In the dark: an experimental study in distinguishing an authentic herbal substance from a sham herbal substance

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CERTIFICATE OF AUTHORSHIP/ORIGINALITY

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Abstract

Background: The randomised controlled trial (RCT) is currently the "gold standard" for assessing the efficacy of a therapeutic intervention. One important aspect of an RCT is the blinding of various parties involved in the trial. Blinding is similar to randomization, in that its goal is to distribute participant expectancies evenly across the two or more groups. The assessment of blinding has been fraught with complications and associated with methodological difficulties. A review of the literature found very poor and limited reporting of the success of blinding in not only pharmaceutical studies, but also many Chinese medicine RCTs. In addition the special qualities of Chinese herbal medicine such as taste, odour, color and texture make it very difficult to blind trial participants. Because of these reasons it was decided to undertake a series of experiments to determine whether a credible sham substance could be developed for use in a clinical trial for Chinese herbal medicine.

Methods: Three separate studies were undertaken in sequential order between 2009 and 2011, with the second and third study attempting to improve on each previous study. In all three studies the primary aim was to evaluate whether a greater proportion of participants could correctly identify an authentic herbal substance when comparing to a sham substance using visual, odour and taste assessments. The first study was a pilot involving eleven participants. The aim of this study was to pilot the data collection process and determine whether the initial materials were suitable for the study.

The second study involved 81 participants. This study improved on the initial pilot

study in several ways. There was an increase in participants and an opaque eye pad was used during the tasting phase to effectively blind the participant. In addition a closer colour matching of the three sham substances to the herbal substance was achieved.

The third and final study involved only two substances, a refined sham substance and the herbal substance. Participants were randomly given only one of the two substances and asked whether they believed the substance to be a' herbal' or 'placebo' substance or 'didn't know' using a questionnaire. In this study there was no comparison with more than one substance as occurred in study one and two. This study improved on the first and second study by improving the taste and colour of the sham substance in order to be comparable to the herbal substance.

Results: For study one a one proportion test found that for both odour ($p=0.484$) and visual appearance ($p=0.077$) the number of participants correctly selecting capsule B as the herbal substance was not significantly different from what may have been selected by chance. This was not the case for taste where significantly more participants correctly selected capsule B as the herbal substance ($p=0.004$) compared to the other three substances.

For study two, in all three assessments (visual, odour and taste) a statistically greater percentage of participants selected the herbal substance than the comparator sham substances ($p<0.0001$ in all three cases).

For the final study a similar proportion of subjects in both groups believed they were observing and smelling an authentic herbal substance ($p=0.435$ for observing

and p=0.443 for odour) suggesting effective blinding. However, similar to the two earlier studies a statistically larger proportion of subjects receiving the authentic herbal substances believing they were tasting a herbal substance (p=0.002) compared to the sham herbal substance group.

Conclusion: This is the first time that a series of studies have been undertaken that have attempted to identify important cues and features that may threaten participant blinding in a herbal medicine trial. It was shown that with refinement it was possible to blind for visual and odour assessments but the sense of taste remained in all three studies a difficult human sense to blind. This obviously presents a problem for any herbal clinical trial where there is the opportunity for participants to break open a capsule and taste the contents. The studies have further demonstrated the need for pre-trial blinding for Chinese herbal studies and that a concerted effort should be made to develop a similar looking, smelling and tasting sham substance compared to the verum intervention arm. If possible inert materials, flavouring and colouring agents should be used. If this is not possible potentially active materials that have been shown to be irrelevant for the particular condition can be an option.

Chapter I

1.1 Background to the studies

1.1.1 The randomized controlled trial (RCT) and its features

The RCT is currently the "gold standard" for assessing the efficacy of a therapeutic intervention (Jadad, 1998). There are three important aspects to the RCT. These are i) randomisation, ii) use of a comparator usually a placebo or sham intervention (but can be an "active control' such as an established treatment) and iii) blinding of various parties involved in the trial. The first of these features, randomization, involves allocating participants in a random fashion to receive either the active therapeutic intervention to be tested or the placebo/sham intervention. This process attempts to distribute evenly across the two or more arms of a RCT any confounding factor. Many methods of randomisation have been developed and are routinely used in RCTs (Lancaster et al 2010).

The second aspect, use of a placebo, is to ensure a comparison can be made with a sham or a placebo intervention that appears to be similar to the therapeutic or active intervention being tested. While all participants appear to receive a therapeutic intervention, only those randomised to the active intervention receive a specific therapeutic component of the treatment being investigated. The use of a placebo intervention in pharmaceutical RCT is a common practice and is uncomplicated as the placebo intervention is easy to produce, for example a sugar pill (Boutron et al 2006). It becomes increasing difficult however when attempting to develop a placebo for surgery, or complex interventions like physiotherapy or acupuncture (Boutron et al 2007). Indeed many placebo interventions have been developed for acupuncture and found to be unconvincing or inappropriate (Lewith, 2010).

The final aspect of blinding is similar to randomisation in that its goal is to distribute participant expectancies evenly across the two or more groups. Various parties involved in an RCT, such as the persons delivering the intervention or the statistician, can be blinded but the most apt concern is the blinding of the actual participants themselves. Participant blinding ensures for example that some participants who have been allocated to the active intervention group believe they are receiving the active intervention, some the placebo comparator and others would be unsure. Conversely some participants in the placebo group would also think they are receiving the active intervention, some the placebo and yet others would also be unsure. Successful blinding therefore ensures that these expectations are distributed evenly across the two or more groups and not necessarily eradicated. Blinding also controls for participant bias such as demand characteristics and motivation (Colagiuri, 2010).

1.1.2 Blinding assessment

The terminology associated with blinding can sometimes be confusing. (eg single, double and triple blind) Single blind often refers to the participant blinding whereby double blind means that both the participant and the researcher are unaware of their allocation. Triple blind includes both participant and researcher but also the data collectors or data analyst (Miller and Stewart, 2010; Minns Lowe et al 2011). When participant blinding fails it can reduce internal validity in double blind placebo controlled trials. If the participant can guess their allocation status better than chance, whether it is to the active intervention or the placebo control arm of the study, then the study would more resemble an open label design.

The assessment of blinding has been fraught with complications and associated with methodological difficulties. Boutron and colleagues (2005) when reviewing the methods of blinding assessment found various approaches were used and that a certain level of

uncertainty was associated with their use. Furthermore there has been a lack of consensus about which quantitative statistical method to use. A common method has been to ask participants at various stages of the trial which group (treatment/placebo/don't know) they think they have been allocated to (Zaslawski et al 1997). Another method involves using a specially designed blinding index (Bang et al 2004; James et al 1996). Still yet another method involves indirectly assessing blinding by asking participants about the credibility of the treatment using a questionnaire (Devilly and Borkovec 2000).

Another contentious issue has been the timing of the assessment. Participants may be asked to guess their allocation status early in the study or at points during the study including at completion. Assessment at the completion may be influenced by the efficacy or side effects associated with the active intervention (Perlis et al, 2010). Yet another approach involves assessing the adequacy of blinding prior to commencing an RCT. This is often termed "pretrial evaluation" (Walter et al, 2005).

Recently, perhaps because of these difficulties, the revised CONSORT statement does not require blinding assessment as the previous version had. They cite "a lack of empirical evidence supporting the practice as well as theoretical concerns about the validity of any such assessment" as the primary reasons behind the revision (Schulz, 2010). Others however have argued the necessity to maintaining blinding assessment as "without blinding the quality of the trial cannot be established, as the readers cannot judge whether the results are due to the treatments received, or from a source of bias." (Hopton and MacPherson 2011; Berger 2011). One potential strategy to resolve many of these issues is to assess the credibility of the placebo by comparison with the active intervention prior to the commencement of the study (pre-trial evaluation). This was the approach taken for these three studies reported in this thesis. Walter et al (2005) has argued that this approach uses participants that can be

"deliberately primed to attempt to discriminate tablets based on specified attributes." and that "this constitutes a more aggressive and stringent test of the notion that blinding will be maintained in the study."

1.1.3 Chinese herbal medicine RCTs

Over the last decade there has been increasing publications of Chinese herbal medicine clinical trials. A search of the Cochrane database for systematic reviews found 24 reviews covering diverse topics such as hepatitis B and measles. A recent published review of Chinese medicine studies involved searching the Chinese Biomedical Database and found 3159 Chinese medicine RCTs that had been published between 1978 and July 2009 (He et al, 2011). They assessed the reporting of randomisation, allocation concealment and more importantly blinding. They found that only 12% (381 RCTs) used adequate randomisation methods, 7% (207 RCTs) used adequate allocation concealment and only 19% (601 RCTs) used adequate blinding. They concluded that the "quality of the current TCM RCTs as judged by their publications is generally poor, especially those published in Chinese journals (He et al, 2011). Another review evaluated the quality of reporting of 46 randomised controlled trials conducted in China on the treatment of cancer pain (Lu et al, 2011). They found a similar situation with only 26% of studies (n=12) deemed to use authentic randomisation and that 78% (n=36) of the studies failed to provide information on blinding of either the participants or investigators. More recently there has been several attempts to improve the quality of herbal medicine research by recommending that authors report the unique information associated with herbal medicine (Gagnier et al 2006a; Gagnier et al 2006b; Bian et al 2006). A follow up study in 2010 found that RCTs frequently do not report these important characteristics (Gagnier et al, 2010). For any successful controlled clinical trial, the use of a placebo plays an important role with respect to controlling for subject expectation.

Indeed an unblinded randomized trial can result in bias treatment effect estimates and lead to erroneous conclusions on the efficacy of the therapeutic intervention (Gotzsche 1996).

A meta-analysis of trials, described as 'double blind' in trial reports, found 14% lower treatment effects on average than similar trials not described as double blind (Boutron et al, 2005). While it has not been customary to check the effectiveness of blinding, a number of researchers have argued that reporting of the success of blinding should be published. Chinese herbal medicines have some additional challenges with blinding. Chinese medicines, in contrast to pharmaceutical substances, have special macroscopic, sensory characteristics including appearance, weight, size of particles; colour, odour and taste as the origin of these constituents are different. To overcome this problem, some investigators have used maize powder, charred germinated barley, food color, and taste additives.

The most common way of ingesting Traditional Chinese herbal medicine involves decocting a tea or soup from the raw herbs. Most Chinese herbs possess special tastes of acrid, bitter, sour or sweet. They have a strong odour when decocting making the fluid difficult to swallow and this may contribute to the difficulty of attracting participants to this kind of research. To overcome this problem researchers often make the herbal substance and placebo substance into tablets or capsules. Even though the encapsulation of the herbal substance is made more palatable the herbal medicines still possess special qualities such as taste, odour, color and texture. A recent Ginger study evaluated the blinding of the encapsulated ginger and placebo substances during the study. They reported that participants were able to identify the ginger capsule in comparison to the placebo tablet by the odour that accumulated in the bottle prior to opening (Zick et al 2005).

