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Research Article

Formulation, Development and Evaluation of Poly-herbal Formulation for Antiulcer and Antispasmodic activity

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ABSTRACT
Formulation of Poly-herbal suspension for Antiulcer and Antispasmodic activity, were identified phytochemical and then Polyherbal suspension was prepared with the extracts of *Abieswebbiana* (Talispatra), *Plumbago zeylanica* (Chitrak), and *Rubiacordiafolia* (Manjistha) with a suitable suspending agent. They were taken in the ratio of 1:2:1. Suspension was prepared by trituration method in mortar and pestle by using the suitable suspending agent of Tween 80 and Sodium Carboxy Methyl Cellulose along with other excipients, The evaluation parameter was studied started with the physical test such as nature, color, odor and texture. The all the three formulations were liquid in nature and dark brown in color with pleasant odor. The physicochemical parameters of formulation studied using sedimentation volume, particle size, viscosity, pH, redispersibility, zeta potential and density, Acute toxicity study of extracts and PHF- F2 were done on basis of OECD guidelines. Results exhibited that the prepared polyherbal formulation was non-toxic to the animals. No any change in behavior, locomotor activity, color and eyes was detected in 14 days study. Thus, lower doses of formulation F2 (200 mg/kg bw) were selected for *in-vivo* studies on animals. The *Plumbagozeylanica* (400mg/kgbw) and PHF-F2. Exhibited a substantial decrease in UI as equated to that of vehicle and standard treated group while the *Abieswebbiana*a nd *Rubiacordiafolia* (400mg/kg bw) didn't exhibit any substantial effect on ulcer index. Biostatistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Bonferroni's t-test. Significant decrease ($p<0.05$) in all test parameters including gastric volume, pH, total acidity, free acidity, and ulcer index was observed in PHF F2 treated animals as compared to induced Control group. Histopathological examination also revealed protective potential of PHF-F2 in gastric mucosa. Based on these observations it is concluded that the PHF F2 comprises significant antiulcer activity. The finding of this experimental study helps the scientific community to further investigate this candidate medicinal plant by initiating advanced studies on formulations of plant source drugs.

KEYWORDS: **Peptic ulcers, Herbal plants, Soxhlet apparatus, *Abieswebbiana* (Talispatra), *Plumbago zeylanica* (Chitrak), and *Rubiacordiafolia* (Manjistha)**

1. INTRODUCTI

Ulcers are painful sores that take a long time to heal and may reoccur. An ulcer is a kind of open wound that can appear in various parts of the body [2]. Ulcers are most commonly found on mucous membranes such as the stomach's surface or within the mouth. When the upper layer of mucous or skin membranes is injured, an ulcer develops. The mucous or skin membrane ruptures as a result, and the burst membrane prevents the affected organ from functioning normally. This causes an ulcer, which is an open sore[3]. Ulcers are a used by injuries, infections, and diseases. The most prevalent cause of ulcers is an injury that does not cure, heals properly due to another health concern, most commonly a blood flow problem. Inflammation of the stomach acid can cause peptic ulcers, which can lead to the progression of cancer. Ulcers are sometimes caused by depression [4].

Herbal medications have gotten a lot of attention recently from researchers all around the world because of their therapeutic worth, patient compliance, and exceptional therapeutic results[5]. patient compliance, and exceptional therapeutic results[5]. Herbal medication delivery systems with NDDS (novel drug-delivery systems) have various advantages over traditional formulations. It involves improving solubility, bioavailability, and toxicity protection, etc[6]. Herbal medications are an alternative to synthetic drugs for treating a variety of medical issues and are thought to be safer. Phytochemicals have been utilised to cure

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many diseases since ancient times, and they are usually thought to be safer than synthetic medications. Various investigations have found that many chemical components extracted from plants have antiulcer effects.
Alkaloids, tannins, flavonoids, terpenoids, glycosides, carotenoids, and saponins are examples of these compounds[8].Herbal medicines are composed of herbal materials, herbs, herbal preparations, and final herbal products that comprise plant parts and other plant components as active ingredients. Pharmaceuticals used in traditional medicine to cure or prevent disease, or to re-store, or modify physiological function, are known as conventional pharmaceuticals[9].

Many synthetic anti-ulcer medicines are on the market, but them any of them are linked to several the adverse side effects, including gastrointestinal irritation, ulceration, and fluid retention. NSAIDS cause hepatotoxicity and nephrotoxicity in addition to aggravating ulcers. Herbal plants are utilized to restore and maintain health since ancient times, either as a preventive or therapeutic agent. Medicinal herbs may play a significant role in drug development[10]. As a result, there is a need for the development of innovative anti-ulcer medicines derived from natural sources that have greater activity and fewer side effects &can be utilized as a viable alternative to chemical treatments.

MATERIAL AND METHOD

Three different herbal plants *Rubiacordifolia*, Family Rubiaceae, Parts used Roots, *Abieswebbiana* Family Pinaceae Parts used Leaves, and *Plumbago zeylanica* Family Plumbaginaceae, Parts used leaves.

Chemicals

All of the chemicals used were analytical grade and obtained from Merck, Sigma, or S.D. Fine Chemicals, respectively.

Processing of plant

Plant samples were collected and rinsed under running tap water for the experiment. All plant materials should be kept in the shade to dry. A blender was used to make a powder from dry plant material. Plant material in powder form was stored in a dry, well-ventilated area for future use.

Extraction

Soxhlet method[12]

Plant material was extracted utilizing the continuous hot percolation method with a Soxhlet apparatus in this investigation. In the thimble of the Soxhlet apparatus, powdered material from *Abieswebbiana* (Talispatra), *Plumbago zeylanica* (Chitrak), and *Rubiacordifolia* (Manjistha) was inserted. Soxhlation was carried out at 60°C in a non-polar solvent, petrole umether[11]. Soxhlation was continued for each solvent untilno visible colour change in th siphon tube was noticed, and the extraction was concluded by the absence of any residual solvent when evaporated. At 40°C, the extracted extracts were evaporated using a rotatory vacuum evaporator (Buchitype). Physical characteristics (colour and odour) of prepared extracts were observed before being stored in an airtight container and labelled for future usage. The percentage yield is essential in determining the extraction efficiency standard for a particular plant, various sections of the same plant, or different solvents.

Determination of Ash Value

In a crucible, a particular amount of sample was carefully weighed. The sample was ignitedfor4 to 5 hours at 550 °C to 600°C, and then allowed to cool to room temperature in a desiccator before being weighed until it reached a steady weight.

Total ash value (%)= (wt. of total ash/wt. of total crude material)*100

Acid-Insoluble Ash

After boiling the whole ash in dilute hydrochloric acid and igniting the residual insoluble matter, residue is obtained. Boiled the ash in 25ml Dil. HCl for 5 to 10 minutes, collected the insoluble material in a crucible or on ash-free filter paper, ignited, and weighed. With the air-dried drug as a reference, the % yield of acid-insoluble ash was estimated.
% Water soluble ash value= (Wt. of acid in soluble ash/ Wt. of crude drug taken) X100

Water soluble Ash

Boiled the total ash for five minutes with 25 ml of water; the soluble materials were collected in a crucible, burned, and weighed. With reference to air dried drug, calculated the percentage of water-soluble ash.
% Water soluble ash value= (Wt. of total ash-Wt. of water insoluble ash/ Wt. of crude drug taken) X100.

Qualitative Phytochemical Estimation of Extracts

Using standard procedures, detailed phytochemical testing was undertaken to determine the presence or absence of various phytoconstituents in *Abieswebbiana* (Talispatra), *Plumbagozeylanica* (Chitrak), and *Rubiacordifolia* (Manjistha) extracts (Kokateetal., 2006).The following tests were performed on the extracts obtained in Petroleum ether and Methanol:

Tests for carbohydrates

Molisch test: To1ml of extract, 2-3 drops of alcoholic α-naphthol solution was added. Along the side of the test tube, conc. $H_2SO_4$was added, till the appearance of purple ring at the junction of 2 liquids. It concludes the appearance of sugar in the test sample.

Fehling test:

The Fehling solution A & B was added to 1ml of plant extract and heated on a water bath for few minutes. The formation of brick red precipitate

confirms the presence of carbohydrates.

Benedict's test:

ThemixtureofequalvolumeofBenedict'sreagent&extractwereaddedinatesttube and boiled in water bath for 5 to 10 minutes. Depending on the amount of reducing sugar present in the test solution the colour of solution changes to green, yellow or red. This change in the colour of solution shows the appearance of reducing sugar[13].

