Herbal Products for Liver Disease: A Therapeutic Challenge for the New Millennium

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Natural Remedies:

- $1.8 billion market in USA;
- $180 million in Germany only for silymarin;
- 3-fold increase between 1992-1996;
- CAM use by liver disease pts. parallels its use in general population;
- nearly 1/3 of outpatients liver patients use herbal remedies;
- USA survey 1999: CAM at least once-41% (33-75%), more common in women, good education, higher income level, no correlation with age, severity or etiology;
- lack of regulations, being classified as “food supplements”;
- occasional severe toxicity;
- lack of randomized, placebo-controlled clinical trials;
Complementary and Alternative Medicine in Chronic Liver Disease

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Conventional vs CAM for Chronic Liver Disease:

Methodology philosophy
prospective RCTs, vs “natural health & healing” paradigm

End-points
morbidity, mortality, surrogate markers of known cause and
natural history of the disease
vs “reinforcing natural healing”, “wellness”

Treatment strategy (ex: for HCV CLD)
sustained virological response by antiviral + BRM
vs “strengthening” small int. > liver > immune system
Conventional vs CAM for Chronic Liver Disease:

R & D Methodology in HCV treatment

identification of an “active” component, purification, quantitation, minimal batch-to-bench variation

vs

ancient, oral tradition, multiple components (active, buffer, diluting active part), time of harvest, soil, climate conditions, methods of drying, processing and extraction

?
Future Directions of CAM for CLD:

• Improve side effects of conventional therapy, thus leading to better compliance and response rate?

• Improve HCV CLD through antioxidant, antifibrotic or immune modulation properties?

• Further proves of such an effect require specific RCTs and possibly liver histology data.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Putative Biological Mechanism*</th>
<th>Targeted Liver Disease†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silymarin (milk thistle)</td>
<td>Biologically active compound—silibinin Acts as an antioxidant and free radical scavenger</td>
<td>Cirrhosis In Europe—chronic liver disease, digestive disorders, and gallbladder disease</td>
</tr>
<tr>
<td></td>
<td>In animals, prevents glutathione depletion free radical formation in the liver May also be antifibrotic through undeterminate mechanism(s)</td>
<td></td>
</tr>
<tr>
<td>Glycyrrhizin</td>
<td>Licorice root—multiple constituents appears to inhibit enzyme 11-beta-hydroxysteroid dehydrogenase, thus anti-inflammatory in inhibiting prostaglandin production and modifies arachidonic acid metabolism Also antioxidant properties—induces glutathione-S-transferase and catalase</td>
<td>Used traditionally for cough, bronchitis, gastritis, liver inflammation Fibrosis</td>
</tr>
<tr>
<td>Plantago asiatica seed</td>
<td>Aucubin—active ingredient, iridoid glycoside Transient inhibition of viral replication</td>
<td>Hepatitis B virus</td>
</tr>
<tr>
<td>Herbal Medicine 861</td>
<td>Herbal mixture, blocks stellate cell activation through inhibiting cell cycle progression</td>
<td>Fibrotic liver disease</td>
</tr>
<tr>
<td>TJ-9 (Sho-saiko-to)</td>
<td>Herbal mixture, blocks stellate cell activation Inhibits lipid peroxidation in hepatocytes and stellate cells</td>
<td>Fibrotic liver disease In Japan, recommended for hepatitis B virus</td>
</tr>
<tr>
<td>TJ-41</td>
<td>Herbal mixture, induces cellular apoptosis via P 53.</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td>TJ-108</td>
<td>Herbal mixture with active compound gomisin A. Antiviral</td>
<td>Hepatitis C virus</td>
</tr>
<tr>
<td>Liv-52</td>
<td>Herbal mixture—hepatoprotective</td>
<td>In India, alcohol-induced liver disease</td>
</tr>
<tr>
<td>Phyllanthus amarus</td>
<td>Extract inhibits hepatitis B viral polymerase by inhibiting the virus enhancer I activity—complexes transcription factors</td>
<td>Hepatitis B virus</td>
</tr>
</tbody>
</table>
some Clinical studies