Because of these reasons it was decided to undertake a series of experiments to determine whether a credible sham substance could be developed for use in a clinical trial for Chinese herbal medicine. Please note the deliberate use of the word sham rather than placebo. This is because while the materials used to make the sham substance were commonly used culinary products they are not inert and could conceivable have some physiological effect in the body when ingested. Therefore the word sham was chosen indicating mock or imitation rather than placebo which refers to an inert substance.

1.2 Aim of the research

The aim of the first two studies was to evaluate whether a participant can identify a herbal intervention capsule (Ganopoly combination) when compared to three types of capsules containing sham material using a visual, odour and taste evaluation.

The aim of the third study was to determine whether a participant could identify a herbal or sham capsule when randomised to receive one or the other. This study therefore did not involve a comparison similar to the real life situation of a clinical trial.

The outcome of this study will assist development of suitable sham substance for encapsulation which will eventually be used in a future clinical trial conducted in the future

1.3 Format of the thesis

Chapter I: Introduction

This chapter will provide the background to the study as well as a brief introduction and rationale for the study, study aims and the format of the thesis.

Chapter II: Literature review

This chapter will review the current published literature concerning blinding, both in pharmaceutical clinical trials as well as herbal medicine trials. The main issues associated with blinding will be identified and discussed.

Chapter III: Methods

This chapter will describe the methods used for each of the three studies.

Chapter IV: Results

The results from the three studies will be presented including descriptive and inferential statistics.

Chapter V: Discussion

This chapter discusses some of the issues and limitations of the study. It also makes suggestions to improve future preclinical blinding assessment for Chinese herbal medicine RCTs.

Chapter VI: Conclusion and Recommendations

The final chapter reviews the findings from the three studies and makes recommendations for future studies including randomized controlled trials of herbal medicine.

References

This lists all the references used in the thesis in alphabetical order.

Appendices

The appendices include the questionnaires that were used in the three studies and a print out of all the statistical operations. Also included is the consent form and information sheet used in all three studies.

Chapter II Literature Review

2.1 Search Strategies

An electronic search was conducted using Pub Med and CINAHL for papers reviewing blinding in randomised controlled trials. These databases were selected because they hold the majority of clinical research in the English speaking world. Databases were searched on 26th March 2010 and again on 10th August 2011. Key words were "blinding assessment" and either "acupuncture" or "herbal medicine". In Pub Med 465 papers were retrieved for the term "blinding assessment", 16 for the terms "blinding assessment and acupuncture" and only 4 for the terms "blinding assessment and herbal medicine". In CINAHL only one paper was retrieved for the term "blinding assessment" none for the terms "blinding assessment and acupuncture" or "blinding assessment and herbal medicine". For brevity sake this literature review will only report the most recent and relevant studies. They include some reports which review the assessment of blinding assessment in biomedicine, including some reviews of blinding in general as well as all retrieved studies that review blinding and herbal medicine.

2.2 Blinding in Randomised Controlled Trials

A number of reviews have been published to determine the quality of reporting of blinding in RCTs. Montori and colleagues (2002) evaluated 200 RCTs. They reported that explicit reporting of blinding status for trial participants was 15%, while for the healthcare providers the reporting of blinding status was only 5%. They also found that for the data collectors' reporting was approximately was 12% and outcome assessors was 23%. In addition data analysts were reported as blinded in only 2.5% of the studies reviewed. Furthermore they found that none of the reviewed studies reported that the manuscript writers were blind.

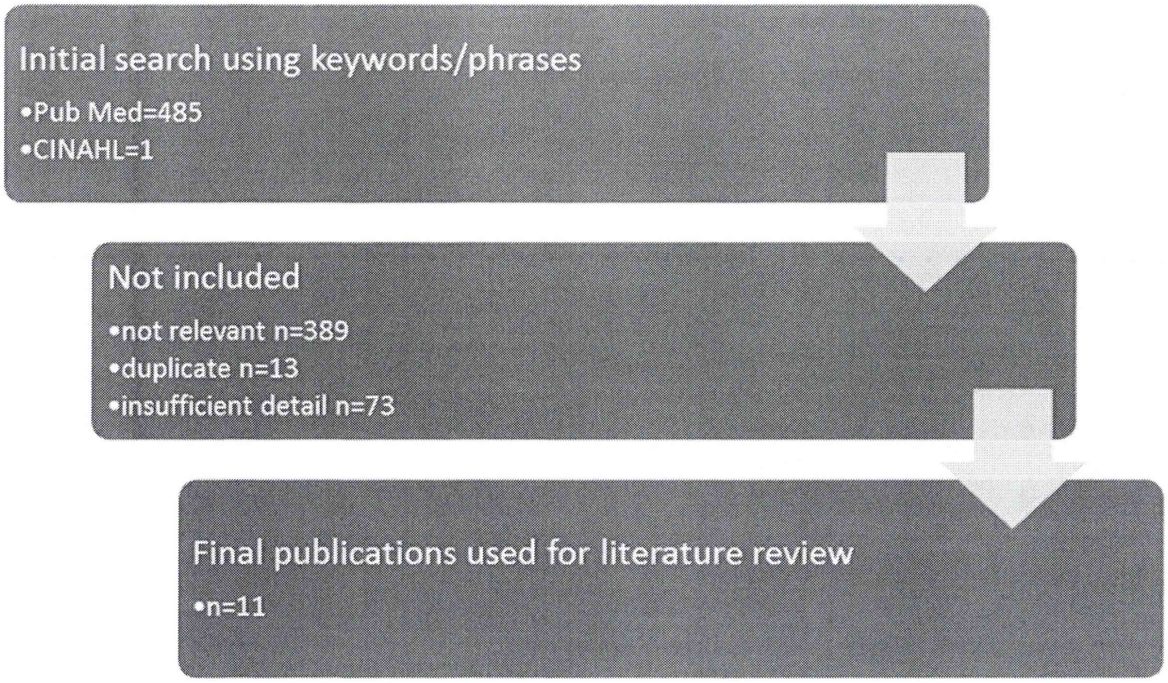


Figure1.1: Flow sequence of literature search

Fergusson and colleagues (2004) evaluated a random sample of 191 trials from leading general medicine and psychiatry journals. They reported that only seven of the 97 general medicine trials provided evidence on the success of blinding, with five reporting the success of blinding was imperfect. In trials from psychiatric journals the success of blinding was reported in eight of the 94 trials (9%) with four reporting the blinding was imperfect.

A more recent study by Boutron et al. (2005) reviewed 90 RCTs and found that 58 of the studies did assess blinding however there was variance in consistency in timing of assessment and mode of answering. They further stated that only 57% used statistical analysis comparing the proportion of correct guesses to those produced by chance.

A latter systematic review of the methods also by Boutron et al. of blinding of 472 trials concluded that more than half of the studies described the blinding but 236 papers gave no details and only 111 gave some data on blinding (Boutron, 2006).

More recently Machado et al (2008) reviewed 126 trials using 25 different placebo interventions. The adequacy of blinding was assessed in only 13% of trials. They also noted that in 20% of trials the placebo intervention was a potentially genuine treatment. To overcome this problem Brinkhaus et al (2008) has proposed a placebo quality checklist for pharmacological trials to help investigators in selecting an appropriate placebo and to help readers interpret the study findings with more care.

Finally a group of researchers assessed a random sample (n=265) of actively recruiting RCTs that were listed on the World Health Organisation International Clinical Trials Registry Platform (ICTRP) search portal in 2008 (Reveiz et al 2010). They found that only 1.4% of the sample adequately reported allocation concealment and only 53% (n=141) were reported as blinded.

2.3 Blinding in Herbal Medicine RCTs

Few studies have looked at the issues that specifically face herbal medicine researchers when designing and conducting a RCT and how successful the studies have been in blinding the participants. Zick (2005) assessed whether 80 trial participants could distinguish between ginger and placebo capsules. They found that of the eighty people in the trial, 42 correctly identified the capsule they were received. Of those that received the placebo capsule, 82% correctly identified their allocation status. Of those subjects who received the ginger, 22.5% correctly identified their capsule. With the increasing evaluation of Chinese herbal substances in RCTs and their specific characteristics, the issue of blinding evaluation should be routine. Herbal substances tend to have a strong odour and powders may be highly coloured due to the colour of the base herbs used in the formulation. More recently Qi and colleagues (2008) evaluated the validity of placebos used in blinded RCTs of Chinese herbal medicine. They reported that of the 77 full length articles they evaluated nearly half did not pay any attention

to the physical quality of the testing drug and placebo. Only two articles specifically validated the comparability of the placebo herbal substance and the testing herb or drug. They concluded that "quality specifications and evaluation of the placebo should deserve attention to reduce the bias in randomised controlled studies of Chinese herbal medicine."

Another recent study involved the assessment of 28 women concerning their group allocation to either a Chinese herbal preparation or a herbal placebo (containing chicory, lemon verbena, coriander, cabbage, sweet corn, turnip, peas and leek) to assess their effect on endometriosis (Flowers et al 2011). The researchers reported that they were successful in blinding participants as three women (20%) in the placebo group (n=15) guessed correctly, eight thought they were on the herbs (53%) and four did not know which group they had been allocated to (27%). In the active herbal group (n=13), two (15%) believed they were receiving a placebo, eight (62%) thought they were given herbs and three participants (23%) did not know which group they had been allocated to.

Another study assessed whether volunteers who received either placebo (n=102) or tee tree oil (n=112) ointment, a strong smelling substance, could determine their treatment allocation (Carson et al 2008). The researchers deceptively told participants that the substances had been modified for the trial with the tea tree oil having a strong odour and the placebo ointment no discernible odour. Of the 100 that presented for treatment assessment approximately 50% correctly identified their allocation status. Of the 114 who did not present for treatment assessment 12 participants did not provide blinding data and another 46 did not open their tube. Of those that did open their tube (n=56) less than half of those who received the tea tree oil (44.4%) and a very small percentage of those who received the placebo

ointment (17%) correctly guessed their treatment allocation. The researchers concluded that "without the use of deception, double blinding would have been virtually impossible."

The development and successful use of a placebo in some clinical trials may be difficult to undertake. This is especially the case when trialing complex interventions such as Chinese herbal medicine or non-pharmaceutical interventions like exercise, psychology or acupuncture (Boutron et al 2007). Chinese medicine preparations carry special macroscopic, sensory characteristics including appearance, weight, size of the particles, color, odour and taste as the origin of their constituents are different, and the complexity of making a perfectly matching placebo nearly impossible.

Placebos that are improperly designed or implemented may introduce bias into trials. The inadvertent use of non-inert placebos in trials may cause the under estimation of treatment effects and as has been pointed out when indistinguishable placebos may consist of potentially genuine substances.

In summary even though blinding is important in RCTs, and thousands of trials are conducted every year, the reporting of blinding and success of double blinding are often inadequate. Many studies and a number of reviews have been conducted to evaluate the current state of play concerning blinding and its evaluation. They have generally concluded that there is minimal assessment of blinding and when there has been it has been poorly reported. Table 2.1 identifies some of the factors and levels of difficulty associated with blinding while table 2.2 shows the perceived risk associated when blinding fails.

