Evaluation of the Laxative Effects of Methanolic Extract of *Spilanthes acmella*

This Thesis Paper Submitted in Partial Fulfillment of the Requirement for the Degree of Bachelor of Pharmacy, East West University

Submitted by

MUSHUMI DAS

ID # 2009-1-70-039

Department of Pharmacy

Research Invigilator

SHAMSUN NAHAR KHAN

Ph. D, Postdoc, Harvard University

Associate Professor

Department of Pharmacy

Submission Date: 19 January, 2014

EAST WEST UNIVERSITY

This Research Paper Is Dedicated

To

My Beloved Parents

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation, entitled "**Evaluation of the Laxative Effects of Methanolic Extract of *Spilanthes acmella***" is an authentic and genuine research work carried out by me under the guidance of Dr. Shamsun Nahar Khan, Associate Professor, Department of Pharmacy, East West University, Dhaka.

Mushumi Das

ID no. 2009-1-70-039

Department of Pharmacy

East West University, Dhaka.

ENDORSEMENT BY HEAD OF THE DEPARTMENT

This is to certify that the dissertation entitled **“Evaluation of the Laxative Effects of Methanolic Extract of *Spilanthes acmella*"** is a genuine research work carried out by Mushumi Das, under the supervision of Shamsun Nahar Khan (Ph. D, Postdoc, University of Harvard, Associate Professor, Department of Pharmacy, East West University, Dhaka). I further certify that no part of the thesis has been submitted for any other degree and all the resources of the information in thus connection are duly acknowledged.

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Prof. Dr. Chowdhury Faiz Hossain

Chairperson

Department of Pharmacy

East West University

CERTIFICATE

This is to certify that, the thesis on **"Evaluation of the Laxative Effects of Methanolic Extract of *Spilanthes acmella*"** submitted to Department of Pharmacy, East West University, Aftabnagar, Dhaka, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (B. Pharm), was carried out by Mushumi das (ID # 2009-1-70-039) under our guidance and supervision and that no part of the thesis has been submitted for any other degree. We further certify that all the sources of information of in this connection are duly acknowledged.

_____ _____

Dr. Shamsun Nahar Khan
Research Supervisor
Associate Professor
Department of Pharmacy
East West University
Aftabnagar, Dhaka

Prof. Dr. Chowdhury Faiz Hossain
Chairperson
Department of Pharmacy
East West University
Aftabnagar, Dhaka

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STATEMENT OF PURPOSE

The research was carried out in order to characterize the pharmacological profile of the methanolic extract of ***Spilanthes acmella.***

The test was done on the following aspect:

Laxative Activity:

- Laxative activity test
- Charcoal meal induced Gastrointestinal Transit Time Test

ABSTRACT

The purpose of this study was to find medicinal use *of Spilanthes acmella* in indigestion and constipation. Laxatives are drugs taken to treat constipation. Laxatives work to increase the movement of feces along the colon. Constipation refers to bowel movements that are infrequent or hard to pass. Constipation is a common cause of painful defecation. Severe constipation can progress to bowel obstruction and become life-threatening. Because constipation is a symptom, not a disease, effective treatment of constipation may require first determining the cause. There are various useful plants plays important role to show antidiarrhoeal activity.

There are various useful plants plays important role to show laxative effects. In this study we are paying attention on the methanolic extract of *Spilanthes acmella* for its laxative effects on animal models. The laxative activity of methanol extract of *Spilanthes acmella* at dose 200, 400 mg/kg was assessed on experimental animal and all these two doses increase the total number feces of experimental animal. It suggests that the crude extract of *Spilanthes acmella* possess laxative activities. It would be help us to identify the pharmacological profile of the plant extract of *Spilanthes acmella.*

INTRODUCTION

1. INTRODUCTION

1.1 Phytomedicine in Global Health Care

Herbal medicines are now in great demand in the developing world for primary healthcare not because they are inexpensive but also for better cultural acceptability, better compatibility with the human body and minimal side effects. Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries for primary healthcare. However among the estimated 250,000-400,000 plants species, only 6% have been studied for biological activity and about 15% have been investigated phytochemically (Calixto et al., 2000).

Researchers isolate different chemical constituents from plants, which have been used to prepare modern medicines. In course of time their synthetic analogues have also been prepared. In this way, ancient uses of Cinchona bark to quinine and quinidine, *Rawolfia serpentina* to reserpine and rescinnamine, *Digitalis purpurea* to digitoxin and digoxin, Opium to morphine and codeine, Ergot to ergotamine and ergometrine, Senna to sennosides, *Catharanthus roseus* to vinblastine and vincristine. Now a day, hundreds of plant metabolites are being successfully used in the treatment of variety of diseases. A few striking examples of plant metabolites include taxol from *Taxus brevifolia,* vincristine and vinblastine from *Vinca roseus.* All of which are important anticancer agents being used clinically. In the current popular field of chemotherapy, cepharanthine, isolated from *Stephania cepharantha* and *Stephania sasaki* is being used as a prophylactic in the management of tuberculosis (Brevoort et al., 1997).

In brief, science might have made in the field of medicine over the years; plants still remain as the primary source of supply of many important drugs, used in modern medicine. Indeed, the potential of obtaining new drugs from plant sources is so great that thousands of substances of plant origin are being studied for activity against such formidable foes as heart diseases, cancer, and AIDS. In this way, modern medicine will continue to be enriched by the introduction of newer and more potent drugs from plant sources.

With the development in techniques and recent researches, it has been proved that certain non-nutritive chemicals in plants such as terpenoids and flavonoids which were earlier thought to be of no importance to human diet possess antioxidant properties (Arias et al., 1999).

1.2 Medicinal Plants and Secondary Metabolites

In addition to providing the animal kingdom its food, fuel and shelter, each of these plants has been synthesizing a large variety of chemical substances since their first day of existence on earth. The substances include, in addition to the basic metabolites, phenolic compounds, alkaloids, glycosides and a lot of other chemical substances referred to as secondary metabolites which are of no apparent importance to the plant's own life. But many of these compounds have prominent effects on the animal systems and some posses' significant therapeutic properties, which can be and have been utilized in the treatment and cure of human and other animal diseases since time immemorial. These secondary metabolites differ from plant to plant. Thus the plant kingdom provides a tremendous reservoir of various chemical substances with potential therapeutic properties. The plants, which produce and accumulate constituents having medicinal values, are generally designated as medicinal plants (Padmavath et al ., 2013).

"A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs." (Sofowora 1982, Medicinal Plant and Traditional Medicine in Africa). This definition of Medicinal Plant has been originated by WHO (World Health Organization).

However, ideally a definition of medicinal plants should include the following:

a) Plants or plant part used medicinally in galenical preparation (e.g. decoctions, infusions etc.
b) Plants used for extraction of pure substances either for direct medicinal use or for the synthesis of medicinal compounds (e.g. synthesis of sex hormones).
c) Food, spice and perfumery plants used medicinally.
d) Microscopic plants, e.g. fungi, actinomyces used for isolation of drugs, especially antibiotics.
e) Fiber plants, e.g. cotton, flax, jute used for the preparation of surgical dressings.

Medicinal plant is an alternative medicine that is still in use and is a popular choice for primary health care. However, if improperly used plants can also be toxic. The World Health

Organization has esti-mated that about 80 % of the population in developing countries are unable to afford drugs and rely on traditional medicines especially those that are plant-based such as India, Sri Lanka , Bangladesh, China and Japan including Thailand (Calixto et al., 2000).

The practice of botanical healing slowly disappeared from western countries with the introduction and advent of science and technology. However, the uses of traditional medicine dramatically increased in Europe and North America in the last 50 years. Herbal medicines have been utilized for many purposes, particularly in medical care as antiasthmatics (86.79 %), anti-rheumatics (62 %), diu-retics (60.22 %), antiinflammation (29.62 %), anticancer (9.75 %), antidiabe-tics (8.33 %), antimicrobials, antifungals, antioxidants, antiallergy, analgesics, anti-obesity and antihypertention. In dental care it has been employed as anticariogenic, analgesic, local anesthetic, wound healing agents, antiinflammation and recurrent aphthous stomatitis treatment . It has also been used for beauty care and as health food e.g. curcumin, ginger, lemon grass, garlic, holy basil, sweet basil, hairy basil. Recently, health foods, herbs as well as dietary supplements enriched with medicinal ingredients such as antioxidants and bioactive metabolites have drawn consid-erable attention worldwide, especially herbs that are used as food and traditional medicine. Our concern centers around medicinal plants bearing bioactive compounds, which are employed as therapeutics and health care. Therefore,Spilanthes acmella Murr. is a plant of great interest owing to its known reputation as an antitoothache plant and hold tremendous medicinal usages. This review focuses on the general background, therapeutic uses, bioactive compounds and large-scale production (Prachayasittikul et al., 2012).

1.3 History: How Medicinal Plants Came Into Human Knowledge

The study of diseases and their treatment exist since the beginning of human civilization. The fear of illness and death as well as the necessity of food and health protection has led man of all times and under all skies to resort to anything that nature can offer them. Plants were such a group of things to which the early man resorted to for preserving his health against diseases. The early man was distinct from his other animal neighbors because of his ability to use rational thought rather than relying on instinct as a basis of his action. Thus he deliberately selected specific plant materials for treatment of his ailments. This selection was not certainly

based on a prior knowledge of its constituents but on certain external factors like seasonal or astronomical, mystical or religious factors or signatures of nature etc., which accepted as influencing his life. Sometimes the healing power of some plants was undoubtedly discovered by accidents. This selection procedure was often a trial and error method, which at times became very dangerous even costing valuable lives. But once he knew the attributes of a plant, beneficial or harmful, he would not normally forget it and could recognize the same plant anytime he came across it. He would then use it as a beneficial one or avoid or discard it as being dangerous. In this way, early man acquired sufficient knowledge about the plants around him and exploited them effectively. This knowledge is transmitted by generation to generation [4], at first orally and latter written form as papyri, backed clay tablets, parchments, manuscripts, herbals and finally printed herbal pharmacopoeias and other works. As civilization progressed, the use of plants and other natural substances has also intensified and increased in volume due to discoveries by successive generations (Newman et al., 2002)

As far as records go, it appears that Babylonians (about 300 B.C) were aware of a large number of medicinal plants and their properties. Some of the plants, they used, are still in the same purpose. As evident from the papyrus Ebres (written in about 1500 B.C), the ancient Egyptians possessed a good knowledge of medicinal properties of hundreds of plants. Important plant drugs like Henbane (*Hyoscyamas* spp.), Mandrake (*Mandrogora officinarum*), Opium (latex of *Papaver somniferum* fruits), Pomegranate (*Punica granatum*), Castor oil (oil of *Ricinus communis* seeds), aloe (juice of *Aloe spp*.), Onion (*Allium cepa*) and many others were in common use in Egypt about 4500 years ago. The use of plants for curing various human ailments figured in ancient manuscripts such as the Bible, the Rig-Vedas, the Iliad, and the Odyssey and the history of Herodotus. The earliest mention of the medicinal use of plants in the Indian subcontinent is found in the Rig-Veda (4500-1600 B.C), which noted that the Indo-Aryans used the Soma plant (*Amania muscaria*, a narcotic and hallucinogenic mushroom) as a medicinal agent. The Vedas made many references to healing plant Sarpogondha (*Rawalfia serpentine*) while a comprehensive Indian herbal, the Charaka, Samhita cites more than 500 medicinal plants (Sawangjaroen et al., 2006)

From the writings of Sumerians (4000 B.C) who inhabited by the river Tigris and Euphrates, we came to know that their medicine included opium, licorice, thyme and mustard. Thousands

of years ago Mexican Indians used peyote cactus. Since the peypote was valued for ots hallucinogenic property and equally possibly for its active medicinal substances, which are still used to heal wounds and are now to have antibiotic property.

