EFFECTS OF ETHANOL EXTRACT OF ZEA MAYS HAIRS ON LIVER FUNCTION TESTS IN ALCOHOL FED RATS

BY

SIM LEE LING

Dissertation submitted in partial fulfillment of the requirements for the degree of Bachelor of Health Sciences (Biomedicine) ÷.

March 2006

CERTIFICATE

This is to certify that the dissertation entitled "Effects of ethanol extract of Zea mays hairs on liver function tests in alcohol fed rats" is a bonafide record of research work done by

s a bonande record of research work done

Miss Sim Lee Ling

Signature of supervisor: _______

Name and address of supervisor: Dr. Prema Sembulingam

Associate Proffesor School of Health Sciences Universiti Sains Malaysia Health Campus 16150 Kubang Kerian, Kelantan 04: 05.06

Date:

Signature of supervisor:

Name and address of supervisor: Dr. K. Sembulingam

Associate Proffesor School of Health Sciences Universiti Sains Malaysia Health Campus 16150 Kubang Kerian, Kelantan O4, OS, 06

Date:

ACKNOWLEDGEMENT

First of all, I would like to express my sincere gratitude to my supervisor Associate Professor Dr. Prema Sembulingam for her caring guidance throughout the period of this study. Under her supervision, I expanded my knowledge in conducting research work and the method of writing dissertation.

My special thanks are due to my co-supervisor, Associate Professor Dr. K. Sembulingam for his great help in this study, especially in teaching me the technique of drawing the blood and maintaining the experimental animals.

I am indebted to Dr. Nik Suriayani, head of Department of Chemical Pathology in PPSP, USM for giving permission to carry out the blood analysis in her laboratory.

I am very thankful to Mr. Rafi and other staffs in Chemical Pathology Department in PPSP for their help in doing the blood analysis.

I would also like to thank Mr. Mohd Zaki bin Selamat, Science Officer in PPSK, for helping me in collecting the plant material.

My sincere thanks also go to all the staff in UKM (Laboratory Equipments Unit), PPSK for helping in the preparation of extract of plant material, with a special thanks to Mr. Nik Fakurudin Nik Ali in providing the necessary instruments during the course of our project. Besides that, I would like to extend my appreciation and thanks to all the staff in Animal House in USM for their co-operation in providing the experimental rats. The RA, Mr. Azri bin Rosely deserves my thanks for his help in feeding and taking care of the experimental rats during the study.

Last but not the least, my sincere thanks are due to my fellow friends and my family members for their valuable support and encouragement throughout the period of my research work.

CONTENTS

No.	Tittle	Page
1.	Certificate	ii
2.	Acknowledgement	iii-iv
3.	Content	v
4.	List of Plates	vi
5.	List of Tables	vii-viii
6.	Abstract in Bahasa Malaysia	ix-x
7.	Abstract in English	xi
8.	Introduction	1-5
9.	Review of Literature	6-13
10.	Objective	14
11.	Materials and Methods	15-21
12.	Results	22-37
13.	Discussion	38-40
14.	Conclusion	41
15.	References	42-47

LIST OF PLATES

- Plate 3.1: Rotavapourization Machine
- Plate 3.2: Sprague dawley rat
- Plate 3.3: Auto Analyzer Hitachi 912
- Plate 3.4: Cardiac Puncture for Blood Collection

LIST OF TABLES

- Table 4.1: Effects of ethanol extract of Zea mays Hairs on Total Protein (g/L) compared with control group
- Table 4.2: Effects of ethanol extract of Zea mays Hairs on Total Protein (g/L) compared with alcohol group
- Table 4.3: Effects of ethanol extract of Zea mays Hairs on Albumin (g/L) compared with control group
- Table 4.4: Effects of ethanol extract of Zea mays Hairs on Albumin (g/L) compared with alcohol group
- Table 4.5: Effects of ethanol extract of Zea mays Hairs on Globulin (g/L) compared with control group
- Table 4.6: Effects of ethanol extract of Zea mays Hairs on Globulin (g/L) compared with alcohol group
- Table 4.7: Effects of ethanol extract of Zea mays Hairs on Albumin/Globulin ratio

 compared with control group
- Table 4.8: Effects of ethanol extract of Zea mays Hairs on Albumin/Globulin ratio compared with alcohol group
- Table 4.9: Effects of ethanol extract of Zea mays Hairs on Bilirubin (µmol/L) compared with control group
- Table 4.10: Effects of ethanol extract of Zea mays Hairs on Bilirubin (µmol/L) compared with alcohol group
- Table 4.11: Effects of ethanol extract of Zea mays Hairs on Alkaline Phosphatas

 Activity (U/L) compared with control group
- Table 4.12: Effects of ethanol extract of Zea mays Hairs on Alkaline Phosphatase

 Activity (U/L) compared with alcohol group

- Table 4.13: Effects of ethanol extract of Zea mays Hairs on Aspartate Aminotransferase

 activity (U/L) compared with control group
- Table 4.14: Effects of ethanol extract of Zea mays Hairs on Aspartate Aminotransferase activity (U/L) compared with alcohol group
- Table 4.15: Effects of ethanol extract of Zea mays Hairs on Alanine Aminotransferase activity (U/L) compared with control group
- Table 4.16: Effects of ethanol extract of Zea mays Hairs on Alanine Aminotransferase activity (U/L) compared with alcohol group

ABSTRAK

Ekstrak etanol rerambut telah dijalankan bahan kajian untuk meninjau kesannya ke atas fungsi hati pada tikus yang diberikan alcohol. 30 ekor *Sprague dawley* jantan telah dibahagikan kepada enam kumpulan di mana setiap kumpulan mengandungi lima ekor tikus. Kumpulan-kumpulan tersebut adalah kumpulan kawalan, kumpulan ekstrak 50mg/kg jisim badan, kumpulan alcohol, kumpulan ekstrak 50mg/kg jisim badan, kumpulan alcohol, kumpulan ekstrak 100mg/kg jisim badan dan kumpulan ekstrak 100mg/kg jis

Ekstrak etanol rerambut Zea mays telah menujukkan peningkatan paras jumlah protein dan globulin secara signifikan pada dos tinggi (100mg). 3ml alkohol 30% juga meningkatkan kedua-dua parameter tersebut (peningkatan jumlah protein adalah tidak signifikan). Peningkatan paras jumlah protein dan globulin adalah lebih tinggi berbanding dengan ekstrak sahaja. Paras albumin juga menunjukkan peningkatan yang tidak signifikan dalam kumpulan 50mg ekstrak, 100mg ekstrak dan 50mg ekstrak bersama dengan alkohol. Tetapi dengan alkohol sahaja, paras albumin menurun secara signifikan.

Untuk enzim hati pula, tiada perubahan yang signifikan bagi alkalin fosfatase (alkaline phosphatase, ALP), aspartat aminotransferase (aspartate aminotransferase, ASP) dan alanin aminotransferase (alanine aminotransferase, ALT).

