

Herbal medicine toxicity: The role of adulterants, contaminants and pharmacokinetic interactions

By

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- P2. Farrington R, Musgrave I, Nash C, Byard RW. (2018) Potential forensic issues in overseas travellers exposed to local herbal products. *J Forensic Leg Med* 60: 1-2.
- P3. Farrington R, Musgrave I, Byard RW. (2018) Potential adverse outcomes of herbal preparation use in childhood. *Acta Paediatr*. doi:10.1111/apa.14595
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- P5. Farrington R, Musgrave IF, Byard RW. Evidence for the efficacy and safety of herbal weight loss preparations. *J Integr Med*. 2019; Epub ahead of print.
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- S7. Farrington R, Byard RW, Musgrave I. Interactions between epigallocatechin-3-gallate (EGCG) and hydroxy citric acid potentiate EGCG hepatotoxicity. *Arch Toxicol* 2019 (under review).

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Abstract

Complementary and alternative medicine (CAM) is a broad set of health care practices that are not part of a country's own medical traditions and are not integrated into the dominant health care system. CAM includes herb medicines, dietary supplements, acupuncture, diet, yoga, Tai chi, meditation, massage, Ayurvedic medicine, Traditional Chinese Medicine, naturopathy and homeopathy. Some have been used for centuries in many cultures and others are more recent innovations. CAM in all its forms is now becoming increasingly popular in Western society. For the purpose of this study, CAMs will be taken to mean only those CAM therapies which involve medications (eg. Herbals, Ayurvedic medicine, Traditional Chinese Medicine) rather than mind-body or physical therapies. Despite the popular belief that CAM is natural and therefore safe, CAMs are continually being linked to adverse reactions caused by adulterants, contaminants and heavy metals, as well as by interactions between pharmaceuticals, other xenobiotics and the phytochemicals in CAMs.

During the 18th century and into the early part of the 20th century it was a very common practice in the United States of America and Australia to include drugs such as cocaine, opium or cannabis in tonics that were used to treat a broad spectrum of conditions and diseases. Mahomet Allum was a herbalist who worked in South Australia in the early part of last century, whose herbal therapies generated some controversy at the time. Chapter 1: "Evaluation of an early 20th century Afghan herbalist's preparations" details the results of analyses of two of his preparations.

With increased interest in natural medicines, health and wellness tourism has grown, as countries in South East Asia promote traditional medicine and sell their herbal products to tourists. Chapter 2 deals with this issue: "Potential forensic issues in overseas travellers exposed to local herbal products" Fourteen processed herbal preparations were randomly selected and purchased from a traditional herbal retailer in Yangon, Myanmar (Burma) and screened for contaminants and adulterants. Toxicological results showed that only one sample contained an adulterant, but that this was yohimbine, a prescription only substance in Australia, and one that it is illegal to import.

Parents are also now taking their children to see CAM practitioners as well as administering herbal products. Chapter 3 reviews this situation: “Potential adverse outcomes of herbal preparation use in childhood” highlighting that children may be more susceptible to harmful effects due to immature physiology and metabolic pathways as well as having different dosage requirements.

Data from a collaborative project which screened 347 herbal products sold in Australia for the presence of adulterants or contaminants was used to randomly select 18 products to test *in vitro* in HepG2 (liver) and Caco2 (intestinal) cell lines. Chapter 4: “Complementary and alternative medicines in Australia: A hidden source of toxicity” demonstrates that toxicity occurs even in the absence of contaminants and adulterants.

With increasing rates of obesity in Australia, CAM products claiming weight loss and anti-obesity effects have become popular in Western society despite a lack of scientific validation. Severe adverse reactions have been reported following the use of these products, including the need for organ transplantation. This background has been addressed in chapter 5: “Evidence for the efficacy and safety of herbal weight loss preparations.”

Three CAM products advertised for weight loss that had caused adverse reactions shortly after consumption were investigated. The three products were added to HepG2 and Caco2 cells. All three supplements caused significant toxicity in both cell lines. Toxicological results showed that there were no contaminants or adulterants present. These results are summarized in chapter 6: “Hepatotoxicity associated with the use of herbal weight loss supplements.”

Epigallocatechin gallate (EGCG) and Hydroxy citrate Acid (HCA) are two common ingredients found in CAMs targeting weight loss. Increasing concentrations (0-100µM) of the two phytochemicals were added to HepG2 and Caco2 cells individually and in the presence of each other to determine if any interactions were occurring. These results are reported in chapter 7: “Interactions between epigallocatechin-3-gallate (EGCG) and hydroxy citric acid potentiate EGCG hepatotoxicity.”

These results demonstrate that despite the perceived perception of safety associated with CAMs, they have the possibility to interact with pharmaceuticals as well as with other herbal compounds. Whilst further work is required to investigate the mechanisms causing these adverse effects, the current work highlights the risk of using these preparations, and at-risk combinations that could be a significant public health concern.

Student Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Abbreviations

ADRs	Adverse drug reactions
BuCl	Butyl chloride
C. aurantium	Citrus aurantium
C. forskohlii	Coleus forskohlii
C.sinensis	Camilla sinensis
CAM	Complementary and Alternative Medicine
CYP	Cytochrome
DMEM	Dulbeccos Modified Eagle Medium
DNA	Deoxyribonucleic acid
EGCG	Epigallocatechin-3-gallate
FCS	Foetal calf serum
G. Cambogia	Garcinia cambogia
GC-NPD MS	Gas chromatography/nitrogen phosphorous detector mass spectrometer
HCA	Hydroxy citrate acid
HPLC	High performance liquid chromatography
LC-QTOF MS	Liquid chromatography/ quadrupole time-of-flight mass spectrometer
LC-UV	Liquid chromatography diode array detector
MTT	3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide
PBS	Phosphate Buffer Solution
SPE	Solid phase extraction

TCM	Traditional Chinese Medicine
TGA	Therapeutic Goods Administration

Statement of Authorship

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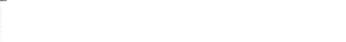
Principal Author

Name of Principal Author (Candidate)	Rachael Farrington		
Contribution to the Paper	Performed toxicological analysis, wrote manuscript, acted as corresponding author.		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	20/01/19

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- the candidate's stated contribution to the publication is accurate (as detailed above);
- permission is granted for the candidate to include the publication in the thesis; and
- the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Ian Musgrave		
Contribution to the Paper	Helped to edit and evaluate manuscript.		
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Name of Co-Author	Christine Nash		
Contribution to the Paper	Assisted in toxicological analysis and quantified toxicological results		
Signature		Date	22/01/19

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Contribution to the Paper	Performed DNA analysis on herbal preparations Interpreted results		
Signature		Date	28/01/2019

Name of Co-Author	Roger Byard		
Contribution to the Paper	Helped to edit and evaluate manuscript.		
Signature		Date	24/1/19

1. Evaluation of an early 20th century Afghan herbalist's preparations.

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1.1 Abstract

Mahomet Allum was a flamboyant philanthropist and herbalist who worked in South Australia in the early part of last century, whose herbal therapies generated some controversy at the time. Two of his preparations have survived to the present day, a general tonic and a treatment for liver and kidney dysfunction. Given the frequent use of pharmaceutical drugs in "tonics" at the time, toxicological analysis was undertaken at Forensic Science SA, Adelaide with liquid chromatography/quadrupole-time-of-flight mass-spectrometer (LC-QTOF MS), liquid-chromatography/ diode array detector (LC/UV) and gas chromatography/ nitrogen phosphorous- detector/mass-spectrometer (GC-NPD/MS), to look for common drugs. In addition DNA analysis was also undertaken at Trace and Environmental DNA (TrEnD) Laboratory (Curtin University) to evaluate the types of plant products used to make these remedies. The general tonic contained genera from the Triticeae (wheat) family as well as the Medicago family (includes alfalfa), possibly as fillers. Other genera found included Urtica (nettle) and Passiflora (passion flower). The preparation for liver and kidney disease also contained genera from the Medicago family as well as genera Arctostaphylos (bear berry) which has traditionally been used for the treatment of dysuria and bladder stones. No common drugs were found. Thus it appears that the two treatments prepared by Mahomet Allum contained only herbal substances and not adulterant pharmaceutical agents. The herbals identified provide an insight into herbalist practices in the early twentieth century

Keywords: Mahomet Allum; herbal preparations; early 20th century; Afghan

1.2 Lessons from the Museum

Mahomet Allum, one of South Australia's most iconic herbalists and philanthropists, was often described as a "a very colourful character" with his dyed hair, eye-catching jewellery and a love of publicity attracting a great deal of attention (Fig. 1.1). Along with this, his practice of alternative medicine caused some controversy within the community [1]. Hanji Mahomet Allum, better known as Mahomet Allum, was born in Kandahar, Afghanistan in 1858. In the late 1880's he sold horses to the British Army earning him enough capital to move through Asia prior to his arrival in Australia in the late 1880's. As he travelled through Australia he used camel teams to deliver goods to isolated farms and townships, as well as working as a miner, rug dealer, station hand, butcher and a storekeeper [1, 2].



Fig 1.1: Image of Mahomet Allum in 1910
State Library of South Australia: B43851

In 1928-29 he moved to Adelaide where dissatisfaction with standard medical treatments enabled him to set up as a herbalist [1-3]. He prepared herbal therapies and according to the Australian dictionary of biography asked for no payments, although he accepted donations and gave freely to charities. His popularity and abilities were written about by his patients (and himself) in testimonials, advertisements in the *South Australian Police Journal* and newspapers (Fig. 1.2), and even in a pamphlet that he published [1, 2]. An article published in 1933 highlighted his popularity and work:

“Mr. Allum's diagnosis of the most obscure malady is as uncannily correct as his treatment is efficacious. His knowledge of herbs, the heritage of an ancient race, handed down from father to son, represents the accumulated wisdom of the ages, and is now applied in a strange land for the benefit of those who, while differing in creed and color, he regards with an all-embracing love as brother man and sister woman” [4].



Figure 1.2: An advertisement and portrait of describing the services and healing powers of Mahomet Allum.
State Library of South Australia B52941

During a court case in 1935 when he was charged with impersonating a medical practitioner while not being registered under the Medical Practitioners Act, forty witnesses stated that he had never claimed to be a doctor. Although in the end he was convicted and fined, it is thought that the publicity of the trial probably brought him more customers [2].

Allum became well-known for his stomach wash, which he called “blackjack” and which was also known as an “Allum bar” [3, 5]. His belief was that most Western ailments were caused by an unclean stomach and so clients were given “blackjack” stomach wash to completely purge their systems. Although the actual composition was kept a secret, it appeared to be comprised of butter, honey and laxative senna pods [3, 5]. Following treatment of ‘blackjack’ customers were then given advice or herbal mixtures for ailments troubling them including lotions (Fig. 1.3) [3, 6].

During the 18th century and into the early part of the 20th century it was a very common practice to include drugs such as cocaine, opium or cannabis in tonics that were used to treat a broad spectrum of conditions and diseases ranging from “blind Piles” (hemorrhoids) and “puerperal after-pains” to childhood teething

problems [7-10] (Fig. 1.4). The entry under opium in the 1796 Encyclopedia Britannica quoting an “*Essay on Diseases of the Viscera*” reveals very clearly how highly the drug was regarded:

“*Opium at present is in great esteem, and is one of the most valuable of the simple medicines. In its effects on the animal system, it is the most extraordinary substance in nature. It touches the nerves as it were by magic, and irresistible power....*” [11].



Figure 1.3: Original packaging of a herbal lotion (Dewyee Remedy) prepared by Mahomet Allum.

Migration Museum collection courtesy R Brown

State Library of South Australia: 2012-49&50.

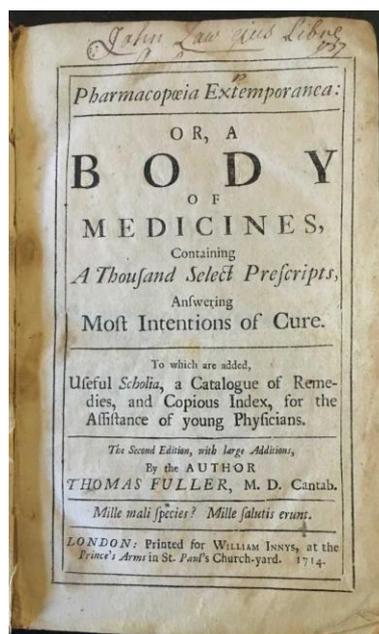


Figure 1.4: The frontispiece of the Pharmacopoeia Extemporanea by Thomas Fuller published in 1714 which contains numerous recipes for laudanum [10].

Given the apparent popularity of Mahomet Allum's potions and the widespread use of additives it was decided to investigate whether the two surviving preparations might contain pharmaceutical adulterants. The pills were made on a hand press as common at the time. Toxicological analysis was undertaken at Forensic Science SA, Aelaide using a liquid chromatography/quadrupole-time-of-flight mass-spectrometer (LC-QTOF MS), liquid-chromatography/ diode array detector (LC/UV) and gas chromatography with nitrogen-phosphorous and detector/mass-spectrometer detectors (GC-NPD/MS) using standard methodology [12]. At the same time next generation DNA characterisation of the samples was performed at Trace and Environmental DNA (TrEnD) Laboratory (Curtin University), again using standard methodology [13] [Table 1.1].

Table 1.1: Biological content of Mahomet Allum Medicines

Sample	Plant DNA	Animal DNA
7X	Medicago (legume family), Passiflora (passion flower/passion vine), Urtica (nettles/stinging nettles), Triticeae (grasses), PACMAD clade	-
P14	Apiaceae (numerous possibilities), Nassauvia (sunflower family), Trixis (threefolds/daisy), Triptilion (sunflower family), Phaenocoma (pussy's-toes tribe/ sunflower family), Arctostaphylos (manzanitas and bearberries), Medicago (legume family), Nasturtium or Cardamine (bittercresses), Coleonema (Diosma) or agathosma, PACMAD clade	-

Note: Apiaceae are the commonest botanical family recommended by Shirazian herbalists for the treatment of kidney stones [15].

No common prescribed or illicit drugs were detected in either preparation. DNA analysis identified genera from the Triticeae (wheat) family as well as Medicago, possibly used as fillers in the general tonic although in Persian traditional medicine *Medicago officinalis* was used as a diuretic *Medicago sativa* was used for kidney stones [14, 15]. Other genera that were found in this tonic included *Urtica* (nettle) and *Passiflora* (passion flower). Herbalists have used *Urtica* leaves in the treatment of numerous systemic and

dermatologic inflammatory conditions [16]. The leaves have also been prepared as a tea with diuretic and antidiabetic therapies, and to treat stomach disorders [17]. *Passiflora* has long been used for its sedative and anxiolytic effects and is commonly added to herbal remedies to treat nervousness and insomnia [18, 19]. Somewhat surprisingly, given Mahomet Allum's beliefs, no purgatives (eg. Senna) were found.

The tonic for liver and kidney function also contained genera from the *Medicago* family; *Medicago sativa* was used for kidney stones as well as *Medicago officinalis* as a diuretic [15]. In addition to genera *Arctostaphylos* (bear berries) which has traditionally been used for the treatment of genitourinary symptoms from cystitis, urethritis, pyelitis and stones [20]. However, there is little published data to support its efficacy. Other families were detected, including Brassicaceae with the closest matching genera being *Nasturtium* or *Cardamine* and the Rutaceae with the closest matching genera being *Coleonema* or *Agathosma*, all four of these genera have been shown to have diuretic properties [21-23]. In particular *Arctostaphylos* and *Nasturtium* form part of the Middle-Eastern herbal tradition to which Mahomet Allum was heir [14, 24].

In conclusion, the two herbal preparations that were tested showed no evidence of pharmaceutical adulteration, although it is possible that age-related degradation may have reduced the likelihood of detection of some substances/herbs. The analysis did, however, identify some of the species of plants used by Mahomet Allum in creating his remedies and gives an insight into the practise of herbalism at this time. After Allum's death in 1964 his estate of £11,218 was given to institutions that cared for children [1] - a very fitting legacy for a remarkable individual.

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Informed consent: Not required

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- iv. the candidate's stated contribution to the publication is accurate (as detailed above);
- v. permission is granted for the candidate to include the publication in the thesis; and
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2. Potential Forensic Issues in Overseas Travellers Exposed to Local Herbal Products

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2.1 Short Communication

Herbal medicines are an important and culturally accepted part of healthcare globally, offering an accessible and affordable way for many to utilize particular therapies. The use of these products is also growing in many Western countries including the United Kingdom, the United States, Canada and Australia. For example, a recent audit of the Australian complementary medicine industry showed that it has generated revenues of \$4.7 billion due to this increase in interest and demand from consumers in Australia and overseas (Comp Med Aust). As part of health and wellness tourism Western travellers to many Asian countries now often participate in visits to herbal centres where free health checks may be performed, and herbal products offered for sale. The problem with this activity is that the composition of many of these products is uncertain, there may be contaminants and pharmaceutical additives, and the interaction with prescription medications may be idiosyncratic (Byard herbs; Ting). Herbal medicines generally are not often considered in medicolegal cases (Byard Musgrave).

While the possibility of occult sepsis has been discussed in the context of recently returned visitors from overseas the potential for forensic issues to arise from herbal medicine has not (Byard travel; dengue). Examples of problems that may be found with traditional preparations were exemplified by Huang et al's study in 1997 of 2609 samples of traditional medicines collected by eight major general hospitals in Taiwan which showed that 618 (23.7%) were adulterated with synthetic therapeutic substances (drugs) and that 52.8% of these contained 2 or more adulterants (Huang). Yee et al's study of 3,320 Chinese medicines screened between 1990-2001 showed that 138 contained toxic heavy metals at levels above legal limits and 41 contained 19 synthetic drugs (Yee, 2005). A study of all cases of herbal antidiabetic products referred to a tertiary centre for toxicological analysis in Hong Kong from 2005-2010 showed that 27 of the cases contained adulterants, eight of which were undeclared registered or banned oral antidiabetic agents such as glipalamide, phenformin, metformin, rosiglitazone, gliclazide, glimepiride, nateglinide and repaglinide. One sample contained four adulterants and 63% of the patients had clinical manifestations, most often arising from hypoglycaemia or lactic acidosis (Ching 2012).

More recently, and of forensic significance, was a study of 61 patients in Hong Kong who had consumed traditional Chinese medicines that had been adulterated with corticosteroids, most often dexamethasone. Seven of the patients (11.5%) required admission to intensive care, two died within 30 days of presentation, and 38 (62.3%) had complications due to the corticosteroid adulterant (Chong). Finally, a recent study of 404 cases referred for analysis to a tertiary referral toxicology laboratory in Hong Kong revealed 1234 adulterants which included “approved drugs, banned drugs, drug analogues and animal thyroid tissue.” Approximately 65% of the patients had adverse clinical effects directly attributable to these substances, with 14 severe and two fatal cases. The three most common presentations were psychosis, Cushing syndrome and hypoglycaemia (Ching 2018).

To investigate this issue further 14 processed herbal preparations in capsule, tablet and powder form were randomly selected and purchased from a traditional herbal retailer in Yangon, Myanmar (Burma). The samples were analysed for the presence of contaminants and adulterants according to established methods (Hoban) using a liquid-chromatography/quadrupole-time-of-flight mass-spectrometer (LC-QTOF), a liquid-chromatography/diode array detector (LC/UV) and a gas-chromatography/nitrogen-phosphorous-detector/mass-spectrometer (GC-NPD/MS). Toxicological results showed for both ethanol and basic extractions that only one sample contained an adulterant, yohimbine.

Yohimbine is an α_2 -adrenergic receptor antagonist which acts on serotonergic and adrenergic receptors in the brain in areas associated with libido and penile erection (Morales; Balon). It is also become popular for athletic performance and weight loss in body-builders (Ostojic). Given that the adulterated product was being marketed to enhance health and vitality, and to treat impotency and erectile dysfunction it was likely a deliberate additive. In Australia yohimbine is a prescription only substance, one that it is illegal to import (FRL 2018; TGA).

The published studies and the reported analysis show that individuals from Western countries who travel through Asia may be exposing themselves to potentially harmful substances or drugs in local herbal preparations that may have a significant negative impact on their health. The possibility of these materials either contributing to, or causing, death should be considered in all medicolegal cases involving recent

overseas travel, particularly to Asian destinations. The lack of cases reported from forensic facilities may merely reflect a failure to check for these products at the time of post mortem assessment.

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3. Potential Adverse Outcomes of Herbal Preparation Use in Childhood

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3.1 Abstract

Aim: Complementary and alternative medicines are becoming increasingly popular worldwide with a variety of purported medicinal uses. These products are generally believed to be natural and therefore safe, with few adverse reactions. With this perception, parents are now taking their children to see practitioners prescribing these medicines as well as self-prescribing. Despite this, there are issues regarding safety, efficacy and regulation, with increasing numbers of reports of adverse reactions to these products. Therefore, a mini-review was conducted to ascertain the potential risks to children.

Methods: A overview of literature was conducted to highlight the current use of complementary and alternative medicines in children and the possible risks associated with their use.

Results: Infants and children may be more susceptible to harmful effects due to their immature physiology and metabolic pathways and different dosage requirements. Adverse reactions may also be caused by interactions with conventional medicines, contamination with heavy metals, and adulteration of filler products including other plant species or pharmaceutical agents.

Conclusion: As complementary and alternative medicines become increasingly used alongside and with conventional drug therapy, there needs to be greater awareness and discussion among parents, complementary practitioners and medical practitioners to ensure the overall health and safety of children being exposed to these products.

Key Notes

- The use of complementary and alternative medicine continues to increase as they are believed to be safer with few side effects.
- Despite perceived safety, adverse events following the use of these products have been documented, with children at greater risk
- To ensure overall health and safety of children, greater awareness and conversation between parents and both medical and complementary practitioners needs to occur.

3.2 Introduction

Complementary and alternative medicines (CAM) have become increasingly popular worldwide with a variety of purported medical uses. CAM is an umbrella term used to describe a wide range of products including medicinal products containing such ingredients as herbs, vitamins, minerals and nutritional supplements, as well as homoeopathic and certain aromatherapy preparations (1). Despite the controversy around these products, including issues of efficacy, safety and dosing, there has been an increased use of CAMs in children (2-5).

This is because it is generally believed that CAMs are safe with fewer side effects compared to conventional medicines; thus more parents are choosing these products for their children (6-7). Despite this belief, there are issues which include lack of clinical research into the possible adverse effects of these products in children, failure to use effective conventional medicines, and possible interactions between pharmaceutical and herbal preparations.

3.3 Usage

A recent systematic review conducted by Italia et al. (8) analysed patterns of CAM usage among children reported in 58 studies from 19 countries. It was found that CAM usage by children varied widely from 10.9 to 87.6% for lifetime use, and from 8 to 48.5% for current use, depending on nationality and CAM modalities. The majority of the studies reviewed included all CAM modalities. Fewer studies reported on CAM practitioners seen or individual CAM treatments. However herbal usage by children varied from 0.8 to 85.5% for lifetime use and homeopathy usage varied from 0.8-39%, with significant differences in usage by country (8).

Frawley and colleagues (9) investigated the prevalence of CAM usage by Australian children over a 12 month period in 2016 and found that 73.8% of parents had taken their child to visit a CAM practitioner and/or had given their children a CAM product in the previous 12 months. In comparison, a study conducted by Smith and Eckert a decade earlier in 2006, investigating the use of CAM in South Australian children found that 18% of children had used a CAM product or consulted with a CAM practitioner in the previous year (10). Another study conducted in 2006 compared the use of CAM amongst children at

hospital outpatient clinics in Australia and Wales and found that 51% of Australian children had used CAM in the previous 12 months (11). Non-prescribed vitamins and minerals were the most common medicinal types of CAM used and aromatherapy and reflexology were the most prevalent non-medicinal CAMs (11). Some of the difference between the Smith and Eckert study (10) and the Crawford Study (11) may be due to Smith and Eckert only considering megadoses of vitamins as CAMs (10). While usage increased from 2006 to 2016, patterns of use were similar, with most CAMs being used for health maintenance and vitamins being used more often than herbals (9,11). These findings demonstrate the increasing popularity of these products in paediatric populations.

3.4 Potential adverse effects

Potential adverse events due to CAMs can range from events precipitated by the intended ingredients (e.g. bleeding and Gingko), events due to adulterants and contaminants or failure to use conventional medicine. A study by Lim et al. (12) investigated adverse events associated with CAM use reported to the Australian Paediatric Surveillance Unit between January 2001 and December 2003. Questionnaires were distributed to Australian paediatricians who had reported a suspected CAM-associated adverse event. Over the study period, there were a total of 46 cases documented. The ages ranged from birth to 16 years, with adverse events from mild to severe, with four fatalities. In 25 of cases (64%), the adverse events were rated as severe, life-threatening or fatal. In 30 cases (77%), the adverse events were either probably or definitely related to CAM. In 17 cases, the paediatricians considered that the child had suffered harm because of the failure to use conventional medicines of proven efficacy. The four fatalities included an 8-month old and a 10-month old who developed septic shock which was treated with CAM therapies rather than conventional medicines (12).

