ฤทธิ์ปกป้องตับของสารสกัดเอธานอลจากเจี่ยวกู้หลาน

(Gynostemma pentaphyllum)

ในหนูขาวที่ทำให้เกิดพิษต่อตับโดยเอธานอล

นางวิไลภรณ์ ตระกูลมุทุตา

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HEPATOPROTECTIVE EFFECTS OF ETHANOL EXTRACT FROM *GYNOSTEMMA PENTAPHYLLUM* IN ETHANOL INDUCED HEPATOTOXIC RATS

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วิไลภรณ์ ตระกูลมุทุตา : ฤทธิ์ปกป้องตับของสารสกัดเอธานอลจากเจี่ยวกู้หลาน *(Gynostemma pentaphyllum)* ในหนูขาวที่ทำให้เกิดพิษต่อตับโดยเอธานอล (HEPATOPROTECTIVE EFFECTS OF ETHANOL EXTRACT FROM *GYNOSTEMMA PENTAPHYLLUM* IN ETHANOL INDUCED HEPATOTOXIC RATS) อ. ที่ปรึกษา : รศ.ดร. พรเพ็ญ เปรมโยธิน, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : ศ.ดร. ราตรี สุดทรวง, ผศ.สพ.ญ.สมลักษณ์ พวงชมภู, 86 หน้า ISBN 974-17-5872-3.

การศึกษาครั้งนี้ทำขึ้นเพื่อพิสูจน์ฤทธิ์ปกป้องตับของสารสกัดเอธานอลจากเจี่ยวกู้หลาน *(Gynostemma pentaphyllum)* ในหนูขาวที่ทำให้เกิดพิษต่อตับจากเอธานอล ในการทดลองพิษเฉียบพลันให้เอธานอลขนาด 5 กรัม/กิโลกรัม ป้อนหนูขาวทางปากเพียงครั้งเดียวมีผลทำให้เกิดการเพิ่มขึ้นของระดับไตรกลีเซอไรด์ในเลือด (STG) และในตับ (HTG) AST และ ALT ในเลือดเมื่อเปรียบเทียบกับหนูขาวกลุ่มควบคุมที่ได้รับแป้งข้าวโพด 5 % ในขนาด 5 มิลลิลิตร/กิโลกรัม ไม่พบผลต่อระดับ GSH และ MDA ในตับ เมื่อให้สารสกัดเจี่ยวกู้หลานที่สกัดด้วยตัวทำละลายเอธานอล 95% ทางปากในขนาด 300, 400 และ 500 มิลลิกรัม/กิโลกรัม มีผลในการลดระดับ AST, ALT และ HTG (โดยเฉพาะในขนาด 300 มิลลิกรัม/กิโลกรัม) ไม่พบผลต่อระดับไตรกลีเซอไรด์ในเลือด GSH และ MDA ในตับ ในการทดลองพิษกึ่งเฉียบพลันให้เอธานอลขนาด 4 กรัม/กิโลกรัม/วัน ป้อนทางปากหนูขาวเป็นเวลา 28 วัน พบว่ามีผลเพิ่มระดับไตรกลีเซอไรด์ในเลือดและในตับและ ALT การป้อนสารสกัดเจี่ยวกู้หลานในขนาด 300 มิลลิกรัม/กิโลกรัม/วัน อย่างต่อเนื่องในหนูขาวที่ทำให้เกิดพิษกึ่งเฉียบพลันต่อตับโดยเอธานอล เป็นเวลา 7, 14 และ 21 วัน พบว่ามีการลดลงของระดับไตรกลีเซอไรด์ในตับ AST และ ALT ในเลือดเมื่อเทียบกับกลุ่มควบคุมที่ได้รับเพียง 5 % ของแป้งข้าวโพดทุกวันหลังจากได้รับเอธานอลมา 28 วัน

ผลการทดลองครั้งนี้ สนับสนุนการใช้สารสกัดเจี่ยวกู้หลานในการปกป้องพิษต่อตับจากเอธานอลในหนูขาว รวมทั้งการศึกษาทางจุลพยาธิวิทยาของตับหนูขาวในการทดลองพิษเฉียบพลันและกึ่งเฉียบพลันต่อตับก็ช่วยยืนยันผลของสารสกัดเจี่ยวกู้หลานในการปกป้องตับจากเอธานอล

ภาควิชา สรีรวิทยา	ลายมือชื่อนิสิต……..…….….
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WILAIPORN TRAKULMUTUTA : HEPATOPROTECTIVE EFFECTS OF ETHANOL EXTRACT FROM *GYNOSTEMMA PENTAPHYLLUM* IN ETHANOL INDUCED HEPATOTOXIC RATS. THESIS ADVISOR : ASSOC. PROF. PORNPEN PRAMYOTHIN, Ph.D., THESIS CO-ADVISOR : PROF. RATREE SUDSUANG, Ph.D., ASST. PROF. SOMLAK POUNGSHOMPOO.86 pp. ISBN 974-17-5872-3.

This study was undertaken to investigate the hepatoprotective effects of ethanol (EtOH) extract from Jiaogulan (*Gynostemma pentaphyllum* (GP)) in EtOH induced hepatotoxic rats. In acute experiment, single dose of EtOH 5 g/kg was administered orally to rats, resulted in the significantly elevated levels of serum triglyceride (STG), hepatic triglyceride (HTG), aspartate transaminase (AST) and alanine transaminase (ALT) as compared with control rats receiving 5% corn flour at 5 ml/kg. Hepatic glutathione (GSH) and malondialdehyde (MDA) were unchanged.Treatment with95 % ethanol extract of Jiaogulan at oral dose of 300, 400 and 500 mg/kg 4 hours before EtOH, lowered the levels of AST, ALT and HTG (only at 300 mg/kg dose), whereas STG, GSH and MDA were unchanged. In sub-acute experiment, EtOH at 4 g/kg/day was administered orally to rats for 28 consecutive days, resulted in the significantly elevated levels of STG, HTG and ALT. Treated rats with Jiaogulan extract at the dose of 300 mg/kg/day in EtOH induced sub-acute hepatotoxic rats for 7, 14 and 21 days, lowered the levels of HTG, AST and ALT compared to control rats receiving dialy with 5 % corn flour after oral feeding with EtOH for 28 days.

Our data indicated that supplementation with Jiaogulan extract can offer protection against EtOH induced hepatotoxicity in rats. In addition, histopathological studies of rat liver in both acute and sub-acute hepatotoxicity studies confirmed the beneficial role of Jiaogulan extract against EtOH.

Department of Physiology Student's signature…….

Field of study Physiology Advisor's signature……

Academic year 2003 Co-advisor's signature……

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CHAPTER I

INTRODUCTION

JIAOGULAN

Jiaogulan (***Gynostemma pentaphyllum*** Makino, family Cucurbitaceae) (Figure 1) is a plant that grows wild in China as well as many other countries throughout asia. Jiaogulan has subterrean shoots (rootstocks) and its items grow like vines. It has curled and thick hairs alternately growing leaves, either of three, five or seven leaves. The leaf is oval shape with sawtooth edges, on which grows fine, white hairs. In China, it has been used for many years as a medicinal and energizing tea in the local regions where it grows. Jiaogulan is sometime called "Southern Ginseng, since it grows in south central China and its similarity to ginseng in chemical composition and function. In Japanese, this plant is called "sweet tea vine" (Amachazuru) and Dungkulcha in Korean (Takemoto et al, 1984). Jiaogulan, is said to be three time effective than ginseang. Takemoto et al (1985) found that it contained chemical constituent called triterepenoid saponins (a high molecular weight glycoside that dissolves in water oil and consisting of two components, aglycons and sugar molecule) similar to the active components of ginseng. They identified and named 82 saponins of the same kind found in ginseng (the so-called ginsenosides, the major active principle of ginseng). These saponins are named gypenoside – Jiaogulan active ingredients (Figure 2). Four gypenosides, i.e., gypenoside 3, 4, 8 and 12 are identical with saponins found in ginseng, i.e., ginsenosides, Rb 1, Rb 3, Rd and F2. Again ginseng has 28 of ginsenosides while Jiaogulan has 82 of gypenosides. Other constituents of Jiaogulan include flavonoids, organic acid, polysaccharides, amino acids, protein, trace minerals, and vitamins (Kuwahara et al., 1989). Four main dammarane – type aglycones of gypenosides extract from the aerial parts of Jiaogulan were identified by gas chromatography mass spectrometry (GC-MS) as shown in (Figure 3) (Cui et al., 1999). Because of this similarity to the expensive ginseng root,

Figure 1 . *Gynostemma pentaphyllum* Makino, (family Cucurbitaceae)

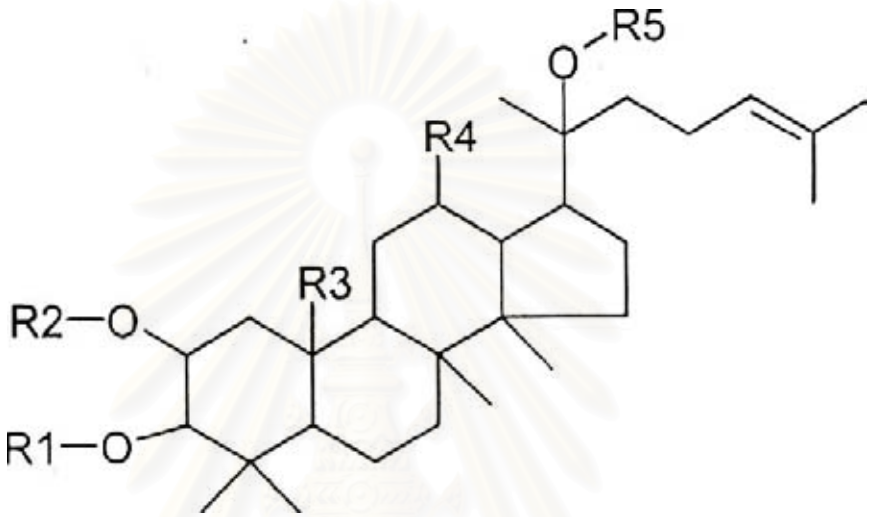


Figure 2. General structure for dammarane-type gypenosides

(where R1 = glucose, rhamnose,etc.

R2 = glucose, rhamnose, etc.

R3 = CH_3, CH_2OH, CHO

R4 = H, CH_2OH

R5 = glucose, xylose, etc.)

(Cui, Eneroth, and Bruhn., 1999)

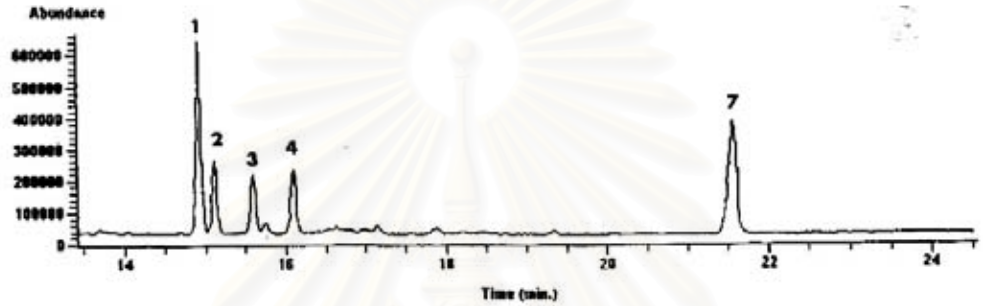


Figure 3. GC-MS chromatograms of the trimethysilyl ether derivatives of aglycones obtained after alkaline clevage of saponins extracted from *Gynostemma pentaphyllum* (Cui, Eneroth, and Bruhn., 1999).

Jiaogulan has attracted much interest as a potential new plant drug. Compare to ginseng, Jiaogulan is cheaper and easily available, and is already being exploited as general tonic.

Pharmacological studies of Jiaogulan and / or other isolated saponins have shown a variety of interesting activities, such as saponin that can increase the immunity in rats by anti the effect of cyclophosphamide, increasing hemolysin, natural killer cells and weight organ of immunity that have the function to destroy carcinogen (Shang et al., 1990). Jiaogulan extracts can inhibit platelet aggregation which stimulated by adenosine diphosphate, inhibit thrombosis, and stimulate erythopoesis (Tan et al., 1993). Water extracts of Jiaogulan are shown to have anti-inflammatory activity against carragenan which induced paw edema in rats, and the action of gypenosides as antioxidant by decreasing superoxide anion and hydrogen peroxide content in human neutrophils (Lin et al., 1993). Fed orally with Jiaogulan extract in dosage of 2% with water two weeks before injection of carcinogen (MANA) in rats, it was found that, in experimental groups the percentage of tumor cells and carcinoma of esophagus were lower than control group with injected only with MANA (Wang., et al 1995). Lacour et al (1995) found that a decoction of Jiaogulan have the effect on the reduction of triglyceride and cholesterol in hyperlipidemic rats. In 1996, Chen et al. reported that Jiaogulan helped to recover the decrease in leukocyte counts, GOT, GPT and IgG in serum and the proliferation of splenocytes stimulated by LPS in the gamma – ray irradiated mice. Experiment of Zhou et al (1998) demonstrated that liquid extract from compound of Jiaogulan and lamp-flower (1:1) could inhibit and reverse the carcinomatous conversion of leukoplasia of golden hamster cheek pouches induced by diethyl benzanthacene (DMBA), indicating its positive anticarcinogenic effect. Gypenoside extracted from Jiaogulan increase the amount of nitric oxide (NO) in the blood, NO relaxes blood vessel walls and increases flow of blood into the tissue down stream (Tanner et al., 1999). In 2001, Zhu et al. reported that Jiaogulan could inhibit

the UV induction of bacteriophage lambda in lysogenic cells. These results indicate that Jiaogulan not only is a scavenger of free radicals, but also possesses the biological function of anti-irradiation. Wang et al (2002) found that treatment of human hepatoma cells with gypenoside induced apoptosis through the up-regulation of Bax and Bak, and down-regulation of Bcl-2, release of mitochondrial cytochrome C and activation of caspase cascade.

The research of effect of Jiaogulan on hepatoprotective effect is found in Lin et al (2000) using water extract of Jiaogulan showing its hepatoprotective activity against single dose carbon tetrachloride (CCl_4) induced liver damage. Chen et al (2000) also demonstrated that gypenosides have the hepatoprotective effect and antifibrotic potential on chronic liver injury induced by injections of CCl_4 for 8 weeks by reducing aspartate transferase (AST) and alanine transferase (ALT) activities and reduced the collagen content by 33%, and in acetaminophen induced liver injury model in rats, water extract (100, 300 and 500 mg/kg) of Jiaogulan enhanced the recovery of liver injury.

Thus, Jiaogulan could have the hepatoprotective effect. In this investigatioon, we selected the rat model for acute (single oral dose of ethanol (EtOH) 5 g / kg) and sub acute (EtOH 4 g / kg / day for 28 days) hepatotoxicity studies (Udomusksorn, 1993). The effects of ethanolic extract of Jiaogulan on EtOH induced liver damage were determined. If Jiaogulan posseses the hepatoprotective activity, it may be one of alternative herbs selected for alcoholic patient and may become the economic plants in the future.

THE LIVER

The liver is the largest gland in the body. It is positioned immediately beneath the diaphargm in the right side of the peritoneal cavity. In human body it has two main lobes, the right is much larger than the left but in rats it has five or seven lobes (Wells, 1964). The lobes of the liver are made up of many functional units called lobules (Figure 4). A lobule consists of specialized epithelial cells, called hepatocytes or parenchymal cells arranged in irregular, branching, interconnected plates around a central vein. Rather than capillaries, the liver has larger spaces lined by endothelium called sinusoids, through which blood passes. The sinusoids are also party lined with stellate reticuloendothelial (Kupffer's) cells. These phagocytes destroy worn-out white and red blood cells, bacteria, and other foreign matter in the blood draining from the gastrointestinal tract (Tortora and Grabowski, 1996).

The liver receives blood from two sources, from the hepatic artery with oxygenated blood, and from the hepatic portal vein with deoxygenated blood containing newly absorbed nutrients, drugs, and possibly microbes and toxins from the gastrointestinal tract. Branches of both the hepatic artery and the hepatic portal vein carry blood into liver sinusoids, where oxygen, most of the nutrients, and certain poisons are extracted by the hepatocytes. Products manufactured by the hepatocytes and nutrients needed by other cells are secreted back into the blood. The blood drains into the central vein. Central veins drain into larger veins often called sublobular veins and these in turn drain into the hepatic veins and empty their blood into the vena cava (Figure 5). Branches of the hepatic portal vein, hepatic artery, and bile duct typically accompany each other in their distribution through the liver. Collectively, these three structures are called a portal triad or portal tract.

The liver is subdividing into functional lobule to regard as a unit of hepatocyte that region which is irrigated by a terminal branch of the distributing veins. This unit is called the hepatic acinus (Rappaport, 1956) (Figure6). Cells in the hepatic acinus

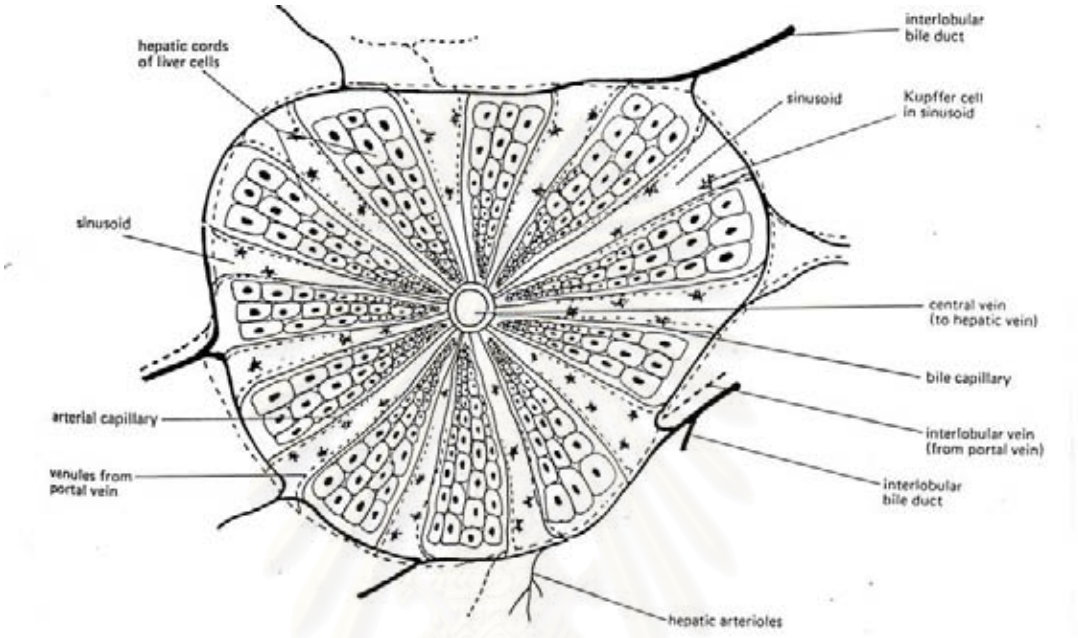


Figure 4. A liver lobule, showing the hepatic cords in the upper-right-hand section. The liver cells that make up the cords are surrounded by sinusoids-wide, blood-filled spaces formed by the capillaries of the portal vein. The arterial capillaries also empty into the sinusoids. The lobule is drained by the central vein, which empties into one of the hepatic veins. The bile capillaries, which also penetrate between the hepatic cells, empty into the bile duct via the common hepatic duct (Tortora and Grabowski, 1996).

