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Ministry of Higher Education

And Scientific research

University of basrah

Collage of Pharmacy

Detection of Active Ingredients in Two Herbal Products From Basrah Market

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Topics

1- Introduction:

1-1 The WHO has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. Traditional medicine is the synthesis of therapeutic experience of generations of practicing physicians of indigenous system of medicine. Traditional preparations comprise medicinal plants, minerals and organic matter etc. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy. The earliest recorded evidence of their use in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years (1).

The consumption of herbal medicines is increasing steadily throughout the world as an alternative treatment for alleviating a number of health problems including heart diseases, diabetes, high blood pressure and even certain types of cancer. In India use of herbal drugs is much more because of their easy accessibility (2).

1-2 Difference of Herbal and Conventional Drugs :

•herbal products are not regulated for purity and potency .There are neither studies on their effectiveness nor control over the quality and safety of these preparations. As per Food and Drug Administration mandates, only medicines have to be proven to be safe before being released into market. Herbalists generally use unpurified plant extracts containing several different constituents. It is claimed that these can work together synergistically so that the effect of the whole herb is greater than the summed effects of its components. It is also claimed that toxicity is reduced when whole herbs are used instead of isolated active ingredients (“buffering”). Although two samples of a particular herbal drug may contain constituent compounds in different proportions, practitioners claim that this does not generally cause clinical problems. There is some experimental evidence for synergy and buffering in certain whole plant

preparations, but how far this is applicable to all herbal products is not known .

•Herb Combining- Often several different herbs are used together. Practitioners say that the principles of synergy and buffering apply to combinations of plants and claim that combining herbs improves efficacy and reduces adverse effect. This contrasts with conventional practice, where polypharmacy is generally avoided whenever possible .

•Diagnosis- Herbal practitioners use different diagnostic principles from conventional practitioners. For example, when treating arthritis, they might observe, “under functioning of a patient’s symptoms of elimination” and decide that the arthritis results from “an accumulation of metabolic waste products”.A diuretic, cholerectic or laxative combination of herbs might then be prescribed alongside herbs with anti-inflammatory properties(1,2) .

1-3 Herbal Medicines: Possible Risks :

Herbal products do not fall under the category of medicine as long as they are not marketed for the prevention of any disease. Herbal drugs are considered as 'food integrators and readily available in the market without prescription. The major driving force for the use of herbal drugs is the perception that ‘they are safe because they are natural and have fewer side effects than prescription drugs'. However, various studies and researchers have high lightened their possible side effects, if taken irregularly, in excessive amounts or in combination with some medicines. A common problem with herb use is that people do not take into consideration how they may interact with any prescription drug . they are taking, or with each other. Interaction between drugs and herbs can result in unexpected concentration of drugs and also cause undesired effects.

Sometimes the use of commonly used herbs with prescription medicines become big barrier for the diagnosis of certain diseases as people do not inform their physicians about their consumption(2) .

Most herbal products on the market today have not been subjected to drug approval process to demonstrate their safety and effectiveness.

Some of them contain mercury, lead, arsenic and corticosteroids and poisonous organic substances in harmful amount. Hepatic failure and even death following ingestion of herbal medicine have been reported. A prospective study shows that 25% of the corneal ulcer in Tanzania and 26% of the childhood blindness in Nigeria and Malawi were associated with the use of traditional eye medicine . Side effect of some medicinal plant is currently reviewed . Medicinal plant materials and possibly herbal tea, if stored improperly allow the growth of Aspergillus flavus a known producer of afalotoxin mycotoxin (1).

1-4 Examples on the risks of herbal products :

***Turmeric**: is known to possess antibacterial, anticancer and antifungal activities. It is well recognized as the best anti-oxidant, hypoglycaemic, colorant, antiseptic and wound healer.

Risks: Turmeric may increase the risk of bleeding or potentiate the effects of warfarin therapy. Also turmeric used with caution with gall bladder problems .