Meinchke et al. (13) used data from VigiBase®, a self-reporting system for medical professionals, to investigate hypersensitivity reactions (type 1) in children (age <18 years) caused by herbal medicines. Data between 1968 and 2014 were reviewed. Inclusion criteria for the study required herbals to be classified as *the suspect* and to have an Herbal Anatomical-Therapeutic-Chemical code. Reaction terms less suggestive of hypersensitivity were excluded, and no cases with gastrointestinal symptoms were

examined. A total of 26,909 unique Individual Case Safety Reports relating to herbal medicines from 42 different countries were identified, 79 cases of which met inclusion criteria, with 107 adverse drug reactions (ADRs) reported. Common ADRs were rashes (22.4%), urticaria (22.4%) and erythema (15.0%). The recovery rate of the patients from one or more ADRs was 72.0%, and there were no reports of fatal events. In the nine ADRs reported as “not recovered,” rash and urticaria were most commonly caused by a variety of herbal medicines.

3.5 Interactions

As herbal medicines become integrated into Western society, concurrent use of pharmaceuticals and herbal products is increasing. In the United States it was found that 20- 30% of patients had used both herbal medicines along with prescription medication (14-15). There is limited information provided to consumers regarding the risk associated with herb-drug interactions associated with the concurrent use of pharmaceuticals and herbal medicines. Several active compounds found in herbal medicines can serve as substrate enzymes involved in the metabolism of xenobiotics. However, little is known regarding the pharmacokinetics of many of the phytochemicals present in herbal materials. Co-administration of herbal and conventional medicine increases the risk of adverse reactions, with children being more vulnerable due to their immature physiology and metabolic pathways and the varying dosages.

3.6 Case studies

Contamination and adulteration of herbal medicines also pose a threat. Table 3.1 provides examples of how potential adverse reactions can occur when children are exposed to herbal preparations with intrinsic toxicity. Heavy metals including lead can be intentionally added to herbal preparations as an essential component or by contamination. Table 3.2 highlights examples and the consequences of heavy metal exposure to children through the use of herbal medicines.

Table 3.1: Adverse events associated with herbal preparations

Herbal Product	Adverse Events	Mechanism of toxicity	Comments
Jin Bu Huan ¹⁶	Lethargy, breathing abnormalities, CNS depression	Incorrect plant used (genus <i>stephanina</i> instead of genus <i>polygala</i>) Contamination with lead	Highlights issues with herb identification, product substitution and mislabelling. Consider use of herbal medicines when idiopathic systems present
Pennyroyal Oil ¹⁷⁻²⁰	8-week-old experienced fulminant liver failure, cerebral oedema and necrosis 6-month-old experienced hepatic dysfunction, epileptic encephalopathy	Pennyroyal contains a hepatotoxic compound: pulegone Pulegone primary compound, menthofuran is highly toxic	Misidentification/substitution of plant <i>Mentha pulegium</i> Traditional use in Hispanic and other cultures if to give infants tea made from boiled mint leaves
Germander ²¹	Abdominal discomfort, dark urine, jaundice and liver enzyme abnormalities	Patient had been consuming 600mg/day of germander along with arginine/betanine supplements	Herbal therapy should be considered in all children who present to hospital with “idiopathic’ hepatic necrosis

Table 3.2: Adverse events associated with heavy metal contamination in herbal preparations.

Herbal product	Adverse events	Mechanism of toxicity	Comments
Local Chinese medicinal herbal spray ²²	5-year old became irritable, continually clearing throat and developed a rash. Sudden onset of motor tics	Patient had been using the spray up to 20 times a day. Recommended dosing was 1-2 times a day. Product contaminated with mercury	
Medicated topical baby powder ²³	3-year old suffered chronic constipation, abdominal pain for 6 months.	Although appearing in good health and no developmental delays the patients blood lead level was 330 µg/l. Sister of patient had a blood lead level of 248 µg/l.	Highlights the importance to examine all family members to investigate exposures Patient and sister were observed over the following years with the blood lead levels slowly dropping.
Various traditional Chinese ²⁴ herbal medicines for minor ailments	4-month-old infant experienced fever, cough, anorexia, vomiting a week prior and seizure before hospital admittance.	Blood lead level was 137µg/l. After testing it was discovered that the herbal preparations contained lead	
Tibetan Herbal Vitamin from India used to promote “brain growth” ²⁵	5-year-old developed encephalopathy, seizures, developmental delay and anaemia	Patients blood lead level was 86 µg/dL. Herbal preparation contained lead and mercury ranging from 54.8- 35,300 mg/kg and 3-12,800 mg/kg respectively.	The patient was estimated to have consumed 63g of lead over four years of taking the vitamin. Over the following four years, patient was readmitted seven times to reduce blood lead levels.

3.7 Conclusion

Although CAMs are becoming an integral part of therapy in the community, there are issues regarding efficacy, safety and regulation of these products. With many children visiting CAM practitioners and/or using CAM products, often bought over the counter in pharmacies or in non-medical settings such as online or health food stores, it is likely that adverse reactions are not being monitored or reported accurately. As the popularity of these products continues to increase there is a need for further discussion and stronger relationships among CAM and general practitioners and parents to ensure that the optimal health and safety of children is achieved.

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4. Complementary and alternative medicine in Australia: a hidden source of toxicity

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4.1 Abstract

Background: Complementary and alternative medicines (CAM) has become an integral part of healthcare for many Australians. Despite the perceived safety of these products, adverse reactions following the use of these products continue to be report.

Aim: To gain further understanding on the quality and safety of herbal preparations on the market.

Methods: A large-scale screening of CAMs currently on the market was conducted between 2014-15 to identify products which has been adulterated or contaminated. Results from this study was used to test these preparations *in vitro* for toxicity using HepG2 and Caco2 cell lines, to identify if adverse reactions are being caused by the presence of contaminants and/or adulterants or are due to the herbal materials themselves.

Results: Ten products from several therapeutic categories showed significant toxicity when added to HepG2 cell cultures. Of these, 8 products showed significant toxicity in both cell lines. Three out of the eight products had the presence of an adulterant or contaminate.

Conclusion The results suggest that even when adulterants and contaminants are not present, toxicity was observed. With the continual growth of this industry within Australia, further research into the safety of these products needs to be conducted to ensure the health of consumers.

4.2 Introduction

Complementary and Alternative Medicine (CAM) is an umbrella term used to describe a wide range of alternative therapies and medicines including products containing vitamins, herbs and minerals, homeopathic medicines, nutritional supplements and aromatherapy products [1]. In Australia, CAMs are fast becoming an integral part of healthcare, with an estimated two out of three Australians using these products [2-3].

The Therapeutic Goods Administration (TGA) is the Australian national regulatory body for all medicinal and therapeutic goods. The current regulation for CAMs uses a two-tiered risk-based approach, based on clinical and scientific information [4]. The status of low risk preparations, which includes most herbal preparations, is determined by ingredients within the supplement and the risk associated with them. Previous studies have highlighted discrepancies between reported, listed and actual composition for herbal medicines which may pose risks to consumers and challenges to regulatory bodies [5-7]. These supplements receive less checking by the TGA which relies on self-assessment by manufacturers and providers [4]. Around 98% of CAMs are listed medicines, needing only to comply with safety and quality criteria, but not efficacy, unlike registered medicines which are considered higher risk preparations and whose efficacy must be proven along with their safety and quality. Safety is regulated by only allowing ingredients that come from a pre-approved list of low risk herbs, requiring manufacturing facilities to hold Good Manufacturing Practice certificates and periodically sample and test listed CAMs. Despite this approach, products that have been contaminated or adulterated can be missed [8-10]

While under-reporting of adverse drug reactions (ADRs) to herbal medicines makes it difficult to gauge the potential risks [11], studies have shown serious ADRs to CAMs, similar to those seen from prescription medicine. These have included acute liver injury, kidney failure, heart toxicity, cancer, pulmonary embolism, psychosis and death [12-16]. Studies have shown substitution and/or contamination with plant materials, pharmaceuticals and heavy metals and other fillers which can cause ADRs [6] [17-19]. Given that there is minimal, or no information known about the pharmacokinetics of many phytochemicals

present in herbs, potential pharmacokinetic interactions may also occur between the numerous ingredients often present in supplements causing ADRs [20].

To gain further understanding of the situation within Australia, a large-scale screening of CAMs currently on the market was conducted between 2014-2015 to identify the percentage of contamination and adulteration of these products. This study used these data to test these preparations *in vitro* for toxicity, to identify if adverse reactions are being caused by the presence of contaminants and/or adulterants or are due to the herbal materials themselves.

4.3 Methods

4.3.1 Sample Collection

A total of 347 CAM products used for a range of therapeutic effects were randomly selected and purchased from pharmacies, health food stores, traditional herbal retailers and online in Australian capital cities during 2014-2015 [21]. Samples were allocated random identification numbers and aliquots were made using sterile DNA free techniques into sterile DNA-free vials for later processing according to standard methodologies [4]. Based on descriptions found on product labels samples were allocated into the following general therapeutic categories: anti-inflammatory and analgesic; psychotropic and sleep; respiratory, cold and influenza; gastrointestinal tract, diet and metabolism; cardiovascular and hypertension; reproductive and fertility.

4.3.2 Pharmaceutical and Adulterant Screening

4.3.2.1 Ethanol Extraction

Samples were crushed to powder, and 50mg was weighed out into test tubes. 25 μ L of internal standard mix and 1mL of Univar Absolute Ethanol (analytical reagent grade) were added to the test tubes, which were then sonicated for 15 minutes, and centrifuged for 10 minutes at 3000rpm (according to standard methodology at Forensic Science SA). The supernatant was transferred into clean glass disposable tubes and evaporated to dryness using a centrifugal evaporator. The extract was reconstituted with 100 μ L of

Univar Absolute Ethanol (analytical reagent grade) and transferred to 2mL autosampler vials containing a tapered insert [22].

4.3.2.2 Basic Extraction

Samples were crushed to powder, and 50mg was weighed out into individual test tubes. 25µL of internal standard mix, 1mL of glass distilled H₂O and 250µL of Chem Supply 30% conc. Ammonia (analytical reagent grade) was added. The test tubes were vortexed for 5-10 seconds. 5mL of Honeywell B&J Brand High Purity Solvent butyl chloride (BuCl) was added to the test tubes, and were then extracted using a mechanical rolling for 15 minutes. Samples were then centrifuged for 10 minutes at 3000rpm. The BuCl layer was transferred into clean glass disposable tubes, and evaporated to dryness using a centrifugal evaporator. The extracts were reconstituted with 100µL of Univar Absolute Ethanol (analytical reagent grade) and transferred to 2mL autosampler vials containing a tapered insert [22-23].

4.3.2.3 Instrumentation

Sample extracts were analysed by an Agilent 1200 LC 6510-QTOF in dual ESI mode fitted with a Waters Acquity BEH C18, 1.7µm, 3.0 mm x 50 mm (Waters part no. 186004660) column, with a guard cartridge (Phenomenex C18 4 x 3mm; part no. AJO-4287 (LC-QTOF)). Analysis was repeated on an Agilent 1100 series High Performance Liquid Chromatography (HPLC) system with diode array detector fitted with an Agilent Eclipse plus C18, 1.8µm, 4.6 x 50mm column with guard cartridge (Phenomenex C18 4.0 x 3.0mm; part no. AJO-4287). Sample extracts underwent further analysis on an Agilent 7890 Gas Chromatograph with nitrogen-phosphorous and mass spectrometer detectors (GC-NPD). Data from the LC-QTOF was processed using the commercially available Agilent Forensic Toxicology PCDL Library (ForTox_AM_PCDL – Broecker, Herre, and Pragst). This library contains accurate mass MS/MS spectra of 3490 pharmaceuticals, drugs of abuse and pesticides and herbicides as well as libraries developed in house (350 pharmaceuticals and drugs of abuse, retention time and MSMS search). Data from LC-UV was processed using libraries developed in house; including a range of compounds including anti-convulsants, analgesics and anti-inflammatories. The GC-NPD/MS data used the NIST/EPA/NIH Mass

Spectral Library (NIST 2008), containing over 200,000 compound spectra as well as libraries developed in house [22].

4.3.2.4 Quantitation

Analytes were extracted from the sample using mixed mode Solid Phase Extraction (SPE) (System48 Cerex Pressure Processor). The analytes were protonated by the addition of a pH 5.7 acetate buffer (2mL) and were retained on the UCT XTRACT®, 200mg/ 3mL. P/N XRDAH203 cartridge (PM Separations Pty Ltd) by cation exchange. Analyte elution was achieved by passing a basified mixture of CH₂Cl₂/i-PrOH through the SPE cartridge. The eluent was evaporated and the residue was reconstituted in methanol. The extract was analysed by LC-MS/MS (ABSciex 4000QTRAP with the Agilent 1200 LC-autosampler system) using electrospray ionisation in the positive mode and multiple reaction monitoring with scheduling [22].

4.3.2.5 Calibration and Quality Control (QC) solutions

A combined working standard solution was prepared by accurately transferring the appropriate volume of each stock solution to a volumetric flask, making up to volume with ethanol [22].

4.3.3 Toxicological screening

Using the data obtained from both pharmaceutical and adulterant screening and DNA screening, CAMs from each therapeutic category were randomly selected for toxicological screening *in vitro*. The preparations were chosen using a random number generator to include two preparations with no evidence of contamination or adulteration, and one preparation which had either a contaminant or adulterant from each therapeutic category.

4.3.3.1 Cell culture

As common adverse events to herbal medicines include hepatotoxicity and issues relating to the gastrointestinal tract two cell lines were chosen to evaluate the toxicity of products. Human liver carcinoma cells (HepG2) and Human colon carcinoma cells (Caco2) were chosen as models of hepatocytes and

intestinal epithelial cells respectively. Both cell lines were maintained in 75cm³ culture flask grown in Dulbecco's Modified Eagle Medium (DMEM) and supplemented with 10% foetal calf serum (FCS), 1% non-essential amino acids and 1% of penicillin streptomycin. Cells were cultured at 37°C with 5% carbon dioxide. Experiments and cell maintenance were performed in a laminar flow hood under sterile conditions. Cells were passaged every 3-4 days and detached from flasks using 1 x trypsin EDTA. Cell counts were performed using trypan blue to stain cells. Ninety-six well plates were used for cell viability experiments, where cells were seeded in DMEM with 10% FCS at a density of 1.2 x 10⁶ cells per well. The cells were then allowed to equilibrate for 48h before the experiment.

4.3.3.2 Bioactive pre-treatments

Metabolism of the phytochemicals in some herbal preparations can cause greater toxicity due to the generation of toxic metabolites [24]. Thus preparations were examined in the presence of absence of cytochrome P450 3A4 induction. The cytochrome P-450 3A (CYP3A) enzyme family is responsible for most of the drug metabolism in the human liver, as well as a number of important phytochemicals with the most common isoform being CYP3A4 [24-25]. Rifampicin at the concentration of 2mM was added to DMEM and plated onto the cells for 48 hours to induce CYP3A4.

4.3.3.3 Effect of CAM preparations on cell lines

Herbal supplements were crushed to a fine consistency. Sterile PBS was added to make a stock solution of 30mg powder/ml for each preparation. Stock solutions were diluted using sterile PBS to final concentrations ranging from 0.1 to 3 mg powder/ml. If there was no observed toxicity observed in HepG2 cell lines, experiments ceased and did not continue in Caco2 cell line.

4.3.3.4 Cell Viability Measures

Cell viability was determined using the thiazolyl blue tetrazolium bromide (MTT) cytotoxicity assay. MTT is taken up by viable cells and converted in the mitochondria to an insoluble blue formazan product. The intensity of colour is proportional to the level of active mitochondria in living cells. After incubation of HepG2 and Caco2 cells with dilutions of herbal preparations for 48 hours, 96-well plates had all media

removed and replaced with serum-free media containing 0.25mg/ml of MTT. The plate was further incubated for 3h at 37°C in a 5% CO₂. atmosphere, then the MTT solution was removed and DMSO was added to lyse cells. The absorbance was then measured at 570nm using a PolarStar Galaxy microplate.

4.3.3.5 Statistical Analysis

Data obtained from the MTT assay was analysed via a one-way analysis of variance (ANOVA) to assess the effects of herbal weight loss preparations against the vehicle, Phosphate-Buffered Saline (PBS). Dunnet's post hoc test was used to determine the P value at each concentration versus the control. A significance value of P <0.05 was used for all experiments. Analysis and production of graphs was performed in GraphPad Prism 7 for Windows (GraphPad Software, San Diego, USA). Graphs show mean ±SEM. Statistical significance is shown as *p<0.05.

4.4 Results

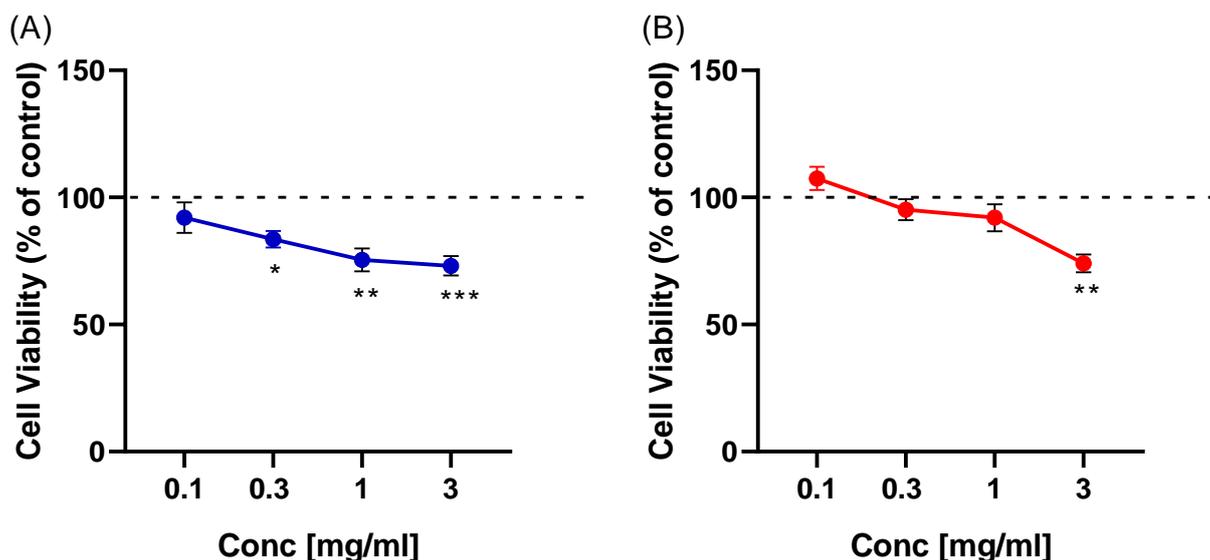


Figure 4.4.1: CAM 5, a supplement to help swelling and bloating. (A). Untreated HepG2 cells shows significant toxicity at concentrations 0.3, 1 and 3 mg/ml. (B). Treated HepG2 cells shows significant toxicity at 3mg/ml. When tested on Caco2 cells, there was no significant toxicity observed in either uninduced or induced cells.

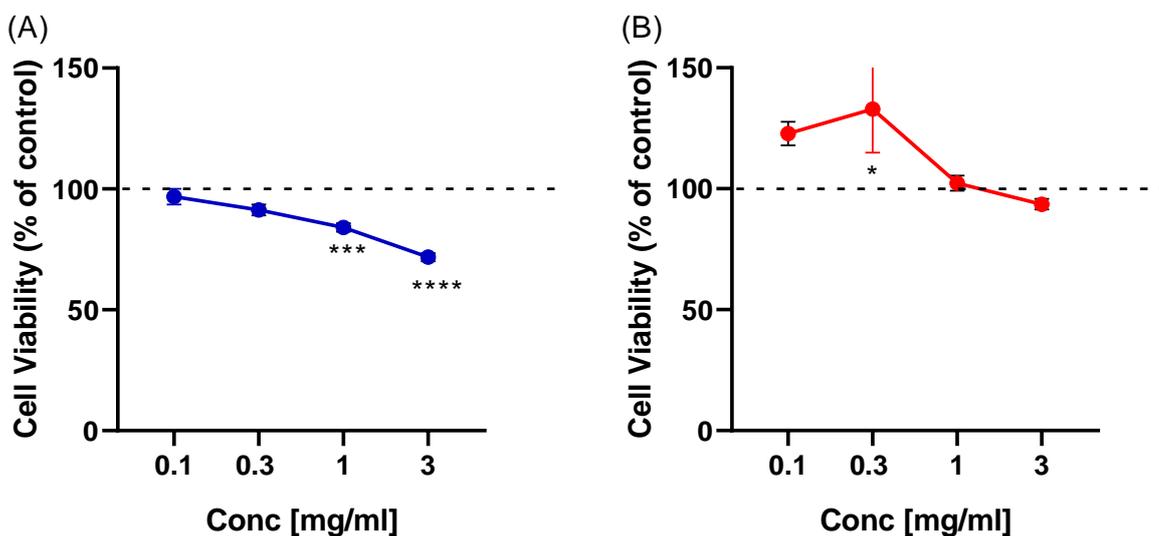


Figure 4.4.2: CAM83, a TCM which claims to alleviate pain. CAM83 had been adulterated with atropine. (A) Uninduced HepG2 cells showed significant toxicity at concentrations 1 and 3mg/ml. (B) When testing in induced HepG2 cells, there was significant toxicity observed at 0.3mg/ml. When tested on Caco2 cells, there was no significant toxicity observed. Means \pm SEM are shown * $P < 0.5$ one way ANOVA followed by Dunnetts test vs control.

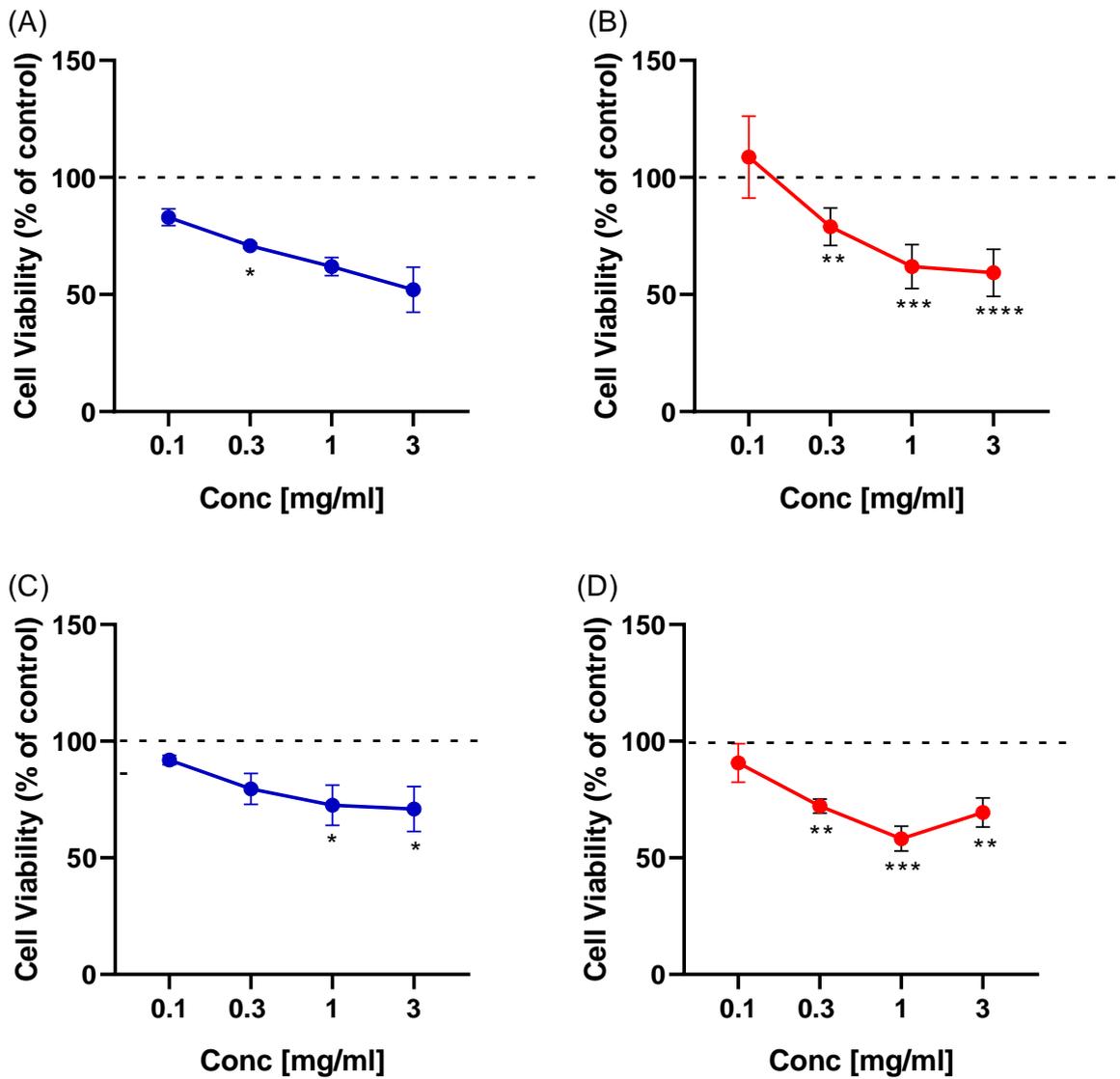


Figure 4.4.3: CAM140, herbal medicine to help elevate arthritis symptoms. Significant toxicity was observed in both HepG2 and Caco2 cells. (A) Uninduced HepG2 cells showed significant toxicity at 0.3mg/ml. (B) In induced HepG2 cells, significant toxicity was observed at concentrations 0.3-3mg/ml. (C) In the Caco2 cells, significant toxicity was seen at concentrations 1 and 3mg/ml in uninduced cells and (D) 0.3-3mg/ml in induced Caco2 cells. Means \pm SEM are shown * P<0.5 one way ANOVA followed by Dunnetts test vs control.

