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Evaluation of Anti Diarrhoeal Activity of Methanolic Leaves Extract of *Artocarpus Heterophyllus* against Castor oil Induced Diarrhoea in Swiss Albino Mice

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ABSTRACT: Diarrhoeal disease is a leading cause of mortality and morbidity, especially among children in the developing countries resulting in a major health care problem. So, the present study was designed to investigate anti-diarrhoeal activity of ethanolic leaves extract of *Artocarpus heterophyllus* using the Swiss mice model of castor oil induced diarrhoea. Acute toxicity of the extract based on Organization for Economic Cooperation and Development-423 guidelines was performed. The anti-diarrhoeal effects of the extract on castor oil-induced diarrhoea in four groups of Swiss Albino mice were determined. The first and second groups received 5 and 200 mg/kg body weight (bw) of the extract, while the third and fourth groups received normal saline (5 mg/kg bw) and loperamide (5 mg/kg bw) as negative and positive controls, respectively. *Artocarpus heterophyllus* extract was used at 1.25% w/v to formulate structured vehicle (carboxyl methylcellulose, polyvinylpyrrolidone and tragacanth) suspensions. The suspensions were tested for pharmacological activity and characterized. Acute toxicity gave a lethal dose 50 (LD50) that is greater than 300 and less than 2,000 mg/kg bw. A reduction in intestinal transit by 0.14 and 0.15% at 5 and 200 mg/kg of the extract was achieved as compared to an inhibition of 0.12% by 5 mg/kg loperamide. There was a dose-dependent decrease in the frequency of watery stool passed in castor oil-induced rats by 35.29% and 64.70% at 5 and 200 mg/kg, respectively. All the suspensions inhibited diarrhoea, exhibiting a dose-dependent pattern and remained stable after 4 weeks. Their pH values ranged from 4.60±2.73 to 4.73±1.91, while viscosity ranged from 3.50±1.23 to 6.75±1.24 Pas at 60 rpm. The results suggest that *Artocarpus heterophyllus* possesses significant anti-diarrhoeal activity. Suspensions of *Artocarpus heterophyllus* were successfully formulated in structured vehicles and were effective in the control of diarrhoea in Swiss Albino mice.

I. GASTRO-PROTECTIVE EFFECT:

Gastrointestinal disorders are one of the severe classes of human ailments causing maximum discomfort, morbidity and mortality. Peptic ulcer is one such gut disorder. Peptic ulcer is a benign lesion of gastric or duodenal mucosa occurring at a site where the mucosal epithelium is exposed to acid and pepsin. There are several causes including, stress, alcohol consumption, cigarette smoking, *H. pylori* infection, ingestion of drugs and chemicals. Especially consumption of alcohol for a prolonged period, smoking of cigarettes, or chronic consumption of NSAIDs are causing peptic ulcers. The role of free radicals in the pathogenesis of peptic ulcer due to mucosal damage is established. The symptoms of peptic ulcer are severe pain and irritation in the upper abdomen. If it is not treated properly, it may result in perforations in the wall of the gastrointestinal tract. Therefore, normally chronic dietary control and pharmacotherapeutic management is adopted for treating peptic ulcer. Several classes of drugs like anti-histamines (H₂-blockers), proton pump inhibitors (Omeprazole, Lansoprazole, Anticholinergic agents (Pirenzepine), Prostaglandins (Misoprostol, Euprostil), Antacids (sodium bicarbonate, magnesium hydroxide gel, etc.),

Peptic Ulcer:

It is a chronic inflammatory condition involving a group of disorders characterized by ulceration in regions of upper gastrointestinal tract where parietal cells secrete pepsin and hydrochloric acid.

Signs and Symptoms:

In peptic ulcer, patients can be asymptomatic or experience anorexia, nausea, vomiting, bleeding and blotting and heart burn or epigastric pain.

Epidemiology:

The life time prevalence of peptic ulcer disease is 5 to 10% in the general population. There are approx 3.9 million patients with peptic ulcer disease in United States with 200,000 to 400,000 new cases reported each year. The peak incidence is between 50 and 70 years of age.

Etimology of Chronic Ulceration:

Heredity:

Patients with peptic ulcer often have a family history of the disease. This is particularly the case with duodenal ulcers, which develop below the age of 20 years. The relatives of gastric ulcer patients have 3 times the expected number of gastric ulcer but duodenal ulcer occurs with the same frequency amongst relatives as in the general population.

Acid-pepsin Vs Mucosal Resistance:

The immediate cause of peptic ulceration is digestion of the mucosa by acid and pepsin of the gastric juice, but the sequence of events leading to this is unknown. Digestion by acid and pepsin can't be the only factor involved, since the normal stomach is obviously capable of resisting digestion by its own secretion. The concept of ulcer aetiology may be written as "acid plus pepsin Vs mucosal resistance".

