Antitumor and neurologically active ingredients from medicinal plants

Prof. Dr. Liu Xinmin

Member, Expert Advisory Panel on Traditional Medicine, WHO Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences (CAMS)/Peking Union Medical College (PUMC), Beijing, 100193, China

EMAIL: LIUXINMIN@HOTMAIL.COM

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Timisoara, Romania
Institute of Medicinal Plant Development

Located in Beijing.

Affiliated with Chinese Academy of Medical Sciences (CAMS) and Peking Union Medical College (PUMC).

WHO collaboration center for traditional medicine.
More about IMPLAD

Staff and structure: 600 staff, including 150 Profs, 5 research centers & 4 branches (Hainan, Yunnan, Guangxi, Xijiang).

Research Areas: Herbal plant cultivation and GACP, Herbal resources, natural product chemistry, pharmacology & toxicology, quality control & drug delivery, and biotechnology.

International Cooperation: with more than 50 countries, Romania, Canada, UK, USA, Luxembourg etc.
Our Research Team

- Toxicology
- Extraction Process
- Compound Isolation
- Drug Metabolism
- Pharmacology
- QC & Drug delivery

- Development of methodology for efficacy evaluation based on behavioral pharmacology with animal models
- Drug Discovery from Chinese herbs on antitumor and neuro-psychotic diseases
Drug discovery strategy

Herb (based on traditional use)

Extraction

Crude extract

In vitro Screening

Active

Not active

Continue in-depth studies
Extraction Process

Extraction and enrichment unit

Equipment of ethanol precipitation

20L Spin steaming
**Compound Isolation and Quality Control**

**Preparative HPLC**

**Chromatographic column**

**HPLC**

**RP C-18**

Standardized extracts were prepared for further pharmacological evaluation
**Cell lines used in vitro**

- PC12
- Primary cell
- MCF-7 (human breast cancer);
- U373 (human astrocytoma);
- U251 and U87 (human glioblastoma cell lines);
- Reh and RS4;11 (acute lymphoblastic leukemia cell lines).
- Caco-2 (caucasian colon adenocarcinoma)
- SH-sy5y (human neuroblastoma)
- A549vp (lung cancer cell line)
- Jurkat (Organism: Homo sapiens)
- Hela, HepG2, Bel-7402, SKOV-3 (Female cancer cell line)
- HEC-1B, NCI-H460 and BGC-823
- HBMEC (human Brain microvascular endothelial cell)
- Astrocytes (control non-cancer cells)
- PBMC (Normal cell)
Efficacy evaluation for Anti-tumor and neuro-protection with animal models

- **Different cancer animal model**
  leukemia, Ovarian, Cervical cancer, etc.

- **Learning and Memory**
  Morris water maze;
  Step-down passive avoidance;
  Step-through passive avoidance;
  Shuttle-box active avoidance
  Reward Directed Operant Conditioning

- **Depression**
  Open-field;
  Tail-suspension;
  Forced swimming;

- **Cerebral ischemic injury**
Drug metabolism and Pharmacokinetics (DMPK)

- The major instruments for DMPK studies.

- Waters UPLC

- Thermo LTQ-Orbitrap LC-MS

- AB 5500-QTrap LC-MS/MS
Drug metabolism and Pharmacokinetics (DMPK)

◆ Study on ADME of active components from medicinal plants
  - *in vivo* animal model (rat, mice, dog)
  - *in situ* small intestinal perfusion model
  - Brain microdialysis model
  - *in vitro* cell monolayer model
    - Caco-2 cells for intestinal permeability
    - BMEC/AC for BBB permeability

◆ Study on Herb-drug interactions (HDI)
  - Metabolism (CYP450 enzymes) based HDI
  - Transport (P-gP) based HDI
Drug Delivery to tumor and CNS

◆ Improve Bioavailability
   Nanocrystals
   Modulating the GIT metabolism
   Inhalation delivery

◆ Target to tumor and CNS
   Polymeric micelles
   Solid lipid nanoparticles
Peptide Array and Anti-body Chip Platform

Peptide Sewing Machine

- A New Way for Biological Research and Drug Development
- Rapid, High Throughput, Time and Labor-Saving
- Drug discovery from medicinal plants, Bio-maker for Cancer diagnosis,
- Signal pathway
Peptide Array

