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The Pharmaceutical Society of Hong Kong
The Practising Pharmacists Association of Hong Kong
The Society of Hospital Pharmacists of Hong Kong

Don't Get Headstrong with Great Leaps on Ultimate Understanding of Traditional Medical Practices in Overnight

Life is one of the most mysterious subjects on this planet. It is a complicate system that has drawn our focus and attention for thousands of year with aims to unveil its underlying phenomenon during the course of seeking knowledge in human history. Because of our curiosity and explorations, we have a better understanding of our body between its structure and functions in only the last 100 years. Indeed, our biological knowledge and medical practices were filled with lots of myths and malpractices three centuries ago. Prevention of health problems and treatment of a disease were based on empirical and non-scientific approaches. Yet we didn't give up nor discontinue our supports such as financial resources, efforts, time and manpower in order to prove which is correct or wrong in our daily practices. We could question what are wrong but won't reject them until they were proved completely wrong.

However, about a year ago, some 400 doctors, medical researchers and scientists formed a lobby group to urge universities to close down all alternative medicine degrees in Australia.[1] The cause of protest from medical professionals was originated from a decision in United Kingdom that no longer is possible to receive a degree from a publicly-funded university in the areas of alternative medicine, including homeopathy and naturopathy because these courses are regarded as quackery and fail to champion evidence-based science and medicine from the western medical point of view. Although the urge to axe alternative medicine courses was eventually unsuccessful,[2] this movement does imply some meanings for those practicing alternative medicines. The urge reflects that lot more scientific efforts and clinical studies are urgently required before they could really find a seat in our modern society and our current education system. Before adequate evidences are established and collected, it will continue to be criticized or attacked by the contemporary medical practitioners.

In order to convince people these para-medical practices are reliable and beneficial to human health, attempts to develop scientific investigations on whatever disciplines should be encouraged. Pun et al via systemic experimental works provides some evidences how purified phytochemicals isolated from herb could give a stronger effect if they were used simultaneously. Their report in p. 25 offers a good piece of work to demonstrate that combinational uses of bioactive compounds give a better efficacy. The work also provides a good explanation why most Chinese medicines are prescribed in compound form instead of a single herb. In their study, a synergetic anti-human promyelocytic leukaemia effect was observed once all-trans retinoic acid was used in combination with p-coumaric acid and caffeic acid.[3] Although no mechanistic information is given in this work, it is still a piece of evidence-base study partially backing up our concurrent uses of different Chinese herbs. Besides this piece of original research work, Al Zaharna and Cheung also produced a mini review falling in the same direction to address the prevention and treatment of cancer by combination use of different phytochemicals. Possible ways in which carcinogenesis is blocked by phytochemicals is outlined in this report albeit not comprehensively covered.[4]

Besides these interesting reports mentioned above, there are announcements about the discontinue use of some conventional drugs due to either negative in their effect or because no evidence for their efficacy after some thoughtful clinical validations. Readers can find these disbanded drugs in the section of News and Communications.

The first quarter of 2014 has been an exciting time for all the Pharmacists in Hong Kong. Firstly, the registration of the Hong Kong Pharmacists Union (HKPU) representing pharmacists working in all sectors was legally approved on February 13 by the Hong Kong Government and the union held its first Annual General Meeting on March 4, 2014 to form the first General Council of the Union. As a registered trade union, the HKPU is empowered to have the legal rights to intervene in situations relating to the work of practicing pharmacists. This is really a big breakthrough for all pharmacists in Hong Kong. To learn about the mission of this new organization, readers are referred to a more detailed report in p34 of this issue.

Secondly, our Annual Hong Kong Pharmacy Conference was successfully held from March 15 to 16 in the Convention and Exhibition Centre, Hong Kong. This two days conference was again a big event for all pharmacists; more than 500 registered members turned up for social contact as well as for continue education of their professional knowledge in this meeting organized once a year by the two local pharmacy schools, DH, HA and the Pharmaceutical Society of Hong Kong, the Practising Pharmacists Association and the Society of Hospital Pharmacists. This year, we had delegates from Mainland China, Australia, Singapore, France and UK. Hence, it was really an international meeting.

A press conference was held by a few veteran pharmacists on March 16, to disclose their findings that there is an obvious need of pharmaceutical services to people dwelling in elderly homes after three years of trials. As the population is aging, the need for more advices and assistances to use medications is expected to continue to increase. Senior citizens, in general, have difficulty to properly use medicines; hence, pharmaceutical services to the elders are obvious. A comprehensive report about this service can be found in p33.

Cheung Hon-Yeung
Editor-in-Chief
1st May, 2014

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INSTRUCTIONS FOR AUTHORS

The Hong Kong Pharmaceutical Journal is a journal of the pharmacists, for the pharmacists and by the pharmacists. Submissions are welcome for the following sections:

- Pharmacy Education & Practice
- Drugs & Therapeutics
- OTC & Health
- Pharmaceutical Techniques & Technology
- Medication Safety
- Herbal Medicines & Nutraceuticals
- Society Activities
- New Products

Comments on any aspects of the profession are also welcome as Letter to the Editor.

There is no restriction on the length of the articles to be submitted. They can be written in English or Chinese. The Editorial Committee may make editorial changes to the articles but major amendments will be communicated with the authors prior to publishing.

It is preferable to have original articles submitted as an electronic file, in Microsoft Word, typed in Arial 9pt. Files can be sent to the following address:

e-mail: ***editor@hkpj.org***
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For detail instructions for authors, please refer to the first issue of each volume of HKPJ.

Singapore: Advisory on the Use of Cyproterone Acetate/Ethinylestradiol

Date: January 4, 2014

It was announced on the website of Health Sciences Authority (HSA) on 4 January 2014 that new restrictions would be imposed to limit the use of products containing cyproterone acetate/ethinylestradiol (CPA/EE) so as to mitigate the risks of venous and arterial thromboembolism in view of the modest benefit derived from treatment with CPA/EE for conditions such as androgenetic alopecia and mild acne.

In Hong Kong, there are ten registered products containing CPA and EE, including the brand Diane -35 Tab (HK-43330). All are prescription only medicines indicated for the treatment of severe acne and moderately severe hirsutism. The Registration Committee of the Pharmacy and Poisons had already discussed the issue in July 2013 and decided that in order to ensure the safe use of pharmaceutical products containing the combination of CPA and EE, the sales pack labels and/or package inserts of combination products containing CPA and EE should be revised as follows:

A. to remove the indication of "androgenetic alopecia";
B. to include new safety information for "indications". Examples of the wordings are:
   - although this product also acts as an oral contraceptive, it should not be used in women solely for contraception, but should be reserved for those women requiring treatment for androgen-dependent conditions only, i.e. moderately severe hirsutism and severe acne;
   - this product should only be used for the treatment of acne when alternative treatments, e.g. topical therapy and oral antibiotic treatment, have failed; and
C. to include new information for "warnings". Examples of the wordings are:
   - there is some epidemiological evidence that the incidence of venous thromboembolism is higher in users of this product when compared to users of combined oral contraceptives with low oestrogen content (<50mcg ethinylestradiol).
   - This product is contraindicated in women with thrombophlebitis, thromboembolic disorders, or a history of these conditions.

Source: www.drugoffice.gov,hk

US: Possible Harm from Exceeding Recommended Dose of Over-the-counter Sodium Phosphate Products to Treat Constipation

Date: January 8, 2014

The Food and Drug Administration (FDA) of the United States (US) warned healthcare professionals about the risk of rare but serious harm to the kidneys and the heart, and even death for products containing sodium phosphate if the recommended dose is exceeded. Sodium phosphate drug products include oral solutions taken by mouth and enemas used rectally, and are indicated for the relief of occasional constipation, and for bowel cleansing before rectal examinations. FDA received reports of severe dehydration and changes in serum electrolytes levels from taking more than the recommended dose of sodium phosphate products, resulting in serious adverse effects on organs, such as the kidneys and heart, and in some cases resulting in death. These serum electrolytes include calcium, sodium, and phosphate. Most reported cases of serious harm occurred with a single dose of sodium phosphate that was larger than recommended or with more than one dose in a day. Some individuals may be at higher risk for potential adverse events when the recommended dose of sodium phosphate is exceeded. These include young children; those older than 55 years; patients who are dehydrated; patients with kidney disease, bowel obstruction, or inflammation of the bowel; and patients who are using medications that may affect kidney function. These medications include diuretics, angiotensin converting enzyme inhibitors, angiotensin receptor blockers and nonsteroidal anti-inflammatory drugs. Healthcare professionals are advised to use these products as recommended on the label, and not exceed the labeled dose. Healthcare professionals are also advised to use the products with caution when recommending such oral products for children 5 years and younger, and that the rectal form of these products should never be given to children younger than 2 years.

Source: http://www.fda.gov/Drugs/DrugSafety/ucm380757.htm

Canada: Health Canada Endorsed Important Safety Information on EFFIENT® (Prasugrel Hydrochloride)

Date: January 18, 2014

Eli Lilly Canada Inc. in collaboration with Health Canada inform healthcare professional regarding the important safety information about EFFIENT® (prasugrel hydrochloride), an antiplatelet agent indicated for the prevention of atherothrombotic events in patients with acute coronary syndromes.

The information concerns the indication related to UA (unstable angina) or NSTEMI (non-ST-segment elevation myocardial infarction). A recent study (ACCOAST) showed an increased risk of bleeding with the use of half loading dose (30 mg) of EFFIENT® prior to coronary angiography followed by the second half loading dose (30mg) at the time of PCI compared to taking the full approved loading dose (60 mg) at the time of PCI. In UA/NSTEMI patients, when coronary angiography is performed within 48 hours after admission, the loading dose of EFFIENT® should generally be given at the time of PCI in order to minimize the risk of bleeding. UA/NSTEMI patients should generally be administered a 60 mg loading dose of EFFIENT® at the time of PCI, followed by a 10 mg maintenance dose.

Source: http://healthycanadians.gc.ca/recall-alert-rappel-avis/hc-sc/../37611a-eng.php

European Union (EU) / Canada: Review of Emergency Contraceptives Started

Date: February 4, 2014

It was noted from the European Medicines Agency (EMA) of the EU on 4 February 2014 that it had started a review of emergency contraceptives to assess whether increased bodyweight and body mass index (BMI) reduce the efficacy of these medicines in preventing an unintended pregnancy following unprotected sexual intercourse or contraceptive failure. Emergency contraceptives act by blocking and/or delaying ovulation.

Available emergency contraceptive medicines in the EU contain levonorgestrel or ulipristal acetate. EMA would evaluate the impact of new data suggesting that a high bodyweight could impair the effectiveness of emergency contraceptives. It would assess whether any changes should be made to the product information for all emergency contraceptive medicines containing levonorgestrel or ulipristal acetate. Emergency contraceptives containing levonorgestrel can be used up to 72 hours after unprotected sexual intercourse or contraceptive failure while ulipristal acetate can be used up to 120 hours. The review of emergency contraceptives started at the request of the Swedish medicines regulatory gency. It follows a procedure finalised in November 2013 for Norlevo, an emergencycontraceptive medicine containing levonorgestrel, to add the following information to the summary of product characteristics: 'In clinical trials, contraceptive efficacy was reduced in women eighing 75 kg or more, and levonorgestrel was not effective in women who weighed more than 80 kg'. This information is currently not reflected in the product information for other emergency contraceptives containing levonorgestrel. For ulipristal acetate, no information regarding the woman's weight or BMI is currently included in the product information. Similar advice was also issued by Health Canada on the same day regarding the effectiveness of levonorgestrel-containing emergency contraception that suggested these pills may be less effective in women over a certain weight. Health Canada will take appropriate action as required, such as working with the manufacturers to update drug labels and notify Canadians of new information. In Hong Kong, there are 28 registered emergency contraceptive medicines containing levonorgestrel and one containing ulipristal. All of the products are prescription only medicines. DH had not received any adverse event report in connection with the use of the products, and will keep vigilant on any safety updates of the drug(s) and actions taken by overseas regulatory authorities for consideration of any action deemed necessary.

Source: www.drugoffice.gov.hk

Canada: New Warnings Regarding Blood Pressure Drugs: Aliskiren, Angiotensin-converting Enzyme Inhibitors or Angiotensin Receptor Blockers

Date: February 5, 2014

Health Canada informed healthcare professionals and patients of the risks associated with combining more than one of the following blood pressure medicines: aliskiren (renin inhibitor), angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs). Recent studies have demonstrated that any combination of aliskiren, ACEIs or ARBs increases the risks of hypotension (low blood pressure), hyperkalemia (high levels of potassium in the blood) and kidney problems. Furthermore, aliskiren should not be taken in combination with ACEIs or with ARBs in patients with diabetes or kidney disease due to the additional risks of stroke and syncope (fainting) in these patients. The product labels have been updated to better reflect the new recommendations regarding the safe use of these medicines. Patients are advised to talk to a healthcare professional if they have any questions or concerns about their blood pressure medication. Patients should not stop treatment without consulting their doctor or healthcare professional. If untreated, high blood pressure can cause serious health effects over time.

Source: http://www.healthycanadians.gc.ca/recall-alert-rappel-avis/hc-sc/../37895a-eng.php

Singapore: New Data Suggest that Individuals Who Were Previously Vaccinated with Mencevax ACWY but Remain at High Risk of Exposure to Serogroups A, W-135 and Y Should Be Considered for Earlier Revaccination

Date: February 17, 2014

GlaxoSmithKline (GSK) would like to inform healthcare professionals of the new antibody persistence data for Mencevax ACWY. The immunity to serogroups W-135 and Y in individuals 11-55 years of age who were vaccinated two years earlier with Mencevax ACWY is 24.0% and 44.0%, respectively. Limited data showed a waning of serum bactericidal antibody titres against serogroup A one year post-vaccination when using human complement in the assay (hSBA), individuals remaining at high risk of exposure to serogroups A, W-135 and Y should be considered for earlier revaccination according to local recommendations. It is to be noted that re-vaccination with group C polysaccharide-containing vaccines may induce lower antibody responses to meningococcal group C polysaccharide compared to primary vaccination. The product information for Mencevax ACWY will be updated to include these new antibody persistence data.

Source: http://www.hsa.gov.sg/publish/hsaportal/../DHCPL.html

EU: Recommendation to Suspend the Use of Protelos/Osseor (Strontium Ranelate)

Date: February 22, 2014

Further to the recommendation on restricting the use of strontium ranelate to reduce the risk of heart problems made by the European Medicines Agency (EMA) on 11 April 2013, the EMA's Pharmacovigilance Risk Assessment Committee (PRAC) had recommended that Protelos/Osseor (strontium ranelate) should no longer be used to treat osteoporosis. PRAC had conducted an in-depth review taking into account available data on the benefits and risks of the medicine. The Committee noted that for every 1,000 patients being treated for 1 year, there were 4 more cases of serious heart problems (including heart attacks) and 4 more cases of blood clots or blockages of blood vessels with Protelos/Osseor than with placebo. In addition, Protelos/Osseor is associated with a number of other risks, such as serious skin reactions, disturbances in consciousness, seizures (fits), liver inflammation and reduced number of blood cells. The Committee also questioned the evidence on the extent to which the restrictions recommended in April 2013 reduced the cardiovascular risk and how well the restrictions work in clinical practice, particularly as the medicine is used for long-term treatment in elderly patients. Besides, with regard to its benefits, Protelos/Osseor had been shown to have a modest effect in osteoporosis, preventing about 5 non-spinal fractures, 15 new spinal fractures and 0.4 hip fractures for every 1,000 patients being treated for 1 year. PRAC weighed the benefits of the medicine against the known risks and concluded that the balance was no longer favourable and recommended Protelos/Osseor be suspended until there are new data showing a favourable balance in a defined patient group. The outcome of PRAC's assessment was sent to the EMA's Committee for Medicinal Products for Human Use (CHMP) for a final opinion.

In the meeting in February 2014, CHMP agreed with the PRAC's overall assessment of the risks of Protelos/Osseor, but noted that study data showed a beneficial effect in preventing fractures, including in patients at high risk of fracture and available data did not show evidence of an increased cardiovascular risk with Protelos/Osseor in patients who did not have a history of heart or circulatory problems. CHMP concluded that Protelos/Osseor remain available but recommended further restricting the use of the medicine to patients who cannot be treated with other medicines approved for osteoporosis. In addition, these patients should be screened and monitored regularly, every 6 to 12 months and treatment should be stopped if patients develop heart or circulatory problems.

Source: http://www.ema.europa.eu/../news_detail_002031.jsp&mid=WC0b01ac058004d5c1

Deregistration of Oral Pharmaceutical Products Containing Ketoconazole

Date: February 27, 2014

The Department of Health (DH) announced on 27 February 2014 the decision of the Registration Committee of the Pharmacy and Poisons Board to deregister oral pharmaceutical products containing ketoconazole with effect from 1 July, 2014, because the benefits of the products no longer outweigh their risks. The Registration Committee's decision was made after taking into consideration the findings from the review conducted by the European Medicines Agency's Committee on Medicinal Products for Human Use, decisions by overseas drug regulatory agencies, and the use of the products in Hong Kong.

Ketoconazole is an anti-fungal agent used orally or topically. It is given orally in chronic mucocutaneous or vaginal candidiasis, fungal infections of the gastrointestinal tract, dermatophyte infections of the skin and fingernails not responding to topical treatment, and systemic fungal infections. The European Medicines Agency concluded that the incidence and seriousness of liver injury with oral ketoconazole were higher than with other antifungals. In light of the increased rate of liver injury and the availability of alternative anti-fungal treatments, the European Medicines Agency concluded that the benefits did not outweigh the risks and recommended suspending oral ketoconazole throughout the European Union. Topical formulations of ketoconazole (such as creams, gels, shampoos and topical solutions) could continue to be used as the amount absorbed throughout the body surface is very low with these formulations.

In Hong Kong, there are currently 21 registered oral pharmaceutical products containing ketoconazole, marketed by 17 manufacturers and wholesalers. They are all prescription-only medicines which can only be sold by pharmacies under the supervision of registered pharmacists upon doctors' prescription. According to local clinical experts in infectious diseases, dermatology and oncology, the use of oral ketoconazole in clinical practice has almost been completely replaced by alternative anti-fungal agents. The DH will issue letters to health-care professionals to inform them of the Registration Committee's decision to deregister oral pharmaceutical products containing ketoconazole, and to advise them to arrange suitable alternative treatments for their patients.

On 1 July, 2014, all drug manufacturers, wholesalers, retailers and health-care professionals must stop selling or supplying oral pharmaceutical products containing ketoconazole. Drug manufacturers and wholesalers are also required to recall all products concerned from the market by June 30, 2014.

Source: www.drugoffice.gov.hk

European Union: PRAC Recommends Product Information of Zolpidem Be Updated with New Advice to Minimise the Risk of Next-morning Impaired Driving Ability and Mental Alertness

Date: March 8, 2014

The European Medicines Agency's Pharmacovigilance Risk Assessment Committee (PRAC) has completed a review of zolpidem-containing medicines, used for the short-term treatment of insomnia (inability to sleep). The benefit-risk balance of these medicines remains positive, but the PRAC recommended changes to the product information, which are aimed at further minimising

the known risks of next-morning impaired driving ability and mental alertness (including somnambulism).

The PRAC has now recommended changes to the product information of zolpidem, including further highlighting the risks of impaired driving and mental alertness and strengthening warnings and precautions aimed at minimising these risks. The PRAC considered that the recommended daily dose should remain at 10 mg of zolpidem, and this dose must not be exceeded. Patients should take the lowest effective dose, in a single intake just before going to bed, and the medicine should not be taken again during the same night. In elderly patients and in patients with reduced liver function, the recommended dose remains 5 mg of zolpidem per day. Furthermore it is recommended not to drive or perform activities that require mental alertness until 8 hours after taking zolpidem. Zolpidem should not be taken together with other medicines that have an effect on the central nervous system (brain and spinal cord). Similarly, alcohol or other substances that affect mental function should not be used when taking zolpidem.

