EVALUATION OF ANTIDIARRHEAL ACTIVITY BY USING ETHANOLIC LEAF EXTRACT OF *Phyllanthus amarusS.*

Dissertation submitted to

THE TAMILNADU Dr.M.G.R.MEDICAL UNIVERSITY,CHENNAI,

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MASTER OF PHARMACY

(PHARMACOLOGY)

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Under the guidance of

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This is to certify that the work embodied in this thesis entitled, "**EVALUATION OFANTIDIARRHEAL ACTIVITY BY USING ETHANOLIC LEAF EXTRACT OF *Phyllanthus amarus*S.**" submitted to the Tamilnadu Dr.M.G.R.Medical university, Chennai was carried out by **Reg.No:261625404,** Department of Pharmacology, Nandha College of Pharmacy, Erode -52. for the partial fulfillment for the award of degree of master of Pharmacy in Pharmacology under my supervision.

This work is original and has not been submitted in part or full for any other degree or diploma of this or any other university.

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EVALUATION CERTIFICATE

This is certify that the work embodied in the thesis entitled **"EVALUATION OF ANTIDIARRHEAL ACTIVITY BY USING ETHANOLIC LEAF EXTRACT OF *Phyllanthus amarus*S."** submitted to the Tamilnadu Dr.M.G.R Medical University Chennai, was carried out by **Reg. No:261625404** in the department of Pharmacology, Nandha College of Pharmacy, Erode-52 for the partial fulfillment of the degree of "Master of Pharmacy" in Pharmacology under the supervision of **Mrs.V.Lalitha,M.Pharm.,**Assistant professor, Department of Pharmacology, Nandha College of Pharmacy, Erode-52.

This work is original and has not been submitted in part or full for the award of any other degree or diploma of any other university.

Internal Examiner **External Examiner**

DECLARATION

DECLARATION

The work presented in this thesis entitled **"EVALUATION OF ANTIDIARRHOEAL ACTIVITY BY USING ETHANOLIC LEAF EXTRACT OF *Phyllanthus amarus*S."** was carried out by me in the Department of Pharmacology, Nandha College of Pharmacy, Erode -52 under the direct supervision of **Mrs.V.LALITHA,M.Pharm.,** Assistant professor,Department of Pharmacology, Nandha College of Pharmacy, Erode-52.

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I would like to express my sincere thanks to librarians Mrs. A. Sasikala and Mrs. P. Chitra and lab attender Mrs. Vijaya.

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"Success is a blessing and a reward from God after entrusting us with the knowledge, strength and the available resources for our use and our service to others for His glory. Giving thanks to anything that happens to you and the people He sends is an honor. Success of any project depends solely on support, guidance and encouragement received from the guide, friends and well-wishers"

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TABLE

ABBREVIATIONS

ABBREVIATIONS

IP	Intraperitoneal
P.O	Per os
EEPA	Ethanolic Extract of *Phyllanthus amarus* S.
IBS	Irritable Bowel Syndrome
SEM	Standard error of the mean

INTRODUCTION

1. INTRODUCTION

ROLE OF MEDICINAL PLANTS [1-2]

Herbal medicine is one of the oldest form of healthcare known in the human race. Plants have the ability to synthesize a variety of chemical compounds that are used to perform important biological functions and to defend against the attack from predators like insects and fungi. The medicinal use of plants seems to have been developed through observation of animals and trial and error.

About 12000 of such compounds are isolated from the plants and estimated to be less than 10% of the total chemical compounds in the plants mediate. Many of the pharmaceutical products available for use by physicians have a long history for use as herbal remedies such like opium, digitalis, quinine, aspirin. Pharmacological studies acknowledge the value of medicinal plants as a potential source of bioactive compounds. The phytochemicals from medicinal plants are lead compounds in drug discovery and design. About 25% of prescribed drugs contain at least one active ingredient derived from plant material. Some synthesised to mimic a natural plant compound and some from plant extracts.

According to World Health Organisation (W.H.O), an estimate of 80% population of some Asian and African countries presently use herbal medicine for some aspect of primary health care. At least, 7000 medicinal compounds in the modern pharmacopeia are derived from different plants whereby in the many medicinal and aromatic plants with significant variation in the soil traits, selective recovery and subsequent release in food of certain elements have been illustrated. Medicinal plants have are used to try to maintain health, to be administered for a specific condition, or both, whether in modern or traditional medicine. Thus the world wide trend towards the use of natural plant remedies has created enormous need for information about the properties and the uses of medicinal plants.

India is the largest producer of medicinal plants (botanical world of the world). The ayurvedic, siddha and unani system of medication is the predominant basis of Indian traditional medication based on the use of plant material. Hence, herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effective. They are the backbone of the traditional medicinal plants. The uses of

herbal medication to treat diseases is almost universal among non-industrialised societies since its more affordable than the modern pharmaceutical medicines. There must be a great attention to choose soil and cropping strategies. This is to obtain satisfactory yields of high quality and best products safe and with high nutritive value. Medicinal plants have curative property due to presence of complex chemical composition found as secondary metabolites in one or more parts of the plant. Many studies have been done on plants that are used in treatment of diarrhea and its effects. Our lifestyles are more responsible for creating problems due to improper eating habits, contamination of food and many more.

In the present review, an attempt has been made to explore a literature survey on the various plants for the anti-diarrhoeal activity. I chose to investigate the anti-diarrhoeal activity of ethanolic leaf extract of *Phyllanthus amarus* because traditionally it has been reported to have the activity but it has not been scientifically proven.

Diarrhea is a condition characterized by an increase in the number of liquidity of a person's stool. Water 60-90% (>90%) is called diarrhea. The frequency of elimination and consistency of stools vary from person to person. It's among the leading death causing diseases especially in developing countries. Approximately millions of people die every year in third world countries. Children are more susceptible to this disease which is accounted as the leading death causes in children especially under 5years. The signs of dehydration often begin with loss of irritable behaviour and the normal stretchness of the skin which can progress to decreased urination, loss of skin colour, a faster heart rate and a decrease in responsiveness as it becomes more severe. It can be acute or chronic and can range in severity from mild to life-threatening and it has many causes and pathophysiologic mechanisms.

Types of diarrhea

Diarrhea occurs due to imbalance between secretion and reabsorption of fluids and electrolytes. Depending on the frequency and the duration of the diarrhea episodes, the types of diarrhea include;

- Secretary diarrhea can be caused by microbial toxins, vasoactive intestinal polypeptides, excessive bile acids, laxatives, unabsorbed fat.
- Mild diarrhea in most cases is self-limiting and may subside within 1-2 days.

- Chronic diarrhea lasts for 14 days or longer and thus it needs more thorough diagnosis to determine the cause and enable selection of appropriate therapy.
- Severe diarrhea caused by bacterial infections and other causes can lead to significant loss of fluid and electrolytes and therefore should be attended to promptly.

Causes of Diarrhea

- Food intolerance: especially lactose
- Bacterial infections: shigella, salmonella, E. coli
- Viral infections
- Functional bowel disorders
- Reaction to medicines: e.g. antibiotics and magnesium containing antacids
- Parasites : e.g. Entamoeba histolytica
- Intestinal diseases: inflammatory bowel syndrome(IBD), celiac disease
- Nutrient malabsorption due to some diseases
- Altered motility
- Secretory tumours of GIT e.g carcinoid, which secrets vasoactive intestinal peptide
- Emotional distress

Mechanism of diarrhea

Irritable Bowel Syndrome (IBS)

This is a motility disorder of the gut that affects approximately 10% of the population. The symptoms are mostly colonic but still the patients with thus syndrome have abnormal motility throughout the gut.

In the management of IBS, first exclude a serious cause of the symptoms and then determine whether exclusion of some of certain foods would be worthwhile. An increase of dietary fibre over a given period of weeks may also reduce the symptoms. Drug treatment for this condition includes:

- Anti-diarrheal drugs such as loperamide
- Anticholinergic drugs e.g hyoscine.The oral use of better absorbed ones like atropine is limited by their side effects.
- Mebeverine. Relaxes intestinal smooth muscles without anticholinergic effects its effects is marginal.
- Peppermint oil: relaxes the intestinal smooth muscles

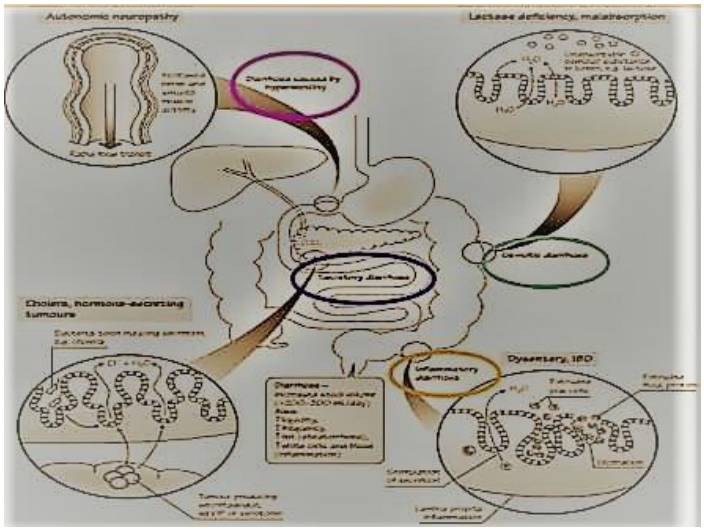
Intestinal movements in diarrhea

The segmentation movement of the intestine helps in mixing the intestinal contents, while peristaltic movement exert propulsive effect. In the case of diarrhea, there is less spontaneous activity of the colon than in people with normal bowel movement l. Patients who have constipation, have more spontaneous activity. During diarrhea, there is loss of segmenting contractions that delay the passage of the contents and thus occasional peristaltic wave may have greater propulsive effect. Hence, anti-diarrhoeal drugs act by increasing segmentation and decreasing peristaltic movements.