Membrane support for peptide synthesis → Spatially addressed peptide synthesis

B-cell epitope mapping directly on membrane

Antibody
Efficacy evaluation based on behavioral pharmacology: Apparatus developed by our team

**Morris water maze test:**
Spatial reference and working memory

**Step down test:**
Fear-motivated avoidance learning and memory

**Step Through test:**
Fear-motivated avoidance
Apparatus developed by our team

Shuttle-Box: Fear-motivated avoidance /escape conditioning

Reward-directed Operant Conditioning: Reward-motivated learning and memory

Novel Object Recognition: Based on animal spontaneous behavior activities, preference for novel over familiar objects in nature
Apparatus developed by our team

Open-field test:
Locomotor activity, novelty-induced hypophagia

Tail-suspension test:
Immobility time
No. of movement
Energy value (Power of movement)

Forced swimming test:
Climbing time
Immobility time
Swimming time
No. of Climbing
Energy value (Power of movement)
Apparatus developed by our team

Gait Analysis system (Cerebral ischemic injury, Parkinson's, Stroke, Spinal cord injury, etc.)
Evaluation Index: Stride Time, Stance Length, Stance Time, Swing Time, Brake Time, Propulsion Time etc)
• About 20 extracts, 80 compounds from Chinese herbs have been screened on anti-cancer in vitro and in vivo
• More than 30 extracts, 40 compounds from Chinese herbs have been screened on neuro-protection in vitro and in vivo

Special attention to: Ginseng, Radix Polygala (Yuan-zhi), Rhizoma Gastrodiae (Tian-ma), Lotus Leaf (He-ye), Radix Bupleuri (Chai-hu), Duchesnea indica (Se Mei), etc.
Duchesnea indica is a traditional Chinese herbal. Phenolic components (DPF) are regarded as the main active components. Exhibiting anti-ovarian cancer and antioxidant activities.
The anticancer effect of *DPF* on *human ovarian cancer cell in vivo*
The effect of DPF on immunological function in transplanted U14 mice

- DPF could inhibit proliferation of human cancer cells *in vitro*.
- DPF significantly reduced the tumor weight, increasing survival days.
- DPF could inhibited tumor cells, and increase cell-mediated immune response.
### Anti-cancer activity of PPD (the metabolite of Rb1, from Chinese Ginseng)

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>(IC_{50} \text{ (μM)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reh</td>
<td>24.45</td>
</tr>
<tr>
<td>RS4;11</td>
<td>27.19</td>
</tr>
<tr>
<td>HL-60</td>
<td>64.37</td>
</tr>
<tr>
<td>Jurkat</td>
<td>47.31</td>
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<tr>
<td>Molt-4</td>
<td>37.13</td>
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<tr>
<td>Astrocyte</td>
<td>35.71</td>
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<tr>
<td>Raji</td>
<td>57.17</td>
</tr>
<tr>
<td>MCF-7</td>
<td>55.49</td>
</tr>
<tr>
<td>U251</td>
<td>52.65</td>
</tr>
<tr>
<td>U87</td>
<td>37.67</td>
</tr>
<tr>
<td>U373</td>
<td>39.75</td>
</tr>
<tr>
<td>CHO</td>
<td>43.22</td>
</tr>
<tr>
<td>L02</td>
<td>53.24</td>
</tr>
<tr>
<td>PBMC</td>
<td>115.76</td>
</tr>
</tbody>
</table>

- Growth inhibitory activity of PPD on the 13 cell lines and PBMC at 48h

The chemical structure of PPD
Effects of PPD on cell cycle distribution. PPD inhibits RS4;11 cell and Reh cell cycle progression in a time dependent manner.
The effect of PPD on the survival time in DBA/2 mice bearing L1210 leukemia (n=8). * P<0.05; **, P<0.01 (significant or highly significant difference between control group and PPD-treated group)
Well known Chinese herbal medicine: Multi-functions (on neuro-psychotic system and anti-cancer). Used as food and drug. Main active components: Ginsenosides and their metabolites sapogenins. More than 40 ginsenosides have been isolated and identified.

