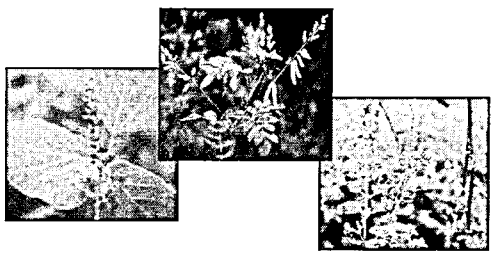
S5

# Development of Health Functional Food for Fatty Liver from Traditional Herb



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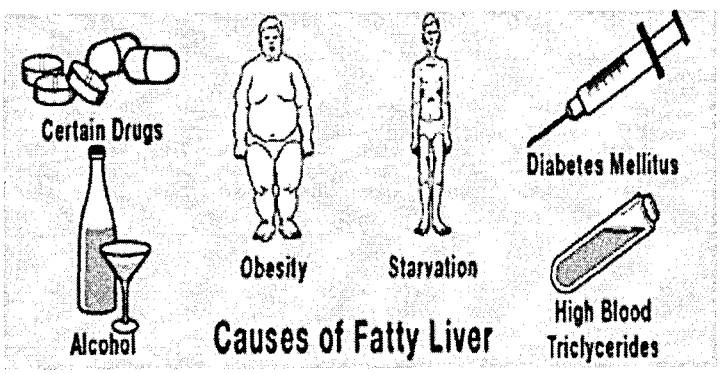
# Differences between health functional food and drug

| | Health functional food | Drug |
|---|---|---|
| Subject | Healthy man | Patient |
| Sample State | Extract, Fraction | Single component |
| Aim | Maintain and improvement of health, Risk reduction | Treatment of disease |
| Effect | Mild | Potent |
| Treatment period | Long | Short |
| Toxicity | Very low or none | Low toxicity (known) |

## Fatty Liver ?

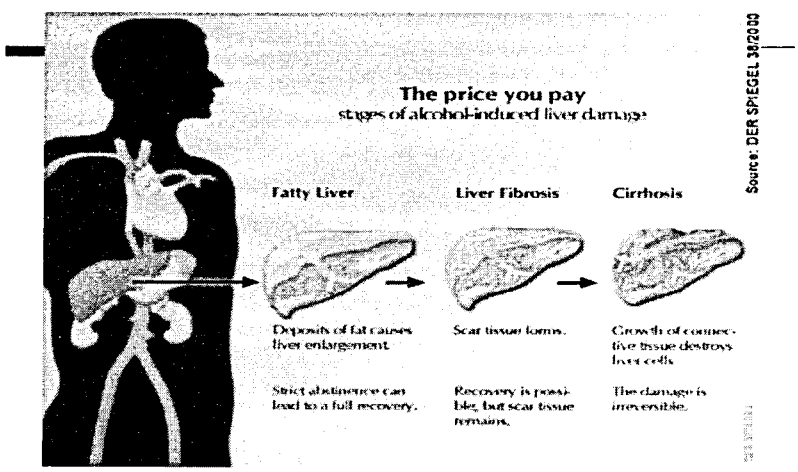
- ❖ **Accumulation of lipid within hepatocytes**
- ❖ **Total lipid exceeding 5% of liver weight**
  **(Over 50% of the excess fat deposit in the liver is TG)**
- ❖ **Adult – Men 30%, Women 15%**
  ***(ref. Korea National Statistical Office '1995)***
- ❖ **In the US - Steatosis affects approximately 25% of the general population**

## Risk Factors

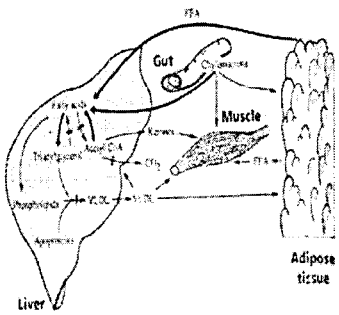


# Alcoholic Fatty Liver?

- **The amount of alcohol consumed (how much and how often) determines the risk and the degree of liver damage**
- **In the US: 15.3 million people in US abuse or depend on alcohol. Fatty liver develops in 90-100% of patients with heavy alcohol use**
- **In northern Italy: Prevalence rates of steatosis in 46.4% of heavy drinkers (>60 g/d of alcohol) and in 94.5% of obese heavy drinkers** *(Bellentani et al. Ann Intern Med. 2000)*