Feasibility of blinding patients and care providers		
Possible	*Difficult*	*Impossible*
The treatment has the same physical characteristics and the same route of administration.	A double dummy for treatments with a different route of administration	The treatments being compared are totally different and the use of double dummy is impossible (e.g., comparison of surgery to physiotherapy)
The procedure and the monitoring are similar	Masked scars	Control treatments are usual care or waiting list
For patients, if surgical interventions with different procedure but performed under general anaesthesia, with similar scars, perioperative care, postoperative complications and no more contact with the unblinded care providers are compared (e.g., total hip arthroplasty with or without cement).	A simulated intervention	Care providers : If care providers are an integral part of the treatment as they perform the treatment (e. g., surgeon for technical operation, physiotherapist for a rehabilitation intervention)
	A sham adapted dosage in the control group	

Table 2.1: Levels of difficulty in blinding and associated factors for each level

The perceived risk of unblinding patients and care providers was considered		
Null if	*Moderate if*	*Important if*
The treatments have the same physical characteristics and route of administration	The treatment does not have the same physical characteristics/route of administration/procedure or monitoring, and blinding suppose creative solutions that have not been previously tested (e. g., sham lavage)	The treatments have the same physical characteristics but never give the same clinical manifestations (e. g., different taste for oral drug, pain after an intramuscular injection)
The procedure and the monitoring are similar	The treatments have the same physical characteristics but some time do not give the same clinical manifestations	Side effects can occur with a high frequency in one group (e. g., bradycardia with beta-blockers)
The treatments being compared have no marked side effects	Side effects that are not specific to a treatment (headache) can occur with different frequencies in each group.	Specific side effects can occur in the group

Table 2.2 Levels of perceived risk of unblinding

Chapter III Methods

3.1 Overview of the three studies

The three separate studies reported in this thesis were undertaken in sequential order between 2009 and 2011, with the second and third study attempting to improve on each previous study. In all three studies the primary aim was to evaluate whether participants could correctly identify a herbal substance from a sham substance.

The first was a pilot study involving eleven participants. The aim of this study was to pilot the data collection process and determine whether the initial materials were suitable for the study.

The second study involved 81 participants. This study improved on the initial pilot study in several ways. The first was an increase in participant number. The second improvement was the use of an opaque eye pad during the tasting phase to effectively blind the participant. This was to ensure participants could not relate their previous responses to vision and odour tests to the substance being taste tested. The third improvement was the closer colour matching of the three sham substances to the herbal substance. This was achieved by using food colouring agents.

The third and final study involved only two substances, a refined sham substance and the herbal substance. Participants were randomly given one of the two substances and asked whether they believed the substance to be a' herbal' or 'placebo' substance or 'didn't know' using a specially designed questionnaire (see appendix 1 and 2). While the participants were blind to their capsule allocation (either herbal or sham) no eye pad was necessary as there

was no comparison with other substances as occurred in study one and two. This study improved on the first and second study by improving the taste and colour of the sham substance in order to be comparable to the herbal substance.

The three studies were designed to recruit only healthy participants who were not currently taking any medication. Participants were excluded if they had any severe food sensitivities or known food allergies.

The person administering the questionnaire was aware of the status of the substances being evaluated and therefore can be considered unblinded. This was due to financial constraints (the employment of someone to administer the task) and time restraints (coordinating two people to the task).

3.2 Recruitment

Participants were recruited by word of mouth and can be regarded as a convenience sample. This involves the sample being drawn from a population that is "close at hand" because they are readily available and convenient (Fowler, 2009).

3.3 Setting

All data was collected on site at the acupuncture/Chinese herb clinic located at the city campus of the University of Technology, Sydney (UTS). The data for the pilot study was collected during the period of May to June 2009 (n=11) while for the larger study (n=81) between the months September to November 2009. The third study (n=62) was conducted during the months of December 2010 to March 2011.

Prior to commencing the study ethical approval was sought and obtained from the Human Research Ethics Committee at UTS (No 2009-070). Participants were given an information sheet and asked to sign a consent form prior to participating in the study.

3.4 Materials

3.4.1 Herbal substance

The herbal substance used in the study is marketed as "Ganopoly combination" and is manufactured by Alpha Healthcare a New Zealand company. The herbal extract (in granule form) is derived from extracted material from two substances, Ganoderma lucidum and Cordyceps sinensis. It is currently approved by the Therapeutic Goods Administration as a herbal supplement and has a listed number-L71644.

Ganoderma lucidum is known in Chinese medicine as *Ling Zhi* (literally "spiritual mushroom). It is an edible mushroom that has been commonly used in ancient China and later in Asia, for the prevention of certain diseases as well as a treatment for insomnia, pain and cough. It contains ganoderma polysaccharides, ganoderma acid, adenosine and other substances.

Over the last 30 years ongoing research and clinical trials have found it possesses the capacity of boosting the immune system, reducing cholesterol and lipids and enhancing liver function. It is widely use in China, Japan and throughout Asia in modern times to treat the cancer, cardiac diseases, asthma, hepatitis and diabetes mellitus.

The other extract is from Cordyceps (known in Chinese medicine as *Dong Chong Xia Cao* which literally means "winter worm summer grass"), a fungus that also has a long usage in

China, over 2000 years, as a treatment for a variety conditions including infectious diseases. Cordyceps contains cordyceps polysaccharides and manitol. A recent study showed cordyceps posed as an activator of innate immune responses, it activates macrophages by engaging Toll-like receptors and inducing mitogen-activated protein kinase pathways characteristic of inflammatory stimuli (Shin et al. 2010). A Korean study found the cordyceps possesses the effects of anti-angiogenetic properties, thus it could prevent the tumour growth and metastasis (Choi et al 2011).

3.4.2 Sham materials

3.4.2.1 Study one

The sham materials for the first study were developed after a trial of different food materials. The material had to have a strong flavor similar to the herbal ingredients but be therapeutically inert as possible. This is always difficult as no substance, even a sugar pill, is physiologically inert once ingested. Therefore we chose commonly used culinary agents (chili powder, curry powder, chocolate cake mixture) that are ingested frequently in daily life. It should be noted that if these sham substances were used in a clinical trial it would be advised to assess that the pharmacologically effect of these substances were irrelevant to the effects desired in the herbal substances being tested.

The base material chosen was wheaten cornflour (Fielders brand). This was chosen as it had a neutral taste and was similar in granule size to the herbal material. To this base material were added some flavouring agents (see figure 3.1). The ingredients for the three sham capsules in study one are listed below:

- Sham capsule A consisted of cornflour flour and curry powder (Master of Spices brand) with a ratio of 100:16 (cornflour:curry powder);

- Sham capsule C consisted of cornflour flour only;
- Sham capsule D consisted of cornflour flour, chilli powder (Master of Spices brand) and chocolate cake mixture (White Wings- Moist chocolate cake) with a ratio of 100: 3:50 (cornflour:chilli powder:chocolate cake mixture).

The materials were mixed thoroughly and then packed inside transparent (00 size) capsules and sealed by bringing the two halves of the capsule together.

3.4.2.2 Study two

For the study two the ingredients were changed because the sham substances were light in colour and did not have the same colour as the herbal compound. Mixtures of food colouring in ratio were used to darken the cornflour and the chocolate cake mixture was removed as it was found that some participants in the pilot study could identify the taste. Below are the ingredients for the three sham capsules for the second study (figure 3.2).

- Sham capsule A consisted of cornflour flour and curry powder (Master of Spices brand) with a ratio of 100:16 (cornflour:curry powder);
- Sham capsule C consisted of cornflour flour, food colouring (red:blue, 4:1) and Black Pepper (McKenzie's Ground Black pepper) with a ratio of 50:10:1 (cornflour:food colouring: black pepper);
- Sham capsule D consisted of cornflour flour, colouring (yellow: red: blue, 6:4:1) and chilli powder with a ratio of 81: 29: 0.7(cornflour:colouring:chilli powder);

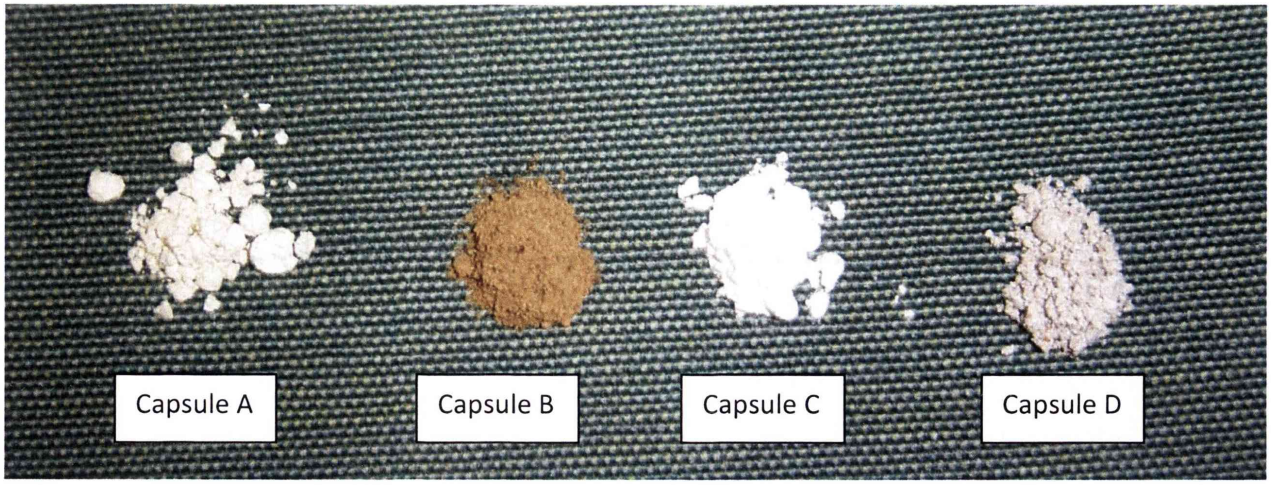


Figure 3.1 Visual appearance of sham substances used in the study 1

Figure 3.2 Visual appearance of sham substances used in study 2

Similarly to study one, the materials were mixed thoroughly and then packed inside transparent (00 size) capsules and sealed by bringing the two halves of the capsule together. Several batches of capsules were made for each substance and placed inside a clear transparent sample jar with a screw lid. This storage method facilitated the odor test whereby when the participant first opened the container a distinct odour could be detected.

3.4.2.3 Study three

While the previous two studies evaluated whether the participant could select the correct capsule from four possible options the final third study used only one of two capsules. This procedure was administered because in a clinical trial the subject is only allocated to one treatment, unless of course it is a cross over design. It makes sense then that the clinical trial subject would only assess one treatment, either the herbal capsule or the control sham capsule. Participants were randomised (using a permutated block of four participants and an envelope method to balance numbers for each type of capsule) to receive either capsule A (sham) or B (herbal substance) and again had to decide on the basis of visual appearance, odour and taste whether the allocated capsule was "herbal substance", a "placebo substance" or "don't know". Similar to the first two studies they also were asked to give a reason for the decision.

