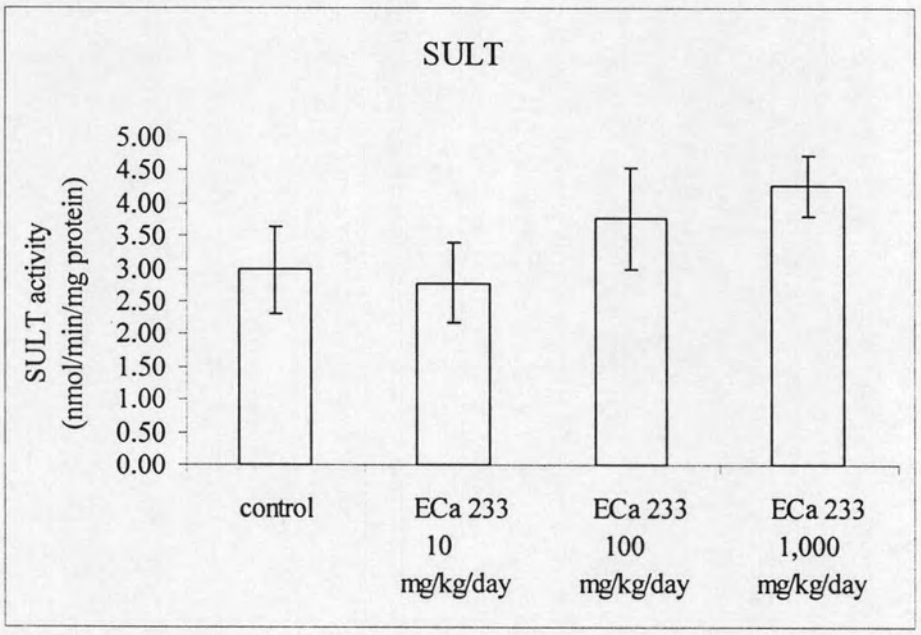

Figure 12 Effects of ECa 233 on liver cytosolic SULT activity in female rats. Rats were treated orally with water (1 ml/kg/day) in the control group, ECa 233 (10, 100 and 1,000 mg/kg/day) for 90 consecutive days. Data were presented as mean ± SEM of 10 rats/group. For statistical analysis, one-way ANOVA and Student-Newman-Keuls test were carried out, $p < 0.05$ was considered statistically significant.