## Mechanism of Fatty Liver




1) Fatty acid beta-oxidation in mitochondrial ↓
2) Endogenous fatty acid synthesis ↑
3) Enhanced delivery of fatty acids to the liver
4) Deficient incorporation or export of triglycerides as VLDL

## SungKangWon

## Composition

- ✓ *Astragalus membranaceus*
- ✓ *Salvia miltiorrhiza*
- ✓ *Pueraria lobata*

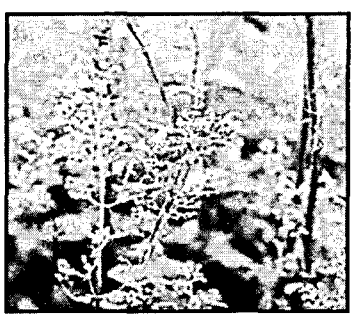
## *Astragalus membranaceus*

- ☐ **Family ; Leguminosae**
- ☐ **Part used ; root**
- ☐ **Common name ; Huang Qi**

## Effects of *Astragalus membranaceus*

- ☐ Liver fibrosis and hepatic collagenolysis by reverse chemical-induced. *(Li et al. Life Sci. 2003)*
- ☐ $CCl_4$ induced liver damage in rats was reduced. *(Fu et al. Zhongguo Zhong Xi Yi Jie He Za Zhi. 1992)*
- ☐ Protecting against liver fibrosis. *(Li et al. Am J Chin Med. 1998)*
- ☐ Reducing blood lipoprotein → antiatherosclerosis *(Lu et al. Zhongguo Zhong Xi Yi Jie He Za Zhi. 1994)*

## *Salvia miltiorrhiza*

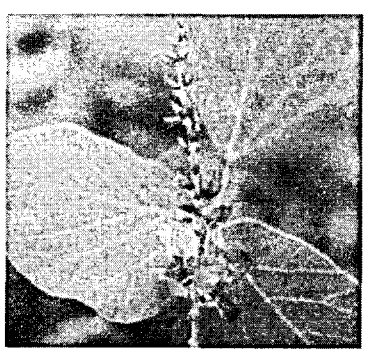


- ☐ **Family ; Lablatae**
- ☐ **Part used ; root**
- ☐ **Common name ; Danshen**

## Effects of *Salvia miltiorrhiza*

- Delay acquisition of alcohol drinking behavior by dose-dependently. *(Brunetti et al. J Ethnopharmacology. 2003)*
- Inhibition of biliary obstruction-induced hepatocyte apoptosis by cytoplasmic sequestration of p53. *(Oh et al. Toxicol Appl Pharmacol. 2002)*
- Amelioration of carbon tetrachloride-induced hepatic fibrosis *(Chen et al. Zhonghua Gan Zang Bing Za Zhi. 2002)*

## *Pueraria lobata*



- **Family ; Leguminosae**
- **Part used ; root**
- **Common name ; Kudzu**

## Effects of *Pueraria lobata*

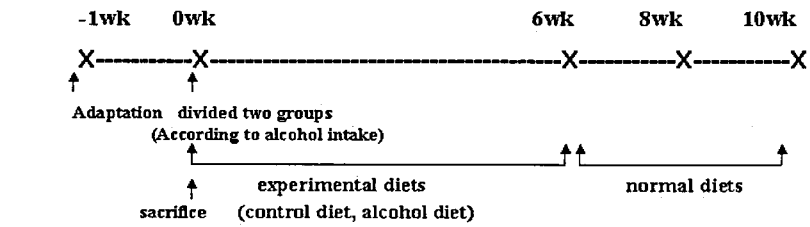
- ☐ Reducing ALT in hepatocytes better than glycyrrhizin
  *(Arao et al. Biol Pharm Bull. 1997)*
- ☐ Daidzin delays and decreases peak blood alcohol levels and shortens sleep time induced by ethanol intoxication.
  *(Xie et al. Alcohol Clin Exp Res. 1994)*
- ☐ Scavenging DPPH radical and inhibiting lipid peroxidation.
  *(Sato et al. Chem Pharm Bull (Tokyo). 1992)*