***Fennel:** It is a highly aromatic and flavourful herb with culinary and medicinal uses. Fennel may be an effective diuretic and a potential drug for treatment of hypertension .

Risks: Fennel is found to show some allergic reaction. Occupational rhinitis and asthma in an atopic individual is reported which mainly involved sensitivity to unique allergens present in fennel .

***Eucalyptus:** belongs to family Myrtaceae and it is used as Stimulant, antiseptic and aromatic.

Risks: Eucalyptus oil is reported to cause dermatological side-effects, which deserves further systematic investigation, as eucalyptus oil is used widely in dermatological preparations. Eucalyptus can cause depression of conscious state, drowsiness, unconsciousness, vomiting or ataxia. Ingestion of eucalyptus oil caused significant morbidity in infants and young children. So it should not be recommended for children .

*__Cloves__: fights germs, viruses and bacteria, and it encourages the loosening of phlegm from the respiratory system.

__Risks:__ Clove may increase the risk of bleeding or potentiate the effects of warfarin therapy .

*__Garlic:__ has been used for its medicinal properties since ages. It is an extensively used herbal medicine.

__Risks__: Inspite of having invaluable applications in the field of medicines, garlic is reported to have interactions with anticoagulant therapy. Garlic may increase the risk of bleeding or potentiate the effects of warfarin therapy . The most common side effects of garlic are it may produce bad breath, heartburn, flatulence, gastrointestinal irritation and nausea.

*__Ginseng__: is used to cure sexual dysfunction in men, also in hair tonics and cosmetic preparations.

__Risks:__ The word ginseng is said to be the wonder of the world, but many adverse effects are also reported. Reports include reactions such as headache, insomnia, anxiety and breast soreness or tenderness. It is also possible that skin rashes may develop as well as asthma attacks, increased blood pressure, diarrhoea, euphoria, nervousness, skin eruptions, heart palpitations, or postmenopausal uterine bleeding.

*__Ginger__: is commonly used to treat various types of “stomach problems,” including motion sickness, morning sickness, colic, upset stomach, gas, diarrhoea, nausea caused by cancer treatment, nausea and vomiting after surgery.

__Risks__: Heartburn or stomach distress can occur if taken in large quantities. Ginger reinforces warfarin action by heterogeneous mechanisms. It should thus not be used in patients on oral anticoagulant and/or antiplatelet therapy.

*__liquorice root__ : (licorice root) or Mulhati is an effective expectorant, and has been used for this purpose since ancient times, especially in Ayurvedic medicine where it is also used in tooth powders.

__Risks__: Licorice may increase the risk of bleeding or potentiate the effects of warfarin therapy. Heavy licorice (glycyrrhizin) consumption has been associated with shorter gestation. Heavy glycyrrhizin exposure was associated with preterm delivery and may be a novel marker of this condition. Licorice should not to be used by people with high blood pressure or kidney failure or who are taking digitalis, unless directed to do so by their physician(2) .

1-5 Bioavailability of Herbal Drugs:

The bioavailability of the active constituents of the herb is another area of considerable importance. Before a compound can act systemically it must pass from the gastrointistinal tract into the blood stream. This is an area in which surprisingly little is known for herbal constituents. Compound, such as berberine and hydrastine in the popular botanical goldenseal (Hydrastic canadensis L.), are essentially not absorbed following oral consumption. Studies showing systemic effect in animal have all involved parenteral administration of these alkaloids (1).

Safety of some herbal ingredients has been recently called into question, in part because of the identification of adverse events associated with their use and, increasingly, because of the demonstration of clinically relevant interactions between herbs and prescription drugs. Adverse events (stroke, heart attacks, heart-rate irregularities, liver toxicity, seizures, psychoses and death) associated with use of ephedra for weight loss, body-building effects and increased energy or kava-kava (also known as kawa), widely used in Europe and increasingly in Canada to treat anxiety, nervousness, insomnia, pain and muscle tension, for example, have caused some countries to issue regulations restricting or banning these products . Only a few herbs in common use have been suspected of causing cancer. These include Aristolochia, Rubia tinctorum, Morinda officinalis and Senecio riddellii (3) .