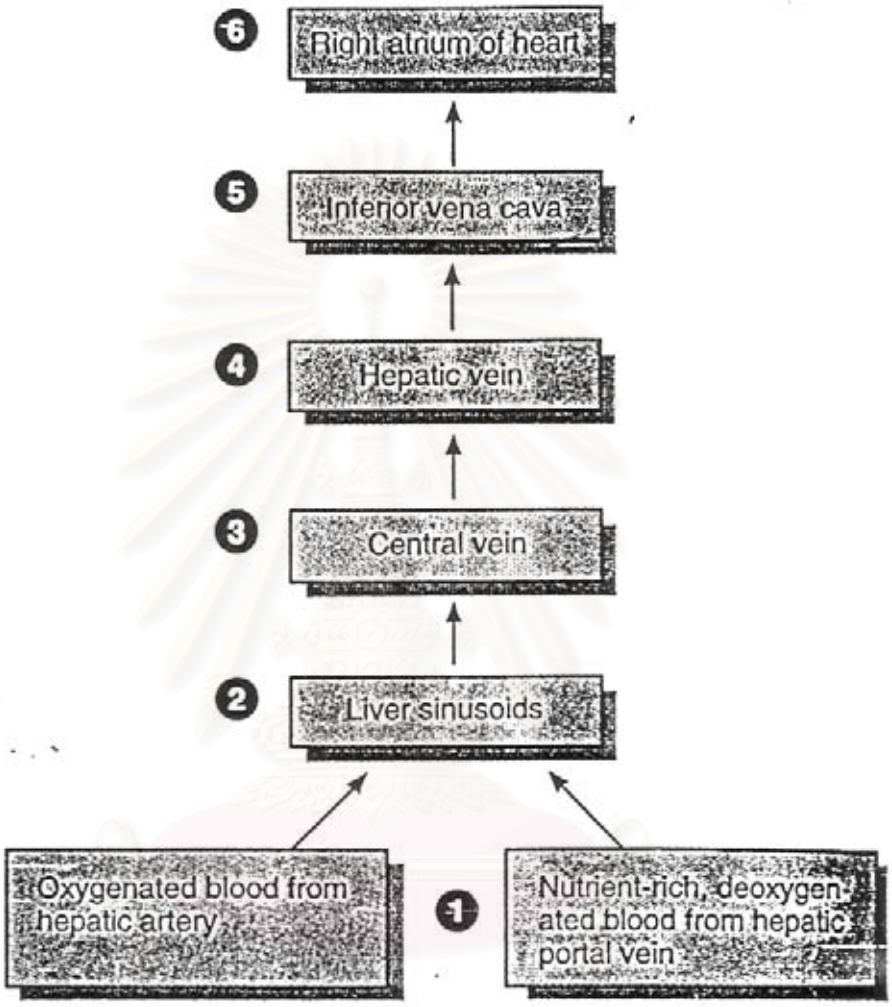


Figure 5. Blood flow through the liver and return to the heart. (Tortora and Grabowski, 1996)

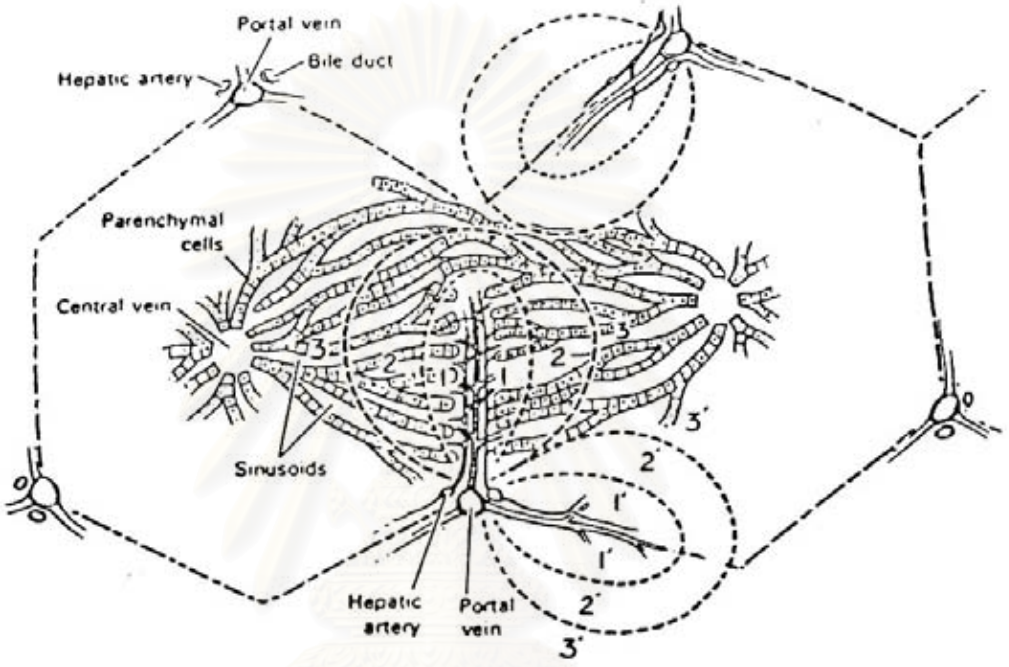


Figure 6. Blood supply through the liver and division of liver acinus zone (Rappaport, 1956).

can be subdivided into zones (Rappaport, 1965). Zone I called periportal area would be closest to the vessel and consequently the first to be affected by or to alter the incoming blood. Cell in zone II called midzone would be second to respond to the blood and zone III called centrilobular or periacinal area would be portal vein blood that has been previously altered by cells in both zone I and II. This zonal arrangement would account for some of the differences in the selective damage of hepatocytes by various noxious agents or in different disease conditions.

Morphological assessment of hepatocyte injury are divided into four stages (Kepple and Proper, 1986). First, if it has mild injury, the hepatocyte can reversibly change called degeneration. The characteristic includes cloudy swelling (cellular swelling, hydropic degeneration, vacuolar degeneration and fatty degeneration) that pre-occurs before cell death. Second, if hepatocyte injury increases and cause irreversible change this stage is called cell death. Third, pre necrotic change occur after equilibium of cell death and fluidity around cells, and finally, cell necrosis occurs after cell death including coagulative necrosis and enzymatic fat necrosis.

Cell death can be occured by either of two distinct mechanisms (Kroemer et al., 1998). **Apoptosis** ("normal" or "programmed" cell death) is the physiological process by which unwanted or useless cells are eliminated during development and other normal biological process. **Necrosis** ("accidental" cell death) is the pathological process which occurs when cells are exposed to a serious physical or chemical insult.

There are many observable morphological and biochemical differences between apoptosis and necrosis (Figure 7). Necrosis occurs when cells are exposed to extreme variance from physiological condition (e.g., hypothemia, hypoxia) which may result in damage to the plasma membrane. Under physiological conditions direct damage to the plasma membrne is evoked by agents like complement and lytic viruses. Necrosis begins with and impairment of the cell's ability to maintain homeostasis,

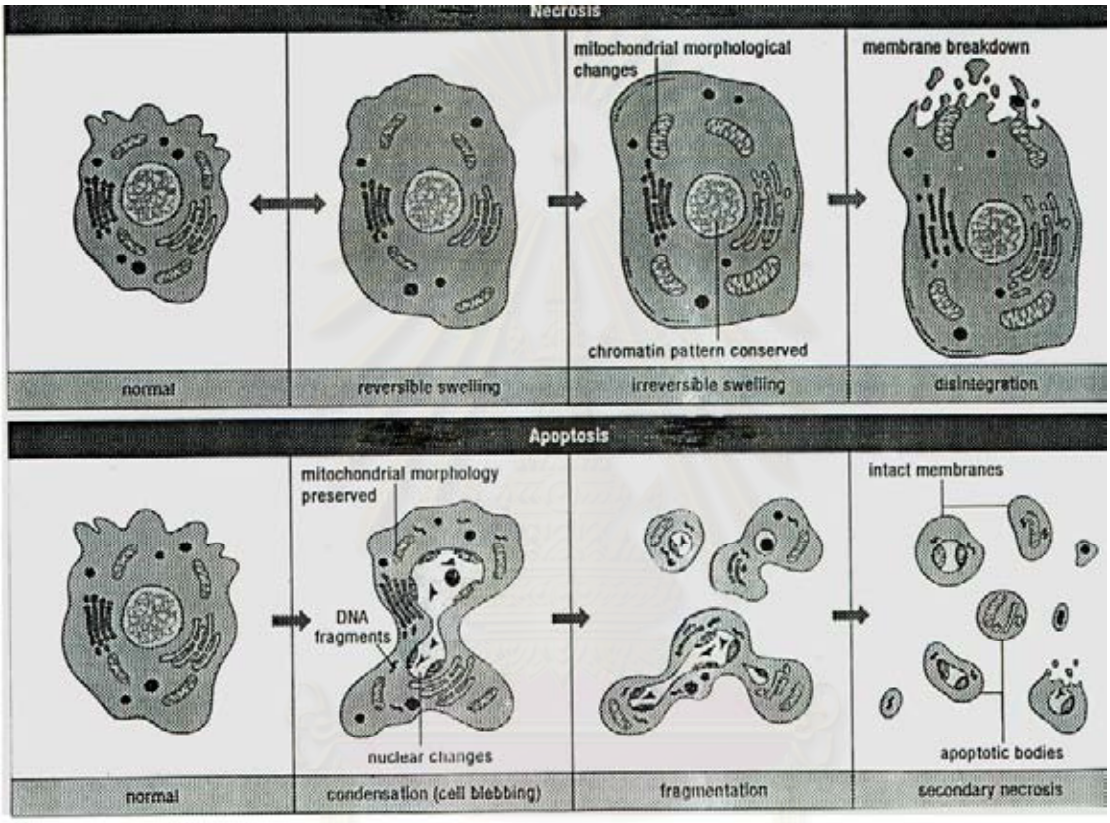


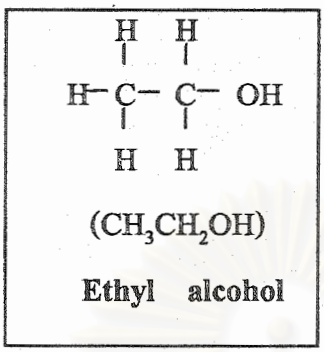
Figure 7. Illustration of the morphological features of necrosis and apoptosis (Guide et al., 1998).

leading to an influx of water and extracellular ions. Intracellular organelles, most notably the mitochondria, and the entire cell swells and ruptures (cell lysis). Due to
the ultimate breakdown of the plasma membrane, the cytoplsmic contents including lysosomal enzymes are released into the extracellular fluid. Therefore, *in vivo*, necrotic cell death is often associated with extensive tissue damage resulting in an intense inflammatory response. Apoptosis, in contrast, is a model of cell death that occurs under normal physiological conditions and the cell is an active participant in its own demise ("cellular suicide"). It is most often found during normal cell turnover and tissue homeostasis, embryogenesis, and development of the nervous system. Cell undergoing apoptosis shows characteristic morphological and biochemical features including chromatin aggregation, nuclear and cytoplasmic condensation, partition of cytoplasm and nucleus into membrane bound-vesicles (apoptotic bodies) which contain ribosomes, morphologically intact mitochondria and nuclear material.

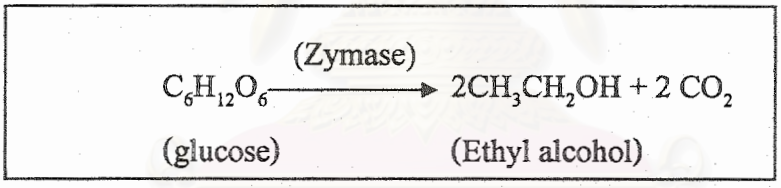
The liver performs many vital functions (Romanes, 1986) : first, metabolizing the products of digestion through the portal vein (principally degradation products of proteins and carbohydrates); second, the storage and release of substances (principally glucose) so as to maintain a constant level in the blood ; and third, the synthesis, conjugation and transformation of substances e.g formation of proteins, detoxification of poisonous substances, production of carbohydrates from proteins. All of these are endocrine functions which alter the composition of blood traversing the liver. The liver also has an exocrine or secretory function including the formation of bile, the important agent in digestion, especially of fats.

ALCOHOLS

Alcohols are hydrocarbon derivatives in which one or more of the hydrogen atoms of the hydrocarbon has been replaced by the OH radical. Alcohols that contain only one OH radical per molecule are called monohydric alcohols. Its may be further classified as primary, secondary or tertiary alcohol.

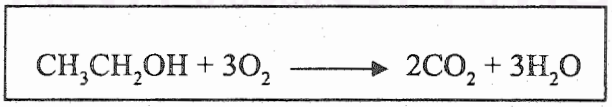


Ethyl alcohol (CH_3CH_2OH) is the ordinary grain alcohol (**ethanol : EtOH**) in wines and liquors. It is volatile and mixed in all proportions with water. It burns with yellow flame. Yeast has a mixture of enzyme, zymase, that catalyzes the conversion of some sugars to alcohol and carbon dioxide.



The reaction is known as fermentation and is used to manufacture alcohol.

Alcohols burn with the formation of carbon dioxide and water. This reaction yields a great deal of heat. When one gram of ethyl alcohol oxidizes completely in the tissue, about 7 calories of heat are produced (Arnow 1966).



ETHANOL METABOLISM

Absorption : Ethanol is absorbed from the gastrointestinal tract by simple diffusion because of its small size and slight lipid solubility. The diffusion is rather slow from the stomach and consequence the main part (70-80%) of ingested EtOH is absorbed from the duodenum and upper jejunum. High EtOH concentration, similar to those of alcoholic beverages, are reached in the stomach and upper duodenum. They are sufficient to engage the high *K*m gastric alcohol dehydrogenate (ADH), resulting in a significant first-pass metabolism (Dipadova et al., 1987). In the small bowel, EtOH concentration rapidly decrease so that in the ileum EtOH levels are parallel to that of the vascular space. The rate of absorption is decreased by delayed gastric emtying and by the intestinal contents. Food delays gastric emptying and intestinal absorption and increase gastric metabolism, thereby producing a slower rise and lower peak value of the blood alcohol in fed than in fasting subjects. The absorption of alcohol is slower from weak alcohols such as beer and wine than from distilled beverages. High EtOH concentration may delay gastric emptying (Newman and Abramson, 1972).

Distribution : The distribution of EtOH is much accelerated by a high blood flow. Organs with a dense vascularization and rich blood supply such as the brain, lungs and liver rapidly equilibrate with the blood. EtOH absorbed into the blood passes almost immediately into the brain tissue. In contrast, the distribution of EtOH to the resting skeletal muscle is particularly slow, because only a part of the capillaries are functioning under normal conditions (Herger and Holpieu, 1956). EtOH is slightly lipid soluble; at body temperature tissue lipids take up only 4% of the quantity of EtOH dissolved in a corresponding volume of water. Consequently the same amount of alcohol per weight gives a higher blood concentration in an obese person than in a skinny one. The distribution of EtOH is mainly related to the water content of various organs and tissues. For instance, urinary EtOH concentration is slightly higher than that of blood or plasma. Owing to its easy diffusiblity, EtOH passes through the

placental membranes and the amniotic fluid to the fetus. Elimination of EtOH from the fetus is regulated primarily by maternal hepatic biotransformation of EtOH. The amniotic fluid may serve as a reservoir for EtOH in utero(Brien et al.,1985). The sex differences in EtOH pharmacokinetics are due to sex differences in body water content. The mean apparent volume of distribution of EtOH is smaller in women than in man. As the result, the peak blood EtOH concentration are higher and mean areas under the EtOH concentration time curves are greater in women than in men(Marshall et al.,1984).

Elimination : The hepatic metabolism of EtOH proceeds in three basic steps (Figure 8). First, EtOH is oxidized within the cytosol of the hepatocyte to acetaldehyde (toxic metabolite); then, acetaldehyde is converted to acetate mainly in the mitochondria and finally the acetate produced in the liver is released into the blood and is oxidized by peripheral tissues to carbon dioxide, fatty acid and water.

The main pathway of EtOH metabolism utilizes the alcohol dehydrogenase (ADH) of the liver. Other metabolic reactions involve catalase and the microsomal ethanol oxidizing system (MEOS). Each metabolic pathway has a different subcellular localization and leads to formation of acetaldehyde. This is further oxidized to acetate and the acetyl CoA which enters into the pool of 2-carbon fragments origination from normal intermediary metabolism.

Alcohol dehydrogenase (ADH)

ADH is a zinc – containing metalloenzyme, which is present in yeast, liver and other animal tissues. In mammals the main portion of ADH activity is present in the liver (80-90% in human). In the liver cell as well as in the cells of other tissues, ADH occurs mainly in the cytosol. In the hepatic lobule the enzyme is generally located in the portal areas (Greenberger et al., 1965).

ADH has a broad substrate specificity ; aliphatic, aromatic, primary and secondary alcohols and their corresponding aldehydes serve as substrates. Three major

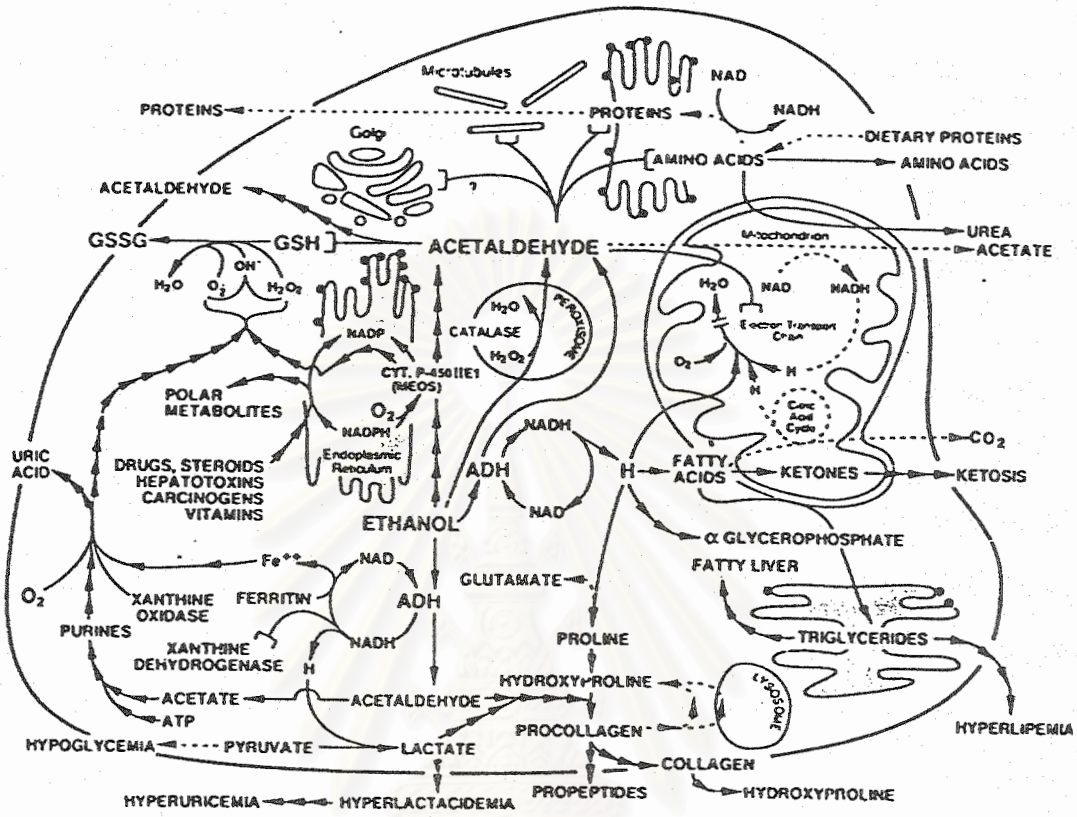


Figure 8. Schematic representation of the metabolism and metabolic effects of ethanol in the hepatocyte.

ADH = alcohol dehydrogenase

MEOS = microsomal ethanol oxidizing system

NAD = nicotinamide-adenine dinucleotide

NADH = nicotinamide-adenine dinucleotide, reduced form

NADP = nicotinamide-adenine dinucleotide phosphate

NADPH = nicotinamide-adenine dinucleotide phosphate,reduced form.

Pathways decreased by acute or chronic administration of ethanol are represented by dotted lines (Lieber, 1985).

classes of ADH have been described on the basis of structure and function. Chromosome mapping studies have revealed that ADH genes are located on the long arm of human chromosome 4 (Cotton and Goldman, 1988). The class isoenzymes are formed by three polypeptide submits alpha, beta and gamma with low *Km* and high V_{max} for EtOH. Three structural gene loci, ADH_1, ADH_2 and ADH_3 are known. Class II ADH isoenzymes has a significantly higher *Km* for EtOH than class I isoenzymes. Class III ADH has a very high *Km* for EtOH and is assumed not to participate in the hepatic EtOH oxidation under physiological conditions. Various ADH forms appear in different frequencies in different racial populations. Furthermore they may explain, at least in part for individual variation in the rate of EtOH elimination. ADH phenotypes have been related to the production of acetaldehyde and to the extent of first-pass elimination of EtOH. There is a possible relationship between ADH genotype and the susceptibility to alcoholism or alcohol – related organ injury. Vonwarth and Buhler (1984) differentiated the normal human liver ADH (pH optimum 10.5) from the so-called atypical type (pH optimum 8.8), which shows a several fold higher activity. This atypical form of ADH occurs in frequencies between 5 and 20 percent in European populations. In Mongoloid populations, the frequency is up to 90 percent.