## Purpose of Study

**To investigate the effect of Sungkangwon (*Astragali membranaceus* Bunge + *Puerariae lobata* Ohwi + *Salviae miltiorrhiza* Bunge ) on fatty liver induced by alcohol diet**

# Materials and Methods

## Experimental Design




**Experimental group ;**
- Control : alcohol free
- Alcohol : alcohol diet
- SKW3000 : Sungkangwon 3g/kg
- SKW1000 : Sungkangwon 1g/kg
- SKW330 : Sungkangwon 0.33g/kg

## Composition of experimental diets

**(Lieber-DeCarli Liquid Diet)**

| Ingredients | Control | Alcohol |
|---|---|---|
| Casein | 41.40 | 41.40 |
| DL-Methionine | 0.30 | 0.30 |
| L-Cystine | 0.50 | 0.50 |
| Cellulose | 10.00 | 10.00 |
| Maltose Dextrin | 25.60 | 115.20 |
| Corn Oil | 8.50 | 8.50 |
| Olive Oil | 28.40 | 28.40 |
| Safflower Oil | 2.70 | 2.70 |
| Mineral Mix [a] | 8.75 | 8.75 |
| Vitamin Mix [b] | 2.50 | 2.50 |
| Choline Bitartrate | 0.53 | 0.53 |
| Xanthan Gum | 3.00 | 3.00 |
| Ethanol | - | 45.5 |

*[a]: AIN-76 Mineral mix; Dyets, Bethlehem, Pennsylvania, USA*
*[b]: AIN-76 Vitamin mix; Dyets, Bethlehem, Pennsylvania, USA*

## Laboratory Analyses

- **Serum**

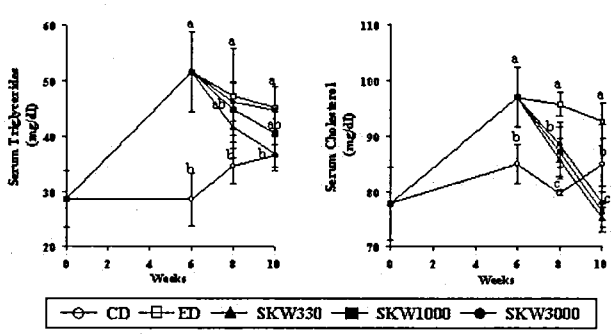
✓ **Triglycerides ; Commercial Kit**
✓ **Total cholesterol ; Commercial Kit**
✓ **HDL-C ; Commercial Kit**
✓ **LDL-C, VLDL-C ; Calculation by Friedewald method**
✓ **AST, ALT ; Commercial Kit**
✓ **Hepatic lipase ; Auto Analysis (COBAS INTEGRA)**

- **Liver**

✓ **Triglycerides ; Commercial Kit**
✓ **Total cholesterol ; Commercial Kit**
✓ **Histology Analysis ; Oil Red O staining**

# Results

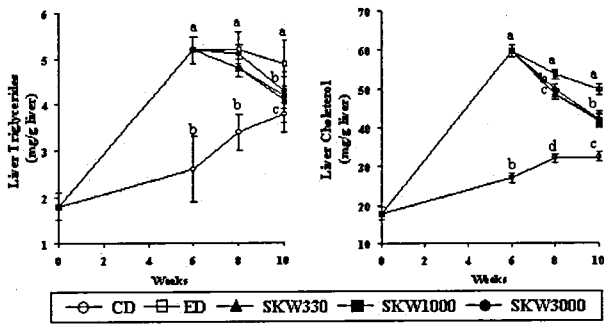
## Serum TG and TC




*1) Values are mean ±S.D.*

*2) Letters with different superscripts are significantly different ($p<0.05$) among the groups by Duncan's multiple range test.*