Gastric Hyper Secretion:

Ulcers occur only in the presence of acid and pepsin they are never found in achlorhydric patients such as those with pernicious anaemia. Acid secretion is more important in the etiology of duodenal than gastric ulcer. Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Considering the several side effects like Arrythmias, impotence, gynacoemastia and hematopoietic changes, of synthetic drugs, hence their usage for a chronic period is restricted. However, comparatively indigenous drugs possessing fewer side effects. Hence, the search for better alternatives for synthetic drugs is on rise. There is evidence concerning the participation of reactive oxygen species in the etiology and pathophysiology and human disease, studies has shown alterations in the antioxidant status following ulceration indicating that free radicals, seems to be associated with the pylorus ligation and ethanol induced ulceration in rats.

Different factors related to acid secretion

1. General factor:

Vagal hormonal effect, histamine and epinephrine, insufficient circulation, shock and general ischemia increase the acid secretion. Constitutional and environmental factors i.e. sex, age, temporarily, family history, social class, geographical different and occupation may also influence the acid release and local factors in stomach.

2. Aggressive Factor:

HCl, pepsin, refluxed bile, NSAIDs, alcohol, pancreatic proteolytic enzymes, ingested irritants, bacterial toxins, physiochemical trauma all of these factors increase the acid secretion.

3. Digestive Factors:

Mucus, bicarbonates, blood flow, resolution of epithelium, the current status of drug therapy.

II. CAUSE OF ULCER STRESS

Stress can arise from prolonged anxiety, tension and emotion, sever physical discomfort, hemorrhagic and surgical shocks, burns and trauma and there by resulting in sever gastric ulceration. The mechanism of gastric ulceration is poorly understood. Recently research has shown that restraint cold stress causes severe haemorrhagic ulcer through derangement of the mucosal antioxidant enzymes such as superoxide dismutase and peroxides. This is the stress condition arising mainly from physiological discomfort and the mechanism of ulceration caused in this case should be different from ulcer caused due to other factors. The stresses generate highly reactive OH- radicals that causes

oxidative damage of the gastric mucosa and that the radical is formed by metal catalysed. Harberweiss reaction between O₂ and H₂O₂ following induction of the superoxide dismutase and oxidative damage of gastric peroxidase.

Alcohol:

Alcohol causes secretion of gastric juice and decrease mucosal resistance. Protein content of gastric juice is significantly increased by ethanol. This could be due to leakage of plasma proteins in to the gastric juice with weakening of mucosal resistance barrier of gastric mucosa. This leads to peptic ulcer.

H. Pylori:

It is a gram negative bacteria found in gastric and duodenal mucosa of most persons particularly the elderly. Bacteria, while in the mucosa, split urea into ammonia and thus elevates the local pH, damage of local region of the mucosa by high alkalinity. In this way they strongly help the peptic ulcer development.

Treatment of Ulcer Antibiotics:

For the eradication of H. Pylori antibiotics like:

- Amoxicillin.
- Clarithromycin.
- Metronidazole and some time colloidal bismuth sub citrate has been used.

Tricyclic Antidepressant:

On theoretical grounds, tricyclic antidepressants should be helpful in treatment of peptic ulcers; few desirable like amitryptiline can block both Ach. & H₂ receptors.

Proton Pump Inhibitors:

Omeprazole, Lansaprazole are the two drugs belonging to the class of proton pump inhibitors omeprazole is prototype. H⁺ ion produced from HOH is pumped into canaliculus within the parietal cell by H⁺ K⁺ ATPase. Therefore, would result in no pumping of H⁺ into the canaliculus (where it unites with Cl⁻ ion to form HCl). There will be no HCl formed. Thus complete inhibition of proton pump must inhibit all HCl formation omeprazole binds with H⁺K⁺ ATPase and inhibits the H⁺K⁺ ATPase i.e. abolition of HCl formation.

Somatostatin Analogue:

Octreotide is a somatostatin analogue. It has been used chiefly against the hormone secreting tumors of GIT.

Carbenoxolone:

It is a derivative glycyrrhic acid (obtained from liquorice) It causes increased

- Secretion of gastric mucus.
- Increased life span of epithelial cells of the stomach and cytoprotective action.

Antacids

- Aluminum compounds.
- Magnesium compounds.
- Calcium carbonate and Sodium bicarbonate.



Mechanism of Action:

Antacids which are weak bases react with the HCl of the stomach to produce a salt plus water (thus removing HCl) raising the pH of gastric content, causing loss of peptic activity, blunting of aggressive factor.

Two types of Antacids:

1) Systemic:

This antacids are absorbed in fair extent by the GIT to produce systemic effect. Thus NaHCO_3 in good deal absorbed and many cans sodium overload syndrome. Thus these antacids are not favoured now a day.

2) Non-systemic Antacids:

These are not absorbed or only slightly absorbed by the GIT. However the daily dose of antacids are heavy, even the so called non systemic antacids.

H₂-Receptor Antagonist:

Cimetidine was first histamine H₂-receptor antagonist available for clinical use. Ranitidine and famotidine are also act as H₂-receptor antagonist like cimetidine. They are used as ulcer healing drugs. Cimetidine is a prototype, it reduces stimulated acid and pepsin by competitive antagonism of the action of histamine on H₂- receptor [1].