A mechanism for the enzyme reaction with EtOH has been firstly proposed by Theorell et al (1955) according to the following reaction

$$\underset{\text{(ethanol)}}{CH_3CH_2OH} + NAD^+ \xrightarrow{ADH} \underset{\text{(acctaldehyde)}}{CH_3CHO} + NADH + H^+$$

The first step in the metabolism of alcohol is the formation of acetaldehyde. The oxidized cofactor nicotinanmide adenine dinucleotide (NAD^+) combines with ADH and EtOH is then bound to this binary complex. After hydrogen has been

transferred from EtOH to NAD^+, coverting it to its reduced form (NADH), acetaldehyde is produced.

Microsomal Ethanol Oxidizing System (MEOS)

The suggestion that the microsomal fraction of the liver could be involved in the metabolism of EtOH was first based on the results of morphological studies. Iseri et al. (1964), lane and lieber (1966), Liber and Rubin (1968) observed that prolong EtOH ingestion resulted in a proliferation of the smooth endoplasmic reticulum (SER) of the liver in both rats and men. An increase in SER is a common feature seen after the administration of drugs that are metabolized by liver microsomes in the electron transport chain known as the drug metabolizing system, the possibility appeared that EtOH like other drugs could be metabolized by liver microsomes.

MEOS was then extensively studied by lieber and Decarli (1968). The system reguires oxygen and is inhibited by CO. The differentiation from ADH owing to the different sub cellular localization (Cytosol for ADH, microsome for MEOS), cofactor reguirements (NAD^+ for ADH, NADPH for MEOS) and pH optimum (10 – 11 for ADH, physiological pH for MEOS).

Catalase

Catalase is a heme – containing enzyme. It is present in most animal tissues (such as liver, kidney, red blood cell). Because of its wide spread distribution in animal tissues, this enzyme, together with the ADH present in tissue other than the liver, certainly represent the main route for extrahepatic EtOH oxidation. Catalase occurs mainly in cytoplasmic organells which have been called peroxisomes by Deduve and Baudbuin (1966).

Catalase catalyses the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen according to the following scheme.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

Whereas the reduction of H_2O_2 to water by electron donors (EtOH) is called peroxidation.

$$CH_3CH_2OH + H_2O_2 \xrightarrow{\text{catalase}} CH_3CHO + 2H_2O$$

(ethanol) (acetaldehyde)

Generally the latter reaction is catalysed by peroxidase, but catalase may also catalyse peroxidative type reactions when hydrogen peroxide is produced and a source of electroen donor is available. In this case catalase promotes the oxidation of some substrates at the expense of H_2O_2. Catalase combines with hydrogen peroxide to form a labile complex which rapidly reacts with an hydrogen donor.

METABOLISM OF ACETALDEHYDE

Acetaldehyde is oxidized further in the liver by the rather non specific group of aldehyde dehydrogenase (ALDH) (Leiber, 1985).

$$CH_3CHO + NAD^+ + H_2O \xrightarrow{\text{ALDH}} CH_3COOH + NADH + H^+$$

The oxidation of acetaldehyde gives rise to acetyl CoA in the reaction catalysed by acetyl thiokinase. This can be further broken down to acetate, which may be oxidized to carbon dioxide and water, or converted by the citric acid cycle to other biochemically important compound including fatty acids.

Two major hepatic ALDH – isoenzymes (ALDH I and II) exist in human (Crabb et al., 1989). ALDH I, with a low *K*m for acetaldehyde, is predominantly of mitochondrial origin and ALDH II which has a relatively higher *K*m, is of cytosolic origin. The oxidation of acetaldehyde to acetate is catalysed by a low-*K*m ALDH located in mitochondrial matrix. Mitochondrial NAD functions as a coenzyme in the reaction. Consequently, oxidation of EtOH via acetaldehyde to acetate results in the reduction of both cytosolic and mitochondrial redox states which are reflected respectively in the increase in ratio of liver and blood lactate to pyruvate and beta hydroxybutyrate to acetoacetate.

Excretion : Most of the EtOH (90-98%) is oxidized and excreted as carbon dioxide and water (Thompson, 1956). Only small amount of the ingested EtOH are excreted ummetabolise in urine, breath and sweat. In man under normal condition 1-3% can be eliminated through the lungs. In small animals with higher metabolic rate, for instance in rats, up to 10% of administered EtOH can be eliminated through the lungs .

ALCOHOL AND THE LIVER

Ethanol -Induced Liver Injury

In both clinical studies (which generally deal with chronic alcoholics) and experimental researches on chronic EtOH intoxication, a curcial and unresolved problem is the pathogenesis of the EtOH induced liver injury. The spectrum of alcoholic liver injury involves fatty liver (steatosis), alcoholic hepatitis and cirrhosis. In this review literature emphases especially for fatty liver.

"**Fatty Liver**" is a pathological condition that is characterized by the appearance within the cytoplasm of liver parenchymal cells of droplets of fat. According to the intralobular distribution, pathologist mainly consider two types of fatty liver, "Centrolobular" and "Peripheral" (Dianzani, 1969). The first type is

characterized by an earlier involment of the cells surrounding the central vein ; this occurs typically after variuos types of poisoning (for example by EtOH or by white phosphorus). The second type is characterized by an earlier involment of the cells lining the portal spaces, is usually encountered in protein difficiencies and in most situations in which protein synthesis is impaired.

The normal liver weighs about 1.5 kg, whereas the alcoholic fatty liver weighs 2.0-2.5 kg (Lieber and Salvolainen, 1984). All possible mechanisms that can be the explanation of hepatic fat accummulation produced by EtOH will be reviews below.

Enhanced mobilization of free fatty acids from depot fat

Mallov (1961) and Brodic reported that a progressive increase in plasma free fatty acids (FFA) occurs after the administration to rats of a single large does of EtOH. Increased amounts of fat can be of exogenous (dietary) origin, can come from adipose tissue being transported to the liver as FFA, or come from lipids synthesized in the liver itself. The origin depends upon the dose of EtOH ingested and the lipid content of the diet. After an acute, isolated ingestion of a large dose of EtOH the fatty acids found in the liver may originate from adipose tissue.

Enhanced hepatic lipid synthesis

Lieber and Schmid (1961) found that in liver slices the fatty acid synthesis was increased by EtOH. EtOH stimulates fatty acid synthesis by the followings: EtOH metabolism increases the production of NADH while NADPH is necessary for lipogenesis. Lieber (1968) had proposed a metabolism according to which the enahnced NADH production from EtOH may lead to an increased production of NADPH through the coupling of EtOH oxidation with the reduction of oxaloacetate to malate in the cytosol, followed by the oxidative decarboxylation of malate to pyruvate ; the latter reaction, which is catalysed by the malic enzyme generated NADPH. Pyruvate enters the mitochondria where it is converted to either acetyl CoA or oxaloacetate (by pyruvate carboxylase), both of which condense to form citrate. In the

presence of EtOH, the NADH linked reduction of oxaloacetate to malate reoxidizes NADH for alcohol and acetaldehyde dehydrogenases. The net result is that pyruvate is converted to acetyl CoA and that the oxidation of NADH produced by EtOH give rise to NADPH. Therefore, through the coupling of EtOH oxidation with the oxaloacetate – malate cycle, EtOH could markedly increase the production of the compounds necessary for fatty acid synthesis; the increased systhesis of fatty acids could be considered "as a mean for disposing of the excess hydrogen produced on EtOH oxidation."

Decreased fatty acid oxidation

Ontko (1973) suggested that EtOH inhibited β-oxidation and citric acid cycle in the mitochondria by competitive oxidation, since the reducing equivalents produced from EtOH in the cytosol are transferred into the mitochondria. As a result of the inhibition of mitochondrial β-oxidation of fatty acids, an increased long chain acyl CoA level in the mitochondrial matrix would occur, thereby decreasing the vectorial translocation of acyl groups occur in the inner mitochondrial membrane and resulting in a greater availability of acyl CoA in the cytosol. Chronic EtOH ingestion has also been shown to cause a profound decrease in the activity of acyl CoA : carnitine acyltransferase activity in the liver . It has also been shown that the decreased conversion of palmitate to CO_2 and ketone bodies is matched by an increased incorporation of palmitate into glycerolipids, especially triglycerides, which may explain the hepatic accumulation of triglycerides after EtOH.

Increase fatty acid esterification

Increased fatty acid esterification could result from a decreased fatty acid oxidation or from an increased fatty acid synthesis or both. A decrease in fatty acid oxidation could result in a greater availability of long chain fatty acids in the cytoplasm which may lead to an enhanced esterification of fatty acids. EtOH induced

the increase in hepatić α - glycerophosphate concentration (Nikkila and Ojala, 1963), secondary to NADH generation which may play a significant role in this respect.

Imparied hepatic secretion of triglyceride as lipoprotein

Scharpiro et al (1964) showed that in rats after acute EtOH intoxication, there was a decreased lipoprotein output from the liver. EtOH induced fatty liver is unlike fatty liver produced by a variety of other hepatotoxic agents. It is not accompanied *in vivo* by a fall in serum triglycerides. On the contrary, the level of circulating triglycerides was generally found to be increased after prolonged EtOH administration to rats or to men.

"Alcoholic hepatitis" develops only in a fraction of heavy drinkers, even after decades of alcohol abuse. Histological characteristics of alcoholic hepatitis are ballooning and great disarrary of hepatocytes predominantly in perivenular areas, parenchymal and portal infiltration with polymorphonuclear leukocytes. Commonly lymphocytes can be demonstrated in perivenular zone or within the parenchyma in direct contract with the plasma membranes of the hepatocytes. Mallory's alcoholic hyalin irregular cytoplasmic bodies can be considered as a diagnositc hallmark but it is not always present (Beckett et al., 1961).

"Cirrhosis" is defined anatomically as a diffuse process with fibrosis and nodule formation. Zone 3 myofibroblastic proliferation and collagen deposition may be the first apparent lesions in the sequence of events leading to alcoholic cirrhosis. Fibrosis follows hepato cellular necrosis. This may follow by interaction with hepatitis in zone 1 leading to portal – portal fibrous bridges. Confluent necrosis in zone 3 leads to central – portal bridging and fibrosis. Focal necrosis is followed by local fibrosis which disturbs the hepatic architecture and a full cirrhosis develops.In conclusion the mechanism of EtOH induced liver injury (Casini et al, 1991) may be occurred from, **first**: Acetaldehyde is extremely reactive, it binds to

phospholipid, amino acid residues and sulphydryl groups. It affects the plasma membrane by depolymerizing proteins and producing altered surface antigens. Lipid peroxidation is flavoured (**Lipid peroxidation** is an autocatalytic mechanism leading to the oxidative destruction of cellular membrane and finally cell death). It composes of three stages namely initiation, propagation and termination. Lipid peroxidation of hepatocytes can be initiated by the interaction of activated free radical products (resulting from the cytochrome P450 catalysed metabolism of a variety of toxic agents) with polyunsaturated fatty acids of the membrane (Jose and Slater, 1973). Lipid peroxidation rate is strongly dependent on temperature (as it is catalysed by enzyme) on the relative abundance of free radical chain terminator, and on the level of polyunsaturated fatty acid in the membrance, a variable that is affected by dietary lipids. The end products of lipid peroxidation are malondialdehyde (MDA) and 4-hydroxy alkenals (4-HAE). **Second**: A marked increase in NADH: NAD ratio in hepatocytes oxidizing alcohol producing profound metabolic consequences. Thus the redox ratio of lactate to pyruvate is markedly increased, leading to lactic acidosis. The altered redox potential has also been implicated in the pathogenesis of fatty liver, and collagen formation.

CHAPTER II

MATERIALS AND METHODS

1. PREPARATION OF CRUDE EXTRACT

Fresh Jiaogulan were purchased from the Five Leaf company, Sansai district, Chiangmai province. The whole herb were dried thoroughly under shade and cutting into small pieces, macerated in 95 % ethanol at room temperature for 1 week with occasional shaking. The process of maceration was repeated. The combined filtrate was filtered through the filter paper No.4 and 1. A concentrated green residue was produced under the reduction of pressure at 45 °C on a rotary evaporator (EYELA Tokyo Rikakikai Co., Ltd.) and lyophilized by a freeze dryer (FTS System Stone Ridge.,U.S.A.) . The yield of lyophilized residue was 13% of the original dry aerial part weight (diagram of extraction was shown in Figure 9). The green extract powder was suspended in 5% corn flour paste and thoroughly by magnetic stirrer 15 min before use.

2. ANIMAL

Male Wistar Albino rats weighing 160-180 g, 6-8 weeks old from the National Laboratory Animal Center, Mahidol University, Salaya,Nakorn Pathom were used. Animals were kept in a controlled temperature room at 23±2 °C under standard conditions (12 hour dark: 12 hour light cycle) and libitum fed with commercial rodent food (Jareorn Pokaphan Co., Ltd.Bangkok) and tap water.

3. INDUCTION OF HEPATIC INJURY

Hepatic injury in rats was individually induced by oral administrtaion of ethanol (E. Merck Germany).

Acute toxicity : Ethanol (5 g / kg) as a single dose

Sub-acute toxicity : Ethanol (4 g / kg/ day) for 28 consecutive days

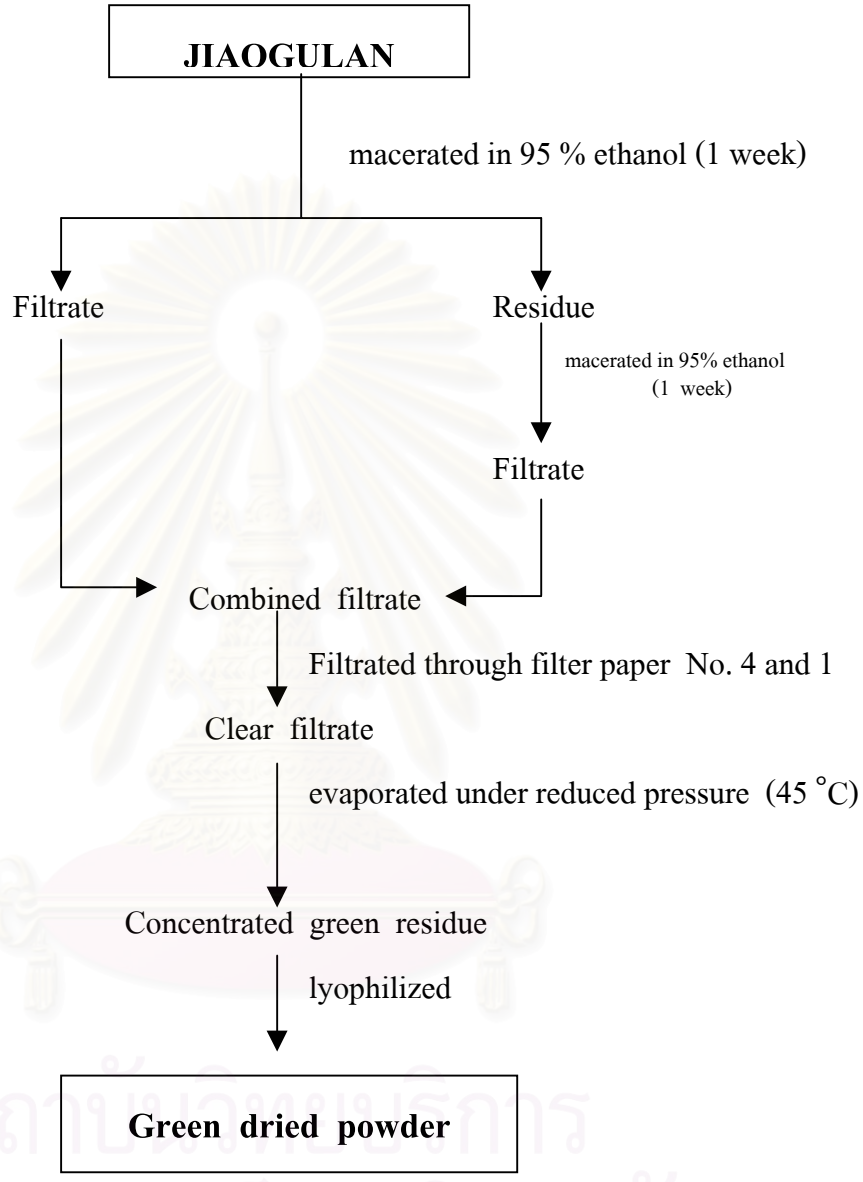


Figure 9. The preparation of ethanol extract of Jiaogulan

4. EXPERIMENTAL PROTOCOLS

EXPERIMENT I: The hepatoprotective effect of Jiaogulan extract against a toxic single dose of ethanol (5g / kg)

Rats were randomly divided into five experimental groups of thirteen rats in each group. **Group 1** served as a negative control and received 5% corn flour (5 ml / kg) orally. **Group 2** served as a control group and received 5% corn flour 4 hr before administration with single dose of EtOH (5 g/kg). **Groups 3, 4 and 5** were orally treated with Jiaogulan extract (300,400 and 500 mg/kg) 4 hr before oral administration of EtOH (diagram illustrated the experiment I was shown in Figure 10).

EXPERIMENT II: Effect of Jiaogulan extract against sub-acute toxic dose of EtOH (4 g/kg/day)

2.1 Sub-acute hepatotoxicity of EtOH

Rats (n=16) were divided randomly into two groups of eight animals. **Group 1** serve as control group fed orally with 5% corn flour (5 ml/kg) single dose. After 4 hrs rats were sacrificed. **Group 2** fed orally with EtOH (4 g/kg/day) for a period of 28 consecutive days (diagram illustrated the experiment 2.1 in Figure 11).

2.2 Reduction of sub-acute EtOH induced hepatotoxicity by Jiaogulan extract given by varying time of treatment

Rats (n = 48) were given EtOH (4 g / kg / day) for 28 consecutive days to induce liver injury. After that rats were divided randomly into two groups of 24 rats each and divided again into three sub-groups of 8 rats each. **The first** group received 5% corn flour (5 ml / kg / day) for 7, 14 and 21 consecutive days. **The second** group received Jiaogulan (300 mg/kg/day) for 7, 14 and 21 consecutive days (diagram illustrated the experiment 2.2 was shown in Figure 12).