## Liver TG and TC

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## Serum HDL-C, LDL-C, VLDL-C

(mg/dl)

| | Wks | Control | Alcohol | SKW3000 | SKW1000 | SKW330 |
|---|---|---|---|---|---|---|
| HDL Chol | 0 | 53.09±3.84 | 53.09±3.84 | 53.09±3.84 | 53.09±3.84 | 53.09±3.84 |
| | 6 | 76.65±4.98$^{b}$ | 52.96±13.70$^{ab}$ | 57.86±5.42$^{a}$ | 56.85±14.24$^{ab}$ | 54.71±4.47$^{a}$ |
| | 8 | 59.32±5.32 | 53.46±15.14 | 61.05±20.12 | 61.74±8.58 | 60.31±8.95 |
| | 10 | 62.26±13.37 | 60.98±3.55 | 67.90±16.79 | 69.09±10.98 | 72.99±9.93 |
| LDL Chol | 0 | 12.14±3.50 | 12.14±3.50 | 12.14±3.50 | 12.14±3.50 | 12.14±3.50 |
| | 6 | 13.28±2.87 | 30.56±16.50 | 19.37±7.68 | 22.04±7.65 | 20.29±8.74 |
| | 8 | 18.19±13.00 | 25.41±16.10 | 14.98±2.13 | 19.82±11.86 | 17.00±5.60 |
| | 10 | 13.67±7.80 | 22.90±11.47 | 14.10±3.85 | 16.54±4.28 | 14.50±6.27 |
| VLDL Chol | 0 | 12.78±0.68 | 12.78±0.68 | 12.78±0.68 | 12.78±0.68 | 12.78±0.68 |
| | 6 | 9.90±0.28 | 3.67±0.31 | 5.85±0.22 | 4.46±0.27 | 4.29±0.22 |
| | 8 | 9.20±0.46 | 4.64±0.34 | 7.24±0.15 | 6.63±0.24 | 6.59±0.34 |
| | 10 | 9.14±0.42 | 6.56±0.37 | 8.00±0.34 | 8.65±0.39 | 9.02±0.18 |

# AST / ALT

(unit/ml)

| | Wks | Control | Alcohol | SKW3000 | SKW1000 | SKW330 |
|---|---|---|---|---|---|---|
| AST | 0 | 113.53±9.32 | 113.53±9.32 | 113.53±9.32 | 113.53±9.32 | 113.53±9.32 |
| | 6 | 105.10±8.11$^{b}$ | 137.82±9.59$^{a}$ | 137.82±9.59$^{a}$ | 137.82±9.60$^{a}$ | 137.82±9.61$^{a}$ |
| | 8 | 113.75±5.59$^{c}$ | 148.15±4.64$^{a}$ | 128.01±8.74$^{b}$ | 124.92±7.69$^{b}$ | 113.44±5.05$^{c}$ |
| | 10 | 105.06±8.30$^{bc}$ | 119.30±5.98$^{a}$ | 114.59±11.66$^{ab}$ | 98.46±6.59$^{c}$ | 93.81±8.69$^{c}$ |
| ALT | 0 | 27.39±4.94 | 27.39±4.94 | 27.39±4.94 | 27.39±4.94 | 27.39±4.94 |
| | 6 | 32.27±5.19$^{b}$ | 65.09±8.55$^{a}$ | 65.09±8.55$^{a}$ | 65.09±8.55$^{a}$ | 65.09±8.55$^{a}$ |
| | 8 | 26.54±3.81$^{c}$ | 45.97±0.28$^{a}$ | 32.85±2.18$^{b}$ | 30.74±4.48$^{bc}$ | 27.40±3.22$^{c}$ |
| | 10 | 35.07±7.84$^{ab}$ | 41.30±2.85$^{a}$ | 29.50±5.27$^{b}$ | 29.27±7.16$^{b}$ | 26.31±5.51$^{b}$ |

*1) Values are mean ±S.D.*

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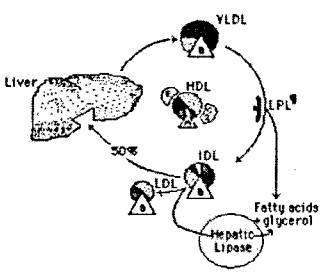
# Hepatic Lipase ?




