CHAPTER 4

DISCUSSION AND CONCLUSION

The aqueous extract of *C. racemosa* var. *cylindracea* (AqCR) at the doses of 100 and 500 mg/kg showed an inhibitory effect against gastric ulcer formation induced in rats by 1) restraint water immersion stress, 2) HCl/Ethanol, 3) indomethacin. Additinally, the AqCR at the dose of 100 and 500 mg/kg exhibited a hepatoprotective effect against CCl_4-induced hepatic damage. The AqCR showed radical scavenging activity when tested in the DPPH assay, anti-lipid peroxidation activity and the presence of phenolic substances.

In most of the cases the etiology of the ulcer is unknown, it is generally accepted that it results from an imbalance between aggressive factors and endogenous defense mechanisms which maintain mucosal integrity (100). To regain the balance, different therapeutic agents including those from natural sources are used to inhibit the gastric secretion or boost the mucosal defense mechanisms by increasing mucus production and stabilizing the surface epithelial cells (101). The experimental models used to evaluate an anti-gastric ulcer activity in the present study were those which are commonly employed to induce gastric ulceration. Stress, indomethacin (nonsteroidal anti-inflammatory drug) and ethanol were used as (13) aggressive factors to induce gastric ulceration.

The AqCR was found to exert an anti-gastric ulcer activity in water-immersion stress-induced ulcers model. Both physical and psychological stress can cause gastric ulcerations in animal and human models. The vagus nerve over activity has been suggested as the principle effecter in stress-induced ulceration (102). An increase of gastric acid secretion that often termed as the aggressive factor is considered to be an important factor in the stress ulcers (103-105). Moreover, acid secretion in stressed animals was completing inhibited by vagotomy (106). Not only vagotomy and/or anti-

muscarinic agent inhibit stress-induced gastric ulcers, but also other anti-secretory agents such as cimetidine, a H_2-receptor antagonist (107). In addition, response in stress mediated by the sympathetic nerve to increase epinephrine level pronouncedly constricts the arteries that cause reduction of the gastric mucosal blood flow (15). Other mechanisms have been propose such as increased gastric motility (108, 109), decreased gastric blood flow (108-110), decreased prostaglandin levels (108, 109), mast cell degranulation (108, 109) and decreased mucus content (111). Recently, ROS has been suggested to play a role in the pathogenesis of stress-induced gastric damage (112, 113).

The AqCR showed protective effect on HCl/Ethanol-induced gastric ulcers. HCl caused severe damage to gastric mucosa (114) whereas ethanol produced necrotic lesions by direct necrotizing action which in turn reduced defensive factor like the secretion of bicarbonate and production of mucus (115). Additionally, ethanol caused gastric ulcers by the decreased gastric mucosal blood flow whereas gastric acid had little part in such lesion formation (116). The products of the 5-lipoxygenase pathway may also play a key role in the development of ulcers, induced by irritant agents such as ethanol (117). Ethanol is not only being a superficial aggressive, but it can also cause releases of tissue-derived such as histamine and LTC4 (118). Leukotriene antagonists and 5-lipoxygenase inhibitors where shown to be capable of inhibiting ethanol and NSAIDs-induced gastric ulceration in rats (119). The involvement of ROS in gastric ulcer formation has been suggested (46, 48, 49). Gazzieri D *et al*. (1987), proposed that the ethanol increases ROS generation in gastric mucosa via transient receptor potential vanilloid (TRPV1) on sensory nerves to release substance P, which stimulates epithelial neurokinin 1 receptors to generate damaging ROS, resulting in tissue damage (120).

The AqCR exhibited an anti-gastric ulcer activity in indomethacin-induced gastric ulcer in rats. NSAIDs such as indomethacin inhibit biosynthesis of cytoprotective prostaglandins, e.g. PGE_2 and PGI_2 in the cyclooxygenase (COX) pathway of arachidonic acid metabolism (121). Prostaglandins which are present in the normal gastric and duodenal mucosa, especially PGE2 and PGI2 prevented gastric injury by stimulation of mucus secretion (122) and inhibition of gastric secretion (25).