Barfoed's test:

To 1ml of plant extract, add few drops of Barfoed's reagent in a test tube & heated on water bath for 2 minutes. The red colour was appeared due to the formation of cupric oxide shows the appearance of monosaccharide.

Test for alkaloids

All the test extracts were first treated with dil. hydrochloric acid separately and filtered. The filtrate of all the test extracts was exposed to following tests:

Mayer's test: To1- 2ml of extract filtrate, equal amount of Mayer's reagent was added along side of tube. The existence of alkaloids was confirmed after development of white or creamy precipitate.

Hager'stest: To1- 2ml of extract filtrate, equal amount of Hager's reagent was added in a test tube. The existence of alkaloids was confirmed after development of yellow colored precipitate.

Wagner's test: To 1-2ml of extract filtrate, equal amount of Wagner's reagent was added in a tube. The presence of alkaloids was confirmed after formation of reddish- brown colored precipitate.

Test for flavonoids

Lead acetate test: Some drops of lead acetate solution were mixed with the plant extract. The presence of flavonoids could be indicated by the formation of yellow precipitate.

Alkaline reagent test: Some drops of NaOH added to the extract of plant in a test tube. The existance of flavonoids is considered by the formation of a bright yellow colour that fades when some drops of dil. acid are added.

Shinoda test: To the plant extract, 5ml ethanol (95%) was added. A few bits of magnesium turning were added to this mixture, followed by drops of strong hydrochloric acid. The presence of flavonoids is indicated by the development of a pink colour[14].

Test for glycoside

Borntrager's test: In3 ml of extract, sulfuric acid was added, boiled for 5minutesand filtered. In cold filtrate, an equal amount of benzene or chloroform was added and stirred considerably. Ammonia was added to the organic solvent layer after it was separated. The existance of anthraquinone glycosides is indicated by the development of a reddish-brown colour in the layer of ammonia[15].

Legal's test: 1 ml of plant extract dissolved in pyridine. 1 ml of solution of sodium nitropruside was mixed with 10% NaOH solution. The development of pink in the blood-red color confirms the existence of cardiac glycosides.

Keller-Killiani test: In 2ml of extract, add glacial acetic acid (3ml) &one dropof5% FeCl3 to the test tube. Carefully add 0.5ml of conc. H_2SO_4 to the wall of the tube. The development of blue color in the layer of CH_3COOH (Acetic acid) shows the existence of cardiac glycosides.

Biuret test: mixed the solution of extract with 1 ml of NaOH solution in a test tube and heat. Add a drop of 0.7% $CuSO_4$ solution to the above solution. The formation of violet or pink confirms the presence of protein.

Ninhydrin Test: 3 ml of extract solution was warmed with 3 drops of Ninhydrin solution in water bath for 10 minutes. The formation of a blue color indicates the existance of amino acids.

Tests for Saponins

Foam test*:* 1ml of extract of plant sample was mixed in 20 ml of distilled water (DW) and allows shaking for 15 min in graduated cylinder. Formation of persistent foam around 1cm layer shows the presence of saponins.

Tests for terpenoids and steroids

Salkowski's test: The test sample was treated equal amount of chloroform and filtered. Few drops of Conc. H_2SO_4were mixed with the collected filtrate, shaken and allow standing. The presence of triterpenes is shown by the development of a golden yellow layer at the bottom, while the presence of sterolis indicated by the lower layer turning red.

Libermann-Burchard's test: The plant extract was boiled and cooled after being mixed with chloroform and some drops of acetic anhydride. The solution was transferred into the test tube, than concentrated H_2SO_4 was mixed through the test tube'ssides.At the junction of two layers appearance of brownring was observed. Thechange in the colour of upper layer changed to green, it confirms the existence of steroids and if colour changes to deep red color concludes the presence of triterpenoids.

Tests for tannins and phenolic compounds

Ferric chloride test: Little amount of plant extract was mixed in DW and add 2ml of solutionof5%$FeCl_3$.The existence of phenol chemicals was shown by the development of blue, green, or violet colour.

Lead acetate test: In distilled water, some amount of plant extract was dissolved, and adds some drops of solution of lead acetate. The development of white precipitate concludes the presence of phenols.

Quantitative Phytochemical Analysis Spectrophotometric Quantification of Total Phenolic Content

The total amount of phenolic extract released was estimated by the reagent of Folin Ciocalteu. Galic acid is utilized as standard & complete phenolicis expressed as mg/g gallic acid equivalent (GAE). A concentration of 10-100 mg / ml of gallic acid or any anther phenolic compound was prepared in methanol. The 1 mg/ml extract was made with 0.2 ml of each test sample in methanol, 2.5 ml of 10 fold dilute folin-Ciocalteu reagent, and 2ml of 7.5 percent sodium carbonate (Na_2CO_3).The tubes are wrapped in parafilm and incubate for 30 minutes at room temperature before the absorption is read at 760 nm in excess. Every determination is made three times. The folin-Ciocalteu reagent is responsive to reducing substances such as poly phenols. They produce a blue color when you react. This blue color was measured spectrophotometrically. The retrospectiveline from Gallic acid has been used to measure unknown phenol content[15].

Spectrophotometric Quantification of Total Flavonoid Content

A colorimetric assay was used to determine total flavonoids. Total flavonoids were measured by a colorimetric assay. An stock solution of sample or rutin (standard) solution was added to a 75µl of $NaNO_2$solution, and incubate for 6min, before adding 0.15 mL $AlCl_3$ (100g/L). After 5 min, 0.5 mL of NaOH was added. With distilled water, the final volume makes up to 2.5ml and thoroughly mixed. Again stab lank, the mixture's absorbance was observed at 510 nm. Rutin's calibration curve was used to calculate total flavonoid contentin plant extract. Three replications of each sample were performed. For the estimation of unknown flavonoid content, aline of regression from rutin was used.

Spectrophotometric Quantification of Total Alkaloid Content

For the standard curve, the 5concentration was used. Atropine or any other alkaloid compound (1 mg/10 ml DW) was used to make a 100 ppm solution& then make 5 concentration, respectively, 0.5, 1, 1.5, 2 and 2.5 ml atropine was added and 5 ml of BCG solution and same amount of phosphate buffer saline (pH4.7)(5mL)was added and shaken vigorously. 5 ml of chloroform was added to each of them. Then removed the 5 to10 mL flask isolated by chloroform phase, & the manner they moved and were filled to volume with chloroform[16]. Each flask's absorption at 470 nm was determined and read before a witness was prepared to absorb. Little balloons & calibration graphs were plotted after reading the absorption recorded uptake. The plant extract (1mg/ml) was dissolved in 2 N HCl and filtered after that. With 0.1 N NaOH, the pH of the phosphate buffer solution was brought to neutral. One milliliter of this mixture was taken to a separating funnel, followed by 5 milliliters of BCG and 5 milliliters of phosphate buffer. The mixture was continuously shaken, and the resulting complex was extracted using chloroform. The extracts were taken in a 10ml of volumetric flask & chloroform was used to dilute the extracts to volume. The complex's absorbance in chloroform was evaluated at 470 nm by UV using spectrophotometer. For the determination of unknown alkaloid content, a line of regression from atropine was employed.

Spectrophotometrically Quantification of Total Saponin Content

The spectrophotometric method was utilized to find the total saponin content. 0.1 mL of the above-mentioned extract was combined with 0.4 mL of 80 percent methanol solution, 0.5 mL of freshly prepared 8 percent (w/v) vanillin solution (prepared in ethanol), and 5.0 Ml of 72 percent sulfuric acid in an ice water bath. The mixture was then heated for10 minutes in a water bath at 60°Cand finally chilled in ice cold water.The reagent blank was made in the same way, but instead of extract, an equivalent volume of 80 percent methanol was used. A UV-Vis spectrophotometer was used to compare the absorbance at 544 nm to the reagent blank. Using a standard curve (diosgenin), the results were calculated and represented as diosgenin equivalents (mg DE/g extract)

Formulation development Preparation of Polyherbal Suspension

Table 1 show how several methanolic extracts of Abieswebbiana (Talispatra), *Plumbago zeylanica* (Chitrak), and *Rubiacordiafolia* (Manjistha) were combined with an appropriate suspending agent to make a poly herbal suspension. They were captured in a 1:2:1 ratio. Suspension was made by triturating several bioactive extracts of selected plant materials in a mortar and pestle with Tween 80 and sodium carboxy methyl cellulose (CMC) assuspending agents, as well as additional excipients. Tween 80, sodium CMC, and extracts of *Abieswebbiana* (Talispatra), *Plumbago zeylanica* (Chitrak), and *Rubiacordiafolia* (Manjistha) were added in equal amounts to a homogenous mixture.

