

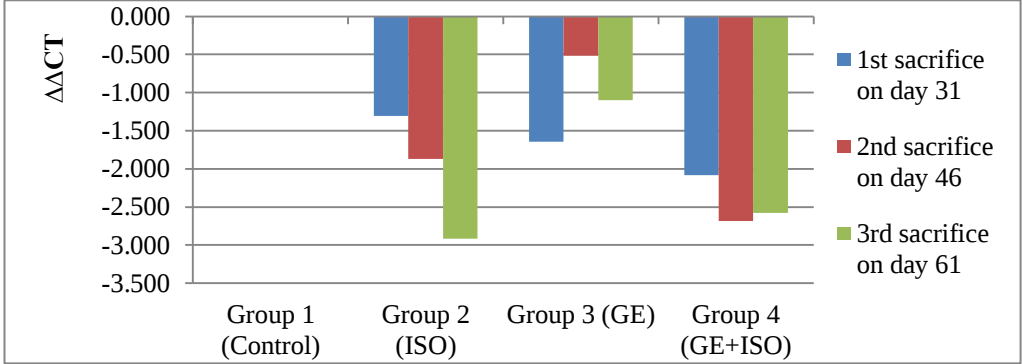
Gene: Caspase3

Double Delta CT Analysis (Female Rat - 2^{-ΔΔCT1})

Sample		Organ	House Keeping Gene	Target Gene	ΔC _{T1}	Avg. ΔC _{T1} of Control Group	ΔΔC _{T1}	2 ^{-ΔΔC_{T1}}
			C _{T1}	C _{T1}				
Group 1	1st Sacrifice	Heart	17.121	24.318	7.197	7.197	0.000	1.000
Group 2	1st Sacrifice	Heart	18.741	24.631	5.890		-1.307	2.474
	2nd Sacrifice	Heart	21.411	26.740	5.329		-1.868	3.650
	3rd Sacrifice	Heart	22.740	27.019	4.279		-2.918	7.558
Group 3	1st Sacrifice	Heart	22.689	28.239	5.550		-1.647	3.132
	2nd Sacrifice	Heart	20.170	26.853	6.683		-0.514	1.428
	3rd Sacrifice	Heart	22.733	28.830	6.097		-1.100	2.144
Group 4	1st Sacrifice	Heart	20.569	25.682	5.113		-2.084	4.240
	2nd Sacrifice	Heart	21.793	26.307	4.514		-2.683	6.422
	3rd Sacrifice	Heart	21.873	26.493	4.620		-2.577	5.967

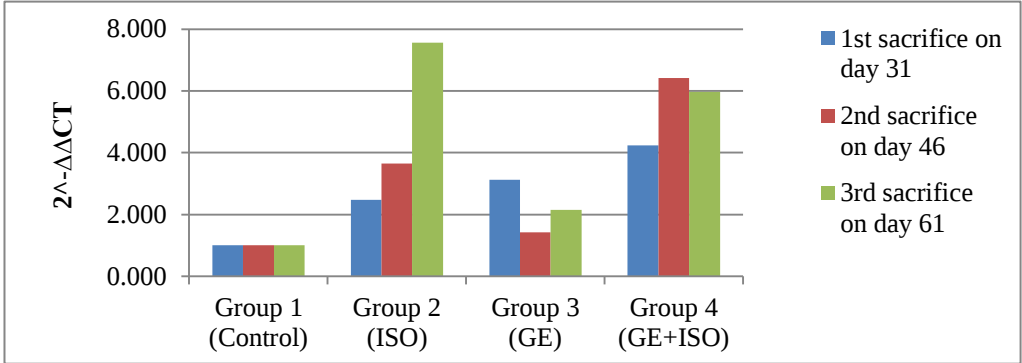
Stimulation / inhibition of gene (ΔΔCT1)

Groups	1st sacrifice on day 31	2nd sacrifice on day 46	3rd sacrifice on day 61
Group 1 (Control)	0.000	0.000	0.000
Group 2 (ISO)	-1.307	-1.868	-2.918
Group 3 (GE)	-1.647	-0.514	-1.100
Group 4 (GE+ISO)	-2.084	-2.683	-2.577



