



## MEDLINE-based assessment of animal studies on Chinese herbal medicine

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### ABSTRACT

**Ethno-pharmacological relevance:** The scientific proof and clinical validation of Chinese herbal medicine (CHM) require a rigorous approach that includes chemical standardization, biological assays, animal studies and clinical trials.

**Aim of the study:** To assess the experimental design of animal studies on the activity of CHM by selection and scrutinizing of a series of papers in some major disease areas.

**Materials and methods:** We have analyzed the English publications reported in MEDLINE (ISI web of knowledge).

**Results:** Our data showed that (i) research of CHM during the last 10 years had been highly intensified and become more accessible worldwide through increased publications in English, although still most authors had Chinese names; (ii) English journals publishing animal research of CHM were comparable to those publishing animal studies of non-Chinese phytotherapy in terms of impact factor; and (iii) published data on authentication and quality control of CHM, as well as research design of animal studies were far from sufficient to meet the criteria needed to support their reproducibility and reliability.

**Conclusions and perspectives:** The recent decade witnessed an increase in CHM research activities and CHM English publications. Based on common problems identified in publications on CHM animal studies, we have proposed a checklist that could help in preliminary selection of publications lacking the most common problems and thus would be useful for a quick search of reproducible CHM regimens that are likely to be effective in a given context. The second application of this checklist is to help avoid the most common problems when designing experiments.

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## 1. Introduction

The classical Chinese pharmacopoeia describes a large number of herbal formulations that are used for the treatment of a wide variety of diseases. However, the adoption of Chinese herbal medicine (CHM) in Western countries is impeded because of several issues. The very limited knowledge on chemical composition, pharmacokinetics and pharmacodynamics is a well recognized problem to be addressed (Zhang, 2000). Another problem is the lack of quality control and scientific and clinical proof of their efficacy and effectiveness (Yuan and Lin, 2000). An editorial in *JAMA* emphasizes that

the fundamental issue is not traditional medicine versus alternative medicine, but medical practice supported by clinical and scientific evidence or not (Fontanarosa and Lundberg, 1998). In the case of CHM, since herbs are often administered as a decoction, i.e., as a combination of multiple herbs in which the individual constituents may be difficult to determine, their 'neither-proven-nor-disproven' medicinal status is likely to prove durable (Chiu et al., 2009). The scientific proof and clinical validation of CHM require a rigorous approach that includes chemical standardization, biological assays, animal studies and clinical trials.

Having in mind these considerations, we have analyzed the English publications in the MEDLINE database (ISI web of knowledge), aiming to assess the state of the art, identify common problems and propose possible solutions, especially focusing on experimental design of CHM studies involving animal models of some major diseases. The authors of this study did not have the intention to read and review all papers in the chosen fields,

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**Table 1**  
CHM in context.

(a)	1950–1999		2000–2011		Total
	Articles	%	Articles	%	
CHM	10732	36.60	18587	63.40	29319
Homeopathy	1978	54.33	1663	45.67	3641
Aspirin	22339	64.26	12425	35.74	34764

  

(b)	Published in English		Published in Chinese		Published in other language	
	Articles	%	Articles	%	Articles	%
1950–1999	4040	28.32	5979	42.58	713	70.52
2000–2011	10225	71.68	8064	57.42	298	29.48
Total	14265	100.00	14043	100.00	1011	100.00

but to see in a limited set of papers from peer-reviewed international journals that are representative for the field what are common shortcomings that seriously affect the reliability of animal experiments

## 2. General features of CHM studies

To get the most relevant characteristics of CHM studies, we searched MEDLINE (ISI web of knowledge) since 1950 by using medical subject headings (MeSH) and the string (Drugs, Chinese herbal or Medicine, Chinese traditional). The results were analyzed by using tools provided by MEDLINE, which allowed us to get statistics on language, source, scientific area and journals more frequently used for publication. We further analyzed the impact factors (IF, 2008 record) of these journals and the names of the authors (Chinese vs non-Chinese). The main features of these CHM studies were as follows.

### 2.1. Number of references and language of publication in 1950–1999 vs 2000–2011

The number of references on CHM in the 12 years from 2000 to 2011 was around 30% higher than that found for the period of 20 years from 1950 to 1999 (Table 1a). In order to ascertain whether this important intensification in the published research is specific for CHM, we analyzed also the number of references in homeopathy (a sample of other alternative medicine) and in aspirin (a sample of a conventional drug) during both periods. There were more references on CHM, but not on aspirin or homeopathy in 2000–2011 than in 1950–1999. These data indicate that research on CHM has specifically increased in the recent years.