The earliest known Chinese Pharmacopoeia appeared around 1122 B.C described the use of chaulmoogra oil to treat leprosy. The material medica of the Greek physician Hippocrates (460-370 B.C) consists of some 300-400 medicinal plants, which included opium, mint, rosemary, sage and verbena. Aristotle, A Greek Philosopher (384-322 B.C) included an effort to catalogue the properties of the various medicinal herbs known at that time. The encyclopedic (1^{st} century A.D) de Materia Medica was the forerunner of all modern pharmacopoeia and an authoritative text on botanical medicine. This work featured about 600 medicinal plants [6]. In the middle ages the great Greek Pharmacist, physician Galen (131-200 A.D) used a large number of medicinal plants in or preparing his recipes which included for the first time in history, ingredients of both plant and animal origin. Arabian Muslim physicians like Al-Razi and Ibne sina (9^{th} to 12^{th} century AD) brought a revolution in the history of medicine by bringing new drugs of plant and mineral origin into general use.

Isolation of natural analgesic drug morphine from the latex of *Papaver somniferum* capsules (Opium) in 1804 probably the first most important example of natural drug, which plant have directly contributed to modern medicine. Isolation of other important plant-derived drug of modern medicine rapidly followed and many useful drugs have since been discovered and introduced into modern medicine. In addition to these natural drugs of modern medicine, plants have also contributed and are still contributing to the development of modern synthetic drug and medicines (Pasquale et al., 1984).

Medicinal plants constitute the major constituents of most indigenous medicines, and a large number of western medicinal preparations contain one or more ingredients of plant origin. Thus the quality and effectiveness of medicinal preparations depend solely in case of indigenous medicine and at least partly in case of many western medicines, on the genuineness and quality of the medicinal plants or products that are used in their preparation. So it is very important to ensure that, these medicinal plants or their products really possess the claimed properties and exert the desired therapeutic effects. But it is not an easy task, because there is hardly any

medicinal plant, which is used for a single therapeutic purpose, and almost all of them are credited with more than one medicinal property and therapeutic use. Again, not all those plants that are designated as medicinal do really possess the claimed therapeutic virtues and medicinal properties.

Moreover, most of these claims are based on old literature, folk sayings, occasional experiences and traditional uses, but not on any significant clinical or pharmacological studies and statistical data. Thus the claims of medicinal properties may not be true and scientifically valid in case of all the so-called medicinal plants. However, although it is true that not every stated virtue of the various medicinal plants is based on scientific study, yet we have no doubt that out of large number of medicinal plants used by traditional systems for centuries past and still in use, there are many that deserve the reputation they have earned as cures (Pasquale et al., 1984).

Efforts for development of new drugs from medicinal plants are still continuing all over the world and new drugs are discovered and developed every day. Since the chemical constituents of medicinal plants, particularly the secondary metabolites (alkaloids, glycosides, tannins, resins, volatile and fixed oils) have pronounced pharmacological actions on animal systems and organs; they are capable of mitigating sufferings, curing ailments and healing wounds, cuts and burns. Before man acquired sufficient knowledge of synthetic chemistry these chemical constituents of medicinal plants served as the major raw material for the preparation of drugs and medicines. Since only about 15% of known medicinal plants have lot more to offer to the field of medicine. Thus there is still ample of scope and possibility for developing new drugs and pharmaceutical raw materials from plants (Goldfrank, L., et al., 1982)

1.4 Medicinal Plants of Bangladesh

Being naturally gifted by a suitable tropical climate and fertile soil, Bangladesh possesses a rich flora of tropical plants. Around 5000 species of phanerogams and pteridophytes grow in its forests, jungles, wastelands and roadsides as indigenous, naturalised and cultivated plants. Out of them, more than a thousand have been claimed to posses medicinal poisonous properties, of which 546 have recently been enumerated with their medicinal properties and therapeutic uses. In addition to possessing various other

medicinal properties, 257 of these medicinal plants have been identified as efficacious remedies for diarrhoeal diseases and 47 for diabetes (Ghani et al., 2003).

Medicinal plants are an accessible, affordable and culturally appropriate source of primary health care system in Bangladesh. Marginalised, rural and indigenous people, who can not afford or access formal health care systems, are especially dependent on these culturally familiar, technically simple, financially affordable and generally effective traditional medicines. As such, there is widespread interest in promoting traditional health systems to meet primary health care needs. This is especially true in this country, as prices of modern medicines spiral and governments find it increasingly difficult to meet the cost of pharmaceutical-based health care.

However, it has been observed that many other medicinal plants growing in the country have not been identified taxonomically and that there are many of them, which have not been chemically examined and no attention has yet been paid to characterise them from the pharmacognostic viewpoint. Thus, it is expected that the number of medicinal plants growing or available in Bangladesh may be more than what has so far been enumerated. It has further been observed that the countless herbs found in Bangladesh should be used for promotion of health and for fighting many diseases. Thus medicinal plants of Bangladesh hold good promises as potential resources for drug development.

However, in order to develop these medicinal plants as drugs, attempts should be first made to certainly identify them and preclinical studies on them should be carried out to establish their claimed therapeutic properties (Ghani et al., 2003)

1.5 Phytochemical Constituents of Medicinal Plants

Plants have been serving the animal kingdom as its source of energy since the dawn of civilization. In addition plants provide a tremendous reservoir of various chemical substances with potential therapeutic properties. The chemical constituents, which are capable of influencing the physiological systems of the animal body by exerting some pharmacological actions, are designated as the active chemical constituents or simply active constituents. In short, it may be said that the chemical constituents present in the medicinal plants constitute the

most important aspect of all medicinal plants. The types of chemical constituents obtained from different medicinal plants are as follows:

i. Alkaloids and amides: Pyridine group, Tropane group, Isoquinoline group, Quinoline group, Indole group, Steroidal group, Imidazole group, Phenylethylamine group and Alkaloid amines.
ii. Antbiotic and Anti-inflammatory principles.
iii. Bitter and pungent principles
iv. Volatile oils and Fixed oils
v. Glycosides: Anthraquinone glycosides, Cardiac glycosides, Saponin glycosides, Thiocyanate glycosides and other glycosides.
vi. Gum-resins and mucilage
vii. Vitamins and Minerals

Table 1: Plants derived drugs widely used in modern medicines

Acetyldigoxin	Narcotine
Aescin	Ouabain
Ajamalicin	Papain
Ailantoin	Papaverine
Atropine	Physostigmine
Bromelain	Picrotoxin
Caffein	Pilocarpine
Codein	Protoverratrins A and B
Colchicine	Pseudoephedrine
Danthron	Quinine
Deserpidine	Quinidine
Digitoxin	Rescinnamine
Digoxin	Reserpine
l-Dopa	Scillaren A and B
Emetine	Spartin

Table 1: Plants derived drugs widely used in modern medicines (continue.)

Ephedrine	Strychnine
Hyoscyamine	Tetrahydrocannabinol
Leurocristine	Tubocurarine
Lobeline	Vincaleukoblastin
Morphine	Xanthotoxin

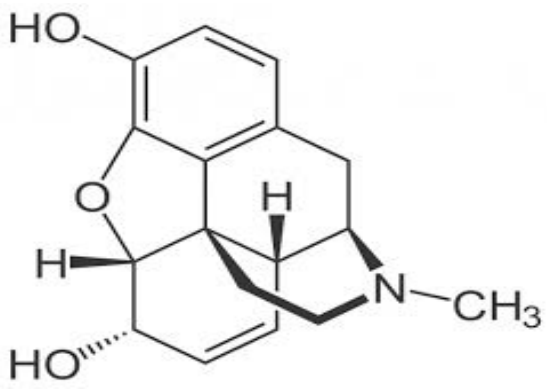


Fig: 1 Structure of Morphine

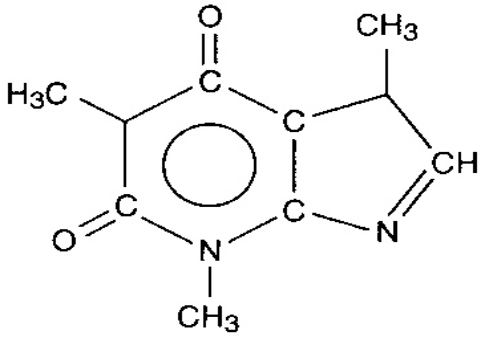


Fig: 2 Structure of Caffeine

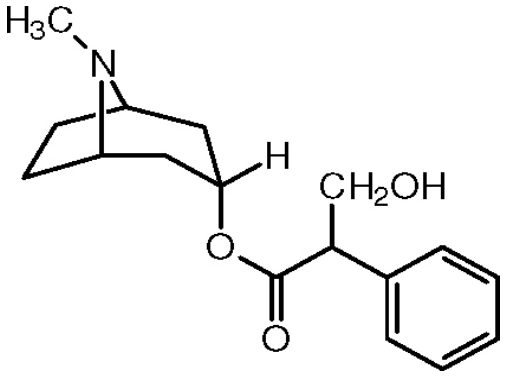


Fig: 3 Structure of Atropine

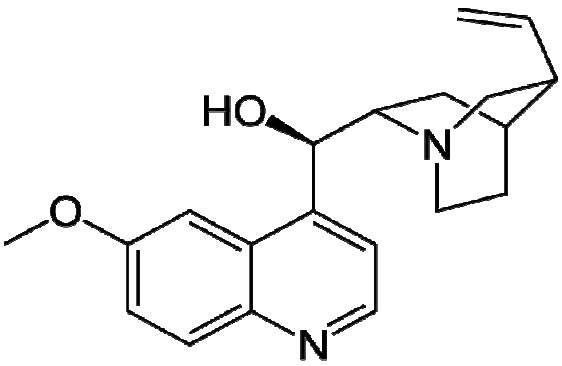
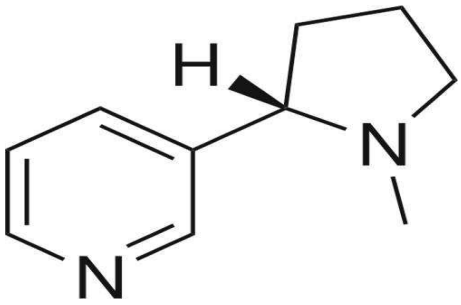