In a mortar and pestle, sodium CMC in an aqueous medium containing specified preservatives was mixed with ethanolic extracts of selected plant materialandtriturated continuously. Three potential Suspension formulations, namely Polyherbal, were created. This was mixed with 10 grammes of sugar. The excipients such as sorbitol, flavouring agent (lemon oil), and preservative were then added, and the mixture was homogenised. Finally, the volume was increased to100ml to obtain a consistent result. After that, all three possible suspensions of *Abieswebbiana* (Talispatra), *Plumbago*

Zeylanica (Chitrak), and
Rubiacordiafolia (Manjistha) extracts
were evaluated according to standards.

Table 1: Formulation composition of poly herbal suspension

Ingredients(%w/w)	F1	F2	F3
Rubiacordifolia	1gm	1gm	2gm
Abieswebbiana	2gm	1gm	1gm
Plumbago zeylanica	1gm	2gm	1gm
Tween80	1%	1%	1%
Sodium CMC	2gm	2gm	2gm
Sucrose	10gm	10gm	10gm
Methylparaben	200mg	200mg	200mg
Lemonoil	0.01%	0.01%	0.01%
Purified water	100ml	100ml	100ml

Organoleptic characters
The organoleptic characters of the Poly herbal Suspension were evaluated by using the following parameters colour, odor and texture etc.

Evaluation parameters of the suspension
Particle size distribution and physicochemical characterization such as pH measurement, Viscosity, redispersibility, Sedimentation Volume VS were carried out[17].

Sedimentation volume
As the suspension settles in a cylinder under proper standard circumstances, the sedimentation volume is the ratio of the ultimate height of the sediment to the initial height of the complete suspension. It was assessed by leaving a measured volume of suspension in a graduated cylinder undisturbed for a length of time and noting that the volume of the sediment was expressed as ultimate height[18].

Redispersibility
The suspension was allowed to settle in a measuring cylinder. The mouth of the cylinder was closed and was inverted through 180º and the number of inversions necessary to restore a homogeneous suspension was determined.

pH
The pH of the suspension was determined by using a pH meter (Eutech).

Particles size analysis
The distribution of particle size in suspension is an important aspect of its stability. Particle size distribution was carried out by using optical microscopy in dilute suspensions.

After shaking, 10 ml of each sample was separately transferred into 200 ml cylinder. Distilled water (150 ml) was then added, mixed, and 10 ml aliquot was removed at a distance of 10 cm below the surface of the mixture and at 1, 5, 10, 15, 20, 25 and 30 min. This was transferred into an evaporating dish and evaporated to dryness in an oven at 105℃ and the residue weighed.

Viscosity
Brookfield viscometer type III, spindle # 2 at 250 rpm, was used to determine the viscosity of suspensions. The slowest permitted spindle speed was employed for each measure mention this study and the reading were collected as soon as the change became slow and steady. A reading may usually be obtained in ten to fifteen seconds. The liquid was placed into a large enough beaker to prevent wall and end effects. A known weight of solid particles was added to the liquid after the viscosity of the liquid was established. To make a "uniform" suspension, the mixture was agitated. In a vacuum chamber, air bubbles that had been injected into the suspension were removed.

Zeta potential
A Zeta photometer III (Melvern) with a rectangular electrophoresis cell comprising a pair of hydrogen ted palladium electrodes, a laser illuminator, and a digital video image capture (CCD camera) and viewing system was used to measure zeta potential. The computer is dope rating system enabled for precise positioning of the camera view field at a stationary layer, allowing for accurate electrophoretic mobility measurement. The electrophoresis cell was filled with around 40ml of the prepared suspension. The movement of 50–100 particles in the stationary layer was tracked 5 times in each direction by alternating positive and negative electrode potentials using the laser- illuminating and video-viewing system. Built-in imagery processing software was used to examine the recorded photos.

Density
Calculate the dry and empty density bottle weights. Fill the density bottle half way with liquid, making sure there are no bubbles. About a third of the ground neck should be covered. Set the temperature of the bottle and its contents to 20 °C in a thermostatic bath. Align the cork with the density bottle's thermometer according to the markings, then carefully insert. The displaced liquid is pushed out of the capillary tube as it fills up. Using tissue, gently dry the exterior sides of the stopper and the density bottle. Calculate the filled density bottle's weight.

Density was calculated from the mass (weight) and the volume of the liquid at the reference temperature of 20°C.The volume is engraved on the bottle. Use the following equation:

$$\text{Density } (\rho) = \text{Mass } (m)/\text{Volume } (V)$$

Isolated rat Ileum Preparation
Preparation of Tyrode solution (mM:): NaCl 136.9, KCl 2.7, $MgCl_2$ 1.0, $CaCl_2$1.8, $NaH_2PO_4$0.4, $NaHCO_3$11.9,and glucose 5.0,
bubbled with a mixture of 95% of oxygen and 5% of carbon dioxide at 37ºC.

Six fasted (over night starved) rats weighing 250-300g weres laughter red and used in the study. Every time a tissue was needed, the animal's

abdominal cavity was opened by a midline incision, and the ileum, which was 2 cm long, was quickly taken and clipped from the surrounding tissues. Tyrode solution, a Physiological Salt Solution (PSS), was used to wash the contents of the colon. Ileum segments were tied at both ends with silk threads (ileum tied in opposing directions) and suspended in a 370 C thermo regulated 25 cc organ baths containing a Tyrode solution. The ileum was connected to the isometric recording device on one end and a tissue holder at the bottom of the organ bath on the other. The tissues were continually bubbled with a 95 percent O_2 and 5% CO2 air combination. Before adding histamine, the specific plant extract, or the conventional medications, the suspended ileum was allowed to equilibrate for 30-45 minutes. Histamine was given to the organ bath after the initial equilibration phase, and the control cumulative concentration-response curve for histamine was built, and the reaction of histamine was recorded using a 5-minute time cycle. Before adding the next concentration of histamine, the previous concentration was left in contact with the tissues for 30 seconds[19].

The tissue was then rinsed twice with Tyrode solution at a 10-minute interval. It was allowed to continue normal contractions. After producing a dose response curve for histamine (10g/ml) on the ileum at doses of (0.1, 0.2, 0.4, 0.8, and1.6ml), extracts and PHF (5, 10, and 20 g/l) were added to the reservoir, and the maximal contraction dose of histamine was repeated in the presence of plant extract. The standard medication Chlorpheniramine maleate (CPM10g/ml) underwent the same treatment as the extract. Spasmogen responses were observed, with results expressed in percent relaxation[20].

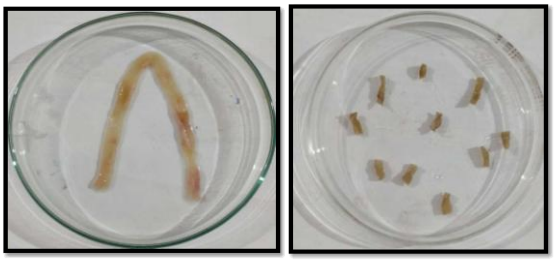


Figure 1: Ileum

***In-vivo* study**

Acute toxicity study Species Rats **Strain** A*lbino Wistar,* **Source** Animal House, Pinnacle Biomedical Research Institute (PBRI), Bhopal **No. of animals/dose level** Three (03), **Acclimatization** One week prior to dosing **Diet** Standard pellets supplied by Golden Feeds, NewDelhi, **Water** Purified water *ad libitum,* **Body weight** 195gm ± 15All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI), Bhopal (CPCSEA Reg. No 1824/po/Rc/S/15/CPCSEA). Protocol Approval Reference No. PBRI/IAEC/08-22/024 Acute oral toxicity will be performed as per OECD423 guidelines. As per annexure 2a of OECD 423 guidelines.

According to OECD (423) rules, an acute toxicity research was conducted on RCM, AWM, and PZM extracts. Four treatment groups with dosages of 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg body weight are included in the study. Individual doses were estimated based on the animal's weight on the day of treatment[21]. Gavage was used to provide the test material in a single dose.Theanimalswerefastedfor3hoursbefore to treatment (only food was withheld, not water) (OECD, 2001). The animals were monitored for behavioural changes, mortality, and appearance for the first 4 hours, then every 24 hours for the next 24 hours, and then every day for the next two weeks until 14 days.

