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Effect of Polyherbal Ethanolic Extract in Treating of Ulcerative Colitis and Inflammation in Wistar Rats

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ABSTRACT

Background: Ulcerative colitis is a chronic & idiopathic inflammatory bowel disease (IBD) of the colon part or area. It starts from the rectum and ended in the proximal colon. Abdominal pain, bloody diarrhea, and fecal urgency are the symptoms that are seen in Ulcerative colitis patients. The six herbal drugs are *Terminalia chebula (*Combretaceae), *Aegle marmelos (*Rutaceae), *Foeniculum vulgare* (Apiaceae), *Cuminum cyminum* (Umbelliferae/Apiaceae), *Asafoetida* (Umbelliferae/Apiaceae), *Plantago ovata* or Isabgol belongs to the family Plantaginaceae. **Objective:** To prepare an ethanolic extract from herbal sources and to evaluate the heal action of the polyherbal ethanolic extract on induced colitis in wistar rats. **Method:** All herbal drugs were collected and uniformly mixed them. The standardization parameters were evolved after the extraction of ethanol by the soxhlet technique. Then extract was subjected to toxicity studies as per OECD guidelines (OECD test guideline 423 and OECD test guideline 407). This study aimed to investigate the effect or therapeutic potential of the ethanolic extract on chemically induced colitis models (TNBS induced colitis & Acetic acid-induced colitis) which are rapid and quite easy to develop. To evaluate these preclinical studies some parameters like the colon weight/length, DAI (bodyweight & stool consistency), Ulcer index & biochemical assessment (SOD, MDA, MPO, GSH & CAT) were used for the certification of the effectiveness of herbal sources. **Conclusions:** Treatment group 1 (300 mg/kg) & treatment group 2 (500 mg/kg) of ethanolic extract of polyherbal showed a significant effect in TNBS & Acetic Acid induced colitis in wistar rats as compared to the standard drug group (Prednisolone drug) and the study shows that the therapeutic activity of ethanolic extract of polyherbal is beneficial in the Ulcerative colitis disease.

INTRODUCTION:

The ulcer can be defined by a discontinuity in the membrane of the body in the form of sores or wounds. In the tract of gastrointestinal ulcer could be divided into two types that are based on their location; Peptic ulcer (Upper part) and Inflammatory Bowel Disease/IBD (lower part). [1] The first biological retaliation of the immune system is irritation and infection. The causes behind the inflammation can be ultraviolet radiation, immune reactions, microbial invasion, or physical damage. It can be identified by the redness swelling, heating, and pain in some part of the skin. The inflammation can be categories into two i.e. chronic and acute inflammations. A continued inflammation is known as chronic inflammations whereas acute inflammation arises only for a few seconds, minutes, or for a day. [2] Ulcerative colitis is a chronic and idiopathic inflammatory bowel disease (IBD) of the colon part. Ulcerative colitis was initiated in the rectum and extended to the proximal colon. Some of the symptoms that have been seen in the patient of ulcerative colitis are abdominal pain, bloody diarrhea, and fecal urgency. [3, 4] The medicines are used for the treatment of ulcerative gastrointestinal disorder like; 5-aminosalicylates (5-ASA) and corticosteroids which also include some immunomodulators such as; 6-mercaptopurine, azathioprine, cyclosporine, and antibody. 5-ASA is well accepted but including abdominal pain and diarrhea are its side effects which may be followed by fever or kidney problems. Corticosteroids have also been used to treat severe ulcerative colitis with well-known side effects, including increased body hair, weight gain, high blood pressure, and diabetes. [5, 6] However, the side-effects of these synthetic medicines are very usual. Based on these side effects, there is a requirement of a more effective and safe pharmacological option for the treatment of gastrointestinal problems. [7] In recent years, herbal medicines or natural products have been used in the treatment of Ulcerative colitis which is the most common and alternative treatment of colitis. [8] The combination of an herbal-herbal drug is a more selected and elongated therapeutic effect. The herbal-herbal combinations are practicing form for many years and also were reported in Traditional literature. Hence, the study was been planned to standardize and formulate the six herbal sources as a polyherbal formulation against ulcerative colitis diseases. [9,10]

