DETERMINATION OF MAXIMUM TOLERATED DOSAGE OF HUANG LIAN IN MICE AND ITS EFFECT OF INTESTINAL LACTOBACILLUS POPULATION

CHONG LEE CHING, JANICE

Dissertation submitted in partial fulfillment of the
requirement for the course
Degree of Bachelor (Hons) of Traditional Chinese Medicine
Faculty of Traditional Chinese Medicine
Inti International University
July 2015

DECLARATION

I hereby declare that the thesis is based on my original work except for the quotation and citation which have been fully acknowledged.

JULY 2015

CHONG LEE CHING, JANICE

I11008963

ACKNOWLEDGEMENTS

I would like to give my upmost gratitude to Miss Yuka Hara, my mentor for her time and commitment in guiding me throughout this project. The knowledge, the advices and techniques I learned from her had been a great value for me. I am truly grateful that I had the opportunity to be under her mentorship during this project.

My sincerity also goes to the Animal House faculty members from University Kebangsaan Malaysia who patiently and earnestly taught us how to handle and care the mice for this experiment. I also want to express my gratitude to all the faculty members for their cooperation and preparation of facilities throughout this experiment.

I also own my gratitude to my university, Inti International University for providing funds and free journals to support this project.

To my teammates, I want to express my thanks for their transportation and cooperation throughout the experiment.

ABSTRACT

Huang Lian also known as Rhizoma Coptidis is a dried rhizome of *Coptis chinensis* which belonged to the Genus *Coptis* and family of Ranunculaceae. It is used to clear Heat and dries Dampness in Traditional Chinese Medicine. *Xiang Sha Liu Jun Zhi Tang* is a formula decoction used to tonify the Spleen and Stomach and improve the digestive functions. *Lactobacillus* is an anaerobic bacterium that belonged to the order of lactic acid bacteria (LAB). It is categorized as probiotics which commercially acts as a treatment for diarrhea, indigestion, urinary tract infections and enhancing immunity. Thus the objectives of this study are to determine the maximum tolerated dosage of *Huang Lian* in mice and analyses its impact on *Lactobacillus* population with and without XSLJZT. In this experiment, 35 mice are divided into 7 groups with 5 mice each group. Three groups received *Huang Lian* with the dosage raging from 65mg/kg, 250mg/kg and 500mg/kg respectively while another 3 groups received *Huang Lian* as described previously combined with 50mg/kg of XSLJZT. The control group of mice was fed with same volume of saline. The weights of the mice were recorded weekly while and the feaces of mice were collected weekly for cultivation of *Lactobacillus* bacteria. Results showed that *Huang Lian* generally inhibit *Lactobacillus* populations while XSLJZT reduce the inhibition effect. *Huang Lian* has little effect on the weight of mice while XSLJZT significantly increase the weight of the mice during the duration of 28 days. In conclusion, *Huang Lian* generally inhibit the *Lactobacillus* while has little effect on the weight of mice.

TABLE OF CONTENTS

CHAPTER 3 RESEARCH METHODOLOGY

CHAPTER 4 RESULTS AND DISCUSSION

CHAPTER 5 CONCLUSION

LIST OF FIGURES

CHAPTER 1 INTRODUCTION

1.1 Research Background

1.1.1 *Huang Lian*

Huang Lian also known as Rhizoma Coptidis is the dried rhizome of *Coptis chinensis* which belonged to the Genus *Coptis* and family of Ranunculaceae (Chen and Chen, 2004). In Traditional Chinese Medicine, it is mainly used to clear Heat and dries Dampness. It can enter into the Heart, Liver, Stomach and Large Intestine Meridians. *Huang Lian* has been used widely used to treat gastrointestinal disease due to Damp Heat or Stomach Fire such as diarrhea, dysentery, gastric reflux, peptic ulcer, nausea and vomiting. *Huang Lian* is effective in treating Heart Disease like palpitation, insomnia, vexation due to Heart Fire (Chen and Chen, 2004). In modern science, *Huang Lian* prove to has a vast antibiotic effect on bacteria such as *Bacillus dysenteriae, Salmonella typhi, E.coli, Vibrio cholera, Staphlococcus aureus* and others (Chen and Chen, 2004). *Huang Lian* also has antifungal and antibacterial properties and has been demonstrated to be effective against influenza and hepatitis viruses (Chen and Chen, 2004).