In-vivo Anti-ulcer activity

Ethanol induced ulcer

A total number of 36 healthy Wistar albino rats either sex weighing (aged12weeks) 200 ± 20 grams were randomly divided into six groups[22]. They were housed in a controlled room temperature environment (22± 20C) and light with alternate 12-hour light/dark cycles. The animals had free access to rat pellets and water adlibitum. After 12 days of acclimatization, rats were and only divided into six experimental groups of six rats in each group. The ethanol control group received a vehicle (distilledwater,10ml/kg, p.o.) and the IInd, IIIrd, IVth groups received test sample (Extract-RCM, AWM, PZM 400mg/kg bodyweight), group Vth received poly herbal formulation (PHF-F2) respectively (200 mg/kg body weight), 60 min prior to the ethanol solution (99% v/v, 1 ml/kg, p. o.). Standard group (VI) was treated with the standard antiulcer drug (ranitidine50 mg/kg, p.o,).The rats were sacrificed after one hours of ethanol administration and the stomach was removed and opened along the greater curvature. The stomachs were photographed for observations of the ulcerated area[23].

Drug induced ulcer

The ulcers were induced by administering indomethacin (20 mg·kg-1, p.o,) to the animals fasted for 36h. Six groups of rats with six rats per group were selected for the present study. The first group was the Indomethacin (20 mg/kg) induced ulcer group where Indomethacin were administered orally and VIth group treated with standard (ranitidine 50mg/kg, p.o,). All treatment (Group II, III, IV) and PHF-F2 (200mg·kg-1) (Group V) was administered with extracts, PHF, standard continued for 21 days. After 4 hours of Indomethacin administration[24], rats were sacrificed, stomach were opened and washed with

normal saline and storedin10% formalin solution.

Parameters of studied

Determination of Ulcer index

The stomach was then cut open along the greater curvature and the mucosa was washed under slow running tap water to remove the gastric contents and blood clots and examined by a 10X magnifier lens to assess the formation of ulcer. The numbers of ulcers were counted. Normal stomach = (0), Red coloration = (0.5), Spot ulcer = (1) Hemorrhagic streak = (1.5), Ulcers = (2), Perforation = (3).

Theme a nulcer score for each animal was expressed as the ulcer index.

Ulcer index (UI) formula:

UI=UN+US+UPX10-1

Where

UI = ulcer index; UN = average number of ulcers per animal; US = Average number of severity score; UP = Percentage of animals with ulcer.

Determination of Acidity of Juice Gastric

Principle:

It is based on simple acid-base titration. Reagents: NAOH (0.01N) Phenolphthale in (1%), 1 mL of gastric juice was taken in a 50 ml conical flask. Two drops of phenolphthalein indicator for total acidity determination and Topfer's reagent for free acidity determination was added to it[25]. It was titrated with0.01N NaOH until a permanent pink color (free acidity determination) was observed.

The volume of 0.01 N NaOH consumed was noted. The total/free acidity is expressed as mEq./L by the following formula:

Acidity=(1+ Vol. of NaOH ×N× 100mEq/L)/0.1

Where n is volume of NaOH consumed, 0.01is normality of NaOH, and 36.45 is molecular weight of NaOH.

Volume of gastric juice

The contents were drained into tubes and centrifuged at 1000 rpm for 10 minutes, and the volume was noted (an aliquot of 1ml gastric juice was diluted with 1ml of distilled water).

Determination of pH

After centrifugation, an aliquot of 1mL gastric juice was diluted with 1mL of distilled water and the pH of the solution was measured using pH meter.

Histopathology

For his tological investigation, the stomach was sub mergedina10% formalin solution. These tissues were treated, dehydrated in various grades of alcohol, cleaned in toluene, and impregnated for specific times in molten paraffin wax. Tissues were processed and imbedded in fresh molten paraffin wax, which was then allowed to harden[26]. To show general tissue structure, sections were frozenat3°C, dried on a hot plate for 15 minutes, and stained with hematoxylin and1% aqueous OS in. Stained slides were dehydrated in various grades of alcohol, and then clean e dinxylene before being mounted in Canada balsam. Using 40 objective lenses, sections were inspected microscopically. Under a microscope, stained slices were inspected for histological alterations.

RESULTS AND DISCUSSION

Extract Percentage yield

Table 2: Percentage extractive yield of various extracts of *Abieswebbiana* (Talispatra), *Plumbagozeylanica* (Chitrak), and *Rubiacordifolia* (Manjistha)

S.No.	Plant	%Yield	
		Pet ether	Methanol
1	*Rubiacordifolia*	0.608	3.69
2	*Abieswebbiana*	0.354	3.040
3	*Plumbago zeylanica*	0.748	4.036

Ash content

Table 3: Total ash, acid in soluble ash, water soluble ash content in *Rubiacordifolia, Abieswebbiana* and *Plumbagozeylanica*

S.No.	Test (2 gm material)	Wt. in gm (*Rubiacordifolia)*	Wt. in gm (*Abieswebbiana)*	Wt. in gm (*Plumbago zeylanica)*
1	Wt. of total ash	0.142	0.113	0.122
2	Wt. of total acid In soluble ash	0.118	0.089	0.096

Solubility analysis

Table 4: Solubility Determination of Rubiacordifolia, Abieswebbiana, Plumbago zeylanica

S.No	Test	*Rubiacordifolia*	*Abieswebbiana*	*Plumbago zeylanica*
1	Total ash value in %	7.1	5.65	6.1
2	Acid in soluble ash in %	5.9	4.45	4.8
3	Water soluble ash in %	0.6	0.6	0.65

Solubility analysis

Table 5: Solubility Determination of Rubiacordifolia, Abieswebbiana, Plumbago zeylanica

S. No.	Solvent	*Rubiacordifolia*		*Abieswebbiana*		*Plumbago zeylanica*	
		Pet. Ether	Methanol	Pet. Ether	Methanol	*Pet. Ether*	*Methanol*
1	Water	Insoluble	Slightly Soluble	Insoluble	Slightly Soluble	*Insoluble*	*Slightly Soluble*
2	Methanol	Insoluble	Soluble	Insoluble	Soluble	*Insoluble*	*Soluble*
3	Ethyl Acetate	Soluble	Slightly Soluble	Soluble	Slightly Soluble	*Soluble*	*Slightly Soluble*
4	DMSO	Soluble	Soluble	Soluble	Soluble	*Soluble*	*Soluble*
5	Petroleum Ether	Soluble	Insoluble	Soluble	Insoluble	*Soluble*	*Insoluble*

Qualitative Phytochemical estimation of extracts

Table 6: Qualitative test of Rubiacordifolia, Abieswebbiana, Plumbagozeylanica

S. No.	Experiment	*Rubiacordifolia*		*Abieswebbiana*		*Plumbagozeylanica*	
		Pet.Ether Extract	Methanol	Pet.Ether Extract	Methanol	Pet.Ether Extract	Methanol
Test for Carbohydrates							
1	Molisch's Test	+	+	-	-	-	-
2	Fehling's Test	+	+	-	-	-	-
3	Benedict's Test	-	+	-	-	-	-
4	Bareford's Test	-	-	-	-	-	-
Test for Alkaloids							
1	Mayer'sTest	-	+	-	+	-	+
2	Hager'sTest	-	+	-	+	-	+
3	Wagner'sTest	-	+	-	+	-	+
Test for Terpenoids							
1	Salkowski Test	-	-	-	+	+	+
2	Libermann-Burchard's Test	-	-	-	+	+	+
Test for Flavonoids							
1	LeadAcetate Test	-	+	+	+	+	+
2	AlkalineReagentTest	-	+	+	+	+	+
3	Shinoda Test	-	+	-	+	+	+
Test for Tannins and Phenolic Compounds							
1	FeCl3Test	+	+	-	+	+	+
2	LeadAcetate Test	+	+	-	+	+	-
Test for Saponins							
1	FrothTest	+	+	+	+	-	+
Test for Protein and Amino acids							
1	Ninhydrin Test	-	-	-	-	-	+
2	Biuret's Test	-	-	-	-	-	+
Test for Glycosides							
1	Legal's Test	-	+	-	-	-	+
2	KellerKillani Test	-	-	-	-	-	+
3	Borntrager's Test	-	+	-	-	-	+