2-Tools:

1-beaker

2-test tube

3-droper

4-funnel

5-filter paper

6-hot plate

7-conical flask

8-electrical balance

Materials:

1- colon herbal medicine

2- UTI herbal medicine

3- concentrated sulphuric acid

4-$FeCl_3$

5-HCl

6-lead acetate solution

7-chloroform

8- mayer's and wagner's reagent

Procedure:

By using aqueous and organic extract for both herbal product we do multiple tests

Test for tannin

About 2 ml of the aqueous extract was stirred with 2 ml of distilled water and few drops of FeCl3 solution were added. The formation of a green precipitate was an indication for the presence of tannins.

Test for phlobatannins

2 ml of the aqueous extract was added to 2 ml of 1 % HCl and the mixture was boiled. Deposition of a red precipitate was an evidence for the presence of phlobatannins .

Test for flavonoids

To 1 ml of aqueous extract was added 1 ml of 10 % lead acetate solution. The formation of a yellow precipitate was taken as a positive test for flavonolds.

Test for terpenoids

2 ml of the organic extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. A greyish colour indicates the presence of terpenoids.

Tests for sterolds

1(A red colour produced in the lower chloroform layer when 2 ml of organic extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid added indicates the presence of steroids

2) The development of a greenish colour when 2 ml of the organic extract was dissolved in 2 ml of chloroform and treated with sulphuric and acetic acids indicates the presence of steroids

Test for alkalolds

3m1 of aqueous extract was stirred with 3 ml of 1 % HCl on a steam bath. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

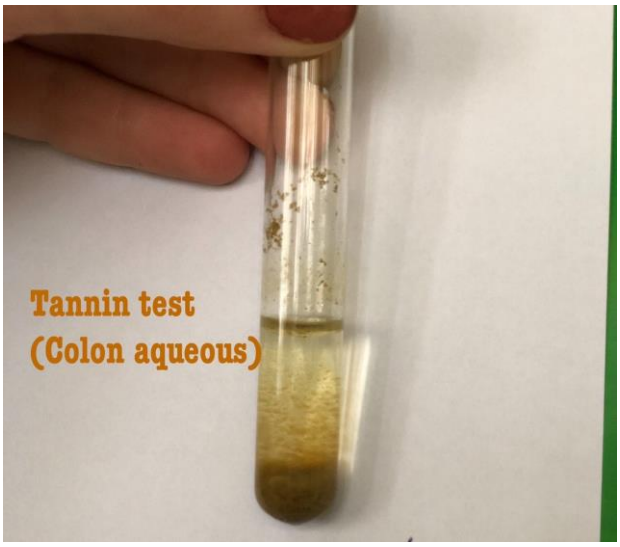
Tests for glycosides

(a) Liebermann's test: 2 ml of the organic extract was dissolved in 2 ml of chloroform and 2 ml of acetic acid was added and the solution cooled well in ice. Sulphuric acid was then added carefully. A colour change from violet to blue to green indicates the presence ofa steroidal nucleus (that is, a glycone portion of glycoside).

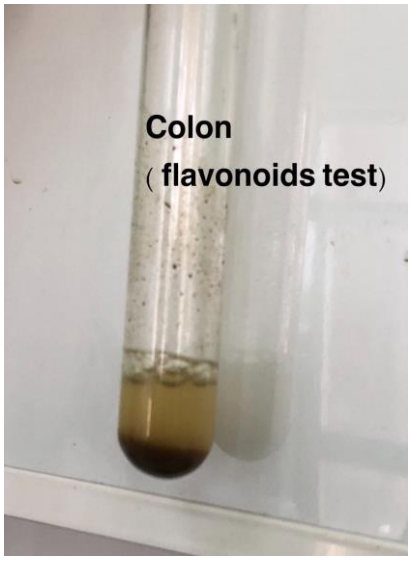
b) Salkowski's test : 2 ml of each extract was dissolved in 2 ml of chloroform . 2 ml of Sulphuric acid was added carefully and shaken gently. A reddish brown colour indicates the presence of a steriodal ring (that is, a glycone portion of glycoside). (4)