Pathophysiology

The absorption and secretion of water and electrolytes happens in the intestine, with the jejunum being freely permeable to salt and water which are passively absorbed secondary to nutrient absorption. Active Na+K+ATPase mediated salt absorption in the colon and ileum occurs, in the mature cells lining the villous tips, water flows isoosmotically. On the other hand, glucose facilitated Na+ absorption takes place in the ileum by Na+-glucose co-transporter. This mechanism remains intact even in severe diarrhoeas.

In secretory diarrhea, the mediators act by stimulating cAMP formation or inhibiting membrane Na+K+ATPase and thus lead to increase in secretion of water and electrolytes by the intestinal mucosa.



Mechanism of action of anti-diarrhoeal drugs.

The anti-diarrhoeal drugs act in the body by various mechanisms to stop or reduce the effects of diarrhea caused in different ways and they include;

Electrolyte solutions are used to replace lost fluids and salts in acute cases.

Absorbents They are colloidal bulk1 forming substances also called fluid absorbants that absorb toxic substances that cause infective diarrhea, they modify the consistency and frequency of stool but do not reduce the water and electrolyte loss e.g methylcellulose, isapghula, psyllium. Fluid adsorbent, like kaolin, are used in short-term treatment of diarrhea and usually given in combination with other drugs. Bulking agents are used for diarrhea in functional bowel disease and to control ileostomy output.

Uses

- Irritable bowel syndrome (IBS)
- Illeostomy/ colostomy diarrhea

Antimotility (Opioids): They have agonist action on the intestinal opioid receptors which when activated cause constipation e.g morphine and codeine, loperamide. They are the most effective in diarrhea treatment by exerting a nonspecific effect that can control diarrhea from almost any cause. They act by inducing a sustained segmental contraction of intestinal smooth muscles, which prevents the rhythmic wave contraction and relaxation of smooth muscle that occur with normal peristalsis. Their effects are mediated by activation of opioid receptors in the smooth muscles. The more potent opioid receptor agonists are effective antidiarrheal agents.

Uses

- Used after anal surgery, colostomy
- Non-infective or mild travelers' diarrhea
- Idiopathic diarrhea in AIDS

Antisecretory and Anticholinergics Agents: Atropinic act by reducing bowel motility and secretions. They may benefit the drug induced diarrhoeas and symptomatic relief in case of dysentery. These class of drugs also act by reducing intestinal movements.

Classification of Anti-diarrhoeal drugs

- Adsorbents: kaolin, pectin, chalk, activated charcoal, isaghula, psyllium.
- Antimotility drugs (Opiates): codeine, loperamide, diphenoxylate, diphenoxin, racecodotril, lactobacillus preparations.
- Antisecretory agents: atropine derivatives, drotaverine, octreotide, racecadotril, sulfasalazine, bismuth subsalicylate.

Absorbents

This includes indigestible vegetable fibre and hydrophilic colloids that increase the volume and lower the viscosity of intestinal contents forming a large soft, solid stool.

The dietary fibre consists of cell walls and other parts of fruits and vegetables that are unabsorbable and produce a perception of decreased stool fluidity.

They modify the consistency and frequency of stool and give impression of improvement. They are of value in condition like diarrhea phase of IBS and to increase the consistency of faeces in colostomy patients.

Uses

Symptomatic relieve of acute and chronic diarrhea

Opiates

Stimulation of the μ receptor by these drugs lead to decrease of tone of longitudinal smooth muscle cells, increase the tone of circular smooth muscle cells, inhibit gastrocolic reflex, reduces the GIT motility and increase transit time.

Uses

- Travellers diarrhea
- Chronic diarrhea

Adverse Effects

- Abdominal pains
- Nausea
- Constipation

Antisecretory agents

They inhibit the release of different hormones like gastrin, secretin, pancreatic polypeptide. Also reduces fluid and electrolyte secretion from the intestines and reduces GIT motility.

Uses

In secretary diarrhea due to hormone secreting tumor of pancreas or intestine.

Sulfasalazine

It has a low solubility, poorly absorbed in the ileum. Though it inhibits both COX and LOX, decreased PG and LT production appears to play a minor role in the therapeutic effect. It is given during an exacerbation it reduces number of stools, abdominal cramps and fever, but is less effective than corticosteroids. Sulfasalazine has also been used as a disease modifying drug in rheumatoid arthritis.

Racecadotril

It is an enkephalinase inhibitor. It prevents degradation of endogenous enkephalins. It is indicated in the short-term treatment of acute secretory diarrhoeas.

Side effects

Vomiting

Nausea

Drowsiness

Codeine

It is an opium alkaloid that has prominent constipating action. The antidiarrhoeal effect is attributed to its peripheral action on small intestine and colon. Its side effects sre nausea, vomiting and drowsiness.

Loperamide

It is an opiate analogue with major peripheral μ opioid. It has more potent constipating action than codeine. Loperamide inhibits secretion, directly interacts with calmodulin that may be responsible for the antidiarrhoeal action.

Adverse effects

Rashes

Abdominal cramps

Diphenoxylate

It used exclusively as constipating agent with action is similar to codeine. The anti-diarrhoeal action is most prominent, but because it is absorbed systemically and crosses blood brain barrier.

Principles of management

This largely depends on the underlying cause and instituting specific therapy if necessary. The importance of each depends on the severity and nature of diarrhea. Therapeutic measures may be grouped into:

- Treatment of fluid depletion, shock and acidosis: rehydration by use of Oral Rehydration Salt
- Maintenance of nutrition.
- Drug therapy.
- Probiotics

Rehydration

Rehydration can be done both orally and i.v.

Intravenous rehydration

This is needed only when there is severe loss of fluid >10 body weight or if the patient is losing >10ml/kg/hr or in case one is unable to take enough oral fluids due to vomiting, weakness or stupor. The composition of i.v. fluid is:

NaCl	5g = 85mM
KCl	1g = 13mM
NaHCO3	4g = 48mM

The salts are mixed in one litre of water or 5% glucose solution. Volume that is equivalent to 10% body weight should be infused over 2-4 hours.

Oral rehydration

Oral Rehydration Therapy (ORT) is done in case there is a mild fluid loss (5-7% BW) or moderate (7.5-10% BW)

The preparation is possible when glucose is added with salt. The general principles of ORS are:

- The molar concentration of glucose should be equal or higher than sodium concentration but not exceed 110mM.The excess glucose will be utilized in absorbing sodium present in the intestinal secretions.
- It should be isotonic (200-310mOsm/L)
- Should have enough potassium (15-25mM) and bicarbonate or citrate (8-12mM). This is to make up the losses in stool.

WHO recommended a standard formula which provided Sodium 90mM, Potassium 20mM, Cl 80mM, citrate base 10mM, glucose 110mM and had a total osmolarity of 310mOsm/L.

New formula WHO-ORS

New formula low sodium glucose was released by WHO in 2002. The recent studies showed that the efficacy of ORS in children with acute diarrhea is improved by reducing sodium Na+ and glucose concentration to 75mM, and total osmolarity to 245 mOsm/L and with this the supplemental i.v. therapy was reduced significantly by 33%.

The new ORS has proven effective in management of cholera both in children and in adults. The WHO and UNICEF have now recommended replacement of standard (310 mOsm/L) ORS formula by new (245mOsm/L).

Content	**Concentration**
NaCl: 2.6g	Na+ - 75mM
KCl: 1.5g	K+ - 20mM
Trisod. Citrate: 2.9g	Cl^- - 65mM
Glucose: 13.5g	citrate – 10mM
Water: 1L	glucose – 75mM
Total osmolarity 245 mOsm/L	

Maintenance of nutrition

Patients with diarrhea should not be starved, this is because fasting decreases disaccharidase enzymes and reduces absorption of water, salt and nutrients which may lead to malnutrition in case the diarrhea prolongs is recurrent. Foods like boiled potato, rice, banana, sago should be given as soon as the patient can eat.

Drug therapy

This consists of:

- Nonspecific anti-diarrhoeal drugs
- Specific antimicrobial drugs

Probiotics

They are microbial cell preparations which either live in lyophilised powders or in cultures that are intended to maintain healthy gut flora.

Diarrhea that may happen due to antibiotics happens by the fact that the drugs may also kill some of the good bacteria, this in turn can upset the normal balance of the intestines leading to diarrhea. Present studies of children and adults show that the chances of having diarrhea can be cut or reduced by taking probiotics before and during treatment and several days after stopping the antibiotics. Examples include; *Saccharomyces boulardii, Lactobacillus acidophilus, Bifidobacterium bifidum.*

GUT MOTILITY MODELS

In vitro models

- [^{125}I]CCK Receptor binding assay
- [^{3}H]GR-113808 Binding assay for 5-HT_4 receptors
- [^{3}H]Zacopride binding assay for 5-HT_3 receptors
- Cascade Superfusion Technique
- Isolated Rat Thoracic Esophageal Muscularis Mucosae

In vivo models

- Charcoal passage test
- In vivo evaluation of spasmolytic activity
- Colonic motility studies in rats
- Gut motility studies in dogs
- Gastrointestinal motor activity in conscious dogs
- Castor oil induced model
- Magnesium sulphate induced model

PLANT PROFILE

2. PLANT PROFILE

Phyllanthus amarus

Scientific name **:** *Phyllanthus amarus Schum. & Thonn.*

Family **:** Euphorbiaceae

Synonyms **:** *Phyllanthus niruri*

Phyllanthus erectus

Phyllanthus rosellus

Common names

English	:	sleeping plant
Hindi	:	jangli amli
Telugu	:	nela usari
Kannada	:	kirunelli
Tamil	:	kikkaynelli
Malayalam	:	kilanelli
Marathi	:	bhooyiaamli

Distribution and description.[3-9]

It is a shrub, perennial which grows in a tropical climate. It grows 50-70 cm tall and bears ascending herbaceous branches. The name 'Phyllanthus' means "leaf and flower" and named so because of its appearance where the flower, fruit and leaf appear fused. It bears pale green or yellowish flowers and its fruits are tiny underneath the branches, small capsules containing seeds which are trigonous, pale brown with longitudinal parallel ribs on the back.