Although it is well known that most published references in the Medicine field are written in English, the number of references on CHM in Chinese is very similar to that of English references on CHM (Table 1b). But again the period 2000–2011 makes a difference, since there are more references in English than in Chinese (the opposite than in 1959–1999, Table 1b). Interestingly, the vast majority (>95%) of the authors of the English references-published either in China or abroad – had Chinese names (results not shown).

As a conclusion, we state that scientists of non-Chinese origin remain a minority among authorship of CHM studies and that this is not due to any voluntary isolation by the Chinese since there were more articles published in English than in Chinese in the period of 2000–2011, reflecting an increasing effort by the Chinese to make the results and knowledge more accessible by the international community. Meanwhile, we must state that the above conclusions do not mean to under-estimate the contributions to CHM studies by institutions outside China: a recently published study on hot topics in CHM in the period of 1998–2007 found 4790 and 2609

references from (corresponding) authors affiliated to institutions in and outside China, respectively (Li et al., 2009).

### 2.2. Most active areas in CHM research

According to MEDLINE, the most active medical areas of CHM research in 2000–2011 were the following (% of total references indicated in brackets): integrative and complementary medicine (13.15%), immunology (11.05%), cardiovascular system and cardiology (9.14%), gastroenterology and hepatology (8.64%), neurosciences and neurology (7.77%), hematology (7.73%), and oncology (7.37%). This was little different from that in the period of 1950–1999. The study on hot topics in CHM in the period 1998–2007 (Li et al., 2009), which identified the MeSH terms more frequently used in the citations on CHM, confirms roughly our data. The authors found the following: (i) active areas of research: anti-inflammatory activity, antioxidant activities, anticancer activity of phytochemical antineoplastic agents, and neuroprotective effects of Chinese herbal drugs and (ii) common diseases treated with Chinese herbal drugs: hepatocirrhosis, diabetes, angina, chronic hepatitis B, asthma and prostate cancer.

### 2.3. CHM studies in animals (with particular reference to cancer)

Around 25% of CHM studies were done in animals (8952 references out of 29319 total references on CHM), these data being similar to those found for animal studies in the conventional medicine field of antineoplastic agents (54276 references in animals out of 236768 total references on antineoplastic agents). Most references of animal studies of CHM were published in 2000–2011 (5813 vs 3139 in 1950–1999), these data showing also a similar distribution to that found in the field of non-Chinese plant medicine studies in animals (6596 references in 2000–2011 vs 604 references in 1950–1999). Taking together, these data indicate that the animal studies in CHM and conventional medicine share the same features in the analyzed parameters.

To perform IF analysis, we took cancer as an example area of focus to illustrate the whole field of animal studies of CHM. We chose cancer because CHM are popularly used by cancer patients, particularly those of East Asian ethnicity and/or those in the palliative phases of advanced disease. Various strategies have evolved for the treatment of tumors with CHM (Beinfeld and Korngold, 2003): (i) reducing pain, inflammation, swelling and tumor mass; (ii) improving host resistance through preserving immune competence and enhancing the function of internal organs to counter chemotherapy-induced immune or myelosuppression; (iii) potentiating the effects of conventional radiation and chemotherapies; and (iv) preventing, controlling and treating the adverse effects of conventional treatment, including fatigue, weakness, gastrointestinal distress, loss of appetite, nausea, emesis and leucopenia. On the other hand, cancer is one of the most active areas in CHM, as indicated above.

To analyze the IF of CHM publications on cancer, we located in MEDLINE the journals more frequently used in 2000–2009 until 0.5% of frequency (for clarity Table 2 summarizes the results found until 1%). These journals had 223 of the 388 articles published between 2000 and 2009. As expected, around 50% of the articles (51.57%) were published in Chinese journals (4 out of the 5 most used journals) and they had no IF. The IF in the journals rated ranged from 4.930 to 1.524 and 2 out of 16 journals had IF > 4, being these values similar to that found for the most used journals publishing phytotherapy studies in animals in the cancer field (results are not shown). Again these data indicate that the animal studies in CHM and non-Chinese phytotherapy share the same features (with the exception of articles published in Chinese).