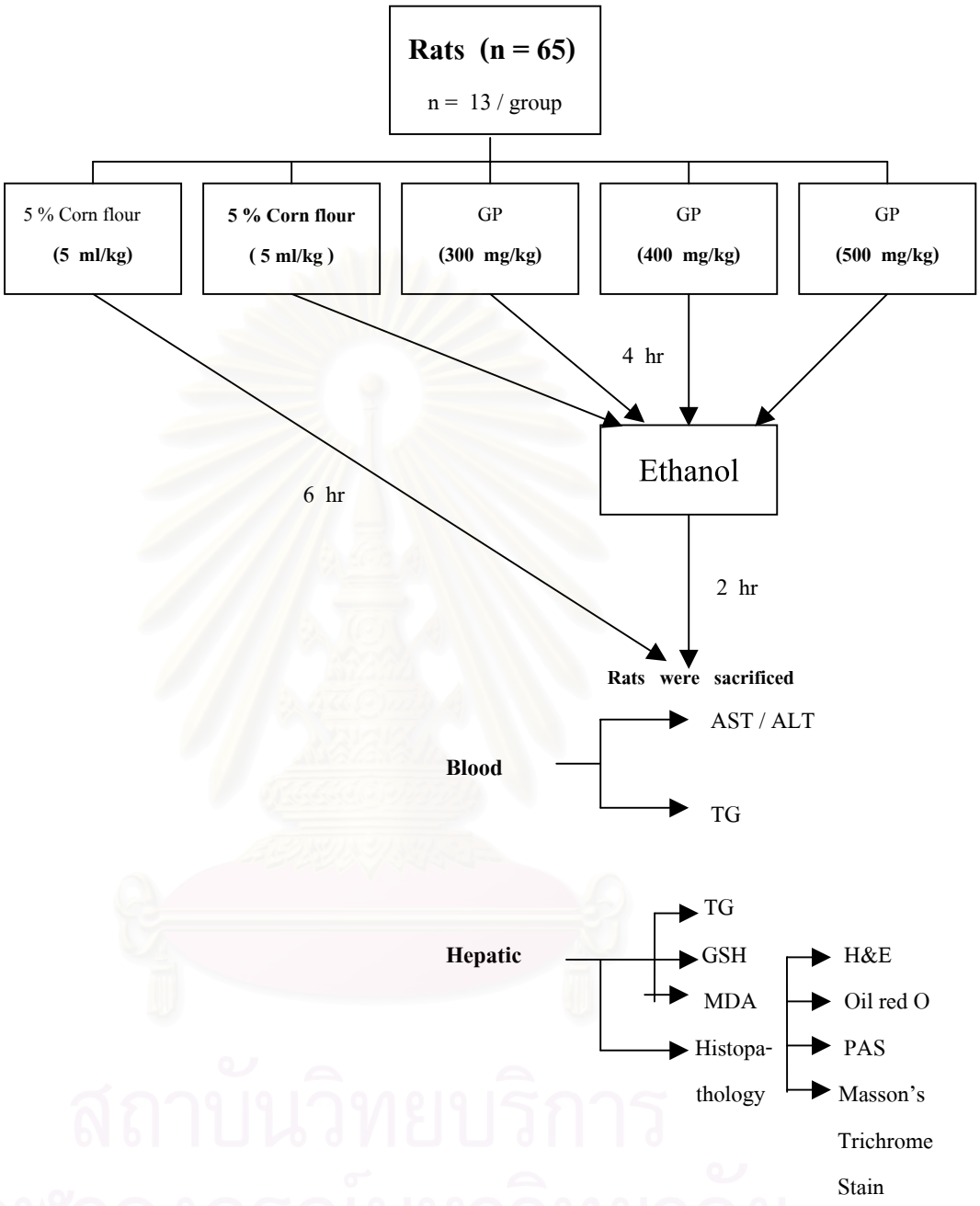


Figure 10. Diagram illustrated the hepatoprotective effect of Jiaogulan extract against toxic single dose of ethanol 5 g/kg

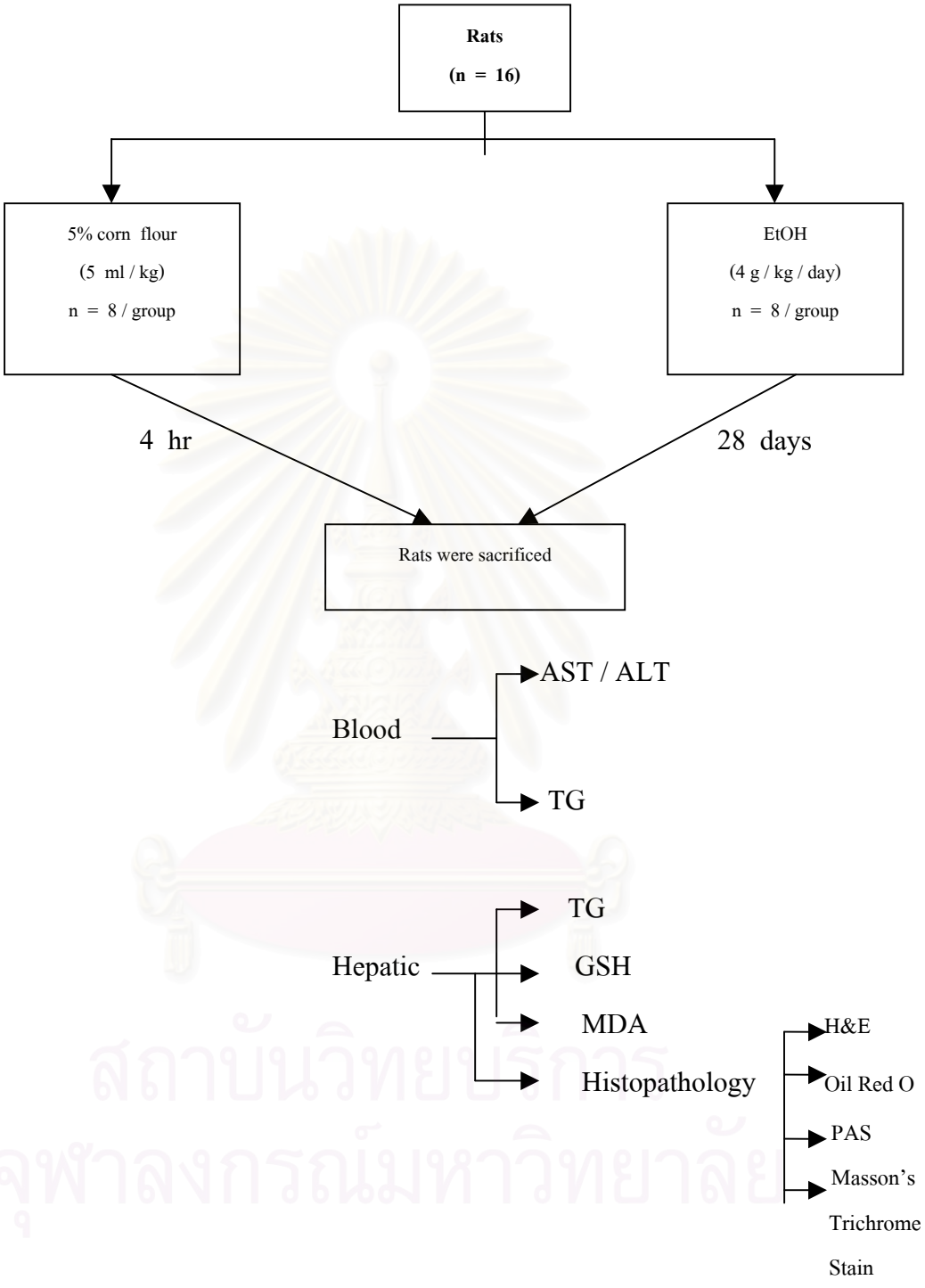


Figure 11. Diagram illustrated sub-acute hepatotoxic effect of EtOH (4 g / kg / day for 28 days)

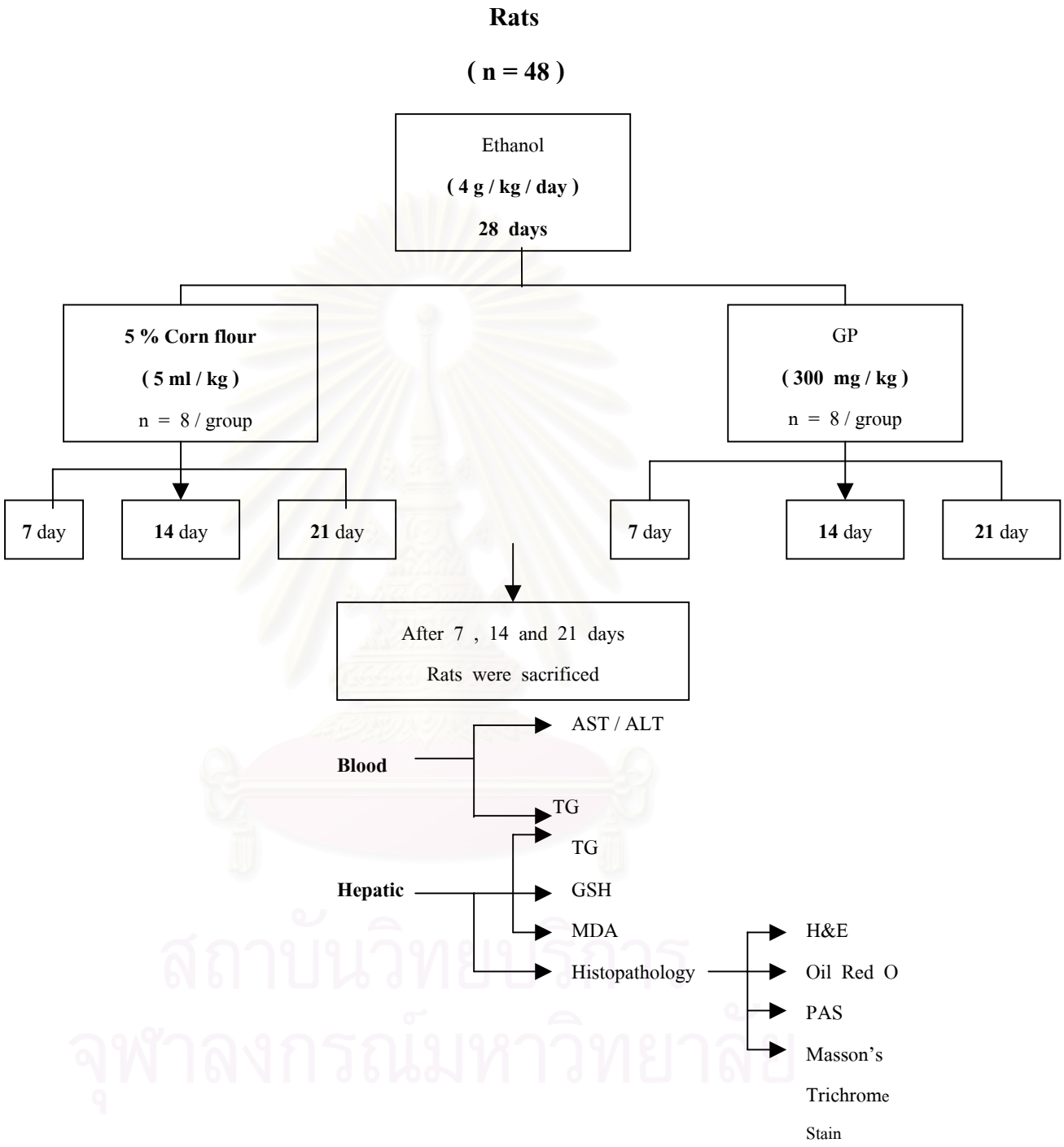


Figure 12. Diagram illustrated the reduction of sub-acute EtOH induced hepatotoxicity by Jiaogulan extract given by varying time of treatment

2.3 Post effect of EtOH and Jiaogulan extract

Rats (n=16) was administered EtOH (4g/kg/day) for a period of 28 days to induce liver injury. After that rats were randomly divided into two groups of eight rat each : control groups received 5% corn flour (5 ml/kg/day) for consecutive 21 days. Experimental group received Jiaogulan extract (300mg/kg/day) for the same period of 21 days. Then control and experimental groups received standard rats diet and tap water ad libitum for 1week before sacrificed (diagram illustrated the experiment 2.3 was shown in Figure 13).

5. DATA COLLECTION

Rats were fasted 12 hr prior to the experiment. After oral administration of the last dose of each experiment, rats were anaesthetized with diethyl ether (C_2H_2O, Merck Germany) and blood was collected (1.5 ml) via inferior vena cava using sterile disposable syringes and needle No.21 and keep in appendrop tube. Then the animal was sacrificed by cutting to the inferior vena cava. The liver was perfused immediately through hepatic portal vein with iced-cold 0.9% NaCl (NSS) 30 ml. Liver were excised quickly, rinsed in iced-cold NSS, trimmed of adherent tissues , blotted dry and weighed. Liver and blood were immediately put on ice.

5.1) Serum enzyme determination

After the whole blood coaglulation serum was separated by automatic high speed refrigerated centrifuge(Himac SCR 20B/18B, Hitachi) at 3000 rpm,4 $^{\circ}$C for 10 min. The activity of serum AST and ALT was measured as a marker of liver injury (Wroblewski and ladue, 1956), using spectrophotometer (Ultraspec II LKB Biochrom Ltd., England) at 340 nm. The reagent kits for the assay was obtained from Clinac Co., Bangkok., Thailand.

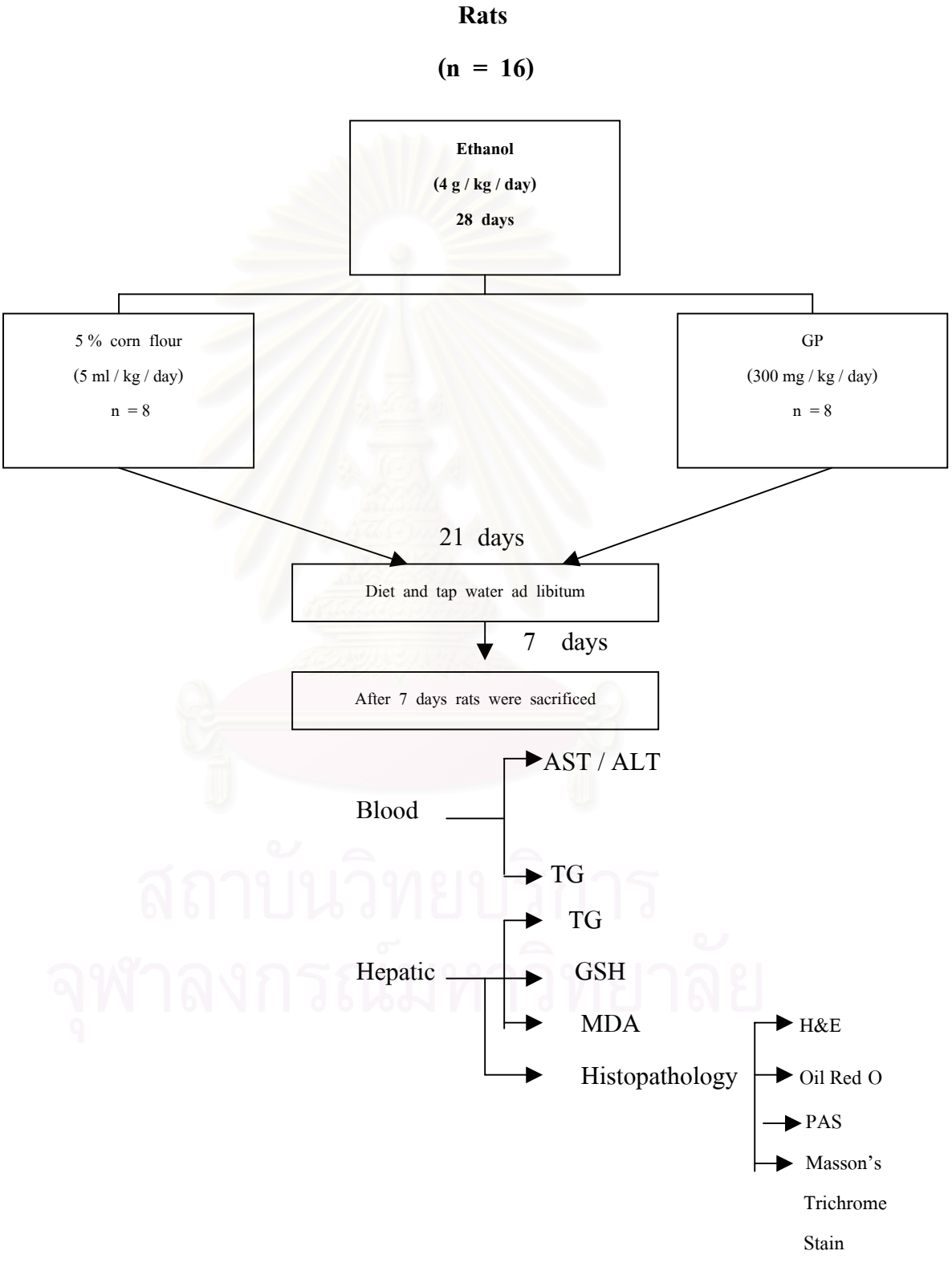


Figure 13. Diagram illustrated post effect of EtOH and Jiaogulan extract

5.2) Serum triglyceride (STG)

Using 10 μl of serum to react with TG reagent kit (Clinac Co., Bangkok., Thailand) 1.0 ml, incubated at 37 °C for 5 min and measured STG by spectrophotometer at 500 nm.

5.3) Hepatic triglyceride (HTG)

After cutting one lobe of rat liver for histopathological examination, the remaining liver sample was minced with 15 ml iced-cold 0.1 M phosphate buffer, pH 7.4 in a motor-diven glass teflon homogenizer. This liver homogenate was used for the determination of HTG, GSH and MDA. HTG was quantified as described by Mendez and Franklin (1957). Aliquot of 0.5 ml liver homogenate was used. Glycerol was removed by *N*-heptane–isopropanol(2:2.5v/v) and 0.04 N sulfuric acid. This glycerol, obtained by sponification of the triglycerides, was oxidized to formaldehyde with sodium metaperiodate. The resulting formaldehyde was converted into diacetyldihydrolutidine with acetyl acetone and ammonium salt in acetic acid solution.Triolein was used for comparison. The yellow amyl alcoholic solution was quantified spectrophotometrically at 415 nm within 45 min.

5.4) **Hepatic glutathione (GSH) determination**

GSH content was measured using method described by Ellman (1959). The protein in an aliquot of 0.5 ml liver homogenate was precipitated by adding an equal volume of 4% sulfosalicylic acid followed by centrifugation at 3000 rpm for 15 min. After centrifugation, 0.5 ml of supernatant was reacted with 4.5 ml of 5,5-Dithiobis (2 – nitrobenzoic acid) (Sigma Chemical Co., St. Louis, U.S.A.). The entire sample was analyzed for absorbance at 412 nm. GSH content was calculated by extinction coefficient ($E = 1.36 \times 10^4\ M^{-1}\ cm^{-1}$) and expressed as micromoles per gram liver (μmol/g liver).

5.5) Hepatic malondialdehyde (MDA) determination

Lipid peroxidation was determined by thiobarbituric acid reactive substances (TBARS) for the estimation of MDA content according to Budge and Aust (1978). One milliliter aliquot of liver homogenate was then added to 3.0 ml of TBA reagent, then heated in boiling water for 15 min and centrifuged at 3000 rpm, 15 min at room temperature. The spectrophotometric absorbance of the supernatant was recorded at 535 nm. MDA content was calculated by extinction coefficient ($E=1.56x10^5\ M^{-1}\ cm^{-1}$) and expressed as nanomoles per gram liver (nmol/g liver).

5.6) Histopathological examination

One lobe of the liver was removed and divided into 2 parts. First part was cut appoximately 1.0x1.5 x 0.5 cm and fixed in 10% buffered formalin and embedded in paraffin. Hematoxylin-Eosin (H&E) staining was performed according to standard histological procedures on 4μ sections. Quantification of necrotic cells was performed by immage analysis under light microscopy. The pathological changes in liver were graded (0, normal; +1, mild degree; +2, moderate degree; +3, severe degree). Second part of fresh liver approximately 0.5x1.0x0.3 cm was immediately cut as a frozen section using cryostat or cryocut (Leica Cm 1800) on 5 μ sections and stained with Oil Red O for detection of fatty deposited in liver tissue (Mallory, 1942), special staining of Periodic Acid Schiff (PAS) and Masson's trichrome stain were used for the detection of glycogen, glycoprotein, soluble polysaccharides and fibrous tissue (Masson, 1929; Lillie, 1948).

6. DATA ANALYSIS

The data are expressed as mean ± standard errors of the mean (S.E.M.) using the SPSS 11.0 for Windows program, (SPSS, Chicago,IL). Statistical comparisons between group were analyzed by one–way analysis of variance (ANOVA) and post

hoc comparisons were done by least-significant difference (LSD) comparison test. Statistical between two groups were analyzed by Student't-test. P values less than 0.05 were considered significant.

CHAPTER III

RESULTS

Experiment I: The hepatoprotective effect of Jiaogulan extract against toxic single dose of EtOH (5 g / kg)

1.1 Hepatic triglyceride (HTG) content

HTG content of control and experimental rats was shown in Table 1. HTG content of EtOH treated group was higher than control ($P < 0.05$). Treatment with GP extract at the dose of 400 and 500 mg/kg 4 hours before EtOH did not change HTG content. Whereas, GP extract at 300 mg/kg significantly reduced HTG content induced by EtOH.

1.2 Serum triglyceride (STG) concentration

STG concentration in control rats was within the low physiological values (60.2 ± 4.5 mg / dl). STG concentration increased rapidly two hours after EtOH administration. GP extract at the dose of 300, 400 and 500 mg/kg 4 hours before EtOH had no effect on STG concentration induced by EtOH (Table 2).

1.3 Liver glutathione (GSH) content

The content of hepatic GSH was not different in rats given EtOH or GP extract plus EtOH compared with control rats (Table 3). The values were in the range of 6.5-7.5 μmol / g liver.

Table 1. Effect of Jiaogulan extract on HTG content in EtOH induced hepatotoxic rats

Groups	HTG (mg / g liver)
Control (5% corn flour ; 5 ml / kg)	4.2 ± 0.3
EtOH (5 g / kg)	6.0 ± 0.4 *
GP (300 mg/kg)	4.7 ± 0.3 #
GP (400 mg/kg)	5.3 ± 0.3 *
GP (500 mg/kg)	5.6 ± 0.3 *

Values expressed as mean ± S.E.M. (n=13)

*Significant difference ($P < 0.05$) compared to control group

Significant difference ($P < 0.05$) compared to EtOH group

Table 2. Effect of Jiaogulan extract on STG concentration in EtOH induced hepatotoxic rats

Groups	STG (mg / dl)
Control (5% corn flour; 5 ml/kg)	60.2 ± 4.5
EtOH (5 g / kg)	181.7 ± 15.7 *
GP (300 mg/kg)	166.1 ± 14.8 *
GP (400 mg/kg)	169.4 ± 13.4 *
GP (500 mg/kg)	151.8 ± 13.8 *

Values expressed as mean ± S.E.M. (n=13)

* Significant difference ($P < 0.05$) compared to control group

Table 3. Effect of Jiaogulan extract on GSH content in EtOH induced hepatotoxic rats

Groups	GSH (μmol / g liver)
Control (5% corn flour; 5 ml/kg)	7.0 ± 0.3
EtOH (5 g/kg)	6.6 ± 0.2
GP (300 mg/kg)	7.1 ± 0.3
GP (400 mg/kg)	7.2 ± 0.2
GP (500 mg/kg)	7.3 ± 0.2

Values expressed as mean ± S.E.M. (n=13)

1.4 Liver malondialdehyde (MDA) content

MDA content in rat liver was unchanged after treating with EtOH and GP extract plus EtOH (Table 4). The values were in the range of 85.0-95.0 nmol / g liver.