Parts used

Whole plant

Chemical constituents

The main active constituents are lignans (phyllanthin, hypophyllanthin in), flavanoids (quercetin, quercetrin,rutin), ellagitannins (amarinic acid, geraniic acid B),tannins (geraniin, amariin, gallocetechin), phenolic compounds (gallic acid,ellagic acid, oleanolic acid etc) terpenes, tripenes, alkaloids etc.

The leaves are rich in phyallanthin (0.7%). The whole plant contains phyllanthin (0.4%), hydrophyllanthin (1.2%), gallic acid (0.4%), geraniin (1-2%), ellagic acid (0.2%).

Traditional uses and pharmacological activities

Phyllanthus amarus has been useful in several health problems like diarrhea, dysentery, jaundice, scabies, dropsy, fevers and in wound healing. It has been used further in treatment of urinary bladder disturbances, kidney problems, chronic dysentery etc. Topically it is used in treatment of skin ulcers, sores, swellings, scabby, ringworm lesions. Young shoots of this plant are used in disorders like dysentery, diarrhea and

constipation. The fresh leaf paste is effective in wound healing, cure of skin infections and jaundice. The herb has been found effective in several female problems such as in leucorrhoea, mammary abscess and can act as galactagogue. The young shoot of the plant administered as infusion is used in treatment of chronic dysentery. The root extract is used to cure stomach pain and the flower paste as an antidote for snake bite. The whole plant is more effective in treatment of urinary tract problems and swelling of liver.

Along with the traditional uses of the plant, the numerous phytocompounds in *Phyllanthus amarus* like alkaloids, flavanoids, phenolic compounds isolated from the plant can be used as antiviral agent, antispasmodic, pain-relieving and anti-inflammatory actions, liver protective and detoxification actions, anticancerous and cellular protective actions, antidiabetic among many more other activities.

LITERATURE REVIEW

3. LITERATURE REVIEW

Verma et al., (2014)[8] reported on a review study on *Phyllanthus amarus*. The herb is widely used in tropical countries including India. 'Phyllanthus' means leaf and flower and its named so because of its appearance as the flower, fruit and leaf are fused. The traditional usefulness of *P.amarus* include treatment of chronic dysentery, diabetes, jaundice, skin rashes, ringworms, scaby, kidney problems. *P.amarus* has numerous of phytocompounds such as tannins, lignans, triterpenoids, alkaloids, volatile oil. In the pharmacological study it is used as an anticancer, antiamnesic, antimicrobial, antinociceptive, antiinflammatory, anticonvulsant.

Mithun et al., (2011)[9] investigated the anti-diarrhea activity of aerial parts of vinca major in experimental animals. The effect of the ethanolic extract of aerial parts of the plant on castor oil induced diarrhea, castor oil and magnesium sulfate induced enteropooling, gastrointestinal motility test using charcoal meal model were examined. This extract (250, 500 and, 1000 mg/kg, p.o.) was evaluated on the latent periods, fecal frequencies in castor oil induced diarrhea. Gastrointestinal transit using charcoal meal, castor oil and magnesium sulfate induced enteropooling assays (500 mg/kg, p.o.) were done. The results showed that there is a significant ($p<0.05$) reduction of the fecal output was observed at a dose of 500 mg/kg,orally.

Dhongaje et al., (2015)[10] reported on the pharmacognostical and phytochemical studies of *Phyllanthus amarus*. The ethno medicine involves the use of different plant extracts of importance in the health application at an affordable cost. Morphological study and pharmacognostical properties like stomatal number, palisade ratio, extractive value, moisture content was studied. Qualitative phytochemical analysis of the plant confirms the presence of various phytochemicals such as alkaloids, tannins, saponins, carbohydrates, glycosides, steroids, phenols and flavanoids. About 1000gms of coarsely powdered *P. amarus* was taken and extracted continuously with water and ethanol by soxhlet extraction. The filtrate was then concentrated and dried in a dessicator. With this microscopic and phytochemical studies will help to authenticate genuine sample of *P. amarus* from its five different species found in India.

Prashant et al., (2012)[11] reported the anti-diarrhoeal activity of piperine in mice. This was evaluated in castor oil and magnesium sulphate induced diarrhea. . Piperine, at a dose of 5 to 20 mg/kg showed anti-diarrhoeal activity in castor oil and magnesium sulphate induced diarrhoea. The animals were divided in to control, positive and test

groups containing six in each group. The piperine was studied for acute oral toxicity as per revised OECD guidelines number 423. Piperine was used for the study at the dose of 5, 10 and 20 mg/kg because it has not shown any toxicity up to 50 mg/kg. The result showed both antimotility and antisecretory activity in castor oil and magnesium induced diarrhea. The parameters observed were: onset of diarrhoea, total weight of stool output, total weight of wet stools, total number of stool output, and number of wet stools.

Lakshminarayana et al.,[12] studied the anti-diarrhoea activity of leaf extract of Moringa Oleifera in experimentally induced diarrhea in rats. The extract was evaluated using animal models of diarrhoea like the castor oil and magnesium sulfate induced gastrointestinal motility, in a model of enteropooling induced by the administration of castor oil and PGE2, Charcoal meal test. Acute toxicity studies indicated that the extract is safe till 2500 mg/kg. The results suggest Moringa oleifera mechanism of action in inhibiting diarrhea involves anti propulsive and antisecretory effects.

Shetti et al., (2012)[13] evaluated the anti diabetic effect of ethanolic leaf extract of P. amarus in alloxan induced diabetic mice. The present study was carried to evaluate the antidiabetic effect of ethanolic extract of *P. amarus* in alloxan induced diabetic mice. The leaves of the plant were dried under shade for about 6 days and reduced to powder by using dry grinder. 100gm of the powder was then packed into soxhlet apparatus and extracted using 95% ethanol for about 40hours. The extract was dried at 45^{o}C in hot air oven till a green colored semi solid mass was obtained. It was then stored in a refrigerator at 4^{o}C until further use. The animals were grouped into four groups of 5 mice. Diabetes was induced by i.p administration of alloxan monohydrate 150mg/kg body weight. Group I served as normal healthy mice and given only vehicle, Group II served as control, Group III diabetic mice received a dose of glibenclamide 600µg/kg body weight and Group IV received plant extract 400mg/kg body weight. The results showed significant ($p<0.05$) reduction on oral administration of *P. amarus.*

Povi Lawson-Evi et al., (2008)[14] reported on the toxicological assessment on extracts of *P. amarus* Schum & Thonn. The purpose of this study is to evaluate its toxicity. The cytotoxicity of its aqueous and hydroalcoholic extracts was performed. Aqueous extract was more cytotoxic than hydroalcoholic extract. The acute and sub acute toxicity of the extracts were evaluated in Swiss mice and Wistar rats. A single oral dose of the extracts at 5 g/kg b.w. did not produce mortality or any significant change in

treated animals over 14 days of observation. *P. amarus* could then be considerd to be safe in animals by oral route (LD 50> 5g/kg).

Srivastava et al., (2015)[15] researched on the HPLC analysis of *P.amarus* sample stored in stability chambers under different conditions and study of the effect on quantification of the phytomarkers phyllanthin and hypophylanthin. The quantification of the active principles is essential before using the crude drug due to the deterioration of active principles during storage. In this study the effect of storage conditions on the quantification of the bioactive markers by HPLC analysis in the crude plant material of *Phyllanthus amarus*. The results showed that active lignans reduce during the storage of plant material. Therefore the *P. amarus* should be used fresh to get the maximum concentration of active lignans or stored under LS conditions up to 6 months.

Karuna et al., (2009)[16] evaluated the antioxidant potential of aqueous extract of *P. amarus* in rats. The study aims at assessing the antioxidant potential of *Phyllanthus amarus* since the increased levels of oxidative stress may be implicated in the etiology of many pathological conditions. Rats were divided into 2 groups: control and *P. amarus* treated. Treated rats received *P. amarus* extract at a dose of 200 mg/kg body weight for 8 weeks. *P. amarus* treated rats showed a significant decrease in lipid peroxidation and a significant increase in plasma vitamin C, uric acid and superoxide dismutase which suggest the non-toxic nature of the extract and can be linked to improved antioxidant status. The extract has been reported to have anti-diarrheal, anti-bacterial, antiviral, anti-plasmodial properties.

Atul et al., (2013)[17] researched on toxicity studies and evaluation of *P. amarus* and *P. freternus* extract on the CNS and musculoskeletal function. In the present study studies of the extracts *P. amarus* and *P. freternus* and also examined their effect on CNS and skeletal muscle function. The toxicity of *P. amarus* and *P. freternus* was carried out by adopting the OECD guidelines 420 (fixed dose procedure) carried out by male mice divided into five groups of four animals. The extracts of Phyllanthus species were administered i.p. (2.0g/kg). the mortality within 72hrs was recorded and the animals were observed for any signs of toxicity, weight variation, consumption of food and water, behavioral changes, locomotion, convulsion and mortality. The results showed no significant sign of toxicity during the study.

Sirajudeen et al., (2006)[18] researched on the safety evaluation of aqueous extract of leaves of a plant *P. amarus* in rat liver. The aim of this study was to determine the

toxic side effects of aqueous extract of leaves of *P. amarus* following oral administration in rats. The extract was administered in both sex rats at doses of 100, 400 and 800 mg/kg body weight for 6 weeks. Acute administration of *P. amarus* extract at a dose of 5g/kg body weight did neither produce any sign of toxicity or mortality nor was observed any effect on the general condition or behavior of the experimental animals. They all appeared normal, survived during the experimental period and none showed any visible signs of toxicity.