Radix Ginseng (人参, Chinese Ginseng)

Ginsenosides’ parent structure: Tetracyclic Triterpene
Molecular structure of PPD (protopanaxadiol)-type ginsenosides (Rb1, Rb2)

Molecular structure of PPT (protopanaxatriol)-type ginsenosides (Rg1, Rh1)

<table>
<thead>
<tr>
<th>Ginsenoside</th>
<th>$R_1$ (C-3)</th>
<th>$R_2$ (C-20)</th>
</tr>
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<tbody>
<tr>
<td>Rb₁</td>
<td>Glu (1→2) Glu-</td>
<td>Glu (1→6) Glu-</td>
</tr>
<tr>
<td>Rb₂</td>
<td>Glu (1→2) Glu-</td>
<td>Arap (1→6) Glu-</td>
</tr>
<tr>
<td>Rc</td>
<td>Glu (1→2) Glu-</td>
<td>Arap (1→6) Glu-</td>
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<tr>
<td>Rd</td>
<td>Glu (1→2) Glu-</td>
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<td>F₂</td>
<td>Glu-</td>
<td>Glu-</td>
</tr>
<tr>
<td>Compound Mc-1</td>
<td>Glu-</td>
<td>Arap (1→6) Glu-</td>
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<tr>
<td>Compound Mc</td>
<td>H-</td>
<td>Arap (1→6) Glu-</td>
</tr>
<tr>
<td>Compound O</td>
<td>Glu-</td>
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<tr>
<td>Compound Y</td>
<td>H-</td>
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<tr>
<td>Compound K</td>
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<td>Glu-</td>
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<tr>
<td>Gypenoside XVII</td>
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<td>Glu (1→6) Glu-</td>
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<tr>
<td>Gypenoside LXXV</td>
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<tr>
<td>Rg₃</td>
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<tr>
<td>Rh₂</td>
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<td>H-</td>
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<tr>
<td>Aglycon-PPD</td>
<td>H-</td>
<td>H-</td>
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</table>

<table>
<thead>
<tr>
<th>Ginsenoside</th>
<th>$R_1$ (C-6)</th>
<th>$R_2$ (C-20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Re</td>
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<td>Glu-</td>
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</tr>
<tr>
<td>Rf</td>
<td>Glu (1→2) Glu-</td>
<td>H-</td>
</tr>
<tr>
<td>Rg₁</td>
<td>Glu-</td>
<td>Glu-</td>
</tr>
<tr>
<td>Rg₂</td>
<td>Rha (1→2) Glu-</td>
<td>H-</td>
</tr>
<tr>
<td>Rh₁</td>
<td>Glu-</td>
<td>H-</td>
</tr>
<tr>
<td>F₁</td>
<td>H-</td>
<td>Glu-</td>
</tr>
<tr>
<td>Aglycon-PPT</td>
<td>H-</td>
<td>H-</td>
</tr>
</tbody>
</table>
The researches on the pharmacological effects of ginseng in our lab

Total Ginsenosides (GTS): Memory enhancement, Anti-depression.

Individual Ginsenosides (Rg1, Rb1): Memory enhancement, Anti-depression.

Ginseng Sapogenins (PPT, PPD, complete removal of side chain glycosyl): Memory enhancement, Anti-cancer

Ginsenosides and Sapogenins Mixture (GSSM): Protection of Myelosuppression
GTS’s Anti-depression and Memory enhancement

Effects of GTS and Rg1 on latency for feeding in CMS rats.

***p<0.001 compared with control; #p<0.05, ##p<0.01 compared with CMS

A 35-day treatment of either fluoxetine or GTS (50,100mg/kg), and Rg1(50-100 mg/kg) significantly decreased the latency of feeding in novel cages.
GTS’s Anti-depression and Memory enhancement

Effects of GTS and Rg1 on the sucrose-preference test *p<0.05, **p<0.01 compared with control; #p<0.05, compared with CMS.
GTS’s Anti-depression and Memory enhancement

Effects of on BDNF expression in the hippocampus of CMS rats

**P<0.01 with respect to CON group, #P<0.05, ##P<0.01 with respect to CMS group.
The effects of Rg1 on the cognitive function in the shuttle box in CMS animal model. *P<0.05; vs Control group, #P<0.05, VS CMS group.

Tips: Fluoxetine did not show effects to improve learning and memory in shuttle box test, while Rg1 could improve memory deficiency induced by CMS.
Rg1’s Memory enhancement

The effect of Rg1 on Corticosterone and ACTH. ##P<0.01; VS CMS group

- Rg1 decrease the levels of ACTH.
- Rg1 decrease the expression of AChE in the hippocampus; 10mg FLU did not influence the expression of AChE.