**Table 2**  
Animal studies of CHM in cancer: journals more frequently used in 2000–2009.

Source	Articles	%	IF (2008)
Zhongguo Zhong Yao Za Zhi	40	10.36	–
Zhong Xi Yi Jie He Xue Bao	28	7.25	–
Zhong Yao Cai	24	6.22	–
World J Gastroenterol	23	5.96	2.031
Zhongguo Zhong Xi Yi Jie He Za Zhi	23	5.96	–
J Nat Prod	16	4.15	2.843
Am J Chin Med	10	2.59	1.058
Biol Pharm Bull	9	2.33	1.765
Cancer Lett	9	2.33	3.504
J Ethnopharmacol	7	1.81	2.260
J Traditional Chin Med	7	1.81	–
Life Sci	7	1.81	2.583
Oncol Rep	7	1.81	1.524
Carcinogenesis	5	1.30	4.930
Acta Pharmacol Sin	4	1.04	1.676
Int J Cancer	4	1.04	4.734

### 3. Animal studies and the need of scientific proof of the efficacy of CHM

#### 3.1. Introductory remarks

The excellent review on scientific proof and clinical validation of CHM formulations (Yuan and Lin, 2000) states in its summary that it requires a rigorous approach that includes chemical standardization, biological assays, animal models, and clinical trials. The review specifically indicates that the effect of the CHM formulation should be tested on animal models for a particular disease or sub-health condition (Yuan and Lin, 2000). Accordingly, we have focused this section analyzing the present state of animal studies in relation to the need of scientific proof of the efficacy of CHM.

Another relevant issue is the type of formulations included in our study. CHMs act multi-systemically and are often administered as decoctions, i.e., as a combination of multiple herbs in which the individual constituents may be difficult to determine. Therefore, in terms of addressing this challenge, researchers on the whole try to research into efficacy of either a single or small combination of a few active ingredients, which cannot fully recapitulate the effect of the complex mixture. This is why we have focused this part of our study on CHM preparations consisting of herbal mixtures of 3 or more herbs prepared following the principles of traditional Chinese medicine (TCM).

Regarding the animal models analyzed, most animal studies of CHM published in English deal with animal models which reproduce the diseases considered in conventional medicine and not with animal models of the human disease patterns considered in TCM (perhaps because the intrinsic problem of replicating in animal models the human disease patterns remains unsolved). Therefore, our analysis of animal studies in CHM has been focused on animal models of diseases described in conventional medicine.

#### 3.2. Efficacy of CHM in animal models of disease

We searched the animal studies of CHM in English in six selected diseases which were chosen as samples of the whole field of animal studies of CHM. Taking into account that the period 2000–2011 (July) comprised more than 50% of the published studies on CHM in the database (Table 1a), we analyzed publications between 2000 and 2011 (July), which included: cancer, 57 studies; diabetes mellitus, 43 studies; Alzheimer studies, 12; psoriasis, 1 study; hypertension, 14 studies; fibrotic diseases, 18 studies. Our purpose was to identify CHM formulations from which there were enough publications to support their efficacy in a given disease. However, when the studies were individually analyzed with that aim, we could not find any CHM formulation that was used in at least 2

different studies of the same animal model of disease. Worth mentioning, our study has an important limitation: it only includes the literature collected in MEDLINE so that we did not analyze the much more abundant Chinese publications. Therefore, we cannot rule out the possibility that the analysis of Chinese journals would render a significant number of studies in which the same CHM formulation is at least used twice in the same animal model of disease.

The absence of CHM formulations which were used in at least two different studies of the same animal model of disease may be reflecting the fact that herbal formulae (Fufang, herbal mixture for decoction) vary based on authors' experience and therefore we should not expect to find many studies using the same Fufang in a given animal model of disease. However, herbal formulae also can be herbal classical products, and in such cases, more pharmacological studies in animal models could be reported for some times. Taking into account these considerations we chose one classical product (based on the publications in MEDLINE) for further analysis. Xiao-qing-long-tang is a CHM also known as So-Cheong-Ryong-Tang in Korea or sho-seiryu-to in Japan. It is a mixture of 2 parts Pinellia tuber and one part each of Glycyrrhiza root, Cinnamon bark, Schizandra fruit, Asiasarum root, Peony root, Ephedra, and Ginger. We found 7 references using the classical product in animal models of allergic pulmonary inflammation. This is a good example which illustrates how the use of classical herbal products may substantially increase the number of animal studies on efficacy in a given model of disease. Furthermore, classical herbal products may significantly increase the reproducibility of these studies, which is another potential problem (see Section 3.3).

In summary, although we have analyzed a sample consisting of six diseases, it is very likely that our results can be extrapolated to other diseases. Therefore, more studies are needed to confirm the efficacy of selected CHM formulations in animal models of disease. Our recommendation is to give priority to classical herbal products or to those CHM which either have shown their efficacy in at least one study or have been successfully tested in controlled clinical trials. These studies should be designed taking into account minimum quality criteria as described in the checklist to be detailed in Table 3.