Formulation evaluation

Table 7: Physical test for herbal suspension

S. No.	Parameter	F1	F2	F3
1	Nature	Liquid	Liquid	Liquid
2	Color	Dark brown	Dark brown	Dark brown
3	Odor	Pleasant	Pleasant	Pleasant
4	Texture	Suspension	Suspension	Suspension

Table 8: Physicochemical Parameters of formulation

Formulation	Sedimentation volume	Particle size (µm)	Viscosity (Cps)	pH	Redispersibility	Zeta potential (mv)	Density (gm/ml)
F1	0.29	16.84	47.3	5.16	Good	-31.2	1.069
F2	0.21	15.54	44.2	5.10	Good	-33.5	1.080
F3	0.26	17.01	43.5	5.27	Good	-29.7	1.140

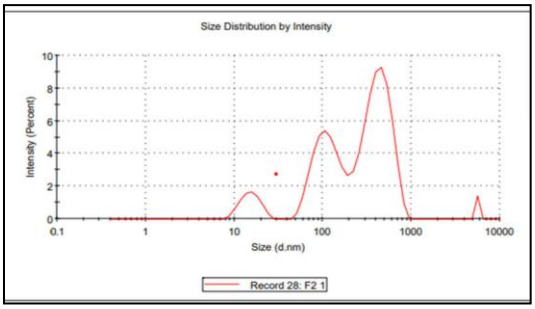


Figure 2: Particle size of F2formulation

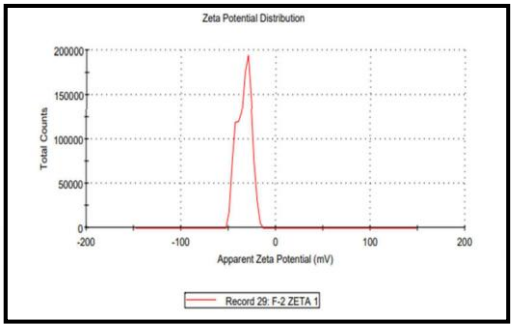


Figure 3: Zeta potential of F2 formulation

The particle size distribution data (Table 8), concluded that the F2 suspension is a coarse dispersion with particle size predominantly 15.54 micrometers.

The trituration process was used to create a polyherbal composition. All three formulations, Polyherbal formulation-F1, Polyherbal formulation-F2, and Polyherbal formulation-F3, were found to exhibit similar organoleptic properties, such as being liquid in nature, having a dark brown colour, and having a nice odour. When comparing[27].

PolyherbalFormulationF2toPolyherbalFormulation F1and F3, it was discovered that the sedimentation volume was reduced to (0.21). It also affected the viscosity (44.2 centipoise), alkaline pH (5.1), and the flow rate of the formulation (15.54 um). F2 formulation has the best qualities based on physiochemical metrics[28].

In vitro anti-spasmolytic activity
Isolated RatIleum Preparation

Table 9: Histamine (10µg/ml) Induced Contraction on Rat Ileum

Dose (ml)	Height of Response(mm)					
	Control	Standard	RCM	AWM	PZM	PHF-F2
0.1 ml	12.83±1.16	12.16±0.983	11.66±1.966	11.66±0.816	14.16±1.471	12.20±2.00
0.2 ml	23.50±1.516	23.50±1.870	24.83±2.483	22.33±1.505	27.16±1.169	24.26±2.714
0.4 ml	35.16±2.316	33.50±3.563	35.83±2.562	32.83±2.316	36.16±2.228	34.33±2.065
0.8 ml	53.33±2.338	52.00±4.427	53.16±2.639	49.66±4.179	52.33±1.861	52.00±1.44
1.6 ml	45.50±1.870	43.83±1.169	45.50±2.738	40.33±3.076	41.66±2.422	43.00±2.529

Table 10: Effect of RCM, AWM, PZM and PHF-F2 on Histamine (10µg/ml) Induced Contraction on Rat Ileum

S. No.	Treatment	Dose (ml)	Height of Response (mm)	% Relaxation
1	Normal Saline +Histamine	0.2 ml + 0.8 ml	54.50+2.26	0
2	Standard CPM +Histamine	0.2 ml + 0.8 ml	2.16 +0.752	96.02
3	RCM (5mg/ml) + Histamine	0.2 ml + 0.8 ml	39.00 +2.607	28.44
4	RCM (10mg/ml) + Histamine	0.2 ml + 0.8 ml	31.66 +3.386	41.9
5	RCM (20mg/ml) + Histamine	0.2 ml + 0.8 ml	23.83 + 2.401	56.26
6	AWM (5mg/ml)+ Histamine	0.2 ml + 0.8 ml	35.66 +2.422	34.56
7	AWM (10mg/ml)+ Histamine	0.2 ml + 0.8 ml	28.00 +1.788	48.62
8	AWM (20mg/ml)+Histamine	0.2 ml + 0.8 ml	15.66 + 1.032	71.26
9	PZM (5mg/ml)+Histamine	0.2 ml + 0.8 ml	14.50 + 1.870	73.39
10	PZM (10mg/ml)+Histamine	0.2 ml + 0.8 ml	13.00 + 1.414	76.14
11	PZM (20mg/ml)+Histamine	0.2 ml + 0.8 ml	6.16 + 1.94	88.68
12	PHFF2(5mg/ml)	0.2 ml + 0.8 ml	14.50 + 1.870	70.94
13	PHFF2(10mg/ml)	0.2 ml + 0.8 ml	13.00 + 1.414	77.67
14	PHFF2(20mg/ml)	0.2 ml + 0.8 ml	6.16 + 1.940	89.9

***In-vivo* study**
Acute oral toxicity of *Abieswebbiana*(AW), *Plumbagozeylanica*(PZ),and *Rubiacordifolia* (RC)

Table11: Body weight changes and Lethality of RC extract

Group	Dose (mg/kg)	Rat No.	Day of Death	Body Weight (gm)			No. dead/Tested
				0 Day	7 Day	14 Day	
A	5 mg/kg	R1	--	196.11	197.65	199.52	0/3
	"	R2	--	191.11	193.90	195.79	
	"	R3	--	197.10	199.01	202.85	
B	50 mg/kg	R1	--	195.41	197.48	199.32	0/3
	"	R2	--	190.67	193.51	195.45	
	"	R3	--	196.86	198.61	200.55	
C	300 mg/kg	R1	--	194.95	196.71	199.83	0/3
	"	R2	--	190.21	193.06	197.97	
	"	R3	--	196.44	198.33	202.21	
D	2000 mg/kg	R1	--	194.67	195.51	199.28	0/3
	"	R2	--	199.84	202.70	205.64	
	"	R3	--	196.13	199.01	202.91	

Table 12: Body weight changes and Lethality of AW extract

Group	Dose (mg/kg)	Rat No.	Day of Death	Body Weight (gm)			No. dead/Tested
				0 Day	7 Day	14 Day	
A	5 mg/kg	R1	--	195.45	197.34	201.14	0/3
	"	R2	--	193.33	195.06	98.87	
	"	R3	--	199.45	201.08	203.84	
B	50 mg/kg	R1	--	198.77	197.50	199.21	0/3
	"	R2	--	195.65	195.45	197.26	
	"	R3	--	198.60	201.46	204.24	
C	300 mg/kg	R1	--	200.05	202.72	205.74	0/3
	"	R2	--	192.05	195.84	198.62	
	"	R3	--	197.11	199.85	203.51	
D	2000 mg/kg	R1	--	206.67	207.46	209.12	0/3
	"	R2	--	201.42	203.11	205.85	
	"	R3	--	196.34	199.12	204.91	

Table 13: Body weight changes and Lethality of PZ extract

Group	Dose (mg/kg)	Rat No.	Day of Death	Body Weight (gm)			No. dead/Tested
				0 Day	7 Day	14Day	
A	5 mg/kg	R1	--	202.45	204.31	206.12	0/3
	"	R2	--	199.21	202.11	203.17	
	"	R3	--	199.22	202.18	205.99	
B	50 mg/kg	R1	--	194.11	196.90	198.31	0/3
	"	R2	--	193.25	195.06	197.28	
	"	R3	--	196.12	198.46	201.15	
C	300 mg/kg	R1	--	203.05	205.32	206.99	0/3
	"	R2	--	193.13	194.89	196.12	
	"	R3	--	194.14	196.13	198.46	
D	2000 mg/kg	R1	--	196.37	197.49	199.10	0/3
	"	R2	--	203.40	205.10	207.66	
	"	R3	--	195.24	196.87	199.10	

Table 14: Body weight changes and Lethality of PHF-F2

Group	Dose (mg/kg)	Rat No.	Day of Death	Body Weight (gm)			No. dead/Tested
				0 Day	7 Day	14 Day	
A	5 mg/kg	R1	--	198.45	202.14	204.92	0/3
	"	R2	--	196.10	198.12	201.08	
	"	R3	--	200.05	202.11	204.99	
B	50 mg/kg	R1	--	195.61	197.98	200.81	0/3
	"	R2	--	195.54	198.63	201.33	
	"	R3	--	198.24	202.11	205.54	
C	300 mg/kg	R1	--	200.41	203.92	205.88	0/3
	"	R2	--	195.44	198.22	201.34	
	"	R3	--	201.77	203.65	205.86	
D	2000 mg/kg	R1	--	200.83	202.53	204.29	0/3

Results exhibited that the extracts and prepared PHF-F2 was non-toxic to the animals. No any change in behavior, locomotor activity, color of fur and eyes were detected in extractsandPHF-F2 till14days study. Thus, lower doses of formulation were elected for *in vivo* studies on animals.