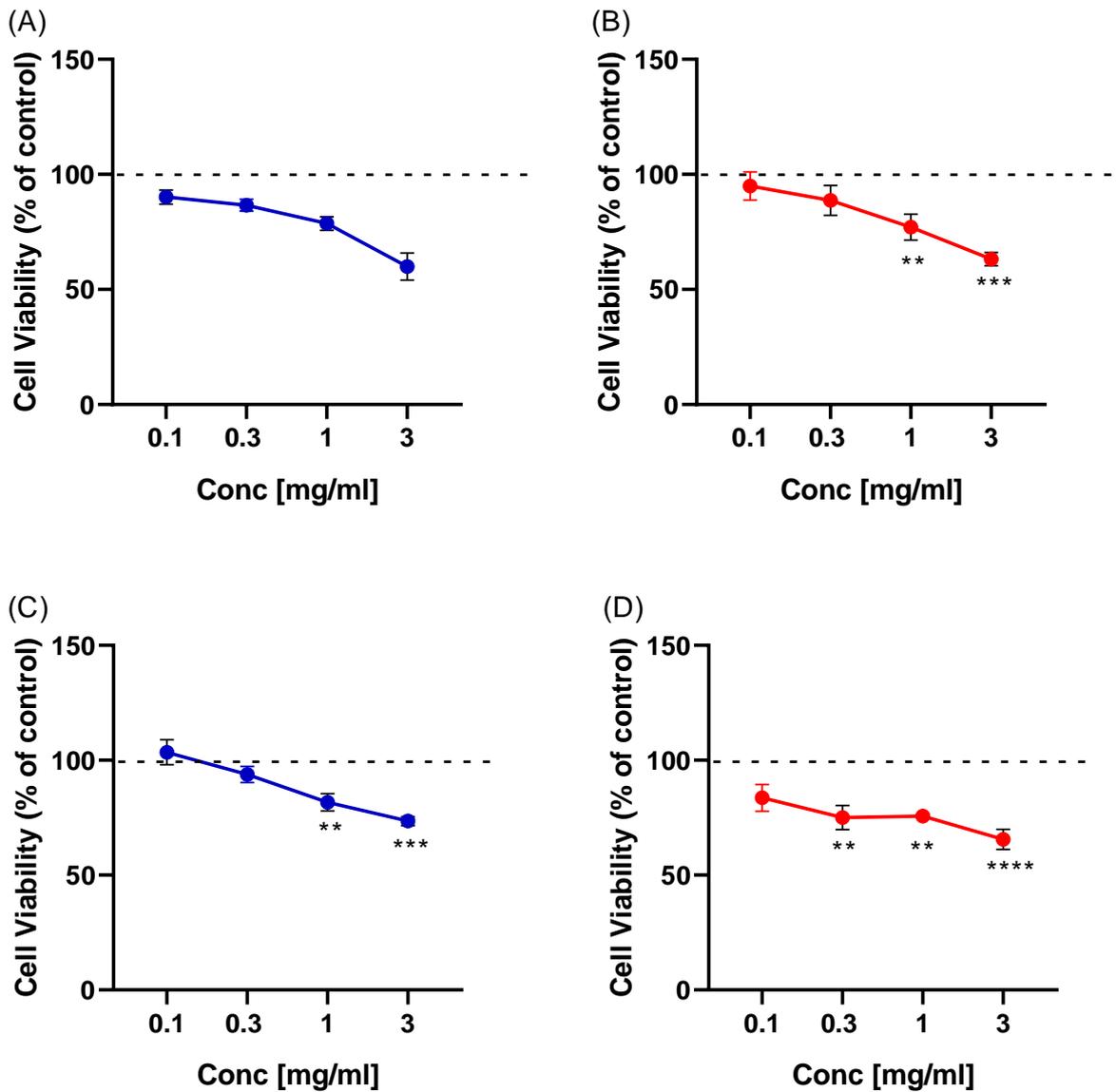


Figure 4.4.4: CAM 26, an Ayurveda preparation used for improved memory. There was toxicity observed in both HepG2 and Caco2 cell lines. (A) Uninduced HepG2 cells showed no significant toxicity. (B) In induced HepG2 cells, toxicity was observed at concentrations 1 and 3mg/ml. (C) When exposed to Caco2 cells, significant toxicity was observed at concentrations 1 and 3mg/ml in uninduced cells and (D) 0.3-3 mg/ml in induced cells. Means \pm SEM are shown * P<0.05 one way ANOVA followed by Dunnetts test vs control.

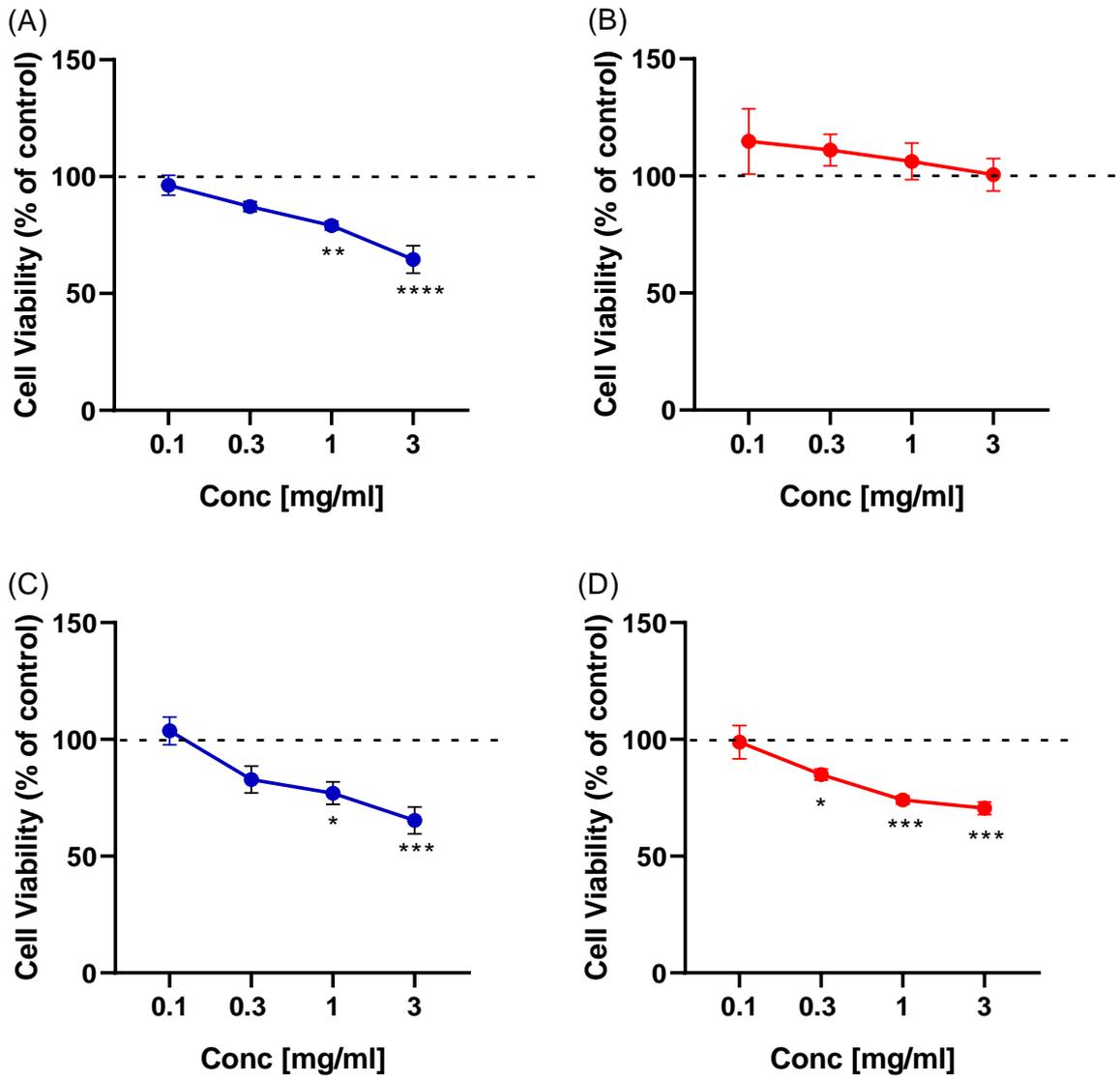


Figure 4.4.5: CAM103, a TCM used to help relieve stress symptoms. Toxicity was seen in both HepG2 and Caco2 cell lines. (A) In uninduced HepG2 cells, toxicity was seen at concentrations 1 and 3mg/ml (B) however no toxicity was seen in treated HepG2 cells. (C) In uninduced Caco2 cells, toxicity was observed at concentrations 1 and 3mg/ml. (D) In induced Caco2 cells, toxicity was observed at concentration 0.3-3mg/ml. Means \pm SEM are shown * $P < 0.5$ one way ANOVA followed by Dunnetts test vs control.

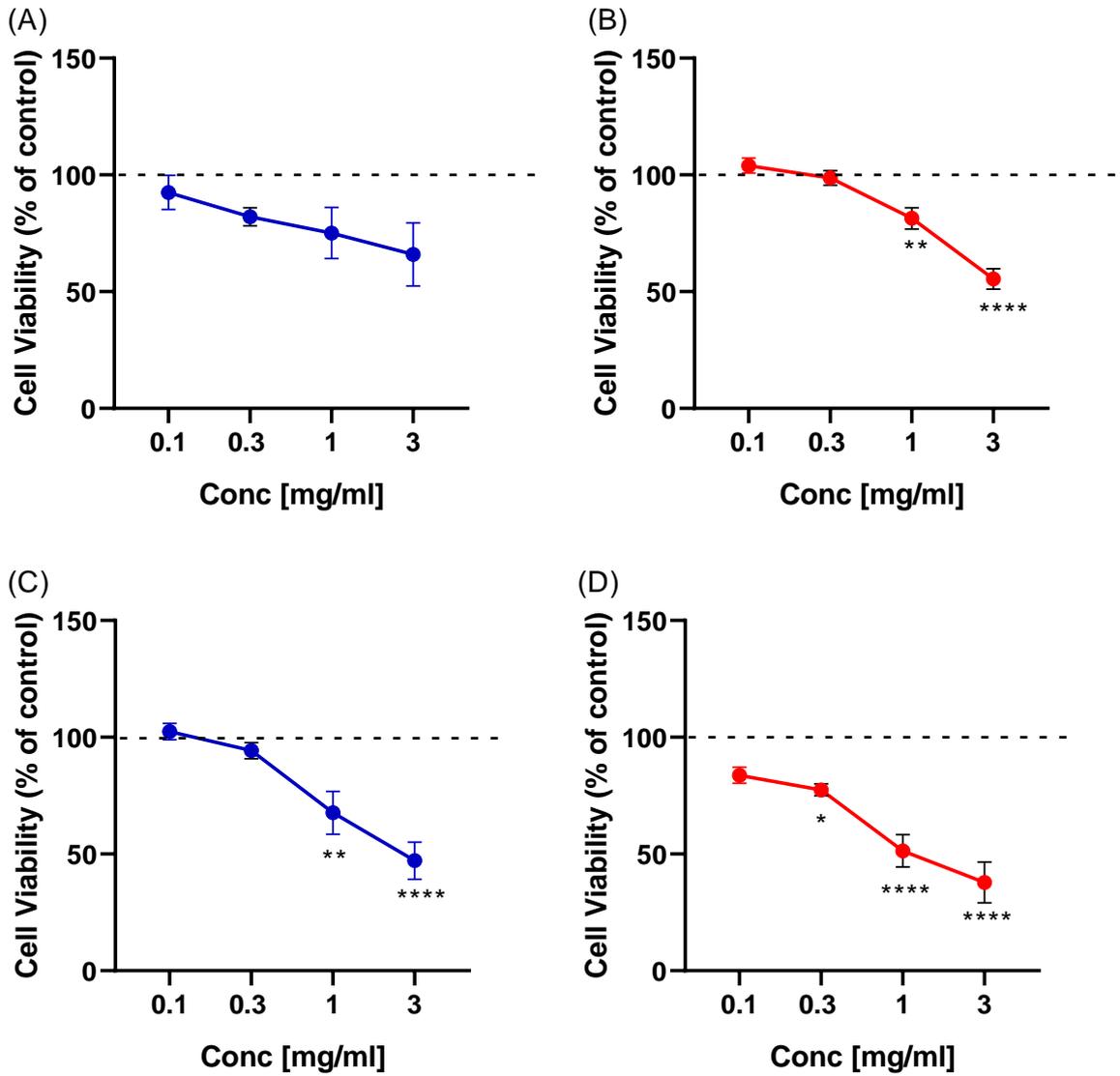


Figure 4.4.6. CAM185, a supplement to boost kids immunity. Significant toxicity was observed in both cell lines. (A) Uninduced HepG2 cells showed significant toxicity at concentrations 1 and 3mg/ml. (B) Induced HepG2 cells shows toxicity at 1 and 3mg/ml. (C) No significant toxicity was observed in uninduced Caco2 cells. (D) In induced Caco2 cells, significant toxicity was observed at 0.3-3mg/ml. Means \pm SEM are shown * $P < 0.5$ one way ANOVA followed by Dunnetts test vs control.

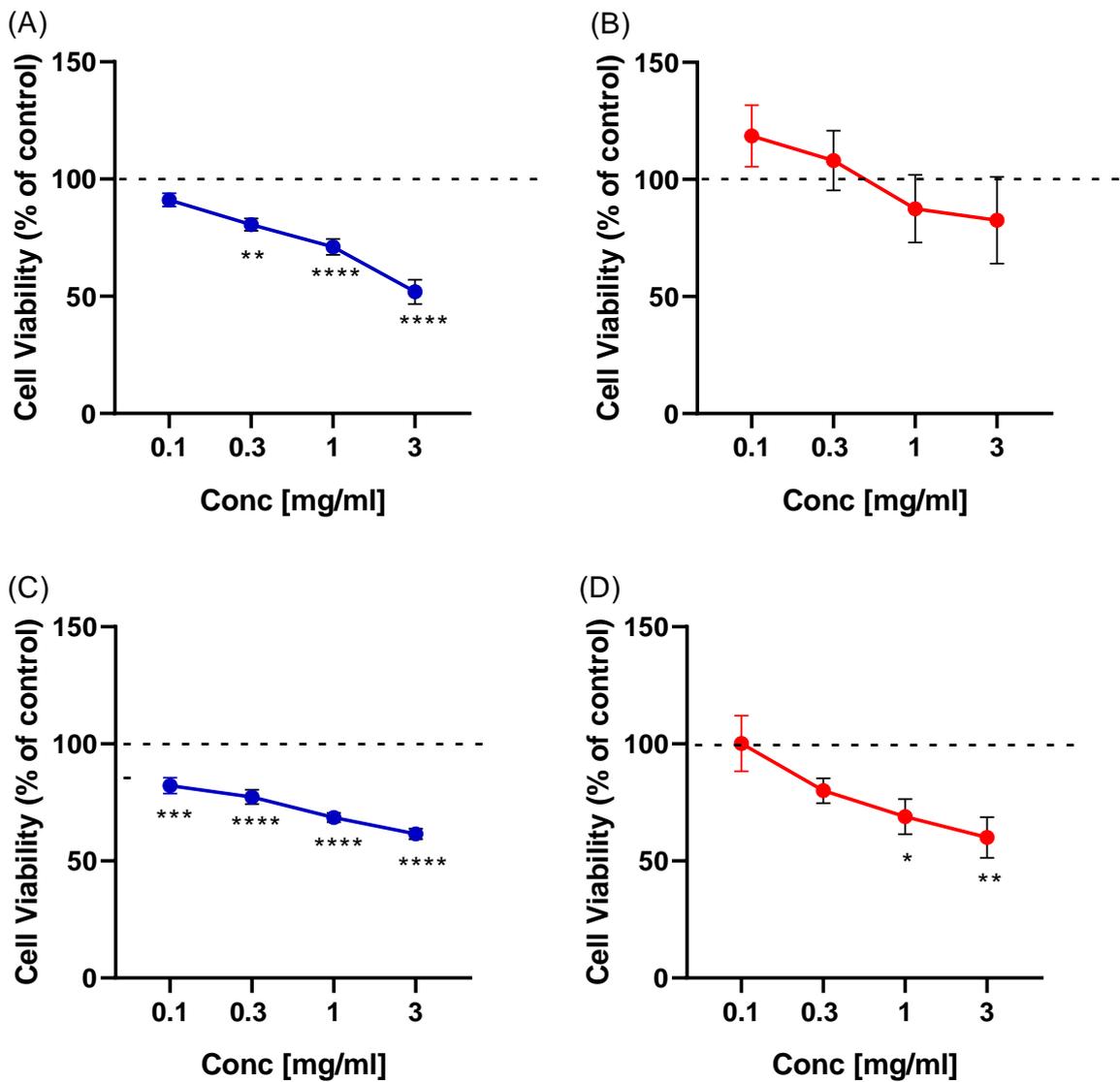


Figure 4.4.7 CAM 227, a herbal medicine used to help weight loss. Significant toxicity was observed in all cell lines. (A) Uninduced HepG2 cells showed significant toxicity at concentrations 0.3-3mg/ml. (B) Induced HepG2 cells showed no significant toxicity. (C) Uninduced Caco2 cells showed significant toxicity at all concentrations (0.1-3mg/ml). (D) Induced Caco2 cells showed toxicity at concentrations 1 and 3 mg/ml. Means \pm SEM are shown * $P < 0.5$ one way ANOVA followed by Dunnetts test vs control.

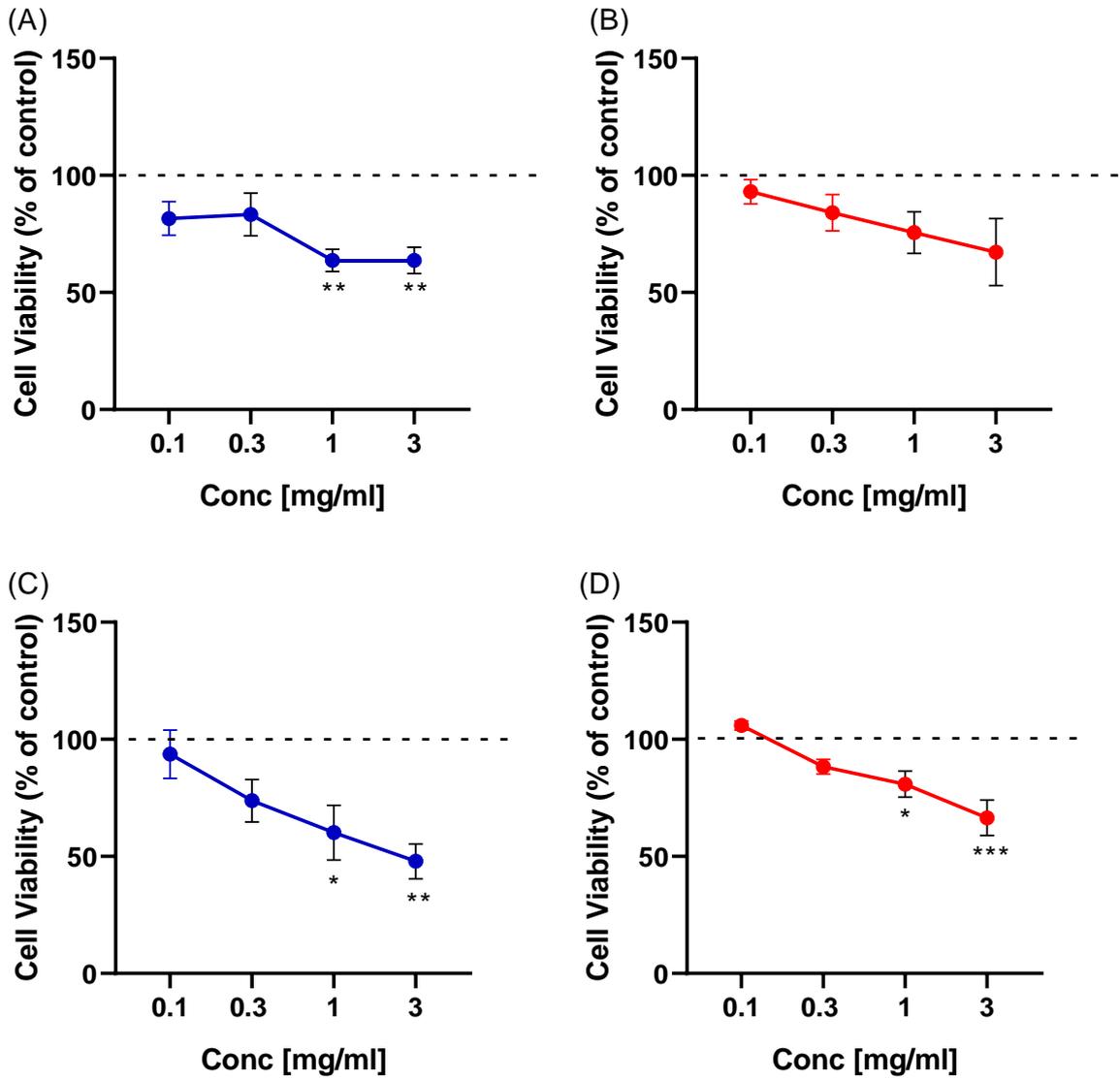


Figure 4.4.8: CAM 323, A herbal medicine used to aid weight loss which contained synephrine. Toxicity was observed in both cell lines. (A) Uninduced HepG2 cells showed significant toxicity at concentrations 1 and 3mg/ml. (B) There was no significant toxicity observed in treated HepG2 cells. (C) Uninduced Caco2 cells showed significant toxicity at concentrations 1 and 3mg/ml. (D) Treated Caco2 cells showed significant toxicity at 1 and 3mg/ml. Means \pm SEM are shown * $P < 0.5$ one way ANOVA followed by Dunnetts test vs control.

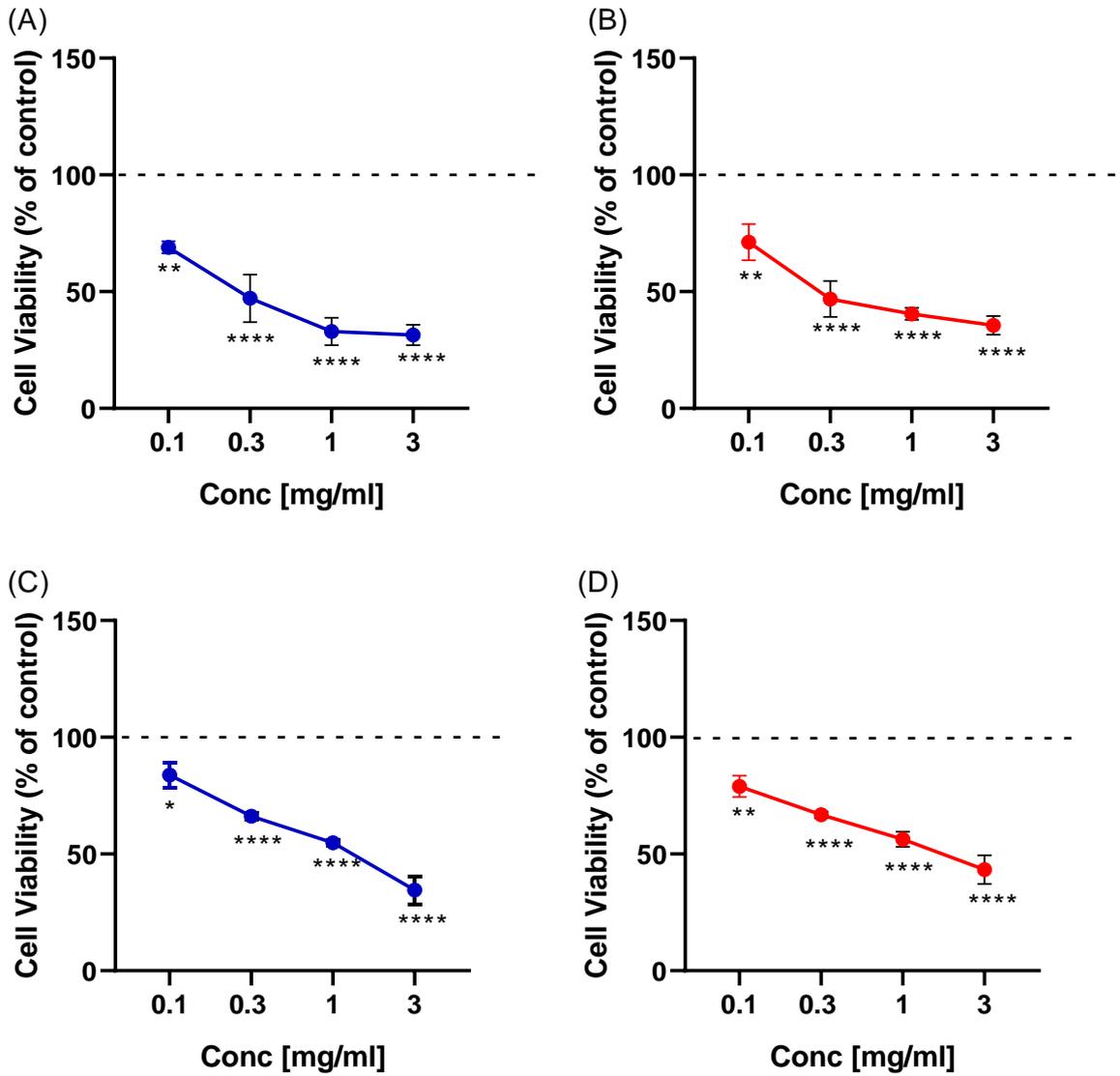


Figure 4.4.9: CAM221, a herbal supplement to improve cardiac health. Significant toxicity was observed at all concentrations (0.1-3 mg/ml) in both Hepg2 and Caco2 cells (A-D). Means \pm SEM are shown * $P < 0.5$ one way ANOVA followed by Dunnetts test vs control.