Glycyrrhizin
1991 4-wks Gly + 4 wks IFN: 70% loss of HbeAg after 6 months;
1994 Gly + IFN vs IFN: 33% vs 13% HCV-RNA negativization;
1997 80mg x 2 weeks → AST, ALT in >60% of CAH pts;
1997 2-7/weekly i.v. Gly x 10 years: 2.5-fold decrease of HCC and 1.5-fold decrease of cirrhosis;

Silymarin
1978 expedites recovery after acute A or B hepatitis;
1980 expedites recovery in alcohol-related hepatitis;
1982 2-fold decrease of death rate due to Amanita intoxication;
1989 41 months follow-up: higher survival in cirrhotics;
1998 previous data not confirmed!

……..lack of reliable formulation, erratic pharmacokinetics
some Clinical studies

Plantago
1997 10mg/kg/day i.v. x 4-month: 10-40% ↓ HBV-DNA;

Compound 861
1995 2-years, CHB: 83% subj. improv., ↓ 41% spleen size,
↓ AST,ALT (73% to normal range), PIIINP;
1998 6-month, CHB: histological improvement (infl. & fibrosis);

CH-100 1998 RCT - HCV pts: significant ALT reduction;

TJ-9 1995 5-year study, 260 cirrhotics, ↑ survival, ↓ HCC;
TJ-108 2000 ↓ HCV-RNA in 21% HCV +ve patients;

YHK/K-17.22 1998-2004 HCV pts.: ↓ ALT;
A novel nutraceutical: YHK

YHK composition:

- *Panax pseudo-ginseng*: 40-60% weight%
- *Eucomiae ulmoides*:
- *Gallic acid-containing rhizome*: 8-12% weight%
- *Glycyrrhiza glabra Linn*: 8-12% weight%

No heavy metals, hyphe or Coliform bacteria
In vitro and in vivo CCl₄ experimental study
In vitro $\text{CCl}_4$-hepatotoxicity assay

|$\text{H.C.}$| $\text{C}$| $\text{Sil}$| $\text{Gly}$| $1\mu\text{g}$| $10\mu\text{g}$| $100\mu\text{g}$| $200\mu\text{g}$

$\text{H.C.}$: healthy control  
$\text{C}$: control
Male albino Wistar rats were allocated into three groups:

A) given a s.c. injection of 0.1ml/100g body wt of CCL\textsubscript{4} in olive oil (1:1 v/v) twice/week for 5 weeks;

B) as A plus oral supplementation with 50mg/kg of YHK dissolved in 5% glucose, \textit{concomitantly} with the CCL\textsubscript{4} injection;

C) as A plus oral supplementation with 50mg/kg of YHK dissolved in 5% glucose, \textit{1 week after} the 1st CCL\textsubscript{4} injection.

Olive oil s.c. injection alone was used in the control group.
Y-protein liver content: effect of YHK

% of control

$ \text{p} < 0.001 \text{ vs control} \quad * \text{ p} < 0.01 \text{ vs A}$
GSH S-transferase activity in Y-fraction: effect of YHK on selected substrates

μmol/min/mg protein

* p<0.01 vs B, C and control
**Plasma level of transaminases: effect of YHK**

IU/l

- Control
- A
- B
- C

* p<0.001 vs Control, B and C
Liver histology score

(necrosis + inflammatory infiltrate)

* $p<0.001$ vs control, B and C

§ $p<0.05$ vs A
**STUDY 3**

*Experimental Ischemia-reperfusion* Liver injury
Male Wistar rats were allocated into three groups which were given a 2-week supplementation with:

A) standard pellet diet;  
B) as A plus added with 30mg of YHK

*Liver ischemia/reperfusion model* (microvessel clipping of left portal venous branch and hepatic artery, 15min liver ischemia, clip release, removal of right lateral and caudate lobes, 60min reperfusion, sacrifice)