***In vivo* antiulcer activity**

Ethanol induced antiulcer activity

Table 15: Ethanol induced antiulcer activity in rats

Groups	Treatment	Dose	The volume of Gastric juice	The Acidity of Gastric juice	The pH of Gastric juice	Ulcer index
Groups I N.C	Inducer ethanol(99% v/v,1 ml/kg, p. o)	10ml/kg, p.o	4.178±0.978	37.166±6.112	2.868±0.0.751	10.77±0.103
Group II RCM treated group	RCM Extract(400 mg/kg bw)	400mg/kg body weight p.o	3.281±1.016^{ns}	24.333±9.003^{ns}	3.358±0.924^{ns}	8.90±0.225**
Group III AWM treated group	AWM Extract(400 mg/kg bw)	400mg/kg body weight p.o	3.645±0.957^{ns}	28.166±9.239^{ns}	3.266±0.734^{ns}	7.25±0.312**
Group IV PZM treated group	PZMExtract (400 mg/kg bw)	400mg/kg body weight p.o	3.453±0.970^{ns}	20.833±5.810**	3.316±0.845^{ns}	5.52±0.183**
Group V Polyherbal formulation PHF-F2	PHFF2(200 mg/kgbw)	200 mg/body weight, p.o	3.905±1.260^{ns}	23.833±5.528 ^{ns}	4.166±1.187^{ns}	3.900±0.225**
Group VI Standard Ranitidine (50mg/kg bw)	Ranitidine 50 mg/kg bodyweight	50mg/kg body weight. p.o	3.048±0.930^{ns}	22.166±9.152**	4.54±1.0008**	1.720±0.112**

Values are expressed as MEAN±SD at n=6,
One-wayANOVAfollowedbyBonferronitest,**P<0.050,comparedtotheinducedcontrol.

Indomethacin induced ulcer in rats

Table 16: Indomethacin induced ulcer in rats

Groups	Treatment	Dose	The volume of Gastric juice	The Acidity of Gastric juice	The pH of Gastric juice	Ulcer index
Groups I N.C	Inducer (Indomethacin)	20 mg/kg, p.o	6.035±0.920	35.66±5.715	2.848±0.545	10.63±0.103**
Group II RCM treated group	RCM Extrac t(400 mg/kg bw)	400 mg/kg body weight p.o	4.671±1.330^{ns}	30.833±9.988^{ns}	2.9±1.163^{ns}	7.27±0.178**
Group III AWM treated group	AWM Extract (400 mg/kg bw)	400 mg/kg body weight p.o	4.113±1.530^{ns}	27.666±8.710^{ns}	3.436±1.110^{ns}	7.20±0.233**
Group IV PZM treated group	PZMExtract (400 mg/kg bw)	400 mg/kg body weight p.o	4.211±0.786^{ns}	29.166±8.376^{ns}	3.181±0.969^{ns}	3.57±0.273**
Group V Polyherbal formulation PHF-F2	PHFF2 (200 mg/kgbw)	200 mg/body weight, p.o	4.126±0.353^{ns}	25.66±5.006^{ns}	4.095±1.311^{ns}	3.52±0.098**
Group VI Standard Ranitidine(50mg/kg bw)	Ranitidine 50mg/kgbody weight	50 mg/kg body Weight. p.o	3.008±0.942**	22.00±4.335^{ns}	5.1883±0.9282**	1.69±0.081**

Values are expressed as MEAN±SD at n=6,
One-way ANOVA followed by Bonferronitest, **P<0.050, compared to the induced control

From biostatistical data analysis, the Group II was found statistically significant (P<0.05) when compared to Standard (Ranitidine), (Extract: RCM, AWM, PZM and PHF-F2).In this data Standard ranitidine was found highly significant when compared to groupie as compared to Test samples treated (Sample-RCM, AWM, PZM and PHF- F2) groups[29].

The results of various acid secretory parameters such as Gastric volume, pH, Free acidity and of extracts-RCM, AWM, PZM and poly herbal formulation PHF-F2 on ethanol induced and indomethacin induced gastric ulcer in rats. Estimation of acid secretory parameters was increased significantly in the control group. Administration of extracts in indomethacin induced model, extracts RCM, AWM, PZM exhibited a significant effect and results were comparable with the standard drug Ran35±0.920ml. Ranitidine, the standard drug decreased the mean gastric volume it idine. In control group the mean volume of gastric juice was 6.0 3.008±0.942 which is statistically significant. Apart from the standard, RCM, AWM, PZM extracts also showed decrease in the mean gastric juice at both the doses 400mg/kg. Similarly, PHF F2 showed significant effect[30,31].

Extract of PZM (at a dose 400mg/kg bw, per oral) exhibited a substantial decrease in ulcer index as equated to that of standard group. The RCM, AWM extracts didn't exhibit substantial decrease in ulcer index as equated to that of standard group. The poly herbal formulation (PHFF2) formulated using the extracts RCM, AWM, PZM extracts exhibited a substantial decrease in ulcer index in the indomethacin induced gastric ulcer models tested[32,33. In current study, we also quantify the total saponin content in all three extracts Abieswebbiana (Talispatra), Plumbago zeylanica (Chitrak), and Rubiacordifolia (Manjistha). The total saponin contents of Abieswebbiana (Talispatra), Plumbago zeylanica (Chitrak), and Rubiacordifolia (Manjistha) extracts was calculated with a regression equation based on a standard curve (y=0.003x-0.028, R2=0.970). The Rubia cordifolia extract showed maximum saponin content (8.88±0.509 mg/g equivalent of Diosgenin).

The total alkaloid content was studied in extracts of Abies webbiana (Talispatra), Plumbago zeylanica (Chitrak), and Rubiacordifolia (Manjistha).The quantification of alkaloid showed that Plumbago zeylanica has highest alkaloid content (56.66±2.081) calculated based on standard curve of atropine with the regression co-efficient R2= 0.983. The plot has a slope of 0.001 and intercept of 0.065[34].

Polyherbal suspension was prepared with the extracts of Abieswebbiana (Talispatra), Plumbago zeylanica (Chitrak), and Rubia cordiafolia (Manjistha) with a suitable suspending agent. They were taken in the ratio of 1:2:1. Suspension was prepared by trituration method in mortar and pestle by using the sui. Suspending agent of Tween 80 and Sodium carboxy methyl cellulose (CMC) along with other excipients[35].

The extracts of Abieswebbiana (Talispatra), Plumbago zeylanica (Chitrak), and Rubiacordiafolia (Manjistha), tween80 and sodium CMC in respective quantities were takenand was made into a homogeneous mixture. The suspending agent[36], sodium CMC in the aqueous medium containing selected preservatives was added in mortar and pestle along with ethanolic extracts of selected plant material with continuous triturating. Three possible formulations of Suspension viz. Polyherbal were prepared. Then the excipients like sorbitol, flavouring agent (Lemon oil) and preservative was added. All three possible forms of suspension of extract coded as F1, F2, F3.

The evaluation parameter was studied started with the physical test such as nature, color, odor and texture. The all the three formulations were liquid in nature and dark brown in color with pleasant odo[37]r. The physicochemical parameters of formulation studied using sedimentation volume, particle size, viscosity, pH, redispersibility, zeta potential and density. When left undisturbed for a long period of time he suspension particles will aggregate, sediment, eventually cake. When a suspension is very well dispersed (i.e., deflocculated), the particles will settle as small individual particles. This settling will be very slow and will result in a low-volume, high-density sediment that may be difficult or impossible to redisperse. When the particles are held together in a loose open structure, the system is said to be in the state of flocculation. Particle size of the active agent plays a key role in the physical stability and bioavailability of the drug product. The rate of sedimentation is affected by particle size[38].