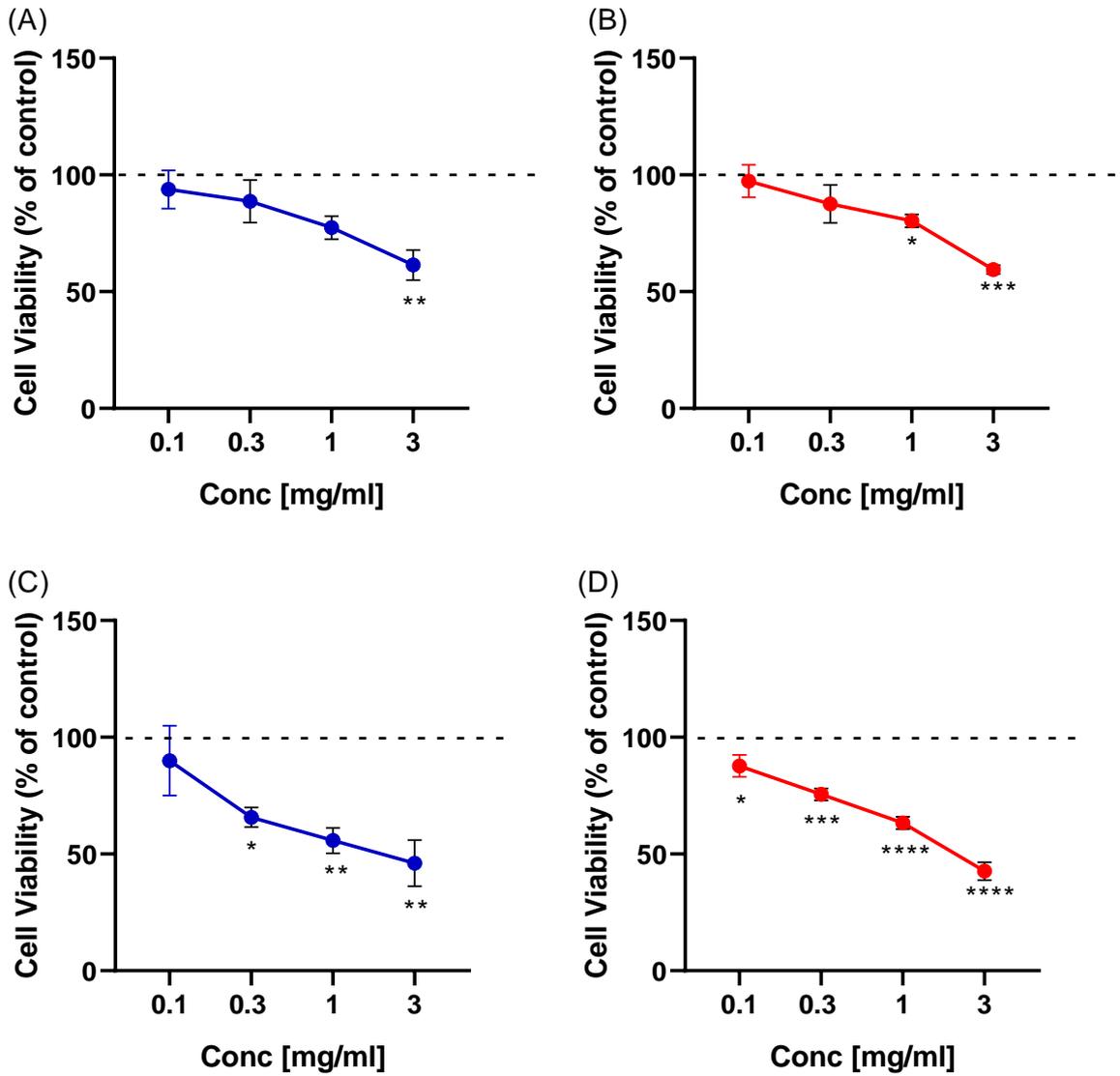


Figure 4.4.10: CAM 311, a herbal supplement used to help period pains. Significant toxicity was observed in both HepG2 and Caco2 cells. (A) HepG2 uninduced cells showed significant toxicity at 3mg/ml. (B) Induced HepG2 cells showed significant toxicity at concentrations 1 and 3mg/ml. (C) Uninduced Caco2 cells showed significant cell death at concentrations 0.3-3 mg/ml. (D) In induced Caco2 cells, there was significant toxicity at all concentrations (0.1-3mg/ml). Means \pm SEM are shown * $P < 0.5$ one way ANOVA followed by Dunnetts test vs control.

4.5 Discussion

The toxicity observed in both HepG2 and Caco2 cell lines following the exposure of CAMs suggests that the *in vitro* action of these CAMs could represent an important public health issue. Ten out of the 18 products from several therapeutic categories tested showed significant toxicity when added to HepG2 cell cultures. Of these, 8 products showed significant toxicity in both cell lines. While screening for potentially toxic compounds has traditionally involved the use of animal models, interspecies differences limit the ability to produce cost effective, rapid and accurate safety assessments [25-26]. Thus, the use of human cell culture systems *in vitro* provides a complementary and informative alternative technique [27-28]. A major function of hepatocytes, the most abundant cells in the liver includes the metabolism of xenobiotics. Therefore, the use of HepG2 cells is becoming more frequent to assess the potential toxicity of chemicals [29-30]. Human intestinal cell line Caco2, are commonly used as a model of the intestinal epithelium in toxicity studies [31-33]. The use of both cell lines provides an insight into potentially harmful chemicals.

Anti-Inflammatory and Analgesics

Chronic pain is a major public health concern in Australia affecting 30% of all adult Australians [34-35]. Over the past few decades the use of prescription opioid analgesics for such pain has been increasing [36]. With increased use, there becomes an issue of dependence, tolerance and toxicity [36]. To combat these issues, there has been an increase in monitoring and control of prescriptions for these drugs, including limitation of over-the-counter pain medications as well as education programs for clinicians to control and review patient progress [37]. However, the increasing large-scale use of herbal medicines to treat lower back and arthritic pain may be partly a result of these regulatory and educational practices [38]. In the current study all three samples tested from this category showed toxicity both in induced and uninduced HepG2 cells (Figure 1-3A-B). When these products were exposed to Caco2 cells significant toxicity was only observed in one supplement that was used for the treatment of arthritis, with no contaminants or adulterants present (Fig 3 C-D). [relate this to actual doses taken by people]

Psychotropic and Sleep

CAM use is widespread amongst those who suffer from anxiety, and sleep and mood disorders [39]. Kessler et al. 2001 found that 54% of those suffering from severe depression and 57% of those suffering from anxiety attacks had used herbal medicine and related therapies during the past 12 months for treatment of their disorders [40]. These products are often sold over-the-counter, with individuals self-prescribing and not consulting experienced herbal medicine practitioners or disclosing this use to health practitioners [41-46]. These actions are concerning as individuals may not be receiving the best treatments, with possible occult ADRs being experienced. Toxicity was observed from all three supplements in this category in the HepG2 cells, with two of the three showing significant toxicity in the Caco2 cell line. The two supplements that were toxic in both cell lines CAM 26 and CAM 103, were from Ayurvedic and Traditional Chinese Medicine traditions, respectively. CAM 103 had been adulterated with ephedrine and pseudoephedrine, which may have contributed to the observed toxicity. Traditional Chinese medicine has been linked to adverse reactions, due to misidentification of plant materials, additional plant materials and the presence of heavy metals [4]. Examples of adverse reactions to herbal remedies include a case study presented by Fahmi et al. detailing a woman developing mania after consuming high dosages of *H. perforatum* for the treatment of depressive symptoms [47]. Other ADRs include liver toxicity following the use of *Piper methysticum* (kava) for the treatment of anxiety, as well as herb-drug interactions [48-49]

Respiratory, Cold and Influenza

Sore throats, nasal congestion, cough, headaches and fever are all manifestations of respiratory tract infections [50]. Diseases can range from the common cold and influenza to tuberculosis and pneumonia, which can be quite serious or life threatening [51]. Out of the three supplements in this category, CAM185, a supplement targeted to children's health was toxic in both HepG2 and Caco2 cell lines despite the absence of adulterants and contaminants. This is especially concerning as children and infants may be more vulnerable to the potential adverse effects due to dosages and children's immature metabolic pathways and immature physiology [52].

Gastrointestinal, Diet and Metabolism

Rising rates of obesity globally have been associated with an increase in the use of herbal preparations for weight control [53]. From the three preparations in this category tested it was found that two of the CAMs caused toxicity in both HepG2 and Caco2 cell lines. CAM 323 was found to contain undisclosed caffeine and CAM 227 had no adulteration or contamination. High doses of caffeine can cause toxicity [54]. When caffeine content is not disclosed in herbal products, individuals may exceed the recommended 400 mg per day intake, increasing risks of adverse reactions [55]. As the popularity of herbal weight loss products increase, there has been an abundance of case studies detailing ADRs to these preparations [56-60]. The severity of the ADRs from herbal weight loss preparation range from mild to life threatening.

Cardiovascular and Hypertension

Cardiovascular disease is the leading cause of morbidity and mortality in Australia [61]. A wide variety of CAMs for treatment are available on the market; which offer both risks and benefits to cardiac patients [62]. The prevalence of CAM use varies significant between 4-61% [62] of this has been noted in other reviews of CAM literature [63]. CAM 221 which is a multivitamin targeted to the elderly showed significant toxicity in both HepG2 and Caco2 cell lines (Fig 9 A-D). Concurrent use of prescription medication and herbal medicines in older adults is concerning as the risk of adverse drug reactions increases with age [64-65]. In addition to this, older people live with co-morbidities that may increase medication-related problems due to the delayed clearance of active compounds [66-67].

Reproductive and Fertility

Despite the increasing use of CAM to relieve menstrual symptoms, enhance fertility and alleviate manifestations of pregnancy little is known about product safety and efficacy [68-69]. The prevalence of CAM usage to treat menstrual pain ranges from 3 and 70% [70]. The high prevalence of CAM use is concerning as results showed one supplement purported to be for this indication (CAM 311) caused significant toxicity in both cell lines and also contained atropine at the concentration of 0.0003 mg/g.

4.6 Conclusion

In Australia the TGA considers complementary and alternative medicines low risk preparations, with no need to prove effectiveness and only a need to show that the ingredients are safe. Despite this, more reports are emerging detailing adverse drug reactions following the use of CAMs. Adverse reactions may be due to adulteration or contamination of these products with pharmaceuticals, heavy metals or misidentified plant materials. However, this study has highlighted that even when these compounds are not present, toxicity was observed in standard toxicological screening models for hepatotoxicity.

CAMs have the ability to cause severe or even life threatening adverse reactions from overuse or misuse, interactions between compounds found within the supplement or with other medications, as well as pre-existing health conditions. With the continual growth of this industry within Australia, further research into the safety of these products needs to be conducted to ensure the health of consumers.

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5. Evidence for the efficacy and safety of herbal weight loss preparations

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5.1 Abstract

Rising rates of global obesity have been associated with an increase in the use of herbal preparations for weight control. However, the mechanisms of action of these substances are often not known and the potential for interaction with other herbal preparations or prescription pharmaceutical drugs is unclear. To investigate the reported efficacy and safety of herbal weight loss preparations, a review of the literature was undertaken focusing on herbs that are most commonly used in weight loss preparations. Specifically: *Garcinia cambogia*, *Camellia sinensis*, *Hoodia gordonii*, *Citrus aurantium* and *Coleus forskohlii*. There was no clear evidence that the above herbal preparations will cause sustained weight loss in humans in the long term. Serious illness and even death have occasionally resulted from the use of herbal weight loss preparations. Limited clinical trials have been undertaken to evaluate the efficacy and/or safety of herbal weight loss preparations. In addition, potential issues of herb-herb and herb-drug interactions are often not considered. Regulation of these products is much less rigorous than for prescription medications despite documentation of cases of a few associated hepatotoxicity.

Keywords: Weight Loss, Hepatotoxicity, Complementary Medicine, Traditional Chinese Medicine

5.2 Introduction

Obesity is characterized by excessive or abnormal fat accumulation that poses a risk to health, impairing quality of life and decreasing longevity and is now a major global public health concern [1]. A review of reported adult body weights during the period of 1980 to 2008 in 199 countries revealed that the mean body mass index had risen globally by 0.4 kg/m² per decade and the term obesity “epidemic” was coined [2]. Overall, the prevalence of overweight and obesity amongst the world’s adult population is 13%, and in children and adolescence is 18% [1]. Australia, New Zealand and the United States had the most significant increases [2]. Obesity is associated with a wide range of disabilities and illnesses, including cardiovascular disease, malignancies and type 2 diabetes mellitus [3].

Sedentary lifestyles decreased physical activity, increased automation of work and an increase of high calorie foods all contribute to this trend [4,5]. Consequently, medicines and dietary supplements are often utilized to help obese individuals lose weight. For example, approximately \$6.6 billion was spent on health and weight loss businesses and products in 2013-2014 in Australia [6]. Of this, \$280.7 million was directed at dietary supplements, including food and drinks [6]. However, there is limited research on the both the safety and effectiveness of these products for weight loss.

Complementary and alternative medicines (CAM) is an umbrella term used to describe a wide range of products that include medicinal preparations containing nutritional supplements, vitamins, minerals, herbs, aromatherapy and homeopathic preparations [7]. Traditional Chinese medicine (TCM), which falls under the umbrella of CAM, forms an intrinsic part of Chinese culture, with approximately 60% of the Chinese population accessing TCM practitioners [8]. Despite the controversy around these products [9] widespread use now also occurs in Western countries because of their perceived effectiveness and accessibility, permitting personal control over treatment regimes. Other reasons for the increase in their use include dissatisfaction with conventional medicine, and rejection of science, technology and pharmaceutical companies [9].

The increase use of CAM in Western societies includes the use of herbal plants for weight loss and have anti-obesity effects. It is common belief that herbal remedies are safe because they are “natural” with fewer side effects compared to conventional medicines [10–12]. However, despite the continued use of traditional medicines for centuries for the prevention and treatment of illnesses, the effectiveness and safety of many of these products is unclear [13]. There is also significant disparity between countries in laws, policies and regulations regarding the quality, safety and efficacy of CAM therapies [14].

Practitioners and consumers may also be unaware of the possible interactions of CAM with prescription drugs and other potential adverse reactions [14]. Inconsistencies in regulatory practice may contribute to problems regarding quality and safety and efficacy of these products, allowing ineffective or unsafe CAM products to be sold [14]. A recent study reported unapproved pharmaceutical ingredients in US dietary supplements in America. From 2007 to 2016, 776 adulterated products were identified by their regulatory body (Food and Drug Administration) with the most adulterated category were products for sexual enhancement (45.5%) followed by weight loss (40.9%) and muscle building (11.9%) [15].

Herbal preparations are available online, which may be the preferred method of purchase as there is an element of privacy for the buyer, as well as it being quick, convenient and sometimes cheaper [16]. However, although many sites appear to be based in the county of purchase, they may in fact locate in other countries where standards and regulations for dietary supplements may be less rigorous. Dietary supplements can also be produced, sold and marketed in the United States, without being required to first demonstrate efficacy and safety, as is the case for pharmaceutical drugs [17].

As the popularity of herbal medicine increases issues have continually arisen regarding quality control, composition and purity [10,11,18]. Studies have shown substitution and/or contamination with plant materials, pharmaceuticals and heavy metals and other fillers which can pose serious health risks [3]. Adulteration of herbal medicines occurs when manufacturers intentionally add undeclared ingredients. Often these ingredients contain pharmaceutical or other compounds which enhance or cause the purported therapeutic effects [19]. Undeclared ingredients may also be introduced accidentally and inappropriate administration, such as injecting rather than ingesting, may result in adverse outcomes [20].

Adverse reactions can be due to the presence of plant toxins, allergenic substances, and pharmaceutically active compounds, and heavy metals such as lead, arsenic and mercury. This was highlighted in a study of dietary supplements by Geller et al. [16] which reported that in the United States an estimated 23,000 emergency department visits every year were attributable to adverse events related to these products. There have also been further case studies identifying undeclared pharmaceuticals such as sibutramine in weight loss supplements [21,22].

In Belgium in the early 1990's at least 100 cases of interstitial fibrosis of the kidneys and renal failure occurred in women after they consumed Chinese herbs as part of a weight loss regimen [23]. Retrospectively, it was discovered that the Chinese herb *Stephania tetrandia* which was prescribed had been unintentionally substituted with the Chinese herb, *Aristolochia fangchi*. *Aristolochia* species contains aristolochic acid which is a nephrotoxin and a carcinogen [24]. It was believed that the effects of the *Aristolochia* were magnified by the presence of the other ingredients within the weight loss supplements such as acetazolamide and fenfluramine.

Despite numerous case reports detailing severe adverse reactions to herbal preparations [22–25], with clinical outcomes including organ transplantation, and even death [26], the underlying biochemical interactions are still not fully understood. Recently a case emerged of a sixteen-year-old girl presenting with acute hepatitis. On initial enquiry she had not taken any prescribed or over-the-counter medications and had not participated in any recent drug trials. After testing ruled out viral, autoimmune and metabolic causes, further questioning, revealed that she had for several months been regularly consuming Chinese green tea for weight loss. This had been ordered over the internet. After ceasing the use of the tea there was rapid and sustained recovery [27].

5.3 Methods

A structured integrative review of the literature was carried out between January 2016 and November 2018. This review focused on five herbal preparations used for weight loss with reports of their safety and

efficacy. PubMed was the main data base used along with Google Scholar, with articles published in English. The search strategy covered review articles, clinical trials, systematic review and meta analyse studies published between 1981 to 2018 (up to October 2018) were considered. Studies published after 2010 were given higher priority. For each database, a specific search strategy was implemented based on themes, as well as a general search of references. A combination of keywords used for this review included: weight loss, complementary and alternative medicine, herbal medicine, obesity treatment, traditional Chinese medicine, adverse reactions, safety, efficacy, *Garcinia cambogia*, *Camellia sinensis*, *Hoodia gordonii*, *Citrus aurantium* and *Coleus forskohlii*.

One of the search strategies used for PubMed was (“herbal medicine” [MeSH Terms] OR (“herbal” [All Fields] AND “medicine” [All Fields]) OR “herbal medicine” [All Fields]) AND (“weight loss” [MeSH Terms] OR (“weight” [All Fields] AND “loss” [All Fields]) OR “weight loss” [All Fields]) AND adverse [All Fields] AND events [All Fields].

5.4 Medicinal plants with purported weight loss effects

Medicinal plants containing phytochemical constituents have long been used for the treatment of chronic illness, as well as to prevent and cure other ailments [28]. However, the use of medicinal plants and their extracts for weight loss is rapidly growing area of therapeutic interest. The burden of obesity on an individual’s health and on society has created interest in the possibility of using natural supplements for the long-term treatment. Despite the growth in the industry, there is a need for further investigation into the efficacy, safety, possibly adverse reactions, and appropriate dosages, as well as mechanisms of action. In addition, another significant problem concerns the unrealistic weight loss claims without appropriate supportive evidence. This review will focus on five common herbs used in plant-derived supplements for weight loss: *G. cambogia*, *C. sinensis*, *H. gordonii*, *C. aurantium* and *C. forskohlii* [13,29,30].

5.4.1. *Garcinia cambogia*

The fruit of *G. cambogia* is a popular ingredient used in cooking in Southern India and now also in herbal products with purported weight loss effects. The active ingredient, hydroxycitric acid (HCA) is extracted

from the rind of *G. cambogia*. HCA inhibits the activity of the ATP-dependent citrate lyase enzyme, causing the breakdown of citrate into oxaloacetate and acetyl-CoA, thus restricting the synthesis of cholesterol and fatty acids in various tissues [31]. This purportedly either induces a sense of satiety or reduces appetite [31]; animal experiments have shown some evidence that there was weight reduction after administration of HCA [32]. On the other hand, several human trials found no significant difference between this and a placebo [33–35]. However, a meta-analysis of nine clinical trials evaluating the effectiveness of HCA in weight reduction suggested that HCA may be relatively more effective than a placebo (mean difference: –0.88 kg; 95% confidence interval (CI): 0.00–1.75 kg) [36].

Thus, there is evidence that *G. cambogia* may act as a weight loss agent in the short-term. As human trials have been short, with small sample sizes, it is unclear how effective *G. cambogia* would be for weight control over a longer period.

Despite evidence supporting *G. cambogia* for weight loss, further investigation is required particularly looking at potential side effects. A study by Cresciolo et al.[37] detailed four case series where adverse reactions occurred following the use of dietary supplements containing *G. cambogia*. Severe adverse events have also been reported in response to using dietary supplements containing *G. cambogia* including acute liver injury, liver failure and hepatotoxicity, cardiomyopathy and myocarditis, as well as psychosis [38–49].

5.4.2 *Camellia sinensis*

Green tea produced from the unfermented dried leaves of *C. sinensis* has been used over the centuries for a variety of reasons and is another popular medicine for weight loss [13]. The bioactive constituents of green tea include caffeine and polyphenols [29]. These polyphenols make up 30% of the dry weight of the fresh leaf with the most prominent being the catechins [50]. Green tea stimulates the sympathetic nervous system leading to increased energy consumption and triggering the oxidation of fat [51]. Alternative mechanism(s) may include downregulation of enzymes responsible for hepatic lipid metabolism, reduced nutrient absorption and appetite suppression [50]. Hepatic fatty acid oxidation has

been studied as a method of reducing food intakes in rats, and the consumption of medium-chain fatty acids has been shown to reduce food intake in humans [52].

Studies done on animal subjects, including rats, of the effect of primary catechins produced by green tea have been inconsistent. Several studies showed no measurable effect on food intake after oral administration of green tea catechins or pure epigallocatechin-3-gallate [53–58]. While Murase et al. [59] found a 5.6% decrease in energy intake in mice fed catechins, and Kao et al. [60] showed that an intraperitoneal injection of epigallocatechin-3-gallate reduced food intake by up to 60% and caused acute weight loss in both male and female Sprague Dawley rats within 2-7 days of treatment.

In humans, studies have failed to demonstrate an effect of *C. sinensis* on appetite suppression and energy consumption [50,61,62], although it has been shown to increase energy expenditure which may assist in weight loss [63–65]. However, a recent review of 15 weight loss trials and three studies on weight maintenance involving the consumption of green tea reported that it was unlikely to be clinically effective, concluding that green tea consumption would not help maintain weight loss [51]. There was also no information on costs, patient's satisfaction or illnesses resulting from green tea preparations, yet Maki et al. [63] has shown that common minor adverse side effects include joint pain, sinusitis and rhinitis.

With increased consumption of green tea and increased use of supplements containing green tea, issues with safety have appeared [66], specifically involving hepatotoxicity [67,68]. Mazzanti et al. [69] discovered 19 cases of hepatotoxicity between 2009 and 2014. There was, however, a large variation between the cases, with treatment ranging from 2 days to more than a year, and latency of reactions varying from 14 days to more than a year. All cases required hospitalisation, with four cases requiring a liver transplant [69].

