SUMMARY OF MY MASTER THESIS

Colon cancer is considered the second common type of malignant tumors worldwide. Diet that contains less carbohydrates, high fats and proteins may be a predisposing factor in the colon cancer occurrence. It is estimated that in 2020, there will be 16 million new cases of colon cancer all over the world. 1,2dimethylhydrazine (DMH) is a pro-carcinogen of the colon. It can undergo a series of activation reactions in the liver to be converted to the active metabolite, methyldiazonium ions, that cause DNA bases mismatch and mutation. Moreover, DMH causes an increase in proliferation and apoptosis. Importantly, DMH exerts the same histopathological and molecular characteristics changes like that of the human colon cancer and therefore it is used extensively to induce colon cancer in the experimental animals.

Probiotics are a group of microorganisms which improve the balance of the normal flora in the intestine. They exert a beneficial effect to the host by adjusting non-specific, cellular or humoral immunity. In some cases, the presence of *lactobacilli* probiotic in the food and medicines could inhibit the attack of pathogenic bacteria and cancer formation. Moreover, Probiotics enhance the gut homeostasis, for example, the barrier function and intestinal epithelial integrity.

Prebiotics are bioactive substances found in foodstuff that can enhance the beneficial microbiota and metabolism of the gastrointestinal tract (GIT). Prebiotics are fermented by the non-pathogenic bacteria in GIT to short chain fatty acids like acetate, butyrate and propionate which prevent the development of colon cancer. Inulin is a prebiotic which contains a polymer of fructooligoosaccharide linked together by β-(2-1)- bond and it is found in many edible plants like chicory. Inulin is not absorbed not metabolized in human small intestine. Remarkably, inulin has an important role in decreasing the pre-cancerous lesion of colon cancer.

Combinations of prebiotics and probiotics are known as synbiotics which provide a synergistic effect that improve the growth of beneficial microbiome along with newly added ones in the colon. For instances, the combination of Bifido-bacterium longum as a probiotic and inulin as a prebiotic previously showed prominent advantages in reducing colonic lesions.

Selenium is one of the trace elements that is found in many dietary products such as cereals, meat, fish and milk. Selenium exhibits both antioxidant and pro oxidant activities in various disease models. Therefore, it showed a protective effect against colon, prostate and lung cancers.

Beta-catenin represents a key element in colon carcinogenesis and tissue homeostasis. In normal conditions, β-catenin signaling is responsible for maintaining cell proliferation in the crypt of small intestine. However, aberrant β-catenin signaling produces an imbalance between the intestinal epithelium differentiation and proliferation and therefore eventually tumor formation. JNK is a member of a large family of kinases called MAPK which are responsible for regulation of apoptosis, cell proliferation and differentiation. Previous studies showed that JNK-1 is reported as a tumor suppressor gene. Interestingly, there is a correlation between JNK-1 and βcatenin. JNK-1 activation leads to activation of GSK3β by inhibiting its phosphorylation on serine amino acid position no 9 (SER9). In turn, the active GSK3β phosphorylates beta catenin and inhibits its actions by forming degradation complex, hence downstream target genes of βcatenin like c-myc is decreased.

Angiogenesis and apoptosis are two essential processes involved in tumor growth and metastasis. VEGF is an angiogenic molecule responsible for tumor progression. Apoptosis or the programmed cell death is responsible for the deletion of unwanted, damaged or infected cells from the body with Caspase 3 acts as an effector molecule

This study aimed to address the role of *Lactobacillus casei*, Inulin, or both in the protection against DMH-induced colon cancer in Swiss mice. In addition, the role of selenium in the treatment of DMH-induced colon cancer in BALB/C mice through influencing apoptosis, angiogenesis and oxidative stress processes was investigated.

For Experiment (1): protection against colon cancer, Swiss mice were divided into five groups: Control group, DMH- treated group, Prebiotic + DMH- treated group, Probiotic + DMH- treated group, and Synbiotic + DMH- treated group. Microbiome analysis of the fecal samples, biochemical measurements, histopathological examination of the colon tissues, immunostaining and Western blotting analysis of β-catenin, GSK3β and JNK-1 were performed.

The results of experiment no 1 showed that there was no change in the body weight between the DMH-treated mice and the control group except after 24 weeks of the experimental period. Administration of inulin, *L.casei* or inulin plus *L.casei* together with DMH significantly decreased the body weight compared to either the control or the DMH groups. Furthermore, pre-, pro- and synbiotic treatments decreased the blood glucose, triglycerides and total cholesterol levels. Moreover, the results showed that (*L. casei* plus inulin plus DMH)- treated group exhibited significantly decreased CEA levels, ACF number as well as other colon cancer features compared to either (*L. casei* plus DMH) or (inulin plus DMH)- treated groups. Interestingly, *L. casei*, inulin or *L.casei* plus inulin exert their protective effects against colon cancer through increasing the expression of phosphorylated JNK-1 while decreasing the expression of phosphorylated GSK3β and ultimately β-catenin expression. The microbiome analysis showed an increased bacterial diversity in the synbiotic- treated animal group. Specific enhancement of the genus *Akkermansia* was noticed in the *L. casei* treated groups (DMH+ *L. casei*) and (DMH+ Inulin+ *L. casei*) compared to non-*lactobacillus* treated groups.

For Experiment (2): Treatment of colon cancer, BALB/c mice were divided into three groups:

Control group, DMH-treated group and DMH plus Selenium treated group. Selenium was given to DMH plus selenium-treated group in the drinking water for 3 months after the induction of colon cancer.

The results of experiment 2 showed that there was no statistically significant difference in the reduced glutathione and malondialdehyde contents between DMH-treated group and the control group after 32 weeks of DMH administration. Moreover, glutathione peroxidase activity was decreased significantly in the DMH-treated group compared to the control group. Interestingly, our results demonstrated that administration of a large dose of selenium for long periods increased the oxidative stress, as evidenced by increasing MDA and decreasing GSH levels and glutathione peroxidase activity. Furthermore, the chronic administration of selenium increased caspase-3 expression while resulted in a significant decrease in VEGF expression. These results point to the apoptotic and anti-angiogenic effect of selenium as well as a pro-oxidant activity against the tumor cells**.**