Sham-op. rats (RL and caudate lobe resection without ischemia): control (C)

40 rats undergoing liver ischemia with/without dietary treatment were used for survival study
Assay of SOD activity in liver tissue: effect of YHK (at 60min after reperfusion)

- **A**
- **B**
- **C**

U/mg protein

- $p<0.001$ vs control
- $p<0.05$ vs A
Hepatic Tissue Blood Flow after ischemia-reperfusion

ml/min/100g

A § p<0.001 vs control
B * p<0.05 vs A
C
Plasma level of transaminases and LDH after ischemia-reperfusion liver injury

IU/l

§ p<0.001 vs control  * p<0.05 vs A

§ x 1000
Assay of Lipid Peroxides in liver tissue: effect of YHK (at 60min after reperfusion)

nmol/mg protein

§ p<0.001 vs control  * p<0.01 vs A
GSH liver content after ischemia-reperfusion

mg/g liver

§ p<0.001 vs control  * p<0.05 vs A
Ischemia-Reperfusion Liver Injury

Untreated

YHK-Treated
YHK and *metal-induced oxidative* liver injury: an anti-xenobiotic therapeutic tool?
It has been shown that metals deplete glutathione and protein-bound sulfhydryl groups, resulting in the production of reactive oxygen species leading to oxidative damage to proteins, loss of enzymatic activity and alteration of protein structure.

There is a growing body of evidences suggesting the role of free radical generation and oxidant injury in the pathogenesis of liver fibrosis, NASH and NAFLD while several synthetic antioxidants may raise concerns over their toxicity.

Thus, the aim of the present study was to further investigate this compound in *in vitro* testing of hepatocytes oxidative damage by iron, copper and vanadium which is also known to trigger oxidative damage to cellular membranes and nuclear DNA.
Isolation and culture of hepatocytes.

- Hepatocytes were isolated by collagenase perfusion method as described by Wolkoff.
- The monolayer of hepatocytes were cultured for 12hr in the medium containing 1.0mM $\alpha$-linolenic acid (LNA)-bovine serum albumin (BSA).
- The control hepatocytes were maintained in culture in the medium without LNA and the amount of cell protein was determined by the method of Lowry et al.
**Hepatocyte culture test.** Hepatocytes were washed twice with Hanks’ medium and further cultured in 60-mm (1.5 x 10⁶ cells/dish) with graded dilution of

**YHK** (panax pseudo-ginseng, Eucommia Ulmoides, polygonati rhizome, glycyrrhiza licorice, panax ginseng, Kyotsu, Tokyo, Japan) sample (100μg/ml and 200μg/ml) or **sylbin** (100μg/ml) dissolved in dimethyl sulfoxide 10min before the addition of metallic salts dissolved in saline a concentration of 100μM each.

Malonildialdehyde (MDA) in the medium was assessed by a slight modification of the Uchiyama and Mihara method.
**Preparation of LNA-BSA complex.** LNA was serially added to 240ml of complete Williams’ medium E 1mM BSA.

**Preparation of lysosomal fractions.** After liver homogenization the supernatants and the lysosome-containing supernatant were centrifuged and the final pellets were resuspended in the sucrose buffer to a protein concentration of ~15 mg/ml to yield a lysosome enriched fraction.

**Lysosome fragility test.** The fraction was incubated with the test compound and each metal ions and β-galactosidase activity was assessed as described by Olsson. The results were expressed as percentage of total β-galactosidase released. **Lactate dehydrogenase leakage** was also measured in the culture medium.