Anti-diarrhoeal property of *Artocarpus Heterophyllus*:

Artocarpus Heterophyllus Lam. belongs to the family Moraceae. *Artocarpus Heterophyllus* is a monoecious evergreen tree grown in several tropical and subtropical regions. Different parts of this tree have been used for ailments; leaves for stimulating lactation in women and animals, seeds for relieving sick, constipation and diarrhoea and the roots for alleviating asthma and fever [2]. The leaves are also useful to treat fever, ulcers, boil wounds, skin diseases and are anti-diarrhoeal, analgesic and immune modulator. The ripe fruits are sweet, cooling, laxative, aphrodisiac and tonic. Hence, in the present investigation, we evaluated the phyto-chemical constituents, free radical scavenging activities and antioxidant properties of the methanol extract of leaves of *Artocarpus Heterophyllus* [3].

III. MATERIALS AND METHODS

Chemicals and Reagents:

All the chemicals used in the study were of analytical grade and procured from Merck India Pvt. Ltd.

Plant Material:

The plant was identified at Leaves of *Artocarpus Heterophyllus* were collected from Local Garden of Ghaziabad Uttar Pradesh, India. The plant (Fig.1) was authenticated by the Department of Botanist, Dr. K.C Bhatt, Principle Scientist at [ICAR-] National Bureau of Plant Genetic Resources New Delhi, India, [Voucher No. (AC-11/2021) Jackfruit is an important tree in home gardens in India. Fresh leaves of *Artocarpus heterophyllus*, milling machine, methanol, electronic balance, rotary evaporator, filter paper, water bath, distilled water, viscometer and pH meter. DMSO, normal saline, castor oil, Swiss albino mice, gelatin, filter paper, and loperamide, pharmaceutical grades of tragacanth, carboxyl methyl cellulose (CMC), polyvinyl pyrrolidone (PVP), polysorbate 80, sodium chloride and glycerine were obtained from a chemical laboratory.

Medicinal Properties of Artocarpus Heterophyllus:

Fig. Artocarpus Heterophyllus



Qualitative Phytochemical Screening:

Chemical tests for screening and identification of bioactive chemical constituents present in the methanol extract of eaves of Artocarpus Heterophyllus were carried out using the standard procedures.

Quantitative Phytochemical Estimation:

Estimation of Total Phenol by Folin-Ciocalteu Reagent Method:

Folin-Ciocalteu method was used to determine the total Phenolic compounds with slight modifications. Methanol extract (0.05ml) of eaves of A. Heterophyllus was mixed with 0.5 mL of Folin Ciocalteu reagent (1:10 diluted with distilled water). After 5 min of aqueous Na₂CO₃ (20%) was added. The mixture 'was then allowed 10 stand for 30 min incubation in dark. The quantification of phenolic compounds was performed spectrophotometrically by measuring the absorbance in UV-VIS spectrophotometer at 765nm. The total phenolic content was expressed in terms of Gallic acid equivalent (mg/g of dry mass), which is a common reference compound.

Estimation of Total Flavonoids by AlCl₃ Method:

Aluminium chloride colorimetric method was used (o determine the total flavonoids with slight 'modifications. Methanol extract (0.1ml) o leaves of A. *Heterophyllus* was mixed with 0.5ml of 10 % aluminium chloride, 0.5ml of 1 M potassium acetate and 0.5ml of distilled water. It was incubated at room temperature for 30min. The absorbance of the reaction mixture was measured by spectrophotometer at 415nm. The total flavonoids content was expressed in terms of quercetin equivalent, which is a common reference standard.

In vitro Antioxidant Assay:

DPPH Radical Scavenging Assay:

The antioxidant activity of methanol extract of leaves of A. *Heterophyllus* was measured on the basis of the scavenging activity of the stable 1, 1-diphenyl 2-picrylhydrazyl (DPPH) free radical with slight modifications. 1mL of 0.1mM DPPH solution in methanol was mixed with L. of various concentrations (10-70g/mL) of methanol extract of leaves of A.heterophyllus. The mixture was then allowed to stand for 30 min incubation in dark. Ascorbic acid was used as the reference standard. Mixer of L methanol and ml DPPH solution was used as the control. The decrease in absorbance was measured using UV-Vis spectrophotometer at 517 nm.

$$\% \text{ of DPPH Radical Inhibition} = \text{Control Sample/Control} \times 100$$

Hydroxyl Radical (OH[•]) Scavenging Activity:

Various concentrations (2-14pg/ml) of methanol extract (1ml) of leaves of *A.heterophyllus* were added with 1.0mL of iron-EDTA solution (0.13% ferrous ammonium sulphate and 0.6% EDTA), 0.5mL of EDTA solution (0.018%), 1.0ml of dimethyl sulphoxide (DMSO) (0.85% /v in 0.1m phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5mL of ascorbic acid (0.22%) and incubated at 80- 90°C for 15min in a water bath. After incubation, the reaction was terminated by the addition of 1.0mL of ice-cold TCA (17.5% w/v). An amount of 0.5ml of Nash reagent (75.0g of ammonium acetate, 3.0ml of glacial acetic acid, and 2ml of acetyl acetone were mixed and made up to 1-L with distilled water) was added and Left at room temperature for 15min. The reaction mixture without sample was used as control. "The intensity of the colour formed was measured spectroscopically at 412nm. Ascorbic acid was used as the reference standard. The percentage of inhibition was calculated using the following formula.