❖ **Hepatic lipase was known to be important in the final steps in the lipolytic conversion of VLDL to LDL by hydrosis of the LDL triglyceride.**

❖ **The triglycerides of the other 50% of the IDL (VLDL remnant) is hydrolyzed by hepatic lipase producing LDL, a lipoprotein that is richer than IDL in cholesterol and its ester.**

❖ **Therefore, with hindering hepatic lipase activity, it can be reduced to remodel lipoprotein into LDL**

## Hepatic Lipase

(U/L)

| Wks | Control | Alcohol | SKW3000 | SKW1000 | SKW330 |
|---|---|---|---|---|---|
| 0 | 51.37±4.55 | 51.37±4.55 | 51.37±4.55 | 51.37±4.55 | 51.37±4.55 |
| 6 | 65.00±7.92$^{b}$ | 426.10±72.43$^{a}$ | 426.10±72.43$^{a}$ | 426.10±72.43$^{a}$ | 426.10±72.43$^{a}$ |
| 8 | 51.20±8.91$^{c}$ | 363.43±123.97$^{a}$ | 90.97±13.94$^{c}$ | 148.58±69.84$^{bc}$ | 246.95±17.89$^{b}$ |
| 10 | 55.20±1.41$^{b}$ | 254.75±22.27$^{a}$ | 48.55±2.14$^{b}$ | 56.38±6.90$^{b}$ | 117.95±68.14$^{b}$ |

*1) Values are mean±S.D.*

*2) Letters with different superscripts in the same raw are significantly different ($p<0.05$) among the groups by Duncan's multiple range test.*

## HB-SungKangWon

## Composition

- ✓ *Astragalus membranaceus*
- ✓ *Salvia miltiorrhiza*
- ✓ *Pueraria lobata*

} *SungKangWon*

- ✓ *Morus alba*
- ✓ *Crataegus pinnatifida*
- ✓ *Alisma oriental*

} *Add*

## *Morus alba*

❖ Family ; Moraceae

❖ Part used ; Folium

❖ Common name ; Sang Ye

## Effects of *Morus alba*

☐ ***Morus alba*** **showed hepatoprotective effects on tacrine-induced cytotoxicity in human liver-derived Hep G2 cells.**

*(Oh et al. Planta Med. 2002)*

## *Crataegus pinnatifida*

❖ **Family ; Rosaceae**

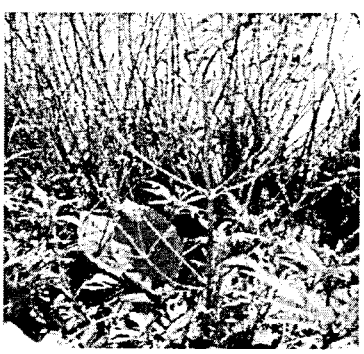
❖ **Part used ; Fructus**

❖ **Common name ; Shan Zha**

## Effects of *Crataegus pinnatifida*

- ☐ **Crataegus reduced the increase of plasma cholesterol and lipid in rats fed a high fat diet.**
  *(Shanthi et al. Indian J. Biochem. Biophy. 1994)*
- ☐ **Pretreatment with Crataegus gave close related protection for isoprenaline – induced damage of liver in rats.**
  *(Ciplea et al. Arzneimittelforschung 1988)*

## *Alisma orientale*



- ❖ **Family ; Alismataceae**
- ❖ **Part used ; Rhizoma**
- ❖ **Common name ; Ze Xie**