Source:http://www.ema.europa.eu/../news_detail_002037.jsp&mid=WC0b01ac058004d5c1

European Union: PRAC Re-examines Diacerein and Recommends that It Remain Available with Restrictions

Date: March 8, 2014

The European Medicines Agency's (EMA) Pharmacovigilance Risk Assessment Committee (PRAC) has re-examined diacerein-containing medicines and is recommending that they remain available but with restrictions to manage the risks of severe diarrhoea and effects on the liver.

Due to the risks associated with severe diarrhoea, diacerein is no longer recommended in patients aged 65 years and above. It is also advised that patients start treatment on half the normal dose (i.e. 50 mg daily instead of 100 mg) and should stop taking diacerein if diarrhoea occurs. In addition, diacerein-containing medicines must now not be used in any patient with liver disease or a history of liver disease, and doctors should be monitoring their patients for early signs of liver problems. The PRAC further recommends that diacerein should only be started by doctors experienced in treating osteoarthritis. Doctors should note that, based on available data, the use of diacerein is to be limited to treating symptoms of osteoarthritis affecting the hip or knee.

Source: http://www.ema.europa.eu/../news_detail_002038.jsp&mid=WC0b01ac058004d5c1

European Union: PRAC Recommends Restricting Use of Domperidone

Date: March 8, 2014

The European Medicines Agency's (EMA) Pharmacovigilance Risk Assessment Committee (PRAC) has completed a review of domperidone-containing medicines and has recommended changes to their use throughout the European Union (EU), including using these medicines only to relieve symptoms of nausea and vomiting, restricting the dose and adjusting doses carefully by weight where it is licensed in children. Reducing the recommended dose and duration of treatment was considered key to minimising its risks.

The PRAC recommended that domperidone-containing medicines should remain available and may continue to be used in the EU for the management of the symptoms of nausea and vomiting, but that the recommended dose should be reduced to 10 mg up to three times daily by mouth for adults and adolescents weighing 35 kg or more. These patients may also be given the medicine as suppositories of 30 mg twice daily. Where the medicine is licensed in children and adolescents weighing less than 35 kg, it should be given by mouth at a dose of 0.25 mg per kg bodyweight up to three times daily. The medicine should not normally be used for longer than one week.

Domperidone should no longer be authorised to treat other conditions such as bloating or heartburn. It must not be given to patients with moderate or severe impairment of liver function, or in those who have existing abnormalities of electrical activity in the heart or heart rhythm, or who are at increased risk of such effects. In addition, it must not be used with other medicines that have similar effects on the heart or reduce the breakdown of domperidone in the body (thus increasing the risk of side effects). Products supplying a dose of 20 mg by mouth, and suppositories of 10 or 60 mg are no longer recommended for use and should be withdrawn.

Source: http://www.ema.europa.eu/../news_detail_002039.jsp&mid=WC0b01ac058004d5c1

Singapore: Concerta™ (Methylphenidate Hydrochloride): New Warning on Priapism

Date: March 13, 2014

The Health Sciences Authority announced that Janssen alerted healthcare professionals to the potential risk of priapism associated with Concerta™ (methylphenidate hydrochloride). Prolonged and painful erections (priapism) requiring immediate medical attention (sometimes requiring surgical intervention), have been reported in people taking methylphenidate-containing medicines, including Concerta™. Cases of priapism have been observed in both paediatric and adult patients. These adverse events may not occur immediately with methylphenidate and can develop after some time, often following an increase in dose. Priapism has also occurred during periods of methylphenidate withdrawal (e.g. during drug holidays or during discontinuation). Patients should be instructed to seek medical attention if they develop abnormally sustained or frequent and painful erections. The local package inserts for Concerta™ will be updated to reflect the new safety information.

Source: http://www.hsa.gov.sg/publish/hsaportal/../DHCPL.html

Canada: Association of IMURAN® (Azathioprine) or PURINETHOL® (Mercaptopurine) with Hepatosplenic T-Cell Lymphoma (HSTCL)

Date: March 27, 2014

Triton Pharma Inc. and Teva Canada Ltd., in consultation with Health Canada, informed healthcare professionals and the public of the association between the use of purine antagonists IMURAN® (azathioprine) or PURINETHOL® (mercaptopurine) and the development of hepatosplenic T-cell lymphoma (HSTCL), a rare but serious cancer, mostly in patients where it is used for inflammatory bowel disease (IBD).

IMURAN® (azathioprine) is a drug used to treat adult rheumatoid arthritis and help prevent kidney transplant rejection. PURINETHOL® (mercaptopurine) is a drug approved to treat cancer (leukemias). IMURAN® (azathioprine) or PURINETHOL® (mercaptopurine) monotherapies are not authorized by Health Canada for the treatment of Inflammatory Bowel Disease (IBD). Cases of HSTCL (including fatalities) have been reported in IBD patients treated with IMURAN® (azathioprine) or PURINETHOL® (mercaptopurine) monotherapy. IMURAN® (azathioprine) and PURINETHOL® (mercaptopurine) labels have been updated for HSTCL and physicians should discuss the currently available information regarding risks and benefits of these treatments with their patients.

Source: http://www.healthycanadians.gc.ca/recall-alert-rappel-avis/hc-sc/../38691a-eng.php

Canada: Association of REMERON® / REMERON RD® (Mirtazapine) with QT Prolongation/Torsades de Pointes

Date: March 29, 2014

Merck Canada Inc., in consultation with Health Canada, informed healthcare professionals and the public of new warnings for REMERON® and REMERON RD® (mirtazapine) regarding post marketing cases of QT prolongation and torsades de pointes reported with the use of mirtazapine. REMERON® / REMERON RD® is indicated for the symptomatic relief of depressive illness.

Most cases occurred in association with drug overdose or in patients with other risk factors for QT prolongation, including concomitant use of QT prolonging medications. The Product Monograph has been updated to include this information and to advise caution in patients with risk factors such as known cardiovascular disease, family history of QT prolongation and concomitant use of QT prolonging medications. Monitoring of vital signs and cardiac rhythm should be undertaken in the management of mirtazapine overdose. Patients with torsades de pointes may present with dizziness, palpitations, syncope, or seizures. If sustained, torsades de pointes can progress to ventricular fibrillation and sudden cardiac death.

Source: http://www.healthycanadians.gc.ca/recall-alert-rappel-avis/hc-sc/../38709a-eng.php

The United States: Drug Safety Communication: FDA Clarifies Warning about Pediatric Use of Revatio (Sildenafil) for Pulmonary Arterial Hypertension

Date: April 1, 2014

FDA is clarifying its previous recommendation related to prescribing Revatio (sildenafil) for children with pulmonary arterial hypertension (PAH). Revatio is FDA-approved only to treat PAH in adults, not in children; however, health care professionals must consider whether the benefits of treatment with the drug are likely to outweigh its potential risks for each patient.

FDA revised the Revatio drug label in August 2012, adding a warning stating that “use of Revatio, particularly chronic use, is not recommended in children.” This recommendation was based on an observation of increasing mortality with increasing Revatio doses in a long-term clinical trial in pediatric patients with PAH. FDA issued a Drug Safety Communication at that time. There may be situations in which the benefit-risk profile of Revatio may be acceptable in individual children, for example, when other treatment options are limited and Revatio can be used with close monitoring. This recommendation was not intended to suggest that **Revatio should never be used in children; however, some health care professionals have interpreted this** information as a contraindication, and have refused to prescribe or administer the drug.

The evidence behind FDAs initial recommendation has not changed, and the communication is clarifying the strength of the warning communicated in the Revatio drug label.

Source: http://www.fda.gov/Safety/MedWatch/../ucm391152.htm

Australia: Safety Advisory - Hydroxyethyl Starch (Voluven and Volulyte) - Increased Risk of Mortality and the Need for Dialysis in Patients with Sepsis

Date: April 4, 2014

Consumers and health professionals are advised that a recent safety review of hydroxyethyl starch has found an increased risk of mortality and the need for dialysis when this medicine is used to treat patients with sepsis.

Hydroxyethyl starch is used in clinical situations, including during surgery, to treat and prevent a condition known as hypovolaemia. The TGA has worked with the Australian sponsor of Voluven and Volulyte, Fresenius Kabi Australia, to update the Product Information (PI) for these medicines. The updated PI, which included new contraindications for 'patients with sepsis' and 'patients with severe liver disease', as well as updated information in the precautions and dosage and administration sections, was approved on 12 July 2013. For non-septic patients, no evidence of increased risk of mortality or the need for dialysis was identified. The TGA will continue to monitor this issue.

Health professionals should ensure that they are familiar with the current PI for hydroxyethyl starch. In particular, note that this medicine is now contraindicated for patients with sepsis and patients with severe liver disease. Hydroxyethyl starch should be discontinued at the first sign of renal injury or if coagulopathy is detected. Use in critically ill patients should only be considered if other therapies have failed, the lowest possible dose is chosen, and the benefits outweigh the risk.

Source: http://www.tga.gov.au/safety/alerts-medicine-hydroxyethyl-starch-140404.htm#.Uz4rQEzyx

My Experience of Voluntary Healthcare Works in India

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BACKGROUND

India is a place where people either hate or love going. People hate going there because of the hygiene **(Plate 1)** and security issues massively reported in global media. People love going there because of the flamboyant and interesting culture. There I was for a month volunteering in a HIV care home.

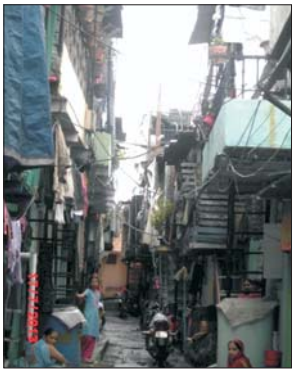

Plate 1. Scenic of Slum area

Before I started my study in pharmacy, I had been dreaming of working in the developing countries to improve the people's health there as a professional healthcare worker. I nearly forgot the dream after a year of intense study of pharmacy. While all my colleagues are planning their internship and thinking about their career, working in developing countries seemed more distant than ever to me.

Taking off

The turning point for me came when I got a chance to study a public health course about the issue in developing countries during an exchange programme held by the University of Hong Kong. I learned how developed countries including the government and NGOs help the people in need and I realized that health was not only affected by the medical facilities and staff in the area but also the culture, the social policies, etc. I felt an urge to know more about the health of those people with much less resources like us as the "privileged ones" who could participate in improving their health. Therefore, when the opportunity for volunteering in India opened to me, I grasped it with both hands as it is a golden opportunity for future healthcare professional like me, to learn, to ask questions, and to make mistakes.

MY VISIT TO SAHARA AALHAD

Sahara Aalhad locates in Pune, India. It is about three hours' drive from Mumbai. Sahara Aalhad runs a care home **(Plate 2)**, which is a drop-in-centre for IV drug users **(Plate 3)**, and a new regional clinic for locals. The care home is a NGO established by local people with funding support from Keep-a-Child-Alive, which is an American NGO for people living with AIDS. This care home has been serving thousands of people living with AIDS (PLHIV) and the families since it was established in 1978. The care home provides around-the-clock inpatient care for very sick PLHIV as well as outpatient services including regular check-ups and patient counselling. Recently, it started more outreach services in the region to extend the target group.

Plate 2. Sahara Aalhad Care Home, where I came to do my voluntary healthcare work in India.

Plate 3. The clients having lunch at Drop-in-Centre for IV drug users.

In India, the fraction of HIV-infected population is much greater than that in Hong Kong. It has 2.4 million known HIV-carrier while Hong Kong has only 2600. The hospital service in Pune is so run-down and primitive. I was told that even the very sick HIV patients would not want to be admitted to the local government hospital unless they were approaching the end of their life. The hospital is severely short of staff and facilities that all patients just lay on shared beds without appropriate treatment and care. Therefore, it would not be difficult to imagine how much a care home, like Sahara Aalhad, would mean to the HIV-positive patients.

SOME VOLUNTARY WORKS ENGAGED

The Care Home

Before my arrival at the Sahara Aalhad, I thought it would be a very sad place as the people staying there would be very ill. However, it turned out to be very different. I was greeted by many curious eyes and friendly smiles at the care home **(Plate 4a-d)**. Some patients who were lying down even sat up to greet me. I could tell they were eager to see strangers and their hearts still beat with excitement to getting to know me. Moreover, the care home's environment and hygiene were actually a lot better than what I expected. The floor was clean and sterilised daily, the staff wore gloves when they administer injectable drugs to patients and the wastes were wrapped and discarded properly. This was something I certainly not anticipated.

Plate 4. Staffs and patients of the Healthcare centre. (A). Friendship among us despite of the difference of language; (B) A local lady drew a beautiful henna on my hand; (C) Sharing photos of Hong Kong to the patients in the care home; and (D) having fun time in the afternoon.

There was a doctor consultation room with cupboards for drug storage, an examination room, a small laboratory for simple tests, a counselling room, a sterilisation room and four wards for in-patients. The care home could accommodate 20-30 patients at a time. And the patients' age varies from five years old to fifty years old. They stay in different wards: male ward, female ward, children ward and family ward. The patients were all tested HIV-positive and could not take care of themselves due to various reasons including the severity of the illness, substance abuse, etc. All of them were taking Highly Active Antiretroviral Therapy (HAART) and a majority of them required Directly Observed Treatment Short Course (DOTS) for tuberculosis (TB) therapy. The HAART and TB medicines were provided to those patients free of charge by the government subsidies. There was an "ambulance" to transport the patients for hospital admission and visits **(Plate 5)**. In contrast to the fancy ambulances in Hong Kong, it was just a van with a small bed and a first-aid box. I would never imagine that I would take the ambulance to work every day. In India, nothing is impossible.

Plate 5. An ambulane which was also my daily transportation tool during my voluntary working period in Sahara Aalhad.

During my stay, the project director asked me to help out in the classifying the drugs and re-organise the drug store in the care home. I also helped out in the medical rounds, medical check-ups, drugs dispensing and food preparation for the patients, just to name a few. Through the engagement in these activities, I got the chances to talk to doctors, the staff members and the patients in the care home. I also got chances in participating in other projects run by the organization, including the opening of a new clinic, a drop-in-centre for substance abusers and outreach services. In the preparation work of the new clinic opening, I was asked to involve in setting up a new drug system and making fact sheets and booklets for staff and patient education in the clinic. I also helped in preparing some documents with the staff on the case profiles we visited in the outreach and the meetings with the clients we had made in the drop-in-centre.

Most of the time, I stayed in the care home. My daily routine usually started with following the doctor in the medical rounds taking records of patients' body temperature, blood pressure, blood oxygen saturation and checking their general conditions in the morning. The doctors were very nice to me by explaining the cases to me in English. On some weekdays, the doctor would do outpatient general consultation at the care home. There were only two doctors and two nurses working on shift in Sahara Aalhad so task redistribution and teamwork were quite effective to overcome the problem of staff shortage.

Due to the limitation in resources, the care home did not have a pharmacy and there was just a cupboard for putting all the drugs in a random order. It was no surprise that finding the drugs become one of the most difficult tasks, only the doctor and the nurse knew the position of drugs well. The care workers knew little English but they were the ones to give out the medicine to patients. The system to record the drugs was inefficient and disorganised. They kept a dispensing book but the record was incomplete so towards the end of every month, the staffs need to stock-take all the drugs in the cupboard. Every afternoon, the care workers would dispense the drugs for the next day by putting the drugs in the small container labelled with the name of each patient **(Plate 6)**. The care worker distributed drugs according to past practice while knowing very little about the drugs, the frequency to be taken, the common adverse effects, and they have limited means to know whether the doses had been changed.

Plate 6. Daily preparation of medicine in cups for the next day's distribution.

Upon these observations, I discussed with the doctor-in-charge and shared Hong Kong drug storage and dispensing system which I think more reasonable. We had arrived at an agreement to sort-out the drugs by their therapeutic drug class and also separated the drugs within each class by formulations available. The new allocation was more user-friendly pharmacy shelves **(Plate 7)**. Since then the healthcare workers know what to take when patient shows certain signs and symptoms and they also know the route of administration immediately.

Plate 7. A "Pharmacy Shelves" in the newly opened clinic was systematically reset

When I had some free time at Sahara Aalhad, I seized the opportunities in talking to the patients by helping the care workers in translation. Most of the patients in the care home knew nothing about their drugs they were taking, let alone of the side effects and the importance of compliance, they were not even aware that they were on HAART or TB treatment. While these people were in-patient, their medication taking were monitored by care workers but no one had the vision of how they would manage their medications after they are discharged. Therefore, I think it is crucially important to build up the medication knowledge in both the patients and the care

workers and to enforce the importance of good lifelong drug compliance.

Generic drugs

I felt that people living in an economically sufficient society like us in Hong Kong, sometimes forget how people from the third world are struggling with their health and cannot even afford to keep it. For instance, one of the doctors showed me two medicines, ondansetron injection and domperidone oral tablet, which costs us only HKD $4 per ampoule and HKD 0.4 per tablet, respectively. But to them, they treasured it very much as they will use it for treating vomiting. To these doctors, they feel it is too expensive to prescribe the drug.

During my time at Sahara Aalhad, I learned to appreciate the value and the importance of generic drug usage in economically deprived societies, and how generic drugs really bring life and hope to the poor. India, which is regarded as 'the pharmacy of the developing word', produces 80% of generic medicine for treating 6.6 millions of people living with HIV/AIDs(PLHIV) in the world. The production of generic drugs makes HAARTs affordable to the poor by reducing the cost of HIV medicine by 99%. Here in the care home, I could see how the lives are changed by the HAART medicine. Patients at Sahara Aalhad Care Home are provided with HAARTs free of charge, subsidised by the government. With this subsidy, people could have a hope of leading a normal live, going to work and having a family. In fact some people working in the care home are also HIV-positive but they can work normally, thanks to the free provision of the medications.

Then the controversy between the patent law for intellectual property protection and the generic drugs comes to my mind. While the drug patent law protects the pharmaceutical companies' intellectual properties and the profits they make on drug sales allow the companies to re-invest on drug development; the patent law also prevents competition from generic drug market and as a result, the prices of medicines remain high and unaffordable for the people who really need them. This is especially the case of HAARTs. HIV is most prevalent in the third world. According to WHO statistics, Sub-Saharan Africa account for 71% of PLHIV in the world and half of PLHIV in Asia are in India.[1] In these developing countries, where people and their governments cannot afford those drugs. The incidence rate of HIV has dropped significantly since 2001 when Indian company Cipla offered the first generic triple therapy in the world as some HAART agents patency went off.[2] I felt that one should bear in mind the importance of striking the right balance between profit making and provision of healthcare to people in need, especially in those developing countries.

The hospital

I also got a chance to visit a local government hospital that I would never forget the scene! It was the worst hospital I have ever visited. There was a long queue waiting to see doctors but other people just jumped in the queue wherever they wanted and some even pushed away the patient who was engaged in a conversation with the doctor. Patients who were waiting and the patient who was seeing the doctor were in the same room. Hence, there was no patient confidentiality at all. The noise was so loud that one would think that he was in an open market.

We waited for an hour to see the doctor, which was already considered fast as we were representing a NGO. The inpatient department was a nightmare! There was no nurse or doctor in sight, no partitions to separate the patients so everybody lay on beds in one big room. Some children had to share beds with others because space was limited. We went to see a patient from our organization whose was admitted a few days ago. She was a three year-old little girl lying in bed, who was so thinned to the bone. She got almost no hair and she looked like someone who was starving and dying, the scene I had seen on many charity advertisements. A week later, I was sad to be told that she passed away.