Thus although there has been evidence to show increased energy consumption following the use of *C. sinensis* there is little evidence to support claims of weight loss. With case studies detailing adverse reactions, these preparations should be consumed with caution.

5.4.3 *Hoodia. gordonii*

Consumed widely for its alleged weight loss effects [70], *H. gordonii* is a member of the milkweed family that has been traditionally used as an appetite suppressant during hunting expeditions by indigenous South Americans [71]. There is, however, limited information on its mechanism of action. The active compound, steroidal glycoside P57, is associated with altering neuropeptide-mediated pathways of the central nervous system and inhibiting appetite by effecting hypothalamic neurons and the hypothalamus [72]. A study in rats found, following an injection of P57 into the central nervous system, that there was a reduction in food intake over the subsequent 24 h [72].

However, despite its popularity as a weight loss product [71], there are limited scientifically based studies on clinical relevance, bioactivity, and *in vivo* biopharmaceutical behaviour of *H. gordonii*.

5.4.4. *Citrus. aurantium*

C. aurantium, commonly known as Seville orange, bitter orange or sour orange, has become a popular ingredient in herbal medicines to suppress appetite although it had been used previously in TCM and South American folk medicine for a number of other purposes [73]. Due to a ban on ephedra in weight loss products in both the United States and Australia, *C. aurantium* has been advertised as a substitute for ephedra without the associated adverse effects. The bioactive constituents in *C. aurantium* include flavonoids such as hesperidin and naringin, and alkaloids. The most important alkaloid is p-synephrine [74] which is believed to be the main component that stimulates weight loss. Synephrine is primarily an α -adrenergic agonist, with some β -adrenergic agonist properties [75].

Synephrine alkaloids are thought to increase energy expenditure, with adrenergic agonistic properties, and decreased food intake through reduction of gastric motility [74,76]. In animal trials using rodents, food intake was significantly reduced by the synephrine alkaloids with dosing over a 10-day period [77]. In humans there are case studies showing positive results of *C. aurantium* for weight loss, ranging from 0.169 to 0.516 kg lost per week compared to placebos [78–80]. However, these studies were looking at a range of synephrine alkaloids and included mixtures of compounds. Recent reviews of *Citrus aurantium*

alone conclude that there is minimal objective evidence that consumption either reduces appetite or causes weight loss in humans [81]. Evidence for adverse reactions is not lacking, with *Citrus aurantium* being associated with diarrhea, cardiovascular disease, hypertension, nausea/vomiting, migraines, insomnia, upper respiratory problems, skin damage, anxiety, and cramping [82].

5.4.5. *C. forskohlii*

C. forskohlii is a *Coleus* plant which grows in arid and semi-arid regions of India and Thailand, the most common species being *C. forskohlii* Briq. Forskolin is a labdane diterpene isolated from the roots of *C. forskohlii* which acts directly on adenylate cyclase [83,84] to produce the second messenger cAMP which then stimulates the breakdown of fat in human and animal fat cells [85]. This stimulation of fat breakdown could lead to weight loss.

Han et al., [86] found that administration of *C. forskohlii* significantly reduced fat accumulation and reduced food intake in rats. In contrast, a study in humans has shown that although *C. forskohlii* may affect appetite, it did not result in weight loss. Similarly, Henderson et al., [87] in evaluating the effects of *C. forskohlii* supplementation on hematological profiles and body composition in overweight women, found no changes in mean energy intake between placebo and *C. forskohlii* groups, although the *C. forskohlii* group had a significant decrease in feelings of fullness. However, Godard et al., [88] has reported that *C. forskohlii* could reduce body fat in obese males, although there was no significant fall in overall weight due to a gain in lean body mass. Similar intakes of *C. forskohlii* were used in all three studies, and it is not clear if the difference in body composition changes were due to sex-based responses.

Despite promotion as a weight loss supplement, there is little evidence to support this. There appear to be no case reports detailing adverse reactions following the use of *C. forskohlii*, however this may be an omission as CAM use is often not disclosed.

5.5 Pharmacokinetic interactions of weight loss herbs

The concurrent use of pharmaceuticals and herbal products is increasing with recent surveys showing concomitant use of prescription drugs with herbal medicines in 20%–30% of patients in the United States

[89,90]. However, there is a risk of interaction between pharmaceuticals and herbal medicines which is poorly understood by the general public. For example, a number of active compounds within herbal medicines can serve as substrate for enzymes involved in the metabolism of xenobiotics (e.g., drugs, pollutants, and food additives) [91]. Given that there is minimal or no information on the pharmacokinetics of many phytochemicals present in herbs, co-administered of herbal preparations and conventional drugs can result in an increased risk of unrecognised herb-drug interactions [92].

Metabolism of xenobiotics normally occurs in two phases: phase 1 (functionalization reactions) and phase 2 (conjugative reactions). Cytochrome P450 (CYP) is a superfamily of enzymes responsible for phase 1 metabolism of various xenobiotics and some endogenously derived substances such as steroids [92]. Although CYP are spread throughout multiple organs, most of drug metabolizing CYP enzymes are found in the liver. Currently over fifty human CYP's have been isolated, the major hepatic CYP's include CYP2C9/19, CYP1A2, CYP2D6, CYP3A4/5/7 and CYP2E1 [93]. As with conventional medicines, in order to be processed and excreted from the body, the phytochemicals in herbal medicines also undergo phases 1 and 2 metabolism. Weight loss herbal medicines may either induce or inhibit the expression or activity of a specific CYP, which may significantly affect the metabolism of a prescription pharmaceutical agent [94]. For example, hesperidin from *C. aurantium* interacts with numerous pharmaceuticals [95]. A major catechin in green tea is epigallocatechin-3-gallate; a study by Abe et al. [96] found that following a single dose of green tea extract, the plasma levels of nadolol were significantly reduced.

5.6 Conclusion

Several issues arise in the assessment of herbal weight loss preparations. Despite their increasing popularity, the mechanisms of action are generally not known and their potential for interaction with other herbal preparations or prescription pharmaceutical drugs is often unclear. Few clinical trials have been performed to prove convincingly that they are firstly, effective, and secondly, safe. Regulation of these preparations is also much less rigorous than for prescription medications [14]. As herbal use is often not inquired about by physicians or pathologists in cases of nonlethal and lethal liver and renal disease, the

contribution of herbal weight loss preparations to community morbidity and mortality is currently not known [8].

Finally, as cases of a few hepatotoxicity and other severe adverse effects have been reported in the literature to be directly linked to the taking of herbal weight loss preparations, considerably more work needs to be undertaken to adequately understand adverse reactions before these products can be endorsed or promoted.

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Conflict of interest

The authors declare that they have no conflict of interest.

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6. Hepatotoxicity Associated with the Use of Herbal Weight Loss Supplements

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6.1 Abstract

Background: As obesity rates continue to rise alongside the lack of conventional medicines for treatment, the use of herbal preparations are gaining popularity with purported claims for weight loss. Despite the growing popularity of these products, there is limited information regarding their efficacy and safety.

Methods: Three herbal preparations for weight loss were tested after following consumption, individuals experienced adverse reactions. The three herbal preparations included a green tea extract, an enzyme preparation and a general marketed herbal weight loss product. To investigate the cause of the adverse effects, drug contamination was suspected and investigated using toxicological analysis undertaken at Forensic Science SA, Adelaide. Methods included liquid chromatography/quadrupole-time-of-flight mass-spectrometer (LC-QTOF MS), liquid-chromatography/ diode array detector (LC/UV) and gas chromatography/ nitrogen phosphorous- detector/mass-spectrometer (GC-NPD/MS). In addition, these products were tested *in vitro* for cytotoxicity using human liver carcinoma cells (HepG2) and human colon carcinoma cells (Caco2).

Results: No contamination or adulteration was found in the three herbal weight loss supplements. However significant toxicity was observed *in vitro* in both HepG2 and Caco2 cell lines for all 3 tested substances. These results are consistent with the reports from those who had consumed the product.

Conclusion: The results from this study have demonstrated drug-induced cellular injury using cell models for both the liver (HepG2) and the gastrointestinal epithelium (Caco2). Using the established cell model can help identify at-risk herbal preparations for pre-clinical assessment of herbal hepatotoxicity to ensure public safety.

6.2 Introduction

Obesity has become a worldwide public health concern characterized by abnormal or excessive accumulation of adipose tissue that poses a risk to health, decreasing longevity and quality of life. Obesity is now categorized as an epidemic following a review of adult body weights from 1980 to 2008 in 199 countries which revealed that the mean body mass index (BMI) has risen globally by 0.4kg/m²/decade [1]. Obesity is associated with many illnesses and disabilities including Type 2 diabetes and cardiovascular disease and has also been linked to at least 2.8 million deaths per year [2-3]. Sedentary lifestyles, an increase in high calorie foods, increased automation of work and a decrease in physical activity all contribute to this trend [4-5]. Due to the lack of conventional medicines for the treatment of obesity, many complementary and alternative medicines (CAM) are gaining popularity with purported claims for weight loss.

CAM is an umbrella term used to describe a range of clinical practices including; acupuncture, naturopathy and treatments such as herbal medicines containing herbs, vitamins, minerals, homeopathic and aromatherapy preparations that are not often considered part of conventional medicine [6]. CAMs have long been an integral part of healthcare in many Eastern countries, with now Western populations are incorporating many aspects into their health practices [7-8]. CAMs are believed to be safe as they are derived from natural sources, with fewer side effects compared to conventional medicine [9-11]. However; as their popularity rises there is an increase in safety concerns regarding their use [12]. This was highlighted by Pajor et al. who found that both users and non-users of dietary supplements believed these products to be safe, and if any adverse effects were experienced, they would attribute this to the consumer status (e.g. being ill or due to excessive use) rather than to the product itself [13].

Despite the use of CAMs worldwide for the treatment and prevention of illness, the safety and efficacy of these preparations is sometime still unclear [14], often with a lack of scientific validation for their effectiveness [15]. There are disparities in the policies, regulations and laws in relation to CAM therapies and their quality, safety and efficacy between countries [16] which has allowed unsafe and ineffective

CAM products to be placed on the market [16]. Consumers and practitioners may be unaware of possible interactions between CAMs and other herbal or conventional medications [16]. This is also a major concern as many who take herbal supplements are self-medicating and not disclosing this to health practitioners [17].

CAMs, including herbal medicines and dietary supplements are becoming a popular method for weight loss. In Australia, approximately \$6.6 billion was spent on weight loss health products and businesses; of this \$280.7 million was spent on dietary supplements [18]. Despite the belief that natural equals safe, several adverse reactions following the use of CAM products have been documented [19-28]. Adverse reactions can be a result of contamination with heavy metals, misidentification of herbs, adulteration with pharmaceuticals, improper preparation, toxicity to phytochemicals or pharmacokinetic interactions [2, 29-31].

The aim of the following study was to identify the contents of three herbal weight loss preparations which caused adverse reactions as well as to test these products for toxicity *in vitro*. Products were exposed to gastrointestinal and hepatic cell lines to gauge their toxicity.

6.3 Methods

6.3.1 Sample Collection

Three samples were provided that had caused adverse reactions shortly after consumption.

6.3.2 Pharmaceutical and Adulterant Screening

6.3.2.1 Ethanol Extraction

Samples were crushed to a powder consistency and 50mg was weighed out into test tubes. 25µL of internal standard mix and 1mL of Univar Absolute Ethanol (analytical reagent grade) were added to the test tubes, which were then sonicated for 15 minutes, and centrifuged for 10 minutes at 3000rpm (according to standard methodology at Forensic Science SA) [32]. The supernatant was transferred into clean glass disposable tubes and evaporated to dryness using a centrifugal evaporator. The extract was

reconstituted with 100 μ L of Univar Absolute Ethanol (analytical reagent grade) and transferred to 2mL autosampler vials containing a tapered insert [32].

6.3.2.2. Basic Extraction

Samples were crushed to powder and 50mg was weighed out into individual test tubes. 25 μ L of internal standard mix, 1mL of glass distilled H₂O and 250 μ L of Chem Supply 30% conc. Ammonia (analytical reagent grade) was added. The test tubes were vortexed for 5-10 seconds. 5mL of Honeywell B&J Brand High Purity Solvent butyl chloride (BuCl) was added to the test tubes, and were then extracted using a mechanical rolling for 15 minutes. Samples were then centrifuged for 10 minutes at 3000rpm. The BuCl layer was transferred into clean glass disposable tubes, and evaporated to dryness using a centrifugal evaporator. The extracts were reconstituted with 100 μ L of Univar Absolute Ethanol (analytical reagent grade) and transferred to 2mL autosampler vials containing a tapered insert [32-33].

6.3.2.3 Instrumentation

Sample extracts were analysed by an Agilent 1200 LC 6510-QTOF in dual ESI mode fitted with a Waters Acquity BEH C18, 1.7 μ m, 3.0 mm x 50 mm (Waters part no. 186004660) column, with a guard cartridge (Phenomenex C18 4 x 3mm; part no. AJO-4287 (LC-QTOF)). Analysis was repeated on an Agilent 1100 series High Performance Liquid Chromatography (HPLC) system with diode array detector fitted with an Agilent Eclipse plus C18, 1.8 μ m, 4.6 x 50mm column with guard cartridge (Phenomenex C18 4.0 x 3.0mm; part no. AJO-4287). Sample extracts underwent further analysis on an Agilent 7890 Gas Chromatograph with nitrogen-phosphorous and mass spectrometer detectors (GC-NPD). Data from the LC-QTOF was processed using the commercially available Agilent Forensic Toxicology PCDL Library (ForTox_AM_PCDL – Broecker, Herre, and Pragst). This library contains accurate mass MS/MS spectra of 3490 pharmaceuticals, drugs of abuse and pesticides and herbicides as well as libraries developed in house (350 pharmaceuticals and drugs of abuse, retention time and MSMS search). Data from LC-UV was processed using libraries developed in house; including a range of compounds including anti-convulsants, analgesics and anti-inflammatories. The GC-NPD/MS data used the NIST/EPA/NIH Mass

Spectral Library (NIST 2008), containing over 200,000 compound spectra as well as libraries developed in house [32].

6.3.2.4. Quantitation

Analytes were extracted from the sample using mixed mode Solid Phase Extraction (SPE) (System48 Cerex Pressure Processor). The analytes were protonated by the addition of a pH 5.7 acetate buffer (2mL) and were retained on the UCT XTRACT®, 200mg/ 3mL. P/N XRDAH203 cartridge (PM Separations Pty Ltd) by cation exchange. Analyte elution was achieved by passing a basified mixture of CH₂Cl₂/i-PrOH through the SPE cartridge. The eluent was evaporated and the residue was reconstituted in methanol. The extract was analysed by LC-MS/MS (ABSciex 4000QTRAP with the Agilent 1200 LC-autosampler system) using electrospray ionisation in the positive mode and multiple reaction monitoring with scheduling [32].

6.3.2.5 Calibration and Quality Control (QC) solutions

A combined working standard solution was prepared by accurately transferring the appropriate volume of each stock solution to a volumetric flask, making up to volume with ethanol [32].

6.3.3 Toxicological screening

6.3.3.1 Cell culture

As common adverse events to herbal medicines include hepatotoxicity and issues relating to the gastrointestinal tract two cell lines were chosen to evaluate the toxicity of products. Human liver carcinoma cells (HepG2) and Human colon carcinoma cells (Caco2) were chosen. Both cell lines were maintained in 75cm³ culture flask grown in Dulbecco's Modified Eagle Medium (DMEM) and supplemented with 10% foetal calf serum, 1% non-essential amino acids and 1% of penicillin streptomycin. Cells were cultured at 37°C with 5% carbon dioxide. Experiments and cell maintenance were performed in a laminar flow hood under sterile conditions. Cells were passaged every 3-4 days and detached from flasks using 1 x trypsin EDTA. Cell counts were performed using trypan blue to stain cells. Ninety-six well plates were used for cell viability experiments, where cells were seeded in DMEM

with 10% FCS at a density of 1.2×10^6 cells per well. The cells were then allowed to equilibrate for 48h before the experiment.

6.3.3.2 Bioactive pre-treatments

Metabolism of the phytochemicals in some herbal preparations can cause greater toxicity due to the generation of toxic metabolites (ref). Thus preparations were examined in the presence of absence of cytochrome P450 3A4 induction. The cytochrome P-450 3A (CYP3A) enzyme family is responsible for most of the drug metabolism in the human liver, as well as a number of important phytochemicals with the most common isoform being CYP3A4 (reference). Rifampicin at the concentration of 2mM was added to DMEM and plated onto the cells for 48 hours to induce CYP3A

6.3.3.3 Effect of weight loss preparations on cell lines

The three herbal supplements were crushed to a fine consistency. Sterile PBS was added to make a stock solution of 30mg/ml for each preparation. Stock solutions were diluted using sterile PBS to final concentrations ranging from 0.1 to 3 mg/ml.

6.3.3.4 Cell Viability Measures

Cell viability was determined using the thiazolyl blue tetrazolium bromide (MTT) cytotoxicity assay. MTT is taken up by viable cells and converted in the mitochondria to an insoluble blue formazan product. The intensity of colour is proportional to the level of active mitochondria in living cells. After incubation of HepG2 and Caco2 cells with dilutions of herbal preparations for 48 hours, 96-well plates had all media removed and replaced with serum-free media containing 0.25mg/ml of MTT. The plate was further incubated for 3h at 37°C in a 5% CO₂ atmosphere, then the MTT solution was removed and DMSO was added to lyse cells. The absorbance was then measured at 570nm using a PolarStar Galaxy microplate.

6.3.3.5 Statistical Analysis

Data obtained from the MTT assay was analysed via a one-way analysis of variance (AVOVA) to assess the effects of herbal weight loss preparations against the vehicle, Phosphate-Buffered Saline

(PBS). Dunnet's post hoc test was used to determine the P value at each concentration versus the control. A significance value of $P < 0.05$ was used for all experiments. Analysis and production of graphs was performed in GraphPad Prism 7 for Windows (GraphPad Software, San Diego, USA). Graphs show mean \pm SEM. Statistical significance is shown as $*p < 0.05$.

6.4 Results

6.4.1 Pharmaceutical and adulterant screening

Two of the three herbal medicine samples were screened using three instruments (LC-QTOF, LC/UV and GC-NPD/MS) to ensure a comprehensive analysis. Toxicological screening found no contaminants or adulterants in both ethanol and basic extracts.

6.4.2 Toxicological screening

6.4.2.1 Sample 1: Green tea supplement

There was a concentration dependent reduction in cell viability with increasing concentrations of sample 1 in both induced and uninduced HepG2 and Caco2 cells (Fig 6.4.2.1 A-D).

6.4.2.2 Sample 2: Enzyme supplement

Significant toxicity was observed in both cell lines. In HepG2 cell lines significant toxicity was seen at 1 and 3 mg/ml in untreated HepG2 and at all concentrations in HepG2 cells which had been treated with rifampicin (Fig 6.4.2.2 A-B). When this supplement was exposed to Caco2 cells, significant cell toxicity was observed at all concentrations when exposed to Caco2 cells which had been induced with rifampicin, there was significant cell toxicity at concentrations 0.3-3mg/ml in untreated Caco2 cells (Fig 6.4.2.2 C-D). Of note, this substance seems to become more toxicity in induced cells.

6.4.2.3 Sample 3: Weight loss supplement

Significant toxicity was observed at concentrations 0.3-3mg/ml in both non-induced and induced HepG2 cells (Fig 6.4.2.3 A-B). In Caco2 cell significant toxicity was observed at concentrations 0.3-3mg/ml in uninduced cells and in all concentrations in induced Caco2 cells (Fig 6.4.2.3 C-D).

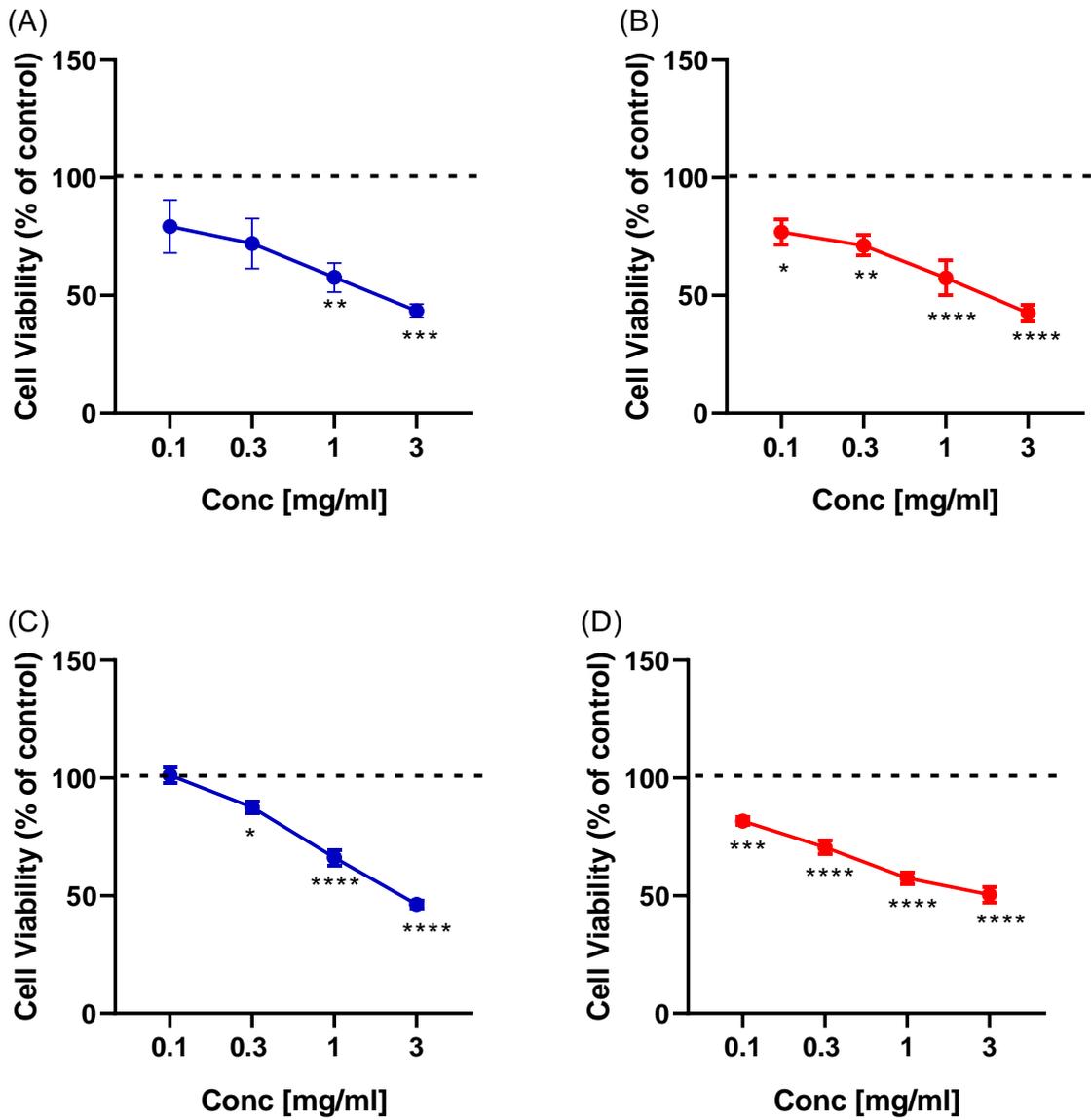


Figure 6.4.2.1. Herbal weight loss supplement containing green tea. Significant toxicity was observed in both HepG2 and Caco2 cell lines. (A) HepG2 untreated showed significant toxicity at concentrations 1 and 3mg/ml. (B) HepG2 cells treated with rifampicin showed significant toxicity in all concentrations (0.1-3mg/ml). (C) Caco2 cells untreated showed significant toxicity at concentrations 0.3-3mg/ml. (D) Toxicity was observed at all concentrations in Caco2 cells which had been treated with rifampicin.