Phospho-Molybdenum Reduction Assay:

'The antioxidant capacity of the methanol extract of leaves of *A. Heterophyllus* was assessed as described by Pricto et al. The methanol extract of leaves of *A. heterophyllus* in dilution from 10 to 70pg/ml was combined with reagent solution containing ammonium molybdate (4mm). Na-phosphate (28mm) and sulphuric acid (600mm). The reaction mixture was incubated in a water bath at 90°C for 90min. The absorbance of the coloured complex was measured at 695nm. Ascorbic acid was used as standard reference. The methanol extract of leaves of *A. Heterophyllus* was taken for various qualitative phytochemical tests and it showed the presence of various phytoconstituents such as terpenoids, flavonoids, steroids, Phenolic compounds, glycosides, carbohydrates and saponins as depicted in Table I.

Table.1: Qualitative Phytochemical screening of methanol extract of leaves of *Artocarpus Heterophyllus*

S. No.	Phytoconstituents	Chemical reagents	Results
1	Terpenoids	CHCl ₃ + cone. H ₂ SO ₄ ;	+++
2	Flavonoids	NaOH solution	+
3	Phenols	FeCl ₃ solution	+
4	Steroids	Acetic anhydride + cone. H ₂ SO ₄	+
5.	Glycosides	5% NaOH + Fehling's solution	+
6	Carbohydrates	α-Naphthol + conc. H ₂ SO ₄	+
7.	Saponins	Foam test	+

+ Present ++ Strongly present

'The total phenolic content (estimated by Folin-Ciocalteu method) in the methanol extract of leaves of *A. heterophyllus* was 524.86 mg/g and the total flavonoid content (estimated by AICI method) was 86.75 mg/g (Table 2). Free radicals are produced under certain environmental conditions and during normal cellular function in the body; these molecules are missing in an electron, giving them an electric charge. To neutralize: this charge, free radicals try to withdraw an electron from, or donate an electron to a neighbouring molecule. 'The newly create free radical, in turn, looks out for another molecule and withdraws or donates an electron, setting off a chain reaction that can damage hundreds of molecules. Antioxidants such as phenolic compounds and flavonoids halt this chain reaction. These antioxidants are themselves free radical, donating electrons to stabilize and neutralize the dangerous free radicals. The phenolic compounds and flavonoids present in the methanol extract of leaves of *A. heterophyllus* work against the molecules that form free radicals, destroying them before they can begin the domino effect that leads to oxidative damage.

Table.2: Quantitative phytochemical estimation of phenols and flavonoids of methanol extract of leaves of *Artocarpus Heterophyllus*

S. No.	Component	Quantity (mg/g of extract)
1	Phenols	524.86
2	Flavonoids	86.75

DPPH is a stable free radical, usually used as a substrate to evaluate antioxidant activity.

Table.3: Hydroxyl (OH[•]) and nitric oxide (NO[•]) radical scavenging activities of methanol extract of leaves of *A. Heterophyllus*.

S. No.	Conc. µg/ml	% of Inhibition	
		OH	NO
1	2	5.91±0.41	10.87±0.76
2	4	11.26±0.78	33.69±2.35
3	6	23.49±1.64	43.48±3.04
4	8	25.34±1.77	55.43±3.88
5	10	30.61±2.14	60.87±4.26
6	12	46.14±3.22	65.22±4.56
7	14	51.60±3.61	68.48±4.79

Table.4: DPPH, OH and nitric oxide (NO[•]) radical scavenging activities of Standard Ascorbic Acid

S. No.	Conc. µg/ml	% of Inhibition		
		DPPH	OH	NO
1	2	11.98±0.83	22.00±1.54	27.14±1.89
2	4	17.70±1.23	36.04±2.52	35.26±2.46
3	6	25.48±1.78	49.14±3.43	48.00±3.36
4	8	37.25±2.60	56.89±3.98	54.28±3.79
5	10	60.62±4.24	62.24±4.35	60.00±4.20
6	12	70.95±4.96	74.23±5.19	65.85±4.60
7	14	79.28±5.54	87.67±6.13	69.14±4.83

Preparation of the Extract:

The collected leaves were subjected to shade drying for 20 days. The dried plant material was powdered mechanically and stored in air tight container for further analysis. Aqueous methanol (1:3) as solvent was used to macerate the milled plant. The extract was evaporated using a rotary evaporator and subsequently freeze-dried. The product obtained was stored at 4°C.

Experimental Animals:

Healthy Swiss albino mice of either sex weighting 15-25g and aged 5-6 weeks were obtained from the Department of Veterinary Jamia Hamdard, New Delhi. The animals were maintained at room temperature and humidity (25°C, 70% relative humidity) and allowed to acclimatize for 2 weeks. All the animals were fed with standard pelleted diet and water *ad libitum*. The study followed ethical guideline of Institutional Animal Ethics Committee (Reg. No: 1678/Po/Re/2021/CPCSEA).