I reflect a lot after this experience at the hospital. Despite of the overloaded Hong Kong public hospital system, the situation I encountered in India would never happen in Hong Kong, thanks to the well-established and organised hospital system. In Hong Kong, public hospitals are overloaded, attributing 73% to the total of hospital expenditure and providing over 90% of in-patient services in Hong Kong.[3] We often hear people complaining about the inefficiency of the Hong Kong healthcare system, I am still grateful that everyone can access healthcare when they have fallen ill, regardless of one's social status. The system makes sure that everyone can see a doctor and get a proper treatment to be healthy again. However, in India, people are deprived of this privilege as the government could not afford healthcare services for everyone.

In the clinic

Apart from the care home, an opportunity came my way to help in the first few days of operation in a new general clinic located in a slum area. I helped by taking information from the new registered patients, measuring their body weights, heights and medication dispensing. It was a more challenging task than I originally imagined. Even designing a registration form was brain teasing because it was necessary to make sure all the essential information required by our NGO was included, so that patients could be properly followed-up. People there were very sensitive in giving away information such as their HIV status, so I also learned how to extract a proper medical history professionally and wisely.

I observed a lot of limitations in the clinic and how difficult it was to build systems to properly keeping medical records, distributing medicine, inventory control of medicine, just to name a few. When a doctor prescribed the medicine, the nurse just handed all the medicine to the patients, unlabelled, and told them the frequency of taking the medicine. Patients were waved to go away before they could remember the pieces of new information given, and no one was there to help them. I decided to suggest a labelling system to the doctor. He happily accepted it and started to implement it straight away.

Another problem faced by the clinic was the limited variety and quantity of the drugs available due to the operation cost. Therefore, they required a good balance keeping system to record dispenses of drug and drug purchase. The clinic tried to record the daily use of every drug and would decide the quantity of drugs to buy for next month depending on the current month's use. This is actually very inefficient way, which took up a lot of time and human resources to deal the balance record of drugs. I had helped in counting the stock for each medicine in which I already found very tedious to do so for 150 items only. I realised this system was not going to work for a larger scale of pharmacy operation and I learned the importance of computerisation like the pharmacy management in Hong Kong.

Drop-in-centre for substance abusers

Sahara Aalhad also operated a drop-in-centre for intravenous (IV) drug users for harm reduction. Harm reduction was a public health approach in an attempt to reduce harmful consequences in the IV drug users rather than to force them to quit drug. They used buprenorphine as the oral substituting agent, and implementing the needle exchange programme free-of-charge for the IV drug users. Drug users have to come to the centre for collecting the drug **(Plate 8)**. I spoke to the manager and explained to him that buprenorphine was preferred to methadone in oral substitution therapy as the former had fewer overdose problems. Consequently it requires less monitoring. A booklet should be given to all IV drug users providing information regarding some practical safety tips in carrying out injection by themselves. For instance, words such as "solid substances should not be injected into the vein" should be included in the booklet. This piece of information may seem so obvious to us, but IV drug users have very poor drug knowledge and it may lead to harmful consequences if they are not provided with these information.

Plate 8. IV drug users on their way to the centre.

Outreach Services

I also made some visits with the outreach team staff to the HIV-positive clients and their families in the slum areas. In one of the visits, an old man diagnosed with HIV told us that he stopped taking his HAARTs because the drugs made him itch. He admitted he often stopped taking his medicine without consulting the doctor. Our care worker suggested him to see the doctor as soon as possible to discuss the problem rather than stopping the medicine by himself. I reminded myself that this case was only a tip of an iceberg, as the residence in the slum areas had very poor knowledge about their disease and their medicines, the importance of compliance and how to deal with adverse drug reactions, something which pharmacists would be most valuable in doing.

CONCLUSION

This outreach experience allowed me to relate to some community visits to the elderly who lives alone. Health literacy remains a major global challenge for health improvement especially for those who are in the poor socio-economical class. In order to reach out to these people, health professionals need to work with each other for the benefit of the patient. For instance, Indian women are usually discriminated against receiving treatment and health information. As a result, they were usually under-diagnosed and under-treated. In reaction to this, multi-disciplinary women support groups are initiated to spread health information to women who also deserve the right in receiving healthcare.

As a Pharmacist-to-be

This experience in India changes my perspective on pharmacists' roles. The importance of pharmacists may often be neglected at the developing countries since many may not perceive pharmacists being a front-line staff, and clinical pharmacy is a luxury in the developed worlds. However, in this volunteer experience, I noticed that there are many places that require the expertise of pharmacists, including drug management and drug information provision to staff and patients, these are duties that doctors and nurses are not trained to do or have time to do.

Humble student like myself, was even asked to help setting up a drug system to keep the balance of drugs but at the time I had only just barely finished my first year at pharmacy school, and therefore I was not equipped to be of much help. How I wish I had more experience before I come to this volunteering! I know very well there are still a lot of things I need to learn in order to be a full competent pharmacist.

It also came to my mind that pharmacists are important members in the front-line team, and should not be considered as a luxury component in a healthcare system. In a developed city like Hong Kong, where clinical pharmacy and community pharmacy practice are still in its infancy; and in a developing country like India, where clinical pharmacy is almost non-existence and pharmacists mainly work for the pharmaceutical manufacturers, there are still a lot of room for the pharmacy profession to grow. We need to believe in ourselves, keep reminding ourselves what we are capable of doing and not to submit to external resistance that inhibit the progression of our profession.

Through this volunteer, I realise that there are many more things that are as important as the study of drugs. I learned about the importance and the power of a multi-disciplinary team approach in direct patient care, such as nutrition and emotional care of patients. I also learned that everyone should have the equal opportunity to receive good healthcare, no matter who they are, whether they are drug addicts or beggars. These people may not choose to lead that kind of live, and everyone has their own story. As a pharmacist, I should always remember to have empathy in my patients, because each life is valuable. This volunteer experience also allows me to reflect on our healthcare system in Hong Kong, and makes me appreciate how lucky we are to be living in such a developed city, but also contemplate on all the jobs that are yet to be done to make the pharmacy profession to prosper. Pharmacists, a profession that I aspire myself to become one day, are like the doctors and nurses, being a part of the healthcare team, that work hard to strive to make Hong Kong a healthier city for everyone to live in.

Author's background

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Time Matters of Medication: Is It Important?

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ABSTRACT

When patients are counselled on their medications, a question often arises as to what time in the day those medications should be taken. For many of them, the administration times are at the preference of the patients or carers as long as the schedule is about the same every day whilst for others, the time in the day and any concurrent medications do influence the therapeutic and/or adverse effects of individual drugs. This article discusses some of the recommendations for the administration time in the day, including the rationale behind and certain practical considerations. Selected drug-drug interactions that warrant separation of administration times are also discussed.

Keywords: *administration time, chronotherapy, drug-drug interactions, drug absorption*

INTRODUCTION

"What time in the day should I take this medication?" and "Can I take these two (or more) medications at the same time?" are two popular questions encountered during patient counselling. These questions arise as some drugs may work better if taken at specific times in the day and some drug pairs would 'crash' if taken together. Recommendations are many but a few examples have been selected to illustrate the basic principles in this article.

TIME IN THE DAY

Drugs taken in the morning

Diuretics, characterised by a few subclasses, are indicated in the treatment of hypertension and oedema in heart failure.[1] Thiazide-type diuretics, such as hydrochlorothiazide, indapamide and metolazone, block sodium reabsorption in the distal convoluted tubule and have duration of action of 12 to 24 hours. Furosemide, a loop diuretic, inhibits sodium and chloride reabsorption in the ascending loop of Henle and diuresis lasts about 6 hours. Considering their duration of action, they are usually taken in the morning if given once daily, or in the morning and late afternoon if given twice daily, to avoid nocturnal diuresis and sleep disturbance. Similarly, once-daily hydrochlorothiazide-based fixed-dose combination, e.g. Dyazide, Moduretic and Micardis Plus, should be taken before 6 p.m. and preferably in the morning.[2]

Bisphosphonates inhibit bone resorption by osteoclasts and are indicated in the prevention and treatment of osteoporosis.[1,3] Oral bisphosphonates are taken in the morning, after an overnight fast and at least 30 minutes (60 minutes for ibandronate) before the first food, beverage or medication of the day with plain water.[1] Compliance is essential as bisphosphonates are poorly absorbed with bioavailabilities in the fasting state ranging from 0.7% to 6%.[3] Absorption is significantly decreased by food and beverages, especially those containing calcium or other polyvalent cations. On the other hand, safety concerns have warranted additional precautions. General gastrointestinal disturbances and severe oesophageal reactions, including oesophagitis and ulceration, have occurred with bisphosphonates. Patients are therefore instructed to take the medication whole with plenty of water and remain upright for 30 minutes (60 minutes for ibandronate) afterwards. It is also important that they do not take it at bedtime or before arising for the day.[1]

Prednisolone is a synthetic glucocorticoid that is used principally for its anti-inflammatory or immunosuppressant properties.[4] In patients with poorly controlled asthma or severe asthma exacerbations, once-daily prednisolone may be prescribed in addition to existing reliever and control medications. It is taken in the morning to reduce the disturbance to circadian cortisol secretion and avoid adverse effects, especially insomnia.[1,5]

Additional examples of medications taken in the morning to exhibit therapeutic effects and avoid adverse effects during daytime are listed in Table 1.

Drugs taken in the evening or at bedtime

Statins inhibit the action of HMG-CoA reductase and therefore interrupt the biosynthesis of cholesterol in the liver.[1] They are indicated in the treatment of hypercholesterolaemia and prevention of cardiovascular events in high-risk patients.[4] Hepatic cholesterol biosynthesis is thought to peak after midnight.[2] Thus, statins with a shorter half-life; namely fluvastatin and simvastatin, should be taken in the evening.[9-11] Meanwhile, the manufacturers of atorvastatin, prolonged-release fluvastatin, pravastatin and rosuvastatin, advise that the medication may be given at any time of day.[12-15]

Montelukast, a leukotriene receptor antagonist, is indicated in the management of asthma and allergic rhinitis.[4] In the management of asthma, efficacy and safety of montelukast were established in clinical trials in which the drug was taken in the evening. It is hypothesised that administration in the evening allows the peak plasma concentrations of the drug to coincide with the peak airway reactivity in the morning. In the management of allergic rhinitis, efficacy was demonstrated when montelukast was taken in the morning or in the evening. Patients with allergic rhinitis can therefore individualize their administration time. Patients with both asthma and allergic rhinitis should take their dose in the evening.[16]

Table 1. Examples of drugs recommended to be taken in the morning					
Class	**Members**	**Indications**	**Therapeutic Effects**	**Adverse Effects**	**Remarks**
Proton Pump Inhibitors[6,7,8]	Dexlansoprazole Esomeprazole Lansoprazole Omeprazole Pantoprazole Rabeprazole	Dyspepsia Gastric and duodenal ulcers Gastro-oesophageal reflux disease	Inhibit daytime gastric acid secretion		Not applicable in patients with nocturnal symptoms
Stimulants[1,4]	Modified-release methylphenidate	Attention deficit hyperactivity disorder	Manage inattentive, hyperactive and impulsive behaviour at school or at work		Possible need for supplemental doses of a short-release preparation
Monoamine-oxidase-B inhibitors[1,4]	Selegiline	Parkinson's disease Symptomatic parkinsonism		Insomnia	With breakfast and lunch if twice daily

Prostaglandin analogues, including latanoprost, tafluprost, travoprost and bimatoprost, lower intraocular pressure by increasing the uveoscleral and trabecular outflow of aqueous humour and are indicated in the treatment of open-angle glaucoma and ocular hypertension as eye drops.[1,9] Results from clinical trials suggest that evening dosing may be more effective in lowering intraocular pressure than morning dosing.[17] It may be due to the fact that prostaglandin analogues demonstrate their peak efficacy 12 to 24 hours after administration, i.e. maximum intraocular pressure control during daytime if dosed in the evening. They are also available in combination with timolol, which is usually given twice daily. The manufacturers recommend that the combination eye drops are to be given once daily and may be instilled either in the morning or in the evening.[18-20]

Some medications are taken in the evening or at bedtime to minimise their adverse effects. An example is donepezil, an acetylcholinesterase inhibitor, which is indicated for the treatment of dementia in Alzheimer's disease.[4] Its major adverse effects are cholinergic and gastrointestinal in nature, such as nausea and diarrhoea.[21] Taking it at bedtime often helps minimise their impact. However, if insomnia or nightmares occur, dosing in the morning should be considered.

Drugs with special administration times

Nitrates have multiple mechanisms of action in the management of angina.[1] They are potent vasodilators that increase myocardial oxygen supply and decrease ventricular preload. The regular use of isosorbide dinitrate or mononitrate may prevent exertional attacks and achieve long-term prophylaxis.[4] However, tolerance to nitrates is observed with prolonged use and appears to be associated with high and/or sustained plasma concentrations and frequent administration. Evidence suggests that the development of tolerance can be minimised by use of the lowest effective dose of nitrates and asymmetric, intermittent dosing with a nitrate-free interval between 8 to 14 hours.[4,9] Once-daily modified-release and transdermal preparations may be administered in the morning to produce a nitrate-free interval at night.

It must be noted that the timing of the nitrate-free interval may vary with the timing of angina.[22] If a patient experiences frequent nocturnal angina, it may be necessary to arrange a daytime nitrate-free interval and adjust the administration time of concomitant beta blockers and calcium channel blockers. Constipation that is not caused by serious pathology will respond to simple measures, most notably dietary modifications and laxatives. Bisacodyl is a common stimulant laxative. It is indicated in the management of constipation or as a bowel preparation for a radiological purpose.[1] It is available in coated tablets and suppositories. Usually tablets are taken at night and suppositories are administered in the morning. The reason behind this difference lies with the mechanism of action. Bisacodyl is not absorbed in the body and is only hydrolysed and locally active in the colon. Consequently, the onset of action for tablets is between 6 to 12 hours and that for suppositories is within 15 to 30 minutes.[23] If a bowel movement is desired in the morning, administration of the tablets at night gives them sufficient time to reach the colon and stimulate peristalsis. Senna is another stimulant laxative that is usually taken at bedtime and acts within 6 to 12 hours.[4]

Table 2. Administration schedules of nitrates to provide a nitrate-free interval		
Route	**Prescribed frequency**	**Administration schedule**
Oral	TDS	Q4-6H after the first dose, e.g. 7 a.m., 12 p.m., 5 p.m.
	BD	Q8H after the first dose, e.g. 7 a.m., 3 p.m.
	Daily	Q24H (in the morning)
Transdermal	Daily	Q24H (apply in the morning, remove in the early evening)

TIME WITH RESPECT TO OTHER DRUGS

General drugs to separate from

Several medications decrease the bioavailability of a number of drugs and therefore are generally recommended to be taken separately from all other drugs. Klean Prep® induces diarrhoea and causes medicines being flushed through the gut without having enough time to be absorbed. All oral medications should be taken at least 1 hour prior to the administration of Klean Prep.[24] Cholestyramine, an anion-exchange resin that is used to reduce diarrhoea and pruritus by forming an insoluble complex with bile acids in the intestine, can bind with other drugs in the gut, thereby reducing their absorption. Patients should be reminded to take other drugs at least 1 hour before or 4 to 6 hours after cholestyramine.[25] The effect of Metamucil® is believed to be comparatively small but there is still a general recommendation from the manufacturer that Metamucil should be separated from other medicines by at least 2 hours.[26]

pH-dependent absorption

Many drugs have pH-dependent absorption and generally need to be taken at least 2 hours apart from antacids. Examples include, not exhaustively, atazanavir, cefuroxime, gabapentin, itraconazole, ketoconazole, mycophenolate and phosphate supplements. In view of the high significance of this interaction to itraconazole,[i] H_2 blockers and proton pump inhibitors should

also be avoided in patients requiring oral itraconazole therapy.[27] The effectiveness of tyrosine kinase inhibitors, an evolutional class of drugs used as targeted therapy for various cancers, is highly associated with its bioavailability, which, for the majority, is dependent on gastric pH. Patients should be counselled to follow strictly the relevant recommendations.

i Itraconazole oral suspension may be less sensitive to the effects of decreased gastric acidity.

Insoluble chelation complexes

When chelation-susceptible drugs are taken together with polyvalent-cation-containing products, insoluble chelation complexes are formed in the gastrointestinal tract and drug absorption is reduced as a result. Drugs liable to this pharmacokinetic interaction should be separated from polyvalent cations such as aluminum- or magnesium-containing antacids and calcium or iron supplements. Dairy products are also rich in calcium and patients should be counselled accordingly.

Interaction with enteral feeds

Apart from drug-drug interactions, medicines also interact with food and enteral feeds. One typical example is the interaction between phenytoin suspension and enteral feeds. When administered concurrently, phenytoin absorption may be reduced by up to 70%.[37] There is still no definitive answer to explain the mechanism behind, but it is generally recommended to give phenytoin as a single daily dose, or at most twice daily, to prevent an interaction. It is also desirable to consider interrupting enteral feeding 2 hours before and after phenytoin administration and flush the enteral tube with plenty of water before and after the administration.[38]

CONCLUSIONS

Based on the pharmacokinetic and pharmacodynamic properties of and interactions between drugs, patients should be counselled to take their medications in an individualised schedule. To take one example, morning dosing of antihypertensive medications is likely to improve daytime blood pressure control but evening dosing may prevent falls due to orthostatic hypotension and dizziness. As for drug-drug interactions, sometimes switching one drug in the pair to a clinical alternative could be another solution. When a recommendation of administration time is evaluated, sufficient consideration should be given to the lifestyle of the patient, their likely compliance and strategies to minimise possible adverse effects.

Table 3. Interactions between tyrosine kinase inhibitors and gastric acid neutralisers or suppressants

	Antacids	H_2 blockers	Proton pump inhibitors
Bosutinib[28]	Separate by at least 2 hours.	Separate by at least 2 hours.	Avoid concurrent use.
Dasatinib[29]	Separate by at least 2 hours.	Avoid concurrent use.	Avoid concurrent use.
Erlotinib[30]	Daily dose of erlotinib should be taken 4 hours after and 2 hours before antacids.	Daily dose of erlotinib should be taken 10 hours after and at least 2 hours before H_2 blockers.	Avoid concurrent use.
Gefitinib[27]	Concurrent use with gastric acid lowering agents may reduce gefitinib plasma concentration but at present there is no recommendation on avoiding these agents.		
Nilotinib[31]	Separate by at least 2 hours.	Nilotinib should be taken 10 hours after and 2 hours before H_2 blockers.	Avoid concurrent use.

Table 4. Recommendations for drug interactions with polyvalent cations[27,33-36]

Drugs	Separation from polyvalent cations
Bisphosphonates - Alendronate - Risedronate - Ibandronate - Clodronate - Etidronate	Avoid administration of polyvalent cations within: 2 hours before or after clodronate/etidronate; 60 minutes after oral ibandronate; or 30 minutes after alendronate/risedronate.
Chloroquine - Hydroxychloroquine	Separate by at least 4 hours.
Deferiprone	Separate from aluminium-based antacids by at least 4 hours.
Deferasirox	Separate from aluminium-based antacids by at least 2 hours
Eltrombopag	Separate by at least 4 hours.
PenicillAMINE	Separate by at least 2 hours.
Quinolones - Ciprofloxacin - Levofloxacin - Moxifloxacin	Ciprofloxacin should be taken at least 2 hours before or 6 hours after polyvalent cations. Levofloxacin should be taken at least 2 hours before or 2 hours after polyvalent cations. Moxifloxacin should be taken at least 4 hours before or 8 hours after administration of aluminum-, iron-, magnesium-, or zinc-containing agents. However, no clinically significant interaction occurs when moxifloxacin is administered concomitantly with milk or calcium carbonate.[32] Stagger the administration times of bismuth and quinolones by 2 hours.
Rosuvastatin	Rosuvastatin should be taken at least 2 hours before any antacids.[ii]
Tetracycline derivatives - Doxycycline - Minocycline - Tetracycline	Separate by at least 2 to 3 hours. Stagger the administration times of bismuth and tetracycline or its derivatives by 2 hours.
Thyroxine	Separate by at least 4 hours.

ii Current evidence only shows significance of the interaction with antacids but not all polyvalent cation-containing products.