Relative gene expression (2^{-ΔΔCT1})

Groups	1st sacrifice on day 31	2nd sacrifice on day 46	3rd sacrifice on day 61
Group 1 (Control)	1.000	1.000	1.000
Group 2 (ISO)	2.474	3.650	7.558
Group 3 (GE)	3.132	1.428	2.144
Group 4 (GE+ISO)	4.240	6.422	5.967



Formulae:

$$\Delta C_T = \text{Target Gene } C_T - \text{Housekeeping Gene } C_T$$
$$\Delta\Delta C_T = \text{Sample } \Delta C_T - \text{Avg. of Control group } \Delta C_T$$

- C_T values are inverse to the amount of nucleic acid which is in your sample, Lower C_T values indicate high amounts of targeted nucleic acid, while higher C_T values mean lower (and even too little) amounts of your target nucleic acid.
- Positive $\Delta\Delta C_T$ values indicate less targeted gene's mRNA after induction or treatment, meaning inhibition of targeted gene's expression. Conversely, negative $\Delta\Delta C_T$ values indicate more mRNA after stimulation, indicating induction of gene expression.
- $2^{-\Delta\Delta C_T}$ is a relative (or normalized) expression.

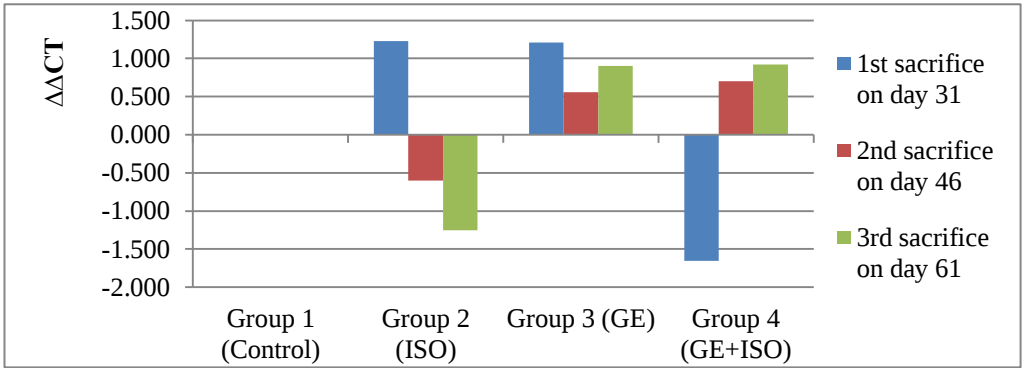
Gene: Caspase3

Double Delta CT Analysis (Female Rat - 2^{-ΔΔCT2})

Sample		Organ	House Keeping Gene	Target Gene	ΔC _{T2}	Avg. ΔC _{T2} of Control Group	ΔΔC _{T2}	2 ^{-ΔΔC_{T2}}
			C _{T2}	C _{T2}				
Group 1	1st Sacrifice	Heart	18.713	24.654	5.941	5.941	0.000	1.000
Group 2	1st Sacrifice	Heart	17.373	24.543	7.170		1.229	0.427
	2nd Sacrifice	Heart	21.883	27.220	5.337		-0.604	1.520
	3rd Sacrifice	Heart	23.220	27.907	4.687		-1.254	2.385
Group 3	1st Sacrifice	Heart	21.417	28.567	7.150		1.209	0.433
	2nd Sacrifice	Heart	20.010	26.509	6.499		0.558	0.679
	3rd Sacrifice	Heart	22.149	28.990	6.841		0.900	0.536
Group 4	1st Sacrifice	Heart	21.057	25.346	4.289		-1.652	3.143
	2nd Sacrifice	Heart	20.329	26.971	6.642		0.701	0.615
	3rd Sacrifice	Heart	20.569	27.429	6.860		0.919	0.529