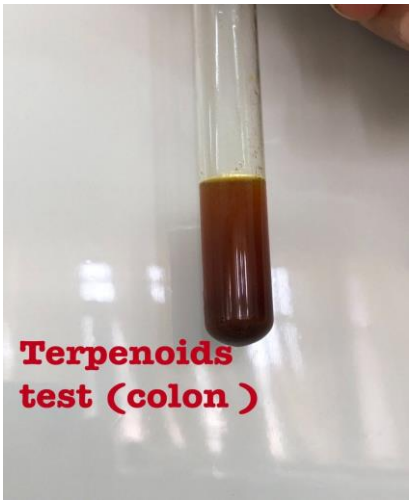
3- Results

Name of test	Colon extract
Tannins test	+
Phlobatannins test	+
Flavonoids test	-
Terpenoids test	-
steroids test	+ +
alkaloids test	+
glycosides test Salkowski's test Libermann's test	

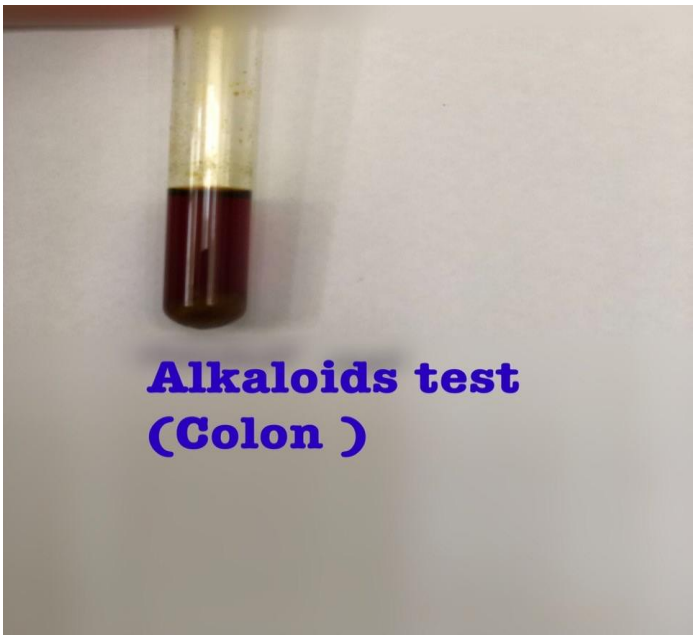

Tannin test
(Colon aqueous)


Colon
(phlobatannins test)


Colon
(flavonoids test)

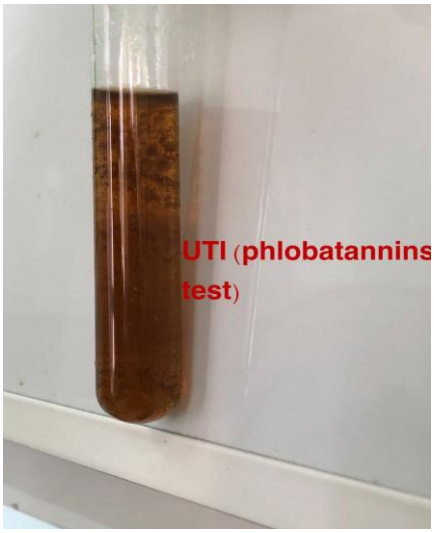

Terpenoids
test (colon)