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Questions for Pharmacy Central Continuing Education Committee Program

(Please be informed that this article and answer sheet will be available on PCCC website concurrently. Members may go to PCCC website (www.pccchk.com) to fill in their answers there.)

2 CE Units

Time Matters of Medication: Is It Important?

1. ***Which of the following statements about the use of nitrates is TRUE?***
   A. A nitrate-free interval in the morning is not a routine practice because of a possible disturbance in the natural circadian rhythm.
   B. Asymmetric, intermittent dosing of nitrates is an effective means of minimising nitrate tolerance.
   C. Glyceryl trinitrate spray preparations should be discarded after 8 weeks in use.
   D. Transdermal nitrate preparations do not give rise to nitrate tolerance and are recommended to be administered without a nitrate-free interval.

2. ***Which of the following medications is administered at specific time(s) in the day in order to follow the natural circadian rhythm?***
   A. Bimatoprost
   B. Furosemide
   C. Methylphenidate
   D. Prednisolone

3. ***Which of the following medications is administered at specific time(s) in the day in order to minimise its adverse effects?***
   A. Donepezil
   B. Methylphenidate
   C. Montelukast
   D. Senna

4. ***Which of the following combination products should be administered in the morning?***
   A. Amoxicillin and clavulanic acid
   B. Telmisartan and hydrochlorothiazide
   C. Timolol and latanoprost
   D. Simvastatin and ezetimibe

5. ***JK works a permanent night shift and is taking multiple medications. Which of the following is an INAPPROPRIATE recommendation for her?***
   A. Take alendronic acid before bed
   B. Take atorvastatin before bed
   C. Take modified-release isosorbide mononitrate before work
   D. Take pantoprazole before work

6. ***How long should cholestyramine be separated from other drugs?***
   A. Other drugs should be taken at least 2 hours before or after cholestyramine.
   B. Other drugs should be taken at least 1 hour before or 4 to 6 hours after cholestyramine.
   C. Other drugs should be taken at least 1 hour before cholestyramine.
   D. It is not necessary to separate choletyramine from other medications.

7. ***According to the current evidence, which of the following tyrosine kinase inhibitors (TKIs) can be taken with proton pump inhibitors (PPI) concurrently?***
   A. Dasatinib
   B. Erlotinib
   C. Nilotinib
   D. None of the above

8. ***According to the current evidence, which of the following tyrosine kinase inhibitors (TKIs) CANNOT be taken with H2 blockers concurrently?***
   A. Dasatinib
   B. Erlotinib
   C. Nilotinib
   D. None of the above

9. ***Which of the following drugs need to be taken separately with antacids due to formation of insoluble chelation complexes?***
   I. Bosutinib;
   II. Deferiprone
   III. Rosuvastatin
   IV. Itraconazole
   A. II only
   B. II & III only
   C. II and IV only
   D. All of the above

10. ***Which of the following statements is FALSE?***
    A. When administered concurrently with enteral feeds, phenytoin absorption may be reduced by up to 70%.
    B. The absorption of itraconazole oral suspension may be less sensitive to the effects of decreased gastric acidity than itraconazole tablet.
    C. Metamucil needs to be separated from other medications by at least 2 hours due to formation of insoluble complexes.
    D. The absorption of mycophenolate is pH-dependent.

Answers will be released in the next issue of HKPJ.

CE Questions Answer for 204(D&T)

An Overview of Contemporary Treatment for Rheumatoid Arthritis

1. D 2. C 3. C 4. D 5. C 6. A 7. C 8. B 9. C 10. C

Achieving Synergistic Effects by Combining Different Phytochemicals for the Prevention and Treatment of Cancer

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ABSTRACT

Cancer is a fatal disease which emerges due to the uncontrolled proliferation of cells. It is considered as a complicated health problem with complex processes and redundant signal transduction pathways. The conventional approach of chemotherapy based on the use of mono-target therapeutic agents has demonstrated that it is an inadequate medical treatment for cancer because it may lead cancer cells to develop acquired drug resistance. Botanical extracts of herbal medicines, on the other hand, have been shown to give effective results in prevention or curing of cancer. Many epidemiological studies and human clinical trials revealed that natural compounds, such as flavonoids, polyphenolic compounds and many other phytochemicals play important roles in cancer chemoprevention and chemotherapy. These phytochemicals have been proved to interfere at different stages of cancer including initiation, promotion and progression by acting on multiple signal transduction pathways of cellular proliferation, differentiation, apoptosis and DNA replication. Many recent studies on combination use of different botanical extracts suggested that some additive or synergistic effects may have taken place. In order to achieve a satisfactory, effective and safe medical treatment, combination of different mechanistic based agents is probably a solution to control multiple aberrant pathways in cancer. This report provides a review on the efficacy by combining various phytochemicals, including flavonoids, diterpene lactones and some other chemotherapeutic drugs, for the treatment of cancer.

Keywords: *Cancer, phytochemicals, combined effects, chemotherapy, efficacy, multiple aberrant pathways; drug resistance*

INTRODUCTION

Cancer, which is not just one but more than hundred distinct types, is considered as a complicated disease with complex processes and is a major health problem due to the uncontrollable proliferation of tumor cells and the potential of invading other tissue through the blood and lymphatic system.[1-2] After more than half a century of research, people now realize that tumorigenesis is a multiple manner and that these process signify alterations in genetic materials that drive the successive transformation of normal cells into highly malignant copies. Nowadays many types of cancers have been diagnosed in human beings with an age-dependent incidence involving five to six rate-limiting, stochastic events. **Figure 1** illustrates how the huge catalog of cancer cell genotypes is a representation of six essential alterations in cell physiology that collectively rule malignant growth; namely, (1) antigrowth (insensitivity to growth-inhibitory signals), (2) self-sufficiency in growth signals, (3) apoptosis (evasion of programmed cell death), (4) susbtained angiogenesis, (5) limitless replicative potential and (6) tissue invasion and metastatsis. Each of these physiological changes, which are acquired during tumor development, represents the successful falling-out of an anticancer defense mechanism hardwired into cells and tissues.

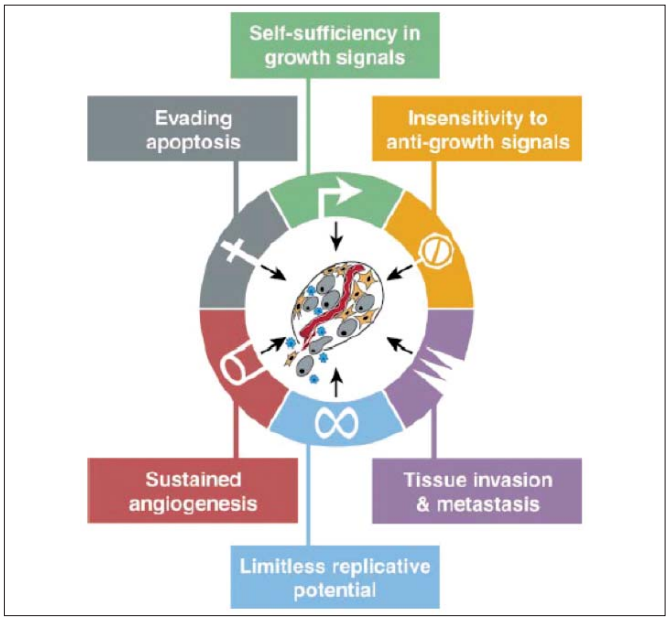

Figure 1. Acquired characteristics of cancer cell. *Most if not all cancers have acquired the same set of functional capabilities during their development, albeit through various mechanistic strategies.*[50]

Conventional Treatment of Cancers

Up to now surgery, radiation and chemotherapy treatments are the main curative therapies for different types of cancer.[3] Surgery is the most common treatment for most types of cancer. Different surgical procedures can be followed to remove the tumor including Mohs surgery or microsurgery, laser surgery, cryosurgery and amputation. Recently, FDA has announced that for any cancer surgery to be successful it should be done at an early stage of cancer and when cancer is still localized. The surgery should be followed by radiation therapy or chemotherapy in order to kill any cancer cells that were left.[4-6]

The second type of cancer treatment is the radiation therapy which uses high-energy x-rays or other types of radiation to kill cancer cells or stop their growth.[7] Two types of radiation therapy are used depending on the stage of the cancer. The external radiation therapy where an outside machine is used to send radiation toward the cancer and the internal radiation therapy where a radioactive substance sealed in seeds or capsules is placed directly into or near the cancer.[7] However, radiotherapy is not effective in cancer cells that survive in an environment with low oxygen tension due to the increase resistant of cells.[8]

The third main type of cancer treatment is the chemotherapy where drugs are used to induce cell death or cell cycle arrest in cancer cells. Chemotherapy can be systemic where route of entry is through mouth or injection into a vein or muscle, topical when chemotherapy is placed directly onto the skin or regional where the drugs mainly affect cancer cells in one area. The way the chemotherapy is given depends on the type and stage of the cancer being treated.

PROBLEM OF CONENTIONAL PRACTICES FOR CANCER TREATMENT

Conventional chemotherapy for treatment of cancers, although quite effective, has been associated with toxicities to normal tissue and organs, which is still a major dose limited factor. Furthermore, chemoresistance is another major obstacle for successful treatment of cancer.[9] There is widespread dissatisfaction with surgery, radiotherapy, and especially chemotherapy and hence, treatment of cancer is being re-evaluated around the world.

The traditional model that the malignant phenotype is driven by a dominant signal transduction pathway is becoming increasingly unacceptable. This is due to the appearance of resistance to target- and mechanism-based drugs, and therefore reflects the genetic flexibility of the cancer cell genome as well as the redundancy in the pathways that govern kinase signal transduction networks.[10] Based on this, the traditional mono-target chemotherapy protocol for cancer treatment is becoming increasingly ineffective and may lead cancer cells to develop acquired drug resistance due to the complex signaling pathways involved in cancer.[11] The multi-component therapy in which more than one drug are used at the same time, is the proven cure for cancers.[12]

ALTERNATIVE APPROACHES TO THE TREATMENT OF CANCER

Different innovative strategies have been adopted to treat cancer in recent years including selective inhibition of anti-apoptotic pathways, antiangiogenic therapy and tissue-selective therapy (including immunotherapy). These overlapping and complementary strategies depend on rational drug combinations aimed at matching targets.[13] For example, reactivation of the apoptotic cascade in apoptosis-reluctant cancer cells where one drug decreases the antiapoptotic block (e.g. inhibitors of apoptosis) allowing the other drug to activate the corresponding apoptotic pathway **(Figure 2)**. Another example is activation of the extrinsic (caspase 8) and intrinsic (caspase 9) apoptotic pathways where both pathways will activate caspase 3 and therefore increase the apoptotic effect. [13-14]

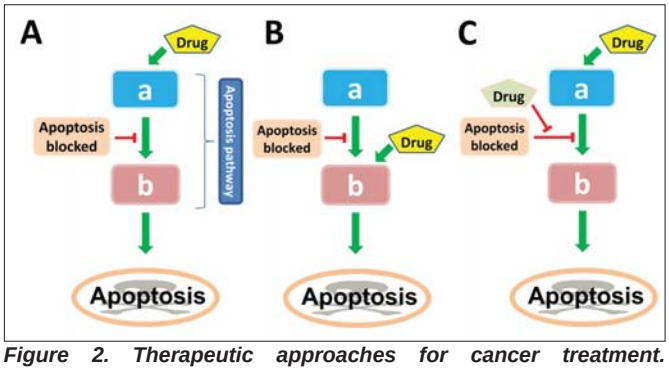

Figure 2. Therapeutic approaches for cancer treatment. *(A) Inhibitors of apoptosis prevent the cell death although the apoptosis pathway is initiated. (B) The use of another drug which bypasses the inhibitors of apoptosis and induces apoptosis downstream of the blockages will lead to cell death. (C) The use of combined drugs where one will initiate the apoptosis pathway while the other one blocks the inhibitors of apoptosis will also lead to cell death.*

The concept of drugs combination, with similar or different modes of action, seeks to result in synergistic or additive therapeutic effects, including increased therapeutic efficacy, decreased host toxicity, and minimal or delayed drug resistance.[14] Drugs that contain several active components have been in use long time ago. Many traditional medicine, including the Chinese medicine, have used mixtures of naturally occurring herbs or herbal extracts.[15] Cancer is a complex disease which involves different signaling pathways and therefore combination therapy in which one or more drugs are used at the same time is the proven cure for cancer.[12]

Polyphenols including flavonoids, diterpene lactones and other phytochemicals have long been known for their antioxidant, anti-inflammatory, antiallergic, antithrombotic, hepatoprotective, antiviral, antibacterial, antiageing and anticarcinogenic activities **(Figure 3)**.[16-21] Epidemiological studies have shown that there is an inverse association between fruits and vegetables consumption, where flavonoids are prominent components, and the risk of various human cancers.[22] The combination effect of flavonoids when combined with other flavonoids, natural compounds or chemotherapeutic drugs was also proved to be a synergistic one by different studies.[22-23]

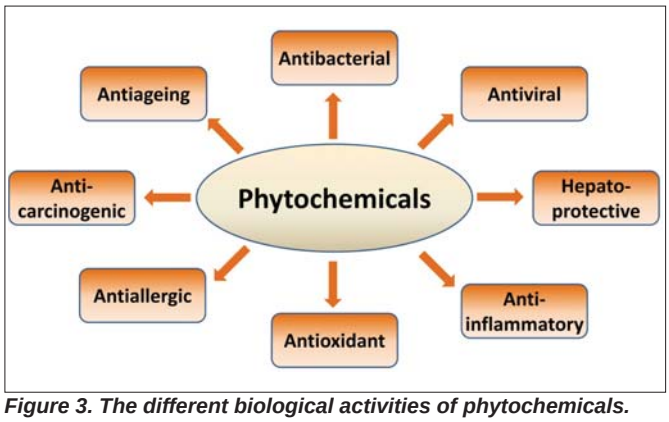

Figure 3. The different biological activities of phytochemicals.

The combination therapy can be approached by combination of different mechanism-based agents to control multiple abnormal pathways seen in the tumor.[10] On the other hand, the development of multitarget molecules also seems to be an increasingly reasonable and attractive option.[24] Therefore, based on both of these options, exploration of newer chemical diversity will be an utmost need.[10] The chemical complexity of botanicals makes them important starting materials for the discovery of newer synergistic combinations and single agent multi-target drugs.[25-27]

EFFECT OF DRUG COMBINATION ON CELL CYCLE

With advancements in our understanding of the basic mechanisms of carcinogenesis, cell cycle physiology, and apoptosis, the effects of chemotherapy on normal and cancerous cells are now better understood. With this knowledge, it is more clear now that a critical role in chemosensitivity is played by the cell cycle in combination chemotherapy.[28] A number of cancers are associated with increase in the activity of Cdks, important molecules for cell cycle regulation, due to gene mutations. Therefore, compounds that can modulate the activity of Cdks are of importance in cancer therapy.[29] Flavonoids are involved in the regulation of many proteins associated with cell growth and differentiation. These include increase in the Cdks inhibitors (p21, p27), or a decrease in cyclins B, D and E and Cdks 2,4 and 6.[30]

Different studies have investigated the effect of drugs combination on the cell cycle arrest. Drugs that block different phases of cell cycle can act in synergy. Shen *et al.* studied the effect of the combination of quercetin (arrest cells at G1 and S phases) and triazofurin (arrest cells at S phase) on the human ovarian carcinoma cells. A synergistic effect on the growth inhibition was observed when the two compounds were used in combination with a CI value of 0.37.[31] Also significant alterations in the cell cycle kinetics induced by the single compounds such as ellagic acid, quercetin and their combinations was observed against human leukemia cells.[23] In one study, the use of Taxifolin in combination with andrographolide increased the percentage of cells arrested at G2/M by increasing the levels of cyclin B and activation of Cdc2.[32] In another study, the combination of silibinin with doxorubicin strongly increased the cell cycle arrest at G2/M compared to the use of single compounds.[33]

PHYTO-COMPOUNDS USED IN COMBINED THERAPY OF CANCER

There is an increasing evidence indicating the effectiveness of using combined phyto-compounds for the treatment of solid tumors.[14] The combination of anticancer agents which have similar or different modes of action can result in synergistic, additive or antagonistic outcome.[34] Experimental techniques to determine the action of combination drugs and to design effective mixtures have not been completely standardized. It is still being practiced as an art using trial and error methods.[35] The pure natural or synthetic compounds used in western medicine usually aims a single target, while the processed crude multi-component natural products are used in Chinese medicine, in various combinations and formulations, aimed at multiple targets and different symptoms.[36]

Many studies have presented the enhanced effects of drugs combination in treatment of cancer cells using western drugs, isolated pure herbal extracts or a combination of both. A number of good chemotherapeutic agent combinations have been developed to treat cancers and they showed positive cytokinetic and biological interaction with reduced toxicity. Examples include ABV (Adriamycin, bleomycin & vinblastine) or BEP (bleomycin, etoposide & Platinol).[37] Compounds isolated from a single herbal component used in combination, two lignans, asarinin and xanthoxylol inhibited the carcinogenesis in mouse skin and pulmonary tumors.[38]

Other studies indicated that selenium act in synergy with retinoids and vitamin E to inhibit carcinogenesis. It was also found to work in synergy with crambene to kill MCF-7 mammary cells.[39] The combination of EGCG and curcumin showed a synergistic effect on growth inhibition of oral cancer cells. There was an increase dose reduction 4.4-8.5 fold for EGCG and 2.2-2.8 fold for curcumin at ED50 as indicated by the dose reduction index (DRI).[40]

ROLES OF COMBINED FLAVONOIDS IN TREATMENT OF CANCER

Many research papers have shown a better outcome in cancer treatment when the conventional drug is combined with a herb and others when combination of herbs is used **(Table 1)**. Flavonoids and other antioxidants when used alone could produce beneficial, detrimental, or insignificant effects in cancer patients while if there are combined with other anticancer compounds (i.e., natural compounds or chemotherapy drugs), their effects are more likely to be beneficial or at least not harmful.[42]

Mertens-Talcott *et al.* investigated the combinational effect of quercetin and ellagic acid on cell death in the MOLT-4 human leukemia cell line. The two compounds together reduced more the proliferation and viability and enhanced the induction of apoptosis compared to each alone.[23] In another study, the combination treatment of human gut (HuTu-80 and Caco-2) and breast cancer cells (PMC42) with quercetin and kaempferol was more effective than the additive effects of each flavonol.[43]

Table 1. Examples of studies on treatment of cancer cells with flavonoids combined with flavonoids, chemotherapy or other phytochemicals

Combined compounds	Cancer cells	Synergistic effect	Reference
Quercetin and ellagic acid	Human leukemia (MOLT-4)	Increase induction of apoptosis	23
Taxifolin and andrographolide	Prostate (DU145)	Increase in cell cycle arrest at G2/M and apoptosis	32
Silibinin and doxorubicin	Prostate (DU145)	Increase in G2/M arrest and apoptosis	33
Quercetin and kaempferol	Gut (HuTu-80, Caco-2) and breast (PMC42)	Decreased expression of nuclear proliferation antigen Ki67 and decreased total protein levels	43
5-Fluorouracil combined with leuteolin or quercetin	Colorectal (CO115)	Activation of the apoptotic mitochondrial pathway	44
Curcumin combined with cisplatin or oxaliplatin	Ovarian cancer cells	Increase induction of apoptosis	46