**Oxidative damage tests of lysosomes.** Assays for the release of acid phosphatase and β- N-acetylglucosaminidase from lysosomes were carried out by incubating lysosomal suspensions with test compounds in the presence of 50mM AAPH or 1mM AMVN. Further, quenching activity of either YHK and sylibin against DPPH radicals was assessed by spectrophotometry.
Inhibiting activity of YHK and silybin on FeSO$_4$-, Cu SO$_4$- and VCl$_3$ -induced lipid peroxidation in normal hepatocytes (mean ± SD)

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>YHK</th>
<th>Silybin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100μM</td>
<td>200μM</td>
</tr>
<tr>
<td>FeSO$_4$</td>
<td>15.6 ± 4.6$§$</td>
<td>12.2 ± 4.4$§$ *</td>
</tr>
<tr>
<td>Cu SO$_4$</td>
<td>7.9 ± 0.3</td>
<td>6.7 ± 0.7</td>
</tr>
<tr>
<td>VCl$_3$</td>
<td>8.7 ± 0.99</td>
<td>9.4 ± 0.85</td>
</tr>
</tbody>
</table>

Values represent the concentrations that inhibit lipid peroxidation by 50% (IC50, μM). IC50 is calculated from the concentration-activity curves.

$§ p<0.05$ vs Cu SO4 and VCl3. * $p<0.05$ vs Silybin
Inhibiting activity of YHK and silybin on FeSO$_4$-, VCl$_3$- and Cu SO$_4$-induced lipid peroxidation in LNA-loaded cells

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>YHK</th>
<th>Silybin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100µM</td>
<td>200µM</td>
</tr>
<tr>
<td>FeSO$_4$</td>
<td>73.4 ± 7.4$^\S$</td>
<td>59.2 ± 9.2$^\S$</td>
</tr>
<tr>
<td>Cu SO$_4$</td>
<td>15.9 ± 2.2</td>
<td>19.8 ± 1.7</td>
</tr>
<tr>
<td>VCl$_3$</td>
<td>16.7 ± 1.2</td>
<td>18.1 ± 0.57</td>
</tr>
</tbody>
</table>

Values represent the concentrations that inhibit lipid peroxidation by 50% (IC50, µM). IC50 is calculated from the concentration-activity curves.

$^\S$ p<0.05 vs Cu SO$_4$ and VCl$_3$. * p<0.05 vs silybin
Effect of YHK on the release of lysosomal enzymes in the presence of hydrophilic or lipophilic radical generators: enzyme activity (% of control ± SE)

<table>
<thead>
<tr>
<th></th>
<th>AAPH-induced release</th>
<th>AMVN-induced release</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acid phosphatase</td>
<td>β-N-acetylglucosaminidase</td>
</tr>
<tr>
<td>YHK 10⁻⁴ M</td>
<td>52.4 ± 6.1*</td>
<td>47.7 ± 4.2*</td>
</tr>
<tr>
<td>Sylbin 10⁻⁴ M</td>
<td>51.9 ± 5.6*</td>
<td>54.6 ± 4.7*</td>
</tr>
<tr>
<td>YHK 10⁻⁴ M</td>
<td>64.4 ± 7.9*§</td>
<td>61.3 ± 8.7*§</td>
</tr>
<tr>
<td>Sylbin 10⁻⁴ M</td>
<td>83.9 ± 10.4*</td>
<td>77.3 ± 7.4*</td>
</tr>
</tbody>
</table>

* p< 0.01 vs DMSO which served as a control compound. § p<0.05 vs. sylbin
EFFECT OF YHK AND SYLIBIN ON LDH LEAKAGE DUE TO METAL IONS DAMAGE IN CULTURED HEPATOCYTES

LDH leakage in medium IU/L/mg protein

control Test YHK 100µM YHK 200µM Sylbin 100µM

Fe Cu V

§

*
EFFECT OF YHK AND SYLIBIN ON METAL IONS-INDUCED β-GALACTOSIDASE RELEASE IN LYPOSOMAL FRACTIONS

supernatant/total %

β-Galactosidase Activity

control  Test  YHK 100μM  YHK 200μM  Sylbibin 100μM

Fe  Cu  V

* §
DPPH RADICALS-SCAVENGING ACTIVITY OF YHK AND SYLIBIN IN LYSOSOMAL FRACTIONS (mean ± SD)

Absorbance of DPPH

% of control

- YHK 100μM
- YHK 200μM
- Sylbin 100μM
It is likely that the antioxidative (Panax pseudo-ginseng, eucommia ulmoides, glycyrrhiza glabra linn, gallic acid) and immunomodulative (gallic acid, glycyrrhiza) properties of the components of this compound have to be advocated for to explain its prospective role in the above studies.