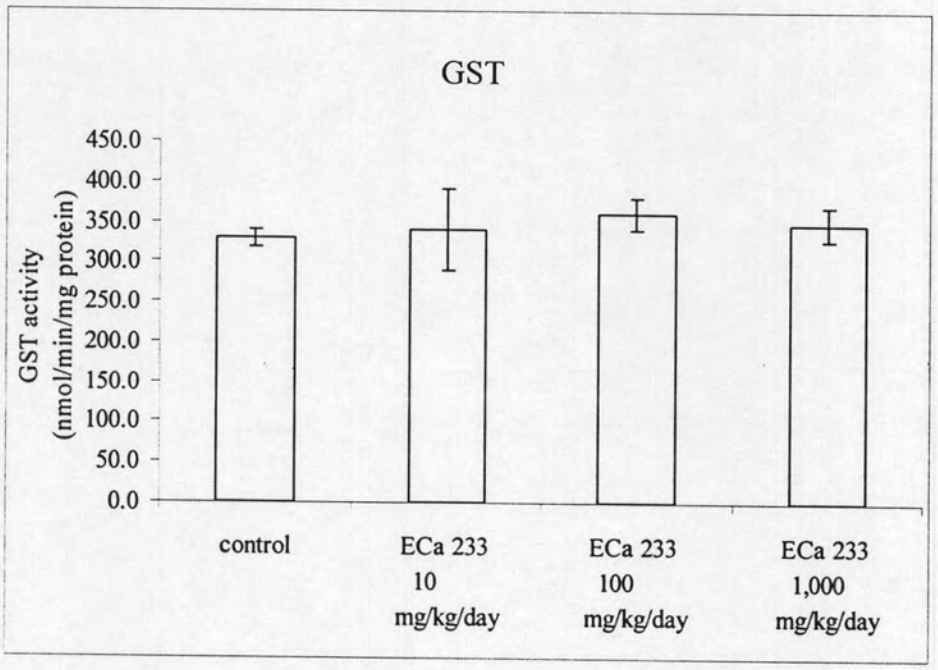

Figure 13 Effects of ECa 233 on liver cytosolic GST activity in male rats. Rats were treated orally with water (1 ml/kg/day) in the control group, ECa 233 (10, 100 and 1,000 mg/kg/day) for 90 consecutive days. Data were presented as mean ± SEM of 10 rats/group. For statistical analysis, one-way ANOVA and Student-Newman-Keuls test were carried out, $p < 0.05$ was considered statistically significant.

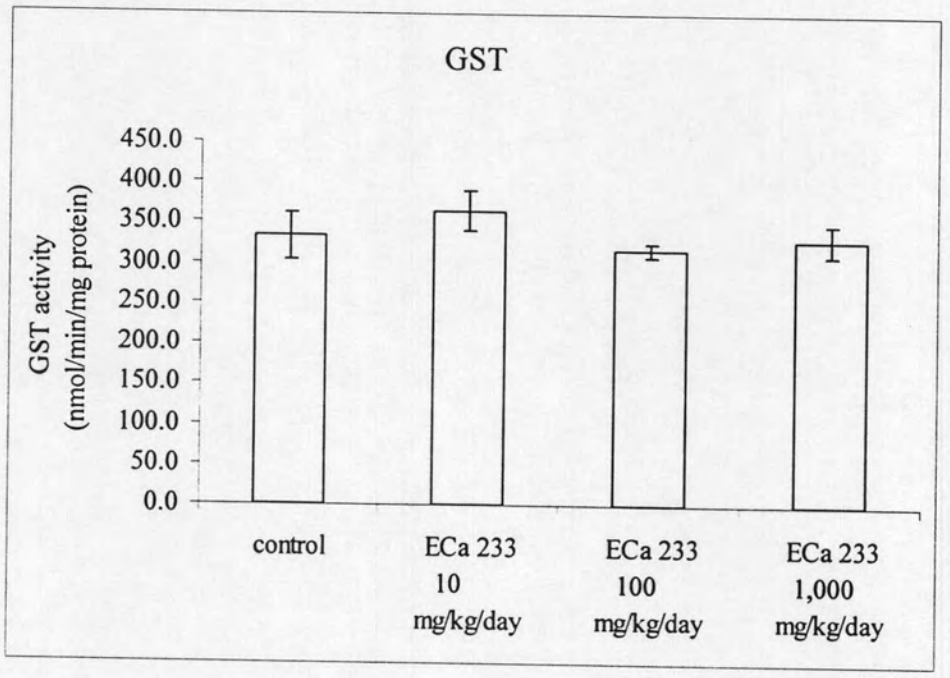

Figure 14 Effects of ECa 233 on liver cytosolic GST activity in female rats. Rats were treated orally with water (1 ml/kg/day) in the control group, ECa 233 (10, 100 and 1,000 mg/kg/day) for 90 consecutive days. Data were presented as mean ± SEM of 10 rats/group. For statistical analysis, one-way ANOVA and Student-Newman-Keuls test were carried out, $p < 0.05$ was considered statistically significant.