#### 3.3. Most relevant problems in animal studies of CHM: the case of cancer

We have stated above the need of animal studies to give evidence-based proof of the efficacy of the CHM formulations. This need led us to identify the more usual problems of the animal studies of CHM in order to give recommendations for further studies. We chose cancer as the focused disease area to assess papers on animal studies of CHM published in English between 2000 and 2009. Most of the papers surveyed did not use randomization (72%) or blinding (98%) to reduce bias in animal selection and outcome assessment. Only 50% of studies used group sizes >5, 40–50% had appropriate statistical analyses and included a relevant control. There was general evidence of efficacy of test CHMs in most papers; the level of efficacy did vary but the CHMs reported were generally shown to be highly efficacious. However, variation in tumor size within experiments was not shown in 41% of studies. In addition there were very few cases where biomarkers linked to mechanisms of action were used. The clinical relevance of these studies was difficult to dissect out and be compared with Western medicines since (i) the majority of studies were performed with treatment starting early in the study (39%), whereas only 19% of studies allowed tumors to become established before clinically relevant treatment of established lesions was initiated. Furthermore, in a large portion of studies it was unclear when treatment was started (42%); (ii) only 20% studies compared test CHM effects with conventional medicine standard of care agents; and (iii) there

**Table 3**

Analysis of methodological aspects of a series of scientific articles on CHM in animal models published in English in the last 10 years (n= 77) in some major areas of diseases.

Animal study quality	Total	
Experimental design	n = 77	%
Administration gavage/IV	42	66.67
Conventional medicine	12	19.05
Randomization	41	65.08
Blinding	1	1.59
Euthanasia process described	17	26.98
Ethical approval granted	30	47.62
Botanical source <sup>a</sup>	n = 48	%
Authentication	16	33.33
Voucher specimen in official herbarium	2	4.17
Physical parameters <sup>b</sup>	1	2.08
Profile the plant material, and the extract applied <sup>c</sup>	7	14.58
Analysis for microbiological contamination	0	0.00
Pesticide residue analysis	1	2.08
Heavy metal analysis	1	2.08
Processing	n = 77	%
Harvesting, post harvest treatment and drying method <sup>d</sup>		
Cleaning	2	2.60
Cutting/grinding fresh/dry material <sup>e</sup>	1	1.30
Further treatment <sup>f</sup>	1	1.30
Storage	1	1.30
Extraction	n = 77	%
Procedure, solvent or preparation of traditionally used formulation	35	45.45
Chemical characterization sample applied	10	12.99

<sup>a</sup> 29 out of the 77 articles analyzed use commercially available proprietary products.

<sup>b</sup> Physical tests include organoleptic evaluation (sensory characters such as taste, appearance, odor, feel of the drug, etc.), viscosity, moisture content, pH, disintegration time, friability, hardness, flowability, sedimentation, and ash value (Zhang, 2000).

<sup>c</sup> Chromatographic/Spectroscopic targeted analysis in case of known actives, or otherwise non-targeted metabolomics.

<sup>d</sup> Heat, sun...

<sup>e</sup> Unless cut in fresh or dry form, crude traditional Chinese drugs should be moistened to soften the drug prior to cutting.

<sup>f</sup> E.g. roasting, etc.; traditional additives (honey, sugar, acid, ash, ...).

was little report of toxicity (where defined, it was associated to weight loss). In addition, only 14% studies stated adherence to animal welfare guidelines and ethical committee compliance.

These issues suggest that the whole field of animal studies of CHM needs to be improved to ensure the subsequent utility and validity of the knowledge base that is used to inform future research. Worth mentioning, most problems identified here are not specific to CHM, as shown by previous surveys of publications describing animal research and assessing specific aspects of experimental design, statistical analysis and reporting (see for instance Kilkenny et al., 2009). But, in view of the need of scientific proof of efficacy, we strongly recommend adherence to the recently published ARRIVE (animal research: reporting in vivo experiments) guidelines (Kilkenny et al., 2010) for future animal studies of CHM. It is also interesting to refer to the use of specific guidelines for particular diseases (e.g., Workman et al., 2010 for animal studies of cancer).