In the present in vitro study, such compound showed to significantly protect hepatocytes from metal ions-induced lipid peroxidation at even better extent than sylbin and it is also conceivable that eucommia ulmoides, among others, might have further contributed to such effect, given its potent antioxidant property.

AAPH and AMVN are azo-compounds which generate radicals after thermal haemolysis in aqueous phase and lipid phase, respectively, and our findings show that YHK significantly protects lysosomal integrity with a mitigated LDH and β-galactosidase release.

This is likely to be the result of its effective DPPH radical-scavenging activity and its activity against lipophilic-generators of free radicals which was stronger than sylbin. Indeed, during metal-induced injury the oxidant stress damage is preferentially targeted to the lysosomal compartment which is particularly rich in low molecular weight redox-active iron and the rupture of lysosomes, followed by relocation of labile iron to the nucleus, could be an important intermediary step in the generation of oxidative DNA damage, as it has been very recently demonstrated.

These latter findings are of interest in view of recent data suggesting that metal-induced lysosome alterations are advocated among the mechanisms of liver steato-hepatitis and carcinogenesis. Taken overall, these experimental data support the potentiality of the clinical application of this compound while well-designed clinical studies are ongoing.
STUDY 5

YHK and *inhibition of implanted tumor growth*  
*(preliminary data)*
**IMPLANTED TUMOR GROWTH: EFFECT OF CHEMOTHERAPY ALONE AND ADDED WITH YHK**

* p<0.05 vs Control

** p<0.05 vs ADM and CP alone and p<0.01 vs Control
Liver changes during chemotherapy

<table>
<thead>
<tr>
<th>Group</th>
<th>Hypertrophy / hyperplasia of Kupffer’s cells</th>
<th>Lymphocytosis</th>
<th>Focal necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control A</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control B</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Adriamycin (ADM)</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cisplatin (CP)</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>YHK + ADM</td>
<td>+</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>YHK + CP</td>
<td>+</td>
<td>+</td>
<td>±</td>
</tr>
</tbody>
</table>
STUDY 6

ANIT-Induced *DNA liver injury*. Which protective role of YHK?
It has been suggested that a number of toxins, drug chemicals and virus infections to the liver can bring about DNA synthesis abnormalities. Such DNA impairments are also of paramount importance in the reparative mechanisms during the recovery phase after the injury;

A number of studies have suggested that a neutrophil-generated lipid peroxidation is likely to be the main mechanism involved in α-naphtyl-isothiocyanate (ANIT)-induced liver injury;

We have recently reported that a controlled herbal remedy, i.e. K-17.22, was able to significantly prevent CCL₄-induced liver disease and such toxic acts also by oxidative damage;
Seventy-two 5-6 weeks Wistar rats were housed in an environmentally-controlled vivarium with free access to deionized water and inert fibers *ad libitum*;

Rats were put on a 2-week dietary supplementation with:

- A) standard diet;
- B) standard diet added with K-17.22 50mg/kg (Kyotsu, Tokyo, Japan)

ANIT-liver injury model was applied (0.04%/day for an average of 35mg/kg b.w./day) to both groups;

Healthy rats served as control;
At one-week observation, blood samples were withdrawn by cardiac puncture under ether anaesthesia and serum was used to measure:

1) Routine biochemistry;
2) Lipid peroxide, MDA;