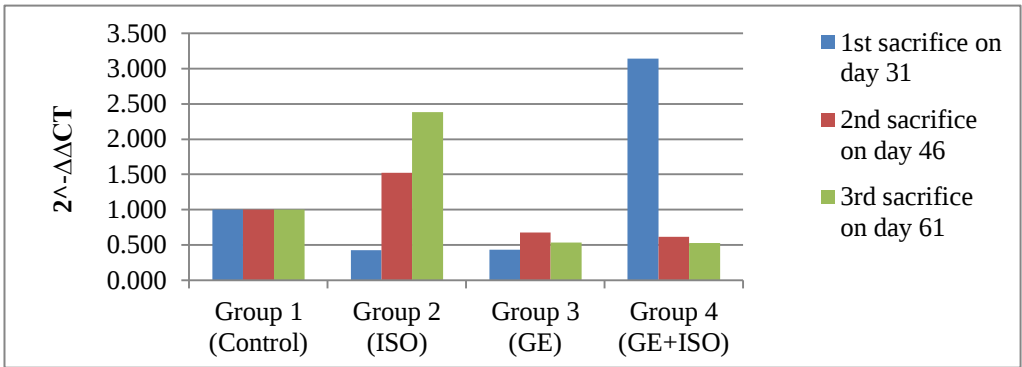
Stimulation / inhibition of gene (ΔΔCT2)

Groups	1st sacrifice on day 31	2nd sacrifice on day 46	3rd sacrifice on day 61
Group 1 (Control)	0.000	0.000	0.000
Group 2 (ISO)	1.229	-0.604	-1.254
Group 3 (GE)	1.209	0.558	0.900
Group 4 (GE+ISO)	-1.652	0.701	0.919



Relative gene expression (2^{-ΔΔCT2})

Groups	1st sacrifice on day 31	2nd sacrifice on day 46	3rd sacrifice on day 61
Group 1 (Control)	1.000	1.000	1.000
Group 2 (ISO)	0.427	1.520	2.385
Group 3 (GE)	0.433	0.679	0.536
Group 4 (GE+ISO)	3.143	0.615	0.529



Formulae:

$$\Delta C_T = \text{Target Gene } C_T - \text{Housekeeping Gene } C_T$$
$$\Delta\Delta C_T = \text{Sample } \Delta C_T - \text{Avg. of Control group } \Delta C_T$$

- C_T values are inverse to the amount of nucleic acid which is in your sample, Lower C_T values indicate high amounts of targeted nucleic acid, while higher C_T values mean lower (and even too little) amounts of your target nucleic acid.
- Positive ΔΔC_T values indicate less targeted gene's mRNA after induction or treatment, meaning inhibition of targeted gene's expression. Conversely, negative ΔΔC_T values indicate more mRNA after stimulation, indicating induction of gene expression.
- 2^{-ΔΔC_T} is a relative (or normalized) expression.

Mean 2 ^{^-ΔΔCT} of Female Rats						
Groups	Sacrifice	2 ^{^-ΔΔCT1}	2 ^{^-ΔΔCT2}	Average 2 ^{^-ΔΔCT}	Standard Deviation	Standard Error of Mean
Group 1	1st Sacrifice (Day 31)	1.000	1.000	1.000	0.000	0.000
	2nd Sacrifice (Day 46)	1.000	1.000	1.000	0.000	0.000
	3rd Sacrifice (Day 61)	1.000	1.000	1.000	0.000	0.000
Group 2	1st Sacrifice (Day 31)	2.474	0.427	1.450	1.448	1.024
	2nd Sacrifice (Day 46)	3.650	1.520	2.585	1.506	1.065
	3rd Sacrifice (Day 61)	7.558	2.385	4.972	3.658	2.586
Group 3	1st Sacrifice (Day 31)	3.132	0.433	1.782	1.909	1.350
	2nd Sacrifice (Day 46)	1.428	0.679	1.054	0.529	0.374
	3rd Sacrifice (Day 61)	2.144	0.536	1.340	1.137	0.804
Group 4	1st Sacrifice (Day 31)	4.240	3.143	3.691	0.776	0.549
	2nd Sacrifice (Day 46)	6.422	0.615	3.519	4.106	2.903
	3rd Sacrifice (Day 61)	5.967	0.529	3.248	3.845	2.719