Prior to conducting study three, based on the materials used for the sham, a refined substance was developed. It differed from the sham materials used in the two previous studies in three ways.

1. Ground rice rather than corn flour was used to give a more grainy texture to the base material. Several participants had previously commented that there was a difference between the corn flour based material and the real herbal substance.
2. A darker colouring agent was used to colour the sham materials giving them a similar colour to the herbal substance.
3. The flavouring agents of black pepper and curry powder were used together in the sham material to give a complex taste. In the previous studies both these flavouring agents had been used separately.

The ingredients for the sham material comprised of;

- ground unprocessed (brown) rice;
- colouring agents (Queen brand) of (yellow: red: blue, 6:9:6);
- a mixture of black pepper (McKenzie's Ground Black pepper), curry powder (Masters of Spice brand) and ground rice in the ratio of 3:4:12 (see figure 3.4 and 3.5).

The materials were packed in the same transparent capsules as the two previous studies and placed in transparent plastic bottles. Similar to the first two studies a questionnaire was administered during the three test of visual appearance, odour and taste (see appendix 2). Also no physical blinding (eg eye pad) was required as there was no need to discriminate taste between several substances.

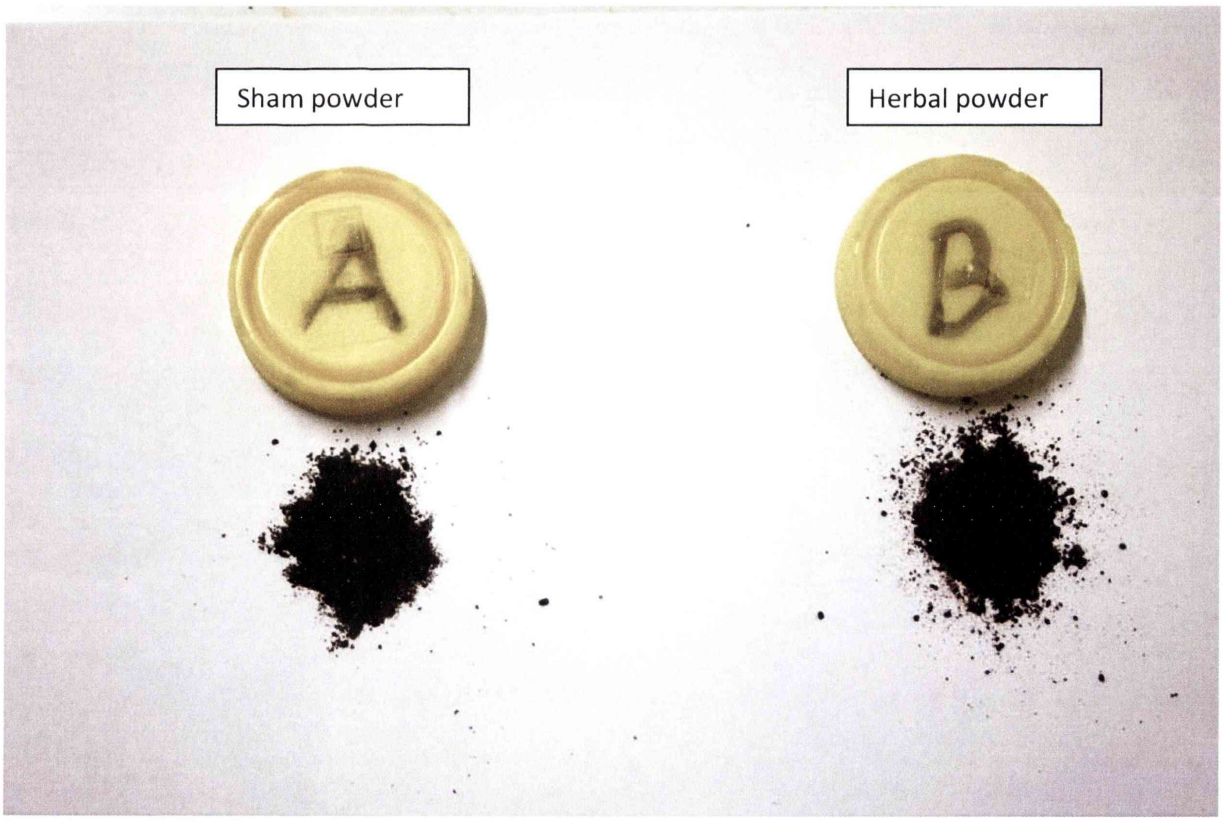


Figure 3.3 Visual appearance of materials used in study 3 (powder form)

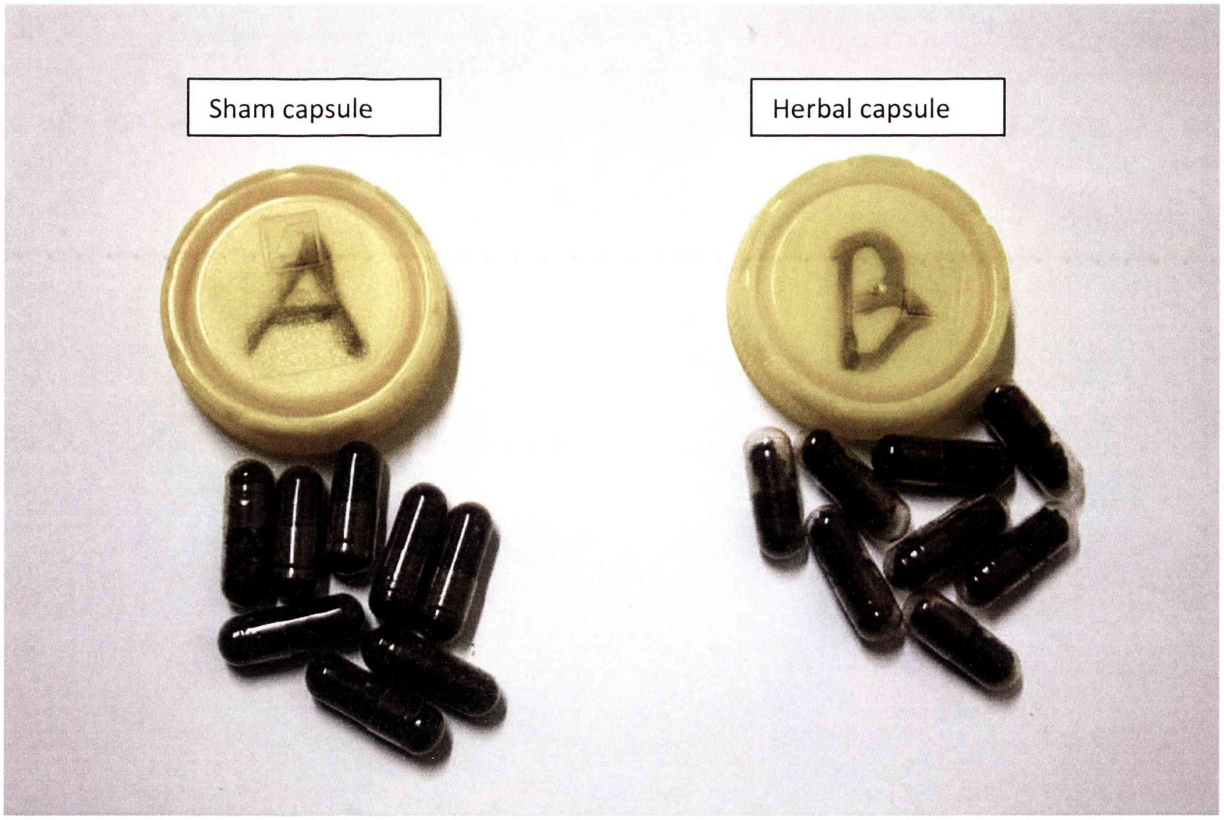


Figure 3.4 Visual appearance of materials used in study 3 (encapsulated form)

3.5 Data collection

In order to evaluate whether a participant could identify a herbal capsule from three sham material capsules a questionnaire was developed and piloted (see appendix 1). Again the questionnaire needed to assess whether participants could detect the herbal materials using the three senses of vision, smell and taste.

For the first two studies the three sections of the questionnaire asked each participant to:

1) Open each of the four bottles and smell the contents;
2) Take out a capsule from each of the four bottles and observe its contents;
3) Open one of each of the four types of capsules (A, B, C, D) and taste its contents.

For studies 1 and 2 participants could select one of the four capsules (A, B, C, D) as the herbal material or select "don't know'. We also asked them the reason as to their choice so that we could identify potential biasing cues. In addition we also requested their age and

gender, whether they had any know food sensitivities or allergies, whether they had previously ingested herbal medicine and if so whether the herbal medicine was a pill, decoction (soup) or in granule form.

3.6 Procedure for evaluating the substances

Subjects were seated in a private room within the UTS Chinese medicine clinic. After being seated they were asked to read the information sheet and sign the consent form. The consent form explained to the participant that they would be asked to distinguish which substance was likely to be a herbal product among four different materials using a visual, odour and taste evaluation. For the visual evaluation participants were asked to observe several transparent capsules in each of the four transparent containers (see figure 3.4). For the odour evaluation, participants were asked to open each of the four the container and smell the contents. Finally for the taste evaluation, participants were asked to wear a blindfold and a small amount (0.1 gm) of the substance from each of the four capsules was fed to the participant using a small spoon. Between each taste the participant was asked to drink a small amount of water to cleanse the mouth.

When conducting the tests, the order of presentation was randomised for both visual appearance and odour however the bottles were marked so as to be identifiable by the researcher. For taste evaluation however participants were visually blinded by wearing an eye mask. The presentation of the substances were randomised, therefore all participants were completely blind to the choice. The randomisation of the presentation order of the substances was conducted to minimise order bias.

For the third study the procedure was different due to the design of the experiment. .For this study each participant was randomly given only one substance rather than having to evaluate four substances as in the previous two studies. To randomize the sequence of testing, an envelope method was used with 31 envelopes having the word sham inside and the remaining 31 having the word herbal. Each participant was asked to select one envelope from a block of four randomly chosen envelopes and the participant was given the material that was indicted inside the envelope they had selected (either A-sham or B-herbal). The questionnaire (see appendix 2) was revised for the third study to respond appropriately as "herbal", "placebo" or "don't know" rather than selecting one substance out of four as was done for the first two studies.

3.7 Statistical Analysis

All data were tabulated into an Excel spreadsheet and then imported into Minitab 15 (Minitab Inc) for analysis. Descriptive statistics were generated and then one proportion hypothesis test was conducted for the first two studies while a two proportion hypothesis test was conducted for the third study. A hypothesis test will determine whether the proportion of trials that produce a certain event is equal to a target value. This statistical procedure tests the null hypothesis that the population proportion (in the case of study one and two the proportion value is 0.25 due to a 1 in 4 chance they will correctly identify the herbal capsule) is equal to a hypothesized value.

For study three a two proportion hypothesis test was used to determine whether participant expectancies were evenly distributed across the two groups, the group receiving the active herbal substance and the group receiving the sham substance. That is, a similar percentage of

subjects in each group should perceive they were observing, smell and tasting a herbal substance. Conversely a similar percentage in each group should believe they were observing, smelling and tasting a sham substance. In the case of study three the proportion value is 0.5 due to a 1 in 2 chance is equal to a hypothesized value.