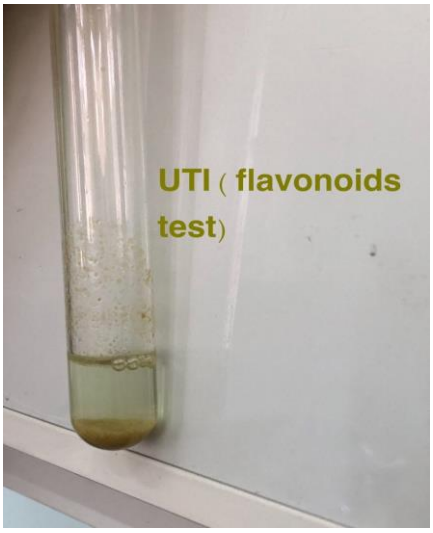

Steroids test
(Organic colon)

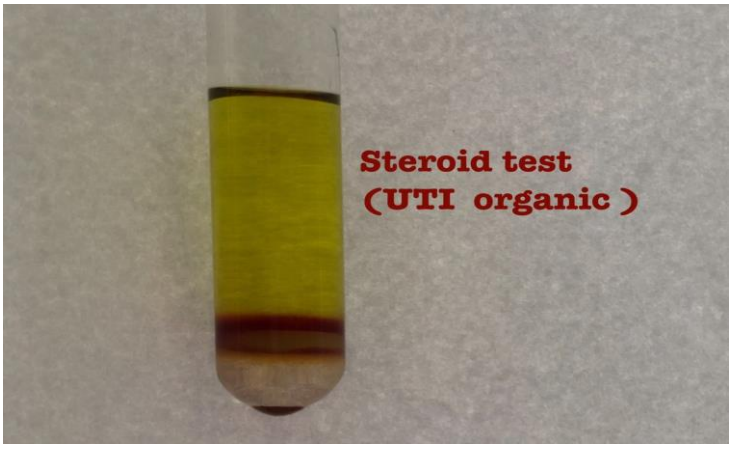

Alkaloids test
(Colon)

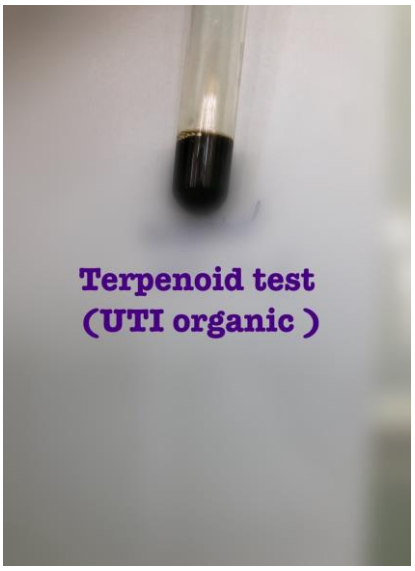
Results

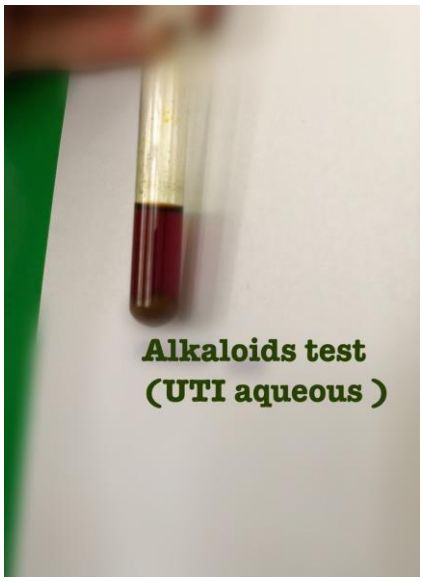
Name of test	UTI extract
Tannin test	
Phlobatannins test	-
Flavonoids test	+
Terpenoid test	-
Steroid test	+
Alkaloids test	+
Glucosides : Salkowski's test Libermann's test	 + +