Other studies have investigated the effect of combination of chemotherapeutic drugs with flavonoids. The treatment of colorectal tumor (CO115) with 5-Fluorouracil combined with leuteolin or quercetin increased apoptosis with a significant effect for quercetin which involved the activation of the apoptotic mitochondrial pathway.[44] In another study, the flavonoid silibinin strongly synergized the antiproliferative effect of doxorubicin in prostate carcinoma DU145 cells. This combination was associated with an increase in G2/M arrest and apoptosis compared with treatment of each compound alone.[33] Silibinin also should synergistic cytotoxic effects when combined with chemotherapeutic drugs against breast and lung cancer cells.[45] Curcumin was also shown to be effective in combination treatment. The combination of curcumin with either cisplatin or oxaliplatin increased significantly the cytotoxic effect on ovarian cancer cells by increasing apoptosis.[46] In a recent study done at our laboratory, the flavonoid Taxifolin synergized the effect of Andrographolide by increasing the cell cycle arrest and apoptosis in DU145 cells.[32]

BENEFITS OF COMBINED USE OF PHYTO-COMPOUNDS FOR PREVENTION AND TREATMENT OF CANCER

Combination or multicomponent therapy, where two or more drugs are used together, usually has one or more of the following objectives: (1) to reduce the frequency of acquired resistance which may arise by combining drugs with minimal cross-resistance; (2) to lower the doses of drugs with getting a similar therapeutic effect so as to achieve efficacy with fewer side effects; (3) to sensitize the cells to the action of one drug through the use of another drug (chemosenstization), this is often achieved by altering the cell-cycle stage or growth properties; and (4) to achieve an enhanced effectiveness through additivity, or better yet, through synergism.[37, 47]

DETERMINATION OF THE COMBINATION EFFECT

Evaluation of the effect of drug combination is important in all areas of medicine particularly in cancer chemotherapy where combination therapy is commonly used. The *in vitro* studies are usually used to determine the nature and quantitative extent of drugs combination.[48] The combination of two drugs can give synergism, antagonism or additive effect. Synergism means that a combination of two drugs produce a therapeutic effect greater than each of the two drugs alone and more than additive effect (greater than the algebraic sum of the parts), whereas antagonism is an effect which is less than additive.[49]

The two methods which are commonly used in the analysis of drug combination effects are the isobologram and the combination index (CI) where the CI method is the most commonly used.[48] The isobologram method is based on the Loewe additivity model which evaluates the interaction at a chosen effect level and is therefore useful to examine the drug interaction at the corresponding concentration, often the median effect concentration.[50] However the CI method is based on the median-effect principle derived by Chou. The median-effect equation correlates the drug dose and cytotoxicity or cytostatic effect.[51] A software program to calculate combination indices (CI) is available and widely used.[49]

CONCLUSION

There is an increasing trend nowadays in cancer research to use a combination therapy for several solid tumors where a growing number of *in vitro* and *in vivo* studies show that combinations of natural agents can result in significant activities at concentrations where any single agent is not effective. This urges us to further explore the synergistic effects of dietary phytochemicals in the field of cancer treatment. With holistic clarity of mechanisms, cancer prognosis and treatment will become a rational science, unrecognizable by current practitioners. It will be possible to understand with precision how and why treatment regimens and specific antitumor drugs succeed or fail. As Hanahan and Weinberg anticipated anticancer drugs targeted to each of the hallmark features of cancer; when some, used in appropriate combinations and in concert with sophisticated technologies, such as nano-delivery, will be able to prevent incipient cancers from developing, while others will cure preexisting cancers.[52] Hence, natural products will continue to provide a broad base for the discovery of new drugs and new substances for combinational treatment of cancers.

Author's background

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Antioxidants Purified from Hedyotis Diffusa Herba Enhanced the Apoptosis of ATRA-induced HL-60 Cells

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ABSTRACT

Antioxidants, such as *p*-coumaric acid and caffeic acid purified from Hedyotis Diffusa Herba, were found to enhance the anti-cancer activity of *all-trans* retinoic acid (ATRA) on human promyelocytic leukaemia cells (HL-60). The medium Lethal Dose (LD_{50}) of the *p*-coumaric acid and caffeic acid was 0.205 and 0.017 mg/ml, respectively, while the LD_{50} of ATRA, which was the lowest amongst the three compounds, was 0.002 mg/ml. When each of these antioxidants was mixed with ATRA in different ratio, different effects were noted. Combining *p*-coumaric acid and ATRA exhibited antagonism at low ratio ranges but became synergetic above 0.4 affected fractions. This situation was the same in different combination of caffeic acid and ATRA. The combination index indicated that synergism took place in higher mixing ratios. Confocal microscopic photos of the cell revealed that some HL-60 cells differentiated into polymorphonuclear leukocytes, which is a typical programmed cell death after treatment with the ATRA. The formation of polymorphonuclear features was more severe whenever these antioxidants were added to the ATRA-induced apoptosis of HL-60 cells. The actual mechanism of synergetic effect, however, was not known.

Keywords: *Hedyotis diffusa, HL-60, p-coumaric, caffeic acid, all-trans retinoic acid, antioxidants, Combination treatment, Synergism, apoptosis*

INTRODUCTION

Chinese medicinal treatment regards the body of human beings as a microcosm of the universe and inherently connected to the nature and to all life.[1,2] Because of this philosophical idea, herbal substances used in Chinese medicinal practices are always dispensed in compound form with an aim to achieve maximal curing effects. Nowadays, Chinese medicines have become an alternative treatment for cancer as it could not be eradicated by conventional therapy. However, supporting scientific data and works are still inadequate. In order to advance the application of Chinese medicines, more scientific data and evidences of their activities are required before evidence-based practices could be employed for the treatment of a specific disease.

Hedyotis diffusa is a Chinese herb that has been used to treat a variety of common diseases. Recently, some researchers had shown that *Hedyotis diffusa* is effective for treating the leukaemia cells.[3] Besides, anti-inflammation and anti-bacterial activities of *Hedyotis diffusa* have also been reported but its traditional use, in particular, in combination with other bioactive compounds or herbs for leukaemia has not been systematically explored.

Therapy of acute promyelocytic leukaemia by ATRA

Hozumi suggested that differentiation therapy is good and effective for patients with the acute promyelocytic leukaemia (APL).[4] Douer proved that retinoic acid enhanced the clonal growth of normal human myeloid and erythroid precursors, and inhibited the clonal growth of fresh leukaemia cells and cell lines from patients with acute myelogenous leukaemia in vitro.[5,6] Other studies indicate that retinoic acid could induce granulocytic differentiation of HL-60 cells and fresh APL cells. More recently, researchers had demonstrated that more than 90% patients with acute promyelocytic leukaemia achieve complete remission after in vivo treatment with ATRA.[7-11] However, undesirable effects are still observed when ATRA is used in treating APL.

Antioxidation properties of ATRA-treated cells

It has been noted that ATRA can simultaneously induce differentiation and apoptosis of APL cells. Some scientific studies reveal that exposure of cells to oxygen-derived free radicals, such as superoxide anions, hydroxyl radicals and peroxyl radicals can lead to apoptosis. The characteristic features of apoptosis are cell shrinkage, chromatin condensation, and internucleosomal degradation of the cell's DNA.[12] As ATRA-induced apoptosis was attributed to inhibitory effect of ATRA on differentiation of the APL cells into polymorphonuclear leukocytes, other antioxidants can be added to achieve a better result. Therefore, it may have advantages to mix antioxidants isolated from *Hedyotis diffusa*

for augmentation of apoptosis in the ATRA pre-treated cells. By combination uses of bioactive compounds, the maximal effects of differentiation therapy using ATRA could be achieved.

DESCRIPTION OF *HEDYOTIS DIFFUSA*

Characteristics

Figure 1. *Photo of Hedyotis diffusa (白花蛇舌草)*

Hedyotis diffusa is Chinese medicinal herb of Ribiaceae. It commonly grows in provinces of the southern part of China. It is a popular Chinese folk medicine for the treatment of various diseases including appendicitis, hepatitis, tonsillitis, sore throat, urethral infection etc. In recent, it is claimed to have anti-tumour activity against human cancers such as hepatoma, cervical, gastric and intestinal carcinoma.[3]

Morphology

Hedyotis diffusa is an annular herb, which is usually 20-50 cm tall. The stem is in oval shape with branch at base. Leaves are in line-shaped with parallel vein and 1 to 3 cm in length. No stalk at the leaves. Flowers are unisexual and length in 2-5 mm. The calyx is divided into four and the crowns of flower are white in colour with four stamens in it. They are often bilabaite and ambricated. Staminodes often present and the anthers are 2-celles. Fruiting period is between July and August.[13]

Main bioactive components of *Hedyotis diffusa*

The mains chemical components in *Hedyotis diffusa* are oleanolic acid, para-coumaric acid, caffeic acid, beta-sitosterol and flavonoid glucose. To investigate the anti-tumor components in *Hedyotis diffusa*, the pure bioactive components were used and used for cytotoxicity effect and combination study on the Human promyelocytic leukemia cells (HL-60). The investigated antioxidants were *p*-coumaric acid and caffeic acid. Both *p*-coumaric acid and caffeic acid can be found in dietary product such as vegetable, cereal etc. Some epidemiological studies also indicated that consumption of fruit and vegetables that contain plant-derived phenolic compounds can lowers cancer risk in humans and its dietary constituents may be effective in preventing colon cancer and inhibit several stages of carcinogensis in vivo.[14,15]

Chemical and physical properties of two antioxidants and tumor-curing drugs

p-coumaric acid

The chemical formula of *p*-coumaric acid is $C_9H_8O_3$, which is one of the bioactive components in *Hedyotis diffusa*. It is a white crystal that has a bitter taste. This substance is a kind of phenolic acid that has molecular weight 164.16. It exists as solid form under room temperature and has melting point at 210ºC. It can readily soluble in polar solvent such as ethanol and water.

Caffeic acid

The chemical formula of caffeic acid is $C_9H_8O_4$. Similar to *p*-coumaric acid it is also a phenolic compound. It is a greenish-yellow powder, which has molecular weight 180.16. It exists as solid form under room temperature and has melting point at 225ºC. It can also readily soluble in polar solvent such as ethanol and water.

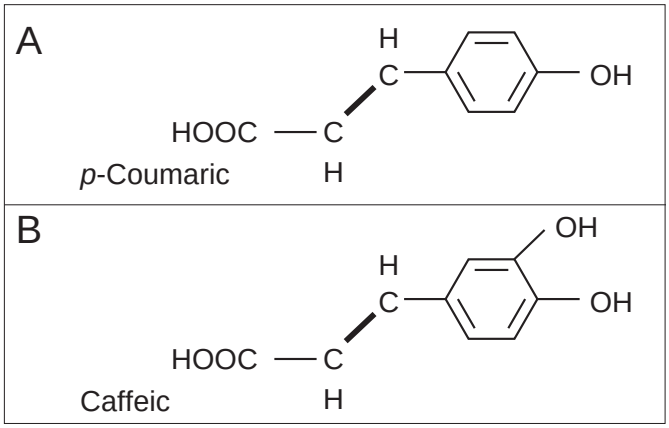

Figure 2. Chemical structure of p-coumaric acid (A) and caffeic acid (B).

All-trans retinoic acid

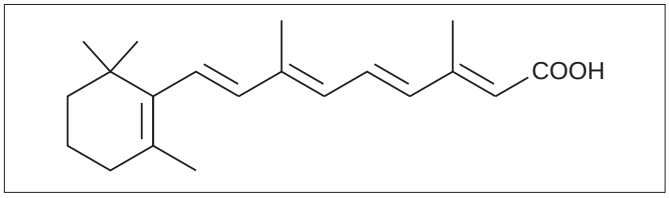

Figure 3. Chemical structure of All-trans retinoic acid (ATRA).

The chemical formula of *all-trans* retinoic acid is $C_{20}H_{30}O$. It is a yellow crystal that has molecular weight 286.46. It exists as solid form under room temperature and has melting point at 63-64ºC. It is the constituent of vitamin A from fish-liver oils, milk, egg-yolk.

ATRA is a medicine that is effective in curing cancer cells. It also a potent inducer of differentiation of acute promyelocytic leukaemia (APL) cells in vitro and in vivo.[16] It has been shown a synergistic effect with interferons on the induction of differentiation and growth inhibition in vitro.[17]

In this study, it was envisaged to investigate and compare the cytotoxicity of individual antioxidants (*p*-coumaric acid, caffeic acid) and the tumour-curing drug, *all-trans* retinoic acid on the viability of HL-60 cells and their combination effect on the HL-60 cells differentiation. The viability of the cells after adding drug was assayed by MTT colorimetric assay and Combination Index. The level of differentiation or apoptosis of HL-60 cells were also assessed under confocal microscopy.

MATERIALS AND METHODS

Materials

Cell lines: The human promyelocytic leukaemia cell (HL-60) was purchased from America Type Culture Collection (Rockville, MD). The cells were grown in suspension and propagated in RPMI-1640 medium supplemented with 10% heat-inactivated bovine serum, 100 units/ml penicillin, 100 µg/ml streptomycin and 2 mM L-glutamine.

Bioactive components and Chemicals

Different batched of Hedyotis Diffusa Herba were purchased from local market. Purified bioactive components, such as caffeic acid, p-coumaric acid and all-trans retinoic acid were obtained from Sigma (MI, USA). The bioactive components were dissolved in absolute ethanol before use. Other chemicals, such as RPMI-1640 medium, antibiotics (penicillin, 100 U/ml; streptomycin, 100 µg/ml), fetal bovine serum (FBS), absolute ethanol, 0.4% trypan blue, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Sodium Hydrogen carbonate and acridine orange-ethidium bromide (dye) also ordered from Gibco (Grand island, NY, USA).

Instruments and Glassware

Insturments used included biosafety cabinet, inverted phase contrast microscope, confocal microscope, CO_2 water-jacket precision automatic incubator, microplate reader and haemocytometer. All glassware used was pyrex grade. All other small apparatus, including micro-centrifuge tube, rack, glass pipettes (1 ml, 2 ml, 5 ml and 10 ml), autopipettes and tips, syringe, membrane filter, tissue culture flask and 96-well plate were all obtained from local suppliers.

Methods

Medium preparation

One litre of milli-Q water was used. RPMI-1640 powder was dissolved in it. Two gram of sodium hydrogen carbonate and 100 ml of fetal bovine serum (FBS) were added into the medium solution. The mixture of medium solution was filtered using medium filter. The filtering process was done in Biosafety cabinet. Sodium hydrogen carbonate was helped in maintaining the pH value in the medium. It interacted with CO_2 in CO_2 incubator and formed the buffer system for maintaining pH value about pH 6-7.

Bioactive components preparation

Each bioactive component (caffeic acid, *p*-coumaric acid) and the tumour-curing drug (*all-trans* retinoic acid) were dissolved in absolute ethanol. The dissolved solutions were filtered by syringes and membrane filters. Each syringe was used for one chemical. The filtered chemicals were drawn into autoclaved micro-centrifuge tubes.

Bioactive components dilution

The stock three chemicals needed to be serial diluted into different concentration. 5 to 6 microcentrifuge tubes were used and 0.2 ml absolute ethanol was added in each of them. 0.2 ml of stock bioactive components was mixed with the first microcentrifuge tube and re-suspended. Then, 0.2 ml of the diluted bioactive components and the drug were further serial diluted in following microcentrifuge tubes.

Cell culture and subculturing

The HL-60 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% of antibiotic (streptomycin). The whole cell culture was kept in autoclaved glass tissue culture flask and incubated in Biosafety cabinet at 37ºC in 5% CO_2. For each subculturing, cells were studied under microscope to see whether they were dense or contaminated. If the cells were dense, 3-5 ml (depend on the density of cells observed under microscope) of cells culture was drawn from the flask and discarded. Same volume of fresh RPMI-1640 medium was load into the culture flask. The neck of the flask was flamed before putting on the cap and kept in incubator. The whole process was done in Biosafety cabinet. Subculture of the cell was done routinely at about 2-3 days intervals.

Cell enumeration

Cell number was enumerated with a haemocytometer. Viable and death cells can be easily distinguished by trypan blue staining solution. 0.2 ml cells culture was mixed well with 0.2 ml of 0.4% trypan blue solution by re-suspension.

Cell dilution

The number of cell in cell culture may be too high for experiment. A standard cell number in each well was necessary. 7500 cells per each well in 96-well plate were prepared in plate preparation by cell dilution. RPMI-1640 medium was used for cell dilution.

96-well plate preparation

Before transferring ingredient into well-plate, the plates needed to be divided into several regions. For individual bioactive components, the Lane 1 to 3, 4 to 6 and 7 to 9 were for each bioactive component respectively and triplicate were done. Lanes 10 to 12 were the control region for each bioactive component. The Row A to F was the different concentration of each bioactive component. Row A was the highest concentration for each bioactive component while Row F was the lowest. 1 µl of each bioactive component were added in each well. Wells G1, G2, G3, H1, H2 and H3 were the control region for whole plate. Only 50 µl of cells culture and 50 µl medium were present. Wells G4, G5, G6, H4, H5 and H6 were the region for studying the effect of absolute ethanol on cells. Therefore, 1 µl of absolute ethanol was added instead of 1 µl of bioactive component. Wells G7, G8, G9, H7, H8 and H9 were the control region for absolute ethanol. The ingredient was same as wells G4, G5, G6, H4, H5 and H6 but no cell was added. Finally, 50 µl of medium added in each wells. For bioactive component combination study, the Lane 1 to 3, 4 to 6 and 7 to 9 were used for different ratio of bioactive component combination such as 1:1, 1:8 and 8:1. The plate was then incubated in 5% CO_2, 37ºC for 3 to 4 days.

Cytotoxicity study: MTT assay

The cytotoxicity of the antioxidants and tumour-curing drug, individually or in combination, were determined in 96-well plates by a quantitative colorimetric assay with a tetrazolium salt was used, which simply called Colorimetric MTT (tetrazolium) assay. MTT is a yellow solution and its chemical name is 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. Tetrazolium salts are attractive candidates for measuring the amount of living cells since it measures the activity of various dehydrogenese enzymes. The tetrazolium rings is cleaved in active mitochondria and so the reaction occurs only in living cells. A resulting dark blue formazan product is shown if it incubated with living cells. The intensity of the colour is proportional to the amount of living cells present. By measuring the optical density of each well, the effect of each bioactive component can be determined easily.

After the plates incubated in 3 to 4 days, 10 µl of MTT solution was added in each well. The well plate was incubated in 37ºC incubator for further 4 hours. After that, 100 µl of stop solution (0.04 N HCl in isopropanol) were added in each well. This solution allowed the cleavage of MTT to ease. Finally, the optical density was measured on multiwell scanning spectrophotometers (ELISA readers) with wavelength 570 nm.

Drug combination analysis[18]

The combination of multiple drug effect can be studied by applying the Median Effect Principle and Combination Index (CI). After reading the optical density of the well-plate, the effect of the bioactive components on cells under different concentration can be shown in graph. The Median Lethal Dose (LD_{50}) of each bioactive component was determined from the graph. When the bioactive components combined with *all-trans* retinoic acid, the Median Lethal Dose (LD_{50}) and combination index were calculated. Combination Indices can indicate whether the combined effect were synergism, summation or antagonism.