Daripada keputusan, ekstrak telah meningkatkan sintesis protein di dalam hati dan pada masa yang sama tiada sebarang kerosakan bertindak atas hati daripada ekstrak tersebut. Apabila 3ml alcohol 30% diberikan selama 14 hari, sintesis protein tiada menunjukkan sebarang kesan buruk. Pada masa yang sama, fungsi enzim hati juga tidak terjejas.

ABSTRACT

The ethanol extract of *Zea mays* hairs was screened for the effects on liver functions in alcohol fed rats. 30 male *Sprague dawley* rats were divided into six groups with five rats in each group. There were control group, 50mg/kg body weight extract group, alcohol group, 50mg/kg body weight extract + alcohol group, 100 mg/kg body weight extract + alcohol group. All six groups were treated for 14 days.

The ethanol extract of Zea mays hairs increased total protein and globulin significantly in high dose (100mg). 3ml of 30% alcohol also increased these two parameters (increase in total protein was non significant). The extract with alcohol, increased the total protein and globulin more than the extract alone. Albumin also showed non significant increase in 50mg, 100mg extract and 50mg extract with alcohol. But alcohol alone decreased albumin significantly.

As far as the liver enzymes are concerned, there was no significant change in alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

The results showed that the extract increased protein synthesis in the liver and did not cause any damage to the liver.

3 ml of 30% alcohol when given for 14 days did not cause any adverse effect on protein synthesis and also did not cause any affect to the enzymatic functions of the liver.

xi

1. INTRODUCTION

For last few years, medicinal plants are gaining more and more popularity in health care system. It is because these herbs produce less harmful effects on human bodies compare to the modern medicines. It is claimed that the herbal plants could at least relieve the symptoms, if not effectively cure the chronic diseases. So, scientists nowadays are working very hard to figure out the medicinal value of different kinds of plants. One of them is *Zea mays*.

Zea mays, known in English as corn or maize, or jagung in the indigenus Malay language, can be found almost anywhere in Malaysia. It is one of the major staple foods which widely cultivated throughout the world other than wheat and rice. Zea mays is a good source of energy for both humans and animals. It is high-yielding, easy to process and readily digested. In some regions such as Africa and Latin America, Zea mays serves as the primary staple food (James A. Duke, 1983).

There are many varieties and the the six major types are as follows:

- Sweetcorn
- Popcom
- Dent corn
- Flint corn
- Flour corn/ Soft corn
- Pod corn

Sweetcorn – has the endosperm translucent and horny in appearance and the starch partially replaced by sugar, making it very sweet, subsequently is particularly

popular for human consumptions. It is of fairly recent development. It has very sweet, soft-skinned grains that can be eaten raw or cooked before they are fully ripe. Kernels become wrinkled when dry.

Popcorn – is characterized by its ability to pop when subjected to high temperatures. It is a primitive form with hard-skinned grains. Two types of kernels are known, one is rice-shaped with a pointed end and the other flat with rounded end; both are small and hard. Popcorn varieties have a relatively impervious layer of flinty endosperm surrounding the outside of the kernels which traps gases as the maize is heated. The higher the content of flinty endosperm in the grain, the greater the popping expansion of the kernels. It is grown mainly for human consumption, accounting for less than 1% of commercial production.

Dent corn – is characterized by wedge-shaped kernels with an indented top and with the soft or floury endosperm extending to the top, while the corneous is confined mainly to the sides of the kernel. It has mostly white to yellow grains. It is more widely grown type that provides millions of tons of grain for oils, cornflour, cereals and is used as live stock feed for animals. The name dent corn is due to its characteristic denting, which is caused by rapid, drying and shrinkage of the soft starch. Upon kernel maturation, moisture is lost from the crown of the kernel, which consists of floury starch. This causes a slight collapse in volume that produces a characteristic dent.

Flint corn – is characterized by its hardness of kernel with a rounded top and small area of soft endosperm around the embryo completely surrounded by corneous endosperm. It shrinks on drying and can have white, yellow, purple, red or blue-black

grains. It is similar to popcorn but with larger grain. It is not so sweet and also takes longer to mature. It is more widely grown in Europe, Asia, Central America and South America where cold torelerance is required or where storage and germination conditions are poor.

Flour corn/ Soft corn - is widely grown in the dry areas of United States and in Andean region of South America. The kernel makes up largely soft starch and has little or no dent, with the shape and outward appearance similar to flint corn but of varying in size. It is easily ground into flour to produce meal that can be consumed directly, or as a flat bread, dumpling or beverage, and used for preparation of variety of dishes. Because of this characteristic feature, it is preferred for direct human consumption. This accounts for 12% of commercial production.

Pod corn – has each kernel as well as the ear covered with a husk, the kernels vary greatly in size and shape. This type of corn is rarely grown (James A. Duke., 1983). It is no doubt that the *Zea mays* is a plant of high nutrient where it is an important sources of carbohydrates, and contains proteins, vitamin A, B_1 , B_2 , B_3 , C as well as minerals such as calcium, phosphorus, potassium and sodium. (David M. R. Culbreth, 1927).

Different parts of Zea mays have different uses. For the seed, it is primarily used as feed for livestock in United States and Canada while in some other parts of the world these are used for the production of corn sweeteners like corn syrup (Anonymous a, 2005). Another common food made from *Zea mays* is corn flakes. The matured seed can be dried and ground into flour. It has a very mild flavor and is used as a thickening agent in food such as custards. The flour is also used to make cornbread and Mexican tortillas. The dried seed of certain varieties can be heated up in an oven when they burst to make 'Popcorn' (Anonymous b, 2005).

Glue is made from the starch of the seed and this starch is also used in cosmetics. Other than that, the roasted seed is a coffee substitute. The seed is diuretic and a mild stimulant. It is a good emollient poultice for ulcers, swellings and rheumatic pains. It contains the cell-proliferant and wound-healing substance called allantonin, which is widely used in the herbal medicine to speed the healing process (Anonymous b, 2005). Decoctions, poultices, cataplasms and the flour of the seed are said to be folk remedies for tumors, warts and corns. The tea made form the seed is said to be a cure for breast cancer (James A. Duke., 1983).

As for the pith of the Zea mays stem, it is chewed like sugar cane and is used in syrup preparation. Besides that, the fibre obtained from the stems is also used in the production of paper, straw hats and small articles such as little baskets (Anonymous b, 2005).

Edible oil can be obtained from seed. It is good quality oil both from a nutritional standpoint and in terms of cooking quality. It contains high level of natural antioxidants. It is rich in the essential polyunsaturated fatty acid linoleic acid, so that it remains as liquid at fairly low temperatures. This polyunsaturated fatty acid and

4

antioxidants are responsible to decrease the risk of cardiovascular diseases (Augustine et al, 1996).

The pollen is used as an ingredient of soups. Rich in protein, it is harvested by tapping the flowering heads over a flat surface such as a bowl (Anonymous b, 2005).