Berberine, one of the active components from *Huang Lian* has been used for the treatment of hyperlipidemia by lowering total cholesterol, triglyceride and low-density-lipoprotein receptor of statins (Kong et al., 2004). Besides, berberine can be used to treat nervous system disease such as neurodegenerative disease, Alzheimer's disease and neurotrosis (Zhu and Qian, 2006) as it can permeate the central nervous system (CNS)(Wang et al., 2005). Berberine is also an effective anti-diabetic agent as it can increase the insulin sensitivity and inhibit the gluconeogenesis process in the liver (Xia et al., 2011). Berberine was also known for its anti-inflammatory effect which may be the mechanism on treating diabetic rats (Tang et al., 2009). Even though berberine demonstrates positive result clinically, the plasma level of berberine is very low and

even less than 1%. The absolute bioavailability of berberine has been reported to be 0.68% (Xie et al., 2011).

However, Huang Lian is bitter, cold and drying in nature. Excessive and prolonged consumption of Huang Lian can cause Deficiency of Spleen and Stomach and may be associated with symptoms such as dizziness, headache, tinnitus, nausea, vomiting, palpitation, abdominal fullness, diarrhea and reduction in red blood cells. Thus, it is important to determine maximum tolerated dosage of Huang Lian in this study. In clinical practice, the cold nature of Huang Lian can be minimized by combining Huang Lian with other warm nature herbs in herbal formulas or using Huang Lian in a small amount. The maximum dosage of Huang Lian used is 20g in clinical practice.

1.1.2 *Xiang Sha Liu Jun Zhi Tang*

Xiang Sha Liu Jun Zi Tang is a frequently used tonic Chinese herbal formula. The formula originated from Gu Jun Ming Yi Fang Lun Volume II, Discussion of Ancient and Modern Famous Doctors' Formula, by Luo Mei in 1675 (Chen and Chen, 2009). Later, the formula was changed into pills in Wan San Gao Dan Ji Cheng (Collection of Medicinal Pills, Powders, Plasters, and Other Chinese Drugs). The formula was then named as *Xiang Sha Liu Jun Zi Wan* in Quan Guo Zhong Yao Cheng Yao Chu Fang Ji which is published in 1965. *Xiang Sha Liu Jun Zi Tang* is composed of *Ren Shen* (Ginseng root), *Bai Zhu* (Atractylodes Rhizome), *Fu Ling* (Poria, China Root), *Gan Cao* (Licorice Root), *Chen Pi* (Tangerine Peel), *Ban Xia* (Pinellia Rhizome), *Sha Ren* (Cardamon), *Mu Xiang* (Costus Root), *Sheng Jiang* (Fresh Ginger Rhizome) (Xie and Preast 2010). This formula is the modification of *Liu Jun Zi Tang* which in turn is the modification of *Si Jun Zi Tang* by adding *Chen Pi* and *Ban Xia*. *Xiang Sha Liu Jun Zi Tang* is yielded by adding *Mu Xiang* and *Sha Ren* to the *Liu Jun Zi Tang*.

The Chinese therapeutic actions of this formula are to tonify Qi, strengthen the Spleen, regulate Qi and harmonize the Stomach (Chen and Chen, 2009). In Traditional

Chinese Medicine theory, Spleen and Stomach are the source of Qi and Blood (Xie and Preast, 2010). It is only when these two organs are strong and the digestive system working properly that the Qi and Blood can be supplied to the rest of the body. Only then our body can be healthy and free of diseases. *Xiang Sha Liu Jun Zi Tang* uses *Ren Shen* (Ginseng root), a warm and sweet herb to tonify the Yuan Qi, Lung and Spleen Qi so that the body is strong enough to ward off the pathogens. *Bai Zhu* (Atractylodes Rhizome) and *Fu Ling* (Poria, China Root) tonify the Spleen, dispels and drains Dampness. *Ban Xia* and *Chen Pi* are both acrid and warm, thus it can regulate Qi, dry Dampness and dispels phlegm. *Mu Xiang* (Costus Root) dries Dampness, regulate Qi, warms and strengthen the Spleen. *Mu Xiang* also had the function to prevent stagnation from tonic herbs in this formula. *Sha Ren* (Cardamon) can dry Dampness, harmonizes the Stomach and descend the Qi. *Zhi Gan Cao* (Licorice Root) is warm and sweet thus can harmonizes the Middle Burner and tonify Qi (Chen and Chen, 2009). This formula is the combinations of four sweet and warm herbs from *Si Jun Zi Tang* and four acrid and warm herbs which are *Sha Ren*, *Mu Xiang*, *Chen Pi* and *Ban Xia*. *Xiang Sha Liu Jun Zhi Tang* is used to treat patients with weak digestive system, stagnation of Qi and Dampness, abdominal distension, weak appetite, fatigue, weight loss, anorexia, diarrhea, weak pulse and white greasy tongue coating (Chen and Chen, 2009). *Xiang Sha Liu Jun Zhi Tang* is combined with *Huang Lian* in this study in hope that the decoctions can minimize the cold nature of *Huang Lian*.