#### 3.4. Reproducibility and comparability of the studies

Crude herbal drugs and particularly Chinese formulae are natural products and their chemical composition varies depending on several factors, such as the geographic source of the plant material, the climate in which the plant was grown, and time of harvest. Commercially available herbal medicinal products also vary in their composition, both quantitatively and qualitatively, from batch to batch. Even when herbal products are standardized for content

of known active or marker compounds there is variation in the concentrations of other constituents that can result in differences in pharmacological activity. Also, preparation methods vary significantly (including the means of extraction, if any; whether the material is heated for a prolonged period, etc.) which could also contribute to variability in chemical composition and hence pharmacological activity. In particular, it is a very frequent practice to use herbs of unknown origin, which are subjected to a variety of extraction procedures to render extracts whose composition is unknown.

Taking into account the IUPAC protocols (Mosihuzzaman and Choudhary, 2008), and the most common problems found in the sample publications on CHM studies in animals, we have written a short list of minimal quality criteria. These criteria should be taken into account to assure a consistent and acceptable quality of herbal entities, which will be used in animal models of disease and are as summarized in the following checklist:

A checklist to assure a consistent and acceptable quality of herbal research materials

1. General records: harvesting time, parts of the plant collected, regional status, details of any voucher specimen;
2. Authentication of the herbal raw material: taxonomic, macroscopic and microscopic, DNA barcoding;
3. Standardization, i.e., to provide quantitative and semiquantitative information about the main active constituents or marker compounds present in the crude drug or herbal products: (i) chromatographic and sophisticated modern techniques: spectroscopic evaluation UV–vis spectrophotometry, TLC, HPTLC, HPLC–mass spectrometry, NMR, etc.; (ii) physical parameters: organoleptic evaluation, viscosity, moisture content, pH, disintegration time, friability, hardness, flowability, sedimentation and ash value.
4. Microbiological contamination;
5. Pesticide residue;
6. Heavy metal analysis;
7. Extraction: solvent used and ratio, time, temperature and yield.

Tested samples should be kept as voucher samples, voucher numbers should be given and preferably the HPLC proofing should be provided.

#### 3.5. A checklist for the preliminary evaluation of the most frequent problems found in publications on animal studies of CHM

The two previous sections (Sections 3.3 and 3.4) gave us the rationale to elaborate a checklist for the preliminary evaluation of the common problems in publications on animal studies of CHM. This checklist includes details of the botanical origin, processing and extraction procedures as well as of the experimental design (Table 3). This checklist was used in a sample of the publications on CHM studies in animals (77 articles published between 2000 and 2011 in four disease areas: cancer, 43; diabetes mellitus, 21; Alzheimer, 3 and fibrotic diseases, 10). Our results indicate that in many cases, it was non-standardized research materials that poorly supported reproducibility and comparability of research on the same CHM. They also are frequent problems regarding the experimental design, particularly in terms of blinding and comparison of test CHM effects with conventional medicine standard of care agents. The mentioned problems could significantly damp the scientific value and impact of these studies and therefore need to be addressed in further animal studies of CHM.

Although we have used it in animal studies of CHM to get a preliminary evaluation of the degree in which those problems are found, this checklist also has other potential applications. For instance, it could help to do a preliminary selection of publications

lacking the most common problems found in the Methods section. This would be useful for a quick search of reproducible CHM treatment that is likely to be effective in a given disease.

A second application of this checklist is to help design experiments devoid of the common problems enlisted in it. In this context, regarding experimental design, the following recommendations complement with the checklist. It is important: (i) *to use software to plan experiments*, in order to avoid that the number of replicates is not sufficient to draw any statistically significant conclusions; (ii) *to review the literature to identify state-of-the-art experiments* which will help to improve the design of the animal study; (iii) *to take into account that the chosen method of administration is relevant for providing activity*; (iv) *to be sure to use proper negative and positive controls* (including conventional medicines); (v) *to ensure the composition of testing extracts is similar to that of traditionally used preparation*; (vi) *to follow the national and international rules of animal experimentation*; and (vii) *to test clinically relevant doses if compared to traditional use and to test more than a single dose*. Finally, two key references that describe how certain standards are set and discuss the misemployment of the concentration–effect paradigm (Cos et al., 2006; Gertsch, 2009) are recommended for further reading.

#### 4. Conclusion

In this work, we have shown that research on CHM during the last 10 years has been highly intensified and has been done more worldwide accessible through increased publications in English. Despite this favorable framework, the data about authentication, quality control and efficacy of CHMs in the animal studies are far from sufficient to meet the criteria needed to support their reproducibility and reliability. In a checklist we have gathered the most important points concerning the experimental design from the point of view of the materials studied. We have not addressed the point of choosing the most appropriate methods and the strategy to step by step build up the evidence for safety and activity. The proposed checklist should be helpful in planning experiments and for reviewers to assess papers.

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