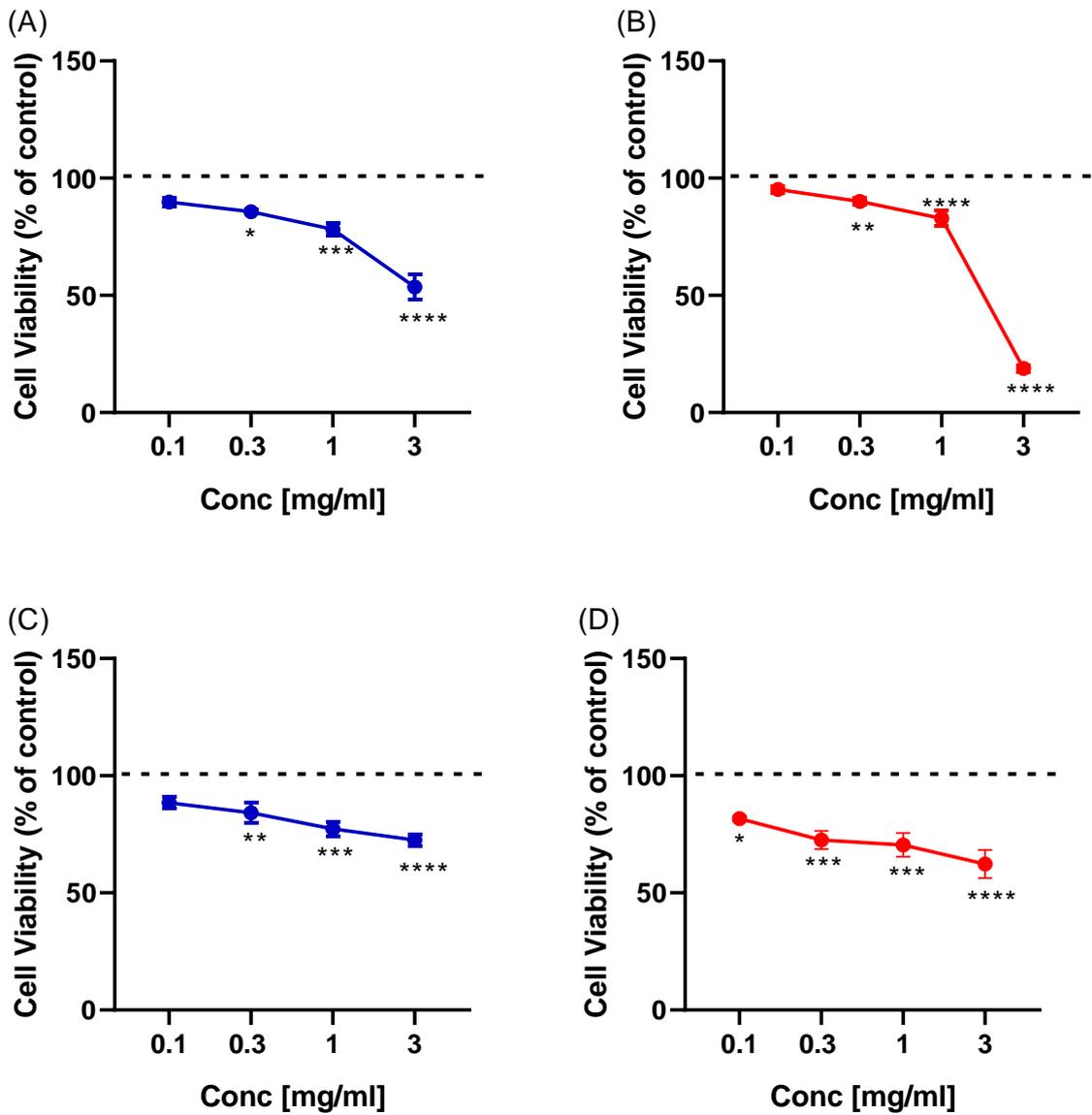


Figure 6.4.2.2 Herbal weight loss supplement containing enzymes. (A) Toxicity was observed at 0.3-3mg/ml in untreated HepG2 cell line. (B) There was significant toxicity observed in treated HepG2 cells at concentrations 0.3-3mg/ml. (C) Concentration dependent toxicity was observed at concentrations 0.3-3mg/ml in untreated Caco2 cells. (D) Toxicity was observed at all concentrations in Caco2 cells treated with rifampicin.

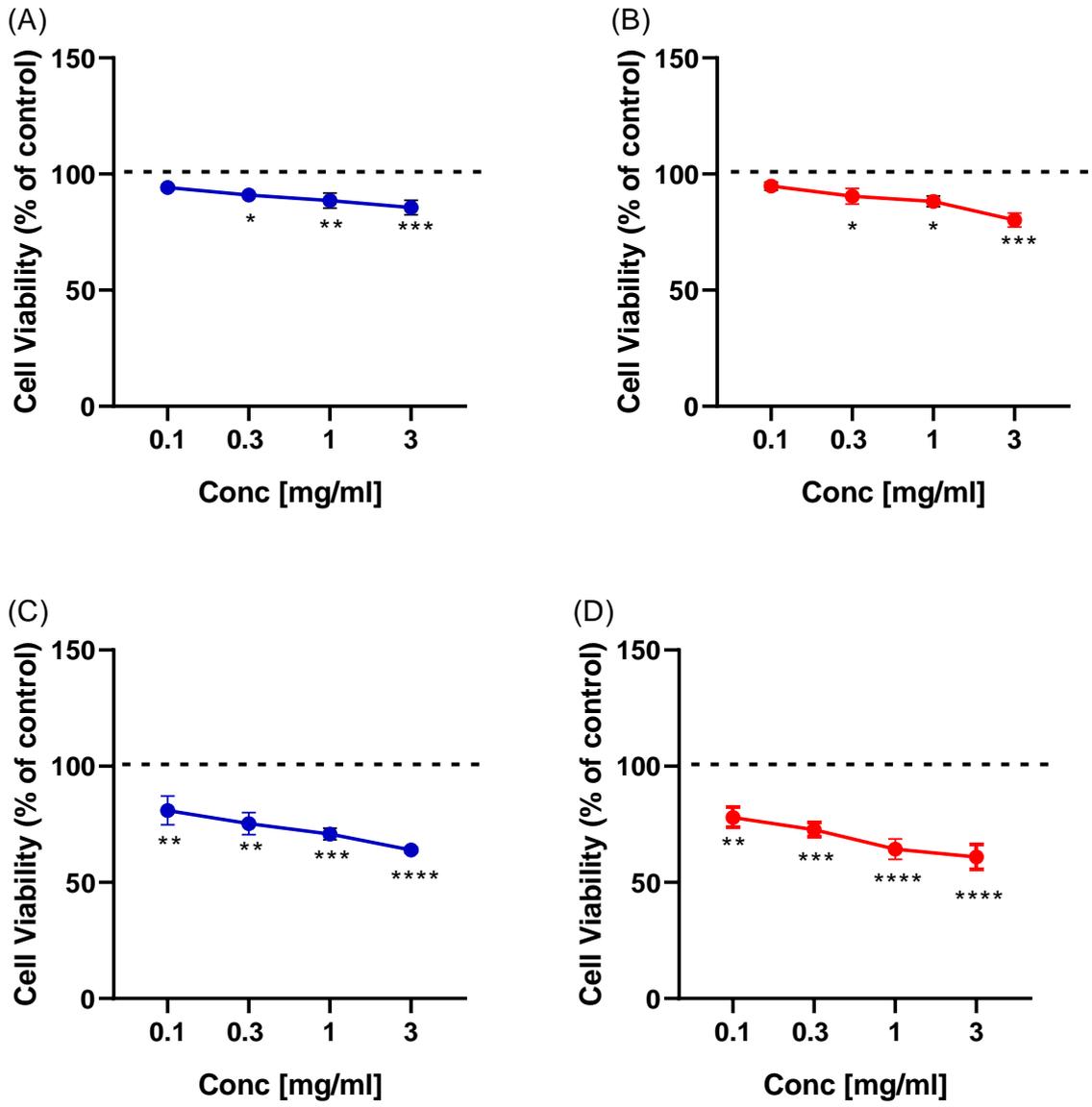


Figure 6.4.2.3. Herbal weight loss supplement. There was significant toxicity observed at concentration 0.3-3mg/ml in the HepG2 cell line (A and B). Significant toxicity was observed in all concentrations in the Caco2 cell lines (C-D).

6.5 Discussion

Certain CAMs have been used for thousands of years; however only recently have these products become popular in Western societies [7-8]. Compared to conventional medicine, limited information is available regarding their safety. Adverse reactions have been linked to the presence of undeclared prescription medications and misidentified plant products which are known hepatotoxins, as well as to the primary phytochemical constituents of the herbs [29-31, 34-35]. With numerous reports emerging detailing liver injury following the use of herbal medicines, in particular herbal weight loss products, there are significant concerns regarding their safety.

The current study examined three herbal weight loss products that had been associated with toxicity. It utilised a two-pronged approach, screening for the presence of potentially toxic contaminants and adulterants and looking for toxicity in model cell lines. No adulterants or contaminants were found. By ruling out toxicity caused by adulterants and contaminants, the reported toxicity is therefore most likely caused by endogenous phytochemicals within the herbal supplements themselves.

Green tea is produced from the unfermented dried leaves of *Camellia sinensis*, which has been used for a variety of health benefits and is now part of a popular treatment regime for weight loss [36]. The bioactive constituents of green tea include polyphenols, which make up 30% of the dry weight of the fresh leaf, with the most abundant catechin, being (-)-epigallocatechin-3-gallate (EGCG) [37-38]. Previous studies have reported adverse reactions following the use of green tea supplements, specifically involving hepatotoxicity [39-41]. The present study has demonstrated a concentration dependent toxicity *in vitro* in both HepG2 cells (liver) and Caco2 cells (gastrointestinal epithelium) (6.4.2.1 A-D). However, in many cases of green tea hepatotoxicity there have been other herbal treatments or pharmaceutical agents implicated.

There is still uncertainty surrounding the role of green tea in hepatotoxicity; it has been hypothesised that it could be linked to catechin EGCG or its metabolite (-) epicatechin gallate which, under fasting conditions can induce oxidative stress leading to liver damage [42]. Sample 1 contained 13.8g of *Camellia sinensis* per tablet with directions to take two 2 tablets. Sample 2, which contained a variety of enzymes showed

significant toxicity in uninduced and induced Hepg2 cells at 0.3-3mg/ml (Fig 6.4.2.2 A-B). In the Caco2 cell line significant toxicity was observed at 0.3mg/ml to 3 mg/ml in uninduced cells and at every concentration in induced cells (Fig 6.4.2.2 C-D). The active ingredients and concentrations for sample 3 are not known. When tested *in vitro* toxicity was observed at concentrations 0.3-3mg/ml in both non induced and induced HepG2 cells and in every concentration for both uninduced and induced Caco2 cell lines (Fig 6.4.2.3 A-D).

Previous pharmacovigilance studies have shown that the liver was one of the major organs involved in herb induced toxicity. A study by Zhou et al. showed that hepatotoxicity caused by CAMs was one of the two most common causes reported amongst 24,112 Chinese patients with drug-induced liver injuries [24]. Medical practitioners are often aware of liver injury caused by conventional medicines, however CAMs as a possible cause have only recently been recognised [43]

Currently, there is no specific diagnostic test or biomarker available to prove or disprove herb-induced hepatic injury [43] [47]. Causality assessment, the process of determining whether it is likely that a drug or herbal medicine is the cause of liver injury, is currently the gold-standard for identification of herb-induced liver injury [48-49]. However, the techniques used during causality assessment do have limitations including poor reporting, incomplete case data, misleading information from patients and herbal misidentification [50-51].

The variability of regulation surrounding CAMs allows for products to enter the market without the need of pre-clinical or clinical studies to test for safety and efficacy [47].

6.6 Conclusion

The present study used *in vitro* techniques to demonstrate drug-induced cellular injury using cell models for both liver (HepG2) and gastrointestinal epithelium (CACO2). Exposing multi-ingredient herbal preparations to HepG2 and Caco2 cells can help identify both hepatotoxic and gastrointestinal toxic products. Despite the absence of contaminants and adulterants found in the three herbal weight loss supplements, significant toxicity was observed *in vitro*. These findings are consistent with the reports of

toxicity from those who had consumed the supplements, but that does not mean the two are always related. Significantly two of the samples showed more toxicity to CACO2 cells than HepG2 cells, suggesting possible gastrointestinal toxicity.

Another potentially concerning issues was that for at least one herbal preparation, inducing metabolizing enzymes increased the toxicity of the preparation. This suggests that the production of toxic metabolites may be an underappreciated source of toxicity in herbal preparations.

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Principal Author

Name of Principal Author (Candidate)	Rachael Farrington		
Contribution to the Paper	Performed analysis on all samples, interpreted data, wrote manuscript and acted as corresponding author		
Overall percentage (%)	85%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	24/1/19

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- xix. the candidate's stated contribution to the publication is accurate (as detailed above);
- xx. permission is granted for the candidate to include the publication in the thesis; and
- xxi. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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7. Interactions Between Epigallocatechin-3-Gallate (EGCG) And Hydroxy Citric Acid Potentiate EGCG Hepatotoxicity

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7.1 Abstract

The rise in popularity of complementary and alternative medicine has seen an increase in concomitant use of herbal medicines with conventional medications as well as the concomitant use of multiple herbal preparations (polyherbacy). Interactions between the components of herbal medicines are poorly understood. However, a recent case report detailing severe hepatotoxicity requiring a liver transplant after the ingestion of supplements with epigallocatechin-3-gallate (EGCG) and hydroxycitric acid (HCA) suggests a possible role for adverse interactions. EGCG has previously been shown to produce hepatotoxicity in humans. Investigation was undertaken to determine whether HCA could produce hepatotoxicity alone, and whether hepatotoxicity might be potentiated by EGCG, in a hepatocyte model cell line HepG2. The toxicity of EGCG and HCA in a model of intestinal epithelium, Caco2 cells was also investigated. EGCG and HCA individually produced concentration-dependent (20 -100 μM) toxicity in HepG2 and Caco2 cells. Combining threshold toxic concentrations of EGCG with HCA potentiated the toxicity of HCA in an additive manner. Similarly combining threshold toxic concentrations of HCA with EGCG potentiated the toxicity of EGCG in an additive manner. Thus, polyherbacy may have significant negative effects on hepatocytes due to the interaction of different plant ingredients.

7.2 Introduction

The use of complementary and alternative medicines (CAMs) has been increasing worldwide over the past few years. In developed countries, such as Australia, these products are becoming an integral part of health care with an estimated two out of three Australians using CAMs [1-4] to promote health and to treat various illnesses including inflammatory conditions, upper respiratory disease, heart disease, obesity and diabetes mellitus [5-6]. It is generally believed that herbal medicines are natural and therefore safe compared to pharmaceuticals, however this is a dangerous oversimplification [7-9]. Adverse reactions following the use of herbal medicines continue to be documented with reports of dietary supplement-induced liver injury increasing [10-12]. Navarro et al. 2017 reported that 20% of liver injury cases from 2013 to 2014 were caused by dietary supplements [10]. The presence of plant toxins, allergenic substances, pharmaceutically active compounds, heavy metals as well as herb-drug and herb-herb interactions may all contribute to these outcomes [5,8,13]. In particular, herb-herb interactions by direct interference with metabolic pathways is an underappreciated source of adverse events.

Cytochrome P450 (CYP) is a superfamily of enzymes responsible for phase 1 metabolism of various xenobiotics, sometimes leading to the formation of toxic compounds [14]. Unlike pharmaceuticals which are extensively tested for the potential to induce or inhibit CYP450, herbal medicines in Australia are placed on the market without testing for possible interactions and are regulated using a risk-based approach centred on the listed ingredients and their potential for adverse reactions [15]. Herbal medicines receive less checking by the regulatory body which relies heavily on self-assessment and reporting by manufacturers and providers [15]. Interactions with other herbal medicines or dietary supplements are rarely considered. Herbal supplements that inhibit or induce CYP450 enzymes are a public health concern as these interactions may inhibit the breakdown of toxic parent compounds to less toxic daughter compounds, as well as affecting therapeutic efficacy [16].

There is also minimal, to no information available about the pharmacokinetics of many phytochemicals present in herbal medicines and their toxicological properties; they are also currently not commercially available for testing [5]. Concurrent use of herbal preparations with conventional drugs can result in an

increased risk of unrecognised herb-drug interactions. This often remains unrecognised as patients tend to not disclose their use of herbal medicines to physicians as they either believe that it is not important, or fear a negative response [17-19]. In a recent survey by Bush et al. 15% of patients receiving conventional medicines also consumed herbal products and, among these, potential drug-herb interactions were observed in 40% [20]. As practitioners and consumers may be unaware of the possible drug interactions and potential adverse reactions when combining prescription medication and herbal medicines, this may lead to substantial harm from hepatotoxicity [21-23].

Polyherbacy is the term used to describe the ingestion of multiple herbal products at the same time [24]. Increasing the number of herbal components consumed increases the risk of herb-herb interactions. Previous studies have classified herb-herb interactions into three types: acute and predictable reactions, cumulative, chronic or delayed toxicity and idiosyncratic effects [25]. Multiple active ingredients, with often many products being used concurrently can complicate identification of active ingredients in the event of a serious adverse reaction [26]. A woman who died of liver failure following the ingestion of 12 Chinese herbs for the treatment of irritable bowel syndrome demonstrates the complexity of identifying toxic ingredients and interactions. [27].

The aim of the current study was to investigate the effects of individual and combined plant constituents on hepatic and intestinal cell lines to gain further understanding of herb-herb interactions. Two common ingredients found in herbal weight loss supplements: epigallocatechin-3-gallate which is the most abundant green tea catechin and hydroxy citric acid were chosen for further investigation as a recent case of hepatotoxicity necessitating liver transplant involved the taking of dietary supplements with epigallocatechin-3-gallate and hydroxy citric acid [28].

7.3 Materials and Methods

7.3.1 Cell culture

Two cell lines were chosen to evaluate the toxicity of products: human liver carcinoma cells (HepG2) and human colon carcinoma cells (Caco2) [9,29]. Both cell lines were maintained in 75cm³ culture flask grown

in Dulbecco's Modified Eagle Medium (DMEM) and supplemented with 10% fetal calf serum, 1% non-essential amino acids and 1% penicillin streptomycin. Cells were cultured at 37°C with 5% carbon dioxide. Experiments and cell maintenance were performed in a laminar flow hood under sterile conditions. Cells were passaged every 3-4 days and detached from flasks using 1 x trypsin EDTA. Cell counts were performed using trypan blue to stain cells. Ninety-six well plates were used for cell viability experiments, where cells were seeded in DMEM with 10% FCS at a density of 1.2×10^6 cells per well. The cells were then allowed to equilibrate for 48h before the experiment.

7.3.2 Bioactive pre-treatments

Metabolism of the phytochemicals in some herbal preparations can cause greater toxicity due to the generation of toxic metabolites [30]. Thus, preparations were examined in the presence and absence of cytochrome P450 3A4 induction. The cytochrome P-450 3A (CYP3A) enzyme family is responsible for most of the drug metabolism in the human liver, as well as a number of important phytochemicals with the most common isoform being CYP3A4 [30-31]. Rifampicin at the concentration of 2mM was added to DMEM and plated onto the cells for 48 hours to induce CYP3A4. Functional induction was confirmed with exposure to paracetamol (data not shown).

7.3.3 Chemicals

DMEM with 4500mg/L D-glucose, L-glutamine, penicillin-streptomycin (10000U/mL), and Trypsin-EDTA (0.5%) were sourced from Gibco, USA. Foetal bovine serum was sourced from Gibco, Australia, non-essential amino acids solution (100X) was from Gibco, UK. All other chemicals were sourced from Sigma Aldrich Australia.

7.3.4 Cell Viability Measures

7.3.4.1 Individual phytochemicals

Cytotoxicity was evaluated using a colorimetric assay. Following 48hrs incubation, the medium was discarded and 100µL of serum free DMEM was added to each well of a 96 well plate. EGCG was added at a concentration of 0-100 µM. After incubation of HepG2 and Caco2 cells with vehicle or dilutions of

phytochemicals for 48 hours, the 96-well plates had all media removed and replaced with serum-free media containing 0.25mg/ml of thiazolyl blue tetrazolium bromide (MTT). MTT is taken up by viable cells and converted in the mitochondria to an insoluble blue formazan product. The intensity of colour is proportional to the level of active mitochondria in living cells. The plate was further incubated for 3h at 37°C in a 5% CO₂ atmosphere, then the MTT solution was removed and DMSO was added to lyse cells. The absorbance was then measured at 570nm using a FLUOstar® Galaxy microplate reader. The same protocol was followed for HCA.

7.3.4.2 Interactions between phytochemicals

Following the previous steps EGCG was added at 20µM to each well except for the control row. In addition, HCA was also added at concentrations of 40-100 µM. The final rows were controls containing 20 µM EGCG, 20 µM EGCG + 40 µM HCA, 20 µM EGCG + 60 µM HCA, 20 µM EGCG + 80 µM HCA and 20 µM EGCG + 100 µM HCA. The experiment was then repeated with 20µM HCA and with EGCG increasing in concentration (40-100 µM).

7.3.5 Statistical Analysis

Data obtained from the MTT assay was analysed via a one-way analysis of variance (ANOVA) for individual phytochemicals and a two-way ANOVA for the interactions data to assess the effects of herbal weight loss preparations against the vehicle, 10% DMSO. Dunnet's post hoc test was used to determine the P value at each concentration versus the control. A significance value of P <0.05 was used for all experiments. Analysis and production of graphs was performed in GraphPad Prism 7 for Windows (GraphPad Software, San Diego, USA). Graphs show mean ±SEM. Statistical significance is shown as *p<0.05.

7.4 Results

7.4.1. Individual phytochemicals

EGCG and HCA were tested for their cytotoxicity in two human cancer cell lines; HepG2 and Caco2. EGCG (20-100µM) showed significant concentration-dependent toxicity in the human hepatic cell line

(HepG2) with approximately 30% cell death at the highest concentration (100 μM). Toxicity was not markedly affected by induction of cytochrome P450s with rifampicin. Similar concentration dependent toxicity to EGCG was observed in the intestinal epithelial cell model (Caco2) (Fig 7.4.1 A-D).

Exposure to HCA (20-100 μM) also produced a significant concentration dependent toxicity in both HepG2 and Caco2 cells which was not significantly affected by induction of cytochrome P450s with rifampicin (Fig 7.4.2 A-D).

7.4 2. Interactions between phytochemicals

When EGCG and HCA were in the presence of each other either a fixed concentration of EGCG (20 μM) and increasing concentrations of HCA (40-100 μM , Fig 7.4.3 A-D), or a fixed concentration of HCA (20 μM) and increasing concentrations of EGCG (40-100 μM , Fig 7.4.4 A-D) there was a concentration-dependent increase in toxicity. This toxicity was simply additive of the individual HCA and EGCG toxicities with no synergism seen. Induction of cytochrome P450s with rifampicin did not affect this pattern of toxicity.

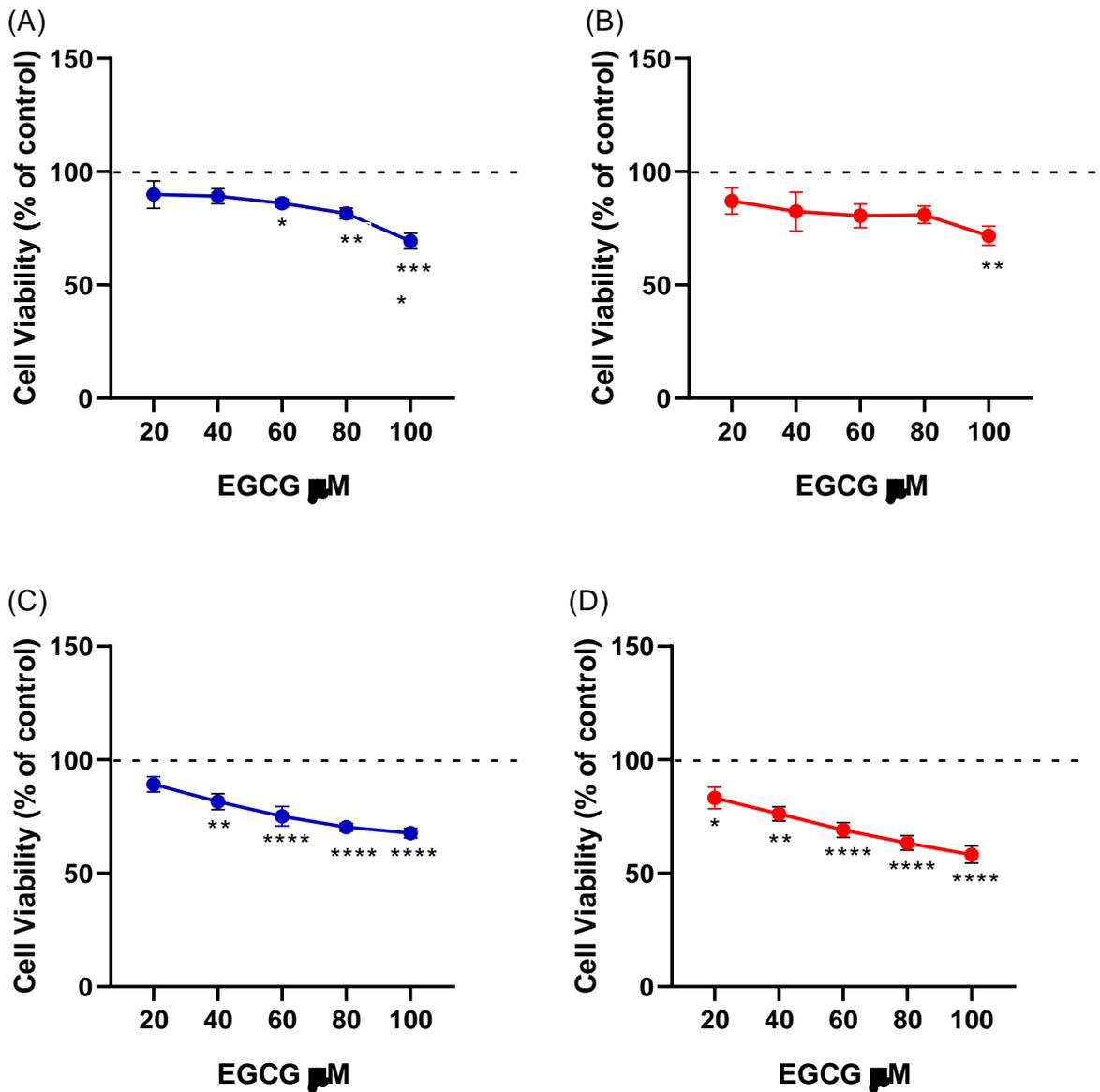


Figure 7.4.1: Exposure of EGCG. Significant toxicity was observed in both HepG2 and Caco2 cell lines. (A) HepG2 untreated showed significant toxicity at concentrations 60-100μM. (B) HepG2 cells treated with rifampicin showed significant toxicity at 100 μM. (C) Caco2 cells untreated showed significant toxicity at concentrations 40-100 μM. (D) Significant toxicity was observed in all concentrations (20-100 μM) in Caco2 cells which had been treated with rifampicin.