Medium Effect Principle

The Medium Effect equation states: $f_a / f_u = (D / D_m)^m$
Where D: the dose

f_a and f_u: the fractions of the system affected and unaffected by the dose D

D_m: the dose required to produce the medium effect (LD_{50})

m: a coefficient signifying the shape of the dose-effect curve

The medium effect equation can be linearized by taking the logarithms of both sides:

$$\log (f_a / f_u) = m \log (D) – m \log (D_m)$$

Then, the Medium Effect Plot of bioactivity can be carried out as shown in Figure 4

The m value can be equal to 1, higher than 1 or smaller than 1, and they indicated a hyperbolic, sigmoidal or negative sigmoidal dose-effect curve respectively.

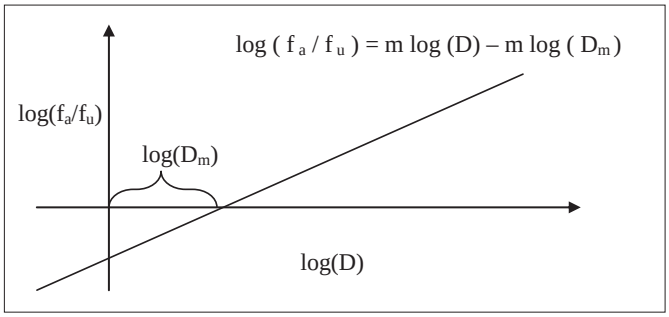

Figure 4. Medium Effect Plot of bioactivity

Combination Index (CI) analysis

After the LD_{50} of each bioactive component were determined, the combined effect between each of them were analysed by the Combination Index.[18] The bioactive components were mixed and added into the cells based on the ratio of LD_{50} of the bioactive components. The mixtures were constant in total amounts but vary in concentration ratio of each bioactive component with *all-trans* retinoic acid. The mixture then further serial diluted in different concentrations.

The equation for determining Combination Index (CI) derived from isobologram is:

$$CI = (D)_1 / (D_m)_1 + (D)_2 / (D_m)_2 + [\alpha (D)_1 (D)_2] / [(D_m)_1(D_m)_2]$$

where,

$(D)_1$: the dose of drug 1 alone required achieving a certain cell killing (f_a)

$(D)_2$: the dose of drug 2 alone required achieving a certain cell killing (f_a)

$(D_m)_1$, $(D_m)_2$: the dose of drug 1 and 2 required to achieve a certain cell killing fraction alone respectively

$[(D_m)_1(D_m)_2]$: the dose of drug 1 and 2 required to achieve a certain cell killing fraction in combination

α: a coefficient indicated the interaction of two drugs.

When the effect of two bioactive components is mutually exclusive, the combined effect is the sum of the two terms (i.e. α = 0)

$$CI = (D)_1 / (D_m)_1 + (D)_2 / (D_m)_2 + 0$$

However, if the effect of two bioactive components is mutually non-exclusive, the combined effect is the sum of three terms (i.e. α = 1)

$$CI = (D)_1 / (D_m)_1 + (D)_2 / (D_m)_2 + [(D)_1 (D)_2] / [(D_m)_1(D_m)_2]$$

The Combination Index (CI) of the two bioactive components may be equal to 1, higher than 1 or smaller than 1 which indicated synergism, summation (additive) and antagonism, respectively (Figure 5). Also, a Combination Index Graph can be constructed and the combination effect of the bioactive components can be determined.

Confocal microscopy

Confocal microscope is one of device used to investigate the differentiation of HL-60 cells after drug adding. Before viewing image under microscope, the cells needed to be labelled with acridine orange and ethidium bromide.[19] The HL-60 cells were incubated with the differentiated component, *all-trans* retinoic

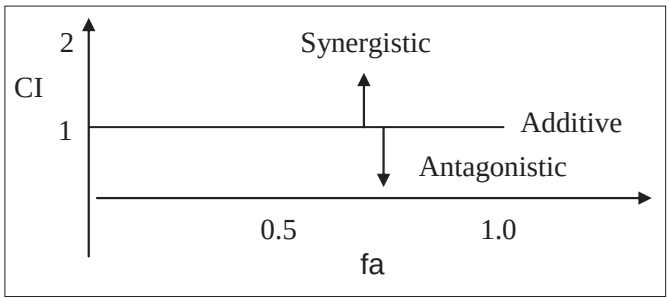

Figure 5. Combination index graph of bioactive substances in simultaneous use

acid, for 3 to 5 days. After, the cells culture was mixed well and poured off into a 15 ml centrifuge tube and centrifuged for 5 minutes in 1000 rpm. Then, the supernatant was removed and the pellet was washed by 1 ml PBS (phosphate buffer saline). The whole mixture was further centrifuged for 5 minutes in 1000 rpm. The supernatant discarded again and 0.5 ml PBS was used to wash it. 0.5 µl of acridine orange-ethidium bromide solution was added into the washed mixture. Then, the mixture can be mounted on a microscope slide and covered with a 22 mm^2 coverslip and examined in oil immersion with a 100x objective. The live cells were determined b the uptake of the acridine orange (green fluorescence) and exclusion of ethidium bromide (red fluorescence) stain. Live and dead apoptotic cells were identified by perinuclear condensation of chromatin stained by the formation of apoptotic bodies. Necrotic cells were identified by uniform labelling of the cells with ethidium bromide.

RESULTS

Effect of caffeic acid, *p*-coumaric acid and *all-trans* retinoic acid on the growth of HL-60 cells by MTT assay

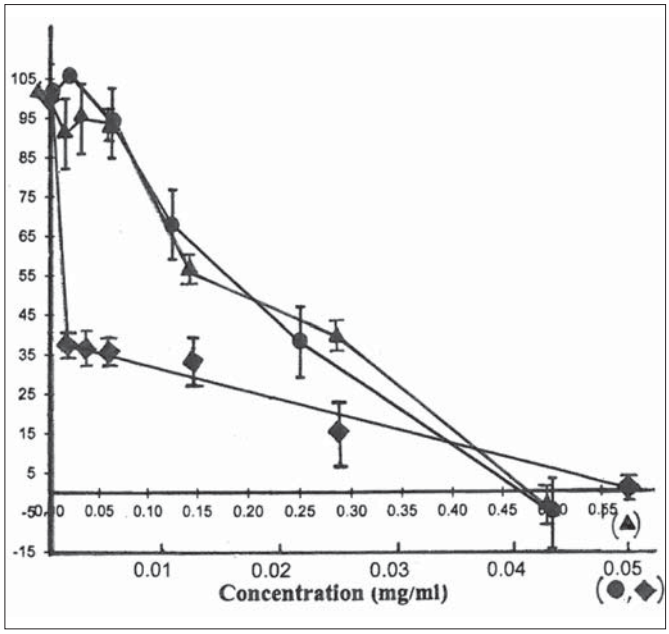

Figure 6. Viability of HL-60 cells in medium supplemented with different concentrations of phytochemical. *HL-60 cells were incubated in RPMI-1640 medium supplemented with 10% fetal bovine serum and the phytochemicals for about 3 days at 37°C in CO_2 incubator. The cytotoxicity of caffeic acid (●), p-coumaric acid (▲) or ATRA (♦), was determined individually or in combination in 96-well plates by MTT colorimetric assay.*

Figure 6 shows the concentration effect of two antioxidants, caffeic acid and *p*-coumaric) and the *all-trans* retinoic acid on the growth of the HL-60 cells. The LD_{50} can be found directly from the graph. The LD_{50} of caffeic acid and *p*-coumaric acid were 0.017 and 0.205 mg/ml respectively. Compared with these values, the LD_{50} of all-trans retinoic acid was the smallest among the three, which is 0.002 mg/ml. The viability of HL-60 cells decrease as the concentration of each chemicals increase except a slightly increase in the first few concentration of caffeic acid and *p*-coumaric acid.

Combination Index of each antioxidants with *all-trans* retinoic acid

Combination of *all-trans* retinoic acid with caffeic acid in 1:1 ratio

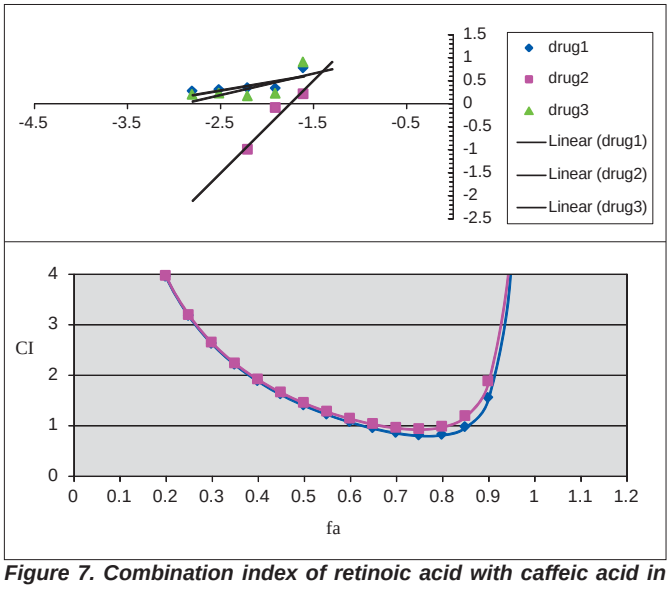

Figure 7. Combination index of retinoic acid with caffeic acid in 1:1 ratio

Combination of *all-trans* retinoic acid with caffeic acid in 1:8 ratio

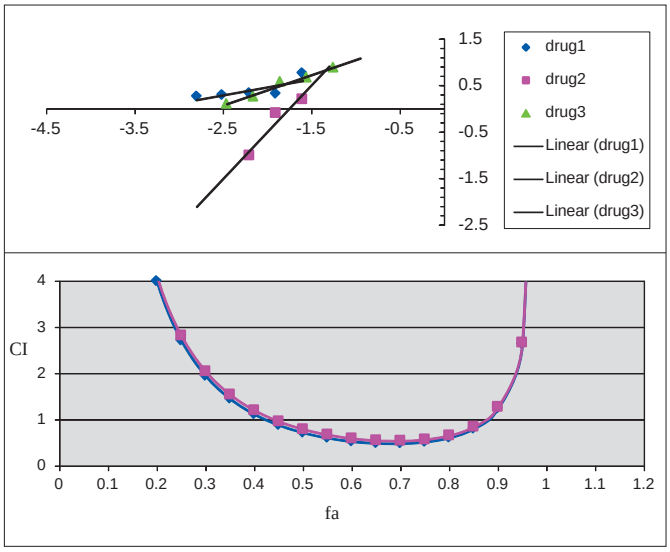

Figure 8. Combination index of retinoic acid with caffeic acid in 1:8 ratio

Combination of *all-trans* retinoic acid with caffeic acid in 8:1 ratio

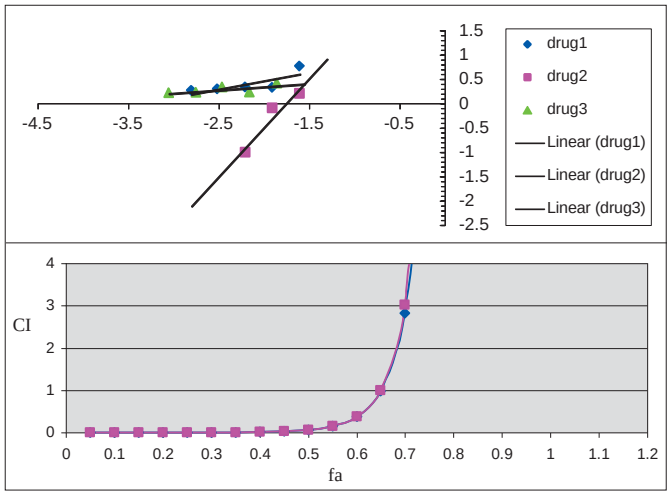

Figure 9. Combination index of retinoic acid with caffeic acid in 8:1 ratio

Combination of *all-trans* retinoic acid with *p*-coumaric acid in 1:1 ratio

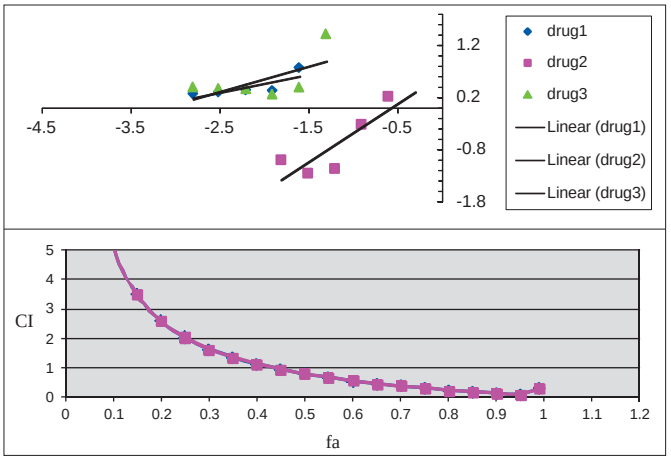

Figure 10. Combination index of retinoic acid with p-coumaric acid in 1:1 ratio

Combination of *all-trans* retinoic acid with *p*-coumaric acid in 1:8 ratio

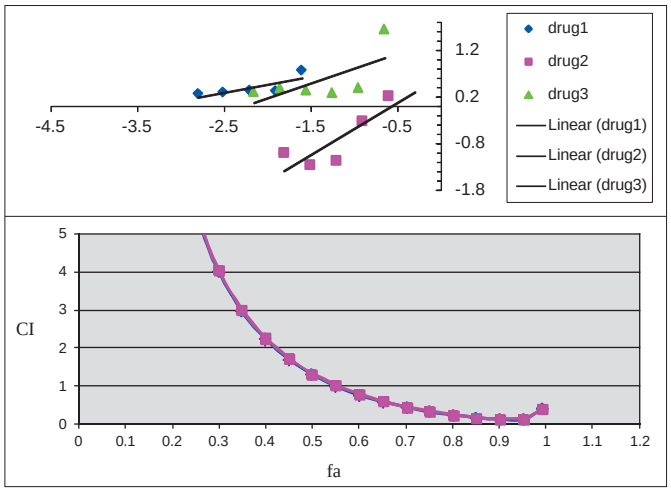

Figure 11. Combination index of retinoic acid with p-coumaric acid in 1:8 ratio

Combination of *all-trans* retinoic acid with *p*-coumaric acid in 8:1 ratio

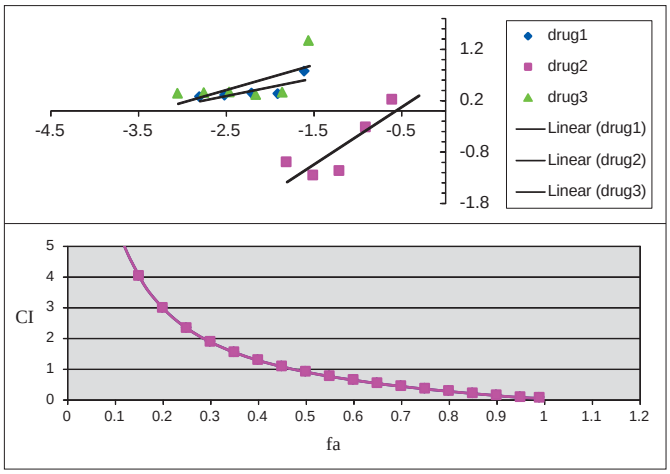

Figure 12. Combination index of retinoic acid with p-coumaric acid in 8:1 ratio

Figure 7 to 12 show the combination index of each antioxidant combined with *all-trans* retinoic acid in different combination ratio. Figure 7 to 9 were the combination of *all-trans* retinoic acid and caffeic acid while figure10 to12 were the combination of *all-trans* retinoic acid and *p*-coumaric acid. The first graph showed the linear relationship of the individual component and combined component on the growth of the HL-60 cells. The second graph showed the combination index under different affected fraction (fa) of cells.

For the combination of *all-trans* retinoic acid and caffeic acid, synergism was found between 0.6 to 0.85 and 0.45 to 0.88 affected fractions in 1:1 and 1:8 combined ratios respectively. For 8:1 ratio, the synergism was found smaller than 0.65 affected fractions. For fractions higher than 0.65, an antagonistic effect was observed.

For the combination of *all-trans* retinoic acid and *p*-coumaric acid, all combination ratios gave a similar curve. They were antagonism at the first few affected fraction and finally synergism. The affected fractions for additive relationship between two components were 0.45, 0.55 and 0.46 for 1:1, 1:8 and 8:1 combination ratio.

Confocal microscopy of ATRA-induced HL-60 cells

Figure 13 is a typical photo of confocal microscopy of HL-60 cells in RPMI-1640 medium. All cells grew normally and retained the typical morphological features of the leukocytes. However, after exposure to ATRA and other antioxidants, differentiation of the cells exhibited differently. Although ATRA facilitated the differentiation, it also induced apoptosis in some cells. Eventually, some cells ended up in necrosis due to the cytotoxic effects of these drugs (Figure 14B & C).

DISCUSSION

There are several methods proposed for curing cancer. The major methods are the enhance differentiation of cancer cells.

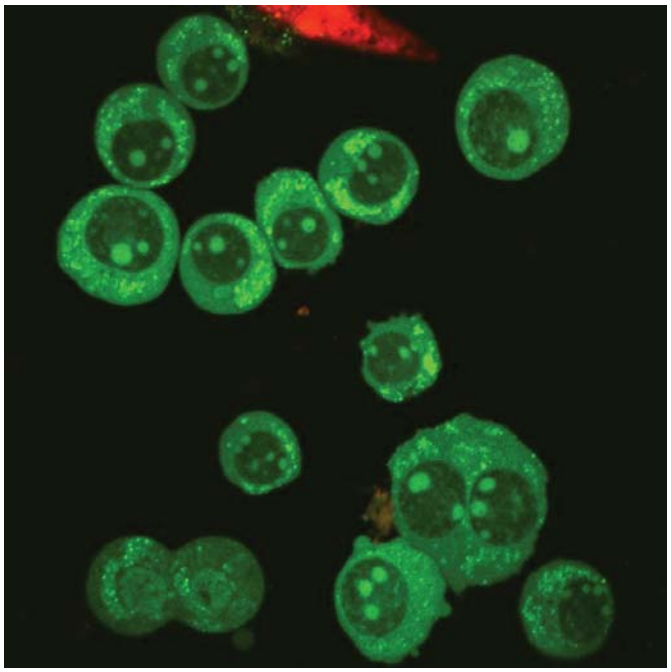

Figure 13. Confocal microscopic image of HL-60 cells. *All cells did not have any apoptotic features.*

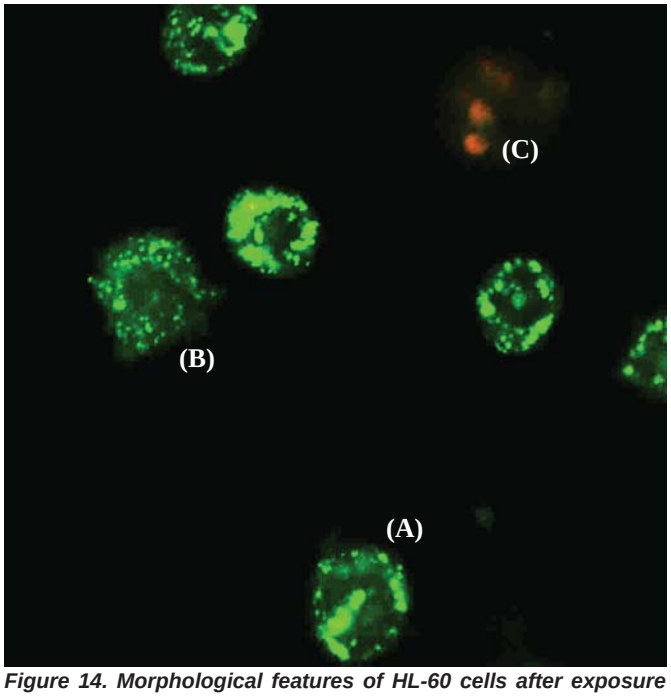

Figure 14. Morphological features of HL-60 cells after exposure to ATRA. *(A): Differentiation of HL-60 cells to Polymorphonuclear leukocytes; (B): HL-60 cells at pre-apoptosis stage; (C) necrosis of HL-60 cells*

The other methods are the programme cell death, apoptosis, and primary necrosis (or oncosis). The two types of cell death can be discriminated on the basis of morphological studies.[20] Apoptosis can be characterized by DNA fragmentation, chromatin condensation, nucleus fragmentation, organelle relocalization and cell fragmentation without increased permeability of the plasma membrane. In contrast, necrosis is characterized by cellular swelling, organelle alternations, rupture of plasma membrane and finally cell lysis and leakage of the cellular components.[21] Dietary phenolic derivatives have been shown to prevent the oxidation induced by the oxygen radical.[22,23] It has been proposed that the two antioxidants,[21] p-coumaric acid and caffeic acid, can act by quenching the oxygen radicals and protect cells against the injurious effect of oxidation.[24]

Cytotoxicity assays was used to measure drug-induced alterations in metabolic pathways or structural integrity which may or may not be related to cell death. In theory, cytotoxicity increases with increasing concentration. This phenomenon can be confirmed by observing tumor cells line treated with purified antioxidants, caffeic acid and *p*-coumaric acid, and the tumor-curing drug, *all-trans* retinoic acid. The LD_{50} obtained from the MTT assay showed that the cytotoxicity of purified *all-trans* retinoic acid much greater than that of two antioxidants. Very low concentration of *all-trans* retinoic acid can already achieve the same inhibitory effects as the two antioxidants on HL-60 cells. This can be shown in the difference in LD50 value (Figure 6). *All-trans* retinoic acid had a LD_{50} at 0.002 mg/ml while 0.205 mg/ml and 0.017 mg/ml for p-coumaric acid and caffeic acid respectively. It was 102.5 times less than *p*-coumaric acid and 8.5 times less than caffeic acid. If in case of caffeic acid and *p*-coumaric acid, the higher concentration *p*-coumaric acid was needed to obtain a LD_{50} compare with the other antioxidant, caffeic acid.