Fig: 4 Structure of Quinine



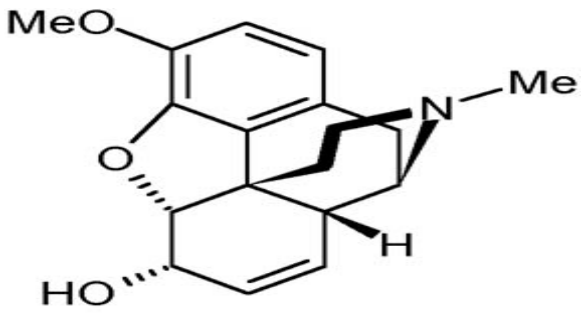


Fig: 5 Structure of Nicotine **Fig: 6 Structure of Codein**

1.6 The Plants: Model of Research and Drug Development

Plant kingdom is one of the major search areas for effective works of recent days. Traditional or folk medicines have been widely employed for centuries, and they remain one important source for the discovery of new bio-active compounds. Medicinal plants are the source of many important scientific drugs of contemporary world. Such as quinine from *Cinchona* bark, reserpine from *Rauwalfia* root, Digitoxin from *Digitalis* leaf, atropine from *Belladona* root and leaf, *Hyoscyamine* from Hyoscyamus and Datura leaves and roots, coniine from Coium fruit, morphine from Opium capsule are a few example of the innumerable modern scientific drugs that are prepared from the medicinal plants. Plants historically seem to have served as models in drug development for three reasons:

- ✓ First, in the industrialized countries, about 27% of all prescription drugs contain active principles that are still extracted from higher plants and this situation has persisted for at least the last 25 years. Further it is generally accepted that as many of the people in the developing countries rely on plants as sources of drugs. It is to say that, plants continue to provide useful drugs for man.
- ✓ Secondly, very often interesting biologically active substance derived from plants may have a poor pharmacological/toxicological profile, at least the purpose of using them as drug in human. Such compounds can often serve as templates for synthetic modification and structure-function studies with the anticipation that useful drugs for human will result.

- ✓ Thirdly, many highly active secondary plant material constituents have unequivocal value as pharmacologic tool that are often found to be useful in studying biological systems and disease proces (Ghisalberti et al., 1993)

1.7 Gastrointestinal (GI) Tract

The GI tract is a series of hollow organs joined in a long, twisting tube from the mouth to the anus. The body digests food using the movement of muscles in the GI tract, along with the release of hormones and enzymes. The GI tract consist of the mouth, esophagus, stomach, small intestine, large intestine which includes the appendix, cecum, colon, and rectum and anus. The last part of the GI tract called the lower GI tract consists of the large intestine and anus. The large intestine absorbs water and any remaining nutrients from partially digested food passed from the small intestine. The large intestine then changes waste from liquid to a solid matter called stool. Stool passes from the colon to the rectum. The rectum is located between the last parts of the colon called the sigmoid colon and the anus. The rectum stores stool prior to a bowel movement. During a bowel movement, stool moves from the rectum to the anus, the opening through which stool leaves the body.

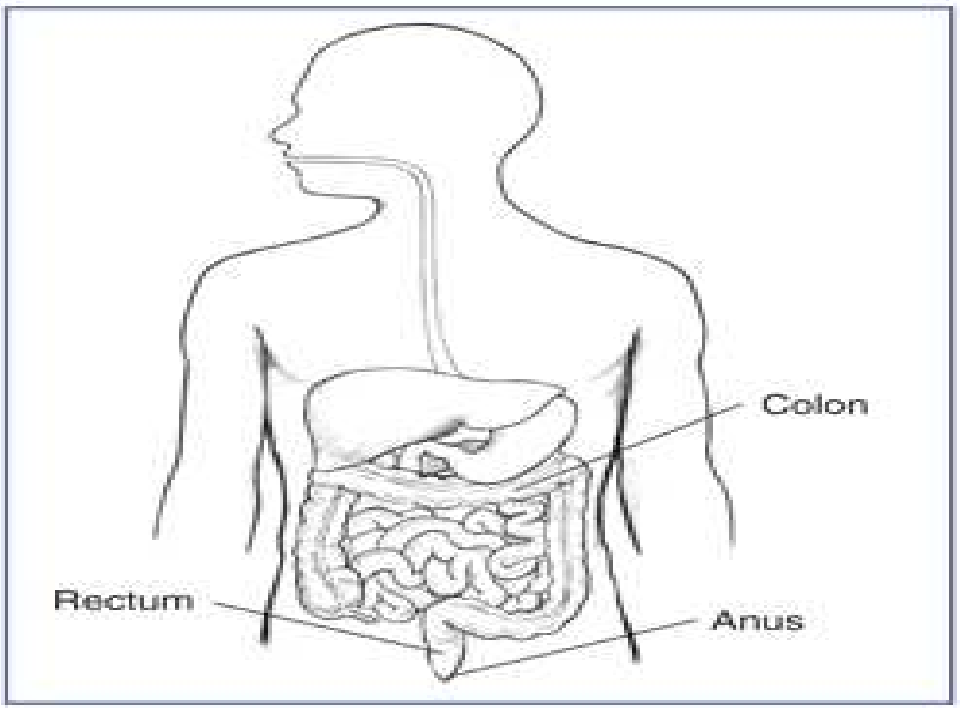


Fig: 7 Pictorial View of the lower GI tract

1.8 Constipation

Constipation is a condition in which a person has fewer than three bowel movements a week or has bowel movements with stools that are hard, dry, and small, making them painful or difficult to pass. People may feel bloated or have pain in their abdomen. Some people think they are constipated if they do not have a bowel movement every day. Most people get constipated at some point in their lives. Constipation can be acute, which means sudden and lasting a short time, or chronic, which means lasting a long time, even years. Most constipation is acute and not dangerous. Understanding the causes, prevention, and treatment of constipation can help many people take steps to find relief.

Constipation is one of the most common gastrointestinal problems. People of any age, race, or gender can get constipated. Constipation most commonly occur in women, adults ages 65 and older, and people in lower socioeconomic classes. Constipation is also a common problem during pregnancy, following childbirth or surgery, or after taking medications to relieve pain from things such as a broken bone, tooth extraction, or back pain (Higgins et al., 2004)

1.9 Causes of Constipation

Constipation is caused by stool spending too much time in the colon. The colon absorbs too much water from the stool, making it hard and dry. Hard, dry stool is more difficult for the muscles of the rectum to push out of the body.

Common factors or disorders that lead to constipation are

- diets low in fiber
- lack of physical activity
- medications
- life changes or daily routine changes
- ignoring the urge to have a bowel movement
- neurological and metabolic disorders
- GI tract problems
- functional GI disorders

1.9.1 Diets Low in Fiber

The most common cause of constipation is a diet with too little fiber. Fiber is a substance in foods that comes from plants. Fiber helps stool stay soft so it moves smoothly through the colon.

1.9.2 Lack of Physical Activity

A lack of physical activity can lead to constipation. For example, constipation often occurs after an accident or during an illness when a person must stay in bed and cannot exercise. Lack of physical activity is thought to be one of the reasons constipation is common in older adults.

1.9.3 Medications

Medications that can cause constipation include

- pain medications, especially narcotics
- antacids that contain aluminum and calcium
- calcium channel blockers, which are used to treat high blood pressure and heart disease
- medications that treat Parkinson's disease—a disorder that affects nerve cells in a part of the brain that controls muscle movement—because these medications also affect the nerves in the colon wall
- antispasmodics—medications that prevent sudden muscle contractions
- some antidepressants
- iron supplements
- diuretics—medications that help the kidneys remove fluid from the blood

Constipation can also be caused by overuse of over-the-counter laxatives. A laxative is medication that loosens stool and increases bowel movements. Although people may feel relief when they use laxatives, they usually must increase the dose over time because the body grows reliant on laxatives to have a bowel movement. Overuse of laxatives can decrease the colon's natural ability to contract and make constipation worse. Continued overuse of laxatives can damage nerves, muscles, and tissues in the large intestine.

1.9.4 Life Changes or Daily Routine Changes

During pregnancy, women may be constipated because of hormonal changes or because the uterus compresses the intestine. Aging can affect bowel regularity, because of a gradual loss of nerves stimulating the muscles in the colon, which results in less intestinal activity. People can also become constipated while traveling, because their normal diet and daily routine are disrupted.

1.9.5 Ignoring the Urge to Have a Bowel Movement

People who ignore the urge to have a bowel movement may eventually stop feeling the need to have one, which can lead to constipation. Some people delay having a bowel movement because they do not want to use toilets outside their home, particularly public restrooms, or they feel they are too busy.

1.9.6 Neurological and Metabolic Disorders

Certain neurological and metabolic disorders can cause food to pass through the digestive system too slowly, leading to constipation. Neurological disorders, such as spinal cord injury and Parkinsonism, affect the brain and spine. Parkinsonism is any condition that leads to the types of movement changes seen in Parkinson's disease. Metabolic disorders, such as diabetes and hypothyroidism, disrupt the process the body uses to get energy from food. Hypothyroidism is a disorder that causes the body to produce too little thyroid hormone, which can cause many of the body's functions to slow down.

1.9.7 GI Tract Problems

Some problems in the GI tract can compress or narrow the colon and rectum, causing constipation.

These problems include

- adhesions—bands of tissue that can connect the loops of the intestines to each other, which may block food or stool from moving through the GI tract

- diverticulosis—a condition that occurs when small pouches, or sacs, form and push outward through weak spots in the colon wall; the pouches are called diverticula
- colon polyps—growths on the surface of the colon that can be raised or flat
- tumors—abnormal masses of tissue that result when cells divide more than they should or do not die when they should
- celiac disease—an immune reaction to gluten, a protein found in wheat, rye, and barley, that causes damage to the lining of the small intestine and prevents absorption of nutrients

1.9.8 Functional GI Disorders

Functional GI disorders are problems caused by changes in the GI tract works. Functional constipation often results from problems with muscle activity in the colon or anus that delay stool movement.

Functional constipation is diagnosed in people who have had symptoms for at least 6 months and meet the following criteria for the last 3 months before diagnosis.

- Two or more of the following symptoms:
    - straining to have a bowel movement at least 25 percent of the time
    - having lumpy or hard stools at least 25 percent of the time
    - feeling as though stool is still in the rectum after a bowel movement at least 25 percent of the time
    - feeling as though something is blocking stool from passing at least 25 percent of the time
    - using their fingers to help with stool passage at least 25 percent of the time
    - having fewer than three bowel movements per week
- Rarely passing loose stools without the use of laxatives
- Not having irritable bowel syndrome (IBS)

BS is a functional GI disorder with symptoms that include abdominal pain or discomfort, often reported as cramping, along with diarrhea, constipation, or both (Longstreth et al., 2006)

1.10 Diagnosis of Constipation

To diagnose the cause of constipation, the health care provider will take a medical history, perform a physical exam, and order specific tests. The tests ordered depend on how long the person has been constipated; how severe the constipation is; the person's age; and whether the person has had blood in stools, recent changes in bowel habits, or weight loss. Most people with constipation do not need extensive testing and can be treated with changes in diet and exercise.