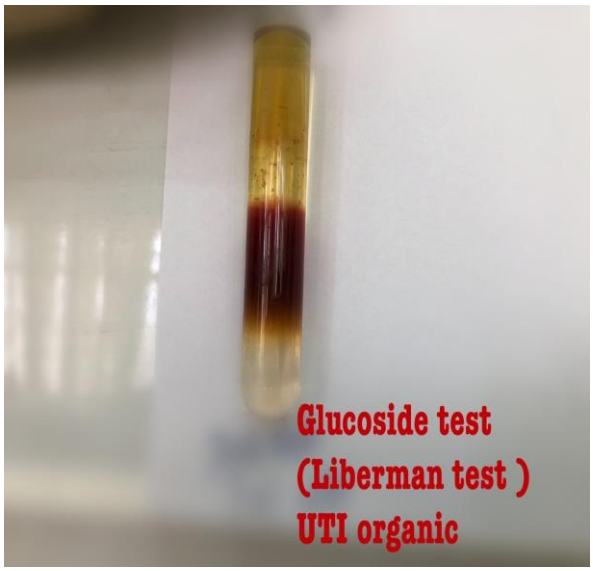

UTI (phlobatannins
test)

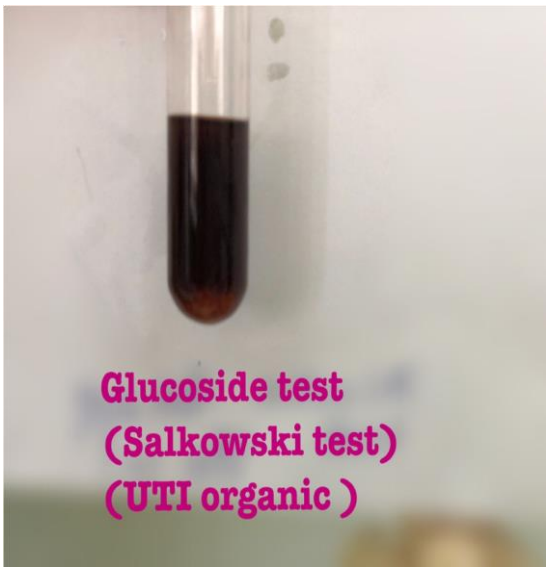

UTI (flavonoids
test)


Steroid test
(UTI organic)


Terpenoid test
(UTI organic)


Alkaloids test
(UTI aqueous)


Glucoside test
(Liberman test)
UTI organic


Glucoside test
(Salkowski test)
(UTI organic)

4-Discussion:

In our presentation we will discuss the abuse of herbal medicine products and it is impact on basra community

These products may contain substances that may interact with other drugs or disease taken by the people that will lead to produce harmful effect or impact on a certain organ inside the body .

We take two herbal medicine product are colon and UTI

And we perform a multiple tests to detect which substances are present in these products.

Some of tests are positive for what we look and other were negative.

A- colon herbe

The tests results show the present of following substance:

Tannin

Ulcerative colitis (UC) is a common inflammatory bowel disease .The disease is characterized by lesions in the colon that are a nonspecific inflammatory response limited to the colonic mucosa and submucosa. Symptoms include bouts of diarrhea, low-grade fever, right lower-quadrant pain, intestinal cramping, weight loss, flatulence, malaise, and bloody stools.

Cause :

GI inflammation that is characteristic of UC has several contributing factors. These factors include the lack of protection from inflammation due to a defect in mucin production, an alteration in GI flora, an increase in MMPs, an increase GI intestinal permeability, and the effect of food allergens.

Advantage:

1. tannins provide a therapeutic option for the major factors in the induction of UC. tannins are a large, diverse group of polyphenolic compounds. They are astringent in nature due to their high polyphenol content has ability to form strong complexes with proteins, starches, and other macromolecules.

2. tannins can modulate inflammation caused by food allergens and pathogenic microflora in the GI tract.

3. condensed tannins can decrease the effect that food allergens have on GI inflammation in UC. Patients with UC display GI increased by the presence of higher levels of mucosal eosinophils and IgE in relation to certain foods. There is an association between UC, tissue eosinophilia, and type-I allergy. tannin has ability to inhibit the development of the oral sensitization; that inhibition could correlate with the rise in the population of T cells in the intestinal intraepithelial cells. but they dramatically inhibit serum specific IgE, IgG1, and histamine. This inhibition decreases the tissue eosinophilia and type-I allergy seen in UC.