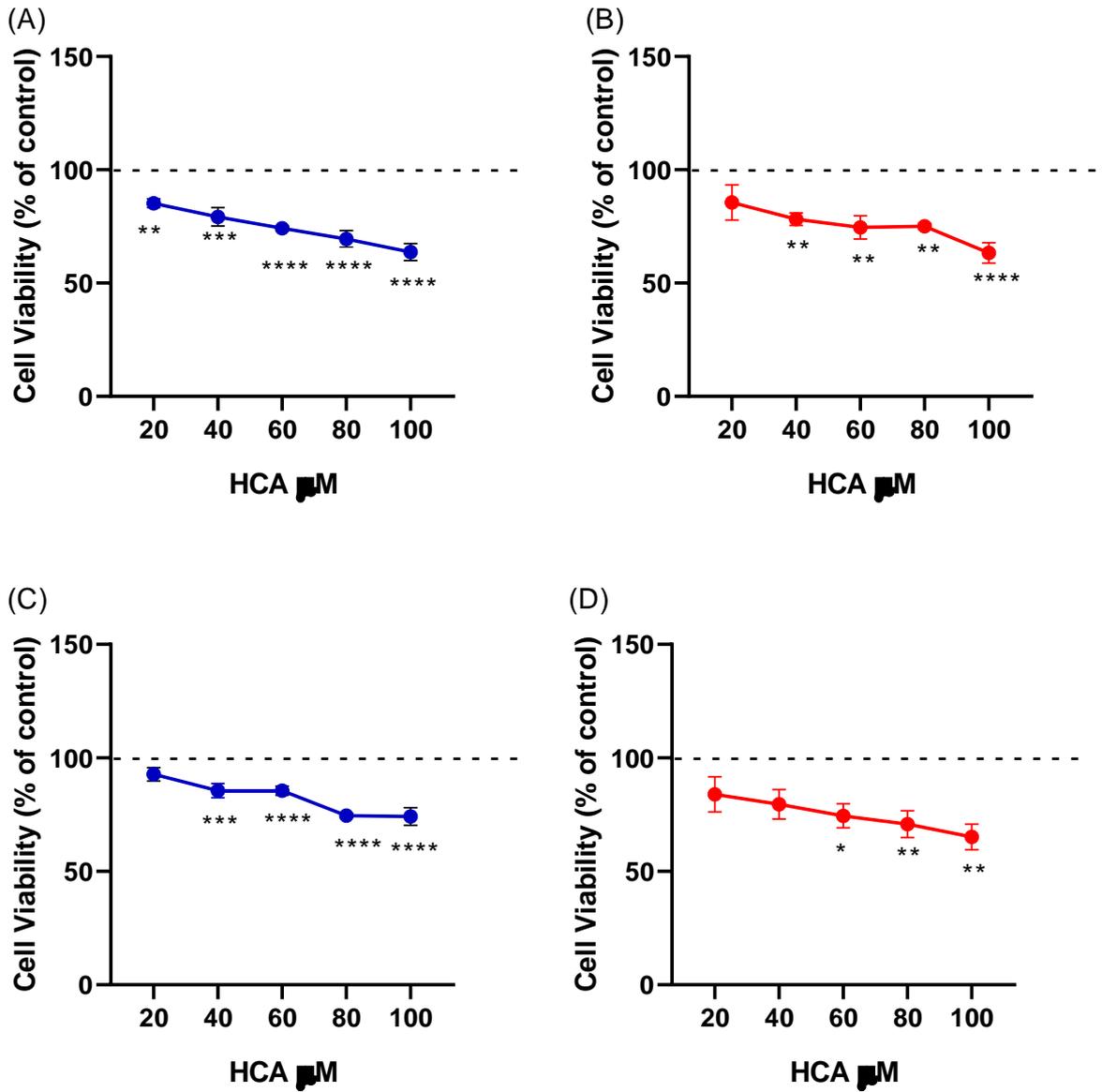


Figure 7.4.2: HCA exposure in HepG2 and Caco2 cell lines. Significant toxicity was observed in both HepG2 and Caco2 cell lines. (A) HepG2 untreated showed significant toxicity at all concentrations 20-100μM. (B) HepG2 cells treated with rifampicin showed significant toxicity at 40-100 μM. (C) Caco2 cells untreated showed significant toxicity at concentrations 40-100 μM. (D) Significant toxicity was observed in at concentrations 60-100 μM in Caco2 cells which had been treated with rifampicin.

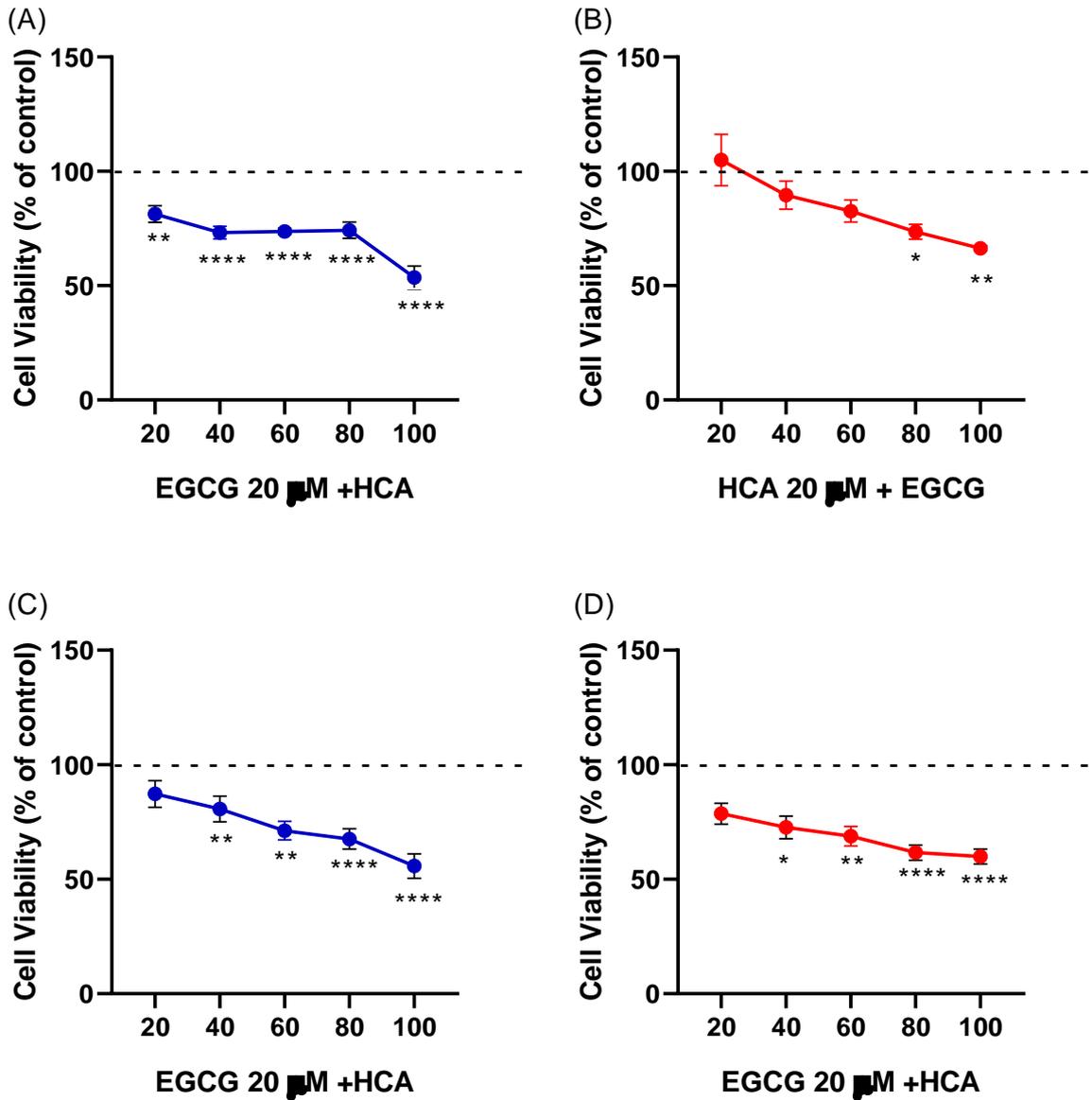


Figure 7.4.3: EGCG at 20 μ M with increasing concentrations of HCA. Significant toxicity was observed in both HepG2 and Caco2 cell lines. (A) HepG2 untreated showed significant toxicity at all concentrations 20-100 μ M. (B) HepG2 cells treated with rifampicin showed significant toxicity only at 100 μ M. (C) Caco2 cells untreated showed significant toxicity at concentrations 40-100 μ M. (D) Significant toxicity was observed in at concentrations 40-100 μ M in Caco2 cells which had been treated with rifampicin.

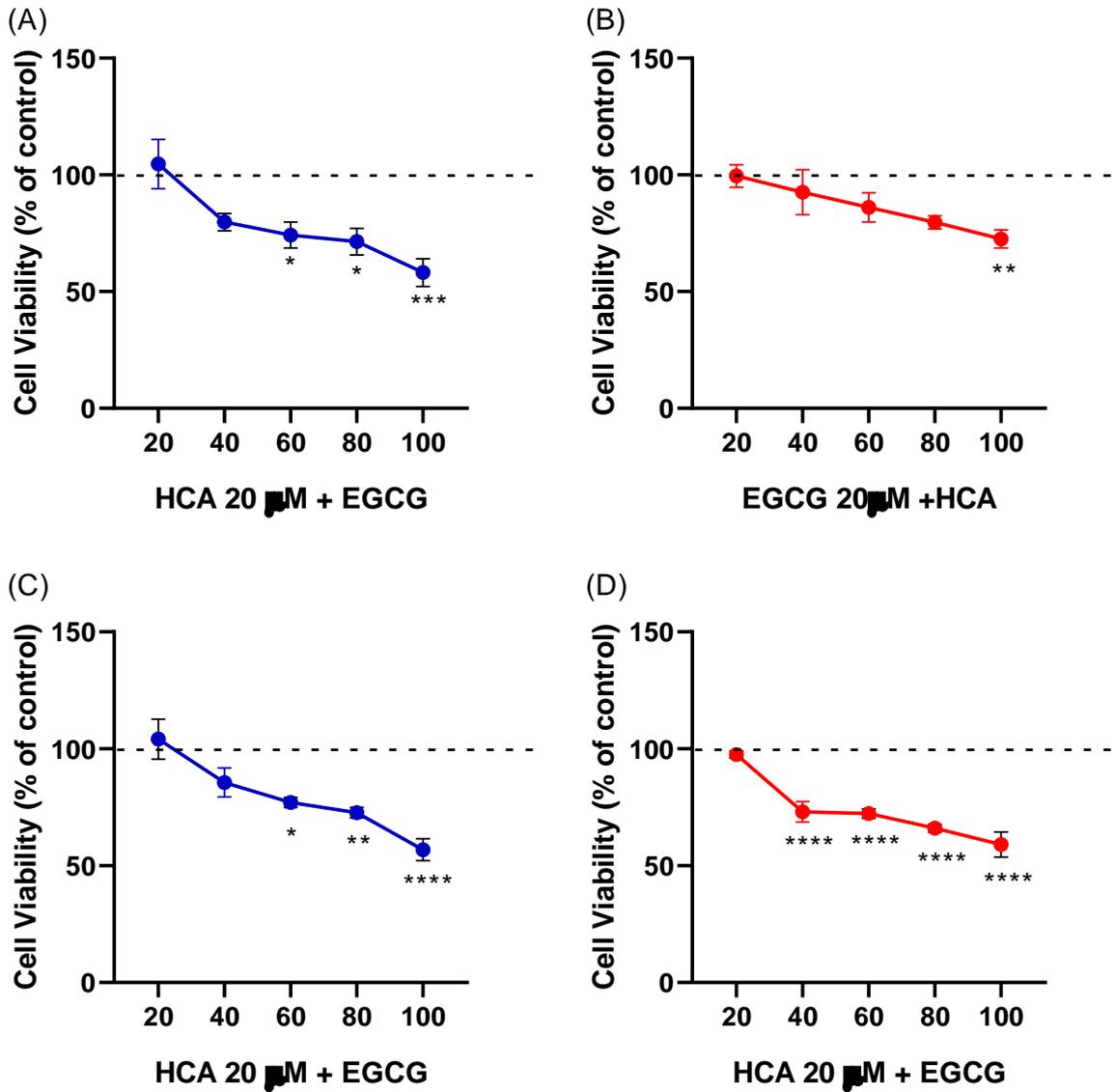


Figure 7.4.4: HCA at 20 μM with increasing concentrations of EGCG. Significant toxicity was observed in both HepG2 and Caco2 cell lines. (A) HepG2 untreated showed significant toxicity at concentrations 60-100 μM . (B) HepG2 cells treated with rifampicin showed significant toxicity only at concentration 80 and 100 μM . (C) Caco2 cells untreated showed significant toxicity at concentrations 60-100 μM . (D) Significant toxicity was observed in at concentrations 40-100 μM in Caco2 cells which had been treated with rifampicin.

7.5 Discussion

CAMs often contain a large number of phytochemicals with unknown toxicological and pharmacokinetic properties that can lead to adverse reactions including interactions with the CYP450 system. The potential of CAMs to inhibit or induce P450 enzymes is not investigated prior to the products being distributed to consumers. Contributing to the problem is the difficulty in reporting adverse events and relating them back to the use of CAMs. Another difficulty occurs in trying to identify the role of specific phytochemicals as there can be multiple supplements, multiple pharmaceuticals, or combinations of both supplements and pharmaceuticals.

The current study focused on two common phytochemicals often found together in herbal weight loss preparations, to evaluate their potential toxicity to the liver and gastro-intestinal tract. For this HepG2 and Caco2 cell lines were chosen. HepG2 cells are popular in drug metabolism and hepatotoxicity studies as they provide many benefits including easy handling, high availability and express differentiated hepatic functions [32]. Caco2 cells are commonly used to evaluate drug absorption and are an intestinal model, which includes the presence of drug metabolizing enzymes [33]. A limiting factor of HepG2 cells is the limited expression of drug metabolizing enzymes. [34]. To overcome this, and to ensure sufficient enzyme activity in both cell lines they were treated with rifampicin for induction on CYP enzymes, in particular CYP3A4. Paracetamol was used to test for function induction.

Popular for its purported weight loss effects, green tea has been associated with hepatotoxicity [11]. Figure 1 (A-D) shows a significant decrease in cell viability in both HepG2 and Caco2 cells. It was found that EGCG was more toxic in Caco2 cells compared to HepG2 cells. However, at the highest concentration, 100 μ M, the average cell death was 31% and 29% untreated and treated respectively in HepG2 cells and 33% and 42% in Caco2 cells.

The toxicity of green tea extract and its catechin EGCG has also been reported in rats following a single injection of green tea extract, severe acute hepatotoxicity developed via oxidative stress to hepatocellular lipids and DNA [35]. Following the consumption of commercially available green tea, the activities of hepatic microsomal CYP450s were decreased in rats [36]. Green tea extract has also been shown to

affect the pharmacokinetics of simvastatin and inhibit the hydroxylation of midazolam by CYP3A [37]. In a purified form, EGCG can inhibit the activity of multiple CYP450 enzymes including CYP3A, CYP2D6, CYP2B6, CYP2C19 and CYP2C8 in human intestinal microsomes and the liver [38]. Abe et al. [39] found that following a single dose of green tea extract, the plasma levels of nadolol, a β -blocker that is not metabolized by CYP450 enzymes but has been reported to be a substrate for several drug transporters, were significantly reduced [39]. Green tea extract and EGCG inhibits multiple CYP450 enzymes and this may contribute to the toxicity observed in Fig 1 and in reported adverse reactions. Additionally, green tea may inhibit the intestinal absorption of other pharmaceuticals taken concurrently.

Hydroxy citric acid (HCA) which is extracted from the rind of *garcinia cambogia* is a common active ingredient in many herbal weight loss preparations. HCA inhibits the activity of the ATP-dependent citrate lyase enzyme, causing the breakdown of citrate into oxaloacetate and acetyl-CoA [40]. Significant toxicity was observed in both HepG2 and Caco2 cell line following the exposure of HCA (Fig 2). Compared to EGCG, HCA was more toxic in HepG2 cells (Fig 2A, B). At the highest concentration of 100 μ M, the average cell death was 38% and 37% in untreated and treated HepG2 cells. The Caco2 cells had a similar result with 26% and 35% cell death following 48h exposure.

Severe adverse events have also been reported in response to dietary supplements containing *garcinia cambogia* [41]. HCA has been linked to adverse reactions, mainly hepatotoxicity with Cresciolo et al. detailing four case series where adverse reactions occurred [42-45]. However, it is important to note that the preparations associated with hepatotoxicity were multicomponent [6], underlining the importance of investigating the interactions between these two popular components.

Less well understood is the possibility of interaction between the multiple phytochemicals often present in CAMs. A recent case of hepatotoxicity necessitating liver transplant involved the taking of dietary supplements with epigallocatechin-3-gallate and hydroxy citric acid [28], suggesting that these phytochemicals may interact to produce toxicity. The results from the current study certainly show interactions between the two targeted phytochemicals. Figure 3 shows 20 μ M of EGCG with increasing concentrations of HCA. In untreated HepG2 cells, there is significant toxicity at all concentrations (Fig 3A)

compared to treated HepG2 cells, where significant toxicity is observed only at 80 μ M and 100 μ M (Fig 3B), suggesting that CYP enzymes are detoxifying EGCG. CYP enzymes found in the liver primary function is to metabolize drugs. Herbs, conventional medications and hepatic disease may inhibit the activity or expression of specific CYPs, which may cause greater risk of toxicity with EGCG.

At the highest concentration of 20 μ M EGCG with 100 μ M of HCA there was 47% and 28% cell death in untreated and treated cells respectively. When compared to the single exposure of EGCG and HCA in untreated HepG2 cells, cell death has increased in an additive manner by 16% and 10% respectively. There was significant toxicity observed at all concentrations (control of 20 μ M EGCG -100 μ M) in both uninduced and induced Caco2 cells (Fig3 C,D), suggesting that cytochrome P450 metabolism was not playing a significant role in detoxifying these phytochemicals in this epithelial cell model. At the highest concentration of 20 μ M EGCG with 100 μ M of HCA there was an additive 44% and 41% cell toxicity observed in uninduced and induced Caco2 cells respectively. Thus, compared to a single exposure to HCA in uninduced Caco2 cells there was an 18% increase and a 6% increase in treated Caco2 cells.

Overall, both EGCG and HCA contributed to each other's toxicity in an additive, rather than synergistic manner in both the hepatocyte model HepG2 and intestinal cell epithelial model Caco2. While both EGCG and HCA inhibit various CYP450 isoforms, EGCG is mostly metabolised by Catechol-O-methyl transferase [46], whereas HCA mostly passes through the citrate lyase pathway. Thus they are unlikely to interfere with each other's metabolism. However, it appears that the toxicity due to EGCG is due to targeting the mitochondrial oxidative stress pathway [47]. While HCA targets the mitochondrial ATP-citrate lyase pathway, the mechanism of HCA toxicity is unclear. The results suggest, the toxicity mechanisms of HCA and EGCG are independent.

7.6 Conclusion

With the rise in popularity of CAMs and dietary supplements, reports of severe adverse reactions following the use of herbal weight loss products in particular are becoming more frequent. Despite a sound knowledge of risks associated with herb-drug interactions, more research needs to be conducted on herb-herb interactions.

In the present study both EGCG and HCA were toxic in both the hepatocyte model HepG2 and intestinal cell epithelial model Caco2. While the effect of EGCG on hepatocyte toxicity is relatively well known, the toxicity of EGCG on Caco2 cells and the toxicity of HCA is less clear. HCA has been reported to produce gastrointestinal symptoms [29] so, the Caco2 results are informative. Induction of CYP enzymes with rifampicin pre-treatment reduced the toxicity of EGCG in HepG2 cells, but not HCA. Importantly, when EGCG and HCA were incubated together, the combined toxicity was potentiated in an additive manner above the individual toxicities of the chemicals. This suggests that EGCG and HCA are causing toxic effects through separate mechanisms. Furthermore, induction of cytochromes with rifampicin did not attenuate this additive toxicity.

The present study highlights the risks associated with multi-component products as well as the potential dangers of consuming multiple herbal products at the same time. These results also may explain underlying mechanisms in recent reports where subjects have developed severe liver failure when consuming multiple products containing EGCG and HCA respectively. Without required evidence of the pharmacokinetics of many of the ingredients contained in supplements before entering the market, consumers are at risk of possible herb-herb interaction. This study has shown that two common ingredients found in herbal weight loss preparations cause toxicity when exposed individually and can cause greater toxicity when they are together.

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Conclusion

The work presented has drawn attention to the associated risks of using CAMs, with particular attention to the roles of adulterants, contaminants and pharmacokinetic interactions in products claiming to aid in weight loss. In chapter one we have examined two existing historical herbal preparations revealing that no adulteration or contamination had occurred.

In chapter two we have demonstrated that tourists travelling to South East Asian countries and visiting herbal medicine facilities should be cautious before buying and consuming products, as there may be adulterants or contaminant which may cause adverse reactions.

In chapter three we have reviewed the currently available literature on the use of CAMs amongst children, highlighting the need for greater awareness and discussion amongst parents and health care providers, as infants and children may be at higher risk to adverse reactions due to their immature physiology. CAMs are fast becoming the most sought-after method for weight loss. In chapter four a literature review was conducted with a focus on five common herbs found in these products. This review also emphasized the risk of herb-herb interactions, which is commonly not considered as the cause of adverse reactions.

In chapter 5 we have shown that even without the presence of contaminants and adulterants in CAM products toxicity *in vitro* was still observed in both hepatocyte and gastrointestinal cell lines.

Following adverse reactions caused by three herbal weight loss products in chapter 6 we demonstrated that despite the lack of adulteration and contamination two out of the three products also caused toxicity *in vitro*.

In chapter seven, we focused on two common ingredients found in herbal weight loss preparations, EGCG and HCA, to gain further understanding of individual and multiple plant constituent toxicity. This study highlighted the risks associated with taking multiple herbal products as well as multi-component products. It has also provided insight into the recent reports of severe liver failure following the consumption of products containing EGCG and HCA.

Whilst the current work does raise awareness of the lack of research into herb-herb interactions, further work is still required, particularly in trying to understand the underlying mechanisms causing the observed toxicity.

In vivo experiments are also required to establish if the adverse effects recorded in cell models are also seen in whole organisms when compounds are not directly applied to cells but instead are subjected to the complex pharmacokinetics of a whole animal.

Modification to the current regulatory system needs to occur. The labelling on herbal medicines should include warnings associated with taking products and use bold text to highlight and ensure consumers are informed.

In conclusion, the work presented contributes new knowledge to the fields of complementary and alternative medicine and public health and raises awareness that the popular belief that it is natural and therefore safe is incorrect.



Evaluation of an early twentieth century Afghan herbalist's preparations

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Abstract

Mahomet Allum was a flamboyant philanthropist and herbalist who worked in South Australia in the early part of last century, whose herbal therapies generated some controversy at the time. Two of his preparations have survived to the present day, a general tonic and a treatment for liver and kidney dysfunction. Given the frequent use of pharmaceutical drugs in “tonics” at the time, toxicological analysis was undertaken at Forensic Science SA, Adelaide with liquid chromatography/quadrupole-time-of-flight mass-spectrometer (LC-QTOF MS), liquid-chromatography/ diode array detector (LC/UV) and gas chromatography/nitrogen phosphorous- detector/mass-spectrometer (GC-NPD/MS), to look for common drugs. In addition DNA analysis was also undertaken at Trace and Environmental DNA (TrEnD) Laboratory (Curtin University) to evaluate the types of plant products used to make these remedies. The general tonic contained genera from the Triticaceae (wheat) family as well as the Medicago family (includes alfalfa), possibly as fillers. Other genera found included *Urtica* (nettle) and *Passiflora* (passion flower). The preparation for liver and kidney disease also contained genera from the Medicago family as well as genera *Arctostaphylos* (bear berry) which has traditionally been used for the treatment of dysuria and bladder stones. No common drugs were found. Thus it appears that the two treatments prepared by Mahomet Allum contained only herbal substances and not adulterant pharmaceutical agents. The herbals identified provide an insight into herbalist practices in the early twentieth century.