Ethical Approval:

The Institutional Ethical Committee of University, approved the protocol for this study.

Acute Toxicity Study:

According to the OECD-423 guidelines, Swiss albino mice were selected by random sampling. The animals were kept fasting overnight and provided with water only. 2,000 mg/kg body weight of the extract was administered orally by gastric intubations, and the rats were observed for 14 days. Mortality was observed in three out of the three animals; therefore, the dose administered was assigned toxic dose. The concentration of the extract administered was stepped down to 300mg/kg body weight, and the rats were observed for 14 days. No mortality was observed. Therefore, the same dose was repeated to confirm the safe dose of the extract.

Grouping and Dosing:

Thirty six mice (18 male and 18 female) were assigned for the study. Male and female mice were separately grouped into six groups each having six mice. The groups were randomly assigned to the study design. All groups were provided their respective treatment orally.

Castor oil-induced Diarrhoea in Rats:

Rats of either sex were divided into four groups of five rats, each. The animals were fasted for 18 hrs prior to the test. Group I rats were treated with normal saline (2ml/kg), which served as control, while Group II received loperamide (5mg/kg). Group III received 5 mg/kg extracts of *Artocarpus Heterophyllus* and Group IV received 200mg/kg extracts of *Artocarpus Heterophyllus*. All doses were administered orally. After 1 hr, all groups received 1ml of castor oil orally. The animals were placed in cages lined with adsorbent papers and observed for 4 hrs for the presence of diarrhoea defined as watery (wet), unformed stool. The control group result was considered as 100%. The activity of each group was expressed as percent inhibition (%) of diarrhoea. The percentage inhibition of defecation was calculated as follows:

$$\% \text{ Inhibition of Defecation} = A-B/A \times 100$$

Where A indicates the mean number of defecations caused by castor oil and B indicates the mean number of defecations caused by drug extract.

Gastrointestinal Motility Test:

For the test, selected rats were divided into four groups of five rats in each. At first, 1ml of castor oil was given orally to every rat of each group to produce diarrhoea. After 1hr, Group I (control group) received saline 2ml/kg orally. Group II received standard drug (loperamide 5mg/kg and Groups III & IV received 5mg/kg and 200mg/kg extract of *Artocarpus Heterophyllus* respectively. After 1hr, all animals received 1ml of charcoal meal (10% charcoal suspension in 5% gum acacia) orally. One hour later, following the charcoal meal administration, all animals were euthanized, and

the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum, was measured and expressed as percentage of the total length of the small intestine from pylorus to the caecum.

$$\text{Intestinal transit \%} = [\text{Length travelled by black marker} / \text{Total length of small intestine}] \times 100$$

$$\text{Percentage of transit inhibition} = (T_0 - T_1) \times 100$$

Where, T_0 = Intestinal transit of saline (control) and T_1 = Intestinal transit of extract.

Methods:

In the current study, we selected the model of diarrhoea induced by castor oil in the Swiss albino mice to examine the effects of preventive treatment containing single as well as multiple oral administrations of probiotic *Bacillus coagulans* Unique IS2 and *Bifido-bacterium bifidum* at dose 1010 CFU/kg and to examine the mechanisms of action of probiotic by studying its co-administration with antagonist L-arginine. During an observation period, the time of onset of diarrhoea, total quantity of faecal output and weight of faeces defecated by the animals were documented.

Preparation of Artocarpus Heterophyllus Suspension:

This was carried out according to the method of Eskanda et al with slight modification. Appropriate quantities of CMC, PVP and traga-canth were weighed as presented in Table 1, sieved with a size 120-mesh sieve and validated according to a standard procedure. A paste of the structured vehicle (CMC, PVP and traga-canth) base was made with the ingredient combination in Table 1 for formulations A–D and allowed to stand for 10 mins. 1.25% w/v of freeze-dried extracts of *Artocarpus hetero-phyllus* was weighed and dispersed into a mixture of the wetting agents, polysorbate 80 and glycerol. The paste was scooped into the extract solution in bits, and the resultant system was mixed systematically for a sufficient duration of time. The flocculating agent, NaCl, was added while the system was being mixed. The mixing was continued until a uniform and consistent suspension was obtained. The formulation was tested for pharmacological action. Three different formulations (A, B and C) were made by varying the composition of the structured vehicle according to Table 5. Formulation D prepared without the extract served as the negative control.

Table.5 Composition of formulated suspensions of methanol extract of *Parquetina nigrescens*

Formulation ID	Tragacanth %	PVP %	CMC %	Polysorbate-80 %	Glycerin %	NaCl %	Extract %
A	0.75	0.5	2	0.35	2.5	0.04	1.25
B	-	-	2	0.35	2.5	0.04	1.25
C	-	-	-	-	-	-	-

Abbreviations: PVP, Polyvinylpyrrolidone; CMC, Carboxymethylcellulose; NaCl, Sodium Chloride.