Neelam et al., (2014)[19] assessed the anti-diarrheal potential of ethanolic leaf extract of *Malvastrum tricuspidatum* in albino rats. The activity was investigated by castor oil induced diarrhea, magnesium sulphate and castor-oil induced enterpooling and gastrointestinal motility by charcoal meal. The animals were divided into five groups of six animals. Group I as control, Group II treated with standard drug loperamide 3 mg/kg and Group III-V received 100, 250 and 500 mg/kg p.o. The 500 mg/kg dose of ethanol extract showed significant ($p < 0.05$) activity against castor oil- induced diarrhea. This dose was selected significantly and used in other models.

Mohammad et al., (2014)[20] did a study on the evaluation of antidiarrheal and antinociceptive activity of methanolic extract of *Alstonia scholaris* Linn. on mice models. The present study designed to investigate the antidiarrhea and antinociceptive activity of methanolic extract of *Alstonia scholaris* Linn. on mice models at different doses of 200 mg/kg and 400 mg/kg. The various models employed for investigating this activities such as castor-oil induced diarrhea, castor oil induced enterpooling and gastrointestinal motility, tail immersion and hot plate methods. The diarrhea episode was inhibited by 50.79% and 57.14% methanol extract at the doses given which showed a highly significant but dose dependent anti-diarrheal and anti-nociceptive activity.

Shanty et al., (2013)[21] studied the nanosuspension of *Phyllanthus amarus* extract for improving oral bioavailability and prevention of paracetamol induced hepatotoxicity in Sprague- dawley rats. The present study was designed to evaluate and compare thr hepatoprotective effects of ethanolic extract of *P. amarus* and its nanoparticles on paracetamol induced acute liver toxicity in rats. An oral dose of *P. amarus* at 125 and 250 mg/kg and *P. amarus* nanoparticles at 25 and 50mg/kg. The results showed a significant hepatoprotective effect relatively to the same extent ($P<0.001$) by reducing levels of aspartate aninotransferase, alanine aminotransferase and bile salts.

Okwute et al., (2014-2015)[22] reported on the biological and chemical evaluation of the extract of the leaf of *P. amarus*. The leaf of *Phyllathus amarus* was investigated for its chemical and biological properties. The powdered leaf was extracted with 95% ethanol and phytochemical analysis was done. The crude ethanol and aqueous methanol extracts were screened for the presence of some classes of natural products using standard procedures. The leaf contains high percentage of carbohydrates 43.30% and crude fibre 21.50%. It also contains 10.1% moisture content, 8.45% protein, 7.15% fat and 6.50% ash content. It was also observed that flavanoids, tannins and terpenoids were present.

Hassan et al., (2017)[23] evaluated the anti-diarrheal action of *Zataria multiflora* hydroalcoholic and hexane extracts in mice. The objective of this study was to investigate the antispasmodic activity of hydroalcoholic and hexane extracts of *Z. multiflora* on intestinal peristaltic movement as well as assessment of its anti-diarrhea action in mice. Animals were fasted overnight and divided into seven groups. Group I and II received oral administration of loperamide 2 mg/kg or vehicle 0.35% ethanol. Group III and IV received the hydroalcoholic extract 20 mg/kg and 40 mg/kg p.o. Animals in Group V and VI received hexane extract 20 and 40 mg/kg. the control Group VII were treated with equivalent amount of the vehicle 7% ethanol. The antispasmodic activity of *Z. multiflora* extracts (20 & 40mg/kg) hydroalcoholic and hexane extracts showed a reduction in the distance travelled by charcoal meal in the small intestines. 20 & 40 mg/kg of the extracts also showed significant reduction in diarrhea in both castor oil and magnesium induced diarrhea.

Sangeetha et al., (2017)[24] studied on the phytochemistry and pharmacological properties of *P. amarus* Schum. This herb is used as traditional medicine in many countries for more than 3000 years. The present review compiles traditional uses, phytochemical constituents and pharmacological actions. The traditional uses include diarrhea, jaundice, dropsy, urinogenital disorders, kidney problems, skin ulcers, wounds, scabies, chronic dysentery. The herb has been found to be effective in several female disorders as in menorrhagia, leucorrhoea and mammary abcess. The stem juice is a wound healer. The root extract is used to cure stomach pain and the flower paste of this plant is applied externally as antidote against skin bite. The phytochemical studies show the presence of tannins, alkaloids, flavanoids, lignins, triterpenoids and many other constituents. Pharmacological activities include anticancer activity, anticonvulsant,

antioxidative activity, anti-inflammatory. These activities establish the therapeautic value of thus herb.

Bharti et al., (2014)[25] reported an overview of important ethnomedical herbs of phyllathus species on the present and future prospects. The genus Phyllanthus consists of more than 1000 species of which many are used as traditional medicine. The modern scientific studies have now confirmed pharmacognostic of Phyllanthus herbs. This review is an attempt to present an overview of the existing studies on the pharmacognostics, phytochemistry, species identification and genetic diversity of Phyllathus herbs.

Aminul et al., (2008)[26] assessed an overview on Phyto-Pharmacology of *Phyllanthus. amarus*. *Phyllanthus amarus* (PA) is a small herb indigenous to Amazon Basin .It is well known for its medicinal properties and widely used by different countries. It is reported to contain lignans, alkaloids, flavonoids, galloatnoids, glycosides and alkaloids. With the constituents *Phyllanthus amarus* possesses, the pharmacological evaluation mentioned in this review establish the therapeutic value which includes antiviral, antiparasitic, antimalarial, antimicrobial, anticancerous, anti-diabetic activities of this herb. Thus activity guided phytochemical and phytoanalytical may leads to development of novel agents for various disorders.

Mainen et al.,[27] studied the effect of Phyllathus amarus aqueous extract on blood glucose in Non Insulin Dependent Diabetic patients.

Arun et al., (2011)[28] reported on the comparative study on hepatoprotective activity of *P. amarus* and *Eclipta prostrate* against alcohol induced in albino rats. Alcohol is one of the most important and commonly used hepatotoxic agents in the experimental study of liver related disorders. The hepatotoxic effects of alcohol are largely due to its active metabolite, trichloromethyl radical these activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. The therapeutic administrations of *Phyllanthus amarus* and Eclipta prostrata leaves fine powder greatly change the biochemical parameters in the ethanol intoxicated rats and maintained well to the normal level. These results clearly suggest that, the *Phyllanthus amarus* and *Eclipta prostrata* have enormous hepatoprotective value.

S. Dinesh et al (2017)[29] studied on molecular docking and simulation studies of P. amarus phytocompounds against structural and nucleocapsid protein of white spot syndrome virus. White spot disease caused by white spot syndrome virus (WSSV) is a

lethal disease for shrimp. Envelope structural proteins play a major role in viral attachment and are believed to be the initial molecules to interact with the host cell. The mobility of protein-ligand binding complex at various time intervals was validated by molecular dynamics and simulation study. Therefore, *P. amarus* phytocompounds were found to be most suitable inhibitors for the antiviral treatment for WSSV infection.

Yakubu et al., (2012)[30] reported on the Antidiarrheal activity of aqueous leaf extract of *Ceratotheca sesamoidesin rats.*The antidiarrheal effects of the aqueous leaf extract of C. sesamoides at 25, 50 and 100 mg/kg body weight was evaluated in female rats using gastrointestinal transit, diarrhea and enteropooling induced by castor oil models. The 25 mg/kg body weight of the extract significantly ($p<0.05$) prolonged the onset time of diarrhea, decreased the fecal parameters with no episode in the animals treated with 50 and 100 mg/kg body weight.

Anjaneyulu et al., (2013)[31] studied on the Pharmacological Evaluation of Anti-diarrheal Activity of Alcoholic extract of Fruits of *Frageria versa* linn. in rats. The purpose of this study was to evaluate the anti-diaaheal effect of *Frageria versa* linn. against castor oil induced diarrhea model. The weight and the volume of intestinal content by castor oil were studied by enterpooling method. Like atropine 3mg/kg there were significant reduction in the fecal output and frequency of droppings when the plant extracts of aqueous 100 and 200 mg/kg doses were administered i.p compared to castor oil treated rats.

Mekonnen et al., (2017)[33] reported on the Evaluation of the anti-diarrheal activity of the leaf extracts of *Myrtus communis* Linn. (Myrtaceae) in mice model. This study aimed at investigation of the anti-diarrheal effect of 80% methanol extract and solvent fractions of leaves of *Myrtus communis* Linn. in mice. The activity was evaluated against castor oil induced diarrhea, charcoal meal and enterpooling tests. For the methanol extract the test groups received 100, 200 and 400 mg/kg of the extract. The results showed that at 200 mg/kg ($p<0.05$) and 400 mg/kg ($p<0.01$). besides the 80ME and both these fractions significantly decreased the frequency and weight of the fecal outputs.

AIM AND OBJECTIVE

4. AIM AND OBJECTIVE

The study aims at investigating the antidiarrheal activity of *Phyllanthus amarus S.* in experimental animal models.

The drug is widely available in major parts of India thus can be used widely in treatment of diarrhea and its effects. With the appreciation of the herbal medicines, opportunities have been created for discovery and study of new drugs has been made possible.

The drug is intended to stop the diarrhea that is induced by different mechanisms after administration. The study will also help production of drugs which are cost effective and also with less or no side effects as compared to conventional drugs.

PLAN OF WORK

5. PLAN OF WORK

I. Collection of plant materials.

II. Authentification of plant materials.

III. Extraction of plant material by soxhlet extraction process.

IV. Preliminary phytochemical analysis.

V. Pharmacological evaluation

a. Acute toxicity studies
b. Antidiarrhoeal studies

Anti-diarrhoeal models:

- Charcoal meal model
- Castor oil induced model
- Castor oil enterpooling
- Magnesium sulphate induced model

Parameters to be assessed in;

Antidiarrheal studies

- Distance travelled by the charcoal meal
- Content of the intestine
- Weight of wet and dry faecal matter
- Onset of diarrhea

VI. Statistical analysis

One-way ANOVA followed by dunnett's.

MATERIALS AND METHODS

6. MATERIALS AND METHODS

Chemical used

Castor oil – Erand Dabur

Loperamide - Torrent pharma Ltd.