1.5 Transaminase enzymes (AST / ALT)

The activities of AST and ALT in control and experimental rats were given in Table 5 and Figure 14. AST and ALT activities were significantly elevated in EtOH treated rats as compared with the control ($P < 0.05$). Treatment with GP extract at 300, 400 and 500 mg/kg 4 hours before EtOH showed the reduction of AST and ALT activities induced by EtOH.

From the acute experiment in rats indicated that administration of oral single dose EtOH 5 g/kg the rats have the effect to increased HTG, STG, AST and ALT but not the hepatic GSH and MDA. GP extract in the doses of 300, 400 and 500 mg/kg reduced AST and ALT activities induced by EtOH with no effect on STG, GSH and MDA levels. GP extract only at dose of 300 mg/kg reduced HTG induced by EtOH. The above observation was inagreement with the pathological changes.

In this acute experiment GP extract at dose of 300 mg/kg seemed to be the effective dose possessing hepatoprotective activity effective dose with no toxic effect to hepatocytes as confirmed by histopathological examination. So it was chosen for further study in sub-acute experiment.

1.6 Histopathological examination

The pathological grading for acute experiment is summarizcd in Table 6. Control rats receiving single dose of 5% corn flour (5 ml/kg) single dose demonstrated normal liver morphology (Figure 15), the round nucleus and homogeneous cytoplasm (H&E). Periodic Acid Schiff (PAS) showed regular pattern of glycogen (Figure 16)

Table 4. Effect of Jiaogulan extract on MDA content in EtOH induced hepatotoxic rats

Groups	MDA (nmol / g liver)
Control (5% corn flour; 5 ml/kg)	86.4 ± 3.7
EtOH (5 g/kg)	91.9 ± 3.9
GP (300 mg/kg)	90.1 ± 5.8
GP (400 mg/kg)	92.7 ± 2.6
GP (500 mg/kg)	93.3 ± 5.0

Values expressed as mean ± S.E.M. (n=13)

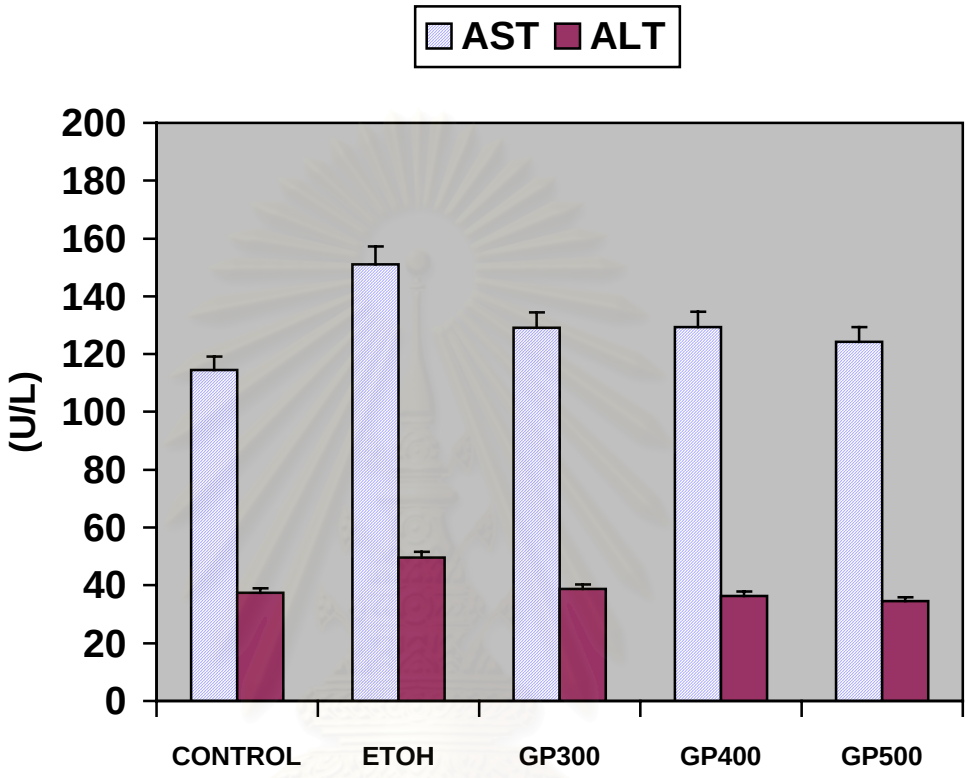
Table 5. Effect of Jiaogulan extract on AST and ALT of EtOH induced hepatotoxic rats

Groups	AST (U /L)	ALT (U/L)
Control (5% corn flour)	114.6 ± 7.4	37.4 ± 0.4
EtOH (5 g/kg)	151.1 ± 7.8*	49.6 ± 2.4 *
GP (300 mg/kg)	129.2 ± 3.3#	38.8 ± 1.5#
GP (400 mg/kg)	129.4 ± 6.9#	36.4 ± 0.8#
GP (500 mg/kg)	124.3 ± 7.9#	34.6 ± 1.1#

Values expressed as mean ± S.E.M. (n=13)

*Significant difference ($P < 0.05$) compared to control group

Significant difference ($P < 0.05$) compared to EtOH group



Figur 14. Effect of jiaogulan on serum AST and ALT of hepatotoxic rats (n=13)

* Significant difference from the control group, $P < 0.05$

Significant difference from the EtOH group, $P < 0.05$

Table 6. The histopathology of single toxic dose of EtOH (5 g/kg) and effect of Jiaogulan extact (300, 400 and 500 mg/kg) in EtOH induced acute hepatotoxicity (n = 13)

Groups	Degree of hepatocyte injury
Control	0
EtOH	+2
GP_{300}	0
GP_{400}	0
Gp_{500}	+1

Table 6. The histopathology of single toxic dose of EtOH (5 g/kg) and effect of Jiaogulan extact (300, 400 and 500 mg/kg) on EtOH induced acute hepatotoxicity (n = 13).

The severity of hepatocyte injury grading by

0 = normal

+ 1 = mild

+ 2 = moderate

+ 3 = severe

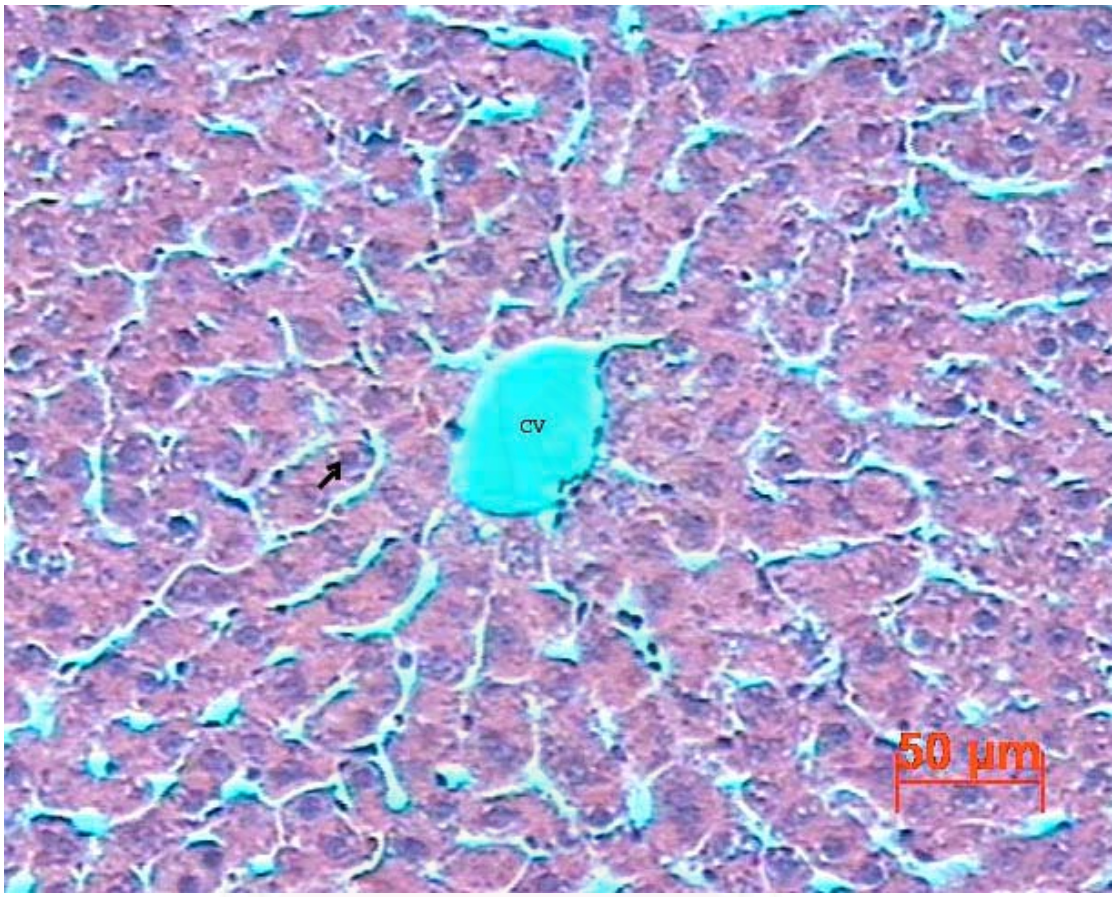


Figure 15. Normal hepatocytes of control rats (5% corn flour; 5 ml/kg)

: Hepatocytes had the round nucleus centrally (arrow) and homogeneous cytoplasm, hepatic cord arrangement as a regular ray pattern from central vein(CV), grading 0 (H&E x 100).

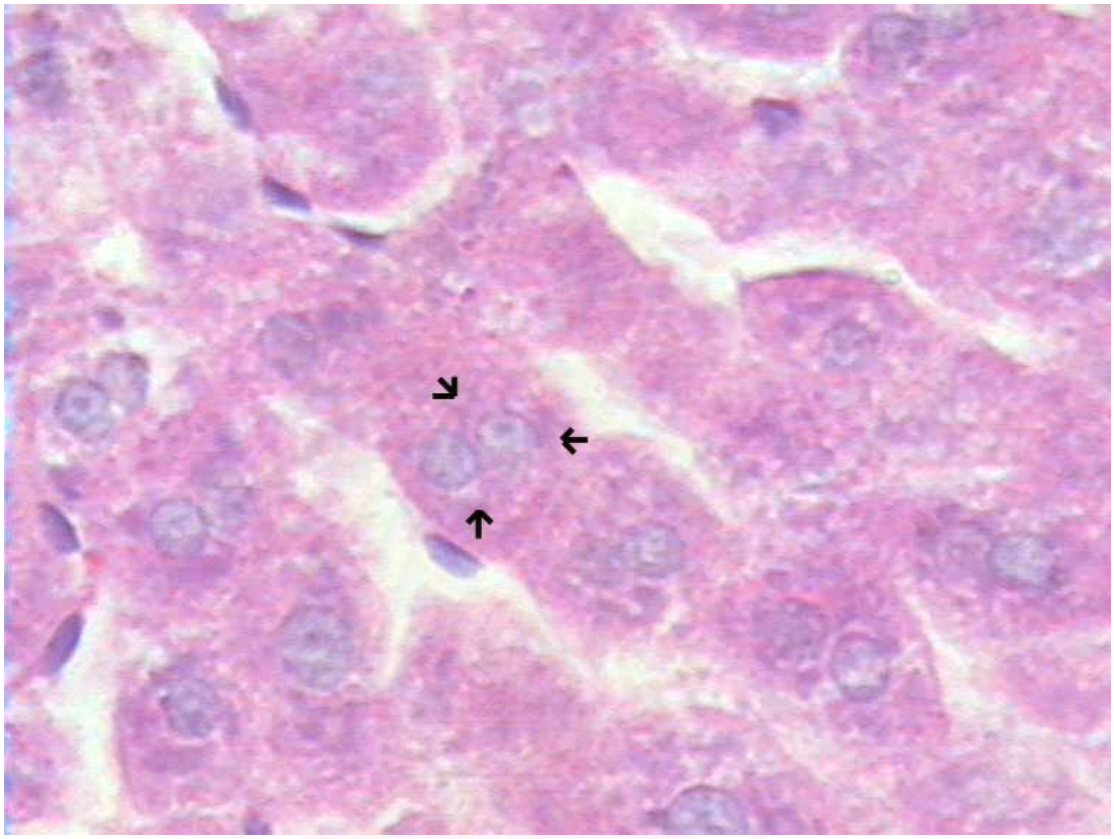


Figure 16. Normal hepatocytes

: Regular distribution of pink – red glycogen globules through cytoplasm of hepatocytes (arrows) (PAS x 400).

and not found fat deposit in hepatocytes, Oil Red O negative (Figure 17), the pathologic score was essentially zero.

The liver of the EtOH treated rats showed fatty changes, vacuolar degeneration at midzonal area and few focal necrosis around centrilobular area. It had extramedullary heamopoiesis adjacent to central vein (Figures 18 and 19).

The liver of EtOH treated rats receiving 300 and 400 mg/kg single dose of Jiaogulan extract showed lymphoid aggregation at portal area, increasing of sinusoidal-space, centrilobular degeneration and very few of focal necrosis (Figures 20 and 21). The pattern of histological changed looked like control group, the grading score was zero. In the dose of 500 mg/kg Jiaogulan extract showed the increasing area of sinusoid han the lower doses, Its had apoptotic hepatocytes estimated about 20 % of the field, midzonal degeneration and periportal vacuolar degeneration (Figures 22).

Experiment II: Effect of Jiaogulan extract against sub-acute toxic dose of EtOH (4 g / kg/ day)

2.1 Sub-acute hepatotoxicity of EtOH

Table 7 demonstrated the sub-acute hepatotoxic effect of EtOH at T_{28} (4 g/kg/day, for 28 days) on serum triglyceride (STG), AST and ALT,hepatic triglyceride (HTG), GSH and MDA there were the increase in triglyceride levels both in serum and liver (STG and HTG), and ALT activity compared to control at T_0 time.

Histopathological study demonstrated that EtOH group had the large vacuolar degeneration, increasing of sinusoidal space, irregular pattern of hepatocytes and focal necrosis (Figure 23), and grading was +3 (Table 8). Special staining of Oil Red O was positive (fat deposit, Figure 24), PAS was positive (glycoprotein aggregation; Figure 25) and Masson's Trichrome was negative (not found connective tissue, Figure 26).

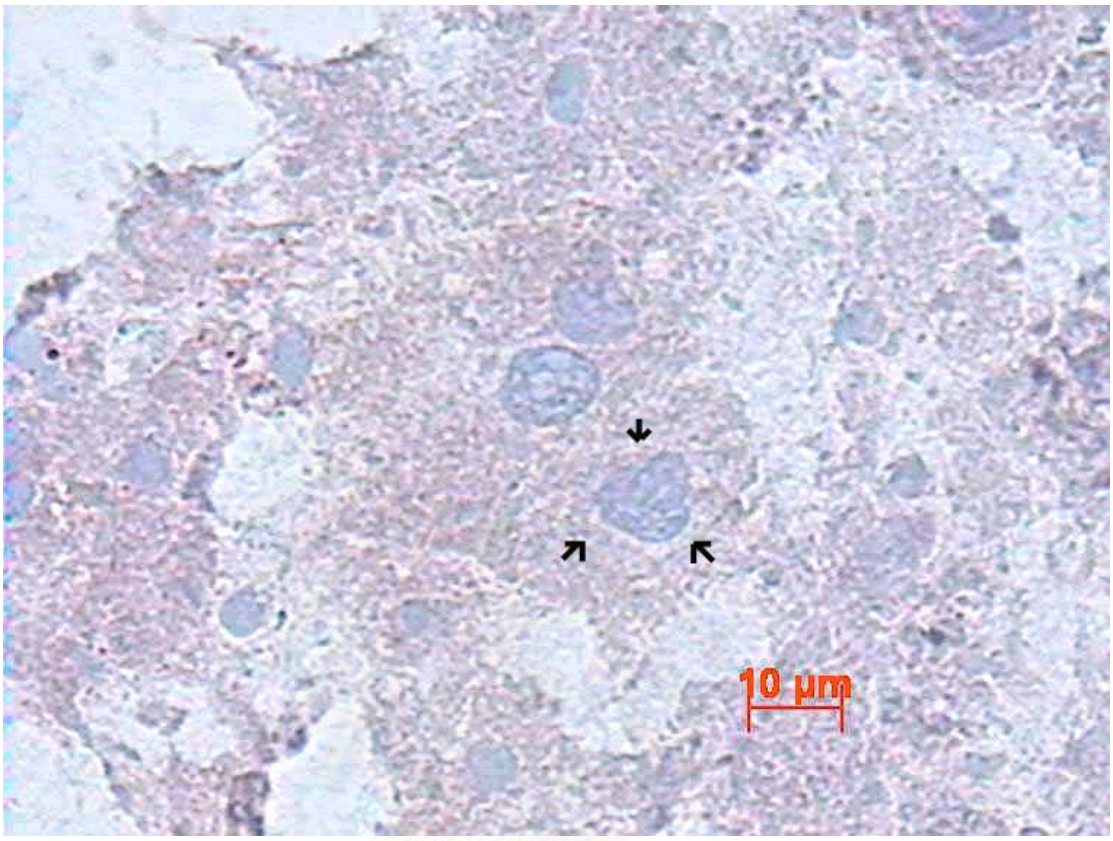


Figure 17. Normal hepatocytes of control rats (5 % corn flour; 5 ml/kg)

: Hepatocytes showed homogeneous cytoplasm (arrows), no sign of intracytoplasmic bright - red fat globules of hepatocytes (Oil Red O x 400).

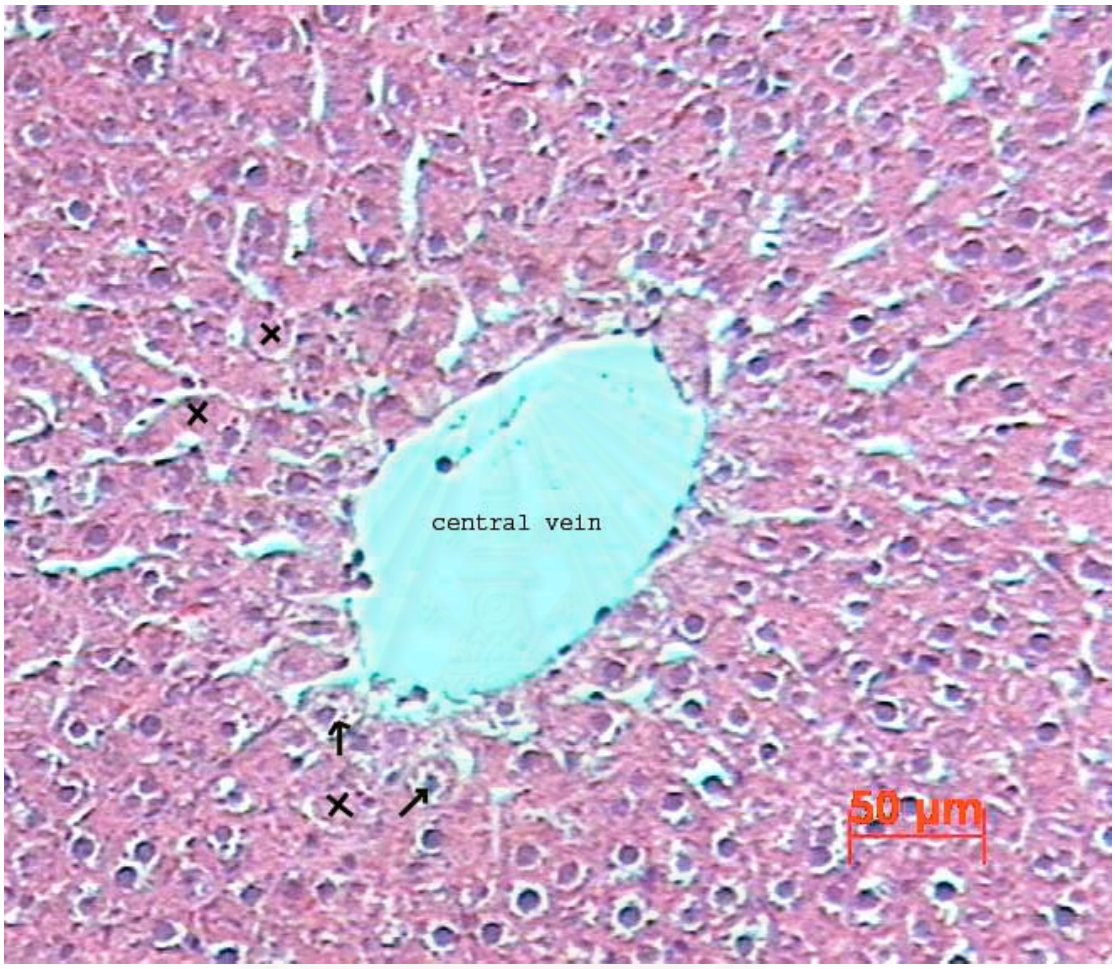


Figure 18. Acute toxicity of EtOH (5 g/kg)

: Periacinar (centrilobular vacuole degeneration) intracytoplasmic vacuolar degeneration (arrows) and a few focal necrosis of hepatocytes (x), grade + 2 (H&E x 100).