1.10.1 Medical History

The health care provider may ask questions about the person's constipation, including how long symptoms have been present, frequency of bowel movements, consistency of stools, and presence of blood in the stool. The health care provider may ask questions about the person's eating habits, medication, and level of physical activity. A record of this information can be prepared before the visit to help the health care provider make a diagnosis.

1.10.2 Physical Exam

A physical exam should include a rectal exam with a gloved, lubricated finger to evaluate the tone of the muscle that closes off the anus called the anal sphincter and to detect tenderness, obstruction, or blood. The health care provider may perform a test for blood in the stool by placing a small sample of the person's stool on a paper card and adding a drop or two of testing solution. A color change is a sign of blood in the stool.

1.10.3 Diagnostic Tests

Additional testing is usually reserved for older adults and people with severe symptoms, sudden changes in the number and consistency of bowel movements, or blood in the stool. Additional tests that may be used to evaluate constipation include

- blood test
- lower GI series
- flexible sigmoidoscopy or colonoscopy

- colorectal transit studies
- anorectal function tests
- defecography

Blood test: A blood test involves drawing blood at a health care provider's office or a commercial facility and sending it to a lab for analysis. The blood test can show if there may be an underlying disease or condition causing constipation. For example, low levels of thyroid hormone may indicate hypothyroidism.

Lower Gastrointestinal series: A lower gastrointestinal series is an x-ray exam that is used to look at the large intestine. The test is performed at a hospital or an outpatient center by a radiologist. The health care provider may give the person written bowel prep instructions to follow at home. The person may be asked to follow a clear liquid diet for 1 to 3 days before the procedure. A laxative or an enema may be used before the test. An enema involves flushing water or laxative into the anus using a special squirt bottle. The medications cause diarrhea, so the person should stay close to a bathroom. For the test, the person will lie on a table while the radiologist inserts a flexible tube into the person's anus. The large intestine is filled with barium, a chalky liquid, making signs of problems that may be causing constipation show up more clearly on x rays.

Flexible sigmoidoscopy or colonoscopy: The tests are similar, but a colonoscopy is used to view the rectum and entire colon, while a flexible sigmoidoscopy is used to view just the rectum and lower colon. These tests are performed at a hospital or an outpatient center by a gastroenterologist. For both tests, a health care provider will give written bowel prep instructions to follow at home. The person may be asked to follow a clear liquid diet for 1 to 3 days before either test. The night before both tests, the person may need to take a laxative. One or more enemas may also be required the night before and about 2 hours before both tests. In most cases, light anesthesia, and possibly pain medication, is used during a flexible sigmoidoscopy or colonoscopy. For either test, the person will lie on a table while the gastroenterologist inserts a flexible tube into the anus. A small camera on the tube sends a video image of the intestinal lining to a computer screen. The test can show signs of problems in the lower gastrointestinal tract.

The gastroenterologist may also perform a biopsy, a procedure that involves taking a small piece of intestinal lining tissue for examination with a microscope. The person will not feel the biopsy. A pathologist examines the tissue in a lab.

Colorectal transit studies: These tests show how well food moves through the colon.

- **Radiopaque markers.** With this technique, the person swallows capsules containing small markers that are visible on an x ray. The markers move through the GI tract just as food and waste do and are passed naturally with stool. During the course of this test, the person eats a high-fiber diet to help stool move through the gastrointestinal tract. Three to 7 days after the person swallows the capsules, abdominal x rays, taken several times, monitor the movement of the markers through the colon. An x-ray technician takes the x rays in a hospital radiology department or health care provider's office, and a radiologist interprets the x rays.
- **Scintigraphy.** This type of nuclear medicine study relies on the detection of small amounts of radiation after a person eats a meal containing radioactive chemicals. The dose of the radioactive chemicals is small; therefore, scintigraphy is not likely to cause damage to cells. Special external cameras and computers are used to create images of the radioactive chemicals as they move through the intestine. To prepare for the test, the person may need to stop taking some medications and should not eat any food after midnight the night before the test.

Anorectal function tests: These tests diagnose constipation caused by anorectal dysfunction, which refers to problems with the anus and rectum.

- **Anal manometry** uses pressure sensors and a balloon. Anal manometry also checks the tightness of the anal sphincter muscles around the anus. For this test, a thin tube with a balloon on its tip and pressure sensors below the balloon is inserted into the anus until the balloon is in the rectum and pressure sensors are inside the anus. The tube is slowly pulled back through the sphincter muscle to measure muscle tone and contractions.
- **Balloon expulsion tests** consist of filling a balloon with varying amounts of water after it has been inserted into the rectum. The person is given a stopwatch and instructed to

go to the restroom and measure the amount of time it takes to expel the balloon. If the person cannot expel a balloon filled with less than 150 milliliters of water or it takes longer than 1 minute to expel the balloon, the person may have a decrease in function for evacuation of stool.

Defecography: This x ray of the anorectal area shows how well the person can hold and evacuate stool. The test also identifies structural changes in the rectum and anus, such as rectocele and rectal prolapse. Rectocele is a condition in which the rectum protrudes through the vagina, and rectal prolapse is a condition in which the rectum drops down through the anus. To prepare for the test, the person uses two enemas and does not eat anything 2 hours prior to the test. During the test, the health care provider fills the rectum with a soft paste that shows up on x rays and is the same consistency as stool. The person sits on a toilet inside an x-ray machine. The person is first asked to pull in and squeeze the sphincter muscles to prevent leakage. Then the person is asked to strain to have a bowel movement. The radiologist studies the x rays for anorectal problems that occurred as the paste was expelled (Longstreth et al., 2006)

1.11 Treatment of Constipation

Treatment for constipation depends on the cause, severity, and duration of the constipation and may include one or more of the following:

- changes in eating, diet, and nutrition
- exercise and lifestyle changes
- medication
- surgery
- biofeedback

First-line treatments for constipation include changes in eating, diet, and nutrition; exercise and lifestyle changes; and laxatives. People who do not respond to these first-line treatments should talk with their health care provider about other treatments.

1.11.1 Eating, Diet, and Nutrition

The Academy of Nutrition and Dietetics recommends consuming 20 to 35 grams of fiber a day for adults. Americans consume only 15 grams a day on average. A list of high-fiber foods is shown below.

Table: 2 Examples of Foods That Have Fiber

Beans, cereals, and breads	**Fiber**
½ cup of beans (navy, pinto, kidney, etc.), cooked	6.2–9.6 grams
½ cup of shredded wheat, ready-to-eat cereal	2.7–3.8 grams
⅓ cup of 100% bran, ready-to-eat cereal	9.1 grams
1 whole-wheat English muffin	4.4 grams
Fruits	
1 small apple, with skin	3.6 grams
½ cup of raspberries	4.0 grams
½ cup of stewed prunes	3.8 grams
Vegetables	
½ cup of winter squash, cooked	2.9 grams
1 small potato, baked, with skin	3.0 grams
½ cup of mixed vegetables, cooked	4.0 grams

Drinking water and other liquids, such as fruit and vegetable juices and clear soups, may make fiber in the diet more effective in normalizing bowel function and maintaining regularity.

1.11.2 Exercise and Lifestyle Changes

Engaging in daily exercise can help people with constipation. Another strategy is to try to have a bowel movement at the same time each day. The best time is 15 to 45 minutes after breakfast because eating helps stimulate the colon (Everhart et al., 2008)

1.11.3 Medication

Classification

I. Luminally active agents

i) Bulk forming - Dietary fibre, psyllium, ispaghula, methyl cellulose

ii) Stoolsoftener - Dioctyl sodiumsulphosuccinate (Docusates, Doss)

iii) Lubricants - Liquid paraffin

iv) Osmotic -Magnesium sulphate, magnesium hydroxide, sodium sulphate, Sodium potassiumtartarate,lactulose, sorbitol, mannitol,

II. Stimulant (Contact) Purgatives

i) Diphenylmethanes - Phenolphthalein, bisacodyl

ii) Anthraquinones - Senna, cascara, rhubarb, aloes, danthron

iii) Fixed oil - Castor oil

III.Prokinetic agents - 5 HT4agonists e.g. Tegaserod

- Opioid receptor antagonists

Bulk Forming: These agents increase the stool mass. Fermentation produces short chain fatty acids which have got prokinetic action. These are contra-indicated in obstructive cases and in patients with megacolon and mega rectum. They cause bloating and abdominal pain. They can increase the Ca2+ load if agents like calcium polycarbophils are used. Sugar containing agents can cause intolerance in diabetics.

Softeners: These are anionic surfactants which lower the surface tension of stool and allow mixing of aqueous and fatty substances thereby softening the stools and allowing easier defaecation. They also increase the cyclic AMP and water secretion.

Osmotic agents: Osmotically mediated water retention can induce peristalsis and prompt catharsis. They may increase nitricoxide synthase and platelet activating factor. There is also production of inflammatory ionic mediator e.g. Mg2+. They are avoided in small children, and in patients with poor renal function. They can cause heart block, neuromuscular block, CNS depression and fluid and electrolyte imbalance. Lactulose contains non-absorbable sugars, which draws water into the lumen. Its fermentation can lead to formation of lactic and acetic acids which are osmotic laxatives and stimulate motility. 15 to 20 ml of 70% solution is used at night and effect is seen after 24 to 48 hours. Lactulose finds important place in treatment of hepatic encephalopathy as it reduces blood ammonia concentrations. 2-3 soft stool evacuation/day at pH of 5.5 is required for its beneficial effects. It is also used in constipation caused by opioids, vincristine, in elderly and debilitated patients. Lactulose can cause flatulence, cramps, diarrhea etc. PEGs are nowadays used in small doses to treat difficult cases of constipation. They are highly osmotically active.

Lubricants: They are viscous liquids which are pharmacologically inert and help in easy passage of stool by coating them. Because of their various side effects and irritant nature they are occasionally used nowadays especially in post operative patients.

Stimulants: These agents have direct effect on enterocytes, neurons, and muscles. They induce low grade inflammation and cause accumulation of water and electrolytes and stimulate intestinal motility by release of mediators like prostaglandins, cAMP, nitric oxide (NO), cGMP and inhibition of Na+K+ATPase. Larger purgation can cause fluid and electrolyte imbalance and hypokalemia. They are routinely used in colonic atony.

They can reflexly stimulate the gravid uterus and are contraindicated in pregnancy and obstructive disorders.

Bisacodyl is given in the dose 10-15 mg in adults and 5-10 mg in children (6-12 years). Enteric coated preparations are used once daily at bed time. Evacuation occurs in 6-8 hours i.e. in the morning. Rectal preparations cause catharsis in 30-60 min. Phenolphthalein is withdrawn from the market because of carcinogenicity seen in mouse.

Castor oil is one of the oldest remedies obtained from seeds of *Ricinus communis*. It has recently lost its usage because of irritant nature and due to side effects of stronger purgation. It contains triglyceride of ricinoleic acid which is a long chain fatty acid and polar in nature. It is hydrolysed to ricinoleic acid and glycerol by pancreatic lipase. Ricinoleic acid is absorbed poorly. It irritates the mucosa and stimulates small intestinal contractions. Most importantly they decrease intestinal absorption of water and electrolytes. Adult dose- 15-25 ml of castor oil may be taken in the morning. It causes purgation within 2-3 hours.