Diethyl ether - Nice chemicals Ltd.

Chloroform - Nice chemicals Ltd.

Selection of animals

Wistar albino rats weighing around 150-350gms were selected for the experiment. The animals were checked to confirm that they were free from any disease. The rats were collected from the animal house of Nandha College of Pharmacy and Research Institute, Erode-52.

Maintenance of animals

The rats that were selected were brought into the laboratory 2 days before the commencement of experiment for acclimatization. They were provided with standard laboratory rodents chow diet obtained from (Pranav agro industries Ltd, Bangalore) and free access to water. A 12 hour day and dark cycle and room temperatures at 27^{o}C were maintained.

Plant collection and authentification

Plant Collection

Phyllanthus amarus was collected in and around Veppampalayam in Erode District.

Authetification

The plant was identified by botanist, Botanical Survey of India, Tamil Nadu Agricultural Campus, Coimbatore bearing reference number 3227 and deposited in herbarium for future reference.

Plant Material

After identification of the plant, the whole plant was washed to remove dust particles and was allowed to air dry in a shade for complete drying. The dried leaves were then powdered in a mixer grinder.

Animal approval

The study was conducted after obtaining the approval from Institutional Animal Ethics Committee (IAEC), and the experimental procedures were in accordance to the guidelines of proposal No: NCP/IEAC/2017-18/06.

EXTRACTION OF PLANT MATERIAL [10-42]

Ethanolic extraction

The coarse powder was packed tightly in the soxhlet apparatus and extracted with 500ml 70% ethanol for 72 hours with occasional shaking maintained at 78°C throughout the extraction process. The extract was concentrated to ¼ of its original volume by evaporation. The resulting extract of *Phyllanthus amarus* was subjected to phytochemical study.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

The concentrated ethanolic extract of *P. amarus* was subjected to qualitative analysis for studying different constituents present in the extract and the following were reported;

Test for Alkaloids:

I. Mayers test: 3 ml ethanolic extract was stirred with 3 ml of 1% HCl on steam bath. Mayers reagent was then added to mixture. Turbidity of the resulting precipitate was taken as an evidence for the presence of alkaloid.
II. Dragendorff Test - To 2 ml of the extract solution, Dragendroff reagent (potassium bismuth iodide solution) was added. Orange brown precipitate is formed.
III. Wagner Test -To 2 mlof the extractsolution, Wagner reagent(iodine potassium iodide solution) is added. Reddishbrown precipitate is observed.
IV. Hager's Test - To 2 ml of the extract solution,Hager's reagent (saturated solution of picric acid) wasadded. Yellowprecipitate is observed.

Test for Tannins: About 2 ml of the ethanolic extract was stirred with 2 ml of distilled water and few drops of FeCl3 Solution were added. Formation of green precipitate was indication of presence of tannins.

Test for Saponins: 5 ml of ethanolic extract was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

Test for Phlobatannins: About 2 ml of ethanolic extract was added to 2 ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatannins.

Test for Flavonoids:

I. Shinoda Tests - To 2 ml of the extract solution, few magnesium turnings, 5 ml 95% ethanol and few drops of conc. HCl was added. Pink to red colour develops.
II. Zn-HCl Reductiontest - To 2 ml of the extract solution mixture of Zn dust and conc. HCl. was added Precipitate develops.

Test for Terpenoids: 2 ml of the 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. Development of a greyish colour indicates the presence of terpenoids.

Tests for glycosides: Liebermann's test: 2 ml of the extract was dissolved in 2 ml of chloroform and then 2 ml of acetic acid was added in it. The solution was cooled well in ice. Sulphuric acid was then added carefully. A colour change from violet to blue to green indicates the presence of a steroidal nucleus.

Tests for steroids: Production of red colour in the lower chloroform layer when 2 ml of the extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid which indicate the presence of steroids. On the other hand, development of a greenish colour when 2 ml of the extract was dissolved in 2 ml of chloroform and treated with sulphuric and acetic acid which indicate the presence of steroids.

Test for carbohydrates: Molish's Test

To 2ml of the extract solution few drops of molish's reagent and conc. sulphuric acid was added in the test tube.Violet colored ring appears at the junction of two liquid.

Test for reducing sugars

I. Fehling Test - To 2 ml of the extract solution few drops of Fehling's solution A and Fehling's solution B was added and boiled for 5 min. on boiling water bath. Yellow or red colour develops.

II. Benedict's Test - To 2 ml of the extract solution few drops of Benedict's reagent was added and the mixture was boiledon water bath for 5 min. Green colour shows the presence of reducing sugars.

Test for Proteins: Biurete test: To 2 ml of the extract solution Biurete reagent was added and boiled for few minutes. Violet colour develops.

Pharmacological screening

Acute toxicity studies

The study was done in two phases, mice of average weight of 20g were divided into 4 groups of three animals each (n=6). 3males and 3 females were selected. Animals received 2g, 5g/kg of the extract. Observation was done over a period of 24 hours for 14 days, mortality and the response of the animals noted too as per OECD guidelines 423. Behavioural changes like excitability, convulsions, lethargy, sleep were monitored at least once daily for 14 days. LD50 was calculated using the formula:

$$LD50 = \sqrt{C \times D}$$

Where;

C= the highest dose at which no death occurred

D= the least dose at which death occurred

During the study, no treatment related effects on the general condition or behaviour was observed on experimental animals in the course of two weeks. So the dose of 250 and 500 mg/kg was selected for my study.

ANTIDIARRHOEAL ACTIVITY

Selection of animals

Wistar albino rats weighing around 150-350gms were selected for the experiment. The animals were checked to confirm that they were free from any disease. The rats were collected from the animal house of Nandha College of Pharmacy and Research Institute, Erode-52.

Maintenance of animals

The rats that were selected were brought into the laboratory 2 days before the commencement of experiment for acclimatization. They were provided with standard laboratory rodents chow diet obtained from (Pranav agro industries Ltd, Bangalore) and free access to water. A 12 hour day and dark cycle and room temperatures at 27^{o}C were maintained.

Charcoal meal test- gastrointestinal motility

The wistar rats weighing 150-350gm were fasted for 18 hours with free access to water and libitum. At first castor oil was administered orally to these animals. Group I normal control received normal saline orally, Group II standard received loperamide 3 mg/kg orally, Group III and IV received test drug (EEPA) 250 and 500 mg/kg respectively.

Group I: Normal control (Normal saline solution and 1ml/kg charcoal meal), orally.

Group II: Standard (Loperamide 3mg/kg and 1ml/kg charcoal meal), orally.

Group III: EEPA (250mg/kg and 1ml/kg charcoal meal), orally.

Group IV: EEPA (500mg/kg and 1ml/kg charcoal meal), orally

One hour after treatment, each rat was given 1ml/kg of 5% charcoal suspension. After 30 minutes the animals were sacrificed by cervical dislocation and were dissected. The entire length of the intestine (from the pylorus to the caecum) was removed and placed length wise on a white paper. The distance travelled by the charcoal meal and the total length of the intestine was measured. The peristaltic index was calculated.

Castor - oil induced diarrhea

The induction of diarrhea with castor oil results from the action of ricimoleic acid formed by hydrolysis of oil which produces changes in the transport of water and eletrolyte results in hypersecretory responses.

Wistar rats weighing 200-350gms were fasted for 18hrs with free access to water and libitum. They were housed individually in cages and divided into 4 groups of six animals. Group I served as control and received normal saline 1ml/kg orally. Group II received standard drug loperamide 3 mg/kg p.o. Group III and IV received ethanolic extract 250 and 500 mg/kg p.o.

Group I: Normal control (Normal saline solution), orally.

Group II: Standard (Loperamide 3mg/kg), orally.

Group III: EEPA (250mg/kg), orally.

Group IV: EEPA (500mg/kg), orally

After 1 hour later, castor oil 0.1 ml/ rat was administered orally. The animals were then caged singly in cages lined with white blotting paper. The total number of both dry and wet feces excreted were measured after 4 hours and compared with the control group.

Castor oil enterpooling

Wistar rats weighing 200-350gms were fasted for 18hrs with free access to water and libitum. They were housed individually in cages and divided into 4 groups of six animals. Group I served as control and received normal saline 1ml/kg orally. Group II received standard drug loperamide 3 mg/kg p.o. Group III and IV received ethanolic extract 250 and 500 mg/kg p.o.

Group I: Normal control (Normal saline solution), orally.

Group II: Standard (Loperamide 3mg/kg), orally.

Group III: EEPA (250mg/kg), orally.

Group IV: EEPA (500mg/kg), orally

After 1 hour later, castor oil 0.1 ml/ rat was administered orally. After 30 min following administration of castor oil, all the rats were sacrificed by overdose of di ethyl ether. The whole length of the intestine from pylorus to caecum was dissected out, its content was collected in measuring cylinder and volume measured.

Magnesium sulphate induced diarrhea

Wistar rats weighing 200-350gms were fasted for 18hrs with free access to water and libitum. They were housed individually in cages and divided into 4 groups of six animals. Group I served as control and received normal saline 1ml/kg orally. Group II received standard drug loperamide 3 mg/kg p.o. Group III and IV received ethanolic extract 250 and 500 mg/kg p.o.

Group I: Normal control (Normal saline solution), orally.

Group II: Standard (Loperamide 3mg/kg), orally.

Group III: EEPA (250mg/kg), orally.

Group IV: EEPA (500mg/kg), orally

After 1 hour later, magnesium sulphate 1 ml/ rat was administered orally. The animals were then caged singly in cages lined with white blotting paper. The total number of both dry and wet feces excreted were measured after 4 hours and compared with the control group.

RESULTS

7. RESULTS

Table 1: Qualitative pytochemical Analysis of *Phyllanthus amarus* S.