4. tannins help decrease the inflammatory molecules that are problematic for UC patients who have a defect in mucin or an increase in intestinal permeability.

5. UC patients show increased levels of MMP-9, which contribute to the intestinal inflammation so Condensed tannins have been shown to suppress the secretion of MMP-2 and MMP-9 in cells.

6. Intestinal permeability is another factor in the inflammation of UC that can be treated with condensed tannins. Procyanidins isolated from cocoa tannins display the ability to inhibit the loss of integrity in Caco-2 cell permeability induced by oxidants. An increase in oxidized molecules and oxidative damage is seen in patients with UC. condensed tannins can decrease intestinal permeability by mitigating GI inflammation caused by oxidative molecules, making them a good therapeutic option for UC.

7. The mode of antimicrobial action of tannins appear to be related to the inhibition of extracellular microbial enzymes

Disadvantage:

tannins inhibit the absorption of iron, which may lead to anemia. Tannins act as metal ion chelators that render the iron unavailable to the body. Tannins only reduce the bioavailability of plant sources of iron; animal sources of iron are left available for absorption. Tannic acid does not affect absorption of other trace minerals such as zinc, copper, and manganese in rats.

Condensed tannins do not interfere with iron absorption.

High doses of tannins lead to excessive astringency on mucous membranes, which produces an irritating effect. This probably led to the practice of adding milk to tea whereby the tannins preferentially bind to proteins in the milk rather than the gut wall.

Summary

If pharmacist not describe this type of drugs that may cause serious problems for many patients like patient with anemia and take iron or patients take Ca supplement; tannin will form complex and decrease the absorption of them so must advice patient to separate between them. (5,6)

Phlobatannins:

Phlobaphenes (or phlobatannins) are reddish-colored, water-insoluble phenolic substances that are believed to be related to co-occurring condensed tannins. The term is also used to describe the red insoluble material produced by treating condensed tannins with mineral acid. The chemical composition of the “natural” phlobaphenes is complex and linked to other extraneous materials in addition to condensed tannins. So that’s mean phlobatannins produced from tannins after treated with mineral acid and also have the same properties accept its in soluble in water , phlobatannins also have the same pharmacological effect of tannins. (7)

B-UTI herbe

The tests results show the present of following substance:

Flavonoid:

Flavonoids have been consumed by humans since , the advent of human life on earth, that is, for about 4 million years.

Advantage :

They have extensive biological properties that promote human health and help reduce the risk of diseases. Oxidative modification of LDL cholesterol is thought to play a key role during atherosclerosis. The isoflavan glabridin, a major polyphenolic compound found in *Glycyrrhiza glabra* (Fabaceae), inhibits LDL oxidation via a mechanism involving scavenging of free radicals.

One of the most importance of flavonoid is: Flavonoids Have Little Toxicity: Long history of consumption with exceptional safety record, extremely large doses used in animal studies; Acute LD_{50} for rats: 2 g / kg BW by direct injection into blood. The margin of safety for the therapeutic use of flavonoids in humans, therefore, is very large and probably not surpassed by any other drug in current use.

Disadvantage :

But the problem when using flavonoid compound is drug interaction for example:
Flavonoid with Paclitaxel (anticancer drug) for example quercetin (type of flavonoid) not recommended with palicitaxel as we know most of cancer patient use herbal drug. Also quercetin+ cyclosporine another type of flavonoid phellamurin+ cyclosporine not recommended (Cyclosporine is used to prevent organ rejection in people who have received a liver, kidney or heart transplantation). (8-9)

These substances present in both UTI and Colon herbe

Alkaloid:

Inflammatory bowel disease (IBD) is a chronic and disrupted inflammation of the gastrointestinal tract. IBD have two main conditions, Crohn's disease and ulcerative colitis

Advantage :

The alkaloids are substances of a very diverse class of plant secondary metabolites; an extensive list of biological activities has been attributed to alkaloids, such as being anticholinergic, antitumor, diuretic, antiviral, antihypertensive, antiulcer, analgesic, and anti-inflammatory.

proved to inhibit the release of proinflammatory cytokines, decreasing the activation of nuclear factor κB (NF-κB), which is important to the inflammatory response in IBD.