A decoction of the leave and roots is used in the treatment of strangury, dysuria and gravel while the decoction of the cob is used in the treatment of nose bleeds and menorrhagia. Last but not the least, the fresh succulent 'silks' (the flowering parts of the cob) can also be eaten (Anonymous b, 2005).

It is no doubt that almost all parts of Zea mays plants are useful. But, most of the time the Zea mays hairs does not attract too much attention from the farmers. Zea mays hairs are rather discarded away as a waste product. Most of us despise these little strings without realizing that on a fresh ear of Zea mays they are sweet and easy to chew.

However, there are some commercial products in the market which claims that they are capable of producing pharmacological effects on human body; but are not proven or documented by researchers. So, this triggered the interest of taking up this project.

5

2. REVIEW OF LITERATURE

Zea mays is the scientific name for the corn or maize. Its scientific classification is as follows:

Kingdom: Plantae Division: Magnoliophyta Class: Liliopsida Order: Graminaceae Family: Poaceae Genus: Zea Species: Z. mays (Anonymous a, 2005)

Zea mays is called as corn in the United States, Canada and Australia but there are further regional differences in terminology. It is also synonyms with Stigmata maydis, Maidis stigmata, Ix-im, Mais, Maiz, Thurah Safrah, Indian corn, Yumixu, Yu Kao Liang, Yu Shu Shu or jagong(Anonymous b, 2005).

Of these crops, Zea mays has the highest average yield per hectare. While the United States produces almost half of the world's harvest, other top producing countries are as widespread as China, India, Brazil, France, Indonesia and South Africa. Its worldwide production was over 600 million metric tons in 2003, just slightly more than rice or wheat (Augustine *et al*, 1996).

Zea mays development is thought to have started since from 7,500 to 12,000 years ago. Archaeologist believed that the remains of the earliest Zea mays cob, was

found at Guila Naquitz Cave in the Oaxaca Valley of Mexico, date back approximately 6,250 years ago (Anonymous a, 2005).

While certain Zea mays varieties grow 7 metres (23 feets) tall at certain locations, commercial Zea mays plants have been bred for a high-end height of 2.5 metres (9 feets). Sweet corn is usually shorter than field corn varieties. Zea mays is essentially a subtropical plant and is grown wherever summer is reasonably warm. Annual rainfall of 750 mm or more is required for adequate moisture. The plant requires light (sandy), medium (loamy) and heavy (clay) soils which should be well-drained. The plant prefers acid and neutral soils with the pH of 5.5 to 6.8 and it is not frost tolerant. It also cannot grow in the shade (James A. Duke. 1983).

Zea mays has a very distinct growth pattern, the lower leaves being like broad flags, 50-100 cm long and 5-10 cm wide (2-4 feet by 2-4 inches); the stems are erect, from 2-3 m (7-10 feet) in height, with many joints, casting off flag-leaves at every joint. The stems look like bamboo cane and the joints are about 40-50 cm (16-20 inches) apart (Anonymous a, 2005).

Its flowers are monoecius. Individual flowers are either male or female, but both sexes can be found on the same plant and are pollinated by wind. It is in flower from July to October, and the seeds ripen from September to October (Anonymous b, 2005). Under the leaves and close to the stem grows the Zea mays corn, covered over by several layers of leaves, and so closed in by them to the stem. The grains are about the size of peas and it does not show itself easily till there bursts out at the end of the ear a number of strings, called silk or Zea mays hairs. Its appearance looks like tufts of horsehair, at first green, and turn red or yellow afterwards (Anonymous a, 2005).

The top of the stem ends in a flower, called the tassle (male spikelets) and with one or more ears (female spikelets) in the axils of leaves below the tassel. For each silk on which pollen from the tassle lands, one kernel of corn is produced. As the plant matures (usually during the summer months) the cob toughens and the silk dries to inedibility (Anonymous a, 2005).

By late August the kernels will be dried out and become difficult to chew without cooking them first in boiling water. The kernel of corn has a pericarp of the fruit fused with the seed coat, typical of the grasses. It is close to a multiple fruit in structure, except in the individual fruits (the kernels) never fuse into a single mass (Anonymous a, 2005).

Although most of the time Zea mays is widely cultivated for its edible seed, but literature has compiled a wide variety of its medicinal value.

Considered as analgesic, anodyne, antiseptic, astringent, choleretic, demulcent, diuretic and litholytic, *Zea mays* plant is a reputed folk remedy for such diverse ailments as amenorrhea, Bright's disease, cystitis, diabetes, dropsy, dysentery, dysmenorrheal, gingivitis, gout, gravel, hepatitis, hypertension, inflammation, influenza, menorrhagia, metritis, nephritis, oliguria, pneumonia, prostatitis, rheumatism, stones, strangury, tumors, urogenital ailments and warts (James A. Duke. 1983).

Herbalists believe that the Zea mays hairs or cornsilk of the plant also has medicinal values. It is gathered when the plant has shed its pollen. These fine soft threads are about 10-20 cm long. They are like silk threads of a light green or yellowbrown colour in fresh. When dry, they resemble fine, dark, crinkled hairs (Anonymous c, 2005). Its taste is sweet, mucilaginous and has a characteristic of corn smell. It makes an effective poultice and has been used in Mayan, Incan and American folk medicine to treat bruises, swellings, sores, boils and similar conditions (Andrew Chevallier, 2005).

Zea mays hairs has often been used as a tea, which is claimed to be an effective, harmless weight-reducer (Anonymous d, 2005). It can also be made into powder, or ointment mixed with corn oil for external applications. It has also been used as a flavor component for some major food products and face powders (David M. R. Culbreth, 1927).

Zea mays hairs is very popular in Chinese herbology. In China, Zea mays hairs is traditionally used to treat oedema and jaundice (Anonymous e, 2005). Studies indicate that it can reduce blood clotting time and reduce high blood pressure (Andrew Chevallier, 2005). Besides that, French herbalists use it to thin the bile and promote bile flow (Anonymous c, 2005).

The Zea mays hairs are sold in United States as a diuretic. The hairs are also believed to be vasodilator (James A. Duke, 1983). Besides that, they act to reduce blood sugar levels and so are used in the treatment of diabetes mellitus as well as cystitis, gonorrhoea, gout etc (Anonymous b, 2005).

9

Besides that, Zea mays hairs contain potassium salts, tannins, saponins (a volatile alkaloid), flavonoids, lipids, glucids and glycoproteins that induce production of interferon, inhibit IgE formation and enhance IgG formation. Thus, it is claimed to have antiviral and antitumor activities (Sharol Tilgner, N.D., 1999). Other constituents of Zea mays hairs are: hordenine (sterols and tigmasterol), volatile oil, fixed oil, resin, sugars, allantonin, maysin and carvacrol.