1.1.3 *Lactobacillus*

Lactobacillus is Gram-positive, anaerobic bacteria that belong to the lactic acid bacteria group which is characterized by their production of lactic acid (Singleton, 2004). They are many types of *Lactobacillus* strain such as *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus equigenerosis, Lactobacillus equi* and among many others (Singleton, 2004). It is categorized as probiotics which according to WHO classification, it is microorganism that contribute to health benefits when an adequate amount is administered to the host (FAO/WHO, 2001).

Lactobacilli colonization of the gut starts even within the first week of life (Salminen et al., 1995). It is essential for the maintenance of a healthy gut intestinal microbial ecosystem (Sandine, 1979). *Lactobacillus acidophilus* and *Lactobacillus casei* are the most common strains of *Lactobacilli* and are used as supplement in drinks such as Yakult and Vitagen.

Lactobacillus has several important role in the gut such as reducing lactose intolerance (Jiang et al., 1996), improving immune system (Aattouri et al., 2001), promote growth in farm animals (Baird, 1977), inhibit putrefactive bacteria by reducing the pH in large intestine (Langhendries, 1995), improving digestion of food, decreasing the rate of infections in the gut, relief constipation (Walker and Duffy, 1998) and lowering cholesterol (Bertazzoni et al., 2001). Numerous studies have been done on *Lactobacilli* in the prevention and treatment of gastrointestinal disease caused by enterobacteria, *E coli* (Oyetayo et al., 2004) and *H pylori* (Coconnier et al., 1998). In addition, *Lactobacillus* was also proven to be effective in treating vaginal and urinary tract infections in numerous clinical studies (Martinez et al., 2009). Despite numerous clinical studies and research done on the benefit of *Lactobacillus*, the Food and Drug Administration (FDA) has not approved the use of *Lactobacillus* as a medical product. It is treated as a supplement (Penn State Hershey, 2015).

1.2 Research Objectives

1.2.1 General Objectives

The general objective of this study is to determine the maximum tolerated dosage of *Huang Lian* in mice and analyse its impact on *Lactobacillus* population.

1.2.2 Specific Objectives

Specific objectives of this study are

(i) To determine the effects of *Huang Lian* on mice at different dosage.

(ii) To determine the difference of *Lactobacillus* population and the weight between mice that is given *Huang Lian* only and *Huang Lian* with *Xiang Sha Liu Jun Zhi* solution.

CHAPTER 2 LITERATURE REVIEW

2.1 *Huang Lian* and Berberine

Meta-analyses or clinical trials on the efficacy of *Huang Lian* have been conducted for a number of common gastrointestinal disease such as *Helicobacter pylori* infection (Lu et al., 2007), diarrhea, dysentery and constipation (Chen and Chen, 2004). These studies have shown that *Huang Lian* have significant effects on the prevention and treatment of gastrointestinal disease. *Huang Lian* also demonstrates anticancer effects as berberine can exert anti-invasion and anti-metastasis characterization on some cancer cells (Tang et al., 2009).

Previous studies also showed that *Huang Lian* efficacy in treating inflammation related and gastrointestinal related symptoms. Current research on *Huang Lian* is focused on its anti-obesity, anti-diabetic effects as well as its anti-microbial effect. Hu et al., 2012 showed that Berberine promotes mild weight loss in obese human subjects. In this study, Berberine has been show to decrease level of triglyceride by 23% and cholesterol level by 12.2% in the subjects. However, other study done using both *Huang Lian* and Berberine on mice demonstrated that *Huang Lian* significantly decreased the triglycerides level in mice while Berberine did not exert such effect on HFD mice (Xie et al., 2011).