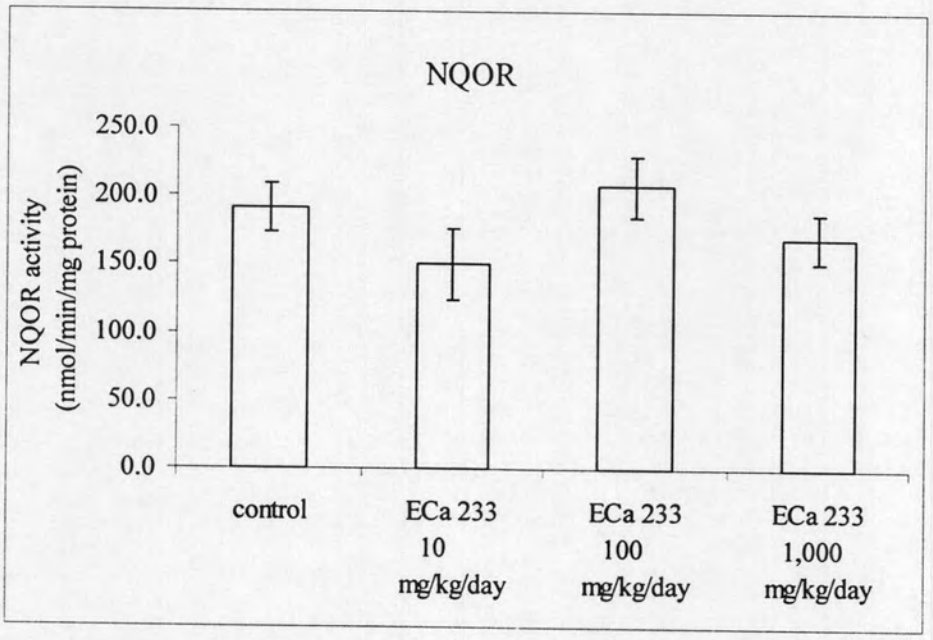

Figure 15 Effects of ECa 233 on liver cytosolic NQOR activity in male rats. Rats were treated orally with water (1 ml/kg/day) in the control group, ECa 233 (10, 100 and 1,000 mg/kg/day) for 90 consecutive days. Data were presented as mean ± SEM of 10 rats/group. For statistical analysis, one-way ANOVA and Student-Newman-Keuls test were carried out, $p < 0.05$ was considered statistically significant.

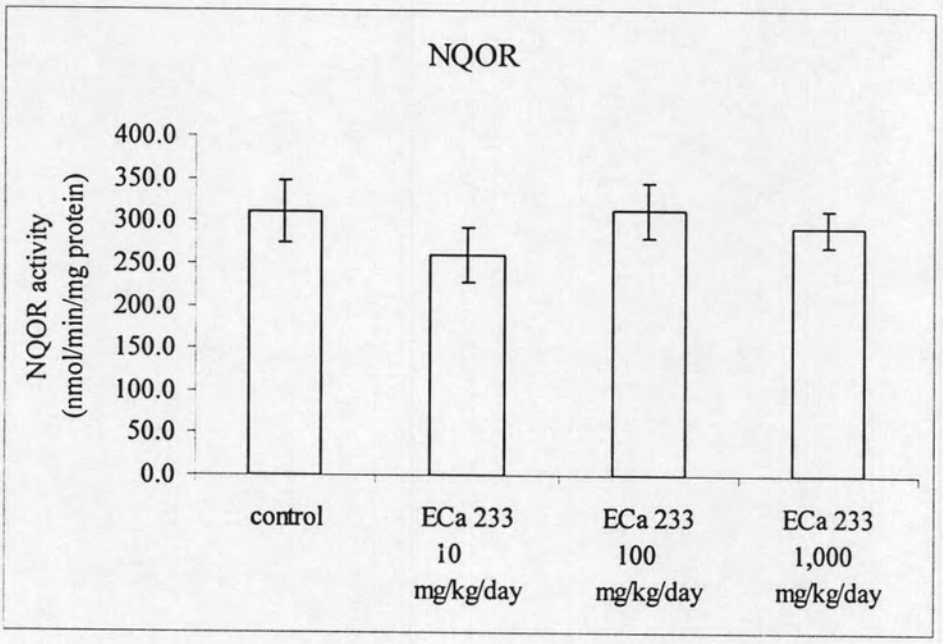

Figure 16 Effects of ECa 233 on liver cytosolic NQOR activity in female rats. Rats were treated orally with water (1 ml/kg/day) in the control group, ECa 233 (10, 100 and 1,000 mg/kg/day) for 90 consecutive days. Data were presented as mean ± SEM of 10 rats/group. For statistical analysis, one-way ANOVA and Student-Newman-Keuls test were carried out, $p < 0.05$ was considered statistically significant.