In all tests a probability value of less than 0.05 was considered statistically significant.

3.8 Statistical power

To establish statistical power for study three the minimum number of participants was determined as 62. This was based on the condition that to test the null hypothesis that the proportion correctly choosing the herbal is 0.5 (i.e. indistinguishable from chance) versus the alternative hypothesis, and that the proportion is 0.7, given a power of 0.9.

Chapter IV Results

4.1 Participants

In total 154 people participated across the three studies, eleven in the pilot study, 81 in the second study and 62 in the third study. For the first study the mean age of the participants was 42.8 years (sd ±4.8, range of 18-63 years). For the second study the mean age of the participants was 34.5 years (sd ±14.1, range of 19-83 years) while for the third study the mean age of the participants was 40.3 years (sd ±14.1, range of 15-68 years). In all three studies there were more females than males.

Table 4.1 shows that for all three studies there were more females than males and that the greater percentage of both genders had previously ingested herbal medicine.

	Study 1 (n=11)		Study 2 (n=81)		Study 3 (n= 62)	
	male	female	male	female	male	female
Had taken herbs previously	4	5	18 (56%)	37 (77%)	17 (81%)	40 (98%)
Never taken herbs previously	1	1	14 (44%)	11 (23%)	4 (19%)	1 (2%)

Table 4.1: Gender and herbal experience status of participants for all three studies

4.2 Participant previous herbal medicine experience

Table 4.2 shows that of the 113 subjects in study 2 and 3 who reported previously taking herbal medicine the greater percentage had used all three forms (pill and, decoction and granule). The remaining participants used other combinations of pill and decoction, pill and granule or decoction and granule or used a single delivery mode.

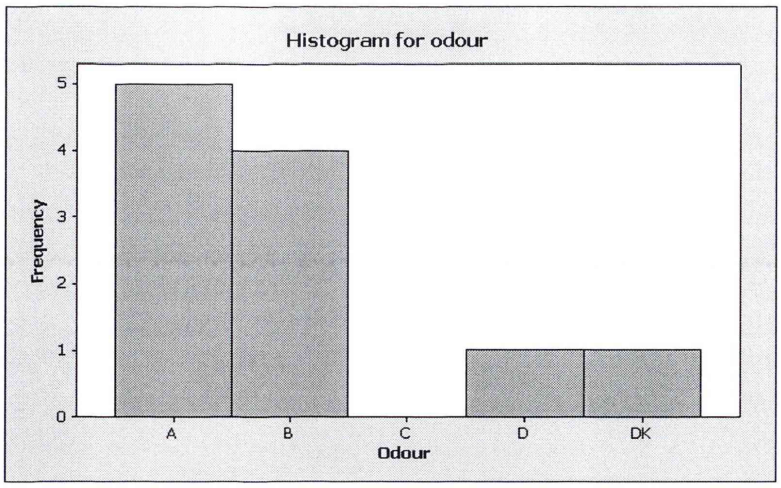
	P, G and D	P only	D only	G only	P and D	P and G	D and G	No answer
tudy 1 (n=11)	3	1	3	0	1	0	0	3
tudy 2 (n=81)	26	7	9	2	3	5	3	26
tudy 3 ı=62)	26	8	12	0	7	4	0	5

Table 4.2: Types of herbal delivery modes previously used.

P= pill; D= decoction; G=granules

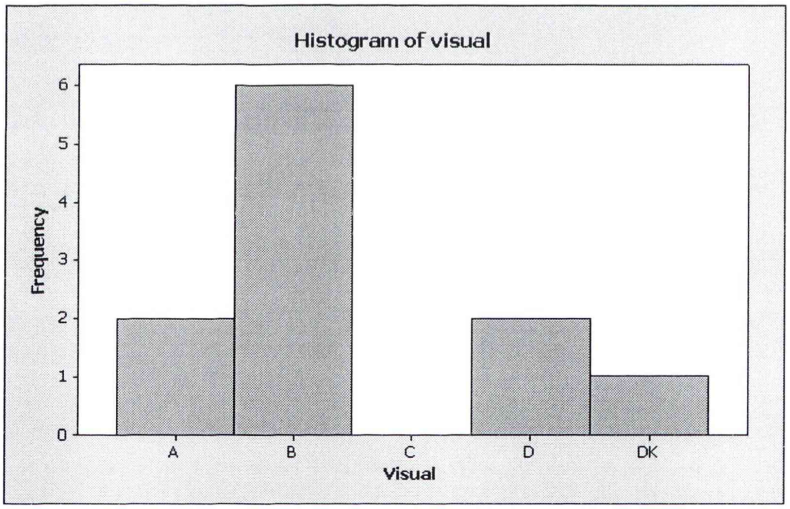
4.3 Choice results for study one (pilot)

Figures 4.1 to 4.3 show the responses by the eleven participants for each of the three tests. For the odour evaluation (figure 4.1) five subjects selected capsule A, four selected capsule B (herbal substance), one selected D and one responded that they did not know.



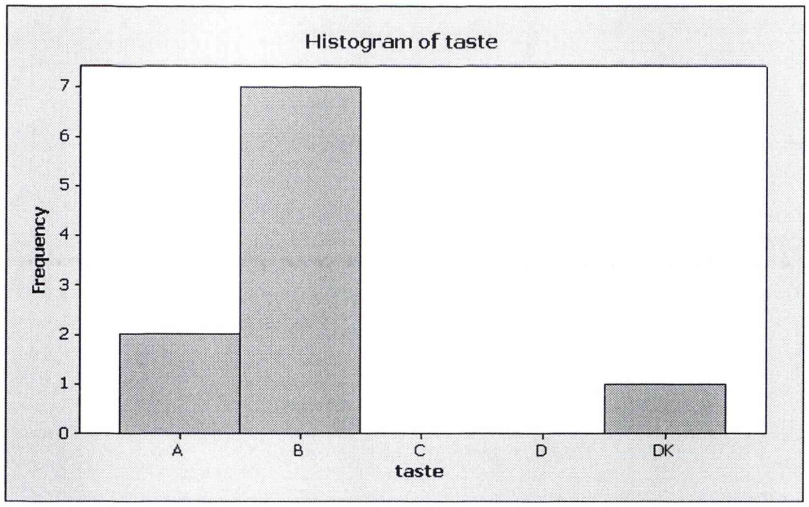
Figures 4.1: Responses to the question concerning odour.

For visual appearance (figure 4.2) two subjects selected capsule A, six selected capsule B and two selected capsule D with one respondent selecting don't know.



Figures 4.2: Responses to the question concerning visual appearance.

For taste evaluation (figure 4.3), two respondents selected capsule A, seven selected capsule B and one replied that they did not know.



Figures 4.3: Responses to the question concerning taste.

A one proportion test found that for both odour (p=0.484) and visual appearance (p=0.077) the number of participants selecting capsule B as the herbal substance was not significantly different from what may have been selected by chance. This was not the case for taste where significantly more participants selected capsule B as the herbal substance (p=0.004).

It must be noted however that there was a small number of participants (n=11) who were involved in the pilot study and the results should be interpreted cautiously. This caution in interpreting these results is evident when larger numbers of participants (n= 81) were involved as reported in the results for study two.

4.4 Choice results for study two

Figures 4.4 to 4.6 show the results for study two which involved 81 subjects. For the question concerning visual appearance (n=81), 13 (16%) selected capsule A, 41 (50%) selected capsule B, 4 selected capsule C (5%), while 8 chose capsule D (10%). Another 15 subjects (18%) replied that they did not know. A one proportion test found significantly more participants selected capsule B the herbal capsule (p<0.0001) than expected by chance. This

indicates that the participant expectancies were not evenly distributed across the four choices and suggests failure to blind.

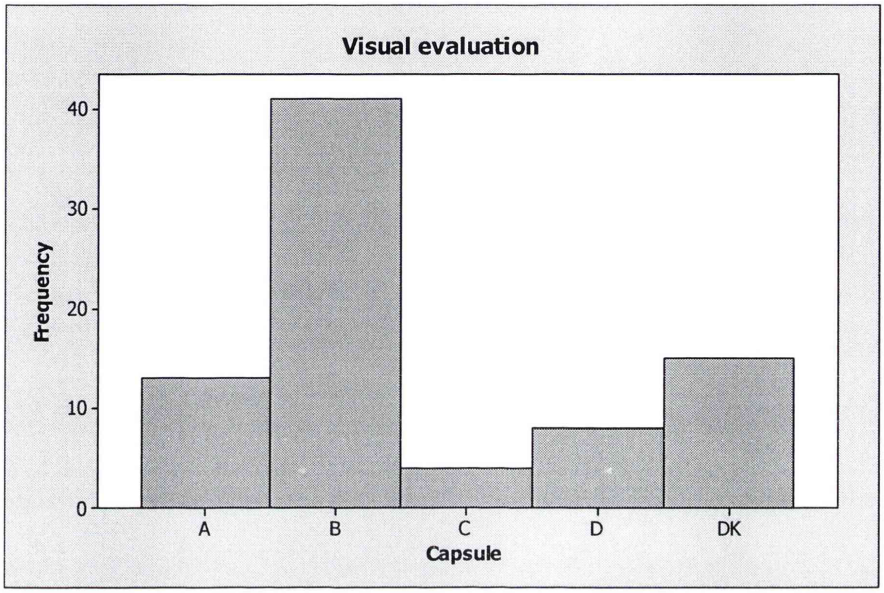


Figure 4.4: Participant choice response for visual inspection

Concerning odour (n=81), figure 4.5 again shows nearly half (n=39: 48%) selected capsule B, while 13 selected capsule A (16%), 8 selected capsule C (10%), 14 selected capsule D (175) and only 7 (9%) replied that they did not know. A one proportion test found significantly more participants selected capsule B the herbal capsule ($p<0.0001$) than expected by chance. This again indicates that the participant expectancies were not evenly distributed across the four choices and again suggests failure to blind.