Phytochemicals	EEPA
1. Flavanoids	+
2. Glycosides	+
3. Carbohydrates	+
4. Alkaloids	+
5. Triterpenoids	+
6. Proteins	+
7. Saponins	+
8. Steroids	+
9. Tannins	++
10. Reducing sugars	+

++: high content, +:moderate, -: negative

From the qualitative pytochemical analysis of the ethanolic leaf extract of *Phyllanthus amarus* S.,flavinoids, glycosides, carbohydrates, alkaloids, triterpenoids, proteins, steroids, saponins, reducing sugars and a high amount of tannins.

Table 2: Effect of EEPA on charcoal meal induced hyperperistalsis

Group	Mean length of small intestine (cm)	Mean distance travelled by charcoal (cm)	Peristalsis index (%)
Control	84.6 ± 0.94	54.2 ± 0.78	64.06 ± 0.72
Castor oil	85.2 ± 0.88	72.6 ± 0.92	85.21 ± 0.56[a]
Castoroil+Loperamide	87.4 ± 0.66	41.9 ± 0.75	47.94 ± 0.62[b]
Castor oil+250 mg/kg	83.9 ± 0.91	50.1 ± 0.84	59.71 ± 0.88 [b]
Castor oil+500 mg/kg	86.2 ± 0.84	48.4 ± 0.81	56.14 ± 0.76 [b]

Values mean ± SEM, n=6, [a]P<0.01 vs control, [b]P<0.01 when compared to castor oil treated group.

The anti-diarrheal activity of the ethanolic leaf extract *Phyllanthus amarus* S. evaluated by charcoal meal hyperperistalsis test. EEPA extract at a dose of 250 and 500 mg/kg showed a significant (P<0.01) anti-diarrheal activity when compared with that of castor oil treated animals. Among the two doses 500 mg showed the maximum anti-diarrheal activity (56.14±0.76) and that of the standard castor oil (47.94± 0.62). The distance travelled by charcoal in castor oil was more. The extracts showed significant (p<0.01) anti-diarrheal activity.

Graph 1: Effect of EEPA on charcoal meal induced hyperperistalsis.

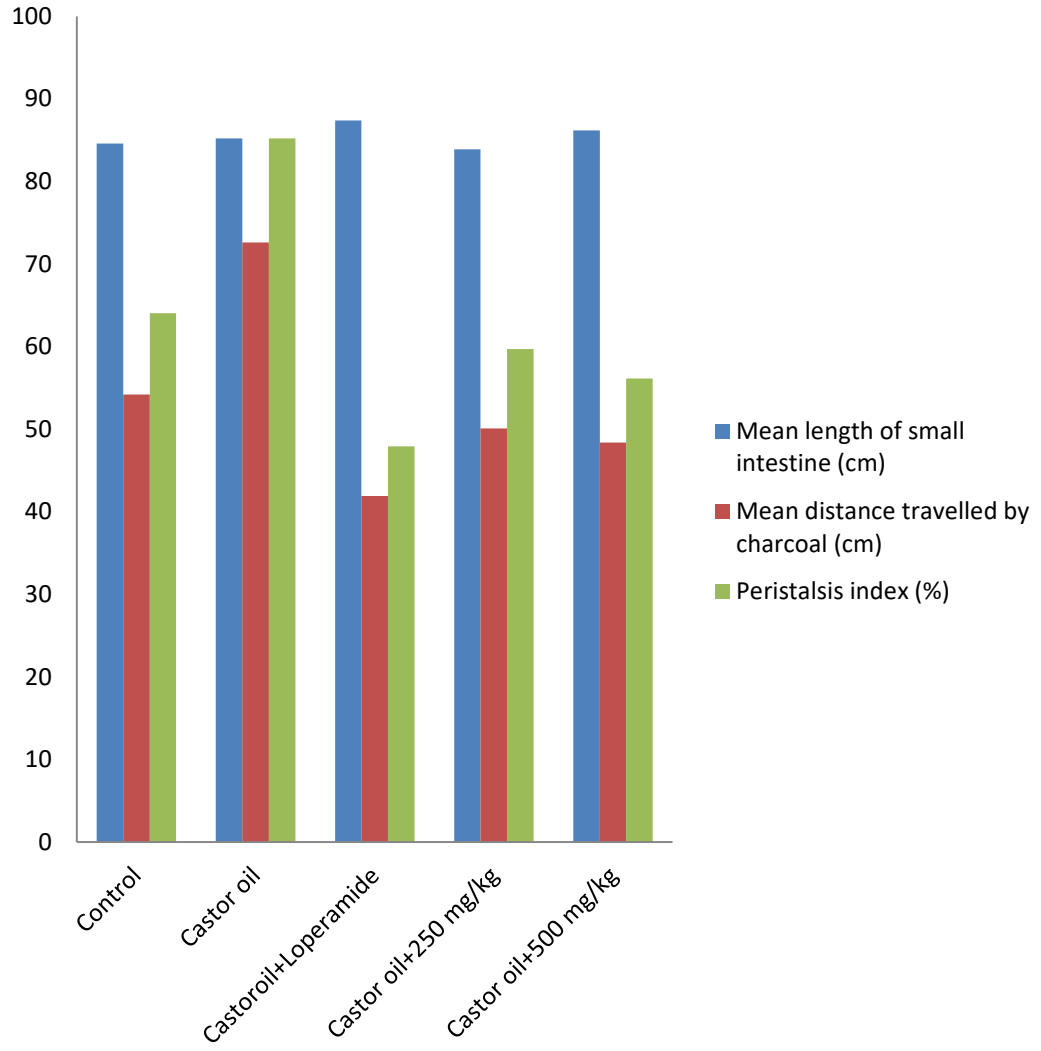


Table 3: Effect of EEPA on anti-diarrhoeal effect on castor oil induced diarrhea in wistar rats

Group	Water intake (ml)	Feed intake (gm)	Weight of fecal matte in 4 hours (gm)
Control	17.8 ± 0.6	16.26 ± 0.5	3.92 ± 0.04
Castor oil	21.9 ± 0.7[a]	17.24 ± 0.4[a]	6.06 ± 0.02[a]
Castoroil+Loperamide	18.7 ± 0.9[c]	15.42 ± 0.5[c]	4.38 ± 0.06[c]
Castor oil+250 mg/kg	20.1 ± 0.8[c]	16.64 ± 0.8 [b]	5.34 ± 0.04[b]
Castor oil+500 mg/kg	19.2 ± 0.7[c]	16.48 ± 0.7 [c]	4.88 ± 0.06[c]

Values mean ± SEM, n=6, [a]P<0.01 vs control, [b]P<0.05, [c]P<0.01 when compared to castor oil treated group

There was a significant anti-diarrheal effect according to the parameters observed and the results analysed. The castor oil treated rats showed a significant (p<0.01) increase in water, feed intake weight of fecal matter when compared to normal animals. The ethanolic leaf extract of *Phyllanthus amarus* S., showed a significant (p<0.01) decrease in water, feed intake weight of fecal matter when compared to castor oil treated rats. The maximum anti-diarrheal effect of the extract 500 mg/kg (4.88 ± 0.06) and that of 250 mg/kg extract (5.34 ± 0.04), but it was lower than standard drug Loperamide treated animals.

Graph 2: Effect of EEPA on anti-diarrhoeal effect on castor oil induced diarrhea in wistar rats.

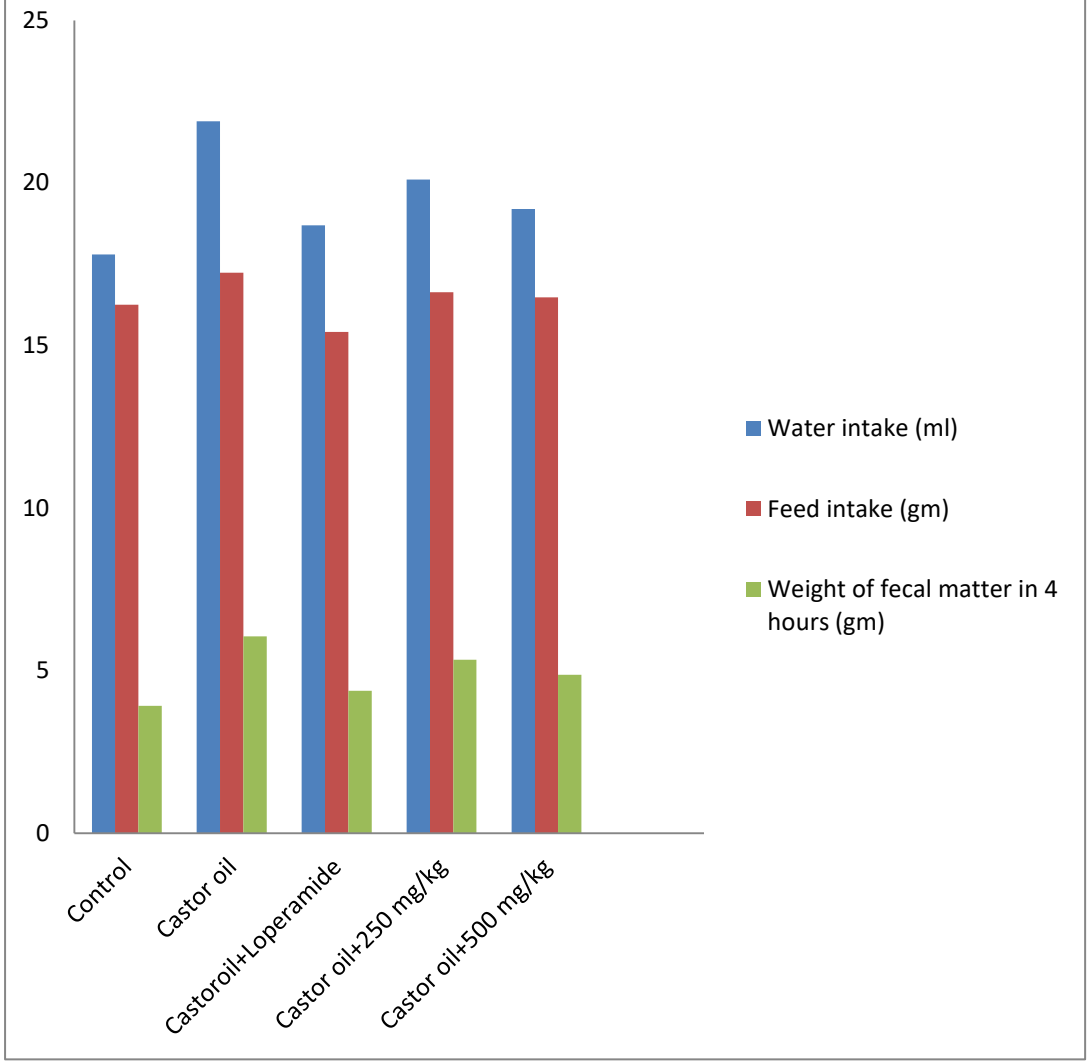


Table 4: Effect of EEPA on Castor oil enterpooling in wistar rats

Group	Weight of intestin content (gm)	% inhibition in wt intestinal content (gm)
Control	4.3 ± 0.6	----
Castor oil	6.9 ± 0.5[a]	----
Castoroil+Loperamide	5.1 ± 0.3[c]	26.08
Castor oil+250 mg/kg	5.8 ± 0.2[c]	15.94
Castor oil+500 mg/kg	5.4 ± 0.4[c]	21.74