The inhibition of COX pathway leads to overproduction of leukotriene and other products of 5-lipoxygenase pathway (123). Increase in leukotriene C4 may induce mucosal vasoconstriction and enhance NSAIDs-induced injury (124). NSAIDs inhibit non- specifically the both rate-limiting isoenzymes, COX-1 (constitutive) and COX-2 (inducible) suppress prostaglandin synthesis thereby causing gastric and intestinal ulceration (125), reduction of gastric mucosal blood flow and delay of gastric healing (126, 127). COX-1 is found in many tissues, and the prostaglandins produced in tissues by COX-1 appear to be important for a variety of normal physiologic process. In contrast, COX-2 is found primarily in inflammatory cells and the products of its actions play a major role in tissue injury, e.g. inflammation (128). NSAIDs which are selective COX-2 inhibitors cause less GI side effects including gastric and intestinal ulceration (129, 130). Other major factors include indomethacin-induced microvascular injury (131), neutrophil infiltration (132), induction of pro- inflammatory TNF-α expression (133, 134), nitric oxide imbalance and apoptosis (134-136) and extracellular matrix damage by modulation of matrix metalloproteinases -9 and -2 (137). ROS caused lipid peroxidation leading to gastric erosions (138-142).

The cytroprotective effects describe the property of prostaglandins (and other compounds which have no structural similarity with prostaglandins) by which cells are rendered defensive to stave off gastric mucosal lesion induced by various necreotiging agents such as ethanol, strong acids or base, and non-steriodal anti-inflammatory drugs (NSAIDs) like indomethacin or aspirin (126, 127).

The 3 models of gastric ulceration used in the study: restraint water immersion stress, HCl/Ethanol and indomethacin represent gastric ulcers occur by stress, irritating substance and NSAIDs, respectively. At the dose of 100 mg/kg, the anti-gastric ulcer activity of the AqCR were in the order of: indomethacin (86.19%) > HCl/Ethanol (81.95%) > stress (59.03%). The AqCR is effective in indomethacin and HCl/Ethanol models thus implying that it has a cytoprotective activity (126, 127) which might be mediated via prostaglandin. Additionally, it is known that polysaccharide with gel formation property is present in *C. racemosa* (83), thus the polysaccharide can coat the gastric mucosa, and protect against aggressive factors. *C. racemosa* has been shown to have an antioxidant activity (10, 92). Hence, the

antioxidant activity of the AqCR is likely to be responsible its anti-gastric ulcer activity. Other mechanisms involved in the anti-gastric ulcer activity of the AqCR such as inhibition of gastric acid secretion, increase of gastric blood flow, inhibition of leukotriene and other 5-lipoxygenase pathway products, etc. could not be proposed in the present study.

The AqCR was evaluated for a hepatoprotective effect in rats with CCl_4-induced liver damage. When the liver is injured as a result of the introduction of infectious agents or chemicals, the serum levels of ALT and AST are raised significantly (143). The increase of ALT level in serum has been attributed to damage the structural integrity of the liver (144). They may be released from the cytoplasm into the blood circulation rapidly after rupture of the plasma membrane and cellular damage (145). Most of the hepatotoxic chemicals damage liver cells mainly by inducing other oxidative damages and lipid peroxidation (58). CCl_4 has long been reported as a hepatotoxin and is commonly used as a model to evaluate hepatotoxicity (78, 143, 146, 147). At a suitable dose, CCl_4 causes extensive necrosis in the liver centrilobular regions around the central veins (78, 148).

CCl_4 is biotransformed by the cytochrome P-450 in the liver endoplasmic reticulum to the highly reactive trichloromethyl free radical ($^{\bullet}CCl_3$). This free radical in turn reacts with oxygen (O_2) to form a trichloromethylperoxy radical ($^{\bullet}OOCCl_3$), which may attack lipids on the membrane of endoplasmic reticulum more readily than the trichloromethyl free radical ($^{\bullet}CCl_3$). The trichloromethylperoxy radical ($^{\bullet}OOCCl_3$) leads to elicit lipid peroxidation, the disruption of Ca^{2+} homeostasis, elevation of hepatic enzymes and finally results in cell death (58, 149). Thus, antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl_4-induced liver injury (58, 150,151).

Silymarin, a flavonoid from milk thistle was used as a standard agent in the study for hepatoprotective activity. It is a widely used hepatoprotective drug, and its strongest active component is silibinin. It has been suggested (152) that silibinin and silymarin act in four different ways: (i) as antioxidants, scavengers and regulators of the intracellular content of glutathione; (ii) as cell membrane stabilisers and

permeability regulators that prevent hepatotoxic agents from entering hepatocytes; (iii) as promoters of ribosomal RNA synthesis, stimulating liver regeneration; and (iv) as inhibitors of the transformation of stellate hepatocytes into myofibroblasts, the process responsible for the deposition of collagen fibers leading to cirrhosis. Thus the key mechanism that ensures hepatoprotection appears to be free radical scavenging.