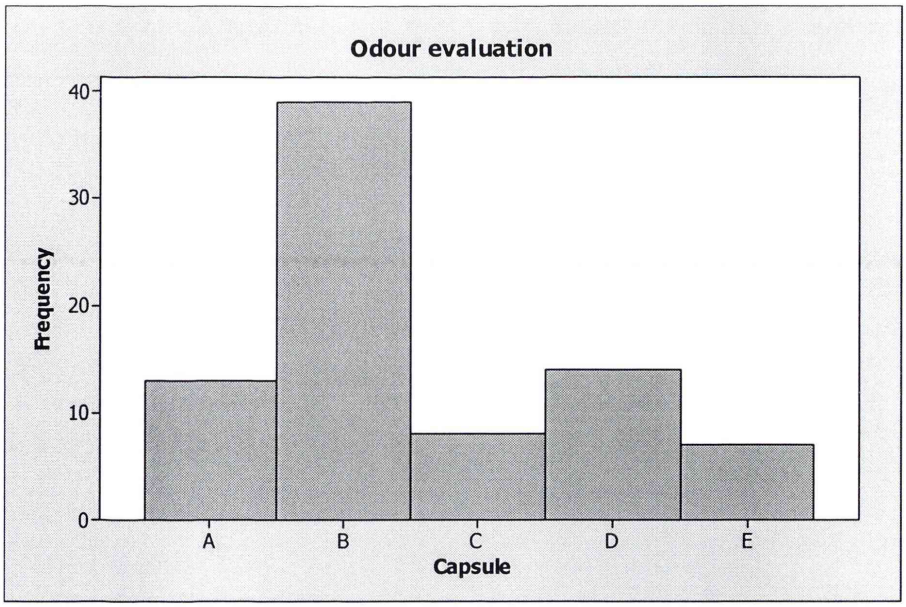


Figure 4.5: Participant choice response for odour

For the taste question (n=79) again as shown in figure 4.6 the largest percentage selected capsule B (n= 50: 63%). For the remaining choices similar number of responses were made for the other three capsules, with 8 (10%) people selected capsule A, 8 (10%) selected capsule D, and nine (11%) selected capsule C. in this case only 4 people said they did not know. Two participants did not give a response. Again a one proportion test found significantly more participants selected capsule B the herbal capsule ($p<0.0001$) again indicating that the participant expectancies were not evenly distributed across the four choices and suggests failure to blind.

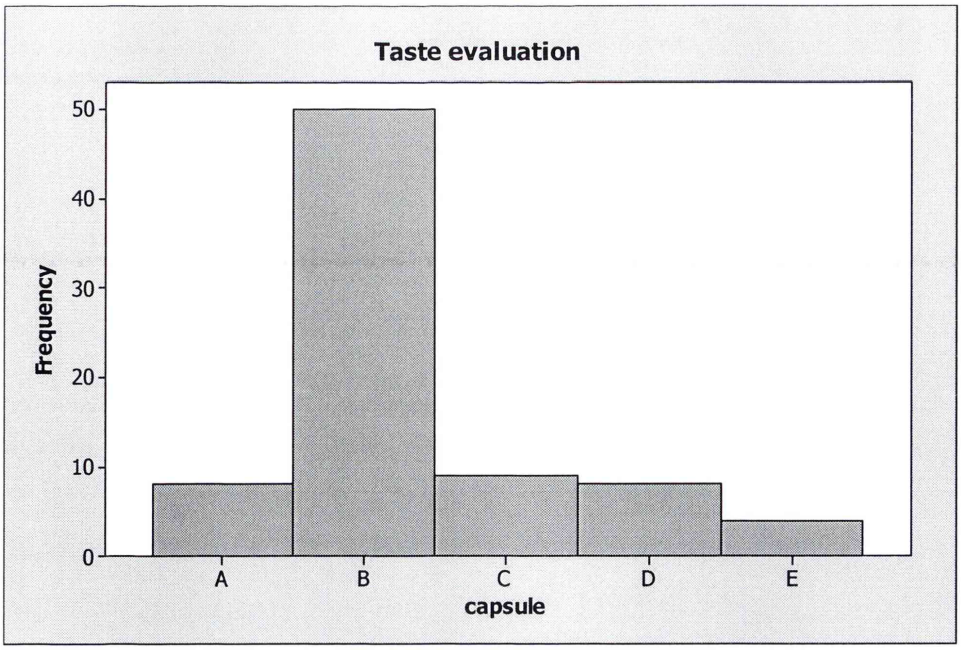


Figure 4.6: Participant choice response for taste

4.2.2 Reasons for choice

In addition to selecting the capsule they believed contained the herbal material concerning the visual appearance, odour and taste cues, subjects were also asked to indicate their reasons for choice. This would assist the researcher in identifying what cues were important for the subjects in discriminating the sham from the herbal material.

4.2.2.1 Visual appearance

The most common reason given for choice concerned the colour of the substance. This was given as the rationale for choice by 34 subjects (42%) of which 20 (59%) had correctly selected the herbal substance (capsule B). The next most common reason (n=16: 20%) given concerned how the substance looked, of which nine (56%) subjects had guessed correctly and seven (44%) selected other capsules. Other responses given concerned colour and texture

(n=7, of which 72% were correct), texture alone (n=2, 50% correctly guessed), with earthy smell, powdery, little particle/irregular shape, guess being the reason for choice for one person each. Sixteen subjects did not give a reason for their choice (20%) of which 15 had selected "don't know".

Reason	Number of participants	Correct	Incorrect
Colour	33	20	13
Look	16	9	7
Colour and texture	7	5	2
Texture	2	1	1
Felt	1		1
Earthy smell	1		1
Powdery	1	1	
Little particle Irregular shape	1	1	
Guess	1	1	
No answer	16	2	1 13 don't know

Table 4.3: Participant choice response for taste

4.2.2.2 Odour

For the odour evaluation, most participants just stated smell (n=48) of which half the participants were incorrect (n=24; 50%). Table 4.4 shows that the remaining respondents gave a particular odour as a reason ranging from "bitter", or "pungent" to "liquorice like" and "cooking like smell". Eleven subjects did not give an answer.

Reason	Frequency	Correct	Incorrect
Smell	48	24	24
Bitter	3	1	2
Pungent	2	1	1
Sweet	2	2	
Natural (no smell?)	1	1	
Herb like smell	3	1	1 (Don't know 1)
From experience of granule intake	1		1
Strong smell	1		1
Bitter and earthy	1	1	
Less appetising	1		1
Most distinct	1	1	
Smell difference	2	1	1
Liquorice like smell	1	1	
Honey like smell	1		1
Vitamin tablet like smell	1	1	
Cooking smell	1		1
No answer	11	3	2 (Don't know 6)

Table 4.4: Reason given by the participants concerning their choice and the odour of the substance

4.2.2.3 Taste

For the taste question the taste of bitter was the rationale for choice most often given (n=26; 32%) as the reason for the participant choice. When these were checked for their capsule choice, 21 had guessed correctly. The rest of the subjects reported varying reasons ranging from "strong taste" (n=9, of which seven guessed correctly), "medicine like" and "herb flavour" (both n=2 each of which half guessed correctly) and the remaining subjects giving a single response only.

Reason	Frequency	Correct	Incorrect
Bitter	13	11	2
Strong taste	9	7	2
Strong and bitter taste	10	8	2
Texture	1		1
Spice	1		1
Taste and texture	1	1	
Medicinal taste	2	1	1
Herb flavour	2	1	1
Strangest taste	1	1	
Cinnamon taste	1		1
Natural taste	1		1
Don't know why	1	1	
Chinese herb smell	1	1	
Bitter and sweet	1	1	
Strong flavour	1	1	
Pungent taste	1	1	

From previous experience of granule intake	1	1	
Guess	1		1
Medicine like taste	2	1	1
Least like food taste	1		1
Stronger taste	1		1
Have some flavour	1		1
Most bitter and unpleasant	1		1
Sweet and spicy	1	1	
The other like cardboard	1		1
Taste and rough texture	1	1	
Bitter and strong taste	1	1	

Table 4.5: Reason given by the participants concerning their choice and the taste of the substance

Because a participant's previous experience with herbal medicine may have influenced their response a chi square was computed to evaluate measures of association between whether they had previously ingested Chinese herbal medicine and the selection of capsule for visual appearance, odour and taste. Tables 4.6, 4.7 and 4.8 show the responses for the participants concerning the visual appearance, odour and taste and their previous experience with Chinese herbal medicine. For both visual appearance and odour no significant association was found (Pearson chi square value=5.4; p=0.25 and Pearson chi square value=5.4; p=0.14 respectively). For taste while no statistical significance was found there was a trend (Pearson chi square value=8.7; p=0.08).

Visual appearance	Capsule A	Capsule B	Capsule C	Capsule D	Don't know
Previous experience with Chinese herbal medicine	7	33	2	5	9
No previous experience with Chinese herbal medicine	6	8	2	3	6

Table 4.6: Participant responses for the participants concerning the visual appearance and their previous experience with Chinese herbal medicine

Odour	Capsule A	Capsule B	Capsule C	Capsule D	Don't know
Previous experience with Chinese herbal medicine	10	31	4	8	3
No previous experience with Chinese herbal medicine	3	8	4	6	4

Table 4.7: Participant responses for the participants (n=81) concerning odour and their previous experience with Chinese herbal medicine

Taste	Capsule A	Capsule B	Capsule C	Capsule D	Don't know
Previous experience with Chinese herbal medicine	4	39	3	5	3
No previous experience with Chinese herbal medicine	4	11	6	3	1

Table 4.8: Participant responses for the participants (n=79) concerning taste and their previous experience with Chinese herbal medicine.

4.3 Consistency of choice across the three tests

The first test involved visual appearance and as previously stated 41 participants selected capsule B the herbal capsule while 25 selected capsule A,C or D and a further 15 selected 'don't know'. Of the 41 that selected capsule B for visual appearance, 19 went on to select odour and taste indicating their choice was consistent over the three tests. A further 10 went onto to select odour in addition to visual appearance and then changed to another capsule for taste. In addition seven selected visual appearance and taste and went onto select another capsule for odour. This meant that five participants after selecting capsule B for visual appearance went on to select other capsules choices for both odour and taste.

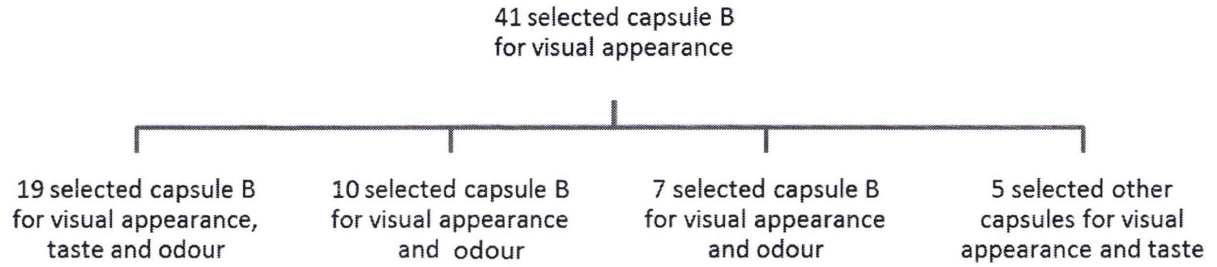


Figure 4.7: Consistency of choice for those participants who initially selected correctly for the visual assessment

The second test involved odour evaluation and 39 participants selected capsule B the herbal capsule of which 19 in addition selected capsule B for visual appearance and taste as well. Five selected capsule B for odour and taste and another capsule for visual appearance while seven participants selected capsule B for odour and visual appearance but not for taste. Eight participants selected other capsule choices for visual appearance and taste.