As example berberine decreased colonic inflammation in UC and CD experimental models and inhibited cytokines release (TNF-α, IL-1β, IL-6, IL-12, and IL17).

isoquinoline alkaloid is showed to decrease symptoms of irritable bowel syndrome , nonalcoholic fatty liver disease, and acute coronary syndrome

This alkaloid improves colon injury and inhibits the increase of inflammatory mediators and oxidative damage. (10)

Disadvantage

Plants naturally synthesize alkaloid compounds based on their needs. Some of the alkaloids like hepatotoxic pyrrolizidine, indolizidine, piperidine and tropane have been documented to cause toxic effects on animals and humans

These alkaloids may enter human body either through inhalation or swallowing. Further, the traditional medication that needs humans consume alkaloid-containing plants without proper medical prescription is a common way they get intoxicated by toxic alkaloids. For animals, grazing activity is a usual way they obtain these poisonous compounds

The ingested plants containing toxic alkaloids can cause injury to the human body, even though the plant extract is taken in a relatively small amount.

The toxic alkaloids may interfere normal functions of human body systems through specific mechanism that involves receptors, transporters, enzymes and genetic materials at specific cells and tissues. The mild toxic effects of consuming toxic alkaloids are itching, nausea, vomiting and mild gastrointestinal perturbation while the chronic effects are psychosis, paralysis, teratogenicity, arrhythmias and sudden death. (11)

Steroid:

Advantage

Corticosteroids are the most commonly used agents in the treatment of acute flares in patients with moderate-to-severe IBD.The anti-inflammatory actions of corticosteroids are well known, but how these translate into their full mechanism of controlling IBD is not completely understood. First-line treatment for moderate-to-severe active UC.(12)

drug interactions

Certain drugs such as , erythromycin and clarithromycin and ketoconazole can reduce the ability of the liver to metabolize corticosteroids and this may lead to an increase in the levels and side effects of steroids in the body. On the other hand, phenobarbital, ephedrine, phenytoin and rifampin may reduce the blood levels of steroids by increasing the breakdown of corticosteroids by the liver.

Estrogens have been shown to increase the effects of steroids possibly by decreasing their breakdown by the liver.

Steroids effects on warfarin can vary; therefore when taking warfarin along with steroids , there may be increased need for monitoring coagulation levels more closely.

Low blood potassium (hypokalemia) and a higher chance of heart failure can result from combining steroid with drugs that reduce potassium in the blood (for example, diuretics, amphotericin B).

Anticholinesterase drugs (for example, physostigmine) may cause severe weakness in some patients with myasthenia gravis when prescribed with steroids .

Corticosteroids can increase blood glucose, so close monitoring of blood sugar and higher doses of diabetes medications may be needed.(13)

Disadvantage

Short-term corticosteroid use is associated with generally mild side effects, including cutaneous effects, electrolyte abnormalities, hypertension, hyperglycemia, pancreatitis, hematologic, immunologic, and neuropsychologic effects, although occasionally, clinically significant side effects may occur. Long-term corticosteroid use may be associated with more serious sequale, including osteoporosis, aseptic joint necrosis, adrenal insufficiency, gastrointestinal, hepatic, and ophthalmologic effects, hyperlipidemia, growth suppression, and possible congenital malformations(14)

5-Summary:

from this presentation we noted the adverse effect of these herbal medicine if give to patient without any information or sell in the market by non health care provider it may cause dangerous to the patient take it .so must educate the community to ensure the correct use

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