Soon after, at sacrifice, liver tissue was used

1) to measure: GSH and GSSG;
2) for \textit{In vitro} study: release of GOT, GPT and ALP
3) for \textbf{DNA synthesis} study (using 1\(\mu\)Ci of thymidine-methyl-\(^3\)H)
DNA SYNTHESIS RATE IN ANIT-INDUCED LIVER DAMAGE:

*Protective effect of YHK*

dpm/incubation

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>ANIT</th>
<th>YHK</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANIT</td>
<td><strong>7000</strong></td>
<td>4000</td>
<td></td>
</tr>
<tr>
<td>YHK</td>
<td></td>
<td><strong>6000</strong></td>
<td>7000</td>
</tr>
</tbody>
</table>
In Vitro ENZYME RELEASE IN ANIT-INDUCED LIVER DAMAGE (GOT, GPT, ALP): Effect of YHK

[Graph showing enzyme release levels for control, ANIT, and YHK]
GSH/GSSG liver content in ANIT injury

mg/g liver

Control  ANIT  YHK

§ p<0.001 vs control  * p<0.05 vs ANIT
These preliminary data suggest that:

YHK does indeed exert a significant protective effect in liver toxicity.

Given that ANIT injury is LP-driven, these data offer a potential tool in an integrative treatment of HCV-chronic liver disease, especially in view of HCC transformation.
Is there an antifibrotic effect of YHK: an experimental study
Chronic liver injury leads to excessive deposition of collagen resulting in the irreversible end-point of cirrhosis, which is one of the most common causes of death worldwide;

Prevention and/or suppression of fibrotic changes in the liver are of vital importance. Further, fibrosis is not simple deposition of excess matrix but it is associated with a change in the type of matrix molecules and in its redistribution;

Therapeutic attempts with antifibrotic drugs are still at an experimental stage and many of such agents have a drawback to be toxic.
150 adult male Sprague-Dowley rats were initially used after one week quarantine. Animals were housed in an environmentally-controlled vivarium with free access to deionized water and non-nutrient fibers ad libitum;

A liver fibrosis model was applied (0.2ml/kg CCL₄ i.p. injection twice/week) and rats allocated into 2 groups:

A) standard diet;

B) standard diet added with YHK 50mg/kg (Kyotsu, Tokyo, Japan)

Healthy rats served as control;
At sacrifice, the liver was immediately removed, weighed and separate samples were sent for:

1) **Routine histology** (5µm-thick section were coded and examined in a blind fashion);

2) **Hydroxyproline determination** (according to the method of Jamail et al.);

3) **Immunohistochemical analysis** of stellate cells;

4) **Northern Blot** of TIMP-1 and α2-procollagen mRNA;
At the same time, blood samples were withdrawn to measure:

1) Routine biochemistry;
2) Type IV collagen 7s;
3) Hyaluronic acid;
### Hydroxyproline content of the liver

<table>
<thead>
<tr>
<th>weeks</th>
<th>Control</th>
<th>CCL\textsubscript{4}</th>
<th>CCL\textsubscript{4} + YHK</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>367 ± 75</td>
<td>344 ± 87</td>
<td>401 ± 110</td>
</tr>
<tr>
<td>10</td>
<td>389 ± 93</td>
<td>839 ± 147*</td>
<td>563 ± 132*§</td>
</tr>
<tr>
<td>20</td>
<td>343 ± 61</td>
<td>1190 ± 205*</td>
<td>718 ± 151*§</td>
</tr>
</tbody>
</table>
## Serum markers of fibrosis