Pharmacological Action of Suspensions of Methanol Extract of Artocarpus Heterophyllus:

Rats of either sex were divided into four groups of three rats each. The animals were fasted for 18 hrs prior to the test. Group I received 5mg/kg suspension of *Artocarpus hetero-phyllus*, Group II received 200mg/kg suspension of *Artocarpus hetero-phyllus*, Group III received loperamide (5mg/kg), while Group IV rats were treated with normal saline (2ml/kg), which served as control. All doses were administered orally. After 1hr, all groups received 1ml of castor oil orally. The animals were placed in cages lined with adsorbent papers and observed for 4 hrs for the presence of diarrhoea defined as watery (wet), unformed stool.

Physical Enhancement of Suspensions of Methanol Extract of Artocarpus Heterophyllus:

On confirmation of pharmacological action, the formulations were enhanced with colouring and flavouring agents in sufficient amounts. The resultant suspensions were dispensed into clear glass bottles and stored for analysis.



Analysis of Organo-leptic Properties and pH of Suspensions of Methanol Extract of Artocarpus Heterophyllus:

The physical properties of the suspensions such as color, odour, appearance, sedimentation and pourability were observed by direct perception and compared to a standard suspension. The suspensions were also observed physically for the presence of sediments over a period of 30 days. The pH value of the formulations was determined at room temperature using a pH meter (Hanna Instruments Limited, Leighton Buzzard,, UK) calibrated to pH 7. The analysis was performed in triplicates.

Evaluation of Viscosity of Suspensions of Methanol Extract of Artocarpus Heterophyllus:

The viscosities of the formulations were determined using an NDJ-5S Digital Display Viscometer (Shanghai Geological Instrument Co. Ltd, Shanghai, China) at 25±1°C. Spindle size 4 was used at speed 6, 12, 30 and 60 rpm.

Stability Studies of Suspensions of Methanol Extract of Artocarpus Heterophyllus:

The formulations were left in clear bottles for 30days at 25°C (room temperature) and 40°C (accelerated temperature).

Statistical Analysis:

The results were expressed as the mean ± SEM. Data obtained were analyzed using graph pad prism. Statistical analysis was carried out using one-way ANOVA. Statistical significance was taken at p<0.05.

Results and Discussion

The oral acute toxicity of the extract LD50 was found to be 300mg/kg body weight > LD50<2000mg/kg body weight. The toxicity indices for agility, tremor, convulsion, breathing pattern, lethargy and coma exhibited by the animal groups are as shown in Table 6.

Table.6 Toxicity index exhibited by the animal groups

Animal Group	Toxicity Index (+) Mild, (++) Moderate, (+++) Severe, (-) Nil					
	Agility	Tremor	Convulsion	Breathing Pattern	Lethargy	Coma
1st Dose (200Mg/Kg)						
Animal-I	++	+	-	-	++	+++
Animal-II	+++	-	-	-	-	+++
Animal-III	++	+	-	-	++	+++
2nd Dose (300Mg/Kg)						
Animal-I	+	-	-	-	-	-
Animal-II	++	-	-	-	-	-
Animal-III	-	-	-	-	-	-
Animal-IV	++	-	-	-	-	-
Animal-V	++	-	-	-	-	-

The number of watery stools passed in castor oil-induced rats and % inhibition of 5 and 200 mg/kg as compared with Loperamide and castor oil-induced (control) groups are presented in Table 7.

Table.7 Effects of Methanol Extract of the leaves of Parquetinanigrescens (Artocarus Heterophyllus) on castor oil-induced Diarrhoea in rats

Treatment	Dose (Mg/Kg)	No. of Watery Diarrheas	% Inhibition
Castor Oil	1.00ml	17.00±1.29	-
Artocarus Heterophyllus Extract	5ml	11.00±0.81	35.29
Artocarus	200ml	6.00±0.39	64.70

Table.8 The intestinal transits of charcoal meal were measured and are presented.

Treatment	Dose (Mg/Kg)	Mean Intestinal Length (Cm)	Mean Distance Travelled by Charcoal (Cm)	Mean Intestinal Transit	% Inhibition
Saline (Control Extract)	2.00ml	99.23±2.56	37.43±3.82	0.38±0.82	-
Artocarus Heterophyllus Extract	5.00ml	9834±1.32	23.37±1.27	0.24±0.24	0.14
Artocarus	-	96.67±1.08	22.66±1.38	0.23±0.17	-

Table.9 Physical properties of suspensions of methanol extract of Artocarus Heterophyllus

Propeerties	Formulation A Extract	Formulation B	Formulation C	Formulation D	Plain Formulation Without
Colour	Sangria	-	-	Crimson	White
Odor	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant
Apperance	Thick, Glossy	-	-	Clear	-
Sedimentation Sedements	No Sediments	No Sediments	No Sediments	No Sediments	No Sediments
Pourability	Not Easy	Easy	Easy	Easy	Easy

In Table.10, the pH of the suspensions ranged from 4.60±2.73 to 4.73±1.91. Viscosity at 6 to 60 revolutions per minute (rpm) was also measured for the suspensions.

