6. Summary and Conclusion

The aim of the present study was running in two parallel aspects. First, to elucidate the possible mechanisms underlying the effects of ethanol consumption on normal colon and on colons with ulcerative colitis (UC). Second, to explore its effects on the heart. To achieve this goal, two sets of experiments were performed; an *in vivo* model on colon, and an *in vitro* model using HL-1 cardiomyocyte cell line.

I. *In vivo* Experiments:

This experiment was performed to demonstrate the effects of ethanol consumption on normal colon and on colons with experimentally-induced UC in rats. UC was induced at the 8th day of the experiment by intracolon injection of 0.1 ml of 2% N-ethylmaleimide (NEM; dissolved in 1% methylcellulose) at a depth of 6 cm from anus via a Nelaton's catheter. Rats were allocated into 4 groups. Animals were divided into a normal control group which received distilled water orally, an ethanol group which received ethanol (2.5 g/kg, orally) once daily for two weeks, an UC control group which received distilled water orally and single dose of 2% NEM via the intracolon route and an ethanol/UC group which received ethanol (2.5 g/kg, orally) once daily for two weeks and a single intracolon dose of 2% NEM. On the last day of the experiment, the rats were sacrificed by cervical dislocation. The colon was excised and the colitis was assessed macroscopically by measuring both colon length and colon mass index. The degree of colonic injury was assessed by measuring colonic reduced glutathione (GSH) content as an oxidative stress biomarker, as well as colonic myeloperoxidase (MPO), tumour necrosis factor-alpha (TNF-α) and total nitrate/nitrite (NOx) contents as inflammatory biomarkers, supported by colon macroscopic and microscopic examinations.

The main findings of the *in vivo* experiments can be summarized as follows:

1. The intracolon injection of NEM led to a significant shortening of colon length as well as an increase in colon mass index. This was associated with a marked increase in the colonic contents of the pro-inflammatory cytokine TNF-α. Colonic MPO activity was also elevated indicating an increase in neutrophilic infiltration of the intestinal mucosa. Colonic tissue GSH was significantly reduced while NOx was significantly increased. Histopathological examination of the colon revealed severe ulcerative changes coupled with neutrophil infiltration.
2. Daily administration of oral ethanol (2.5 mg/kg/day) significantly decreased the colon length but did not significantly affect the colon mass index of normal animals, and markedly increased the colonic contents of the pro-inflammatory cytokine TNF-α. Colonic tissue GSH was significantly reduced while NO_X was significantly increased compared with normal rats. The harmful effect of the drug was substantiated histologically as marked degenerative changes in the colon.
3. Daily administration of oral ethanol (2.5 mg/kg/day) worsened UC disease progression. Treatment of ethanol in UC rats led to a marked shortening of colon length as well as an increase in colon mass index compared to the UC control values. This was associated with a marked increase in the colonic contents of TNF-α. Colonic MPO activity was also elevated indicating an increase in neutrophilic infiltration of the intestinal mucosa. Colonic GSH was significantly reduced while NO_X content was significantly increased. The inflammatory condition of the colon was also confirmed histologically.

II. *In vitro* Experiments:

The effect of ethanol was performed on isolated cardiac myocytes *in vitro*, through using the atrial cell cultures of HL-1 cells. In order to characterize the effects of ethanol on the calcium dependent steps of cardiac excitation-contraction coupling (EC-coupling), cells were exposed to 25 mM and 100 mM ethanol acutely, where Ca^{2+} spikes were recorded before and after cell exposure to 25 or 100 mM of ethanol for 5-10 minutes. In order to investigate the effect of chronic ethanol exposure on cardiac atrial cells, cells were plated and a time course of treatment was developed to characterize the changes associated with 25 mM and 100 mM ethanol exposure up to 72 hours by using western blotting technique to determine the expression of several proteins level as total L-type Ca^{2+} channel (LTCC), phosphorylation of the LTCC (phospho LTCC), T-type Ca^{2+} channel (TTCC) and Ryanodine receptors (RyRs).