<table>
<thead>
<tr>
<th>weeks</th>
<th>Control</th>
<th>CCL$_4$</th>
<th>CCL$_4$ + YHK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Hyaluronic acid</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>8.3 ± 4.3</td>
<td>4.6 ± 3.7</td>
<td>6.2 ± 4.0</td>
</tr>
<tr>
<td>10</td>
<td>6.7 ± 3.6</td>
<td>133.8 ± 55.6*</td>
<td>67.8 ± 24.7*§</td>
</tr>
<tr>
<td>20</td>
<td>11.3 ± 5.4</td>
<td>224.6 ± 77.5*</td>
<td>15.5 ± 7.2§</td>
</tr>
<tr>
<td></td>
<td><strong>Type IV collagen 7s</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4.3 ± 0.2</td>
<td>4.4 ± 0.2</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>10</td>
<td>4.2 ± 0.2</td>
<td>4.2 ± 0.5</td>
<td>4.1 ± 0.5</td>
</tr>
<tr>
<td>20</td>
<td>4.3 ± 0.6</td>
<td>4.9 ± 0.4</td>
<td>4.7 ± 0.1</td>
</tr>
</tbody>
</table>
$\alpha_2$-procollagen mRNA and TIMP-1 mRNA (x10)
Plasma level of transaminases: effect of YHK

IU/l

0 50 100 150 200 250 300 350 400

Control A B

* p<0.001 vs Control and B

AST ALT
Plasma level of biliary stasis parameters: effect of YHK

IU/l

* p<0.001 vs Control and B

GGT
T. Bil.
These preliminary data suggest that:

The present novel phytotherapeutic compound (YHK) exerts a potent anti-fibrotic effect and further studies are awaited to corroborate its clinical potential.
STUDY 8

A pilot clinical study of YHK in *HCV-related Chronic Liver Disease*

HARADA M, MAROTTA F et al.

Med. Corp. Harada Hospital, Tokyo, Japan

Hepato-GI Dept. & WHO-Cntr. For Biotech, University of Milano, Italy

Japan Inst. Health Care with Oriental Herbs & Medicine, Tokyo, Japan
Chronic liver injury leads to excessive deposition of collagen resulting in the irreversible end-point of cirrhosis, which is one of the most common causes of death worldwide;

Related medical therapies are often difficult to handle, expensive and limited in its efficacy and other treatments, either to complement or to replace standard care are often sought;

Potential inhibitors of hepatic fibrosis, including dexamethasone, interferon-gamma, colchicine and 16,16-dimethyl PGE$_2$ have been reported but none of them has been successfully tested in clinical practice
The aim of the present pilot study was:

to test a novel natural compound (YHK),
which had preliminarily shown in clinical practice to
significantly lower transaminases level in HCV-
positive chronic liver disease patients,
in a protocol- and biopsy-controlled manner.
Six patients (age 55-69) with HCV-Chronic Liver Disease participated to the study after an informed consent;

A verified HCV diagnosis history was dating back 2 to 18 years;

All patients had transaminases level 2- to 4-fold increased;

5 patients were on UDCA and glycyrrhizin acid (SNMC) therapy and one had just finished a 6 month IFN-treatment;
At the entry into the study all patients underwent a liver biopsy which was blindly assessed by an experienced pathologist, according to Maruyama Classification;

After a wash-out period, patients were instructed to stop any prior treatment and any other health supplement or vitamin;

Patients were then put on the following treatment:

- YHK, 4 tabs t.i.d. for 10 days, followed by:
- YHK, 2-3 tabs t.i.d. throughout the study period.
<table>
<thead>
<tr>
<th>Fibrosis score</th>
<th>Necro-Inflamm. score</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₁ → F₀⁻¹</td>
<td>A₂ → A₀</td>
<td>improved</td>
</tr>
<tr>
<td>F₂ → F₀⁻¹</td>
<td>A₃ → A₂</td>
<td>improved</td>
</tr>
<tr>
<td>F₁ → F₁</td>
<td>A₂ → A₂</td>
<td>no change</td>
</tr>
<tr>
<td>F₀ → F₀</td>
<td>A₂ → A₀⁻¹</td>
<td>improved</td>
</tr>
<tr>
<td>F₂ → F₃</td>
<td>A₂ → A₂</td>
<td>worse</td>
</tr>
<tr>
<td>F₁⁻² → F₁⁻¹</td>
<td>A₃ → A₁⁻²</td>
<td>improved</td>
</tr>
</tbody>
</table>
TIME-COURSE OF GOT LEVEL DURING YHK-TREATMENT
TIME-COURSE OF GPT LEVEL DURING YHK-TREATMENT