Laboratory analysis reveals high amounts of silicon, potassium, calcium, magnesium, iron, phosphorus minerals in Zea mays hairs. It is also an excellent source of B vitamins, C, K and *p*-aminobenzoic acid (PABA). Along with chlorophyll, resin and a fixed oil, maizenic acid is the active principle in Zea mays hairs (Anonymous f, 2005).

Zea mays hairs is highly valued in herbology as a support to the urinary system. It has been used to soothe irritation to the kidneys and bladder, which often caused by burning and painful urination. It alleviates the irritation of the bladder and urinary tract by coating the membranes lining the urinary system walls. Zea mays hairs are used for bladder complaints because of its cleansing effect on the urea as it circulates (Sharol Tilgner, N. D., 1999). In Mexico, the silky filaments are sold in bulk to anyone desiring safe nutrients for the urinary system (David M. R. Culbreth, 1927).

It is used as a urinary demulcent combined with other appropriate herb in the treatment of cystitis, urethritis and prostatitis (Anonymous e, 2005). It is also believed to be capable of reducing the formation of sediments in the kidneys, help reduce water retention in the body (Anonymous g, 2005). It may also alleviate prostate disorders

including difficulty in the beginning of urination. It can also be employed successfully in bedwetting of children or older people. The diuretic action is in part due to the high concentration of potassium. Herbalists often recommend *Zea mays* hairs for treatment of premenstrual syndrome (Anonymous h, 2005).

There are a lot of evidences in the literature to show the adverse effects of alcohol on the liver. And also, lot of work has been carried on to show that there are many plant materials which can prevent the adverse effect of alcohol on liver.

From animal studies it has been shown that there are three immediate effects of alcohol on the liver. First of all, there is proliferation of the smooth endoplasmic reticulum of hepatocytes; secondly, there is increased activity of the hepatic metabolizing enzymes mostly alcohol dehydrogenase and thirdly is increased activity of the microsomal ethanol oxidizing system (Iain *et al*, 1983).

Generally, three distinct pathological changes are produced in the liver by alcohol. There are: fatty liver, alcoholic hepatitis and cirrhosis. Fatty liver occurs as a consequence of increasing hepatic NADH/NAD ratio which favours fatty acid synthesis and triglyceride accumulation in the liver (lain *et al*, 1983). Alcoholics tend to develop alcoholic hepatitis due to liver cell necrosis and fibrosis. By that time, increase in serum of Aspartate Aminotransferease (AST) and Alkaline Phosphatase (ALP) occur although not usually marked. Following this is alcoholic cirrhosis which is often considered to be a sequel to alcoholic hepatitis. By this stage, there is a continued liver injury progressing to irreversible fibrosis and accompanied by nodular regeneration (lain *et al*, 1983; Gabriele *et al*, 1999; Craig *et al*, 1997).

11

There are many herbal plants which are believed to be able to prevent the effect of alcohol on liver functions. Among these, milk thistle (*Silybum marianum*) is perhaps the most highly valued of all plant remedies used to treat liver disease especially in Europe (Blumenthal M, 1988, 2000; Weiss RF. 1988). It was found to be useful in treating alcohol-related cirrhosis, chronic hepatitis and toxic fatty liver deposits (Ferenci *et al*, 1989; Lang *et al*, 1990; Saller *et al*, 2001). It was effective in liver healing by retarding and perhaps decreasing collagen accumulation (Schuppan *et al*, 1995; Boigk *et al*, 1997).

This is even supported by the study of Salmi and Sarna (1982) that there was a highly significant decrease of ALT and AST in the patients with alcoholic liver disease after silymrin treatment from *Silybum marianum* compared to the control groups. Serum total and conjugated bilirubin decreased more in the treated than in controls.

Other than that, yellow root of turmeric (*Curcuma longa*) is also a common naturopathic medicine. The curcumin and its analogs are hepatoprotective, antiinflammatory and antiviral. It also reduces liver enzyme and acts as choloretic (stimulating production of bile by liver), increase bile solubility and its excretion, along with cholesterol, bilirubin and bile salts (Elvin, 2002).

Besides that, the yellow wood of the tree turmeric, *Berberis aristata* of the Northwestern Himalayas, is known to contain berberine, which probably confers a hepatoprotective capacity (Gilan, 1992).

12

Physicians of the nineteenth century used yellow-flowered dandelion roots (*Taraxacum officinale*) to treat chronic disease of the liver, and recently, a naturopathic physician wrote that there is abundant evidence to show that common dandelion supplies substance that can be utilized by the liver (Powel, 1972).

However, the reports on the effects of Zea mays extract on alcohol induced changes on the liver function are scanty. So, this raised the interest of doing this project.

OBJECTIVE

The objective of the study is to assess the effects of ethanol extract of Zea mays Hairs on Liver Function Tests in alcohol fed rats.

3. MATERIALS AND METHODS

The protocol of the study was approved by the Animal Ethnical Committee of the university.

3.1 PREPARATION OF THE ZEA MAYS HAIRS EXTRACT

First of all, the Zea mays hairs were collected from the supplier in Kelantan. The hairs were dried in the shade and then ground into coarse powder form by using a blender. After this, the coarse powder was soaked in 95% ethanol for 24 hours. This step was repeated twice for two days.

Then, the extract was filtered out and rotavapourised (Plate 3.1) to obtain the concentrated extract. This concentrated extract was later dried in an oven. For storage, the extract was kept in the refrigerator at the temperature below $4^{\circ}C-6^{\circ}C$. This final extract was then diluted in distilled water and was ready for administration. The dilution ratio of the ethanol extract and distilled water was 1:10.

3.2 PREPARATION OF 30% ALCOHOL

The absolute alcohol was diluted into 30 % using double distilled water.

3.3 GROUPING OF ANIMALS

The species of *Sprague dawley* rats (Plate 3.2) weighing 180g-200g about 4-6 weeks age were used in this research. Only male rats are chosen as estrous cycle in the female rats might affect the accuracy of the results. All the rats were maintained in the good conditions which adequate amount of food and water available all the time.

The rats were divided into 6 groups with 5 rats in each group. The extract was given to the animals in two doses of 50mg/kg body weight and 100mg/kg body weight for 14 days. The groupings were as follows:

Group 1 - Control group with normal diet

- Group 2 50mg/kg body weight of extract administration
- Group 3 3ml of 30% ethanol administration
- Group 4 50mg/kg body weight of extract and 3ml of 30% ethanol administration
- Group 5 100mg/kg body weight of extract administration

Group 6 – 100mg/kg body weight of extract and 3ml of 30% ethanol administration

All the feedings were done orally by gavage. Amount of extract which was administered to a rat depends on the weight of the rat by the formula below:

(50mg / 1000g) X weight of rat in gram

3.4 PARAMETERS

Parameters studied in the liver function tests included:

- Total protein
- Albumin
- Globulin
- Albumin/Globulin ratio
- Bilirubin
- Alkaline phosphatase (ALP)
- Alanine aminotransferase (ALT)
- Aspartate aminotransferase (AST)

3.5 EXPERIMENTAL PROTOCOL

- 1. The rats in group 1 were control rats while the rats in group 3 were treated with alcohol alone for 14 days.
- 2. The rats in group 2 and group 5 were given the extract doses of 50 and 100mg/kg body weight respectively for 14 days.
- 3. The rats in 4 and group 6 were given the extract doses of 50 and 100mg/kg body weight along with 3 ml of 30% alcohol respectively for 14 days.
- 4. All the rats were sacrificed on the 15^{th} day.
- 5. 5 to 6 ml of blood was collected by cardiac puncture under mild ether anesthesia.
- 6. Blood sample of each rat was collected separately in plain tubes.
- 7. The samples were analyzed in chemical pathology laboratory in PPSP.
- 8. Sample tubes were centrifuged at 4000 rpm for 4 minutes for serum separation.