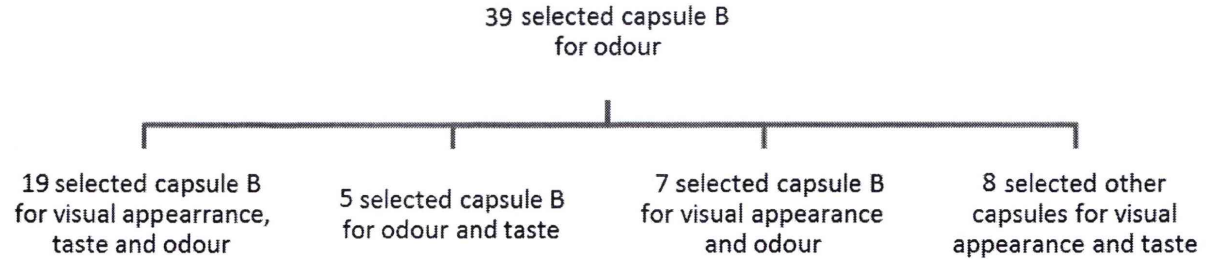


Figure 4.8: Consistency of choice for those participants who initially selected correctly for the odour assessment

The third evaluation involved taste and 50 participants selected capsule B the herbal capsule of which 19 in addition selected capsule B for taste and odour as well. Five participants selected capsule B odour and taste while 7 selected capsule B visual appearance and taste. Nineteen participants selected other capsule choices for visual appearance and taste.

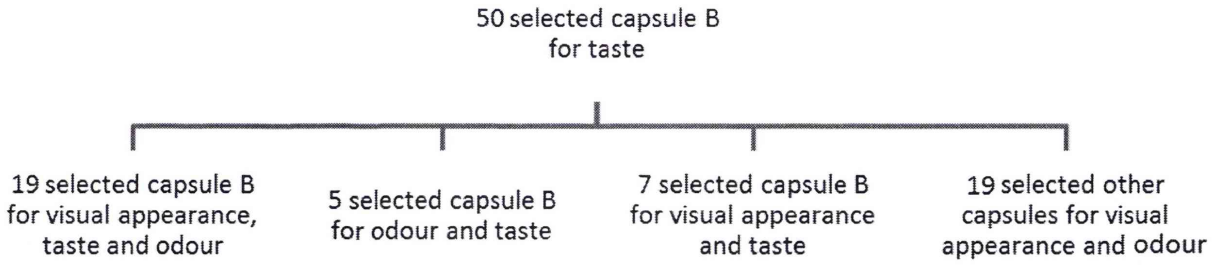


Figure 4.9: Consistency of choice for those participants who initially selected correctly for the taste assessment

The results from the examining choice consistency across the three assessment tasks indicate that the taste test was the most difficult to deceive participants followed by visual appearance and then odour. This indicated that the researcher had to modify the taste of sham substance to more closely mimic the herbal substance. Second the appearance of the sham substance also had to be modified to appear similar to the herbal substance. Both of the modifications were made and are described in section 3.4.2.3. This modified sham substance was then employed in study three.

4.4 Choice results for study three

When interpreting the results for this study using a two proportion test it is important to understand that the successful blinding requires that expectations are distributed evenly across the two groups, for example similar proportions of participants who have been allocated to the herbal group should believe they are receiving the herbal intervention and some the sham comparator. Conversely similar proportions of participants in the sham herbal group would also think they are receiving the herbal intervention and some the sham herbal

intervention. Therefore a similar proportion in both groups would infer that blinding has been successful.

4.4.1 Results for visual appearance

Tables 4.9 and 4.10 show the results for study three which involved 62 subjects for the question concerning visual appearance (n=62). A two proportion hypothesis test showed that a similar proportion of subjects (20 cf 17) in both groups believed they were receiving a herbal substance (p=0.435).

	Herbal (n=31)	Sham (n=31)
Correct choice	20	5
Incorrect choice	3	17
Don't know	8	9

Table 4.9: Frequency of correct and incorrect responses from participants receiving the sham and herbal substance for the visual appearance evaluation

		Received herbal			Received sham		
Look	**n**	**correct**	**incorrect**	**Don't know**	**correct**	**incorrect**	**Don't know**
Herbal look	6	1				5	
Colour	4	2		1		1	
Look	2	2					
Not sure	2			1			1
Dirt look	1				1		
Unable identify	1						1
Herbal fragrant	1					1	
Grain size	1					1	
Crushed tea	1						1
Ground plant	1	1					
Not look smooth	1	1					
Different granule	1				1		
Normal or other	1			1			
Hard to tell	1						1
Like bark	1				1		
Capsule content	1					1	
Plant matter	1					1	
Not normal	1						1
Unlike herbal	1			1			
Vegetable look	1	1					

Granule	2	1				1	
Too dark	1				1		
Fake look	1		1				
Not herbal look	2				2		
Brown granular	1	1					
Colour & texture	1	1					
Coffee	1		1				
Pound herbal	1					1	
Not feel as	1	1					
Smell like herb	1	1					
No reason given	12	2	1	3		3	3
Total	54						

Table 4.10: Reason given by the participants concerning their choice and the visual appearance of the substance and their choice response.

4.4.2 Results for Odour

Tables 4.11 and 4.12 show the results for study three which involved 61 subjects for the question concerning odour (n=61). A two proportion hypothesis test revealed that a similar proportion of subjects in both groups believed they were smelling a herbal substance (p=0.443). This means that the sham substance was successful in blinding an equal proportion of participants in groups, those that received the herbal material and those that received the sham material (13 cf 16).

	Herbal (n=31)	Sham (n=30)
Correct choice	13	11
Incorrect choice	13	16
Don't know	5	4

Table 4.11: Frequency of incorrect and correct responses from participants receiving the sham and herbal substance for the odour evaluation

		Received herbal			Received sham		
Odour	**n**	**correct**	**incorrect**	**Don't know**	**correct**	**incorrect**	**Don't know**
Herbal smell	9	1				8	
Spicy smell	2	1				1	
Coffee smell	2					2	
Smell like it	1					1	
Unable identify	1						1
Too sweet	1				1		
Not as strong	2				1		1
Similar to herbs	1					1	
No herbal	1				1		
Wet grass smell	1	1					
Less acrid	1		1				
Like food more	1		1				
Not Chinese	2		1		1		
Chicken stock	1				1		
Not sure	1			1			
Herbal clinic	1	1					
Liquorice smell	1	1					
Can't tell	1						1
Coffee or	1					1	
Remind of some	1					1	
Not strong herb	1				1		
Mild smell	1				1		
No scent	1		1				
Not smell	1			1			
Not smell bad	1	1					
Not herbal smell	2				2		
Slight pungent	1	1					
Too strong smell	1		1				
Don't know	2			1			1
Not fill in reason	10	3	3			3	1
Total	53						

Table 4.12: Reason given by the participants concerning their choice and the odour of the substance and their choice response

4.4.3 Results for Taste

Tables 4.13 and 4.14 show the results for the 61 subjects for the question concerning taste (n=61), Unlike the first two evaluations concerning visual inspection and odour, the taste test resulted in a statistically larger proportion of subjects in the herbal group believing they were tasting a herbal substance (p=0.002) compared to the sham herbal substance (22 cf 10).

	Herbal (n=31)	Sham (n=30)
Correct choice	22	15
Incorrect choice	7	10
Don't know	2	5

Table 4.13: Frequency of incorrect and correct responses from participants receiving the sham and herbal substance for the taste evaluation

		Received herbal			Received sham		
Taste	**n**	**correct**	**incorrect**	**Don't know**	**correct**	**incorrect**	**Don't know**
Herbal flavour/taste	6	3				2	`1
Pepper taste	5		1		4		
Coffee taste	2	1	1				
Bitter taste	4	4					
Plain taste	1						1
Cooking spice/herb	1				1		
Dried Crumb	1				1		
Similar to herb/bitter	1	1					
Nutmeg pepper	1				1		
Spicy curry	1	1					
Food or flavouring	1		1				
Completely lose	1			1			
Different herbs	1	1					
Cooking herbs	1				1		
Gritty organic taste	1		1				
Taste horrible	1					1	
Not strong taste	1					1	
Not Chinese herb taste	1						1
Spicy	1				1		

Not over bitter	1		1				
Medicine herb taste	1					1	
Woody texture	1	1					
Taste	1					1	
Chilli taste	2					1	1
Don't know	1						1
Acrid pepper	1					1	
Hint of pepper	1					1	
Distinct taste	1	1					
Curry	1				1		
Not herb taste	1				1		
Not give reason	8	4	2		1		1
Total	52						

Table 4.14: Reason given by the participants concerning their choice and the taste of the substance and their choice response

Chapter V Discussion

The blinding of participants is an important aspect of a clinical trial. This is the first time that a series of studies have been undertaken that have attempted to identify important cues and features that may threaten participant blinding in a herbal medicine trial. The three studies used specific assessment approaches including a visual assessment as well as odour and taste evaluation. In addition each succeeding study attempted to improve upon the previous investigation.

Study one, while involving only eleven participants did find that a statistically greater percentage of participants selected the capsule containing the herbal substance in preference to the other three capsules containing the sham material when comparing taste. This was also the case for study two where in all three assessments (visual, odour and taste) a statistically greater percentage of participants selected the herbal substance than the comparator sham substances. A similar situation occurred in in study three where again a larger proportion of subjects in the herbal group believing they were tasting a herbal substance compared to the group that received the sham herbal substance. The sense of taste remained in all three studies a difficult human sense to blind. This obviously presents a problem for any herbal clinical trial where there is the ability for participants to break open a capsule and taste the contents. Despite the culinary flavouring agents having a "herbal taste" participants given the sham substance could identify the pepper and curry flavours in some instances.

Concerning the visual and odour assessments, several modifications were made to the sham substances in the second and third study to mimic the herbal substance more closely. One such modification relating to visual appearance was the addition of colouring agents to minimise difference between the different shades of the substances. Prior to commencing the

project it was not known which colour would be perceived as being "herbal" especially for those naïve to Chinese herbal medicine. A variety of colours were used in the first and the second study but it became apparent at the completion of the second study that a dark brown colour was more frequently selected as suggestive of what herbal granules would look like. Thus in the third study we used a blend of colouring agents to achieve a match with the herbal material.

It should be noted that in the first study most of the sham substances were lighter in colour that the herbal substance while in the second larger study an attempt was made to colour two of the sham substance darker (capsule C and capsule D). This resulted in a larger percentage of participants picking these two capsules compared to the pilot study (15% cf 9%). Obviously colouring these substances darker resulted in a closer visual match thereby prompting a greater percentage of participants to select these two capsules.

The other issue for visual identification was the size of the granules. The herbal substance had a large granule size and clumped together more easily compared to the sham substances. In the first two studies the sham substances all used commercially produced corn flour which had been processed to have small granule size. For the final study we used ground rice which had a much coarser appearance in respect to granule size when compared to the herbal substance.

Three possible strategies to maintain visual blinding should be undertaken. The first is that the sham substances need to be coloured to be exactly the same colour as the herbal substance. This can be easily achieved using the right blend of colouring agents. Another possible solution could be to encapsulate the substances in opaque capsules which would

shield the colour of the materials. While this might be effective for visual blinding, participants could always break open the capsule and smell and taste the substances. Finally for the granule size a more coarse material should be used that has a similar size to the herbal substance.