Table.10. pH and Viscosity of Suspensions of Methanol Extract of Artocarus Heterophyllus

Formulation	pH	Viscosity (Pas)			
		60 rpm	30 rpm	12 rpm	6 rpm
A	4.60±2.73	6.21±0.18	3.80±2.29	2.54±1.04	4.90±4.22
B	4.64±1.64	5.70±3.22	3.40±0.23	1.80±3.33	4.40±2.67
C	4.73±1.91	3.50±1.23	1.80±1.19	3.00±2.23	1.54±0.35
D	6.50±0.23	6.75±1.24	4.14±0.01	2.35±3.58	8.50±1.92

Pharmacological actions of the suspensions of methanol extract of Artocarus heterophyllus demonstrated by the number and amount of stool passed by the test animals are presented in Table 11.

Table.11. Pharmacological Action of the Suspensions of Methanol Extract of Artocarus Heterophyllus

Test Animals	Group-I (Suspension) 5Mg/Kg	Group-II (Suspension) 200Mg/Kg	Group-III (Positive Control)	Group-IV (Negative Control)
Rat-1	2 Loose Stools	1 Loose Stools	3 Loose Stools	4 Loose Stools
Rat-2	3 Loose Stools	2 Loose Stools	3 Loose Stools	4 Loose Stools
Rat-3	2 Loose Stools	1 Loose Stools	4 Loose Stools	4 Loose Stools

While the onset of pharmacological action due to administered suspension of methanol extract of Artocarus Heterophyllus on the test animal groups is presented in Table 12.

Table.12. Onset of Pharmacological Action due to Administered Suspension of Methanol Extract of Artocarus Heterophyllus

Test Group	0-15mins	15-30	30mins-1hrs	1-2hrs	1-3hrs	3-4hrs
Group-1		*	*	*	*	
Group-2		*	*	*	*	
Group-3		*	*	*	*	
Group-4		*	*	*	*	

IV. DISCUSSION

Herbal medicine as an alternative and complementary therapy is now gaining grounds in both developing and advanced countries. From literature, Artocarus heterophyllus has shown potential for the treatment of a variety of diseases. One such disease is diarrhoea, which is one of the leading causes of death in both children and adults in Africa. Herbal medicines are commonly used today due to their limited side effects as compared to conventional medicines. From the result of the study, a single-dose oral administration of the extract at 2,000 mg/kg body weight caused mortality in the



rats. A concentration of 300 mg/kg body weight had no effect on mortality in the rats. According to Global Harmonized System of Classification and Labelling of Chemicals (GHS),¹⁹ the extract is classified in category 4 defined by oral LD₅₀ in the range of 300 to 2,000 mg/kg body weight and the toxicity indices are presented in Table 2. In Table 3, there was a dose-dependent decrease in the number of watery stools passed in castor oil-induced rats by 35.29% and 64.70% at 5 and 200 mg/kg, respectively.

However, at equivalent concentration of the extract and loperamide, 5 mg/kg body weight, loperamide appeared to produce a greater inhibition. This may be attributed to the long duration of action of loperamide. The results obtained showed that the extract of *Artocarpus heterophyllus* has a great potential to reduce the frequency of stool at a dose comparable to that of the conventional drug used as control. In the presence of castor oil in the gut, prostaglandins are secreted, which in turn induces gastrointestinal motility. Hypermotility is one of the different pathophysiological conditions that characterize diarrhoea. The charcoal meal test was carried out to determine the effect of *Artocarpus heterophyllus* extract on gut motility. Loperamide was used as a control as it has been known to inhibit castor oil-induced diarrhoea. As shown in Table 4, it was observed that *Artocarpus heterophyllus* extract reduced the intestinal transit of charcoal meal by 0.14% and 0.15% by 5 and 200 mg/kg of the extract, respectively, as compared to an inhibition of 0.12% by 5 mg/kg loperamide. A reduction in intestinal transit time shows that the extract was able to reduce the motility of the gut and thus reduce diarrhoea. Normal saline, which was the negative control, showed no inhibition, as it will normally not have effects on diarrhoea. The result also showed that at 5 mg/kg body weight of extract and loperamide, the levels of inhibition produced by the extract were greater than that produced by loperamide. It can, therefore, be said that only a small concentration of extract is required for antidiarrhoeal effect and thus less amount of drug in the system. This also implies less toxic effect. Having established the antidiarrhoeal effect of *Artocarpus heterophyllus*, it is pertinent to formulate the extract into dosage forms that can ease administration, mask the characteristic odor and color of the plant and elicit a faster onset of action, hence, the choice of a suspension.

This research was carried out to formulate the extract of *Artocarpus heterophyllus* into a suspension, validate its antidiarrhoeal effect as a suspension for oral administration and characterize the suspension. All the suspensions inhibited diarrhoea in a dose-dependent pattern. As the concentration of extract increased, there was a remarkable decrease in the number of loose stools passed by the test animals. A relatively good level of compatibility was observed between the Ingredients of the suspension and plant extract as there was only a slight decrease in the pharmacological activity of the suspension as compared to the extract only. The choice of a structured vehicle was to improve the stability of the product and aid easy re-dispersion at the time of administration. In a structured vehicle, the viscosity of the preparation approaches infinity on storage under conditions of very low shear. The vehicle can maintain the suspended particles, thereby conferring stability.