## Effects of *Alisma orientale*

- ☐ **Potential liver-protective activities of the isolated alisol compounds were evaluated against $CCl_4$-induced liver damage.**

  *(Chang et al. Kor. J. Pharmacog. 1982)*

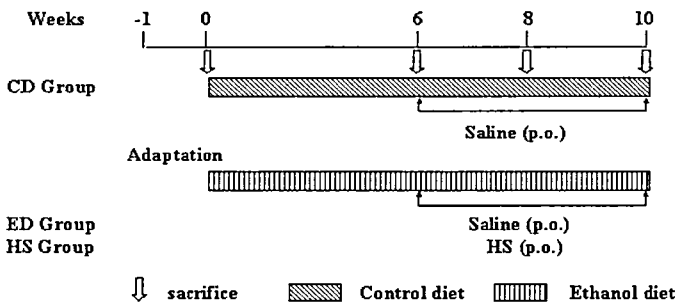
- ☐ **The serum lipid levels of Alisma groups were significantly lower.**

  *(Lim et al. Korean J. Food Sci. Technol. 2003)*

## Purpose of Study

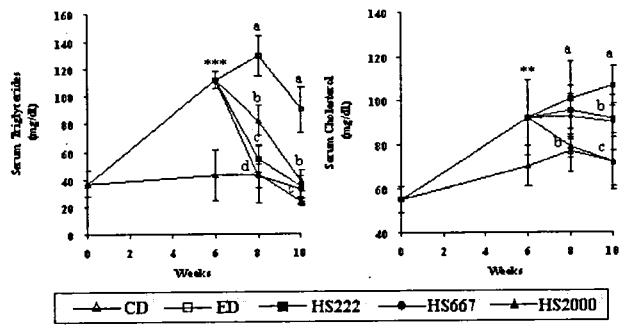
**To investigate the effect of HB-Sungkangwon (*Astragali membranaceus, Puerariae lobata, Salviae miltiorrhiza* + *Morus alba, Crataegus pinnatifida, Alisma oriental* ) on fatty liver induced by alcohol diet**

# Experimental Design




# Results

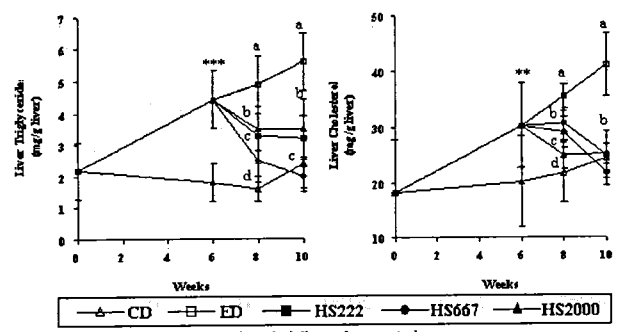
## Serum TG and TC




*1) ** : p<0.01, *** : p<0.0001. Significantly different from control group*

*2) Letters with different superscripts in the same raw are significantly different (p<0.05) among the groups by Duncan's multiple range test.*

## Liver TG and TC




*1) ** : p<0.01, *** : p<0.0001. Significantly different from control group*

*2) Letters with different superscripts in the same raw are significantly different (p<0.05) among the groups by Duncan's multiple range test.*