Newer and more potent prokinetic agents like tegaserod may be useful for the treatment of chronic constipation. Prostaglandin analogues like misoprostol and RU-0211 (under trial) stimulate colonic contraction. Other agent like colchicine has also been shown to be effective in constipation. A novel biologic agent, neurotrophin-3 has shown to be effective in improving the stool consistency and frequency by some unknown mechanism.

Purgative Abuse

It is a psychological problem and drugs used or misused to get full evacuation. The dangers associated can be flaring ofintestinal pathology, rupture of inflamed appendix, fluid electrolyte imbalance, steatorrhea and malabsorption syndromes, protein losing enteropathy and spastic colitis.

1.11.4 Surgery

Surgery may be needed to correct an anorectal blockage caused by rectal prolapse. Surgical removal of the colon may be an option for people, whose colon muscles do not work properly, causing severe symptoms that do not respond to treatment.

1.11.5 Biofeedback

People with chronic constipation caused by problems with the anorectal muscles can use biofeedback to retrain the muscles. Biofeedback uses special sensors to measure bodily functions. The measurements are displayed on a video screen as line graphs and sounds indicate when the person is using the correct muscles (Slavin et al., 2008)

1.12 Complications of Constipation

1.12.1 Hemorrhoids are swollen and inflamed veins around the anus or in the lower rectum that can be caused by straining to have a bowel movement. People with hemorrhoids may have rectal bleeding that appears bright red on the surface of stool, on toilet paper, or in the toilet after a bowel movement. Treatment for hemorrhoids may include making dietary changes to prevent constipation, taking warm tub baths, and applying special cream to the affected area or using suppositories before bedtime.

1.12.2 Anal fissures are small tears in the anus that may cause itching, pain, or bleeding. Treatment for anal fissures may include making dietary changes to prevent constipation, applying cream to numb the area or relax the muscles, using stool softeners, or taking warm tub baths.

1.12.3 Rectal prolapse can be caused by straining during bowel movements. The condition may lead to mucus leaking from the anus. Eliminating the cause of the prolapse, such as straining or coughing, is usually the only treatment needed. Severe or chronic prolapse requires surgery to strengthen and tighten the anal sphincter muscle or to repair the prolapsed lining.

1.12.4 Fecal impaction occurs when hard stool packs the intestine and rectum so tightly that the normal pushing action of the colon is not enough to expel the stool. This condition occurs most often in children and older adults. An impaction can be softened with mineral oil taken by mouth or through an enema. After softening the impaction, the health care provider may break up and remove part of the hardened stool by inserting one or two fingers into the anus (Goodman et al., 2011).

1.13 *Spilanthes acmella*

1.13.1 Description

Spilanthes (Compositae or Asteraceae) is a genus comprising of over 60 species that are widely distributed in tropical and subtropical regions of the world, such as Africa, America, Borneo, India, Sri Lanka and Asia (Sahu et al., 2011; Tiwari et al., 2011). S. acmellais native to Brazil and is cultivated throughout the year as ornamental or medicinal plant. It is an annual or short-lived herb that is 40-60 centimeters tall. It is grown in damp area (Tiwari et al., 2011; Wongsawatkul etal., 2008) and has low rate of germination or poor vegetative propagation (Tiwari et al., 2011). Its flowers and leaves have pungent taste and when touched it is accompanied by tingling sensation and numbness (Wongsawatkul et al., 2008).The plant species has been used commonly as a folk remedy, e.g. for toothache, rheumatic and fever (Wongsawatkul et al., 2008), as fresh vegetable (Tiwari et al., 2011) as well as spice for Japanese appetizer (Leng et al., 2011)

Fig: 8 Pictorial View of *Spilanthes acmella*

The genus contains a wide array of compounds with a diverse range of bioactivity. One such compound, scopoletin (6-methoxy-7-hydroxycoumarin), has attracted the most attention because of its use in cardiovascular disease, and antitumor and antithyroid treatment.. In addition to this, scopoletin also possesses antioxidant, antimicrobial, anti-inflammatory, antipyretic and hepatoprotective properties. It is one of the major phytoalexins reported in tobacco plants. Its production is mainly seen upon pathogenic infection and is considered as an important defense mechanism against bacteria and fungi (Smith 1996). Recently, scopoletin has also been detected in Spilanthes flower buds, but quantitative analysis has not so far been done. S. acmellais conventionally propagated through seeds which lose their viability within a short period of time. Dependence on season and slow germination rates are some of the other major limiting factors in conventional propagation. Moreover, propagation by seeds is also undesirable because of the highly heterozygous nature of the plant due to protandry, which prevents self-pollination. Many small, bright-colored flowers are aggregated into a capitulum (flower head) that make them attractive to insects, thus paving the way for entomophily. The genetic variation due to insect pollination may result into high heterogeneity in quality and quantity of the chemical makeup of the plant (Prachayasittikul et al., 2013)

1.13.2 Arrangement of *Spilanthes acmelle* from Domain to Botanical name

Kingdom Plantae – Plants

Subkingdom Tracheobionta – Vascular plants

Superdivision Spermatophyta – Seed plants

Division Magnoliophyta – Flowering plants

Class Magnoliopsida – Dicotyledons

Subclass – Asteridae

Order – Asterales

Family Asteraceae – Aster family

Genus Spilanthes Jacq. – spilanthes

1.13.3 Bioactive metabolites

Spilanthes acmella constitutes a diverse group of compounds. Major isolates were lipophilic alkylamides or alkamides bearing different number of unsaturated hydrocarbons (alkenes and alkynes), such as spilanthol and amide derivatives. In general, when alkamides are chewed, a pungent taste is released and causes itchand salivation. Alkamides are structurally related to animal endocannabinoids and is highly active in the central nervous system. Particularly, anandamide (N-arachidonoylethanolamine, 9) is an endogenous cannabinoid cerebral neurotransmitter. Spilanthol was first isolated in 1945 from the flower head ethanol (EtOH) extract of S. acmella. In early 1903, it was first obtained from the different plant species,S. acmellaL.var. oleraceaClarke .The synthesis of spilanthol was reported in multistep and afforded low overall yields. The spilanthol is commercially available in form of alcoholic (65 % EtOH) extract or A. Vogel Spilanthes. In addition, phytosterols (e.g. β-sitosterol, stigmasterol, α- and β-amyrins), essential oils (e.g. limonene and β-caryophyllene), sesquiterpenes, α- and β-bisabolenes and cadinenes, flavonoid glucoside and a mixture of long chain hydrocarbons (C22-C35) . In recent years, other bioactive metabolites 10 – 15 have been isolated from the aerial part of S. acmella, namely vanillic acid, trans-ferulic acid, trans-isoferulic acid, scopolelin , 3-acetylaleuritolic acid and β-sitostenone (Prachayasittikul et al., 2009).

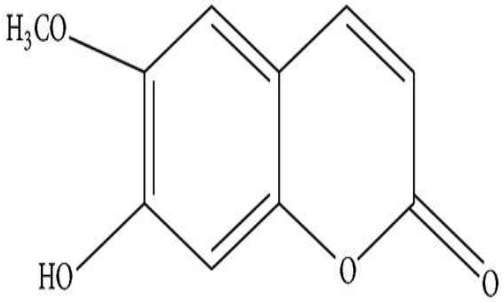


Fig: 9 Chemical structure of scopoletin

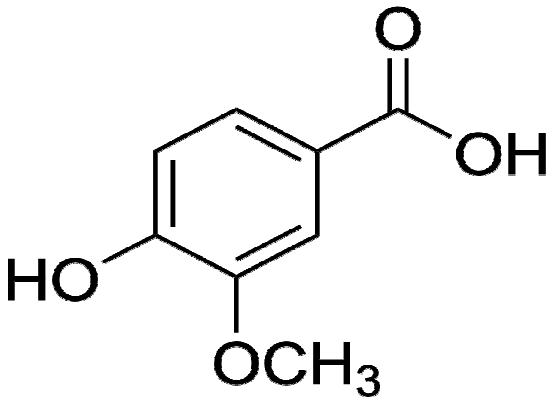


Fig: 10 Chemical structure of Vanillic acid

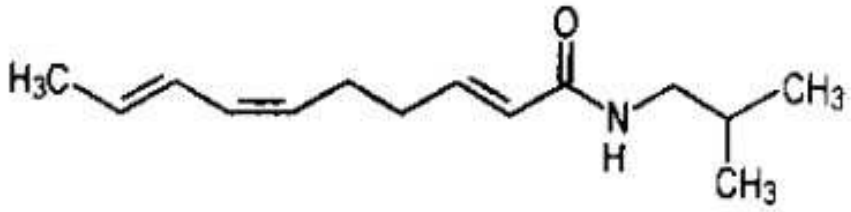


Fig: 11 Chemical structure of spilanthol

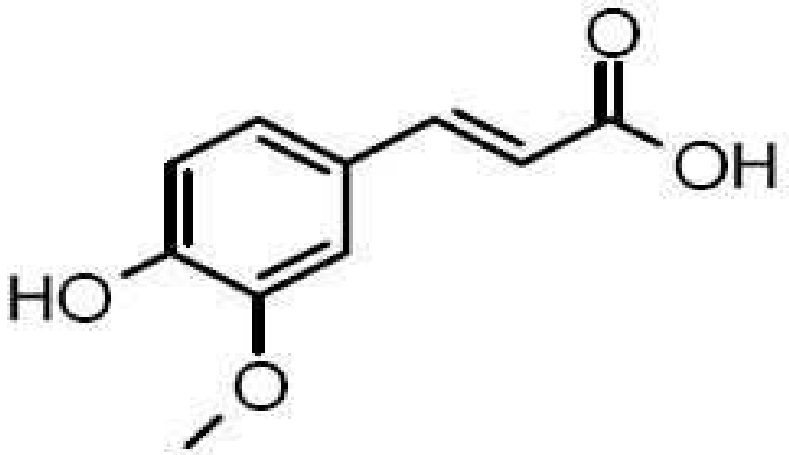


Fig: 12 Chemical structure of trans-isoferulic acid

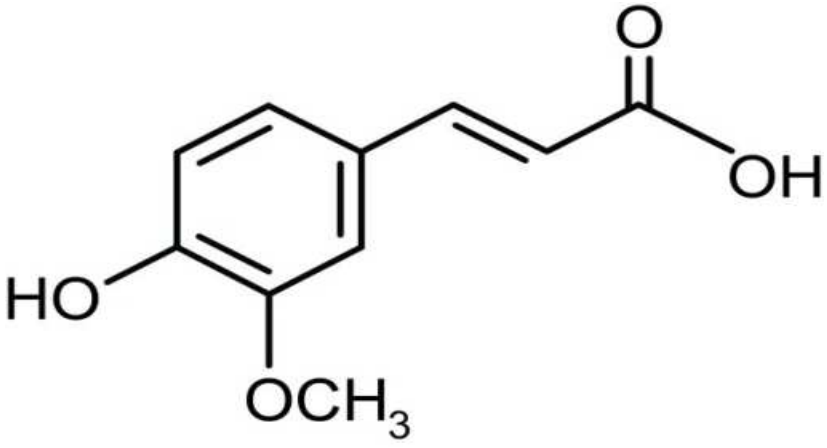


Fig: 13 Chemical structure of trans-ferulic acid

1.13.4 Traditional uses

The whole plants (e.g. flowers, leaves, roots, stems and aerial parts) of Spilanthes have been used in health care and food Particularly, *S. acmellaor S. oleracea* (paracress or eyeball plant), is a well-known antitoothache plant and has been used as traditional medicine for many purposes (Prachayasittikul et al., 2009).