Berberine, an active component of *Huang Lian* tends to reduce the weight of normal chow diet (NCD)-fed rat to a lesser extent than high fat diet (HFD)-fed rat. The adiposity index of the HFD-fed mice was significantly decreased compared with the NCD-fed mice (Zhang et al., 2012). Berberine also significantly reduces fasting blood glucose (FBG) in HFD mice but not significantly in NCD mice (Zhang et al., 2012). Previous clinical trial showed that oral administration of berberine at a dose of 1g per day for the duration of 3 months is effective in treatment of type 2 diabetic (Zhang et al., 2012). No obvious side effects were reported in this study. Liver, cardiovascular, kidney

function and blood tests were done after the treatment indicate that 500mg of berberine fed three times per day did not exert any harmful effect on the subjects (Hu et al., 2012).

In the study done by (Xie et al., 2011) berberine decreases both the Firmicutes and Bacteroidetes in the gut of HFD-fed mice which further proving that the antimicrobial activity of berberine lead to anti-obesity effects. Lactobacillus sp. (CICC21024) a species of Firmicutes was also found to be significantly inhibited by berberine in vitro. However in another study, it is showed that Berberine had no or weak inhibition effect on *Lactobacillus acidophilus and Lactobacillus casei* (Chae et al., 1999)

The richness of the gut microbiota was also significantly reduced by berberine. Berberine significantly decreases the weight and the bacterial diversity of the gut microbiota in both NCD- and HFD-fed rats (Zhang et al., 2012). In a study done by (Gong et al., 2014), it is reported that the plasma berberine concentration increase significantly in post inflammation irritable bowel syndrome rats compared to the control group. Thus, this lead to the hypothesis that pharmacokinetics of berberine may be different in the normal control group compared to the pathological group.

However, berberine has low oral bioavailability. For animal model, the maximum concentration of berberine in plasma was 4ng/ml after oral administration of 100mg/kg berberine (Liu et al., 2009). As for humans, the maximum concentration of berberine in plasma was 0.4ng/ml after oral administration of 400mg of berberine in a single dose (Hua et al., 2007). Berberine is also poorly absorbed into the bloodstream from the gut thus researchers deducted that berberine exerts its effect through modulating gut microbiota (Han et al., 2011). This hypothesis had been suggested to other traditional Chinese Medicine herbs (Zhang et al., 2011). However, there is another study done on the tissue distribution of berberine which reported that the concentration of berberine is much higher in organs and tissues compared to plasma (Tan et al., 2013). The metabolites of berberine were found in liver, kidneys, lungs, muscles and pancreas after 4 hours of oral administration. This result might lead to the hypothesis of the

mechanism of berberine on human diseases even though the plasma concentration of berberine is very low.

2.2 *Xiang Sha Liu Jun Zi Tang*

XSLJZT is known to promote gastrointestinal motility and gastric emptying, decrease gastric sensitivity, and regulate gastrointestinal hormone (Hayakawa, T et al. 1999). Many clinical research shows that *Xiang Sha Liu Jun Zi Tang* can effectively treat functional dyspepsia through improving gastrointestinal hormone secretion. The study also demonstrated that *Xiang Sha Liu Jun Zi Tang* is more effective in treating functional dyspepsia than prokinetic drugs (Ya Xiao et al., 2012). Another study demonstrated that XSLJZT can alleviate stomach pain, diarrhea and vomiting in dyspepsia patients (Zhang, Ma and Xie, 2014). Studies done on gastric cancer patients with Spleen and Stomach Deficiency Cold showed that XSLJZT can relieve the symptoms of gastric cancer and Spleen and Stomach Deficiency Cold (Zhang, Ma and Xie, 2014). Another study demonstrated that dyspepsia patient initially had increased number of mast cells. After treatment of XSLJZT for 28 days, the mast cells decreased significantly. Thus, the possible explanation on the mechanism of XSLJZT is related to reducing the mast cell numbers.

In addition, the tonifying Qi ingredients of XSLJZT causes the excitatory effect while the regulating Qi ingredients had causes inhibitory effect on gastrin muscles at different locations (Zhang, Ma and Xie, 2014).

One research by (Chang et al., 2014) demonstrated that *Huang Lian* and *Si Jun Zi* can significantly exert anti-microbial effect on Salmonella. Previous research also showed that *Si Jun Zi Tang* in XSLJZT can improve immunity (Liu et al., 2005). Another study done using *Si Jun Zi Tang*, *Huo Xiang Zhen Qi San*, *Ge Gen Qin Lian Tang* and *Sheng Ling Bai Zhu San* showed that only *Si Jun Zi Tang* can prevent weight loss in diarrhea (Zhang et al., 2012).