For drug combination, the combination index of *all-trans* retinoic acid combined with different ratio of *p*-coumaric acid and caffeic acid was determined (Figure 7-12). From the combination index, if the combination index greater than one, it means the two drugs are synergistic each other. Therefore, the killing of APL cells are more effective as used in combination since they can combine the specific effect of each drug and enhance the effectiveness of them. In this experiment, synergism happened when the affected fraction (fa) was higher than 0.4 in all ratios of combination of *all-trans* retinoic acid and *p*-coumaric acid (Figure 10-12). Synergism was happened in middle affected fraction (fa) in 1:1 and 1:8 ratios of combination of *all-trans* retinoic acid and caffeic acid (Figure 7 and 8). For 8:1 ratio, the synergism was found when fa lower than 0.6 (Figure 9). The duration of synergism was longer in combination of *all-trans* retinoic acid with *p*-coumaric acid than with caffeic acid. It means the *p*-coumaric acid is more effective in ATRA-induced differentiation as a role of antioxidant. It was more effective in getting rid of the oxygen radicals in HL-60 differentiation.

Degree of differentiation, apoptosis and necrosis were observed under confocal microscope (Figure 13 and 14). In these two figures, dead cells appeared as red fluorescence while live cells shown green fluorescence. The HL-60 cells was treated with *all-trans* retinoic acid and incubated for three day. Although the degree of differentiation was not yet complete, some differentiation can still be found. The dead cells were necrotic cells owing the necrotic effect from the ATRA. Some cells differentiated into polymorphonuclear leukocyte (PMN). It can be distinguished easily as multi-nuclei will be observed under confocal microscope. Some cells were irregular in shape because some apoptotic bodies attach on the surface of the cells. Those cells entered pre-apoptosis stage.

MTT colorimetric assay is a reliable method in determining the amount of viable cells. This method has certain advantages like reproducible with low interassay coefficient of variation.[25] Also, it is sensitive so that influence of very low concentration of the test compounds on cell viability can be detected for *in vivo* treatments. While on the other hand, MTT assay has certain drawbacks. First of all, the dye used may have variation. Stability of dye has a recommend storage time ranging from fresh preparation to at last 6 weeks. MTT is a mutagenic agent. Also, some nonviable cells could metabolise tetrazolium salts. Dark blue formazan is the product of functional mitochondria, non-viable cells could also reduce MTT and thus absorbance of each wells were influenced, i.e. give a positive MTT reaction. Also, the cells which are actively growing will reduce MTT to a greater extent than cells which are not,[26] this combine with inherent differences in the activity of MTT reducing enzymes in each HL-60 cells.

CONCLUSION

Effects of caffeic acid and *p*-coumaric acid, extracted from *Hedyotis diffusa*, on ATRA-induced acute promyelocytic leukaemia (APL) cells was studied. The two antioxidants have played an important role in assisting the differentiation of APL by *all-trans* retinoic acid. *p*-coumaric acid has a higher effectiveness than caffeic acid as an antioxidant in differentiation. The synergism and antagonism effect happened depended on different drug combination. The combination of *all-trans* retinoic acid with *p*-coumaric acid was more effective than with caffeic acid. Apoptosis, necrosis and differentiation were induced by *all-trans* retinoic acid on tumor cell killing process. Antioxidant in the crude extract of *Hedyotis diffusa* has important role to assist the anti-tumor drugs for therapeutic use.

Author's background

Mr PUN Chi Keung *was a BSc degree student. This scientific article was written based on his final year project report submitted in partial fulfillment of the requirements for the Bachelor Degree of Science (Hons) in Applied Biology, City University of Hong Kong.* ***Miss ZHAO Chunyan*** *is an exchange student from the Chengdu University of Traditional Chinese Medicine in Sichuan. She is doing her MSc project in CityU under Dr. Cheung's supervision.* ***Dr. CHEUNG Hon Yeung****, who is an associate professor of Pharmaceutical Microbiology & Biotechnology at the City University of Hong Kong since 1989, is a manufacturing pharmacist and biotechnologist. He has more than 40 years of work experiences in industries, academic and consultancy. He was an expert witness in court and a member of the Biotechnology Committee for Hong Kong and Shenzhen Government. Dr. Cheung has published more than 220 papers and articles in many prestigious international journals. His email address: bhhonyun@cityu.edu.hk*

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人口老化增醫療體系壓力
藥進基層, 優化安老院舍藥物管理助紓困

於2014年3月16日，基層藥劑師工作小組：香港執業藥劑師協會，香港藥學會，香港醫院藥劑師學會，香港中文大學藥劑學院，香港大學李嘉誠醫學院藥理及藥劑學系，萬寧專業藥劑師團隊，屈臣氏專業藥劑師團隊合辦了新聞發佈會。目的是由來自不同機構的藥劑師組成，旨在提升藥劑服務水平，以完善基層醫療的不足。

香港及全球人口壽命增長，人口老化加劇了醫療體系的壓力。根據統計資料顯示，65歲或以上長者人口預計至2017年將超過120萬，未來五年老年人口的增長為非老年人口的三倍，令本港的人口結構出現重大的轉變。

藥劑師於基層醫療上擔當把關角色

醫管局數據顯示，66%的老人患有慢性疾病（如糖尿病、高血壓等），對醫療服務的渴求亦有不斷上升的趨勢。研究同時顯示長者有多重用藥的習慣，每次覆診完畢多會提取3至6個月的藥物；以入住安老院舍的長者為例，他們普遍服用5至10種藥物，但不論病人本身或安老院舍的職員，對於藥物儲存、藥物間會否相沖等問題均可能認知有限，對長者的健康構成不必要的威脅。

香港執業藥劑師協會會長鄭綺雯藥劑師表示：「要減輕人口老化所引申的醫療負擔，進行相關問題的風險管理對整體醫療系統有著極之重要的作用，藥劑師在此議題上可擔當最前線把關的角色。」

開創藥物管理系統　改善醫療服務減用錯藥風險

為促進基層醫療把關的使命，一眾藥劑業同儕認為提升安老院舍的藥物管理系統，長遠可有助紓解香港醫療體系的壓力。

由見及此，香港藥學會早在2000年已認為有必要在安老院舍建立一套安全和質量更好的藥物管理模式，並於2000年於志蓮淨苑安老院設立一個試點項目，提供一套備藥系統和用藥管理程序。

志蓮項目經歷各個階段，現在已建立為世界一流的質量藥物管理系統。自2008年起更由社區藥房開展安老院舍包括備藥在內的藥物管理服務 (Pharmacist Run Medication Management Service Utilizing a Monitored Dosage System, MDS)，從安老院舍收集院友的藥物至藥物管理中心，然後藥劑師根據藥物處方進行覆核，確保安全合理用藥。其後透過電腦及智能技術，將藥物重新包裝並送回安老院舍。此模式相比由人手派藥更為安全有效，減低備錯藥及派錯藥的風險；清晰用藥指示亦有助提升長者服藥依從性，更可同時減低安老院舍醫護人員的工作量。

香港藥學會會長鄭陳佩華藥劑師指出：「有見安老院舍人手短缺，派藥繁複，我們希望能協助提升藥物管理系統，為院友不同來源的藥物作出歸納，制訂確切的服藥清單，令長者得到妥善照顧。經過5年的努力，目前已有22間安老院舍參與藥物管理服務，令3,600位老人受惠。計劃中安老院舍只需為每位院友每月支付港幣$200，即可大大減少派錯藥的危險，作好風險管理。」

勞工及福利局指定合作夥伴

除了包括備藥在內的藥物管理服務之外，政府勞工及福利局自2010年6月起推行了為期3年的「藥劑師到訪服務」(Visiting Pharmacists Service, VPS)，透過非牟利團體「香港藥學服務基金」安排社區藥劑師親身到訪安老院舍，提供藥物管理服務，減輕員工處理藥物時的壓力。

香港醫院藥劑師學會會長崔俊明藥劑師解釋：「安老院舍需要處理大量的藥物，優化藥物管理十分重要。『藥劑師到訪服務』的主要服務範圍包括：

1) 藥物整合 (Medication Reconciliation)；
2) 核實藥物劑量 (Medical Verification) 及
3) 為安老院舍員工提供專業的教育及訓練，指導他們有關藥物管理的知識，包括藥物儲存、棄置等。」

計劃推行至今，已有逾100間安老院舍，合共超過10,000位長者受惠於是項服務，共整合了逾8,000個藥物紀錄及核實了超過6,000個藥物劑量的個案。為確保「藥劑師到訪服務」的質素，參與服務的藥劑師均需接受藥物管理的培訓，獲取有關證書，其實「藥劑師到訪服務」在外國已非常普遍，早被視為基層醫療服務的一部份。

強化基層醫護服務

事實上，連鎖社區藥房對整體基層醫療亦發揮非常重要的角色。萬寧高級藥劑師趙國亮藥劑師及屈臣氏藥劑師總監劉寶珠藥劑師表示：「社區藥劑師能提供即時及免費的藥物資訊和基層藥劑服務，是市民方便接觸到的前線醫護人員。個別連鎖社區藥房更會提供量血壓服務及與安老院舍合作舉辦健康講座。一些沒有入住安老院舍的長者，若對藥物有任何疑問，亦可向社區藥劑師查詢。」

香港中文大學藥劑學院高級講師李翠萍博士及香港大學李嘉誠醫學院藥理及藥劑學系助理教授陳慧賢博士均表示，「本港兩所提供藥劑學培訓的大學學府與本港藥劑專業組織緊密合作，提供專才培訓、持續進修等，確保有足夠及高質素的專業藥劑師配合香港醫療系統的發展及需要。」

總結及展望

目前「藥物管理服務」及「藥劑師到訪服務」在安老院舍推行的成效已獲得肯定及支持，未來藥劑專業展望可與政府有更多合作，發揮社區藥劑師在社區所扮演的角色，強化基層醫護服務，協助舒緩整個醫療系統的壓力。

(左起) 萬寧高級藥劑師趙國亮、屈臣氏藥劑師總監劉寶珠、香港執業藥劑師協會會長鄭綺雯、香港藥學會會長鄭陳佩華及香港醫院藥劑師學會會長崔俊明

Announcing the Registration of the Hong Kong Pharmacists Union

Kevin Cheung, Chairman, Hong Kong Pharmacists Union

In Hong Kong, the Trade Union Ordinance CAP 332 provides the right for members of a certain profession or trade to register as an official trade union to represent the interests of members of their profession or trade in discussions with government bodies, employers, and other stakeholder groups. Many healthcare professionals have recognized the need to have an officially registered trade union to represent their professions including doctors, nurses, dietitians, physiotherapists, dispensers, and others. After decades of discussion about the need to establish an official union to represent pharmacists, The Hong Kong Pharmacists Union obtained its official registration on 13 February 2014 to be legally recognized to represent pharmacists working in all sectors and the first Annual General Meeting was successfully held on 4 March 2014 to elect the first General Council of the Hong Kong Pharmacists Union. (Please refer to the "Results of Election of 1st Annual General Meeting of Hong Kong Pharmacists Union" for more details.)

The aim of the Hong Kong Pharmacists Union is to unite the pharmacy profession and to provide an opportunity for pharmacists working in all sectors to communicate and collaborate. As a registered trade union, the Hong Kong Pharmacists Union is empowered to have more legal rights than any other professional pharmacy society currently in existence in Hong Kong, to intervene in situations relating to the work of practising pharmacists.

The registration of the Hong Kong Pharmacists Union is indeed timely in view of the current ongoing discussions with government and the legislative council relating to the amendment of pharmacy legislation and the development of the various sets of Code of Conduct and Code of Practice affecting the work requirements of pharmacists working across the sectors of manufacturing, wholesaling, and retail pharmacy practice.

All practising pharmacists in Hong Kong from every sector including manufacturing, wholesaling, community, hospital, academia, government, and others may apply to join the Hong Kong Pharmacists Union and the application form may be obtained at **www.hkphu.org**.

Recent Important Discussion Activities of the Hong Kong Pharmacists Union Include:

- 18 February 2014 - Attended the Briefing of the Drug Office, Department of Health Drug Office on the proposed legislative amendments of the Pharmacy and Poisons Ordinance to Legislative Council Health Panel.
- 10 April 2014 - Attended and had active discussion on the proposed amendments of the Pharmacy and Poisons Amendment Bill 2014 with the Asst. Director of Health, Chief Pharmacists, and Senior Pharmacists of the Drug Office.
- 11 April 2014 - Invited the leadership of the three professional societies (Pharmaceutical Society of Hong Kong, The Society of Hospital Pharmacists, and The Practising Pharmacists Association of Hong Kong) to join together with the Hong Kong Pharmacists Union to form the Working Committee on the Pharmacy and Poisons Amendment Bill 2014 to discuss and agree on a common stance in response to the Pharmacy and Poisons Amendment Bill 2014.

The 1st Annual General Meeting of Hong Kong Pharmacists Union
(First row, left to right) Yu Chun AW, Founder PPAHK; Joseph CHEUNG, Council Members HKPhU (HK Pharmacists Union); Margaret KAN, Council Member, HKPhU; Kevin CHEUNG, Chairman, HKPhU; Philip CHAN, Vice-Chairman, HKPhU; Kelvin LAW, Vice-Chairman, Pharmaceutical Trade Alliance; Iris CHANG, President, PPAHK; Alex CHEUNG, Council Member, HK General Chamber of Pharmacy.

Elected Council Members:

Chairman:	Kevin CHEUNG Kin-man
Vice-Chairman:	Philip CHAN Cho-hung
Honorary Treasurer:	Anna YUNG Wai-lan
Honorary Secretary:	Margaret KAN Pik-sheung
Council Members:	Joseph CHEUNG Ching-por
	Carmen FONG Ka-man
	CHEUNG Wan-sze
Auditor:	Mr. Calvin HO, CPA

Active Ingredient:
Each Edarbyclor tablet contains 42.68 mg of azilsartan kamedoxomil, which is equivalent to containing azilsartan medoxomil 40 mg plus 12.5 or 25 mg of chlorthalidone.

Pharmacological Properties:
The active ingredients of Edarbyclor target two separate mechanisms involved in blood pressure regulation. Azilsartan blocks the vasoconstriction and sodium retaining effects of angiotensin II on cardiac, vascular smooth muscle, adrenal and renal cells. Chlorthalidone produces diuresis with increased excretion of sodium and chloride at the cortical diluting segment of the ascending limb of Henle's loop of the nephron. The diuretic effects of chlorthalildone lead to decreased extracellular fluid volume, plasma volume, cardiac output, total exchangeable sodium, glomerular filtration rate, and renal plasma flow. Although the mechanism of action of chlorthalidone and related drugs is not wholly clear, sodium and water depletion appear to provide a basis for its antihypertensive effects.

Indication:
Edarbyclor contains an angiotensin II receptor blocker (ARB) and a thiazide-like diuretic and is indicated for the treatment of hypertension, to lower blood pressure.
Edarbyclor may be used in patients whose blood pressure is not adequately controlled on monotherapy. Edarbyclor may be used as initial therapy if a patient is likely to need multiple drugs to achieve blood pressure goals.

Dosage and Administration:
Edarbyclor is for oral use and may be taken with or without food. The recommended starting dose of Edarbyclor is 40/12.5 mg taken orally once daily. Most of the antihypertensive effect is apparent within 1 to 2 weeks. The dosage may be increased to 40/25 mg after 2 to 4 weeks as needed to achieve blood pressure goals. Edarbyclor doses above 40/25 mg are probably not useful.
Edarbyclor may be used to provide additional blood pressure lowering for patients not adequately controlled on ARB or diuretic monotherapy treatment. Patients not controlled with azilsartan medoxomil 80 mg may have an additional systolic / diastolic clinic blood pressure reduction of 13/6 mm Hg when switched to Edarbyclor 40/12.5 mg. Patients not controlled with chlorthalidone 25 mg may have an additional clinic blood pressure reduction of 10/7 mm Hg when switched to Edarbyclor 40/12.5 mg.
Edarbyclor may be used as initial therapy if a patient is likely to need multiple drugs to achieve blood pressure goals.

Contraindications:
Patients with anuria. Do not co administer Aliskiren with Edarbyclor in patients with diabetes. Second and third trimester of pregnancy.

Warnings & Precautions:
Use of drugs that act on the renin-angiotensin system during the second and third trimesters of pregnancy reduces fetal renal function and increases fetal and neonatal morbidity and death. In patients with an activated renin-angiotensin system, such as volume- or salt-depleted patients (e.g., those being treated with high doses of diuretics), symptomatic hypotension may occur after initiation of treatment with Edarbyclor. Monitor for worsening renal function in patients with renal impairment. Consider withholding or discontinuing Edarbyclor if progressive renal impairment becomes evident. Hypokalemia is a dose-dependent adverse reaction that may develop with chlorthalidone. Co- administration of digitalis may exacerbate the adverse effects of hypokalemia. Hyperuricemia may occur or frank gout may be precipitated in certain patients receiving chlorthalidone or other thiazide diuretics.

Drug Interactions:
Edarbyclor
The pharmacokinetics of azilsartan medoxomil and chlorthalidone are not altered when the drugs are co-administered. No drug interaction studies have been conducted with other drugs and Edarbyclor, although studies have been conducted with azilsartan medoxomil and chlorthalidone.

Azilsartan medoxomil
No clinically significant drug interactions have been observed in studies of azilsartan medoxomil or azilsartan given with amlodipine, antacids, chlorthalidone, digoxin, fluconazole, glyburide, ketoconazole, metformin, pioglitazone, and warfarin. Therefore, azilsartan medoxomil may be used concomitantly with these medications.