- 9. The serum samples were put into auto analyzer Hitachi 912 (Plate 3.3) for further analysis.
- 10. The total protein level was estimated by biuret reagent method using Randox kit.
- 11. Albumin level was estimated by Bromocresol Green method using Randox kit.
- 12. ALP level was estimated by UV method using Roche kit.
- 13. ALT level was estimated by UV method using Trace kit.
- 14. AST level was estimated by UV method using Roche kit.

FLOW CHART



3.6 STATISTICAL ANALYSIS

The results of the data were analyzed using computerized statistical software, the SPSS 11.5 software programme by using Independent Students' Paired t-test.

The values of the control rats were compared with both extract-administrated and alcohol-administered rats. p<0.05 is considered statistically significant.



Plate 3.1: Rotavapourization Machine



Plate 3.2: Sprague dawley rat



Plate 3.3: Auto Analyzer Hitachi 912



Plate 3.4: Cardiac Puncture for Blood Collection

4. RESULTS

Total proteins

When compared with control group:

50mg/kg body weight treatment

There was non significant decrease (very mild) in the total proteins.

100mg/kg body weight treatment:

There was significant increase in the total proteins.

3ml of 30% alcohol administration:

There was non significant increase in the total proteins.

<u>3ml of 30% alcohol administration + 50mg/kg body weight treatment:</u> There was significant increase in the total proteins.

<u>3ml of 30% alcohol administration + 100mg/kg body weight treatment:</u> There was significant increase in the total proteins.

(Table 4.1)

When compared with 3ml of 30% alcohol treated group:

<u>3ml of 30% alcohol administration + 50mg/kg body weight treatment:</u> There was non significant increase in the total proteins.

<u>3ml of 30% alcohol administration + 100mg/kg body weight treatment:</u> There was significant increase in the total proteins.

(Table 4.2)

Albumin

When compared with control group:

50mg/kg body weight treatment:

There was non significant increase in the albumin.

100mg/kg body weight treatment:

There was non significant increase in the albumin.

3ml of 30% alcohol administration:

There was significant decrease in the albumin.

<u>3ml of 30% alcohol administration + 50mg/kg body weight treatment:</u> There was non significant increase in the albumin.

<u>3ml of 30% alcohol administration + 100mg/kg body weight treatment:</u> There was non significant increase in the albumin.

(Table 4.3)

When compared with 3ml of 30% alcohol treated group:

<u>3ml of 30% alcohol administration + 50mg/kg body weight treatment:</u> There was significant increase in the albumin.

<u>3ml of 30% alcohol administration + 100mg/kg body weight treatment:</u> There was significant increase in the albumin.

(Table 4.4)

Globulin

When compared with control group:

50mg/kg body weight treatment:

There was non significant decrease in the globulin.

100mg/kg body weight treatment:

There was significant increase in the globulin.

3ml of 30% alcohol administration:

There was significant increase in the globulin.

<u>3ml of 30% alcohol administration + 50mg/kg body weight treatment:</u> There was significant increase in the globulin.

<u>3ml of 30% alcohol administration + 100mg/kg body weight treatment:</u> There was significant increase in the globulin.

(Table 4.5)

When compared with 3ml of 30% alcohol treated group:

<u>3ml of 30% alcohol administration + 50mg/kg body weight treatment:</u> There was no significant change in the globulin.

<u>3ml of 30% alcohol administration + 100mg/kg body weight treatment:</u> There was no significant change in the globulin.

(Table 4.6)

Albumin/Globulin ratio

71

When compared with control group:

50mg/kg body weight treatment:

There was slight increase (non significant) in the albumin/globulin ratio.

100mg/kg body weight treatment:

There was slight decrease (non significant) in the albumin/globulin ratio.

3ml of 30% alcohol administration:

There was significant decrease in the albumin/globulin ratio.

<u>3ml of 30% alcohol administration + 50mg/kg body weight treatment:</u> There was significant decrease in the albumin/globulin ratio.

<u>3ml of 30% alcohol administration + 100mg/kg body weight treatment:</u> There was significant decrease in the albumin/globulin ratio.

(Table 4.7)

When compared with 3ml of 30% alcohol treated group:

<u>3ml of 30% alcohol administration + 50mg/kg body weight treatment:</u>

There was slight decrease (non significant) in the albumin/globulin ratio.

3ml of 30% alcohol administration + 100mg/kg body weight treatment:

There was slight decrease (non significant) decrease in the albumin/globulin ratio.

(Table 4.8)

Bilirubin

When compared with control group:

50mg/kg body weight treatment:

There was non significant decrease in the bilirubin.

100mg/kg body weight treatment:

There was non significant increase in the bilirubin.

3ml of 30% alcohol administration:

There was non significant decrease in the bilirubin.

<u>3ml of 30% alcohol administration + 50mg/kg body weight treatment:</u> There was non significant increase in the bilirubin.

<u>3ml of 30% alcohol administration + 100mg/kg body weight treatment:</u> There was non significant decrease in the bilirubin.

(Table 4.9)

When compared with 3ml of 30% alcohol treated group:

<u>3ml of 30% alcohol administration + 50mg/kg body weight treatment:</u> There was non significant increase in the bilirubin.

<u>3ml of 30% alcohol administration + 100mg/kg body weight treatment:</u> There was non significant decrease in the bilirubin.

(Table 4.10)

Alkaline Phosphatase

When compared with control group:

50mg/kg body weight treatment:

There was non significant decrease in the alkaline phosphatase.

100mg/kg body weight treatment:

There was non significant decrease in the alkaline phosphatase. The decrease here was more than in 50mg dose.

3ml of 30% alcohol administration:

ł

• • •

There was non significant increase in the alkaline phosphatase.

<u>3ml of 30% alcohol administration + 50mg/kg body weight treatment:</u>

There was slight significant increase (non significant) in the alkaline phosphatase. The increase was less compared to the increase in alcohol treated group.

3ml of 30% alcohol administration + 100mg/kg body weight treatment:

There was no significant decrease in the alkaline phosphatase.

(Table 4.11)

When compared with 3ml of 30% alcohol treated group:

<u>3ml of 30% alcohol administration + 50mg/kg body weight treatment:</u> There was non significant decrease in the alkaline phosphatase.