IU/L

ENTRY 1 6 9 12 16 18
week
Overall ongoing study population: 6 of them in the present report

**GPT の推移（C型）20例**

<table>
<thead>
<tr>
<th>前</th>
<th>10日</th>
<th>20日</th>
<th>1M</th>
<th>2M</th>
<th>3M</th>
<th>4M</th>
<th>5M</th>
<th>6M</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
</tbody>
</table>

- 正常範囲
- 平均値
These preliminary data further support:

the potential use of YHK in clinical practice while larger clinical trials together with experimental investigations are awaited
Evolution Stages of Neoplasia

- **Initiation**: Oncogene/suppressor gene mutations

- **Promotion**
  - Altered patterns of gene expression,
  - Hyperproliferation, hyperplasia,
  - Inflammatory changes

- **Genotoxicity**: DNA Adducts

- **Procarcinogen**
  - Ultimate carcinogen
  - Electrophile
  - Direct acting carcinogen

- **Adduct Removal**
  - Non-Enzymatic Inactivation
  - Enzymatic Inactivation

- **Cancer cell**
  - Promotion to Neoplasia

- **Mutation or Transformation**

- **Excreted**

*Nutrition and Cancer. 41 (1&2), 17-28, 2001*
Food Components as Modifiers of Cancer Process

- Flavonoids
- Phenols
- Sterols
- Sulfhydryls
- Terpenes
- Fibers
- Allyl compounds
- Indols
- Glucosinolates
- Carotenoids
- Vitamins C, D, E
- Selenium
- Isothiocyanates (Cabbage)
- Catechins (Tea)
Initiation

Bioactive dietary constituents

- Alcohol
- Smoking

Phase I metabolising enzymes - P450s etc.

Bioactive dietary constituents e.g. isothiocyanates

Phase II metabolising enzymes

Genes

Procarcinogen

EXCRETION

EXCRETION
Progression

Dietary factors

Smoking and other exposures

DNA repair genes

DNA damage

Immune system

Growth factors

Hormones

DNA damage

Precancerous lesions/dysplasia

Cancer

Metastasis
Nutritional Modulation of Carcinogenesis

Prevention Strategies
- Alter carcinogen metabolism
- Enhance carcinogen detoxification
- Scavenge electrophiles/ROS
- Enhance DNA repair

Prevention Strategies
- Scavenge ROS
- Alter gene expression
- Decrease inflammation
- Suppress proliferation
- Induce differentiation
- Encourage apoptosis

Nutritional Oncology pp. 91, 1999
Nutritional Genomics And Biomarker Discovery

Regulation by Diet
Gene Expression Process
Functional Genomic Techniques

DNA
Transcription, RNA Processing and Stability
Transcriptomics (DNA Arrays)

RNA
Translation, Modification, and Stability
Proteomics

Protein
Metabolomics, Metabonomics, Bioassays

Cell
Metabolites
Health

Nutrients to Modify Cancer Risk
- Vitamins A
- Vitamin D
- Vitamin E
- Vitamin C
- Folic Acid
- Calcium
- Selenium
- Lycopene
- Resveratrol
- Tea Polyphenols
- Curcumin
- Genistein
- Sulforaphane
- Macronutrients
  - Carbohydrates
  - Fat
  - Protein
  - Fiber & Water

Nutritional Genomics And Biomarker Discovery

BMJ 2002; 324:1438.
• Macronutrients- carbohydrates, fats, and proteins

• Micronutrients- vitamins and minerals

• Phytochemicals (YHK?)

• Dietary fiber

• Water