The odour of herbal medicines especially Chinese herbal medicine can be very pungent. While we did attempt to add pungent flavouring agents to the base mixture the majority of participants still selected the herbal substance in the herbal group compared to the sham herbal group for study two. It was obvious that the herbal substance had a unique odour and when combined with the visual cues made blinding more difficult. For study two it was interesting to note that while capsule A remained at a similar proportion for both the visual and odour tests (13 participants for both the visual and odour test respectively), more participants selected capsule C and D for the odour test compared to the visual test (4 and 8 for the visual test compared to 8 and 14 for the odour tests). This infers that the additives used in both capsule C and D (ground black pepper and the chilli powder) did mislead more participants into thinking these substances were herbal.

There are several limitations to this project. One of the difficulties of this study was to develop an inert material that had no therapeutic effect yet looked smelt and tasted like a herbal substance. In fact it could be argued that the culinary herbs that were used had some therapeutic value. Curry contains both curcumin and turmeric and both have been shown to have powerful effects invivo (Hossain et al, 2012; Mythri and Bharath, 2012). The other additive was black pepper which contains pepperine and again has been shown to have therapeutic effects when ingested (Hlavackova et al, 22012). This study has demonstrated

that it is extremely difficult to identify and use truly inert substances and the idea of a sham substance that has similar sensory characteristics to the herbal medicine being used in a study may be impossible. It may be that irrelevant, rather than inert substances are used. While it may be unavoidable to use substances that have a physiological effect, effort should be made in ensuring the sham substance does not have identifiable physiological effect on the condition being evaluated.

Another limitation was the failure to blind the individual administering the task and collecting the questionnaire data. This situation was due to financial a constraint, which is lack of funding to employ someone to undertake the task. Any pre-trial evaluation should use a blinded data collector to administer the blinding questionnaire to minimise potential bias. This would be equivalent to the double blind (patient/practitioner) protocol used in RCTs.

In conclusion, the three studies have identified the difficulties with blinding in a herbal medicine clinical trial. At no stage was it possible to blind for taste. However when both the herbal and sham substance were matched for colour and granule size it was shown that blinding could be achieved for visual assessment. Similarly when appropriate culinary substances were used as scent agents blinding could be achieved for odour assessment.

Chapter VI Summary and future directions

The studies reported in this thesis have demonstrated the need for pre-trial blinding when using Chinese herbal medicine in a controlled trial. Both visual, odour and taste cues need to be assessed for both the intervention as well as the sham control arm. Effort should be made to develop a similar looking, smelling and tasting sham substance compared to the verum intervention arm. If possible inert materials, flavouring and colouring agents should be used. If this is not possible potentially active materials that have been shown to be irrelevant for the particular condition can be an option.

The blinding evaluation should use only the substances used in each arm (active herbal substance and sham substance) and should follow the design of study three. The results of the blinding should be published when the larger clinical trial is published so that readers are able to evaluate whether bias may have been introduced due to failure to blind.

Finally it is suggested that to improve the internal validity of the blinding assessment the administrator testing the blinding evaluation should be unaware of the allocation of substances so as to minimise any potential bias.

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Appendices

UNIVERSITY OF TECHNOLOGY SYDNEY

This questionnaire is to assess whether you can distinguish a herbal substance (Ganopoly manufactured by Alpha NZ P/L) from three other placebo substances. The herbal product is listed with the Therapeutic Goods Administration as a listed supplement AUSTL 71644. It is used for general health purposes. Please follow the directions and tick the most correct response.

Age..... Gender.....

Do you have any food sensitivities or allergies? YES ☐ NO ☐

Have you previously ingested herbal medicine before? YES ☐ NO ☐

If yes did it involve a pill, decoction (soup) or granules .....

After examining the four bottles of capsules, which bottle do you think contains the herbal substance? Indicate by ticking.

☐ A ☐ B ☐ C ☐ D ☐ Don't know

Why did you choose that particular bottle? (please give a reason if possible)

.....

.....

After smelling the four individual capsules, which capsule do you think contains the herbal substance? Indicate by ticking.

☐ A ☐ B ☐ C ☐ D ☐ Don't know

Why did you choose that particular capsule? (please give a reason if possible)

.....

.....

Open the capsule, taste each capsule's content. Which capsule do you think is the herbal substance. Indicate by ticking. Wash your mouth with water after taste each capsule's content.

☐ A ☐ B ☐ C ☐ D ☐ Don't know

Why did you choose that particular substance? (please give a reason if possible)

.....

Appendix 2 : Study 3 questionnaire

UNIVERSITY OF TECHNOLOGY SYDNEY

This questionnaire is to assess whether you can distinguish a herbal substance (Ganopoly manufactured by Alpha NZ P/L) from a placebo substance. The herbal product is listed with the Therapeutic Goods Administration as a listed supplement AUSTL 71644. It is used for general health purposes. Please follow the directions and tick the most correct response.

Age..... Gender.....

Do you have any food sensitivities or allergies? YES ☐ NO ☐

Have you previously ingested herbal medicine before? YES ☐ NO ☐

If yes, did it involve a (please tick which methods of delivery you have used)

Pill ☐

Granules ☐

Decoction (soup) ☐

After <u>examining</u> the capsule, do you think it is a (Indicate by tick).

☐ Herbal substance ☐ Placebo substance ☐ Don't know

Why did you choose that response? (please give a reason if possible)

.....

. .....

After <u>smelling</u> the capsule, do you think it is a (Indicate by tick)

☐ Herbal substance ☐ Placebo substance ☐ Don't know

Why did you choose that response? (please give a reason if possible)

.....

. .....

After <u>tasting</u> the contents of the capsule, do you think it is a (Indicate by tick)

☐ Herbal substance ☐ Placebo substance ☐ Don't know

Why did you choose that response? (please give a reason if possible)

Appendix 3- Statistical printouts

Study 1

TEST FOR ODOUR

1. Test and CI for One Proportion Capsule A

```
Test of p = 0.25 vs p not = 0.25

                                                    Exact
Sample  X   N  Sample p          95% CI          P-Value
1       5  11  0.454545  (0.167488, 0.766206)     0.157
```

2. Test and CI for One Proportion Capsule B

```
Test of p = 0.25 vs p not = 0.25

                                                    Exact
Sample  X   N  Sample p          95% CI          P-Value
1       4  11  0.363636  (0.109263, 0.692095)     0.484
```

3. Test and CI for One Proportion Capsule D

```
Test of p = 0.25 vs p not = 0.25

                                                    Exact
Sample  X   N  Sample p          95% CI          P-Value
1       1  11  0.090909  (0.002299, 0.412780)     0.312
```

TEST FOR VISUAL APPEARANCE

1. Test and CI for One Proportion Capsule A

```
Test of p = 0.25 vs p not = 0.25

                                                    Exact
Sample  X   N  Sample p          95% CI          P-Value
1       2  11  0.181818  (0.022831, 0.517756)     0.742
```

2. Test and CI for One Proportion Capsule B

```
Test of p = 0.25 vs p not = 0.25

                                                    Exact
Sample  X   N  Sample p          95% CI          P-Value
1       6  11  0.545455  (0.233794, 0.832512)     0.077
```

3. Test and CI for One Proportion Capsule D

```
Test of p = 0.25 vs p not = 0.25


                                                    Exact
Sample  X   N  Sample p          95% CI          P-Value
1       2  11  0.181818  (0.022831, 0.517756)     0.742
```

TEST FOR TASTE

1. Test and CI for One Proportion Capsule A

```
Test of p = 0.25 vs p not = 0.25

                                                    Exact
Sample  X   N  Sample p          95% CI          P-Value
1       2  10  0.200000  (0.025211, 0.556095)     0.750
```

2. Test and CI for One Proportion Capsule B

```
Test of p = 0.25 vs p not = 0.25

                                                    Exact
Sample  X   N  Sample p          95% CI          P-Value
1       7  10  0.700000  (0.347547, 0.933260)     0.004
```

Study 2

Test and CI for One Proportion CAPS B VISUAL APPEARANCE

```
Test of p = 0.25 vs p not = 0.25

                                                    Exact
Sample   X   N  Sample p          95% CI          P-Value
1       41  66  0.621212  (0.493357, 0.737815)     0.000
```

Test and CI for One Proportion CAP B ODOUR

```
Test of p = 0.25 vs p not = 0.25

                                                   Exact
Sample   X   N  Sample p          95% CI          P-Value
1       39  74  0.527027  (0.407498, 0.644326)     0.000
```

Test and CI for One Proportion CAP B TASTE

```
Test of p = 0.25 vs p not = 0.25

                                                   Exact
Sample   X   N  Sample p          95% CI          P-Value
1       50  75  0.666667  (0.548349, 0.771368)     0.000
```

Study 3

Power and Sample Size

```
Test for One Proportion


Testing proportion = 0.5 (versus not = 0.5)
Alpha = 0.05

Alternative  Sample  Target
 Proportion    Size   Power  Actual Power
        0.7      62     0.9      0.902857
```

That is, sample size of 62 is required.

TEST FOR VISUAL APPEARANCE

Tabulated statistics: capsule, look Cor=1 inc=2 DK=3

Rows: capsule Columns: look Cor=1 inc=2 DK=3

	Corr	Inco	DK	All
Sham	5	17	9	31
Herbal	20	3	8	31
All	25	20	17	62

Cell Contents: Count

Test and CI for Two Proportions

Sample	X	N	Sample p
1	20	31	0.645161
2	17	31	0.548387

Difference = p (1) - p (2)

Estimate for difference: 0.0967742

95% CI for difference: (-0.146244, 0.339792)

Test for difference = 0 (vs not = 0): Z = 0.78 P-Value = 0.435

Fisher's exact test: P-Value = 0.605

TEST FOR ODOUR

Tabulated statistics: capsule, odour

Rows: capsule Columns: odour

	Corr	Inco	DK	All
Sham	11	16	4	31
Herbal	13	13	5	31
All	24	29	9	62

Test and CI for Two Proportions

```
Sample   X   N  Sample p

1       13  31  0.419355

2       16  31  0.516129

Difference = p (1) - p (2)

Estimate for difference:  -0.0967742

95% CI for difference:  (-0.344001, 0.150452)

Test for difference = 0 (vs not = 0):  Z = -0.77  P-Value = 0.443

Fisher's exact test: P-Value = 0.611
```

TEST FOR TASTE

Tabulated statistics: capsule, taste

```
Rows: capsule   Columns: taste

            Corr  Inco DK         All


Sham         15   10   5         1   30

Herbal       22   7    2         0   31

All          37   17   7         *   61

Cell Contents:      Count
```

Test and CI for Two Proportions

```
Sample   X   N  Sample p

1       22  31  0.709677

2       10  30  0.333333

Difference = p (1) - p (2)

Estimate for difference:  0.376344

95% CI for difference:  (0.143994, 0.608695)

Test for difference = 0 (vs not = 0):  Z = 3.17  P-Value = 0.002

Fisher's exact test: P-Value = 0.005
```