Values mean ± SEM, n=6, [a]P<0.01 vs control, [c]P<0.01 when compared to castor oil treated group

The anti-diarrheal activity of the ethanolic leaf extract of *Phyllanthus amarus* S. evaluated by castor oil enterpooling test. The castor oil treated rats revealed significant (P<0.01) increase in weight of intestinal content when compared to normal rats. The EEPA at a dose of 250 and 500 mg/kg treated rats showed significant (P<0.01) decrease (5.4 ± 0.4 and 5.8 ± 0.2) in weight of the intestinal content and 15.94 and 21.74 % inhibition in weight of intestinal content compared to castor oil treated rats. The castor oil and standard drug loperamide treated rats showed maximum (21.74 %) inhibition of weight of intestinal content. EEPA extract showed anti diarrheal effect in dose depentant manner, the 500mg/kg showed maximam response and it was lower than loperamide treated rats.

Graph 3: Effect of EEPA on Castor oil enterpooling in wistar rats

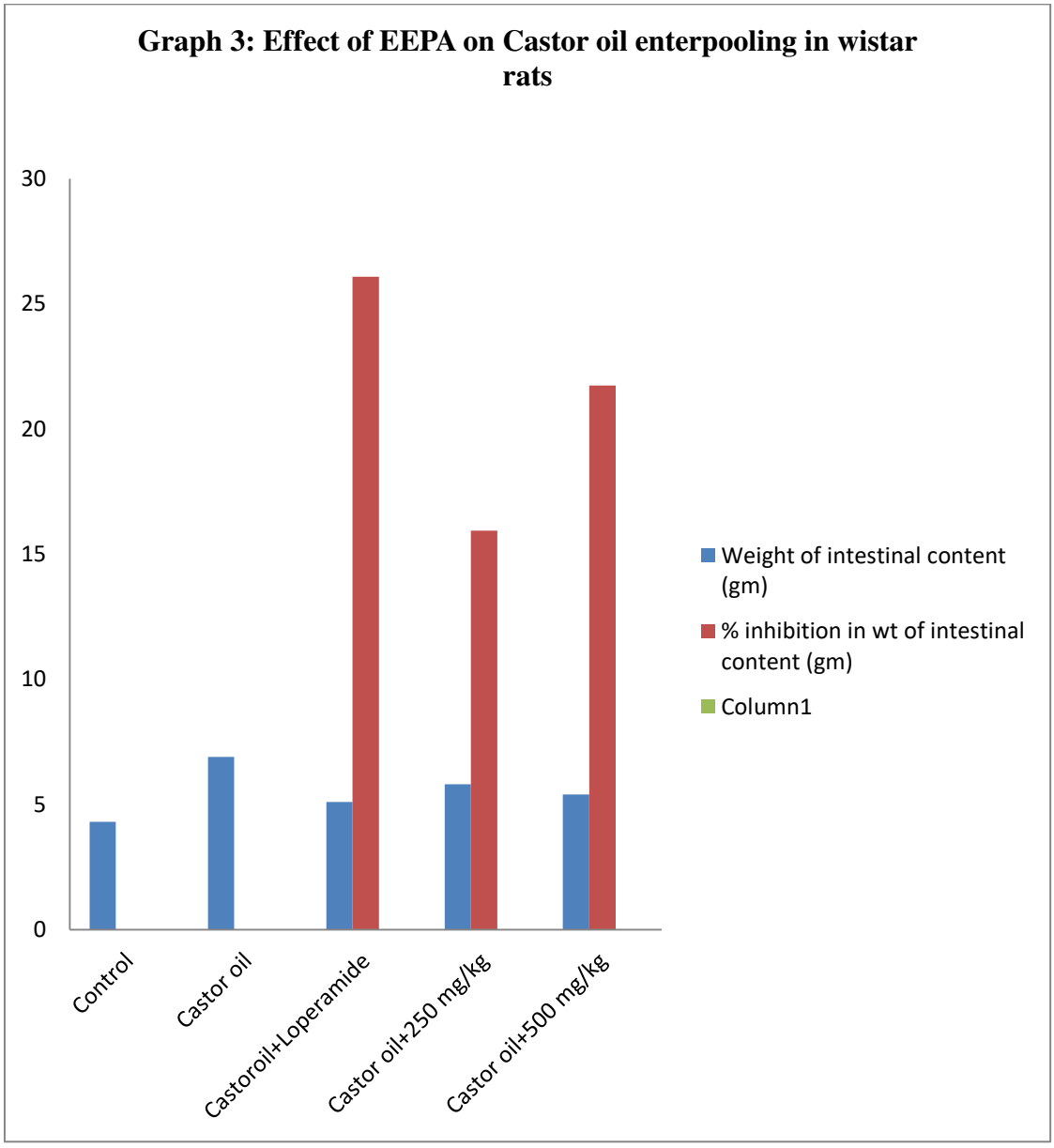


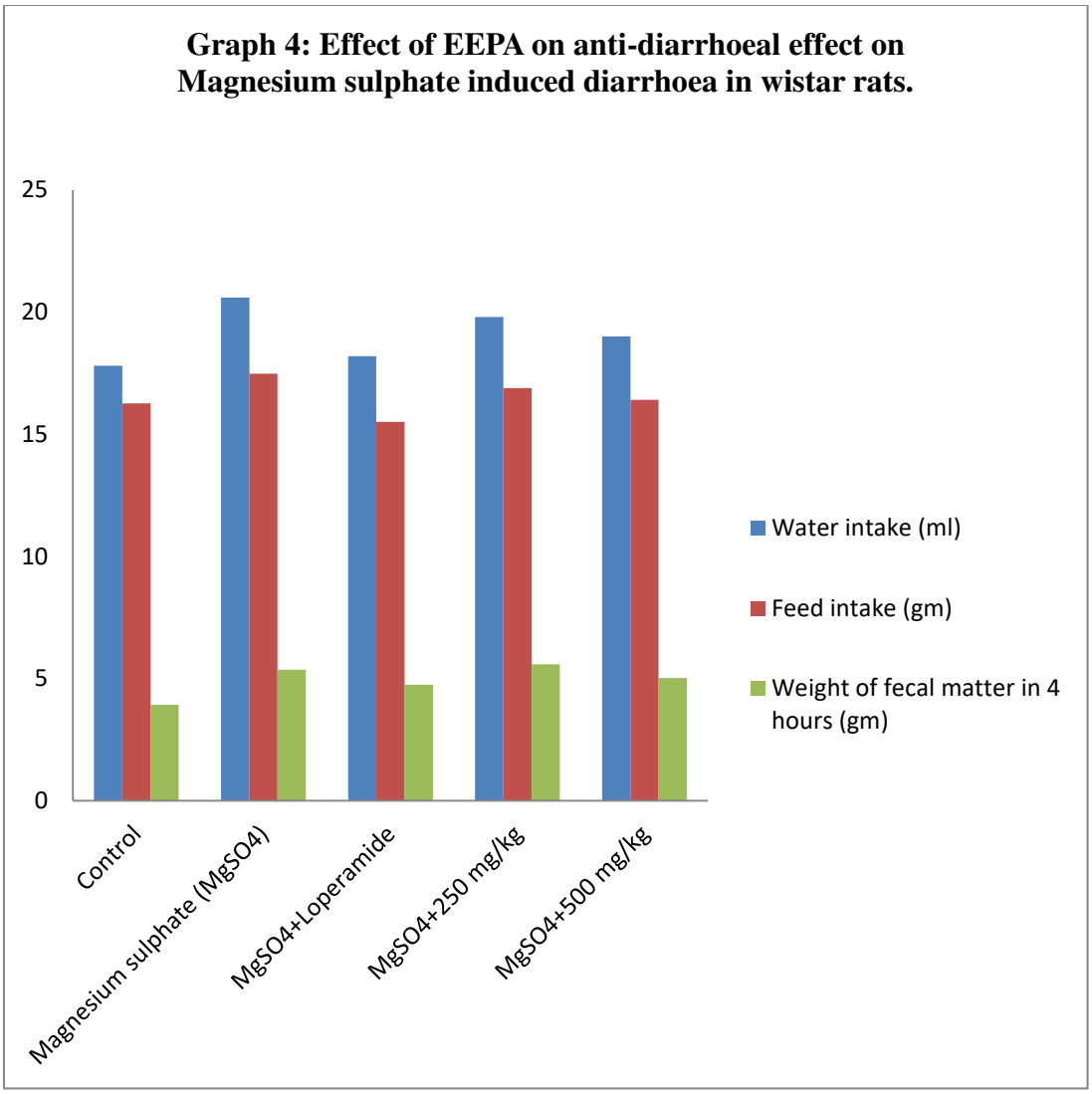
Table 5: Effect of EEPA on anti-diarrhoeal effect on Magnesium sulphate induced diarrhoea in wistar rats