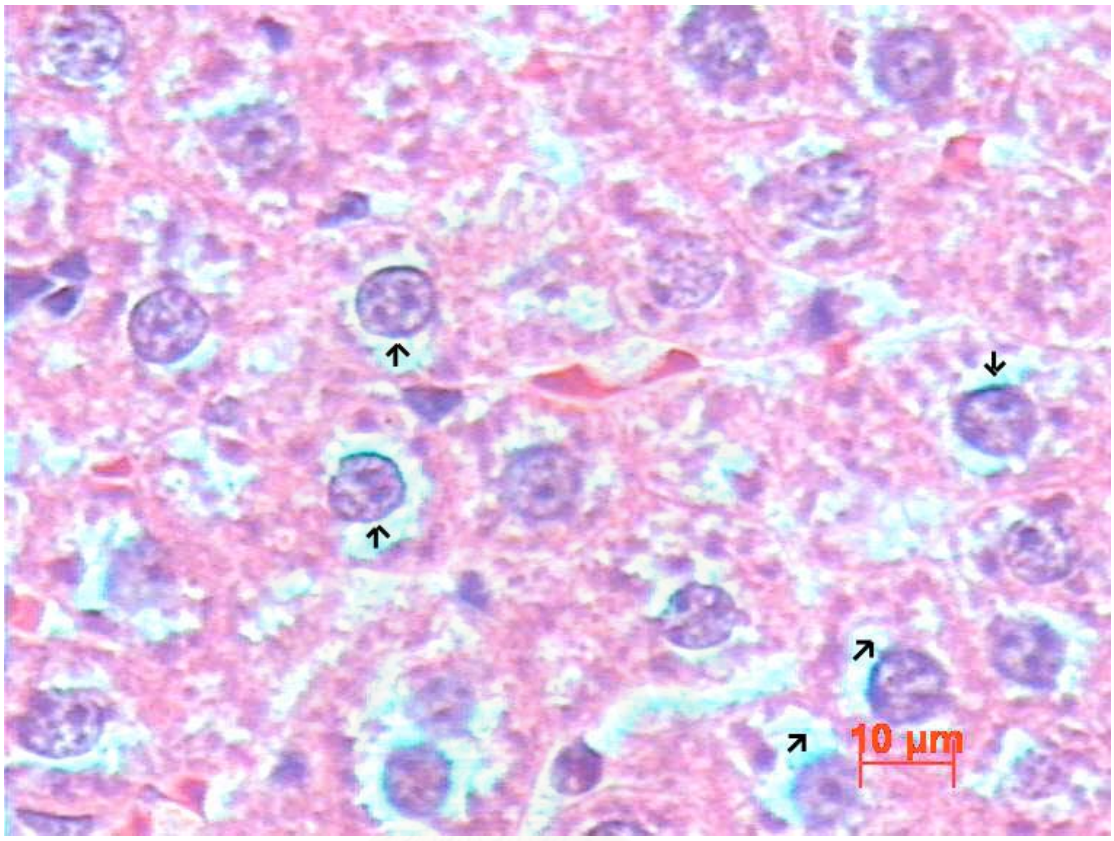


Figure 19. Acute toxicity of EtOH (5 g/kg)

: Higher magnification of liver cells from Figure 18 showed intracytoplasmic vacuolar hepatocytic degeneration(arrows) (H&E X 400).

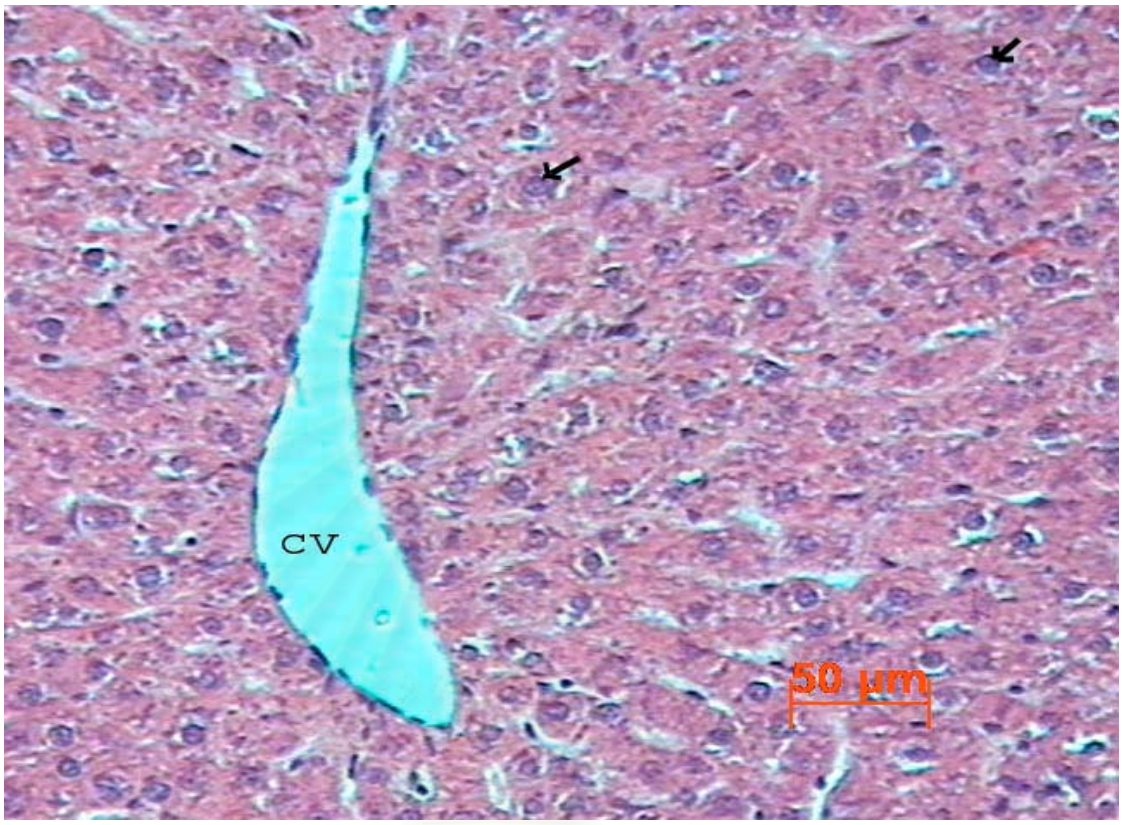


Figure 20. Acute toxic dose of EtOH (5 g/kg) treated with Jiaogulan extract (300 mg/kg)

: Reversible regeneration of hepatocytes. Hepatocytes showed large dense nuclei at a prophase stage(arrows) of the cellular division (H&E x 100).

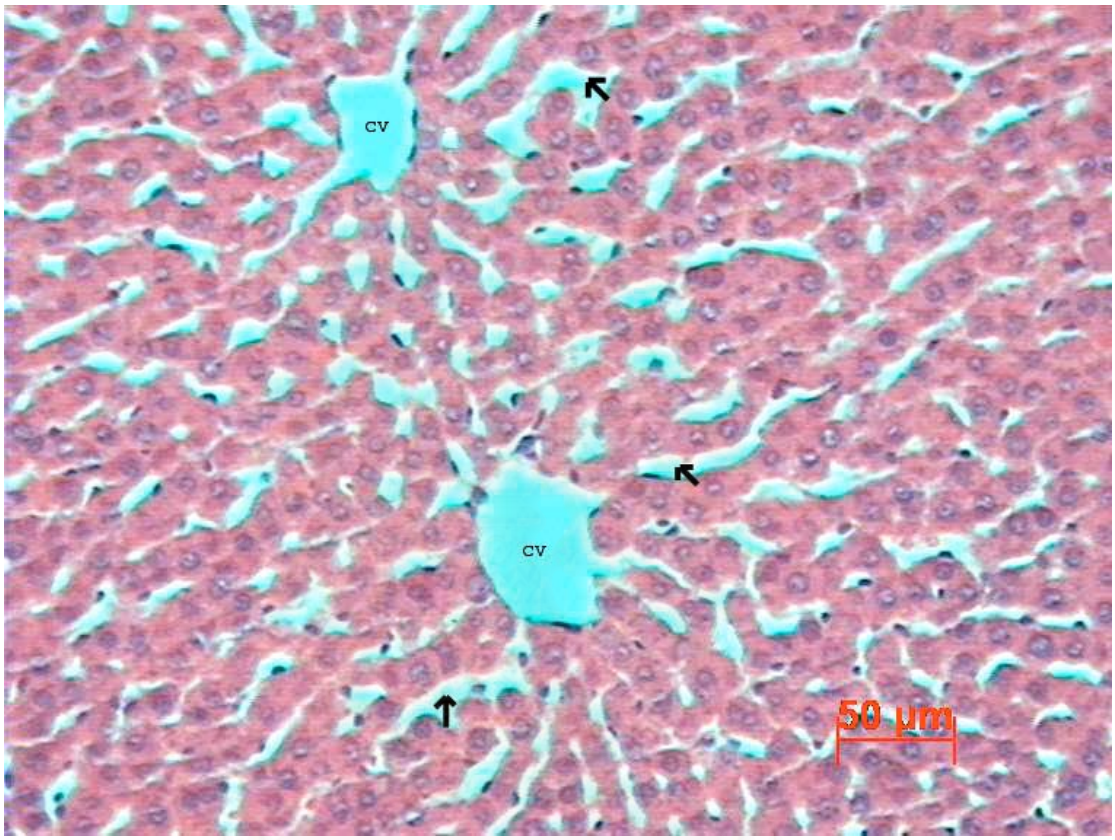


Figure 21. Acute toxic dose of EtOH (5 g/kg) treated with Jiaogulan extract (400 mg / kg)

: Increasing areas of sinusoidal spaces(arrows) was notice, grade 0 (H&E x 100).

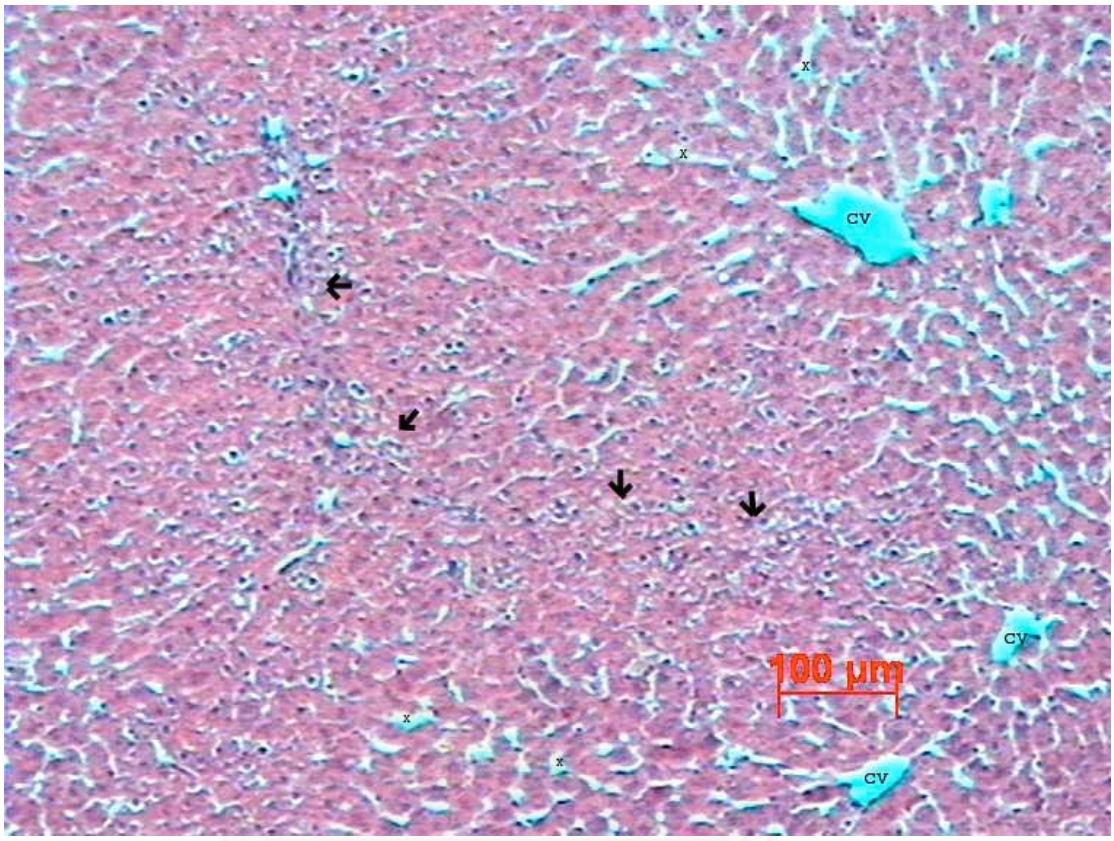


Figure 22. Acute toxic dose of EtOH (5 g/kg) treated with Jiaogulan extract (500 mg/kg)

: Midzonal intracytoplasmic vacuolar degeneration of hepatocytes (arrows) together with increasing areas of sinusoidal spaces(x), grade + 1 (H&E x 50).

Table 7. Effect of sub – acute toxic dose of EtOH (4 g / kg / day) for a period of 28 consecutive days compared with control group (5 % corn flour; 5 ml / kg) at T_O time

Parameters	Groups	
	Control (T_0) (5% corn flour; 5 ml / kg)	**EtOH (T_{28}) (4 g / kg)**
HTG (mg / g liver)	4.1 ± 0.2	5.2 ± 0.4*
STG (mg / dl)	57. ± 3.3	196.9 ± 5.3*
GSH (μmol / g liver)	7.0 ± 0.3	6.0 ± 0.2
MDA (nmol / g liver)	84.0 ± 2.7	65.3 ± 3.1*
AST (U / L)	113.0 ± 8.1	127.4 ± 7.7
ALT (U / L)	37.7 ± 0.5	52.3 ± 3.0*

Values expressed as mean ± S.E.M. (n = 8)

* Significant difference ($P < 0.05$) compared to control group

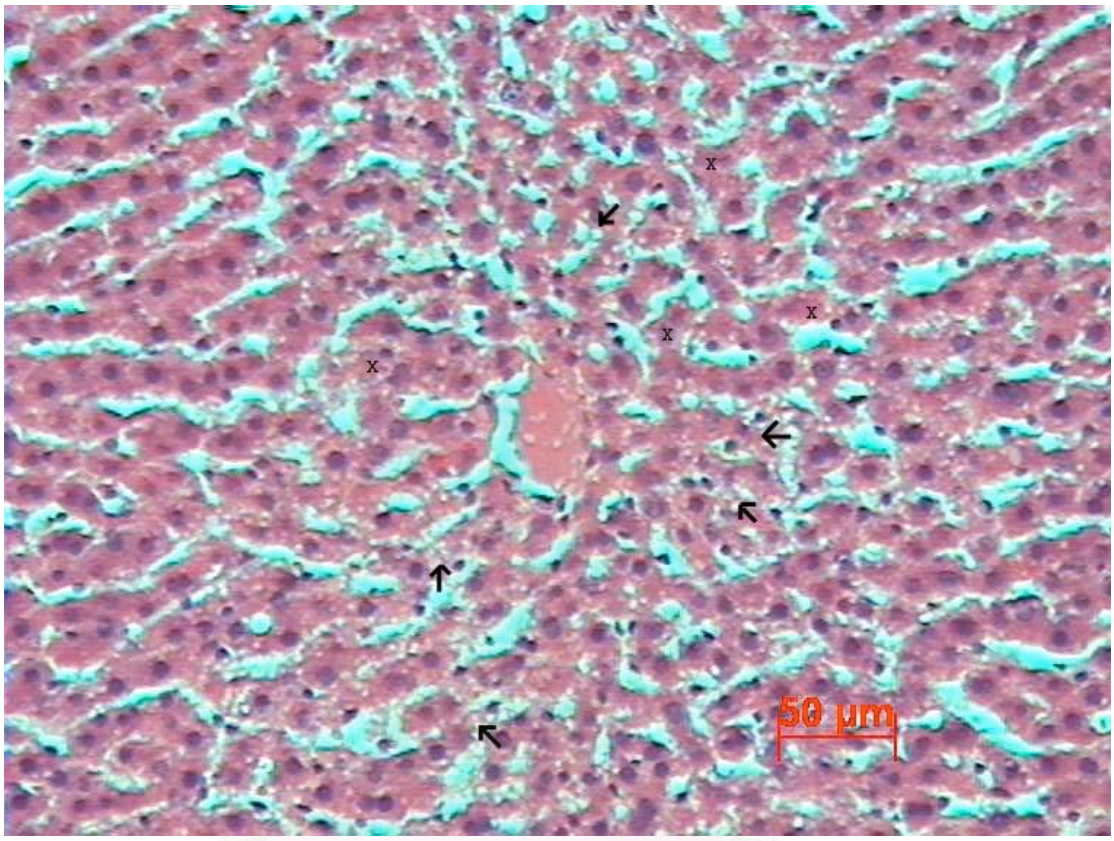


Figure 23. Sub – acute toxic dose of EtOH (4 g/kg) for a period of 28 consecutive days

: Diffuse large intracytoplasmic vacuolar hepatocytic degeneration(arrows) and necrosis(x). Irregular patterns and variety sizes of hepatocytes were marked, grade + 3 (H&Ex100).

Table 8. The histopathology of sub acute toxic dose of EtOH (4 g / kg / day) for a period of 28 consecutive days (T_{28}) compared with control group (5 % corn flour; 5 ml / kg) at T_o (n = 8)

Time / Groups	Degree of hepatocyte injury
T_O Control	0
T_{28} EtOH	+ 3

The severity of hepatocyte injury grading by

0 = normal

+ 1 = mild

+ 2 = moderate

+ 3 = severe

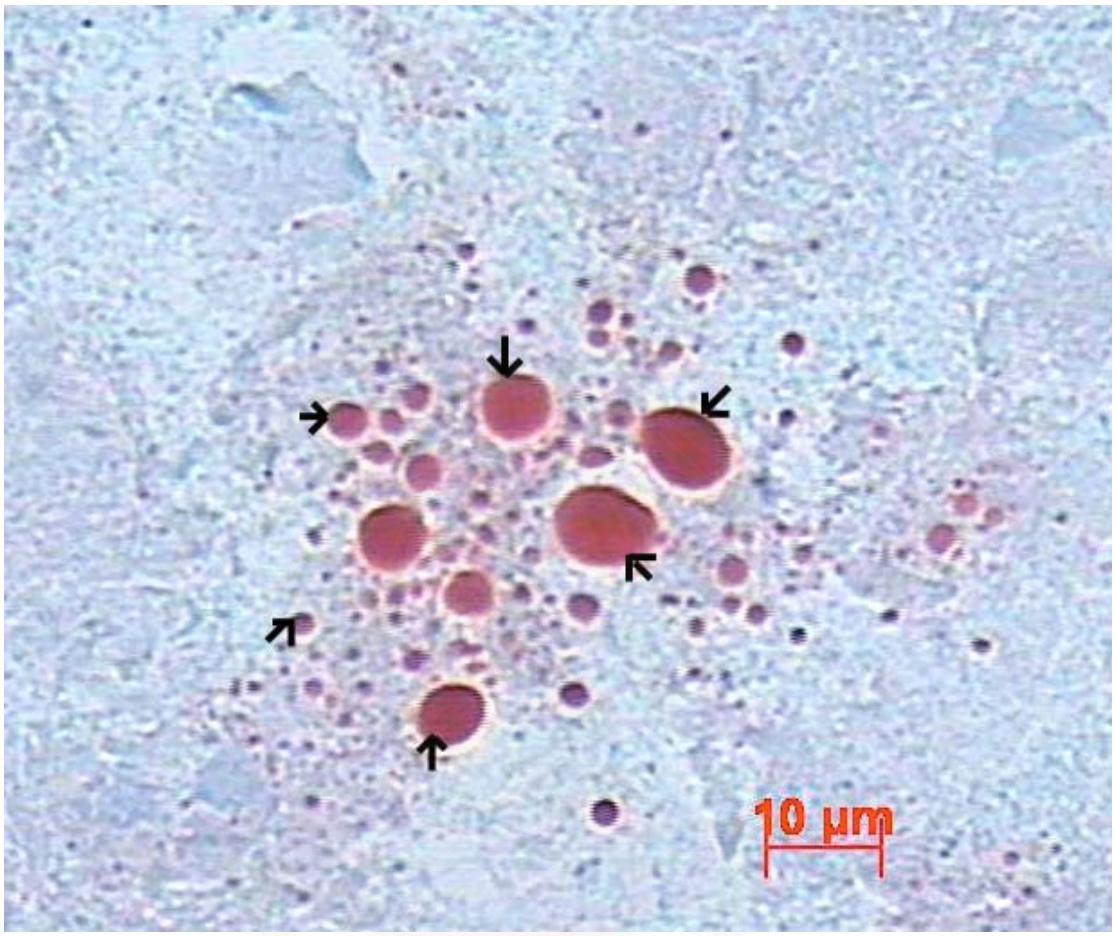


Figure 24. Sub – acute toxic dose of EtOH (4 g/kg) for a period of 28 consecutive days

: Intracytoplasmic bright - red fat globules(arrows) of hepatocytes (Oil Red O x 400).