Non-Steroidal Anti-Inflammatory Agents including Selective Cyclooxygenase-2 Inhibitors (COX-2 Inhibitors)
In patients who are elderly, volume-depleted (including those on diuretic therapy), or who have compromised renal function, co-administration of NSAIDs, including selective COX-2 inhibitors, with angiotensin II receptor antagonists, including azilsartan, may result in deterioration of renal function, including possible acute renal failure. These effects are usually reversible. Monitor renal function periodically in patients receiving Edarbyclor and NSAID therapy. The antihypertensive effect of Edarbyclor may be attenuated by NSAIDs, including selective COX-2 inhibitors.

Adverse Reactions:
Adverse reactions associated with treatment with Edarbyclor have generally been mild and transient in nature. The most common adverse reactions were dizziness and fatigue.

Forensic Classification:
P1S1S3

Active Ingredient:
Combination of fluticasone furoate and vilanterol (as trifenatate)

Presentation:
Relvar Ellipta 100 micrograms/25 micrograms inhalation powder, pre-dispensed
Relvar Ellipta 200 micrograms/25 micrograms inhalation powder, pre-dispensed

Pharmacological Properties:
It is a fixed-dose combination of the active substance fluticasone furoate, a synthetic corticosteroid with potent anti-inflammatory activity, and the active substance vilanterol, a selective long-acting beta2-receptor agonist (LABA). Beta2-receptor agonists stimulate intracellular adenylate cyclase which converts ATP into cyclic AMP. Increased cyclic AMP levels cause relaxation of bronchial smooth muscle and inhibition of release of mediators of immediate hypersensitivity from cells, especially from mast cells.

Indications:
Asthma
Relvar Ellipta is indicated for the regular treatment of asthma in adults and adolescents aged 12 years and older where use of a combination medicinal product (long-acting beta2-agonist and inhaled corticosteroid) is appropriate:
• patients not adequately controlled with inhaled corticosteroids and 'as needed' inhaled short acting beta2-agonists.

COPD (Chronic Obstructive Pulmonary Disease)
Relvar Ellipta is indicated for the symptomatic treatment of adults with COPD with a FEV1<70% predicted normal (post-bronchodilator) with an exacerbation history despite regular bronchodilator therapy.

Dosage and Administration:
Asthma
Adults and adolescents aged 12 years and over
One inhalation of Relvar Ellipta 100/25 micrograms once daily.
Patients usually experience an improvement in lung function within 15 minutes of inhaling Relvar Ellipta.
A starting dose of Relvar Ellipta 100/25 micrograms should be considered for adults and adolescents 12 years and over who require a low to mild dose of inhaled corticosteroid in combination with a long-acting beta2-agonist. If patients are inadequately controlled on Relvar Ellipta 100/25 micrograms, the dose can be increased to 200/25 micrograms, which may provide additional improvement in asthma control.
Relvar Ellipta 200/25 micrograms should be considered for adults and adolescents 12 years and over who require a higher dose of inhaled corticosteroid in combination with a long-acting beta2-agonist.
Patients with asthma should be given the strength of Relvar Ellipta containing the appropriate fluticasone furoate (FF) dosage for the severity of their disease. Prescribers should be aware that in patients with asthma, fluticasone furoate (FF) 100 micrograms once daily is approximately equivalent to fluticasone propionate (FP) 250 micrograms twice daily, while FF 200 micrograms once daily is approximately equivalent to FP 500 micrograms twice daily.

COPD
Adults aged 18 years and over
One inhalation of Relvar Ellipta 100/25 micrograms once daily.
Relvar Ellipta 200/25 micrograms is not indicated for patients with COPD. There is no additional benefit of the 200/25 micrograms dose compared to the 100/25 micrograms dose and there is a potential increased risk of pneumonia and systemic corticosteroid-related adverse reactions.
Patients usually experience an improvement in lung function within 16-17 minutes of inhaling Relvar Ellipta.

Contraindications:
Hypersensitivity to the active substance or to any of the excipients is contraindicated.

Precautions:
Deterioration of disease
Fluticasone furoate/vilanterol should not be used to treat acute asthma symptoms or an acute exacerbation in COPD, for which a short-acting bronchodilator is required. Increasing use of short-acting bronchodilators to relieve symptoms indicates deterioration of control and patients should be reviewed by a physician.

Paradoxical bronchospasm
Paradoxical bronchospasm may occur with an immediate increase in wheezing after dosing. This should be treated immediately with a short-acting inhaled bronchodilator. Relvar Ellipta should be discontinued immediately, the patient assessed and alternative therapy instituted if necessary.

Cardiovascular effects
Cardiovascular effects, such as cardiac arrhythmias e.g. supraventricular tachycardia and extrasystoles may be seen with sympathomimetic medicinal products including Relvar Ellipta. Therefore fluticasone furoate/vilanterol should be used with caution in patients with severe cardiovascular disease.

Drug Interactions:
Clinically significant drug interactions mediated by fluticasone furoate/vilanterol at clinical doses are considered unlikely due to the low plasma concentrations achieved after inhaled dosing.

Interaction with beta-blockers
Beta2-adrenergic blockers may weaken or antagonise the effect of beta2-adrenergic agonists. Concurrent use of both non-selective and selective beta2-adrenergic blockers should be avoided unless there are compelling reasons for their use.

Interaction with CYP3A4 inhibitors
Fluticasone furoate and vilanterol are both rapidly cleared by extensive first pass metabolism mediated by the liver enzyme CYP3A4. Caution is advised when co-administering with strong CYP 3A4 inhibitors (e.g. ketoconazole, ritonavir) as there is potential for increased systemic exposure to both fluticasone furoate and vilanterol, and concomitant use should be avoided.

Side Effects:
Nervous system disorders: Headache
Respiratory, thoracic and mediastinal disorders: Nasopharyngitis

Forensic Classification:
P1S1S3

Hong Kong Pharmaceutical Journal: For Detailed Instructions for Authors

INTRODUCTION

Hong Kong Pharmaceutical Journal (HKPJ) is the official publication of the Pharmaceutical Society of Hong Kong, the Practising Pharmacists Association of Hong Kong and the Society of Hospital Pharmacists of Hong Kong. It is a journal of the pharmacists, for the pharmacists and by the pharmacists. The Journal is currently divided into several sections: ***Editorial Comment; News & Short Communications; Pharmacy Practice; Over-the-Counter & Health; Drugs & Therapeutics; Herbal Medicines & Nutraceuticals; Pharmaceutical Technology*** and ***New Products***. It publishes review articles or original papers relevant to these different fields of pharmacy. In addition to the regular four issues of the Journal per year, there are issues dedicated solely to reports on special function of the society. The Aims and Scope of the Journal are published on the inside back cover of each issue.

Submission of Manuscript

Submission of a paper implies that it has not been published previously, that it is not under consideration for publication elsewhere, and that if accepted it will not be published elsewhere in the same form, in English or in any other language, without the written consent of the publisher. Authors are specifically discouraged from submitting papers as fragmented studies of a particular topic. A manuscript must be indicated which section it is belonged. Upon received, it will be screened by a **Sectional Editor** of HKPJ for initial consideration before it is sent out for further review or comment.

For online submission:
Authors are encouraged to submit manuscripts using the online submission system. Access to the system, and full instructions on its use, can be found on the HKPS website at: http://www.HKPS.org/HKPJ/Guidelines. In creating the electronic version of their manuscript, authors are requested to follow the guidelines for submitting files. The paper should be submitted as a single file, prepared with a standard word-processor such as Microsoft Word, with embedded tables and graphics. Please note that any embedded graphics must also be submitted as separate, original files. The preferred formats for graphics files are tiff or postscript. All correspondence between Editor and author is performed by email. Authors are reminded that the copyright of their article or paper is automatically transferred to HKPJ once it is accepted for publication in the journal.

For hardcopy submission:
Three copies of the manuscript are required on either 8.5"x11" or A4 paper (two copies are used for review purposes and the original is kept on file at the Section Editor). Copies must be produced on a high-quality printer, and originals and copies of all Figures and Schemes must be fully legible. Initially only send hard copies of the paper; when it has been refereed, revised if necessary, and accepted, you will be requested to send a disk containing the final version with the final hard copy to the appropriate Editor. Make sure that the disk and the hard copy match exactly. The revised manuscript must be returned to the Editors within one month, otherwise it may be deemed to be new and subject to further review. When submitting the final version with a disk please label all disks with "HKPJ", your name, software (e.g. word 2000), hardware used (e.g. PC or Macintosh) and file names with the correct extension (e.g. Fig 1.cdx, Table 1-6. xls). Save text on a separate disk from the graphics, include the text and tables in one file, and provide graphics and structures in separate numbered files. Please remember to keep a backup copy of both the electronic files and original manuscript for reference and safety since we cannot accept responsibility for damage or loss of papers. Original manuscripts are discarded three months after publication unless the Publisher is asked to return original material after use.

Suggested Referees

Please submit, with your manuscript, the names and addresses of 2 potential referees. You may also mention persons who you would prefer not to review your paper.

Editorial Authority

The Editors of HKPJ reserve the right to make alterations to manuscripts submitted for publication. Such alterations will be made if manuscripts do not conform with accepted scientific standards or if they contain matter which in the opinion of the Editors is unnecessarily verbose or unclear. Alterations may be queried, but this will inevitably delay publication.

Preparation of manuscript

The manuscript is required to be written in English, with numbered pages, single-spaced, using Arial 9 point font, and in a suitable word-processing format. Each page should have adequate margins (4 cm) and liberal spaces at top and bottom of the manuscript. All textual elements should begin flush left, with the second paragraphs onwards indent, and should use the wrap-around end-of-line feature, i.e. no returns at the end of each line. Place two returns after every element such as title, headings, paragraphs, figure and table call-outs. Most formatting codes will be removed or replaced on processing your article. Please do not use options such as automatic word breaking, justified layout, double columns or automatic paragraph numbering (especially for numbered references). However do use bold face, italic, subscripts, superscripts etc. The Editors reserve the right to adjust style to certain standards of uniformity. If authors are unfamiliar with HKPJ, they should consult a recent copy (or the free online sample copy available from www.HKPS.com/HKPJ) to see the conventions currently followed for guidance in preparing submissions.

The content of manuscripts must be arranged as follows: (1) a Title Page with authors name(s) and address(es); (2) an Abstract, in which contents are briefly stated; (3) a 4 to 6 Key Word Index, (4) Introduction, and (5) the Results and Discussion (preferably combined). Although each section may be separated by headings, they should form one continuous narrative and only include details essential to the arguments presented. If a discussion is separately provided, it should not include a repetition of the results, but only indicate conclusions reached on the basis of them, and those from other referred works; (6) Conclusions or Concluding Remarks; (7) the Experimental should include brief details of the methods used such that a competent researcher in the field may be able to repeat the work; (8) Acknowledgments; (9) References; (10) Legends, Formulae, Tables and Figures.

Title Page and Author Names: Titles must be as brief as possible, consistent with clarity, and should not exceed 10 words in length. Uninformative phrases such as "Chemical examination of", "Studies on", "Survey of", "New", "Novel" etc. will be deleted. If a paper is part of a series, this must not be given in the heading, but referred to in a footnote in the form: *Part 9 in the series "The Role of Pharmacists in Medical Care of Patients" followed by a numbered reference to the previous part. Author names should be typed right underneath the article title. Each author should identify himself or herself with Surname in capital letters, followed by the first name. All names are separated by a semicolon (;). An asterisk should be placed following the name of the author to whom correspondence inquiries should be made. Full postal addresses must be given for all co-authors. Superscript letters; a, b, c should be used to identify authors located at different addresses.

An **Author's background box** at the end of each article is mandatory to include the author's job title and the affiliated institutle or organization. Full details of telephone, fax numbers and e-mail address should also be indicated for the corresponding authors. No academic or professional membership title is allowed.

ABSTRACT: The abstract should be on a separate page and briefly describe the results obtained and conclusions reached, not the methods used, or speculations on any other matter. They are not expected to be a complete summary but only an outline of the main findings. The abstract should be contained within 250 words and should be readable without reference to the rest of the paper.

Key Words: Authors must give four to six "key words" or phrases, which identify the most important subjects covered by the paper.

INTRODUCTION should give the minimum historical data needed to give appropriate context to the author's investigation and its relationship to other similar research previously or currently being conducted. Only information essential to the arguments should be presented. Much data can be taken for granted or quoted in abbreviated form. Specific term (genus, species, authority) of all experimental works must be given at first mention and preferably be in the form adopted by the International Scientific Community.

RESULTS AND DISCUSSION: These sections should be carefully prepared with discussions of the results being compared with existing and/or previous knowledge within the field. Authors are, however, encouraged to combine the Results and Discussion sections wherever possible.

EXPERIMENTAL: Subsections on the Experimental Procedures should be italicized and inserted as part of the first line of the text to which they apply. HKPJ encourages an extensive use of abbreviations (these are listed at the back of the Instructions to Authors, or the reader is referred to other sources). The Experimental should begin with a subsection entitled General Experimental Procedures. This subsection will typically contain brief details of instruments used, and identification of sources of specialized chemicals, biochemicals and molecular biology kits. The next subsection describes the source(s) and documentation of biological materials used, whether in reference to whole plants or parts there from, crude drugs, or any other plant material from which identifiable chemical substances are obtained for the first time. Documentation must also include a reference to voucher specimen(s) and voucher number(s) of the compounds, plants or other material examined. If available, authors should quote the name and address of the authority who identified each sample investigated. Specimens should preferentially be deposited in a major regional herbarium where the collection is maintained by state or private institution and which permits loan of such materials. With other microorganisms, the culture collection from which they were either accessed and/or deposited should be included, together with identification of the strain designation code. The Experimental Procedures employed should be concise but sufficiently detailed that a qualified researcher will be able to repeat the studies undertaken, and these should emphasize either truly new procedures or essential modifications of existing procedures. Experimental details normally omitted include: (I) method of preparation of common chemical and biochemical derivatives, (2) excessive details of separation of compounds, proteins and enzymes, e.g. preparation of columns, TLC plates, column and fraction size. Compound Characterization: Physical and spectroscopic data for new compounds must be comprehensive, and follow the order shown below: compound name (and assigned number in text); physical state of compound (e.g. oil, crystal, liquid, etc.), melting and/or boiling point; optical rotation and/or circular dichroism measurements, if optically active; UV; JR, IH NMR; 13C NMR; MS. For all new compounds, either high-resolution mass spectral or elemental analysis data is required. See later section for method of data presentation.

Nomenclature: Chemical nomenclature, abbreviations and symbols must follow IUPAC rules. Whenever possible, avoid coining new trivial names; every effort should be made to modify an existing name. For example, when a new compound is described, it should be given a full systematic name according to IUPAC nomenclature and this should be cited in the Abstract or in the Experimental section.

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(2) Mabry T, Markham KR, Thomas MB. (1970). *The Systematic Identification of Flavonoids.* 2nd Ed, pp. 79-105. Springer Verlag, New York.

(3) Harborne JB. (1999). Plant chemical ecology. In: Barton D, Nakanishi K, Meth-Cohn 0, (Eds.), *Comprehensive Natural Products Chemistry,* Vol. 8. pp. 137-196. Pergamon, Oxford.

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Abbreviations
About, approximately: ca.
Anhydrous: dry (not anhyd.)
Aqueous: aq.
Circular dichroism: CD
Concentrated (or mineral acids): conc.
Concentrations: ppm (or ppb), μM, mM, M, %, mol
Dry weight: dry wt; fresh weight: fr. wt
Electricity: V, mA, eV
Force due to gravity (centrifugation): g; rpm (revolutions min^{-1})
Gas chromatography: GC
Gas chromatography-mass spectrometry: GC-MS Trimethylsilyl derivative: TMSi (TMS cannot be used as this refers to the internal standard tetramethylsilane used in ^{1}H NMR)
High performance liquid chromatography: HPLC
Infrared spectrophotometry: IR
Length: nm, μm, mm, cm, m
Literature: lit.
Mass spectrometry: m/z $[M]^{+}$ (molecular ion, parent ion)
Melting points: uncorr. (uncorrected)
Molecular mass: Da (daltons), kDa
Molecular weight: M_r
Nuclear magnetic resonance: ^{1}H NMR, ^{13}C NMR, Hz, δ
Numbers: e.g. 1, 10, 100, 1000, 10000; per or $^{-1}$
Optical rotatory dispersion: ORD
Paper chromatography: PC
Precipitate: ppt.
Preparative thin-layer chromatography: prep. TLC
Radioactivity: dpm (disintegrations per min), Ci (Curie), sp. act (specific activity), Bq (1 becquerel = 1 nuclear transformation sec^{-1})
Repetitive manipulations: once, twice, x3, x4, etc.
RR_t (relative retention time), R_1 (Kovat's retention index), ECL (equivalent chain length- term frequently used in fatty acid work)
Saturated: satd.
Solution: soln.
Solvent mixtures including chromatographic solvents: abbreviate as follows n-BuOH-HOAc-H_2O (4:1:5)
Statistics: LSD (least significant difference), s.d. (standard deviation), s.e. (standard error)
Temperature: (with centigrade), mp, mps, mmp, bp
Temperature: temp.
Thin-layer chromatography: TLC, R_f
Time: s, min, h, day, week, month, year
Ultraviolet spectrophotometry: UV, A (absorbance, not aD-optical density)
Volume: 1, (litre), μ1, ml
Weight: wt, pg, ng, μg, mg, g, kg

Inorganics, e.g. $AlCl_3$ (aluminum chloride), BF_3 (boron trifluoride), Cl., CO_3, H_2, HCl, $HClO_4$ (perchloric acid), HNO_3. H_2O, H_2O_2, H_2SO_4, H_3B0_3 (boric acid), He, $KHCO_3$ (potassium bicarbonate), $KMn0_4$ (potassium permanganate;), KOH, K-Pi buffer (potassium phosphate buffer), $LiAlH_4$ (lithium aluminium hydride), Mg^{2+}, $MgCl_2$, N_2, NH_3, $(NH_4)_2S0_4$, Na^{+}, $NaBH_4$ (sodium borohydride), NaCl, $NaI0_4$ (sodium periodate), NaOH, Na_2S0_3 (sodium sulphite), Na_2S0_4 (sodium sulphate), $Na_2S_20_3$ (sodium thiosulphate), 0_3, PPi (inorganic phosphate), SO_4^{2-}., Tris (buffer).

Organics, e.g. Ac_20 (acetic anhydride), n-BuOH (butanol), C_6H_6 (benzene), CCl_4 (carbon tetrachloride), CH_2Cl_2 (methylene chloride), $CHCl_3$ (chloroform), CH_2N_2 (diazo-methane), CM (carboxymethyl), DEAE (diethylaminoethyl), DMF (dimethylformamide), DMSO (dimethyl sulphoxide), EDTA (ethylene-diaminetetra-acetic acid), Et_20 (diethyl ether), EtOAc (ethyl acetate), EtOH (ethanol), $HC0_2H$ (formic acid), HOAc (acetic acid), iso-PrOH (iso-propanol), Me_2CO (acetone), MeCOEt (methyl ethyl ketone), MeOH (methanol), NaOAc (sodium acetate), NaOMe (sodium methoxide), petrol (not light-petroleum or petroleum ether), PhOH (phenol), PrOH (propanol), PVP (polyvinylpyrrolidone), TCA (trichloroacetic acid), TFA (trifluoroacetic acid), THF (tetrahydrofuran). ^{1}H NMR solvents and standards: $CDCl_3$ (deutero-chloroform), D_2O, DMSO-d_6 [deuterodimethylsulphoxide not $(CD_3)_2S0$], pyridine-d_5 (deuteropyridine), TMS (tetramethylsilane).

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