Keywords Mahomet Allum · Herbal preparations · Early twentieth century · Afghan

Mahomet Allum, one of South Australia's most iconic herbalists and philanthropists, was often described as a “a very colorful character” with his dyed hair, eye-catching jewelry and a love of publicity attracting a great deal of attention (Fig. 1). Along with this, his practice of alternative medicine caused some controversy within the community [1]. Hanji Mahomet Allum, better known as Mahomet Allum, was born in Kandahar, Afghanistan in 1858. In the late 1880's he sold horses to the British Army, earning him enough capital to move through Asia prior to his arrival in Australia. As he travelled through Australia he used camel teams to deliver

goods to isolated farms and townships, as well as working as a miner, rug dealer, station hand, butcher and a storekeeper [1, 2] (Table 1).

In 1928–29 he moved to Adelaide where dissatisfaction with standard medical treatments enabled him to set up as a herbalist [1–3]. He prepared herbal therapies and according to the Australian dictionary of biography asked for no payments, although he accepted donations and gave freely to charities. His popularity and abilities were written about by his patients (and himself) in testimonials, advertisements in the *South Australian Police Journal* and newspapers (Fig. 2), and even in a pamphlet that he published [1, 2]. An article published in 1933 highlighted his popularity and work:

“Mr Allum's diagnosis of the most obscure malady is as uncannily correct as his treatment is efficacious. His knowledge of herbs, the heritage of an ancient race, handed down from father to son, represents the accumulated wisdom of the ages, and is now applied in a strange land for the benefit of those who, while differing in creed and color, he regards with an all-embracing love as brother man and sister woman” [4].

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Fig. 1 Image of Mahomet Allum in 1910 State Library of South Australia B43851

During a court case in 1935 when he was charged with impersonating a medical practitioner while not being registered under the Medical Practitioners Act, 40 witnesses stated that he had never claimed to be a doctor. Although in the end he was convicted and fined, it is thought that the publicity of the trial probably brought him more customers [2].

Allum became well-known for his stomach wash, which he called “blackjack” and which was also known as an “Allum bar” [3, 5]. His belief was that most Western ailments were caused by an unclean stomach and so clients were given “blackjack” stomach wash to completely purge their systems. Although the actual composition was kept a secret, it appeared to be comprised of butter, honey and laxative senna pods [3, 5]. Following treatment of ‘blackjack’ customers were then given advice or herbal mixtures, including lotions, for ailments that were troubling them (Fig. 3) [3, 6].

During the eighteenth century and into the early part of the twentieth century it was a very common practice to include drugs such as cocaine, opium or cannabis in tonics that were used to treat a broad spectrum of conditions and diseases ranging from “blind piles” (hemorrhoids) and “puerperal after-pains”, to childhood teething problems [7–10] (Fig. 4). The entry under opium in the 1796 *Encyclopedia Britannica* quoting an “*Essay on Diseases of the Viscera*” reveals very clearly how highly the drug was regarded:

“*Opium at present is in great esteem, and is one of the most valuable of the simple medicines. In its effects on the animal system, it is the most extraordinary substance in nature. It touches the nerves as it were by magic, and irresistible power....*” [11].

Given the apparent popularity of Mahomet Allum’s potions and the widespread use of additives it was decided to investigate whether the two surviving preparations might contain pharmaceutical adulterants. The pills were made on a hand press as was common at the time. Toxicological analysis was undertaken at Forensic Science SA, Adelaide using a liquid chromatography/quadrupole-time-of-flight mass-spectrometer (LC-QTOF MS), liquid-chromatography/ diode array detector (LC/UV) and gas chromatography with nitrogen-phosphorous and detector/mass-spectrometer detectors (GC-NPD/MS) using standard methodology [12]. At the same time next generation DNA characterization of the samples was performed at Trace and Environmental DNA (TrEnD) Laboratory (Curtin University), again using standard methodology [13].

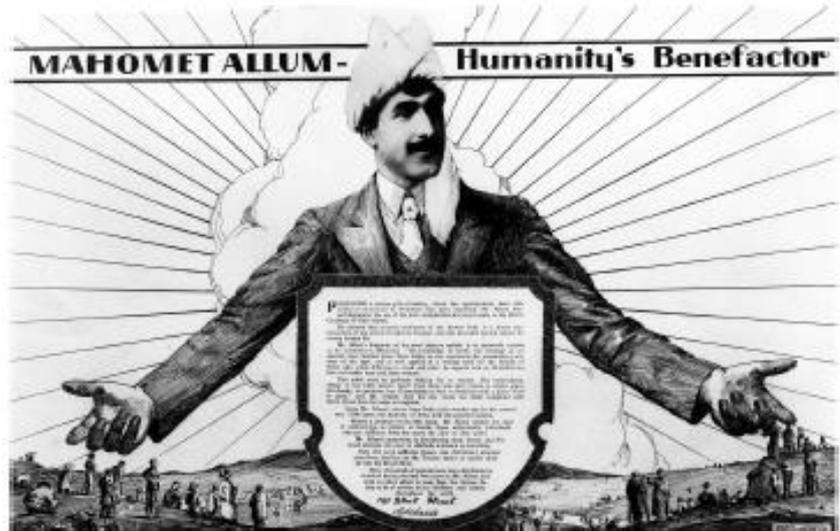
No common prescribed or illicit drugs were detected in either preparation. DNA analysis identified genera from the *Triticeae* (wheat) family as well as *Medicago*, possibly used as fillers in the general tonic, although in Persian traditional medicine *Medicago officinalis* was used as a diuretic and *Medicago sativa* was used for kidney stones [14, 15]. Other genera that were found

Table 1 Biological content of Mahomet Allum medicines

Sample	Plant DNA	Animal DNA
7X	<i>Medicago</i> (legume family), <i>Passiflora</i> (passion flower/passion vine), <i>Urtica</i> (netles/stinging nettles), <i>Triticeae</i> (grasses), PACMAD clade	–
P14	<i>Apiaceae</i> (numerous possibilities), <i>Nassauvia</i> (sunflower family), <i>Trixis</i> (threefolds/daisy), <i>Triptilion</i> (sunflower family), <i>Phaenocoma</i> (pussy’s-toes tribe/sunflower family), <i>Arctostaphylos</i> (manzanitas and bearberries), <i>Medicago</i> (legume family), <i>Nasturtium</i> or <i>Cardamine</i> (bittercresses), <i>Coleonema</i> (<i>Diosma</i>) or <i>agathosma</i> , PACMAD clade	–

Apiaceae are the commonest botanical family recommended by Shirazian herbalists for the treatment of kidney stones [15]

Fig. 2 An advertisement and portrait of describing the services and healing powers of Mahomet Allum. State Library of South Australia B52941



in this tonic included *Urtica* (nettle) and *Passiflora* (passion flower). Herbalists have used *Urtica* leaves in the treatment of numerous systemic and dermatologic inflammatory conditions [16]. The leaves have also been prepared as a tea with diuretic and antidiabetic therapies, and to treat stomach disorders [17]. *Passiflora* has long been used for its sedative and anxiolytic effects and is commonly added to herbal remedies to treat nervousness and insomnia [18, 19]. Somewhat surprisingly, given Mahomet Allum's beliefs, no purgatives (eg. Senna) were found.

The tonic for liver and kidney function also contained genera from the *Medicago* family; *Medicago sativa* was used for kidney stones, as well as *Medicago officinalis* as a diuretic [15]. In addition the genera *Arctostaphylos* (bear berries), which has traditionally been used for the treatment of genitourinary symptoms from cystitis, urethritis, pyelitis and stones was present [20]. However, there is little published data to support its efficacy. Other families were detected, including Brassicaceae, with the closest matching genera being *Nasturtium* or *Cardamine* and the Rutaceae, with the closest matching genera being *Coleonema* or *Agathosma*. All four of

Fig. 3 Original packaging of a herbal lotion (Dewyee Remedy) prepared by Mahomet Allum. Migration Museum collection courtesy R Brown



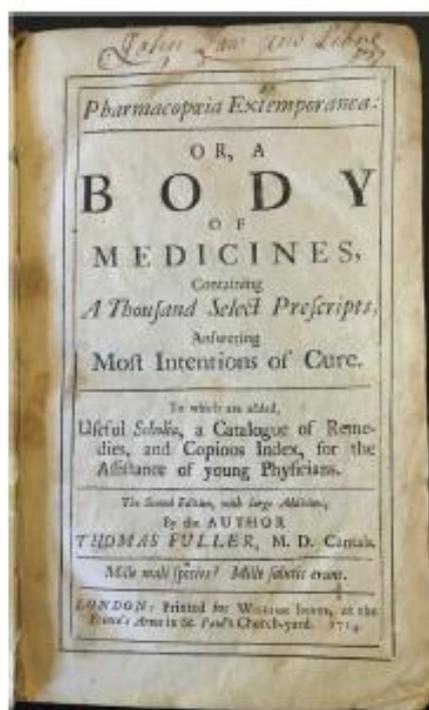


Fig. 4 The frontispiece of the *Pharmacopœia Extemporanea* by Thomas Fuller published in 1714 which contains numerous recipes

these genera have been shown to have diuretic properties [21–23]. In particular *Arctostaphylos* and *Nasturtium* form part of the Middle-Eastern herbal tradition to which Mahomet Allum was heir [14, 24].

In conclusion, the two herbal preparations that were tested showed no evidence of pharmaceutical adulteration, although it is possible that age-related degradation may have reduced the likelihood of detection of some substances/herbs. The analysis did, however, identify some of the species of plants used by Mahomet Allum in creating his remedies and gives an insight into the practice of herbalism at this time. After Allum's death in 1964 his estate of £11,218 was given to institutions that cared for children [1] — a very fitting legacy for a remarkable individual.

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Compliance with ethical standards

Ethical approval Not required.

Conflict of interest The authors declare that they have no conflicts of interest.

Informed consent Not required.

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Short communication

Potential forensic issues in overseas travellers exposed to local herbal products

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Herbal medicines are an important and culturally accepted part of healthcare globally, offering an accessible and affordable way for many to utilize particular therapies. The use of these products is also growing in many Western countries including the United Kingdom, the United States, Canada and Australia. For example, a recent audit of the Australian complementary medicine industry showed that it has generated revenues of \$4.7 billion due to this increase in interest and demand from consumers in Australia and abroad.¹ As part of health and wellness tourism Western travellers to many Asian countries now often visit herbal centres where free health checks may be performed and herbal products are offered for sale. This type of tourism is “based upon learning about and consuming traditional medicinal herbs”,² and is “an important part of the worldwide medical tourism industry” as “it treats patients without using harmful chemicals or drugs”.³ The problem with this activity is that latter statement is simply not correct as the composition of many of these products is uncertain, there may be contaminants and pharmaceutical additives, and the interaction with prescription medications may be idiosyncratic.^{4,5} However, the potential role and impact of herbal medicines is usually not considered in medical cases.⁶

While the possibility of occult sepsis has been discussed in the context of recently returned visitors from overseas the potential for forensic issues to arise from herbal medicines has not.^{7,8} Examples of problems that may be found with traditional preparations were exemplified by Huang et al’s study in 1997 of 2609 samples of traditional medicines collected by eight major general hospitals in Taiwan which showed that 618 (23.7%) were adulterated with synthetic therapeutic substances (drugs) and that 52.8% of these contained two or more adulterants.⁹ Yee et al’s study of 3320 Chinese medicines screened between 1990 and 2001 showed that 138 contained toxic heavy metals at levels above legal limits and 41 contained 19 synthetic drugs.¹⁰ A study of all cases of herbal antidiabetic products referred to a tertiary centre for toxicological analysis in Hong Kong from 2005 to 2010 showed that 27 of the cases contained adulterants, eight of which were undeclared registered or banned oral antidiabetic agents such as glibenclamide, phenformin, metformin, rosiglitazone, gliclazide, glimepiride, nateglinide and repaglinide. One sample contained four

adulterants and 63% of the patients had clinical manifestations, most often arising from hypoglycaemia or lactic acidosis.¹¹

More recently, and of forensic significance, was a study of 61 patients in Hong Kong who had consumed traditional Chinese medicines that had been adulterated with corticosteroids, most often dexamethasone. Seven of the patients (11.5%) required admission to intensive care, two died within 30 days of presentation, and 38 (62.3%) had complications due to the steroid adulterant.¹² Finally, a recent study of 404 cases referred for analysis to a tertiary referral toxicology laboratory in Hong Kong revealed 1234 adulterants which included “approved drugs, banned drugs, drug analogues and animal thyroid tissue.” Approximately 65% of the patients had adverse clinical effects directly attributable to these substances, with 14 severe and two fatal cases. The three most common presentations were psychosis, Cushing syndrome and hypoglycaemia.¹³

To investigate this issue further 14 processed herbal preparations in capsule, tablet and powder form were randomly selected and purchased from a traditional herbal retailer in Yangon, Myanmar (Burma) during a university lecturing visit. The samples were analysed in the toxicology laboratory at Forensic Science SA for the presence of contaminants and adulterants according to established methods¹⁴ using a liquid-chromatography/quadrupole-time-of-flight mass spectrometer (LC-QTOF), a liquid-chromatography/diode array detector (LC/UV) and a gas-chromatography/mass spectrometer (GC/MS). Toxicological results showed that only one sample contained an adulterant, but that this was yohimbine.

Yohimbine is an α 2-adrenergic receptor antagonist alkaloid which acts on serotonergic and adrenergic receptors in the brain in areas associated with libido and penile erection.^{15,16} It is also popular for athletic performance and weight loss in body-builders.¹⁷ Given that the adulterated product was being marketed to enhance health and vitality, and to treat impotency and erectile dysfunction it was likely a deliberate additive. Yohimbine is derived from the bark of the African tree *Pausinystalia yohimbe* and has been associated with a wide range of severe side effects including hyper and hypotension, cardiac tachyarrhythmias, coma, seizures, chest pain, bronchospasm and psychiatric disturbances including mania, as well as significant interaction with

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prescription medications such as antidepressants.^{18–22} For these reasons yohimbine is a prescription only substance in Australia, and one that it is illegal to import.^{23,24}

The published studies and the reported analysis show that individuals from Western countries who travel through Asia may be exposing themselves to potentially harmful substances or drugs in local herbal preparations that may have a significant negative impact on their health. The possibility of these materials either contributing to, or causing, death should be considered in all medicolegal cases involving recent overseas travel, particularly to Asian destinations. The lack of cases reported from forensic facilities may merely reflect a failure to check for these products at the time of post mortem assessment.

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MINI REVIEW

Potential adverse outcomes of herbal preparation use in childhood

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**Keywords**

Adverse drug reactions, Children and adolescents, Complementary and alternative medicine

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ABSTRACT**Aim:** Complementary and alternative medicines are becoming increasingly popular worldwide with a variety of purported medicinal uses. These products are generally believed to be natural and therefore safe, with few adverse reactions. With this perception, parents are now taking their children to see practitioners prescribing these medicines as well as self-prescribing. Despite this, there are issues regarding safety, efficacy and regulation, with increasing numbers of reports of adverse reactions to these products. Therefore, a mini-review was conducted to ascertain the potential risks to children.**Methods:** A overview of literature was conducted to highlight the current use of complementary and alternative medicines in children and the possible risks associated with their use.**Results:** Infants and children may be more susceptible to harmful effects due to their immature physiology and metabolic pathways and different dosage requirements. Adverse reactions may also be caused by interactions with conventional medicines, contamination with heavy metals, and adulteration of filler products including other plant species or pharmaceutical agents.**Conclusion:** As complementary and alternative medicines become increasingly used alongside and with conventional drug therapy, there needs to be greater awareness and discussion among parents, complementary practitioners and medical practitioners to ensure the overall health and safety of children being exposed to these products.**INTRODUCTION**

Complementary and alternative medicines (CAM) have become increasingly popular worldwide with a variety of purported medical uses. CAM is an umbrella term used to describe a wide range of products including medicinal products containing such ingredients as herbs, vitamins, minerals and nutritional supplements, as well as homoeopathic and certain aromatherapy preparations (1). Despite the controversy around these products, including issues of efficacy, safety and dosing, there has been an increased use of CAMs in children (2–5).

This is because it is generally believed that CAMs are safe with fewer side effects compared to conventional

medicines; thus more parents are choosing these products for their children (6,7). Despite this belief, there are issues which include lack of clinical research into the possible adverse effects of these products in children, failure to use

Abbreviations

ADR, Adverse drug reaction; CAM, Complementary and alternative medicine.

Key notes

- The use of complementary and alternative medicine continues to increase as they are believed to be safer with few side effects.
- Despite perceived safety, adverse events following the use of these products have been documented, with children at greater risk.
- To ensure overall health and safety of children, greater awareness and conversation between parents and both medical and complementary practitioners need to occur.

effective conventional medicines, and possible interactions between pharmaceutical and herbal preparations.

USAGE

A recent systematic review conducted by Italia et al. (8) analysed patterns of CAM usage among children reported in 58 studies from 19 countries. It was found that CAM usage by children varied widely from 10.9 to 87.6% for lifetime use, and from 8 to 48.5% for current use, depending on nationality and CAM modalities. The majority of the studies reviewed included all CAM modalities. Fewer studies reported on CAM practitioners seen or individual CAM treatments. However, herbal usage by children varied from 0.8 to 85.5% for lifetime use and homeopathy usage varied from 0.8 to 39%, with significant differences in usage by country (8).

Frawley et al. (9) investigated the prevalence of CAM usage by Australian children over a 12 months period in 2016 and found that 73.8% of parents had taken their child to visit a CAM practitioner and/or had given their children a CAM product in the previous 12 months. In comparison, a study conducted by Smith and Eckert a decade earlier in 2006, investigating the use of CAM in South Australian children found that 18% of children had used a CAM product or consulted with a CAM practitioner in the previous year (10). Another study conducted in 2006 compared the use of CAM amongst children at hospital outpatient clinics in Australia and Wales and found that 51% of Australian children had used CAM in the previous 12 months (4). Nonprescribed vitamins and minerals were the most common medicinal types of CAM used and aromatherapy and reflexology were the most prevalent nonmedicinal CAMs (4). Some of the difference between the Smith and Eckert study (10) and the Crawford Study (4) may be due to Smith and Eckert only considering megadoses of vitamins as CAMs (10). While usage increased from 2006 to 2016, patterns of use were similar, with most CAMs being used for health maintenance and vitamins being used more often than herbals (4,9). These findings demonstrate the increasing popularity of these products in paediatric populations.

POTENTIAL ADVERSE EFFECTS

Potential adverse events due to CAMs can range from events precipitated by the intended ingredients (e.g., bleeding and Ginkgo), events due to adulterants and contaminants or failure to use conventional medicine. A study by Lim et al. (11) investigated adverse events associated with CAM use reported to the Australian Paediatric Surveillance Unit between January 2001 and December 2003. Questionnaires were distributed to Australian paediatricians who had reported a suspected CAM-associated adverse event. Over the study period, there were a total of 46 cases documented. The ages ranged from birth to 16 years, with adverse events from mild to severe, with four fatalities. In 25 of cases (64%), the adverse events were rated as severe, life-threatening or fatal. In 30 cases (77%), the adverse events were either probably or definitely related to CAM. In 17 cases, the paediatricians considered that the child had suffered harm because of the failure to use conventional medicines of proven efficacy. The four fatalities included an 8 months old and a 10 months old who developed septic shock which was treated with CAM therapies rather than conventional medicines (11).

Meinckke et al. (12) used data from Vigibase®, a self-reporting system for medical professionals, to investigate hypersensitivity reactions (type 1) in children (age < 18 years) caused by herbal medicines. Data between 1968 and 2014 were reviewed. Inclusion criteria for the study required herbals to be classified as *the suspect* and to have an Herbal Anatomical-Therapeutic-Chemical code. Reaction terms less suggestive of hypersensitivity were excluded, and no cases with gastrointestinal symptoms were examined. A total of 26 909 unique Individual Case Safety Reports relating to herbal medicines from 42 different countries were identified, 79 cases of which met inclusion criteria, with 107 adverse drug reactions (ADRs) reported. Common ADRs were rashes (22.4%), urticaria (22.4%) and erythema (15.0%). The recovery rate of the patients from one or more ADRs was 72.0%, and there were no reports of fatal events. In the nine ADRs reported as 'not recovered,' rash and urticaria were most commonly caused by a variety of herbal medicines.

Table 1 Adverse events associated with herbal preparations

Herbal product	Adverse events	Mechanism of toxicity	Comments
In Bu Huan (15)	Lethargy, breathing abnormalities, CNS depression	Incorrect plant used (genus <i>stephania</i> instead of genus <i>polygala</i>) Contamination with lead	Highlights issues with herb identification, product substitution and mislabelling. Consider use of herbal medicines when idiopathic systems present
Pennyroyal Oil (16–19)	Eight weeks old experienced fulminant liver failure, cerebral oedema and necrosis six months old experienced hepatic dysfunction, epileptic encephalopathy	Pennyroyal contains a hepatotoxic compound: pulegone Pulegone primary compound, menthofuran is highly toxic	Misidentification/substitution of plant <i>Mentha pulegium</i> Traditional use in Hispanic and other cultures if to give infants tea made from boiled mint leaves
Germanier (20)	Abdominal discomfort, dark urine, jaundice and liver enzyme abnormalities	Patient had been consuming 600 mg/day of germanier along with arginine/betaine supplements	Herbal therapy should be considered in all children who present to hospital with 'idiopathic' hepatic necrosis

Table 2 Adverse events associated with heavy metal contamination in herbal preparations

Herbal product	Adverse events	Mechanism of toxicity	Comments
Local Chinese medicinal herbal spray (21)	A five years old became irritable, continually clearing throat and developed a rash. Sudden onset of motor tics	Patient had been using the spray up to 20 times a day. Recommended dosing was 1–2 times a day. Product contaminated with mercury	
Medicated topical baby powder (22)	A three years old suffered chronic constipation, abdominal pain for six months	Although appearing in good health and no developmental delays the patient's blood lead level was 330 µg/L. Sister of patient had a blood lead level of 248 µg/L	Highlights the importance to examine all family members to investigate exposures. Patient and sister were observed over the following years with the blood lead levels slowly dropping
Various traditional Chinese (23) herbal medicines for minor ailments	A four-month-old infant experienced fever, cough, anorexia, vomiting a week prior and seizure before hospital admittance	Blood lead level was 137 µg/L. After testing it was discovered that the herbal preparations contained lead	
Tibetan Herbal Vitamin from India used to promote 'brain growth' (24)	A five years old developed encephalopathy, seizures, developmental delay and anaemia	Patients blood lead level was 86 µg/dL. Herbal preparation contained lead and mercury ranging from 54.8–35 300 mg/kg and 3–12 800 mg/kg respectively	The patient was estimated to have consumed 63 g of lead over four years of taking the vitamin. Over the following four years, patient was readmitted seven times to reduce blood lead levels

INTERACTIONS

As herbal medicines become integrated into Western society, concurrent use of pharmaceuticals and herbal products is increasing. In the United States, it was found that 20–30% of patients had used both herbal medicines along with prescription medication (13,14). There is limited information provided to consumers regarding the risk associated with herb–drug interactions associated with the concurrent use of pharmaceuticals and herbal medicines. Several active compounds found in herbal medicines can serve as substrate enzymes involved in the metabolism of xenobiotics. However, little is known regarding the pharmacokinetics of many of the phytochemicals present in herbal materials. Co-administration of herbal and conventional medicine increases the risk of adverse reactions, with children being more vulnerable due to their immature physiology and metabolic pathways and the varying dosages.

CASE STUDIES

Contamination and adulteration of herbal medicines also pose a threat. Table 1 provides examples of how potential adverse reactions can occur when children are exposed to herbal preparations with intrinsic toxicity. Heavy metals including lead can be intentionally added to herbal preparations as an essential component or by contamination. Table 2 highlights examples and the consequences of heavy metal exposure to children through the use of herbal medicines.

CONCLUSION

Although CAMs are becoming an integral part of therapy in the community, there are issues regarding efficacy, safety and regulation of these products. With many children visiting CAM practitioners and/or using CAM products, often bought over the counter in pharmacies or in nonmedical settings such as online or health food stores, it is likely that adverse reactions are not being monitored or reported accurately. As the popularity of these products continues to increase there is a need for further discussion and stronger relationships among CAM and general practitioners and parents to ensure that the optimal health and safety of children is achieved.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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