Table 3: Traditional uses and applications of *S. acmella*

Health care	Treatment	Plant extract
Medical	Rheumatism, fever , Diuretics, Flu, cough, rabies diseases, Tuberculosis, antimalarials, Antibacterials	leaves, flowers
	Antifungals, skin diseases, Immunomodulatory, Antiscorbutic, Local anesthetics, Digestive	leaves,
	Obesity control (lipase inhibitor)	flowers
	Snake bite	whole plant,
Dental	Toothache	leaves, flowers,
	Toothpaste	leaves
	Periodontal disease	flower heads, roots
	Recurrent aphthous stomatitis	leaves
Beauty care cosmetics	Fast acting muscle relaxant Anti wrinkle	whole plant

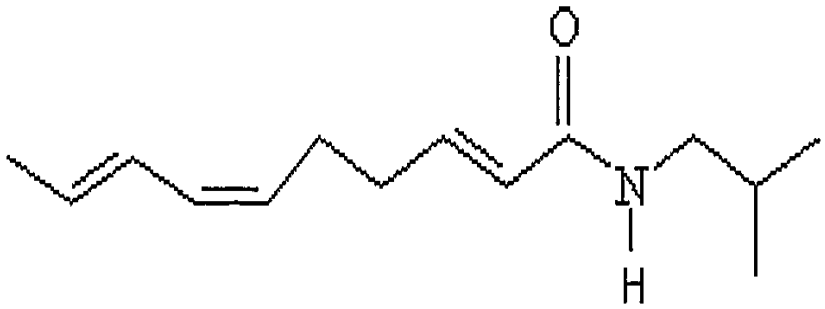
The present study was aimed to evaluate the Laxative activity test of methanolic extract of *Spilanthes acmella* on swiss albino mice.

2. LITERATURE REVIEW

Although plants of asteraceae have potential to work against various pathogenic conditions of humans, there are few studies to conduct their activity. Antimalarial, antiviral,analgesic, antioxidant, insecticidial activity of different plants of this family is already reported. Isolation and purification of compound from the plant of this family is not conducted in large extent. Among them study related to *Spilanthes acmella* is very few. But for other species of this family has proved biological activity like *Spilanthes calva*, has antimulagenic activity, *Spilanthes mauritiana* has antimalarial, larvicidal activity, and *Spilanthes mauritiana* antimicrobial activity.

2.1 Bioactive metabolites of *Spilenthes acmella*

Extensive phytochemical investigations of *Spilanthes acmella* had been reported a diverse group of compounds. Major isolates were lipophilic alkylamides or alkamides bearing different number of unsaturated hydrocarbons such as spilanthol (**1**) or affinin *(2E, 6Z, 8E)-N*-isobutyl-2, 6, 8- decatrienamide (Gokhale and Bhide, 1945; Ramsewak et al., 1999) and amide derivatives **2**–**6** (Figure 1). In general, when alkamides are chewed, a pungent taste is released and causes itch and salivation (Rios, 2012). Alkamides are structurally related to animal endocannabinoids and is highly active in the central nervous system. Particularly, anandamide (*N*-arachidonoyl - ethanolamine, **7**) is an endogenous cannabinoid cerebral neurotransmitter (Figure 1). In addition, phytosterols (e.g. *β*- sitosterol, stigmasterol, *α*- and *β*-amyrins), essential oils (e.g. limonene and *β*-caryophyllene), sesquiterpenes, *α*- and *β*-bisabolenes and cadinenes, flavonoid glucoside and a mixture of long chain hydrocarbons (C22-C35) were reported (Sahu et al., 2011; Tiwari et al., 2011). In recent years, other bioactive metabolites **8 – 13** (Figure 2) have been isolated from the aerial part of *Spilanthes acmella*, namely vanillic acid (**8**), *trans*-ferulic acid (**9**), *trans*-isoferulic acid (**10**), scopolelin (**11**), 3-acetylaleuritolic acid (**12**) and *β*-sitostenone (**13**) (Prachayasittikul et al., 2009b).



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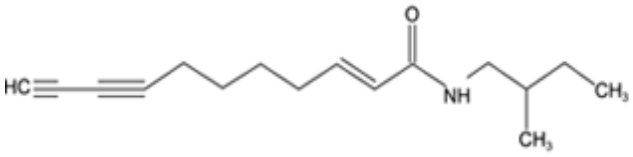
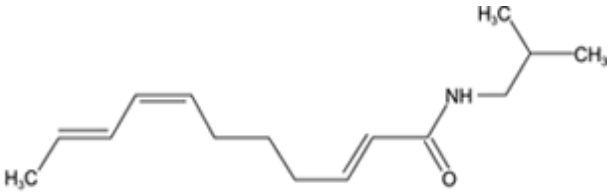
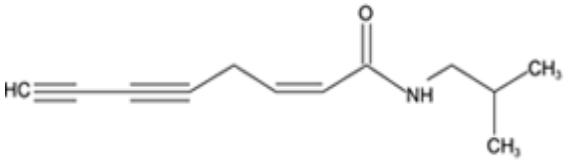


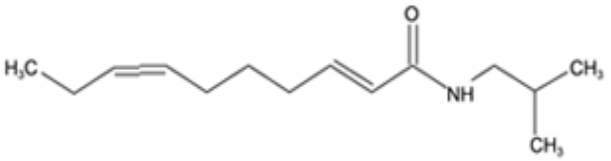
(2E,4Z)-N-isobutyl-2,4-undecadiene-8,10-diynamide



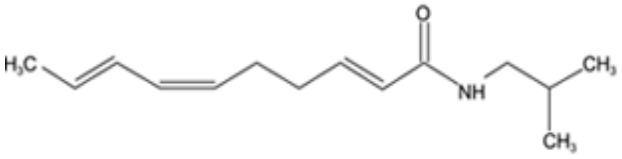
Undeca-2*E*,7*Z*,9*E*-trienoic acid



(2Z)-N-isobutyl-2-nonene-6,8-diynamide



(2E, 7Z)-N-isobutyl-2,7-decadienamide



(2*E*,6*Z*,8*E*)-N-(2-methylbutyl)-2,6,8-decatrienamide (spilanthol)

2-6

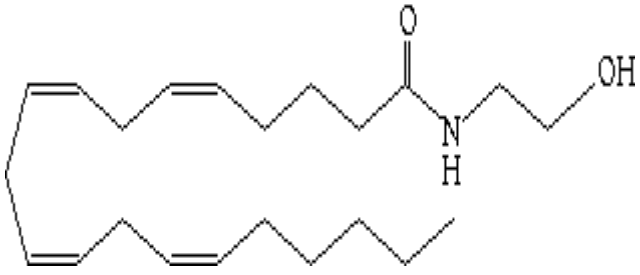


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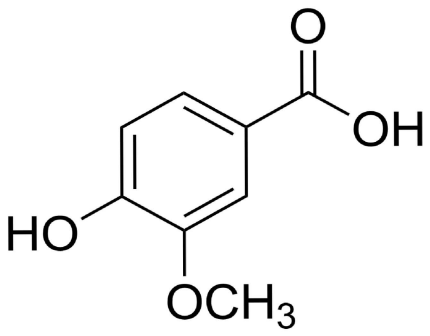
Figure 14: Structure of spilanthol and derivatives



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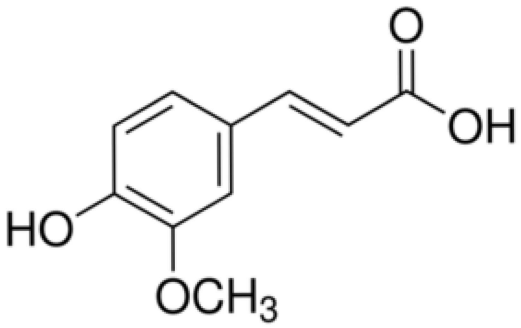

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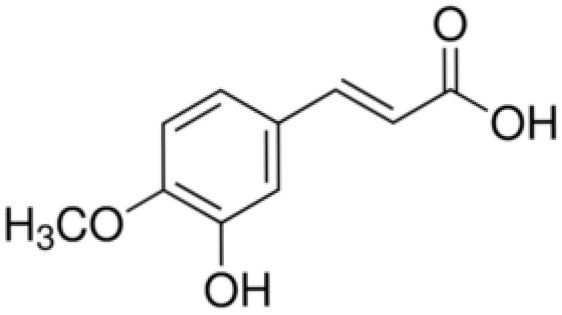

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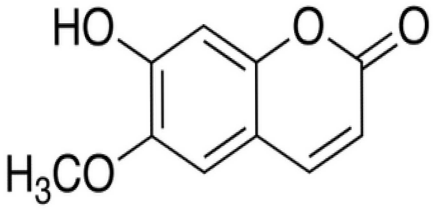

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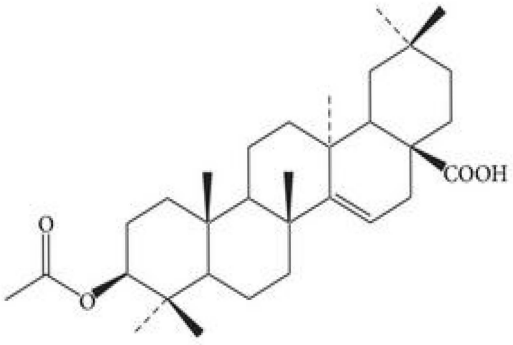

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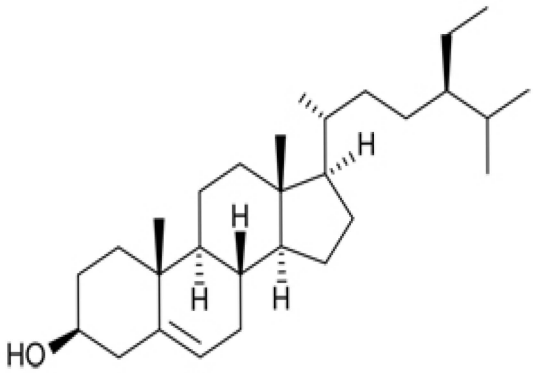

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Figure 15: Bioactive metabolites isolated from *Spilanthes acmella*

2.2 Bioactivity

The *Spilanthes* genera have been used for the treatment of various disorders including life-threatening diseases. Selected bioactivities of *Spilanthes acmella* are summarized below.