<u>3ml of 30% alcohol administration + 100mg/kg body weight treatment:</u> There was non significant decrease in the alkaline phosphatase.

(Table 4.12)

Aspartate Aminotransferase

When compared with control group:

50mg/kg body weight treatment:

There was non significant increase in the aspartate aminotransferase.

100mg/kg body weight treatment:

There was non significant increase in the aspartate aminotransferase.

3ml of 30% alcohol administration:

There was non significant decrease in the aspartate aminotransferase.

<u>3ml of 30% alcohol administration + 50mg/kg body weight treatment:</u>

There was non significant increase in the aspartate aminotransferase.

<u>3ml of 30% alcohol administration + 100mg/kg body weight treatment:</u> There was significant increase in the aspartate aminotransferase.

(Table 4.13)

When compared with 3ml of 30% alcohol treated group:

3ml of 30% alcohol administration + 50mg/kg body weight treatment:

There was non significant increase in the aspartate aminotransferase.

<u>3ml of 30% alcohol administration + 100mg/kg body weight treatment:</u> There was significant increase in the aspartate aminotransferase.

(Table 4.14)

Alanine Aminotrasferase

When compared with control group:

50mg/kg body weight treatment:

There was non significant increase in the alanine aminotransferase.

100mg/kg body weight treatment:

There was very little (non significant) increase in the alanine aminotransferase.

3ml of 30% alcohol administration:

There was very little (non significant) increase in the alanine aminotransferase.

3ml of 30% alcohol administration + 50mg/kg body weight treatment:

There was non significant decrease in the alanine aminotransferase.

<u>3ml of 30% alcohol administration + 100mg/kg body weight treatment:</u>

There was non significant increase in the alanine aminotransferase.

(Table 4.15)

When compared with 3ml of 30% alcohol treated group:

<u>3ml of 30% alcohol administration + 50mg/kg body weight treatment:</u> There was non significant decrease in the alanine aminotransferase.

<u>3ml of 30% alcohol administration + 100mg/kg body weight treatment:</u> There was non significant increase in the alanine aminotransferase.

(Table 4.16)

Table 4.1: Effects of ethanol extract of Zea mays Hairs on Total Protein (g/L) compared with control group

Group	Mean <u>+</u> SEM	t	Significance: p value
Control	65.400 <u>+</u> 0.678	-	-
Alcohol	69.000 <u>+</u> 1.304	-2.092	0.105 NS
50mg extract	64.200 <u>+</u> 0.663	1.238	0.284 NS
50mg + alcohol	73.600 <u>+</u> 1.806	-4.954	0.008*
100mg extract	69.000 <u>+</u> 0.837	-4.431	0.011*
100mg + alcohol	73.000 <u>+</u> 0.894	-6.290	0.003*

Table 4.2: Effects of ethanol extract of Zea mays Hairs on Total Protein (g/L) compared with alcohol group

Group	Mean <u>+</u> SEM	t	Significance: p value
Alcohol	69.000 <u>+</u> 1.303	-	-
50mg + alcohol	73.600 <u>+</u> 1.806	-2.720	0.053 NS
100mg + alcohol	73.000 <u>+</u> 0.894	-4.781	0.009*

NS- No Significance

^{*} - Significance

Table 4.3: Effects of ethanol extract of Zea mays Hairs on Albumin (g/L) compared with control group

Group	Mean <u>+</u> SEM	Т	Significance: p value
Control	32.400 <u>+</u> 0.510	-	-
Alcohol	30.000 <u>+</u> 0.447	6.000	0.004*
50mg extract	33.000 <u>+</u> 0.316	-0.802	0.468 NS
50mg + alcohol	33.000 <u>+</u> 0.548	-1.177	0.305 NS
100mg extract	33.200 <u>+</u> 0.490	-1.633	0.178 NS
100mg + alcohol	32.400 <u>+</u> 0.245	0.000	1.000 NS

Table 4.4: Effects of ethanol extract of Zea mays Hairs on Albumin (g/L) compared with alcohol group

Group	Mean <u>+</u> SEM	t	Significance: p value
Alcohol	30.000 <u>+</u> 0.447	-	-
50mg + alcohol	33.000 <u>+</u> 0.548	-5.477	0.005*
100mg + alcohol	32.400 <u>+</u> 0.245	-6.000	0.004*

NS- No Significance

* - Significance

 Table 4.5: Effects of ethanol extract of Zea mays Hairs on Globulin (g/L) compared with control group

Group	Mean <u>+</u> SEM	t	Significance: p value
Control	32.200 <u>+</u> 0.735	-	-
Alcohol	39.000 <u>+</u> 1.342	-3.721	0.020*
50mg extract	30.600 <u>+</u> 0.812	1.486	0.212 NS
50mg + alcohol	40.600 <u>+</u> 1.503	-5.356	0.006*
100mg extract	35.000 <u>+</u> 0.447	-3.055	0.038*
100mg + alcohol	39.600 <u>+</u> 1.435	-3.816	0.019*

Table 4.6: Effects of ethanol extract of Zea mays Hairs on Globulin (g/L) compared with alcohol group

Group	Mean <u>+</u> SEM	t	Significance: p value
Alcohol	39.000 <u>+</u> 1.342	-	-
50mg + alcohol	40.600 <u>+</u> 1.503	-1.064	0.347 NS
100mg + alcohol	39.600 <u>+</u> 1.435	-0.497	0.646 NS

NS- No Significance

^{*} - Significance

 Table 4.7: Effects of ethanol extract of Zea mays Hairs on Albumin/Globulin ratio

 compared with control group

Group	Mean <u>+</u> SEM	t	Significance: p value
Control	1.038 <u>+</u> 0.124	-	-
Alcohol	0.814 <u>+</u> 0.129	19.956	0.000*
50mg extract	1.080 <u>+</u> 0.294	-1.085	0.339 NS
50mg + alcohol	0.782 <u>+</u> 0.186	9.635	0.001*
100mg extract	0.972 <u>+</u> 0.393	1.870	0.135 NS
100mg + alcohol	0.788 <u>+</u> 0.174	9.806	0.001*

Table 4.8: Effects of ethanol extract of Zea mays Hairs on Albumin/Globulin ratio compared with alcohol group

Group	Mean <u>+</u> SEM	t	Significance: p value
Alcohol	0.814 <u>+</u> 0.129	-	-
50mg + alcohol	0.782 <u>+</u> 0.186	1.095	0.335 NS
100mg + alcohol	0.788 <u>+</u> 0.174	1.093	0.336 NS

NS- No Significance

* - Significance

Table 4.9: Effects of ethanol extract of Zea mays Hairs on Bilirubin (µmol/L) compared with control group