All the suspensions were appealing to sight, smelt good and showed no visible sediments as presented in Table 5. Formulation A was very thick and did not flow easily from the container, while the other formulations flowed easily. The pH of the suspensions ranged from 4.60±2.73 to 4.73±1.91 as presented in Table 6. The pH value of an aqueous solution represents its degree of acidity or alkalinity, and the pH of a preparation must be optimum as they are administered. The slightly acidic pH values obtained are optimal for formulations intended for oral administration. The viscosity of the suspensions was dependent on the components of structured vehicle used. Formulation A containing all three components of the structured vehicle had the highest viscosity as shown in Table 6. However, the control suspension gave viscosity values higher than the test suspensions. This implies that the presence of plant extracts lowered the viscosity of the formulations. At the end of the stability studies, the suspension showed a slight change in pH considered statistically insignificant. The color and odor remained unchanged with no visible sediments. This result shows that the particles of the extract will continue to remain suspended in the vehicle, thereby remaining stable.

Pharmacological analysis was carried out which validated the antidiarrhoeal properties of *Artocarpus heterophyllus* suspension when administered orally. This was in line with the pharmacological effects obtained when the extract was analyzed. There was a dose-dependent decrease in the number and amount of stool passed by the test animals as presented in Table 7. The effect obtained at a concentration of 5 mg/kg body weight of formulated suspension was comparable to that of the control drug used, with 200 mg/kg having a greater effect. This implies that optimal diarrhoea control can be achieved between the doses of 5 and 200 mg/kg. From Table 8, 200 mg/kg concentration of the formulated suspension produced diarrhoea in the test group between 2 and 3 hrs while the group treated with 5 mg/kg produced diarrhoea between 1 and 2 hrs. An equivalent concentration of the control drug produced diarrhoea in the test group between 30 mins to an hour. Therefore, it can be said that in addition to inhibiting the passage of loose stools in test animals, suspensions of *Artocarpus heterophyllus* were able to maintain the inhibition for up to an hour, thereby

confirming a longer duration of pharmacological effect and thus reducing the need for repeated dosing. All numerical data obtained are reported as averages. An ANOVA for the different mean results gave “p-values” <0.05 at every instance, implying that each average is statistically significant and different from the other on comparison.

V. CONCLUSION

The results of the study suggest that methanol extract of the leaves of *Artocarpus heterophyllus* possesses antidiarrhoeal properties. Suspensions of *Artocarpus heterophyllus* were successfully formulated in structured vehicles of a combination of CMC, PVP or tragacanth. The suspension exhibited antidiarrhoeal activity by inhibiting gastric motility in mice. Further investigations will be carried out to validate the dose of the plant extract that produced the best activity in formulation and develop the drug moiety into dosage forms that can be subjected to clinical evaluation.

REFERENCES

1. Richie M-D, Sabrina C, Christiane M-S, et al. Development of an oral suspension containing dry extract of *Aleurites moluccanus* with anti-inflammatory activity. *Res Bras Farmacogn.* 2016; 26(1):68–76. doi:10.1016/j.bjp.2015.06.011.
2. Liu L, Johnson HL, Cousens S, et al.; Child Health Epidemiology Reference Group of WHO and UNICEF. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. *Lancet.* 2012;379 (9832):2151–2161.
3. Khalilur R, Ashraf UC, Mohammed TI, et al. Evaluation of anti-diarrheal activity of methanolic extract of *Maranta arundinacea* (Linn) leaves. *Adv Pharmacol Sci.* 2015. ID 257057. doi:10.1155/2015/ 257057.
4. Kola-Mustapha A, Ghazali Y. Formulation of *Vernonia amygdalina* and *Calotropis procera* Methanol Leaf Extracts into a Cream for the Management of Skin Infections. *West African Journal of Pharmacy.* 2017;28(4):85–95.
5. Ghazali Y, Bello I, Kola-Mustapha A. The Use of Herbal Medicines among Outpatients at the University of Ilorin Teaching Hospital (UITH), Ilorin, Kwara State-Nigeria. *Complementary Therapies in Medicine.* 2019;42:158–163.
6. Alvarez Cruz NS. *Parquetina nigrescens* (Afzel.) Bullock. In: Schmelzer GH, Gurib-Fakim A, editors. *Prota 11(2): Medicinal plants/Plantes Medicinales.* Wageningen, Netherlands: PROTA; 2012;11:2.
7. Baerts M, Lehmann J. *Parquetina Nigrescens and Periploca Nigrescens.* Tervuren, and Belgium: Prelude Medicinal Plants Database, Metafro-Infosys, Royal Museum for Central Africa; 2011.
8. Kesisoglou F, Panmai S, Wu Y. Nanosizing-Oral formulation development and biopharmaceutical evaluation. *Adv Drug Deliv Rev.* 2007;59(7):631–644.
9. Kulshreshtha AK, Singh ON, Wall GM. *Pharmaceutical Suspensions from Formulation Development to Manufacturing.* New York: Springer; 2010.
10. Ogodu OJ, Akubue PI, Okide GB. The kinetics of reversal of pre-sickled erythrocytes by the aqueous extract of *Cajanus Cajan* seeds. *Phytother Res.* 2002;16:1–3.



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