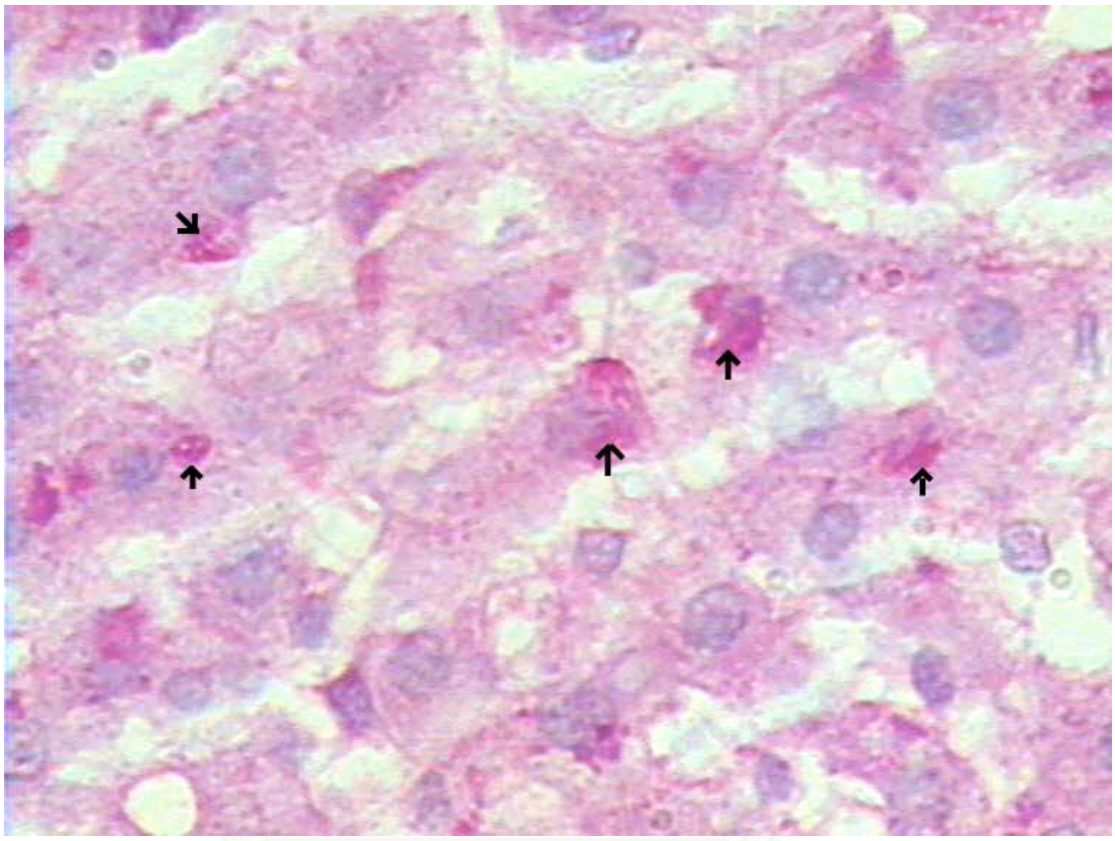


Figure 25. Sub – acute toxic dose of EtOH (4 g/kg) for a period of 28 consecutive days

: Intracytoplasmic pink – red glycoprotein aggregation(arrows) in hepatocytes (PAS x 400).

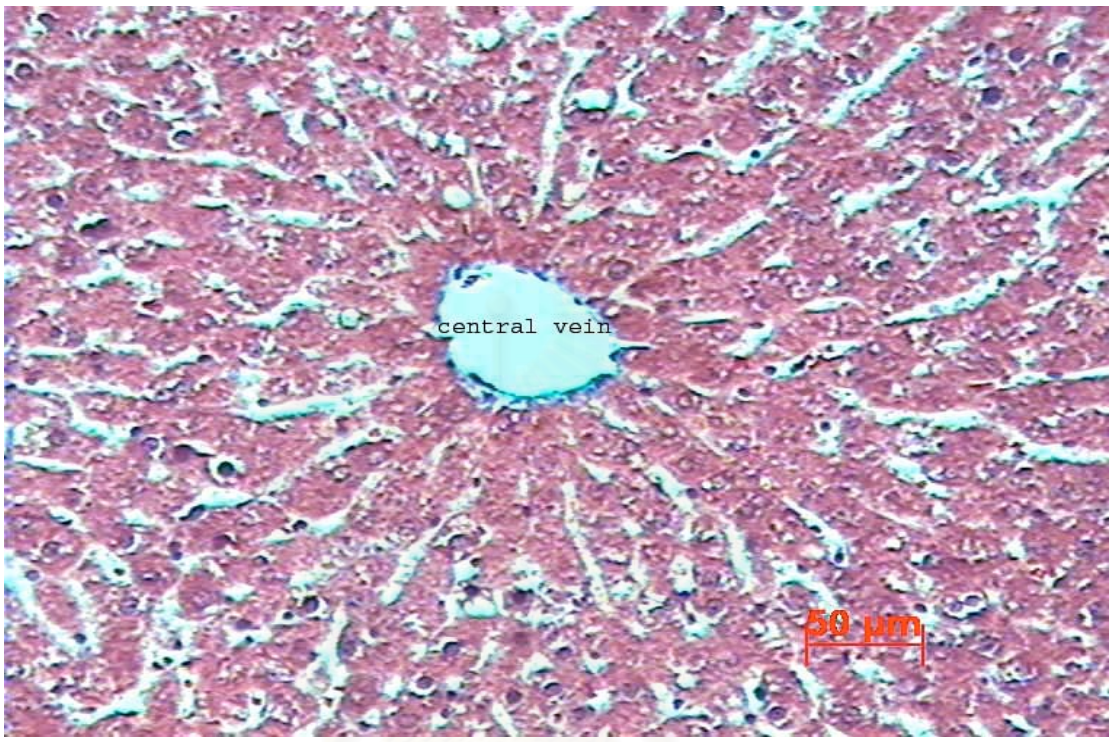


Figure 26 Sub – acute toxic dose of EtOH (4 g/kg) for a period of 28 consecutive days

: No fibrosis, collagen in perivenule, pericellular and perisinusoidal (Masson's trichrome x 100).

2.2 Reduction of sub-acute EtOH induced hepatotoxicity by Jiaogulan extract given by varying time of treatment

Prolonged treatment of EtOH (4 g/kg/day for 28 days) caused hepatotoxicity (Tables 7 and 8, Figures 23-26). Results of tratment with Jiaogulan extract (300 mg/kg/day) for a period of 7, 14 and 21 days were shown in Table 9.

After 7, 14 and 21 days of treatment with 300 mg/kg/day Jiaogulan extract, HTG, AST and ALT levels were smaller than control group treated only with 5 % corn flour (Table 9, Figures 27, 28). The decrease in HTG and AST was shown in 14 days and ALT in 7, 14 and 21 days. The levels of STG, GSH and MDA in both treated with Jiaogulan extract and control groups (Table 9, Figures 29, 30, 31) were quite similar to values found in normal rat.

Histopathological examination of hepatocytes showed slightly decreasing of the severity from 7 to 21 days (Table 10). In control group, it showed vacuolar and hydropic degeneration in midzonal area at 7 and 14 days. After that there was a slightly decreasing of vacuolar degeneration and increasing of cell regeneration in 21 days. In the experimental group, the reversions of hepatocyte are slightly increasing in 7 to 21 days with regular pattern of cytoplasm (H&E). Special staining of control and treatment group were negative.

2.3 Post effect of EtOH and Jiaogulan extract

Control and treatment groups of EtOH treated rats receiving 5 % corn flour (5 ml/kg/day) and Jiaogulan extract (300 mg/kg/day) for a period of 21 consecutive days, followed by diet and tap water ad libitum for 7 days before sacrificed, data was shown in table 11. All parameters (HTG, STG, GSH, MDA, AST and ALT) were not different between control and treated groups. The level of each parameter was the same as normal value.

Table 9. Effect of Jiaogulan extract on sub-acute toxic dose of EtOH (4 g/kg/ day, for 28 days) at varying time of treatment.

CONTROL : 5% Corn flour (5 ml / kg / day)

JIAOGULAN EXTRACT : GP (300 mg / kg / day)

Parameters	Group	7 days	14 days	21 days
HTG (mg / g liver)	Control	3.2 ± 0.2	4.1 ± 0.1	5.4 ± 0.4
	GP.	3.0 ± 0.1	3.2 ± 0.1*	4.9 ± 0.5
STG (mg / dl)	Control	56.2 ± 7.7	42.9 ± 5.1	77.4 ± 20.6
	GP.	45.8 ± 4.0	37.8 ± 2.5	32.0 ± 5.7
GSH (μmol / g liver)	Control	5.7 ± 0.2	5.8 ± 0.2	6.3 ± 0.1
	GP.	6.4 ± 0.2	6.1 ± 0.1	6.8 ± 7.4
MDA (nmol /g liver)	Control	71.1 ± 3.1	73.5 ± 2.7	77.0 ± 1.1
	GP.	69.1 ± 1.5	69.9 ± 1.1	75.6 ± 1.3
AST (U/L)	Control	128.3 ± 7.6	161.3 ± 7.7	155.5 ± 6.6
	GP.	114.9 ± 2.8	145.1 ± 5.6*	95.8 ± 7.0
ALT (U/L)	Control	31.1 ± 1.2	29.0 ± 1.5	33.7 ± 1.7
	GP.	25.5 ± 1.9*	21.3 ± 1.2*	29.3 ± 0.8*

Values expressed as mean + S.E.M. (n = 8)

*Significant difference ($P < 0.05$) compared to control group at the same period of time

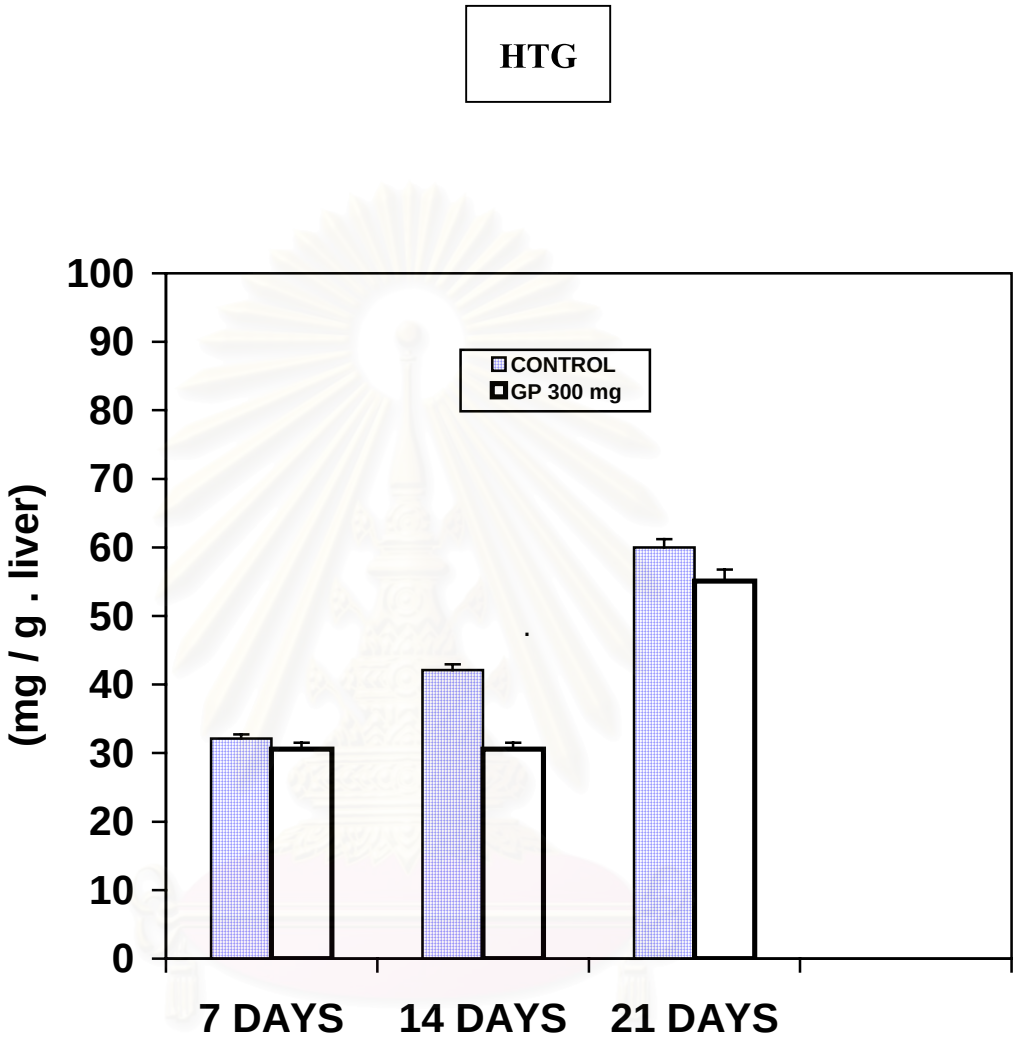


Figure 27. Effect of Jiaogulan extract (300 mg / kg / day) on HTG in ethanol induced hepatotoxic rats (n = 8)

* Significant difference ($P < 0.05$) compared to control group at the same period of time

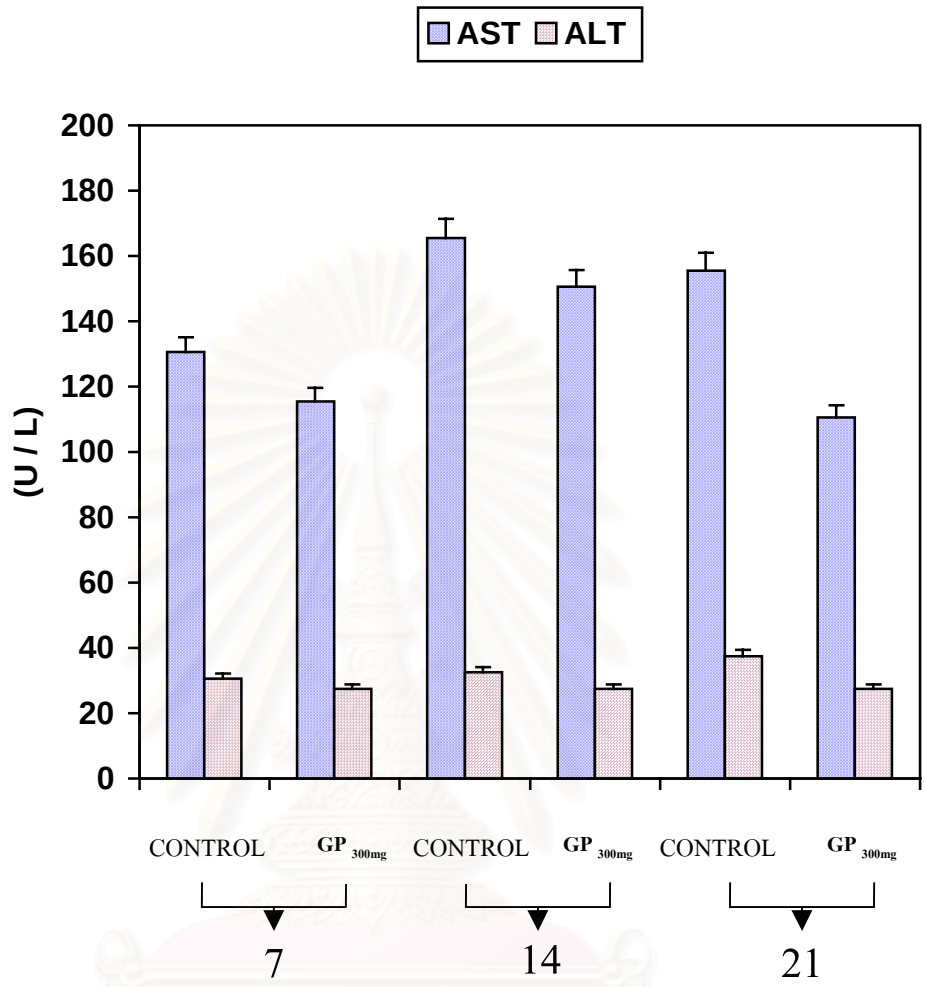


Figure 28 Effect of Jiaogulan extract (300 mg / kg / day) on AST and ALT in ethanol induced hepatotoxic rats (n = 8)

* Significant difference ($P < 0.05$) compared to control group at the same period of time

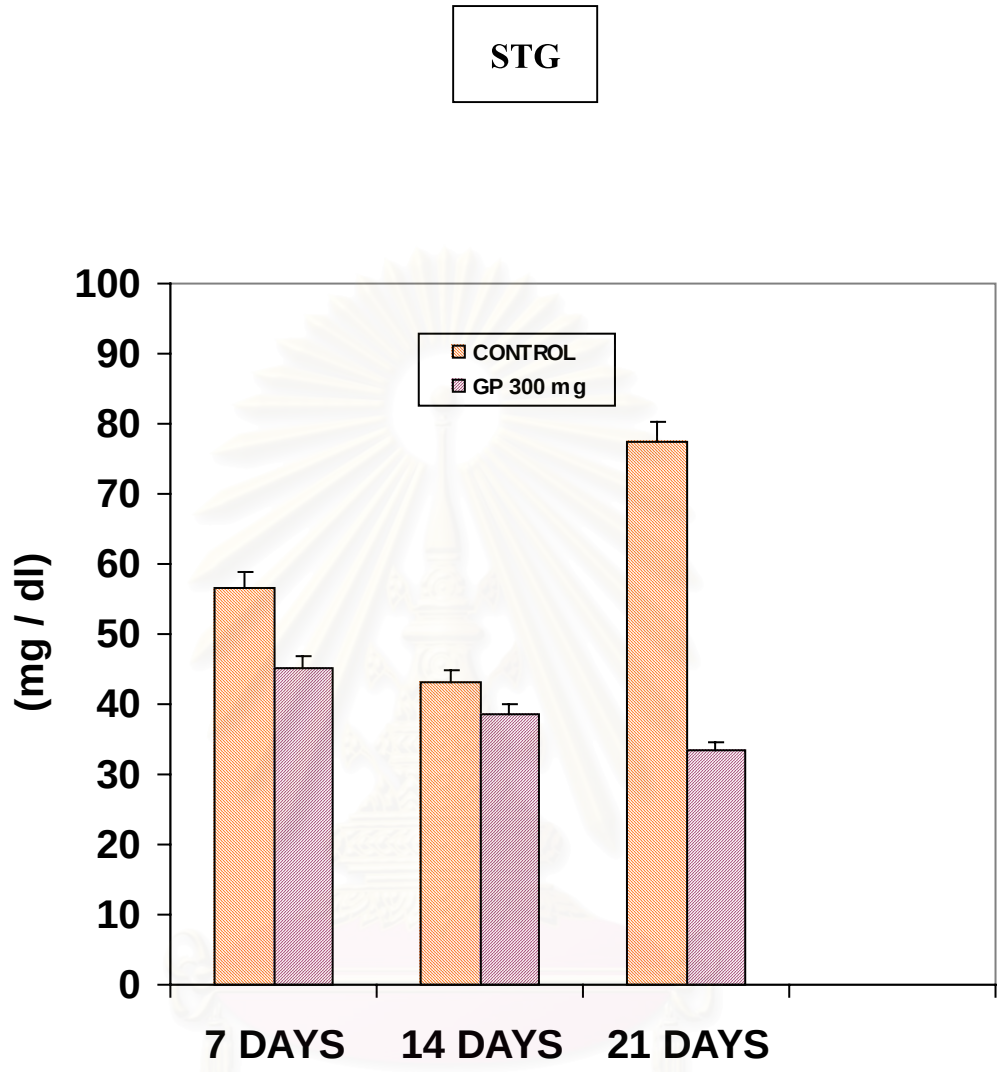


Figure 29. Effect of Jiaogulan extract (300 mg / kg / day) on STG in EtOH induced hepatotoxic rats (n = 8)