The studies showed that *Spilanthes acmella* (aerial aqueous extract) displayed antipyretic activity against Brewer's yeastinduced pyrexia. The antipyretic activity of the plant species can be attributed to flavonoids, which were predominant inhibitors of either cyclooxygenase (COX) or lipoxygenase (LOX) (Sadavongvivad and Supavilai, 1977).

Spilanthol is the main constituent isolated from many parts of *Spilanthes acmella* such as flower 85 % EtOH extract root hexane extract. Traditional usages of *Spilanthes acmella* flowers have been reported as anti-inflammatory agent (Sharma, 2003). Previous investigations demonstrated that spilanthol exerted anti-inflammatory action *via* inhibition of NF-κB pathway; afforded reduction in mRNA level and protein expression of COX-2 and iNOS; and also induced free radical scavenging activity (Wu et al., 2008).

Spilanthes acmella EtOH leaves extracts exerted significant centrally and peripherally (e.g. Writhing test) analgesic activities. The mechanism of action was possibly due to the presence of flavonoids in the plant extract which decreases prostaglandins, PGE2 and PGF2 that are known to be involved in pain perception. In addition, cold aqueous extract of *Spilanthes acmella* flowers also displayed antinociceptive activity against persistent pain and antihyperalgesic activity. The mechanism of action was possibly through inhibition of prostaglandins by spilanthol-containing extract (Ratnasooriya and Pieris, 2005)

Spilanthes acmella is known to be constituted of pungent alkamide-like spilanthol that causes numbness and tingle that show local anesthetic activity.

Ethyl acetate (EtOAc) and methanol (MeOH) extracts from the leaves of *Spilanthes acmella* exhibited the strongest antimicrobial activity among the tested extracts using the well diffusion method against *Klebsiella pneumonia*e (Arora et al., 2011). Several parts of *Spilanthes acmella* were tested for antifungal activity and the studies showed that *Spilanthes acmella* leaves

(EtOAc and aqueous) extracts exhibited better antifungal activity than the standard drug (fluconazole) against *Rhizopus arrhigus* and *Rhizopus stolonifer* (Arora et al., 2011).

Spilanthes acmella is a traditional medicine used in Africa and India for the treatment of malaria. Pharmacological study showed that spilanthol and acetylenic alkamide (undeca-2*E*-ene-8, 10- diynoic acid isobutylamide or UDA), isolated from the root EtOH extract of *Spilanthes acmella*, displayed antimalarial activity against two strains of *Plasmodium falciparum*. Both compounds had a reported antimalarial activity with IC50 in the range of 5.8-41.4 μg/mL in which the spilanthol was the most potent compound.

Antioxidant activity of *Spianthes acmella* extracts obtained from polar and nonpolar solvents were investigated. It was found that *Spilanthes acmella* flower EtOAc extract displayed the highest free radical scavenging activity (DPPH and ABTS assays) when compared to the other tested extracts (Wu et al., 2008). On the other hand, leaves and flowers of *Spilanthe acmella* MeOH extracts showed weak antioxidant activity (Nanasombat and Teckchuen, 2009). The aerial parts of *Spilanthes acmella* were also investigated (Prachayasittikul et al., 2009b; Wongsawatkul et al., 2008). The tested extracts (hexane, CHCl3, EtOAc and MeOH) exhibited antioxidant activity as indicated by DPPH and SOD assays. The EtOAc and MeOH extracts were shown to be the most potent antioxidants (DPPH). This could be due to the presence of phenolic and coumarin compounds that are present in the extracts (Prachayasittikul et al., 2009).

Spilanthes acmella extracts were studied for their vascular effects using rat thoracic aorta. The results showed that the tested extracts exhibited vasorelaxant activity *via* partial endothelium-induced NO and PGI2 in dose dependent manner. EtOAc extract displayed immediate vasorelaxant and the most potent antioxidant (DPPH) activities. Similar vasorelaxant and antioxidant (SOD) activities were also observed in the CHCl3 extract of the plant species. These bioactivities can be attributed to the presence of phenolic and triterpenoids (Prachayasittikul et al., 2009b).

The study of *Spilanthes acmella* EtOH leaves extract revealed diuretic effect possibly arising from tannin, steroid and carotenoid (Vanamala et al., 2012). In addition, flower cold aqueous extract of the plant species exhibited strong diuretic activity (Kumar et al., 2010).

The EtOH leaves extract showed significant immunomodulatory activity by increasing macrophage count with the maximum number of cells on the 15th day. The leaves of *Spilanthes acmella* contained various compounds such as alkamides, pungent amides, carbohydrates, tannins, steroids, carotenoids, essential oils, sesquiterpenes and amino acids. It was reported that spilanthol was involved in immune stimulation and attenuation of inflammatory response in murine Raw 264.7 macrophages (Wu et al., 2008).

4. RESULTS AND DISCUSSIONS OF LAXATIVE ACTIVITY TEST

4.1 Laxative Activity Test of the Extract of *Spillanthes acmella*

4.1.1 Laxative activity test:

The test was carried out to determine whether the extract of *Spillanthes acmella* had any anti-laxative effect, which indirectly correlates with constipation property. The experimental findings that are noted are below-

Total Stool Counts:

Negative Control Group

This group of animals only received vehicles (5% CMC) 10ml/kg through orally. The observed total stool count was followed with a mean value of 4.17±0.48 (Mean ±SEM) in this phase.

Test Group-1

In this case of the test group of the mice received the plant extract of 200 mg/kg through orally. The total stool count was followed with a mean value of 12.50±0.77 (Mean ±SEM) in this phase. The observed P value was **0.000.** The 200 mg/kg dose showed a highly significantly increased the total stool count. There was also significantly increased the total stool count found for the Bisacodyl.

Test Group-2

In this case of the test group of the mice received the plant extract of 400 mg/kg through orally. The total stool count was followed with a mean value of 17.7±0.60 (Mean ±SEM) in this phase. The observed P value was **0.000.** The 400 mg/kg dose showed a highly significantly increased the total stool count. There was also significantly increased the total stool count found for the Bisacodyl.

Test Group-3

In this case of the test group of the mice received the plant extract of 200 mg/kg and atropine (10mg/kg) through intra peritoneal. The total stool count was followed with a mean value of 9.00±0.064 (Mean ±SEM) in this phase. The observed P value was **0.02.** The 200 mg/kg dose and atropine showed a significantly increased the total stool count. Here atropine acts by reducing gastrointestinal motility.

Table 4: Laxative Effect of Crude Extract of *Spillanthes acmella*

Group No.	Treatment	Dose (mg/kg)	Mean defecation/group	Mean no of wet feces/group	Mean % of wet feces
1	5% CMC(p.o ml/kg)	10	4.17±0.48	0.33±0.21	3.33±3.33
2	Bisacodyl(i.p)	5	18.67±1.65***	8.83±1.018**	47.66±3.60***
3	Sp.Ac(p.o)	200	17.17±0.60***	5.50±0.43**	32.33±2.31***
4	Sp.Ac(p.o)	400	12.50±0.77***	3±0.37*	24.83±3.19***
5	Sp.Ac+Atropine(i.p)	200+10	9.00±0.064*	1.16±0.31	13.83±3.05
6	Sp.Ac+Atropine(i.p)	400+10	5.17±0.30	0.5±0.22	9±4.04

Values shown are mean ±S.E.M of 6 animals per group . *p<0.5 ,**p<0.01,***p<0.001 show a comparison of group 2,3,4 vs group 1(One way ANOVA followed by Dunnett's test), group 5 vs group 3, group 6 group 4(unpaired t-test). The term p.o represents per oral, while i.p is for intraperitoneal injection.;

Test Group-4

In this case of the test group of the mice received the plant extract of 400 mg/kg and atropine (10mg/kg) through intra peritonial. The total stool count was followed with a mean value of 5.17±0.0.30 (Mean ±SEM) in this phase. The observed P value was **0.000.** The 400 mg/kg dose and atropine did not show a significantly increased the total stool count. Here atropine acts by reducing gastrointestinal motility.

Positive Control Group

In this case of the mice received the standard drug Bisacodyl of 5mg/kg through orally. The observed total stool count was followed with a mean value of 18.67±1.65 (Mean ±SEM) in this phase.. The observed P value was 0.000. The positive control showed a highly significantly increased the total stool count.

Mean no. of Wet Stool Counts:

Negative Control Group

This group of animals only received vehicles (5% CMC) 10ml/kg through orally. The observed wet stool count was followed with a mean value of 0.33±0.21 (Mean ±SEM) in this phase.

Test Group-1

In this case of the test group of the mice received the plant extract of 200 mg/kg through orally. The wet stool count was followed with a mean value of 5.5±0.43 (Mean ±SEM) in this phase. The observed P value was **0.007.** The 200 mg/kg dose showed a significantly increased the wet stool count. There was also significantly increased the wet stool count found for the Bisacodyl.

Test Group-2

In this case of the test group of the mice received the plant extract of 400 mg/kg through orally. The wet stool count was followed with a mean value of 3±0.37 (Mean ±SEM) in this phase. The observed P value was **0.008.** The 400 mg/kg dose showed a significantly increased the wet stool count. There was also significantly increased the wet stool count found for the Bisacodyl.

Test Group-3

In this case of the test group of the mice received the plant extract of 200 mg/kg dose and atropine(10mg/kg) through intra peritonial. the wet stool count was 1.16±0.31 (Mean ±SEM). The 200 mg/kg dose and atropine did not show to significantly increase the wet stool count. Here atropine acts by antimuscurinic agent which reducing gastrointestinal motility. The observed p value was 0.630.

Test Group-4

In this case of the test group of the mice received the plant extract of 400 mg/kg dose and atropine(10mg/kg) through intra peritonial the wet stool count was 0.5±0.0.22 (Mean ±SEM). The 400 mg/kg dose and atropine did not show to significantly increase the wet stool count. Here atropine acts by antimuscurinic agent which reducing GIT motility. The observed p value was 0.492.

Positive Control Group

In this case of the mice received the standard drug Bisacodyl of 5mg/kg through orally. The observed total wet stool count was followed with a mean value of 8.83±1.018 (Mean ±SEM) in this phase. The observed P value was 0.001. The positive control showed a significantly increased the wet stool count.

Mean % of Wet Stool Counts:

Negative Control Group

This group of animals only received vehicles (5% CMC) 10ml/kg through p.o. The mean % of wet stool count was 3.33±3.33 (Mean ±SEM) in this phase.

Test Group-1

In this case of the test group of mice received the plant extract 200 mg/kg through orally. the mean % of wet stool count was 32.33±2.31 (Mean ±SEM). The observed P value was 0.000. The 200 mg/kg dose showed a highly significantly increased the mean % of wet stool count. There was also significantly increased the mean % of wet stool count found for the Bisacodyl.

Test Group-2

There was over all increased the total stool count, 24.83±3.19 (Mean ±SEM), in case of the mice received plant extract 400 mg/kg dose through orally. The observed P value was 0.000. The 400 mg/kg dose showed a highly significantly increased the mean % of wet stool count. There was also significantly increased the mean % of wet stool count found for the Bisacodyl.