Group	Water intake (ml)	Feed intake (gm)	Weight of fec matter in 4 hou (gm)
Control	17.8 ± 0.6	16.26 ± 0.5	3.92 ± 0.04
Magnesium sulpha ($MgSO_4$)	20.6 ± 0.5[a]	17.48 ± 0.3[a]	5.36 ± 0.06[a]
$MgSO_4$+Loperamide	18.2 ± 0.8[c]	15.51 ± 0.4[c]	4.74 ± 0.03[c]
$MgSO_4$+250 mg/kg	19.8 ± 0.9[c]	16.89 ± 0.6[b]	5.58 ± 0.06[b]
$MgSO_4$+500 mg/kg	19.0 ± 0.7[c]	16.41 ± 0.4[c]	5.02 ± 0.05[c]

Values mean ± SEM, n=6, [a]P<0.01 vs control, [b]P<0.05, [c]P<0.01 when compared to $MgSO_4$ treated group

The anti-diarrheal activity of the ethanolic leaf extract of *Phyllanthus amarus* S. evluated by magnesium sulphate induced diarrhea in wistar rats. The anti-diarrheal activity was analysed by evaluating the parameters such as water intake, feed intake and weight of fecal matter. The magnesium sulphate induced diarrhea was confirmed by significant (P<0.01) elevation in water intake, feed intake and weight of fecal matter when compared to normal rats. The EEPA at 250 and 500 mg/kg treatment showed significant decrease in water intake, feed intake and weight of fecal matter when compared to magnesium sulphate treated rats. The weight of the fecal matter in 4 hours reduced significantly (5.02 ± 0.05) at 500 mg/kg as compared to (5.58 ± 0.06) EEPA treatment. The extracts showed significant (p<0.01) antidiarreal activity but it was lesser than standard drug loperamide treated animals.

Graph 4: Effect of EEPA on anti-diarrhoeal effect on Magnesium sulphate induced diarrhoea in wistar rats.



DISCUSSION

8.DISCUSSION

Diarrheas have long been recognised as one of the most common health problems in the developing countries. Imbalance in the absorption and secretion mechanisms in the intestinal tract result in frequent loss of watery stool (diarrhea). There are several mechanisms proposed to explain the diarrheal effect of castor oil including inhibition of intestinal Na+ K+ ATPase activity, consequently reducing normal fluid absorption, activation of adenylatecyclase or mucosal cAMP-mediated active secretion, and stimulation of prostaglandin formation and platelet activating factor. Prolong onset of diarrhea, reduction of gastrointestinal motility and inhibition of synthesis of prostaglandins observed in this study to support traditional uses of *Phyllanthus amarus.*[62]

Phyllanthus amarus isan ancient herb with utmost medicinal value. The herb has been proved to be of importance in hepato-toxicity, hyperglycaemia and in microbial and bacterial infections. Its been also proved to be excellent in wound healing due to the anti-oxidant nature of its phytoconstituents.

Results obtained from the study showed that the leaf of *Pyllanthus amarus* contained alkaloids, flavonoids, saponins, steroids, terpenoids and other constituents. These phytoconstituents may account for the traditional medicinal uses of the leaves of this herb to manage a number of bacterial and fungal infections. Tannins which are the major anti-diarrheal constituents denature the proteins forming tannates which reduce the intestinal mucosa permeability making the intestinal mucosa more resistant and reduce secretion.[5-6]

This study involved Evaluation of anti-diarrheal activity of the Ethanolic Leaf Extract of *Phyllanthus amarus* preliminary of castor oil induced diarrhea in albino rats using loperamide as the positive control. The use of castor oil induced diarrhea model is because of the autocoids and prostaglandins are involved as they implicate in the causation of diarrhoeas. Ricinoleic acid from castor oil results in irritation and inflammation of intestinal mucosa leading to release of prostaglandins which stimulate motility and secretion.There was a significant anti-diarrheal effect according to the parameters observed and the results analysed. The castor oil treated rats showed a significant increase in water, feed intake weight of fecal matter when compared to normal animals. The ethanolic leaf extract of *Phyllanthus amarus* S., showed a

significantdecrease in water, feed intake weight of fecal matter when compared to castor oil treated rats. This illustrates that the plant extract has the effectiveness of the anti-diarrheal activity.[52-53]

The significant inhibition of castor oil induced enterpooling in rats show that *Phyllanthus amarus* leaf extract produce relief in diarrhea by spasmolytic activity, overall mechanism being inhibition of release of autocoids and prostaglandins this inhibiting secretion by castor oil. The parameters that were observed in this model were the weight of intestinal content and the percentage inhibition of the intestinal content. The castor oil treated rats revealed significant increase in weight of intestinal content when compared to normal rats. The EEPA at a dose of 250 and 500 mg/kg treated rats showed significant decrease and in weight of the intestinal content.[25-26]

Charcoal meal hyper-peristasis model was used to investigate the anti-diarrheal activity of EEPA by using the length of the small intestines, distance travelled by charcoal meal parameters to find the peristalsis index. The reduction of gastrointestinal motility is one of the mechanisms by which antidiarrheal agents can act. It was observed that the EEPA significantly suppressed the propulsion of charcoal meal at all tested doses. This finding suggests that this extract has the ability to influence the peristaltic movement of intestine thereby indicating the presence of an antimotility activity.[27]

Magnesium sulphate is an osmotic laxative. It induces diarrhea by promoting cholecystokinin release from the duodenal mucosawhich thus preventing the reabsorption of sodium chloride and water from the lumen. Loperamide had anti-diarrheal activities of both castor oil and magnesium induced diarrhea. The parameters observed were the water and feed intake and the weight of the fecal matter in a range of 4 hours. The results showed elevation in water intake, feed intake and weight of fecal matter when compared to normal rats. . The EEPA at 250 and 500 mg/kg treatment showed significant decrease in water intake, feed intake and weight of fecal matter when compared to magnesium sulphate treated rats. The weight of the fecal matter in 4 hours reduced significantly at 500 mg/kg as compared to EEPA treatment. The extracts showed significant antidiarreal activity but it was lesser than standard drug loperamide treated animals. This shows the EEPA was effective in the treatment and management of diarrhea.[7]

Standard drug loperamide is one of the most efficient and widely used drug. Loperamide is an mμ opiate receptor agonist that extends the oroecal and colonic transit

time by increasing the gut activity, disarranging electrical activity in the gut and delaying the passage of fluid via the ileum. The anti-diarrheal activity of the plant was compared with that of the standard drug. Loperamide reduces the diarrhea induced by castor oil, prostaglandins effectively. It also slows the intestinal transit, colon flow rate and colonic motility. Reduction in the intestinal motility is associated with inactivating calcium ion influx through voltage gated calcium channels.

Loperamide being a synthetic drug which is widely used in the treatment and management of diarrhea, although it is relatively safe at therapeutic doses, life threatening loperamide toxicity can result in new clinical syndrome of loperamide-induced toxicity. Others may be dizziness, stomach pain, discomfort or enlragement, vomiting, dry mouth and dizziness. The plant extract of *Phyllanthus amarus* S. is equally used in the treatment of diarrhea as per the present as it may have lesser side effects than those of the synthetic drug loperamide.

SUMMARY

9. SUMMARY

The current thesis entitled 'Evaluation of anti-diarrhoeal activity of ethanolic leaf extract of *Phyllanthus amarus* S'. deals with the exploration of pharmacological and phytochemical screening of the selected Indian medicinal plant *Phyllanthus amarus* S. belonging to family Euphorbiaceae. Preliminary phytochemical screening of *Phyllanthus amarus* S. was done and the results obtained revealed the presence of saponins, tannins, triterpenoids, steroids, flavonoids, alkaloids, proteins, carbohydrates.

The plant was collected and got authentification from Botanical Survey of India Southern regional center, Coimbatore with the voucher number 3227. Approval was obtained from the committee for the purpose of control and supervision of experimental animals (CPCSEA) and Institutional Animal Ethics Committee (IAEC), proposal number NCP/IAEC/2017-2018/06.

After identification, the whole plant was washed to remove dust particles and was allowed to air dry in a shade for complete drying. The dried leaves were then powdered in a mixer grinder. The coarse powder was packed tightly in the soxhlet apparatus and extracted with 500ml 70% ethanol for 72 hours with occasional shaking maintained at 78^{o}C throughout the extraction process. The extract was concentrated to ¼ of its original volume by evaporation. It was then stored in refrigerator for further use. The acute toxicity studies of the plant were done using mice with oral doses of 2g and 5g/kg. The animals were observed for toxic symptoms like behavioural changes, locomotion, convulsions and mortality for 14 days. Thus, the dose was identifies as per OECD 423 guidelines.

The models used to assess the ant-diarrheal activity include charcoal meal test or gastrointestinal motility test, castor-oil induced diarrhea, castor-oil enterpooling and magnesium induced diarrhea. The EEPA appeared to act inhibition of diarrhea by reducing the absorption of water from the intestines that lead to the increase in defaecation. This is due to the presence of tannins present in *P. amarus.*

The findings in the present study confirm the anti-diarrheal activity of the ethanolic leaf extract of *Phyllanthus amarus* S. thus provide the scientific basis for the traditional use of this plant in the treatment of diarrhea and its effects.

CONCLUSION

10. CONCLUSION

The present study "Evaluation of anti-diarrheal activity by using ethanolic leaf extract of *Phyllanthus amarus* S." in experimental animal models was found effective in the anti-diarrhea activity due to the presence of tannins. This study strongly implies that the ethanolic leaf extract of *Phyllanthus amarus* can be used to treat diarrhea and its effects.

Further studies are required to evaluate the anti-diarrhea activity of *Phyllanthus amarus* S. ethanolic leaf extract in other models. The isolation of the active compound, its evaluation in experimental model and study of mechanisms of action will eventually yield and develop new drugs.

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