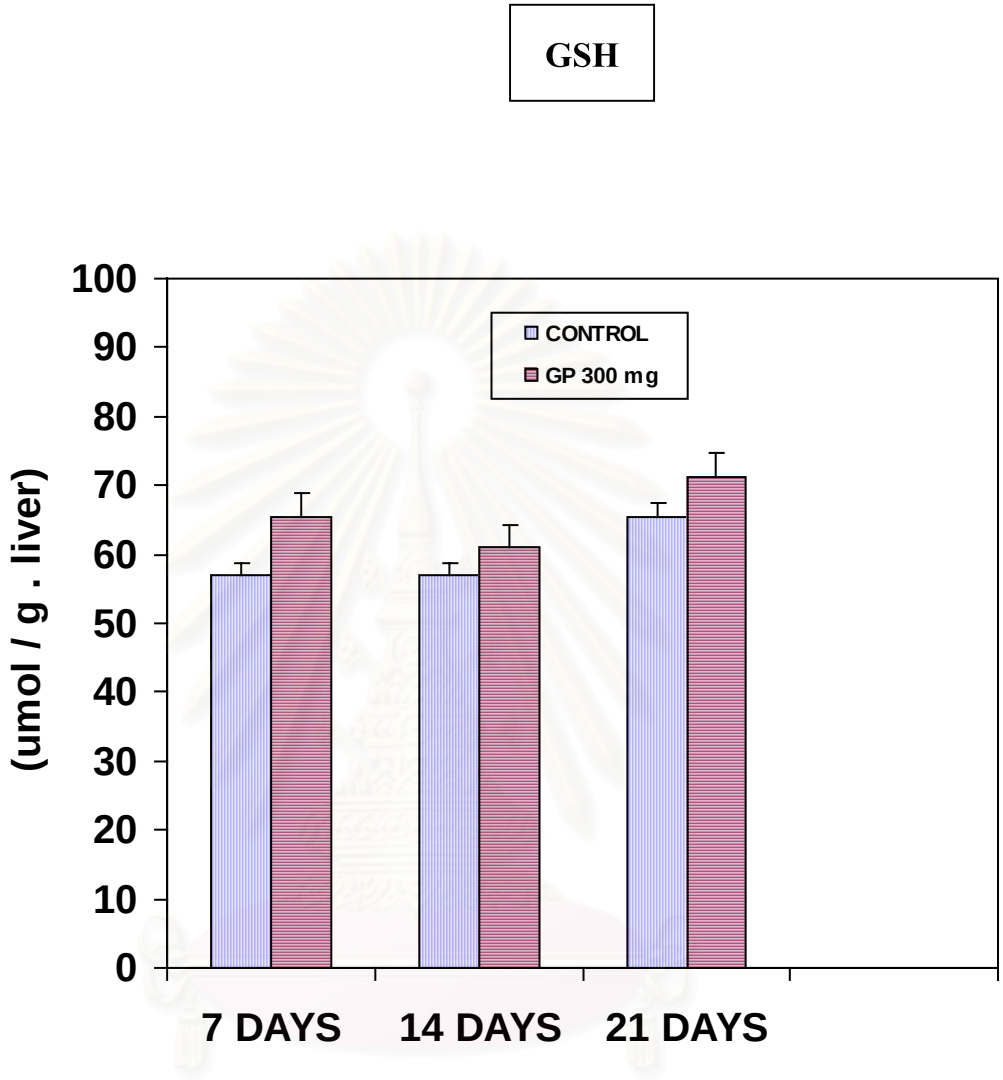


Figure 30. Effect of Jiaogulan extract (300 mg / kg / day) on GSH in EtOH induced hepatotoxic rats (n = 8)

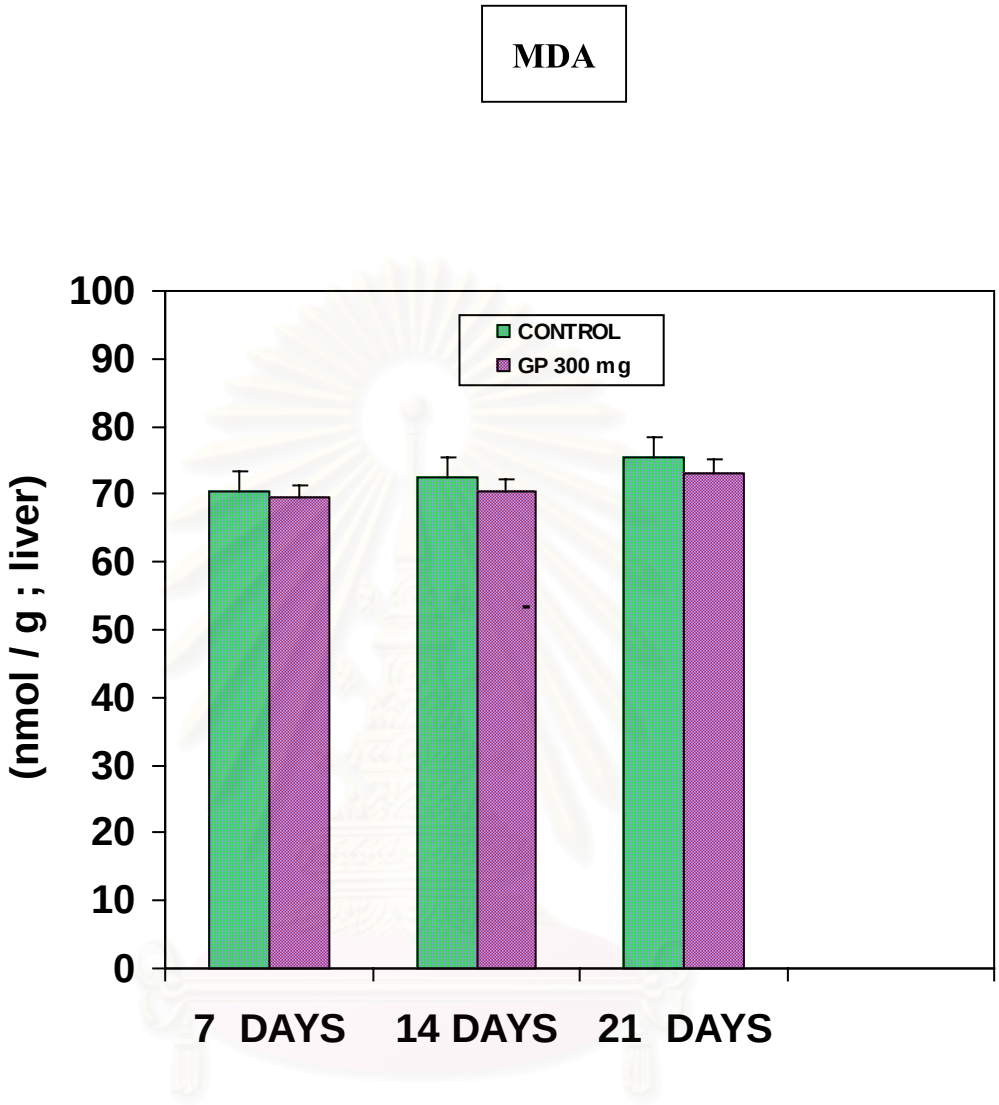


Figure 31. Effect of Jiaogulan extract (300 mg/ kg/day) on MDA in EtOH induced hepatotoxic rats (n = 8)

Table 10. The histopathology of the effect of Jiaogulan extract on sub-acute toxic dose of EtOH (4 g / kg / day, for 28 days) at varying time of treatment (n = 8)

Day	Groups	Degree of hepatocyte injury
7	Control	+1
	GP_{300}	0
14	Control	+1
	GP_{300}	0
21	Control	0
	GP_{300}	0

The severity of hepatocyte injury grading by

0 = normal

+ 1 = mild

+ 2 = moderate

+ 3 = severe

Table 11. Post effect of treatment with corn flour and Jiaogulan extract for 7 days with diet and tap water in EtOH induced hepatotoxic rats

Animal: ethanol induced hepatotoxic rats (4 g/kg/day, for 28 days)

Control: 5 % corn flour, 5 ml/kg/day, 21 days + 7 days of diet and tap water

GP: 300 mg/kg/day, 21 days + 7 days of diet and tap water

Parameters	Groups	
	Control (corn flour) (7 day of diet and tap water)	**GP (7 days of diet and tap water)**
HTG (mg / g liver)	3.4 ± 8.4	3.5 ± 8.3
STG (mg / dl)	38.4 ± 3.7	37.8 ± 3.5
GSH (μmol / g liver)	6.5 ± 0.2	6.9 ± 0.2
MDA (nmol / g liver)	83.8 ± 1.4	77.3 ± 2.5
AST (U/L)	77.3 ± 5.0	73.7 ± 4.8
ALT (U/L)	25.8 ± 1.8	24.0 ± 1.8

Values expressed as mean ± S.E.M. (n = 8)

Pathological study showed in Table 12. The pattern of pathological change between control and treatment group such as nucleus, cytoplasm, sinusoid etc. were in the same pattern, grading was 0.

Table 12. The histopathology of post effect of treatment with corn flour and Jiaogulan extract for 7 days with diet and tap water

Groups	Degree of hepatocyte injury
Control	0
GP_{300}	0

The severity of hepatocyte injury grading by

0 = normal

+ 1 = mild

+ 2 = moderate

+ 3 = severe

CHAPTER IV

DISCUSSIONS AND CONCLUSIONS

Ethanol (EtOH) was chosen as hepatotoxin to induce hepatotoxicity in wistar Albino rats, due to the fact that EtOH can cause liver toxicity in man after overdose or chronic consumption (Addison, 1963). In addition alcohol abuse is now the major health and social problems worldwide. The severity of hepatotoxicity varies from fatty liver, alcoholic hepatitis and cirrhosis. In this experiment EtOH induced hepatotoxicity is studied in the same pattern of pathology as that in man.

Udomusksorn (1995) found that EtOH 4 g/kg administered orally as single dose and repeated doses for 21 consecutive days caused hepatotoxicity in rats and EtOH 4 g/kg was the optimum dose to induce hepatotoxicity, the severity increased when increasing dose to 5 g/kg. Thus, in this experiment EtOH 5 g/kg was used for acute experiment and 4 g/kg for sub-acute experiment in a period of 28 consecutive days.

In normal condition EtOH pathway pass to oxidation by several enzyme systems; alcoholdehydrogenase (ADH), microsomal ethanol oxidizing system (MEOS) and catalase to acetaldehyde (toxic metabolite) that changes to acetate by enzyme aldehyde dehydrogenase (ALDH) finally to CO_2 and H_2O. EtOH overdose or prolonged consumption of EtOH generates more acetaldehyde by a microsomal cytochrome P-450-mediated enzyme system (Lieber and Decarli, 1968). Over producttion of acetaldehyde can destroy hepatocyte mitochondria and endoplasmic reticulum causing liver cell necrosis (Zimmerman, 1978). EtOH or its metabolites can also cause auto-oxidation of hepatic cells either by acting as a pro-oxidant or by reducing the anti-oxidation levels resulting in marked hepatotoxicity (Mitchell and Jollow, 1975).

In the year 2000, Chen et al. demonstrated that gypenoside extract from Jiaogulan has hepatoprotective effect against $CC1_4$-induced liver damage in Wistar rats Lin et al, (2000) also found that Jiaogulan extract has antioxidant and hepatoprotective effects in acetaminophen intoxicated rats. Thus, in this experiment it should be interesting to investigate the effect of 95% ethanol extract of Jiaogulan for its hepatoprotective activity against EtOH in rats. The selection of EtOH as the hepatotoxin may lead to the application of Jiaogulan as the alternative medicine in man intoxicated by ethanol in human.

The protective effect of Jiaogulan extract against toxic single dose of EtOH (5 g/kg)

EtOH group (5 g/kg, single dose) HTG, STG, AST and ALT increased significantly ($P < 0.05$) compared with control rats receiving 5% corn flour (5 ml/kg), while GSH and MDA levels were unchanged. The increase in serum AST and ALT is the sensitive indicates for liver cell damage (normal values in human: AST, 5-40 U/L; ALT, 5-35 U/L and male wistas rat: AST, 62.5 $\pm$ 8.4 IU/L: ALT, 25.2 $\pm$ 2.05 IU/L) (Gad and Chengelis, 1992). Enzyme transaminases contained in hepatocyte can not diffuse out of cell in normal situation. When hepatocyte is injured by drug or toxin that can disrupt plasma membrane, crossing transaminase leakage through extracellular fluid, that then serum transaminase enzyme can be detected at abnormal levels. The change of enzyme levels correlates with hepatocyte necrosis (Robbins, 1974).

The increase in HTG and STG levels in acute toxic dose of EtOH treated rats is inagreement with Daher et al (2003) who demonstrated that 3 hours after the administration of 8 % (v/v) alcohol in form of ethanol, beer or whisky in rats, STG was increased compared with control group receiving 5 ml emulsion of 30 % (w/w) olive oil (2.23 $\pm$ 0.31; 3.71 $\pm$ 0.41; 3.84 $\pm$ 0.31 and 4.42 $\pm$ 0.49 m.mol/l). Galembus (1985) suggeted that the increase in HTG and STG may occurs from metabolic dysfunction of lipid. EtOH oxidation oxidized by ADH to acetaldehyde and ALDH to

acetate, using NAD which converted to NADH (reduced from). The generated NADH is transported to mitochondria by shuttle mechanism and oxidized in respiratory to NAD. EtOH over dose causes over production of toxic metabolite leading to the disturbance of lipid metabolism in hepatocyte. The mechanism of lipid metabolism dysfunction may due to the increase in fatty acid from the decrease in its oxidation. The increased fatty acid from esterification can enhance TG levels. Elevation of STG and HTG can be used as marker for hepatocyte damage or fatty liver.

EtOH (5g/kg, single dose) did not affect GSH and MDA levels. From review literature,there is a very few research study involving the effect of GSH and MDA from acute toxic dose of EtOH. Results from this experiment is similar to Sato and Leiber (1981) showing that acute toxic dose of EtOH (6g/kg) administered to male Sprague-Dawleys rats do not change the levels of GSH and MDA. It may due to the amount of EtOH toxic metabolite formed which is not sufficient to reduce GSH (body antioxidant) level or increase MDA formation (Lipid peroxidation). Many researchers are interested in studying hepatotoxicity induced by paracetamol (APAP) or carbon tetra chloride (CCl_4) by study the change of GSH and MDA levels (Chen et al., 2000; Kim and Lee, 1998 ; Sato et al.,1981). The pathways of APAP and CCl_4 metabolism correlate directly to GSH and MDA levels. APAP induced hepatotoxicity is throught to cause by N-acetyl-p-benzoquinone-imine (NAPQI), a cytochrome p-450-mediated intermediate metabolite (Mitchell et al., 1973). NAPQI can react with sulfhydryl group such as GSH and protein thiols. The covalent binding of NAPQI to cell proteins is considered the initial step in a chain events eventually leading to cell necrosis (Jollow et al., 1973). CCl_4 is metabolized by cytochrome P-450 2E1 (CYP 2E1) to the trichloromethyl radical ($CCl_3^{\bullet}$) which is assumed to initiate free radical mediated lipid peroxidation leading to the accumulation of lipid derived oxidation products that cause liver injury (Recknage et al., 1989). In this experiment acute toxic dose of EtOH cause no sign of lipid peroxidation reaction.

Pre-treatment of Jiaogulan extract at 300, 400 and 500 mg/kg single dose 4 hr before the administration of EtOH 5 g/kg showed that Jiaogulan extract at 300 mg/kg reduced HTG and normalized the level of AST and ALT which was found also with the doses of Jiaogulan 400 and 500 mg/kg. These results are in agreement with Chen et al (2000) and Lin et al (2000) showing that, water and n-butanol extract of Jiaogulan at 100, 300 and 500 mg/kg can reduce AST and ALT induced by APAP and CCl_4 in rats. In this experiment, it may conclude that extract of Jiaogulan at 300-500 mg/kg may have hepatoprotective effect due to some of its active ingredients that functioned as antioxidant against toxic metabolites of EtOH.

Histopathological examination correlated with the biochemical parameters. EtOH treated group had the vacuolar degeneration in centrilobular orea (H&E) and fatty degeneration due to fat deposit grading +2. Pre-treatment with Jiaogulan extract at 300, 400 and 500 mg/kg reduced the severity of hepatocyte injury to 0, 0 and +1.

Pre-treatment of Jiaogulan extract may have hepatoprotective activity against EtOH especially at 300 to 400 mg/kg, in certain dosage range Jiaogulan at 500 mg/kg seemed to be less effective than 300 and 400 mg/kg and may affect the hepatocyte as confirm by histopathological study. We should be aware in selection of optimum dose for safety reason. Overdose may be effective for short treatment but may induce hepatotoxicity in the long term.

To study chronic effect of EtOH and treatment with Jiaogulan extract,we chose the dose of 300 mg/kg due to its rarely safety compared to 400 and 500 mg/kg in prolong treatment and EtOH 4 g/kg.

Effect of Jiaogulan extract against sub-acute toxic dose of EtOH (4 g / kg / day)

Chronic EtOH consumption results in the increased activities of various microsomal drug metabolizing enzymes (Rubin and Lieber. 1968; Misra et al., 1971). Administering EtOH (4g / kg / day) to rats for 28 consecutive days (T_{28}) resulted in significantly elevated levels of HTG, STG and ALT as compared with control rats (T_0)

receiving 5% corn flour, 5 ml / kg. The change of parameters correlated with acute toxic dose of EtOH (5g/kg). It is interesting to see the drop in MDA content after 28 days of ethanol treatment indicating the antioxidant potential of liver cells in protecting themselves by destroying the radicals generated by EtOH metabolism. It seems to be that taken EtOH for short term the severity of lipid peroxidation is very few and GSH is not depleted.

Pathological examination of EtOH treated rats (4 g / kg / day, 28 days) showed a large vacuolar degeneration and focal necrosis, grading +3 which was higher than the severity of acute dose (5 g / kg). Oil red O staining was positive indicating that sub-acute toxic dose of EtOH given for 28 consecutive days can cause fatty liver.

Treatment with Jiaogulan extract at a dose of 300 mg/kg/day in EtOH induced hepatotoxic rats by varying time of treatment at 7, 14 and 21 compared with control group receiving only 5% corn flour given for the same period of time. There were the lower level of HTG and AST in day 14 and ALT in day 7, 14 and 21 in Jiaogulan treatment group, indicating the effect of Jiaogulan in increasing the recovery of liver cell injury. Pathological study also confirmed that after 7, 14 and 21 days of Jiaogulan treatment hepatocyte damage was improved such as a few local necrosis and lesser vacuolar degeneration, the morphology was closed to normal at 14 and 21 days. The special staining are negative. All parameters are approaching the normal values.

After treatment with Jiaogulan extract and corn flour for 21 days in ethanol induced hepatotoxic rats (4 g/kg/day, 28 days), the animals were given only diet and tap water for 7 days to see the after effect of Jiaogulan. Since all parameters are approaching normal values at day 21 of Jiaogulan treatment, there was no difference among these two groups. Indicating the auto-recovery power of hepatocytes, confirmed by these results from histopathological examination.

In conclusions, EtOH overdose or sub-acute toxic dose resulted in hepatotoxicity in rats. Jiaogulan extract may have the hepatoprotective activity given both pre - and post - treatment against EtOH induced liver cell damage by lowering the levels of HTG, STG, AST and ALT with unchanged GSH and MDA. In addition, histopathological studies of rat liver confirmed this beneficial role of Jiaogulan extract.

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