The effect of Rg1 on AChE expression in the hippocampus. ##P<0.01 VS CMS group,
Rg1 improves the impaired memory performance induced by scopolamine with Step-down test, * p<0.05, **p<0.01, VS Scopolamine group.
Effects of Rg1 on memory deficit mice induced by scopolamine (MWM test. * p<0.05, **p<0.01, VS Scopolamine group)

Rg1 reduced the escape latency, more effective than Rb1 in the escape acquisition (navigation).
Effects of Rg1 and Rb1 on Ach and AChE activity in hippocampus in mice.
* p < 0.05; ** p < 0.01, vs scopolamine; # p < 0.05, ## p < 0.01, vs Rg1 group.

Rg1 showed stronger inhibition on acetylcholine esterase than Rb1, while Rb1 improved hippocampal dopamine and serotonin level more than that of Rg1.
Achievements - 7 Chinese patents
More than 30 Papers have been published in international academic journals

- Yanyan Yang, Shu Ping Xu, Qiuxia Xu, Xinmin Liu*, Yue Gao, Andre Steinmetz, Ning Wang, Bo Song Qu. Protective Effect of Dammarane Sapogenins Against Chemotherapy-Induced Myelosuppression in Mice. EXP BIOL MED (2011)


Achievements—about 15 research programmes, including 3 EU-FP7 programmes have been funded

• GP-TCM (Good Practice in Traditional Chinese Medicine Research in the Post-genomic Era), Funding No. 223154; Budget: 1 Million Euro.

• TCMCANCER (Traditional Chinese Medicine in the Post-genomic era: identifying lead therapeutic compounds against cancer), Funding NO. 230232. Budget: 500 thousands Euro.

• PlantLIBRA (PLANT food supplements: Levels of Intake, Benefit and Risk Assessment), Funding No. 2451199; Budget: 10 Million Euro.

Our group has been invited to involve as Coordinator in China.
GP-TCM
More than 200 scientists, clinicians and TCM practitioners from 23 countries,
15 EU Member States and Partners from China
TCMCANCER（Traditional Chinese Medicine in the Post-genomic era: identifying lead therapeutic compounds against cancer）

International Research Staff Exchange Scheme (2008.01-2011.12)

Coordinator: Dr. Andre steinmetz, Centre de Recherche Public de la Santé (CRP-Santé), Luxembourg
Prof. Karl-Henning, University of Bergen, Bergen, Norway
Prof. Zhang Weidong, Modern Research Center for Traditional Chinese Medicine (MRCTCM), Shanghai
Prof. Liu Xinmin, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, China
Senior Scientists between Luxembourg, Norway and China have been exchanged

Left: Dr. Andre visit IMPLAD. (Jan., 2009, Oct., 2011)

Right: Drs. Andre And Ning visit Sino-Bioway Group Co., the top one of biomedicine pharmaceutical company in China. Jan., 2009
PlantLIBRA (PLANT food supplements: Levels of Intake, Benefit and Risk Assessment).
Prof. Liu Xinmin from IMPLAD, gave brief introduction to Mr Yu Wenmin, the Deputy General-Director of State Administration of Traditional Chinese Medicine (SATCM), China, and Ms. Wang Xiaoping, Director of Department of international cooperation of SATCM, China in Beijing on Nov.1, 2011. Both of Chinese Ambassador in Luxembourg and Luxembourg Ambassador in Beijing present this meeting.
Prof. Liu Xinmin gave brief introduction about TCM-Cancer to Philippe Vialatte from EU Delegation, Science and Technology Section, along with Deputy Director Xing Jijun, Exchange Center of Science and Technology, MOST (16 May, 2012, Beijing)
Foreigner Partner from different countries

Left: Presentation in Karachi University, Pakistan (2004).
Right: Prof. Asana Dar from Karachi University visit our labs (2009).
International cooperation with UBC, SFU in Canada have been formed

Left: visit Prof. Antony’s labs in UBC, Vancouver, Canada (2007)
Right: Visit Prof. Russell’s labs in SFU, Vancouver, Canada (2010)
Acknowledgments

All members from our research tram
Ministry of Science & Technology
State Administration of TCM
Astronaut Center China
Sino-Bioway Company

Thanks!