This dimentation volume ranged from 0.21 to 0.29. The pH of formulations F1 to F3 ranged from 5.10 to 5.27 and the viscosity ranged from 43 to 47 cps. The maximum viscosity was observed in F1 formulation (i.e. 47.3 cps). Zeta potential values were in the order of F2>F1>F3. The density of suspensions was in range of 1.06 to 1.14.On basis of these physiochemical parameters F2 formulation have best properties.

The spas molytic activity of Abieswebbiana (Talispatra), Plumbagozeylanica (Chitrak), Rubiacordiafolia (Manjistha) and polyherbal formulation (F1, F2, F3) was studied in isolated rat ileum model using histamine as agonist. Histamine when inhaled causes hypoxia and leads to convulsion in the rats and causes very strong smooth muscle contraction, profound hypotension, and capillary dilation in the cardio vascular system. Bronchodilators can delay the occurrence of these symptoms[39].

The histamine (10µg/ml) produced dose dependent contraction of rat ileum as indicated in the. The results of the study demonstrated that polyherbal formulation F2 showed significant reduction of spasm induced by Histamine. PHF F2 significantly

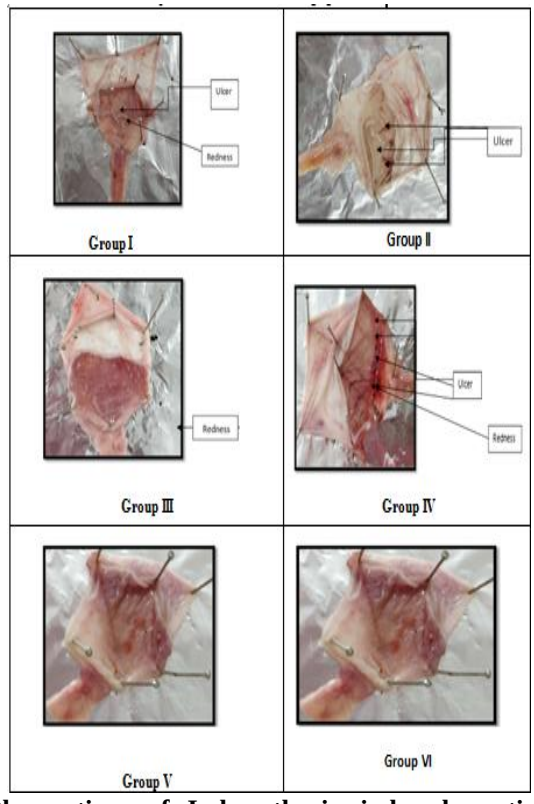
in habited ($p<0.05$) the contract ile effect of histamine on isolated rat ileum preparation. The percentage relaxation of PZM on histamine induced contraction was found to be 88.68%. Similarly, the percentage relaxation of F2 Formulation on histamine induced contraction was found to be 89.90% in comparison with Chlorpheniramine CPM (10µg/ml) 96.02%. Therefore, the PHF-F2 showed almost similar antihistaminic effect that of Chlorpheniramine maleate. Mechanism of the antispasmodic activity of polyherbal formulation F3 is non-specific and may be mediated by inhibiting histamine receptors[40].

Acute toxicity study of extracts Abieswebbiana (Talispatra), Plumbago zeylanica (Chitrak), Rubiacordiafolia (Manjistha and PHF- F2 were done on basis of OECD guidelines.Results exhibited that the prepared polyherbal formulation was non-toxic to the animals. No any change in behavior, locomotor activity, color of furan deyes was detected in 14 days study. Thus, lower doses of formulation F2 (200 mg/kg bw) were selected for in-vivo studies on animals.

In vivo antiulcer activity of Abieswebbiana (Talispatra), Plum bagozeylanica (Chitrak), Rubiacordiafolia (Manjistha was per formed utilizing ethanol and indo methacin induced ulcer model. Study was conducted utilizing male Albino Wistar rats (6 per group). Ranitidine was used as the standard. In the study, we have induced ulceronrats (Wistar rats) and tested the extracts activity on ulcer induced rats. In ethanol induced model it was observed that extract of Abieswebbiana (Talispatra), Plumbago zeylanica (Chitrak), Rubiacordiafolia (Manjistha play important role in anti-ulcer activity and significantly inhibited lesion formation in the glandular stomach and reduced acid secretory parameter and volume of gastric secretions) in comparison to ranitidine. Here, wistar rats were divided into six groups: (normal control, n=6), (standard ranitidine group, n=6), (extract treated groups i.e. Abieswebbiana (Talispatra), Plumbago zeylanica (Chitrak), Rubiacordiafolia (Manjistha, n=6) and PHF-F2 treated group. Each of control, ranitidine, extracts Abies webbiana (Talispatra), Plumbagozeylanica (Chitrak), Rubiacordiafolia (Manjistha and PHF were administered orally to the rats daily for 21 days before the induction of ulcer with the help of indomethacin. The observation significantly increased ($p < 0.05$) value that reduced the ulcer by the treatment with extract groups, Abieswebbiana (Talispatra), Plumbago zeylanica (Chitrak), Rubiacordiafolia (Manjistha) and PHF-F2.There sultindicatestheanti ulcer effect of the Abieswebbiana (Talispatra), Plumbago zeylanica (Chitrak), Rubiacordiafolia (Manjistha) extracts and PHF-F2 showed significant effect when compared with control and standard group. The data of study revealed that the PHFF2 proved to show control over the model of indomethacin-induced gastric ulceration which gives potent activity against ulceration[41]. The Plumbagozeylanica (400mg/kgbw) and PHF-F2. exhibited an substantial decrease in UI as equated to that of vehicle and standard treated group while the Abies webbiana and Rubiacordiafolia (400mg/kg bw) didn't exhibit any substantial effect on ulcer index[42].

Biostatistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Bonferroni's t-test. Significant decrease ($p<0.05$) in all test parameters including gastric volume, pH, total acidity, free acidity, and ulcer index was observed in PHF F2 treated animals as compared to induced Control group. Histopathological examination also revealed protective potential ofPHF-F2 in gastric mucosa. Based on these observations it is concluded that the PHF F2 comprises significant antiulcer activity[43].

This study verified the claims of previous reports on the know ledge of native traditional medicine practitioners. The finding of this experimental study helps the scientific community to further investigate this candidate medicinal plant by initiating advanced studies on formulations of plant source drugs.



Observations of Indomethacin induced peptic ulcer

Figure 4: Photograph of rat stomach showing protective effect of RCM, AWM, PZM and PHF F2 extract on the indomethacin induced ulceration in rat exposed to an experimental model as; Group I.N.C Distilled water, Group IIRCM treated, Group III AWM treated, Group IV PZM treated, Group V Poly herbal formulation, Group VI Standard.

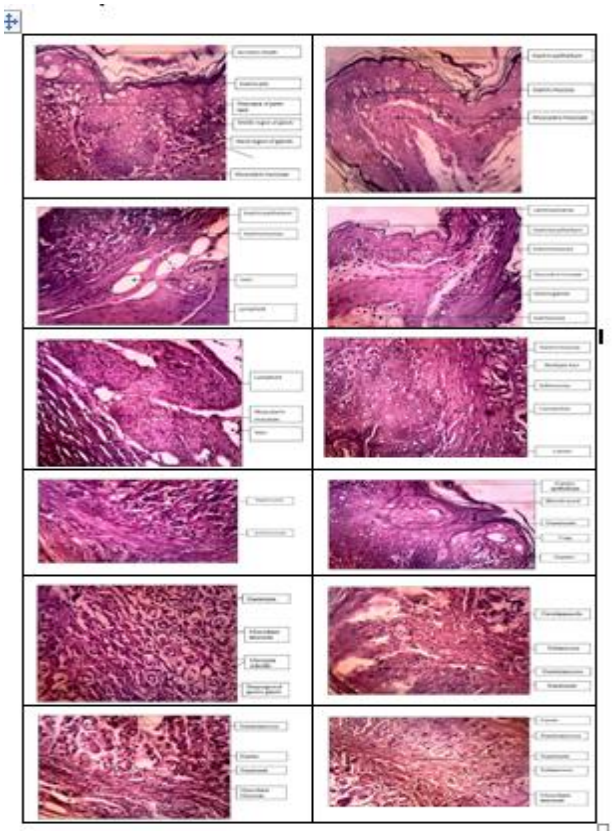


Figure 5: Histopathology: Group I showed severe surface epithelium with necrosis of mucosal glands, early epithelial defects, and hyperplasia Groups II- showed haemorrhages and congested submucosa vessels, Group III- showed multiple foci of moderately deep ulcers with intact muscularis mucosa. Group IV Showed moderate hyperplasia and oedema, Group V showed mild to moderate erosion of surface epithelium Group VI showed normal tissue with no ulcer lesion.