Group	Mean <u>+</u> SEM	t	Significance: p value
Control	3.200 <u>+</u> 0.583	-	-
Alcohol	2.400 <u>+</u> 0.245	1.000	0.374 NS
50mg extract	2.800 <u>+</u> 0.860	0.343	0.749 NS
50mg + alcohol	3.400 <u>+</u> 1.249	-0.131	0.902 NS
100mg extract	3.400 <u>+</u> 0.245	-2.720	0.779 NS
100mg + alcohol	2.000 <u>+</u> 0.447	2.449	0.070 NS

Table 4.10: Effects of ethanol extract of Zea mays Hairs on Bilirubin (µmol/L)

compared	with	alcohol	group
----------	------	---------	-------

Group	Mean <u>+</u> SEM	t	Significance: p value
Alcohol	2.400 <u>+</u> 0.245	-	-
50mg + alcohol	3.400 <u>+</u> 1.249	-7.670	0.486 NS
100mg + alcohol	2.000 <u>+</u> 0.447	0.590	0.587 NS

NS- No Significance

* - Significance

,

 Table 4.11: Effects of ethanol extract of Zea mays Hairs on Alkaline Phosphatase

 activity (U/L) compared with control group

Group	Mean <u>+</u> SEM	t	Significance: p value
Control	264.200 <u>+</u> 12.820	-	-
Alcohol	278.600 <u>+</u> 24.238	-0.573	0.598 NS
50mg extract	251.400 <u>+</u> 16.216	0.573	0.598 NS
50mg + alcohol	269.800 <u>+</u> 23.829	-0.164	0.878 NS
100mg extract	235.400 <u>+</u> 8.078	1.576	0.190 NS
100mg + alcohol	257.600 <u>+</u> 41.679	0.143	0.885 NS

Table 4.12: Effects of ethanol extract of Zea mays Hairs on Alkaline Phosphatase activity (U/L) compared with alcohol group

Group	Mean <u>+</u> SEM	t	Significance: p value
Alcohol	278.600 <u>+</u> 24.238	-	-
50mg + alcohol	269.800 <u>+</u> 23.827	0.208	0.845 NS
100mg + alcohol	257.600 <u>+</u> 41.679	0.767	0.486 NS

NS- No Significance

1

* - Significance

 Table 4.13: Effects of ethanol extract of Zea mays Hairs on Aspartate Aminotransferase

 activity (U/L) compared with control group

Group	Mean <u>+</u> SEM	t	Significance: p value
Control	170.000 <u>+</u> 19.071	-	-
Alcohol	159.800 <u>+</u> 10.190	0.529	0.625 NS
50mg extract	176.400 <u>+</u> 13.948	-2.760	0.796 NS
50mg + alcohol	225.800 <u>+</u> 20.098	-1.820	0.143 NS
100mg extract	175.200 <u>+</u> 10.166	-2.110	0.843 NS
100mg + alcohol	241.400 <u>+</u> 10.980	-3.820	0.019*

Table 4.14: Effects of ethanol extract of Zea mays Hairs on Aspartate Aminotransferase activity (U/L) compared with alcohol group

Group	Mean <u>+</u> SEM	t	Significance: p value
Alcohol	159.800 <u>+</u> 10.190	-	-
50mg + alcohol	225.800 <u>+</u> 20.098	-2.371	0.077 NS
100mg + alcohol	241.400 <u>+</u> 10.980	-4.366	0.012*

NS- No Significance

^{*} - Significance

 Table 4.15: Effects of ethanol extract of Zea mays Hairs on Alanine Aminotransferase

 activity (U/L) compared with control group

Group	Mean <u>+</u> SEM	t	Significance: p value
Control	70.600 <u>+</u> 4.584	-	-
Alcohol	72.600 <u>+</u> 5.706	-0.198	0.852 NS
50mg extract	76.200 <u>+</u> 3.942	-0.825	0.456 NS
50mg + alcohol	62.400 <u>+</u> 6.585	0.915	0.412 NS
100mg extract	71.800 <u>+</u> 2.557	-0.164	0.878 NS
100mg + alcohol	74.400 <u>+</u> 3.187	-0.544	0.615 NS

Table 4.16: Effects of ethanol extract of Zea mays Hairs on Alanine Aminotransferase activity (U/L) compared with alcohol group

Group	Mean <u>+</u> SEM	t	Significance: p value
Alcohol	72.600 <u>+</u> 5.706	-	-
50mg + alcohol	62.400 <u>+</u> 6.585	1.110	0.329 NS
100mg + alcohol	74.400 <u>+</u> 3.187	0.413	0.701 NS

NS- No Significance

* - Significance

5.0 DISCUSSION

The liver is an important organ because it performs many functions: it helps to detoxify the toxins in the body, synthesizes blood clotting proteins and other proteins that help draw fluid into the blood vessels (e.g. albumin) (Anonymous i, 2006; K. Sembuligam and Prema Sembulingam, 2005).

The liver functions are accessed by the liver function tests (LFTs). These are blood tests that are used to evaluate the various functions of the liver such as metabolism, storage, filtration, excretion and synthesis (Anonymous i, 2006).

The commonly used LFTs in routine practice include total protein, serum albumin, serum globulin, bilirubin and the enzymes namely ALT, AST (formerly known as SPGT and SGOT) and ALP (Anonymous i, 2006).

Among these, the most commonly used indicators of liver damage are ALT and AST. These enzymes are normally found in the liver cells and the level of these enzymes increases in the blood when liver cells are damaged. ALP is an indicator of biliary obstruction. This enzyme increases in the blood due to various reasons. Alcohol abuse is one among them (Anonymous i, 2006).

In the present study we have attempted to see whether 3ml of 30% alcohol produced liver damage and if there is liver damage, whether the ethanol extract of Zea mays hairs was capable of reversing the effect back to normal, and also the beneficial effects of these extract on liver.

38

We found some positive results here. The total protein were found to be increased in alcohol fed rats (non significant) and alcohol + high dose of ethanol extract fed rats (significant). 50mg extract alone decreased total protein slightly and 100mg extract increased total protein significantly. However, the degree of increase was more than the degree of decrease.

The increase in total proteins is the reflection of increase in serum albumin and serum globulin. These two protein fractions showed trend of increase with and without alcohol (except in 50mg extract). The levels of total proteins and serum protein fractions reflect the functional status of the liver (Anonymous j, 2006). So, the extract seemed to enhance the liver function, especially the synthesis function.

The bilirubin did not deviate very much from the normal control level either by alcohol or by the extract in the present study. This shows that the liver functions were not affected by these agents.

As far as the enzymes are concerned, the ALP was found to be elevated (non significant) by alcohol (278.600 ± 24.238) (control 264.200 ± 12.820). The extract in 50mg and 100mg doses decreased the ALP levels $(251.400\pm16.216 \text{ and } 235.400\pm8.078 \text{ respectively})$ (non significant). But when alcohol group (278.600 ± 24.238) was compared with the extract + alcohol groups, there was good decrease in ALP (non significant) in the latter groups. The decrease was more in 100mg + alcohol group than in 50mg + alcohol group. These results revealed that alcohol would have done mild damage to the liver which would have been counteracted by the extract.