Mean Relative gene expression (2^{^-ΔΔCT})

Groups	1st sacrifice on day 31	2nd sacrifice on day 46	3rd sacrifice on day 61
Group 1 (Control)	1.000	1.000	1.000
Group 2 (ISO)	1.450	2.585	4.972
Group 3 (GE)	1.782	1.054	1.340
Group 4 (GE+ISO)	3.691	3.519	3.248

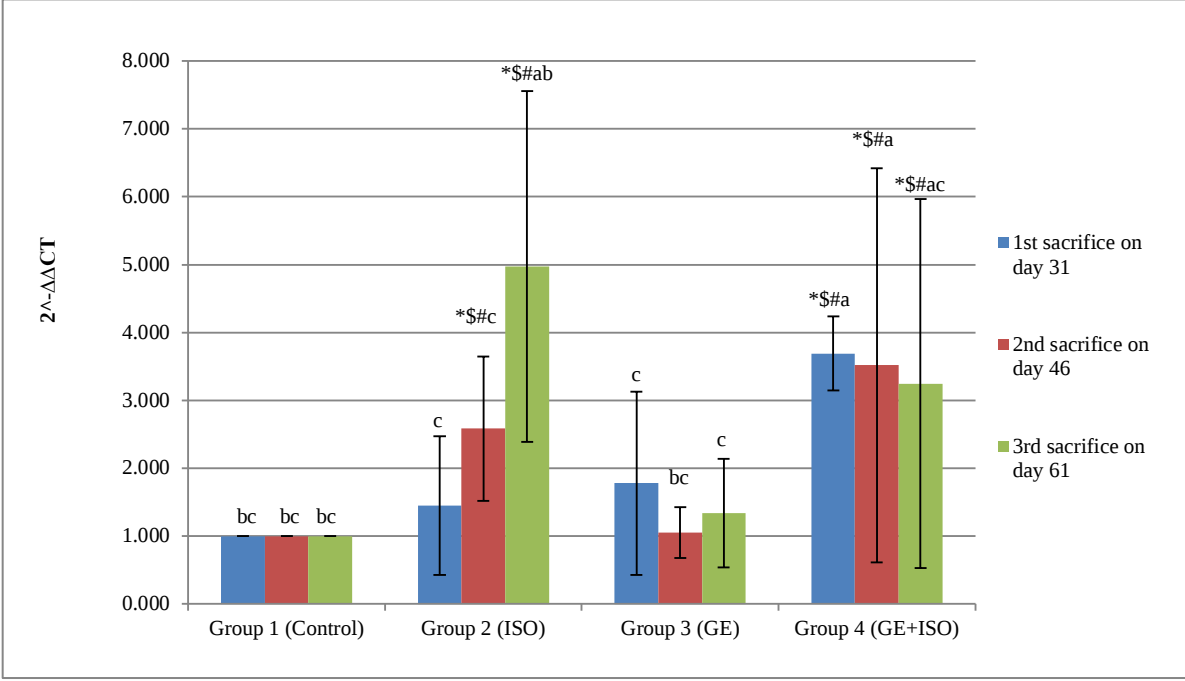


Fig: Relative expression (2^{^-ΔΔCT}) of CASP3 gene. In Group 1 the same data of the day 31 was used for day 46 and 61 as this group was neither GE nor ISO administrated. Data are presented as mean fold change with fold difference from the control group. One-way ANOVA (analysis of variance) along with LSD post hoc test was used to analyze mean±SEM values between different groups. The findings were considered as statistically significant, if *P*<0.5. *, \$ and # indicate the fold change of CASP3 gene significantly different from Group 1 (control group) on the day of rat sacrifice *i.e.* day 31, 46 and 61 respectively and similarly a, b and c indicate fold change of CASP3 gene significantly different from Group 2 on the day of rat sacrifice *i.e.* day 31, 46 and 61 respectively.