Test Group-3

In this case of the test group of mice received the plant extract 200 mg/kg dose and atropine (10mg/kg) through intra peritoneal. the mean % of wet stool count was 13.83±0.31 (Mean ±SEM). The 200 mg/kg dose and atropine showed to increase the mean % of wet stool count. Here atropine acts by antimuscurinic agent which reducing GIT motility. The observed p value was 0.206.

Test Group-4

In this case of the test group of mice received the plant extract 400 mg/kg dose and atropine (10mg/kg) through intra peritoneal. the mean % of wet stool count was 0.5±0.0.22 (Mean ±SEM)**.** The 400 mg/kg dose and atropine did not show to significantly increase the mean % of wet stool count. Here atropine acts by reducing GIT motility. Here atropine acts by Here atropine acts by antimuscurinic agent which reducing GIT motility. The observed p value was 0.629.

Positive Control Group:

In this case of the mice received the standard drug Bisacodyl of 5mg/kg through orally. The observed mean % of wet stool count was followed with a mean value of 47.66±3.60 (Mean ±SEM) in this phase. The observed P value was 0.000. The positive control showed a significantly increased the mean % of wet stool count.

4.1.2 Charcoal meal induced GI Transit Time Test:

The test was carried out to find out the effects of extract on the transit of the gastrointestinal tract. Comparative evaluation of the extract with the reference motility drug, bisacodyl, and Negative control group showed that the extract significantly increase gastrointestinal motility in mice (Table: 5). A total 4 doses, e.g. 200 mg, 400 mg per kg body weight, 200mg/kg + atropine, and 400mg/kg + atropine were used for the gastrointestinal transit time test.

Negative Control Group

This group of animals received vehicles 5% CMC (10ml/kg). After 15 min, the animals were given 0.3 ml of charcoal meal of distilled water suspension containing 10% gum acacia and 20% starch through orally. And then the length of the small intestine and the distance between the pylorus region and the front of the charcoal meal was measured to obtain the charcoal transport percentage. Observed percentage of the intestinal length traversed by the charcoal was 16.50±1.48 (Mean±SEM) in this phase.

Test Group-1

In this case of the test group of mice received the plant extract 200 mg/kg through orally after 15 min, the animals were given 0.3 ml of charcoal meal of distilled water suspension containing 10% gum acacia and 20% starch through orally. And then the length of the small

intestine and the distance between the pylorus region and the front of the charcoal meal was measured to obtain the charcoal transport percentage. Observed percentage of the intestinal length traversed by the charcoal was 72.67±1.48 (Mean±SEM) in this phase. The observed P value was **0.673.**

Test Group-2

In this case of the test group of mice received the plant extract 400 mg/kg through orally. After 15 min, the animals were given 0.3 ml of charcoal meal of distilled water suspension containing 10% gum acacia and 20% starch through orally. And then the length of the small intestine and the distance between the pylorus region and the front of the charcoal meal was measured to obtain the charcoal transport percentage. Observed percentage of the intestinal length traversed by the charcoal was 86.67±0.77 (Mean±SEM). The observed P value was **0.002.** The 400 mg/kg dose showed a highly significant increased transit time by the charcoal through the intestine.

Test Group-3

In this case of the test group of mice received the plant extract 200 mg/kg dose and atropine (10mg/kg) through i.p. after 15 min, the animals were given 0.3 ml of charcoal meal of distilled water suspension containing 10% gum acacia and 20% starch through orally. And then the length of the small intestine and the distance between the pylorus region and the front of the charcoal meal was measured to obtain the charcoal transport percentage. Observed percentage of the intestinal length traversed by the charcoal was 59.0±1.94 (Mean±SEM). The observed P value was **0.001** The 200 mg/kg dose and atropine showed a highly significant increased transit time by the charcoal through the intestine. Here atropine acts by antimuscurinic agent which reducing GIT motility.

Test Group-4

In this case of the test group of mice received the plant extract 400 mg/kg dose and atropine (10mg/kg) through intra peritoneal. After 15 min, the animals were given 0.3 ml of charcoal meal of distilled water suspension containing 10% gum acacia and 20% starch through orally. And then the length of the small intestine and the distance between the pylorus region and the front of the charcoal meal was measured to obtain the charcoal transport percentage. Observed

percentage of the intestinal length traversed by the charcoal was 68.67±1.77 (Mean±SEM). The observed P value was **0.001.** The 400 mg/kg dose and atropine showed a highly significant increased transit time by the charcoal through the intestine. Here atropine acts by antimuscurinic agent which reducing GIT motility.

Positive Control Group

In this case of the mice received the standard drug Bisacodyl of 5mg/kg through intra peritoneal. after 15 min, the animals were given 0.3 ml of charcoal meal of distilled water suspension containing 10% gum acacia and 20% starch through p.o. And then the length of the small intestine and the distance between the pylorus region and the front of the charcoal meal was measured to obtain the charcoal transport percentage. Observed percentage of the intestinal length traversed by the charcoal was found 87.50±0.77 (Mean±SEM) where the observed p value was 0.06. As a positive control bisocodyl showed a significant increased transit by the charcoal through the intestine.

Table 5: Effects of extract on the Gastrointestinal Transit Time Test

Group No.	Treatment	Dose (mg/kg)	Mean of % length of small intestine
1	**5% CMC(p.o ml/kg)**	**10**	**16.50±1.48**
2	**Bisacodyl(i.p)**	**5**	**87.50±0.77***
3	**Sp.Ac(p.o)**	**200**	**72.67±1.48**
4	**Sp.Ac(p.o)**	**400**	**86.67±0.77****
5	**Sp.Ac+Atropine(i.p)**	**200+10**	**59.0±1.94****
6	**Sp.Ac+Atropine(i.p)**	**400+10**	**68.67±1.77****

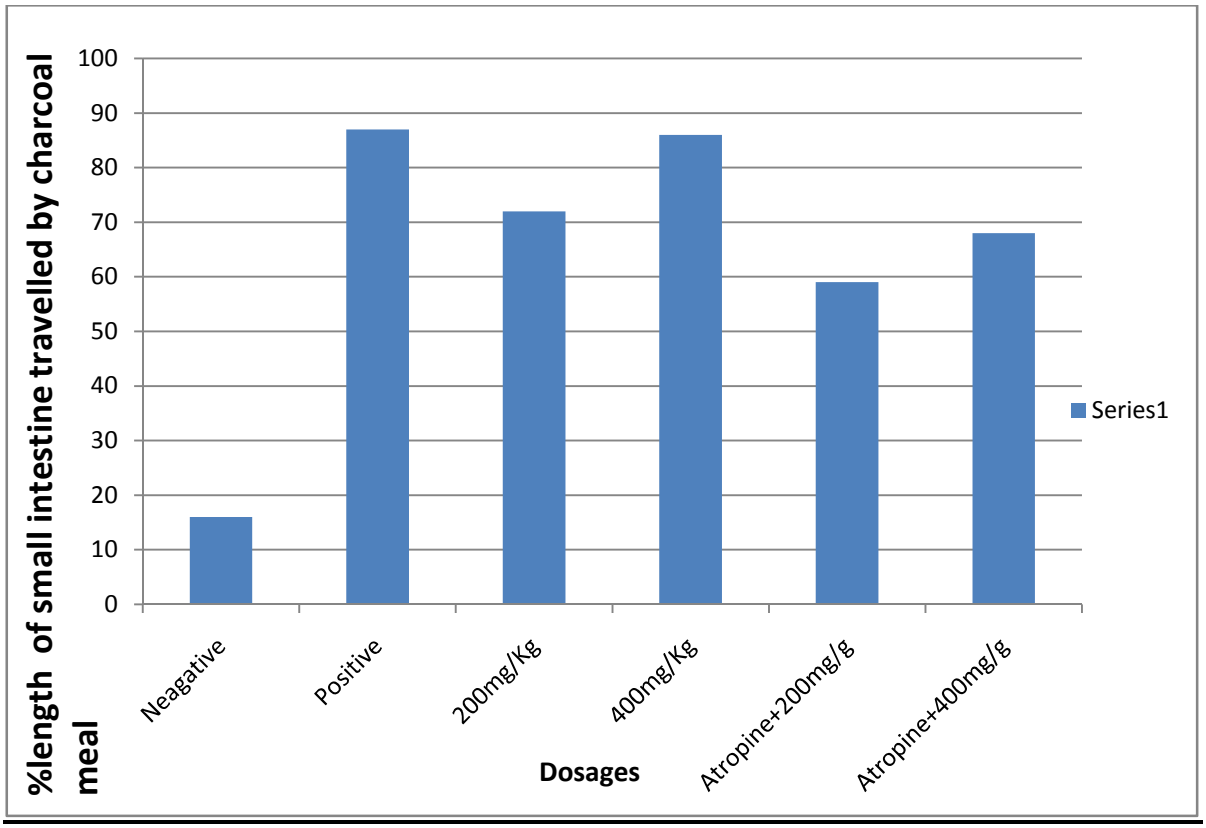

Fig 17: Bar diagram showing the Dose dependent effect of *Spillanthes acmella* crude extract on the travel of charcoal meal through small intestine of mice, in the absence and presence of Atropine. *p<0.05 ,**p<0.01,***p<0.001 show a comparison of group 2,3,4 vs group 1(One way ANOVA followed by Dunnett's test). Each bar shown represents mean± SEM of 6 animals per group.

DISCUSSION

Our results revealed that the extract of *Spillanthes acmella* appear to contain substance(s) that possess significant laxative activity. Bisacodyl is widely used stimulant laxative in the management of constipation, which effectively antagonized constipation induced by atropine. The therapeutic effect of bisacodyl is believed to be due to its motility and secretory properties (Katzung et al., 2011). From the study, it is likely that the extract may mediate its effects through similar mechanisms.

The extract, at the dose 200 mg/kg, 400 mg/kg, exerted a significant laxative effect on the feces count of laxative and charcoal meal induced GI transit test. In the laxative test extract 200 and 400 mg/kg increase the diarrhoeal feces output 24.83±3.19% (***p<0.001), and 32.33±2.31% (***p<0.001) respectively. And the increase of diarrhoeal faeces was highly significant as compared to the negative control group. The standard drug bisacodyl increase the diarrhoeal faeces 47.66±3.60% (***p<0.001), while *Spilanthes acemella* (200 and 400mg/kg) showed positive influence on wet feces in mice pretreated with atropine, the effect decline to 9±4.04% and 13.83±3.05% respectively.

Also in the charcoal meal gastrointestinal transit test, the extract for all doses very significantly increase the gastrointestinal motility by the charcoal. The percentage of the intestinal length traversed by the charcoal was found 72.67±1.48 (*p<0.5) for 200mg/kg & 86.67±0.77 (**p<0.01) for 400mg/kg crude extract respectively. Where the standard drug bisacodyl increase the percentage of transit time of 87.50±0.77 (*p<0.5), when the plant extract (200 and 400mg/kg) groups were restudied for their influence on transit of charcoal meal, in mice pretreated with atropine.

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