Histopathology study of stomach of Indomethacin induced ulcer model Group I

In current study, we also quantify the total saponin content in all three extracts *Abieswebbiana* (Talispatra), *Plumbago zeylanica* (Chitrak), and *Rubiacordifolia* (Manjistha). The total saponin contents of *Abieswebbiana* (Talispatra), *Plumbago zeylanica* (Chitrak), and *Rubiacordifolia* (Manjistha) extracts was calculated with a regression equation based on a standard curve (y=0.003x-0.028, R2=0.970). The *Rubiacordifolia* extract showed maximum saponin content (8.88±0.509 mg/g equivalent of Diosgenin).

The total alkaloid content was studied in extracts of *Abieswebbiana* (Talispatra), *Plumbago zeylanica* (Chitrak), and *Rubiacordifolia* (Manjistha).The quantification of alkaloid showed that *Plumbago zeylanica* has highest alkaloid content (56.66±2.081) calculated based on standard curve of atropine with the regression co-efficient R2= 0.983. The plot has a slope of 0.001 and intercept of 0.065.

Polyherbal suspension was prepared with the extracts of *Abieswebbiana* (Talispatra), *Plumbago zeylanica* (Chitrak), and *Rubiacordiafolia* (Manjistha)with a suitable suspending agent. They were taken in the ratio of 1:2:1. Suspension was prepared by trituration method in mortar and pestle by using the suitable suspending agent of Tween 80 and Sodium carboxy methyl cellulose (CMC) along with other excipients.

The extracts of *Abieswebbiana* (Talispatra), *Plumbago zeylanica* (Chitrak), and *Rubiacordiafolia* (Manjistha), tween80 and sodium CMC in respective quantities were takenandwasmadeintoahomogeneousmixture.Thesuspendingagent,sodiumCMC in the aqueous medium containing selected preservatives was added in mortar and pestle along with ethanolic extracts of selected plant material with continuous triturating. Three possible formulations of Suspension viz. Polyherbal were prepared. Then the excipients like sorbitol, flavouring agent (Lemon oil) and preservative was added. All three possible forms of suspension of extract coded as F1, F2, F3.

The evaluation parameter was studied started with the physical test such as nature, color, odor and texture. The all the three formulations were liquid in nature and dark brown in color with pleasant odor. The physicochemical parameters of formulation studied using sedimentation volume, particle size, viscosity, pH, redispersibility, zeta potential and density. When left undisturbed for a long period of time he suspension particles will aggregate, sediment, eventually cake. When a suspension is very well dispersed (i.e., deflocculated), the particles will settle as small individual particles. This settling will be very slow and will result in a low-volume, high-density sediment that may be difficult or impossible to redisperse. When the particles are held together in a loose open structure, the system is said to be in the state of flocculation. Particle size of the active agent plays a key role in the physical stability and bioavailability of the drug product. The rate of sedimentation is affected by particle size.

This sedimentation volume ranged from 0.21 to 0.29. The pH of formulations F1 to F3 ranged from 5.10 to 5.27 and the viscosity ranged from 43 to 47 cps. The maximum viscosity was observed in F1 formulation (i.e. 47.3 cps). Zeta potential values were in the order of F2>F1>F3. The density of suspensions was in range of 1.06 to 1.14.On basis of these physiochemical parameters F2 formulation have best properties.

The spas molytic activity of Abieswebbiana (Talispatra), Plumbagozeylanica (Chitrak), Rubiacordiafolia (Manjistha) and polyherbal formulation (F1, F2, F3) was studied in isolated rat ileum model using histamine as agonist. Histamine when inhaled causes hypoxia and leads to convulsion in the rats and causes very strong

smooth muscle contraction, profound hypotension, and capillary dilation in the cardio vascular system. Bronchodilators can delay the occurrence of these symptoms.

The histamine (10µg/ml) produced dose dependent contraction of rat ileum as indicated in the table. The results of the study demonstrated that polyherbal formulation F2 showed significant reduction of spasm induced by Histamine. PHF F2 significantly in habited ($p<0.05$) the contract ile effect of histamine on isolatedrat ileum preparation. The percentage relaxation of PZM on histamine induced contraction was found to be 88.68%. Similarly, the percentage relaxation of F2 Formulation on histamine induced contraction was found to be 89.90% in comparison with Chlorpheniramine CPM (10µg/ml) 96.02%. Therefore, the PHF-F2 showed almost similar antihistaminic effect that of Chlorpheniramine maleate. Mechanism of the antispasmodic activity of polyherbal formulation F3 is non-specific and may be mediated by inhibiting histamine receptors.

Acute toxicity study of extracts Abieswebbiana (Talispatra), Plumbago zeylanica (Chitrak), Rubiacordiafolia (Manjistha and PHF- F2 were done on basis of OECD guidelines.Results exhibited that the prepared polyherbal formulation was non-toxic to the animals. No any change in behavior, locomotor activity, color of furandeyes was detected in 14 days study. Thus, lower doses of formulation F2 (200 mg/kg bw) were selected for in-vivo studies on animals.

In vivo antiulcer activity of Abieswebbiana (Talispatra), Plum bagozeylanica (Chitrak), Rubiacordiafolia (Manjistha was per formed utilizing ethanol and indo methacin induced ulcer model. Study was conducted utilizing male Albino Wistar rats (6 per group). Ranitidine was used as the standard. In the study, we have induced ulceronrats (Wistar rats) and tested the extracts activity on ulcer induced rats. In ethanol induced model it was observed that extract of Abieswebbiana (Talispatra), Plumbago zeylanica (Chitrak), Rubiacordiafolia (Manjistha play important role in anti-ulcer activity and significantly inhibited lesion formation in the glandular stomach and reduced acid secretory parameter and volume of gastric secretions) in comparison to ranitidine. Here, wistar rats were divided into six groups: (normal control, n=6), (standard ranitidine group, n=6), (extract treated groups i.e. Abieswebbiana (Talispatra), Plumbago zeylanica (Chitrak), Rubiacordiafolia (Manjistha, n=6) and PHF-F2 treated group. Each of control, ranitidine, extracts Abies webbiana (Talispatra), Plumbagozeylanica (Chitrak), Rubiacordiafolia (Manjistha and PHF were administered orally to the rats daily for 21 days before the induction of ulcer with the help of indomethacin. The observation significantly increased ($p < 0.05$) value that reduced the ulcer by the treatment with extract groups, Abieswebbiana (Talispatra), Plumbago zeylanica (Chitrak), Rubiacordiafolia (Manjistha) and PHF-F2.There sult indicates the anti ulcer effect of the Abieswebbiana (Talispatra), Plumbago zeylanica (Chitrak), Rubiacordiafolia (Manjistha) extracts and PHF-F2 showed significant effect when compared with control and standard group. The data of study revealed that the PHFF2 proved to show control over the model of indomethacin-induced gastric ulceration which gives potent activity against ulceration. The Plumbagozeyl anica (400mg/kgbw) and PHF-F2. exhibited an substantial decrease in UI as equated to that of vehicle and standard treated group while the Abieswebbianaa nd Rubiacordiafolia (400mg/kg bw) didn't exhibit any substantial effect on ulcer index.

Biostatistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Bonferroni's t-test. Significant decrease ($p<0.05$) in all test parameters including gastric volume, pH, total acidity, free acidity, and ulcer index was observed in PHF F2 treated animals as compared to induced Control group. Histopathological examination also revealed protective potential ofPHF-F2 in gastric mucosa. Based on these observations it is concluded that the PHF F2 comprises significant antiulcer activity.

CONCLUSION

This study verified the claims of previous reports on the knowledge of native traditional medicine practitioners. The finding of this experimental study helps the scientific community to further investigate this candidate medicinal plant by initiating advanced studies on formulations of plant source drugs.

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