39

However, AST and ALT showed a different picture. Alcohol decreased AST (non significant) (control group 170.000 ± 19.071 ; alcohol group 159.800 ± 10.190). But the extracts in 50mg (176.400 ± 13.948) and 100mg (175.200 ± 10.166) increased AST levels (non significant). The increase was still more in ethanol + extract groups (50mg 225.800\pm20.098; 100mg 241.400\pm10.980). ALT seemed to be increased by alcohol and the extract. The increase was non significant. From this, we are not in a position to say whether liver was damage at all because there was no histological evidence for this. Elevated enzyme levels may be indicative of liver damage. But slight AST or/and ALT elevations do not necessarily indicate liver disease or damage and normal LFTs do not always mean that liver is normal (Anonymous j, 2006).

Further study is necessary to consolidate the positive and negative indications of alcohol and the extract.

6. CONCLUSION

In general, the results of this study showed no observable changes found between control and extract/alcohol treated groups in the (Liver Function Tests) parameters studied.

To confirm the results, further study is required with increase in dose (extract/alcohol) and duration of treatment.

7. REFERENCES

1. Anonymous a, (2005)

Maize

http://en.wikipedia.org/wiki/Maiz

Accessed on 7th August, 2005

2. Anonymous b, (2005)

Zea mays

http://www.agrifor.ac.uk/browse/cabi.html

Accessed on 7th August, 2005

3. Anonymous c, (2005)

Zea mays

http://www/ibiblio.org/pfaf/cgi-bin/arr html

Accessed on 7th August, 2005

4. Anonymous d, (2005)

Cornsilk

http://www.magdalin.com/herbal/plants-pages/c/cornsilk

Accessed on 7th August, 2005

5. Anonymous e, (2005)

Cornsilk

http://www.phytochemicals.info.plants/cornsilk

Accessed on 7th August, 2005

6. Anonymous f, (2005)

Cornsilk

http://www.1001herbs.com/cornsilk

Accessed on 23rd August, 2005

7. Anonymous g, (2005)

Cornsilk

http://www.herbalmanac.com/cornsilk

Accessed on 23rd August, 2005

8. Anonymous h, (2005)

Cornsilk

http://www.evitamins.com/productasp?pid=1943

Accessed on 23th August, 2005

9. Anonymous i, (2006)

Liver enzymes (Elevated)

http://ww3.komotv.com/global/story.asp?s=1230520

Accessed on 28th March, 2006

10. Anonymous j, (2006)

Special Considerations in Interpreting Liver Function Tests

http://www.aafp.org/afp/990415ap/2223.html

Accessed on 28th March, 2006

11. Andrew Chevallier, (2005)

Cornsilk

The Encyclopedia of Medical Plant

http://www.alchemybotanicas.com/CORNSILK.html

Accessed on 23th August, 2005

 Augustine E. Okoruwa; Jennifer G. Kling (1996) Nutrition and Quality of Maize http://www.iita.org/infa/tm_mat/irg33/irg33.html

Accessed on 15th August, 2005

 Bluementhal M (1998). The Complete German Commission E Monographs: Therapeutic Guide to Herbal Medicines. American Botanical Council, Austin, TX, and Integrative Medicine Communities, Boston. pp685.

- 14. Boigk G, Stroedter L, Herbst H, et al. (1997). Silymarin retards collagen accumulation in early and advanced biliary firbosis secondary to complete bile duct obliteration in rats. Hepatology 26:643-649.
- Craig J. McClain, M.D., Steven Shedlofsky, M.D., Shirish Barve, Ph.D., and Daniell B. Hill, M.D., (1997). Cytokine and Alcoholic Liver Disease, Research Update, Vol. 21, No. 4.
- 16. David M. R. Culbreth, Ph.D., M.D., Lea & Febriger, Philadelphia, Pa. (1927)
 A Manual of Materia Medica and Pharmacology.
 <u>http://theherbsplace.com/cornsilk</u>
 Accessed on 15th August, 2005
- 17. Elvin-Lewis, M. (2002). Therapeutic evaluation of hepatitis remedies: The usefulness of ethnomedical focusing techniques. In Stepp JR, Wyndam FS, Zarger RK (eds), Proceedings of the 7th International Congress of Ethnobiology, University of Georgia, Piess, Athens, GA.
- 18. Ferenci P, Dragosics B, Dittrich H, et al. (1989). Randomized controlled trial of silymarin treatment in patients with cirrhosis of liver. J Hepato 9:105-113.
- Gabriele Bianchi Porro, MD, PhD. (1999). Gastroenterology and Hepatology. McGraw-Hill International (UK) Limited. pp512-517.

- 20. Gilani AH, Janbaz KH. (1992). Prevention of acetaminophen-induced liver damage by *Berberis aristata* leaves. Biochem Soc Trans 20:347 S.
- Ian E. Gillespie, T.J. Thompson (1983). Gastroenteroloyg an integrated course, 3rd edition. Churchill Livingstone Edinburgh London Melbourne and New York. pp184.
- 22. James A. Duke. (1983)
 Handbook of Energy Crops
 <u>http://www.hort.purdue.edu/newcrop/duke_energy/Zea_mays</u>
 Accessed on 7th August, 2005
- 23. K. Sembulingam, Prema Sembulingam. (2005). Essentials of Medical Physiology, 3rd edi, Jaypee Brothers, Medical Publisher (P) Ltd, New Delhi. pp46,198.
- Lang I, Nekam K, Gonzalez. Cabello R, et al. (1990). Hepatoprotective and immunological effects of antioxidant drugs. Tokai J Exp Clin Med 15:123-127.
- 25. Lieber CS, Salaspuro MP. (1992). Alcoholic liver disease. In: Sadler-Millward GH, Wright R, Arthur MJP eds. Wright's Liver and Biliary Disease, 3rd ed, London: Saunders. pp899-964.

26. Powel EFW. (1972). About Dandelions. Thorsons Publishers, London. pp63.

- 27. Saller R, Meider R, Brignoli R. (2001). The use of silymarin in the treatment of liver disease. Drugs 61:2035-2063.
- 28. Salmi HA, Sarna S. (1982). Effect of silymarin on chemical, functional and morphological alterations of the liver. double-blind controlled study. Scand J Gastroenterol Jun;17(4):517-21.
- 29. Schuppan D, Atkinson J, Ruehl M, Riecken EO. (1995). Alcohol and liver fibrosis: pathobiochemistry and treatment. Z Gastroenterol 33:546-550.
- 30. Sharol Tilgner, N. D.

Materia medica, 1999.

http://www.herbaltransitions.com/materiamedica/Zea.htm Accessed on 7th August, 2005

31. Weiss RF. (1988). Herbal Medicine. Translated from the Sixth German Edition of Lehrbuch der Phytotherapie by AR Meuss. Beaconsfield Publishers, Beaconfield, Buckinghamshire, England. pp362.