# Serum LDL-C, VLDL-C, HDL-C

| Wks | Group | LDL-C | VLDL-C | HDL-C |
|---|---|---|---|---|
| | | mg/dl | | |
| 0 | | 16.66 ± 9.58 | 7.41 ± 1.87 | 31.20 ± 9.73 |
| 6 | CD | 26.46 ± 13.45 | 8.62 ± 3.67 | 35.21 ± 6.98 |
| | ED | 41.39 ± 17.36 * | 22.33 ± 1.33 *** | 28.65 ± 7.68 * |
| 8 | CD | 20.21 ± 9.15 c | 8.72 ± 4.21 c | 48.36 ± 4.47 a |
| | ED | 49.53 ± 17.02 a | 25.90 ± 2.88 a | 24.94 ± 7.93 c |
| | HS222 | 45.55 ± 9.66 a | 10.94 ± 1.88 c | 38.67 ± 5.65 b |
| | HS667 | 44.78 ± 10.26 a | 8.47 ± 1.71 c | 32.94 ± 6.71 b |
| | HS2000 | 30.15 ± 4.21 b | 16.43 ± 2.23 b | 31.70 ± 3.74 b |
| 10 | CD | 12.02 ± 8.78 d | 4.75 ± 0.60 c | 55.36 ± 10.48 a |
| | ED | 69.94 ± 11.90 a | 18.05 ± 3.30 a | 20.42 ± 6.21 c |
| | HS222 | 39.14 ± 8.47 b | 6.95 ± 1.57 b | 35.67 ± 3.04 b |
| | HS667 | 35.59 ± 8.91 b | 6.59 ± 1.93 b | 37.71 ± 6.74 b |
| | HS2000 | 26.23 ± 9.99 c | 7.78 ± 1.54 b | 38.27 ± 9.33 b |

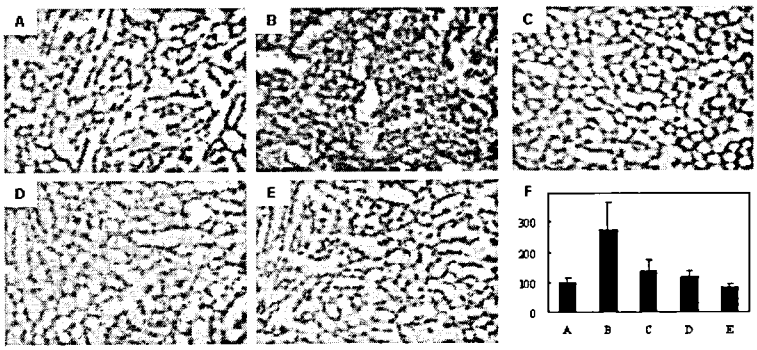
** : p<0.1, *** : p<0.0001. Significantly different from control group*

# AST / ALT

| Wks | Group | AST | ALT |
|---|---|---|---|
| | | Unit / ml | |
| 0 | | 71.79 ± 9.39 | 32.67 ± 8.52 |
| 6 | CD | 89.72 ± 6.17 | 28.00 ± 6.63 |
| | ED | 133.30 ± 12.78 *** | 68.96 ± 9.22 *** |
| 8 | CD | 94.85 ± 8.12 bc | 38.45 ± 8.97 b |
| | ED | 119.52 ± 14.73 a | 62.68 ± 9.48 a |
| | TF222 | 103.61 ± 6.92 b | 59.37 ± 7.07 a |
| | TF667 | 99.21 ± 6.41 b | 29.84 ± 9.44 c |
| | TF2000 | 86.20 ± 9.89 c | 33.92 ± 9.26 bc |
| 10 | CD | 96.97 ± 9.97 ab | 23.50 ± 3.02 c |
| | ED | 105.24 ± 12.06 a | 56.74 ± 9.76 a |
| | TF222 | 88.34 ± 6.72 bc | 40.80 ± 9.44 b |
| | TF667 | 40.80 ± 9.44 b | 27.73 ± 9.87 c |
| | TF2000 | 86.13 ± 9.71 c | 36.23 ± 9.92 b |

** : p<0.1, *** : p<0.0001. Significantly different from control group*

## Liver Histology




Frozen sections of liver (x40) taken from (A) CD + saline (B) ED + saline (C) ED + HS222 (D) ED + HS667 (E) ED + HS2000 (F) Densitometer analysis – treated rats on 10 weeks. Frozen sections were stained with Oil Red O and counterstained with Mayer's haematoxylin. Lipid droplets were stained red.

## What is Found

Sungkangwon → HB-Sungkangwon

TG , Total-C ⇩ (in blood and liver)

By hepatic lipase activity ⇩

⇨ Effects to remedy on fatty liver induced by alcohol intake