CCl_4 treatment caused in significantly increases of the levels of ALT and AST enzymes. The pretreatment with silymarin or the AqCR at the dose of 100 and 500 mg/kg could protect the increases of ALT and AST enzymes of rats with CCl_4-induced hepatic damage. It is therefore suggested that the AqCR exhibits a hepatoprotective activity. In CCl_4-induced hepatic damage, enzyme levels of the group pretreated with 500 mg/kg the AqCR were significantly higher than those of 100 mg/kg the AqCR suggesting that activity is not increase with increasing dose. However, the AqCR at the dose of 500 mg/kg does not produce liver damage since no significant difference of ALT levels between the normal and the 500 mg/kg the AqCR groups. According to histopathological examination of the livers, less degenerative of hepatocytes and fatty changes were seen in rats with the AqCR pretreatment. The results therefore are in accordance with the biochemical enzymatic analysis. Marine algae such as *Gracilaria edulis* (79), *Myagropsis myagroides* (78), *Sargussum henslowinum* (78), *S. polycystum* (76, 77) *S. siliquastrum* (78) and *Ulva lactuca* (69) have been reported to exhibit a hepatoprotective activity and suggested to be due to their antioxidant properties. *C. racemosa* has been reported to have an antioxidant activity (10, 92). It is therefore possible that the hepatoprotective activity of the AqCR is due to its antioxidant activity.

The AqCR was assessed for an antioxidant activity by using DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay. DPPH is a compound that possesses a nitrogen free radical and is readily destroyed by a free radical scavenger. This assay was used to test the ability of the antioxidative compounds functioning as proton radical scavengers or hydrogen donors (153). The AqCR exhibits an antioxidant activity which confirms the report by Chew *et al.* (2007) (10) and Cavas *et al.* (2005) (92).

The AqCR showed an ability to inhibit lipid peroxidation, when tested on rat liver homogenate. Lipid peroxidation is recognized as an important deteriorative reaction in biological membrance. In living animal cells peroxidized membranes lose their permeability, becoming rigid, reactive and nonfunctional. Lipid peroxidation produces singlet oxygen, hydroperoxides and lipid epoxides (43) which destroy polyunsaturated fatty acid and leading to a production of a major metabolite, malondialdehyde (MDA). MDA is a marker of cell membrane lipid peroxidation. (43, 45) In the test for anti-lipid peroxidation activity, Thiobarbituric acid (TBA) is used to determine MDA formation, which is the basis for evaluating the extent of lipid peroxidation. It has been reported that, *C. racemosa* showed a presence of antioxidant enzymes and anti-lipid peroxidation activity (92). Ability to scavenge hydroxyl radical is suggested to be related to the anti-lipid peroxidation activity.

The AqCR showed the presence of phenolic compounds. The estimation of phenolic content of the AqCR was done by using Folin–Ciocalteu reagent that produced blue color by reducing yellow hetero polyphosphomolybdatetungstate anions (154). More was the number of hydrogen donating groups in the phenolic compounds; more was the intensity of blue coloured complex that indicated the higher total phenol content (155-157). Phenolic compounds which are present in marine algae, e.g *Caulerpa racemosa* (10), *Kappaphy alvarezzi* (10), *Sargussum ringgoldianum* (11) and *Padina natillarum* (10), are suggested to function as antioxidative components. The antioxidant activities of phenolics are mainly due to their redox properties that allow them to act as a reducing agent, hydrogen donors and singlet oxygen quenchers (157-160). In addition, they have a metal chelation potential (157, 161-163). Therefore, it is possible that phenolic compounds which are present in the AqCR are responsible for its antioxidant activity.

Oxidative stress has been proposed to play roles in the gastric damage (42, 51, 52) and hepatic damage (54-58). The AqCR has been shown to possess and antioxidant activity. The anti-gastric ulcer and hepatoprotective activities of the AqCR are mediated by its antioxidant activity.

In conclusion, the study has revealed an anti-gastric ulcer, hepatoprotective and antioxidant activities of the AqCR. The presence of phenolic compounds in the AqCR is responsible for its antioxidant activity which may play a role in the anti-gastric ulcer and hepatoprotective activities. Gel formation capacity due the presence of polysaccharide in the AqCR might provide a cytoprotective effect against gastric ulceration. Hence, a green seaweed *C. racemosa* could be considered as a source of natural antioxidant and may be useful for alleviating diseases arising from over free radical production.