The main findings of the *in vitro* experiments can be summarized as follows:

1. HL-1 cells treated with 25 mM ethanol showed a reduction in Ca^{2+} transient amplitude and frequency. The reduction of Ca^{2+} transients was magnified upon increasing ethanol concentration to 100 mM.
2. HL-1 cells treated with 25 mM ethanol showed an increase in level of total LTCC after 24, 48 and 72 hours. Cyclic AMP stimulation also enhanced the protein level of LTCC in comparison to the cells without treatment. When Cyclic AMP stimulation treated to the cells with 25 mM ethanol for 72 hours also enhanced the level of LTCC as compared to treatment of 25 mM ethanol for 72 hours alone.
3. Concerning to phosphorylation of the LTCC, HL-1 cells treated with 25 mM ethanol revealed a significantly higher level of basal phosphorylation after 24, 48 and 72 hours. Cyclic AMP stimulation also enhanced the phosphorylation of the LTCC in comparison to the cells without treatment.

4. Treatment of HL-1 cells with 100 mM ethanol caused an increase in LTCC level after 24, 48 and 72 hours. The highest level of LTCC was after 48 hours of treatment, and then the level decreased after treatment of 100 mM ethanol for 72 hours as compared to 48 hour of treatment. Concerning to phosphorylation of the LTCC, the present study showed that HL-1 cells treated with 100 mM ethanol showed a significantly higher level of basal phosphorylation after 48 hours of treatment.
5. HL-1 cells treated with 25 mM ethanol showed a slight increase in TTCC expression after 24, 48 and 72 hours. Cyclic AMP stimulation also enhanced the expression of TTCC in comparison to the cells not subjected to ethanol treatment, and in comparison to the cells treated with 25 mM ethanol for 72 hours alone.
6. Treatment of the cells with 100 mM ethanol markedly increased the level of TTCC after 24, 48 and 72 hours. The level increased by increasing the duration of treatment to 72 hours. Cyclic AMP stimulation also enhanced the TTCC level in comparison to the cells without treatment, but not when compared to the cells treated with 100 mM ethanol for 72 hours alone.
7. HL-1 cells treated with 25 mM ethanol and 100 mM ethanol showed a significantly higher level of expression of RyR level after 24, 48 and 72 hours of treatment. Cyclic AMP stimulation also enhanced the expression of RyR level in comparison to the cells without treatment, but not when compared to the cells treated with 25 mM and 100 mM ethanol for 72 hours alone.

In conclusion, the present study suggests that ethanol consumption has harmful effects on normal colon and may worsen UC disease progression through decrease in endogenous antioxidant capacity coupled with an increase in inflammatory

progression. Additionally, ethanol consumption has harmful effects on the heart, where acute negative inotropic effects of ethanol were found on isolated atrial cells, while a positive inotropic phase and enhanced EC-coupling were found as increased the proteins expression of LTCC, phospho LTCC, TTCC and RyR after chronic treatment of ethanol, eventually leading to the development of alcoholic cardiomyopathy.

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reduced while NO_X content was significantly increased. The inflammatory condition of the colon was also confirmed histologically.

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The main findings of the *in vitro* experiments can be summarized as follows:

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12. HL-1 cells treated with 25 mM ethanol showed a slight increase in TTCC expression after 24, 48 and 72 hours. Cyclic AMP stimulation also enhanced the expression of TTCC in comparison to the cells not subjected to ethanol treatment, and in comparison to the cells treated with 25 mM ethanol for 72 hours alone.

13. Treatment of the cells with 100 mM ethanol markedly increased the level of TTCC after 24, 48 and 72 hours. The level increased by increasing the duration of treatment to 72 hours. Cyclic AMP stimulation also enhanced the TTCC level in comparison to the cells without treatment, but not when compared to the cells treated with 100 mM ethanol for 72 hours alone.

14. HL-1 cells treated with 25 mM ethanol and 100 mM ethanol showed a significantly higher level of expression of RyR level after 24, 48 and 72 hours of treatment. Cyclic AMP stimulation also enhanced the expression of RyR level in comparison to the cells without treatment, but not when compared to the cells treated with 25 mM and 100 mM ethanol for 72 hours alone.

In conclusion, the present study suggests that ethanol consumption has harmful effects on normal colon and may worsen UC disease progression through decrease in endogenous antioxidant capacity coupled with an increase in inflammatory progression. Additionally, ethanol consumption has harmful effects on the heart, where acute negative inotropic effects of ethanol were found on isolated atrial cells, while a positive inotropic phase and enhanced EC-coupling were found as increased the proteins expression of LTCC, phospho LTCC, TTCC and RyR after chronic treatment of ethanol, eventually leading to the development of alcoholic cardiomyopathy.