Terminalia chebula is highly considered an herbal drug for its characteristic of preventing and curing diseases. *Terminalia chebula* is known as ‘king of Medicines’ because of its remarkable therapeutic benefits. The local name of *T. chebula* is ‘Haritaki’, ‘Harad’ and belongs to the Combretaceae family. *Terminalia c*hebula contains Tannin (non-hydrolyzable and hydrolyzable tannins). The main components of *T. chebula* are hydrolyzable tannins

which consist of Gallotannins and Ellagitannins. Anti-inflammatory, antioxidant, antimicrobial, and immunomodulatory are medicinal properties. [11, 12, 13]

Aegle marmelos in Ayurveda fruits of *Aegle M.* is a highly potent herbal plant. The most common name of *Aegle M.* is known as bael. The *Aegle marmelos* Family is Rutaceae. The components that which having diverse bioactive importance in bael fruits are aeglinosides, aegeline, aegelenine, marmelosin, malondialdehyde, marmelin, marmelide, lupeol, imperation, xanthorrhizol. The *Aegle M.* having a pharmacological activity like antioxidant, anti-diarrheal, anti-inflammatory, anti-bacterial, antimicrobial, antiulcer, antidyslipidemic, gastric mucosal protective, antiviral, antifungal.[14, 15, 16]

Foeniculum vulgare is commonly known as 'Fennel' which is an aromatic and medicinal herb and it's generally used in gastrointestinal and digestive problems. *Foeniculum vulgare* belongs to the Apiaceae family. *Foeniculum vulgare* is mainly known for its essential oils. The important in *Foeniculum V.* is seeds essential oil which consists of trans-anethole, estragole, fenchone, alpha-phellandrene, limonene, and alpha-pinene. *Foeniculum V.* is used as anti-inflammatory, anti-viral, anti-microbial, antispasmodic, antipyretic, antitumor, hypolipidemic, and hypoglycemic, including antibacterial, antifungal, antioxidant. [17, 18, 19]

Cuminum cyminum's common name is known as 'Cumin' and family Umbelliferae (Apiaceae). The seeds of cumin contain fixed and volatile oil. Cuminaldehyde is an important bioactive component of cumin. Alpha-pinenes, beta-pinenes, eugenol, and limonene are other components that are found in cumin. Two sesquiterpenoids glycosides i.e. cuminoside A and Cuminoside B with two alkyl glycosides are also found in seeds. Pharmacological activities of cumin were used as medicines such as stimulants, astringents, flatulence, diarrhea, and carminative. The essential oil of cumin is a source of antimicrobial, antibacterial, antioxidant, and antifungal. [20, 21]

Asafoetida is an oleo-gum resin that has a strong odor and this spicy herb also used in Indian foods as spicy ingredients. It is also known as the source of medicine in Ayurveda and the most effective remedy for the stomach. It is obtained from the Ferula plant which belongs to the family Apiaceae (Umbelliferae). *Asafoetida* shows the properties of protein, carbohydrate, moisture, minerals, fibers, and fats. The main compounds of *Asafoetida* include resin, gum, and essential oil. The pharmacological activity of *Asafoetida* is anti-diabetic, anti-oxidant, anti-cancer, laxative, anti-fungal, and antiviral including aromatic, antispasmodic,

digestive, sedative, expectorant, carminative, and analgesic agent. *Asafoetida* also used to treat various intestinal problems like influenza, intestinal parasites, stomachache, epilepsy, weak digestion, and flatulence. [22, 23, 24]

Plantago ovata is used in both commercial and traditional medicine. *Plantago ovata* is also known as 'Psyllium Husk', 'Isabgol', 'Sand Plantain', and 'Sapogel'. Isabgol belongs to the Family Plantaginaceae. The bioactive compounds found in the seed of *Plantago ovata* are fatty acid, polyphenols, flavonoids, amino acid, and tannin. Hemicellulose is also found in the seed of *Plantago ovata* which is composed of Xylan and linked with rhamnose, arabinose, and galacturonic acid. Arabinoxylans (highly gel-forming property) is a polysaccharide and it is an important bioactive compound of *Plantago ovata*. This herbal source was used in constipation, Inflammatory Bowel Disease (Ulcerative Colitis), and colon cancer. [25, 26]These herbal drugs are shown in figure 1.

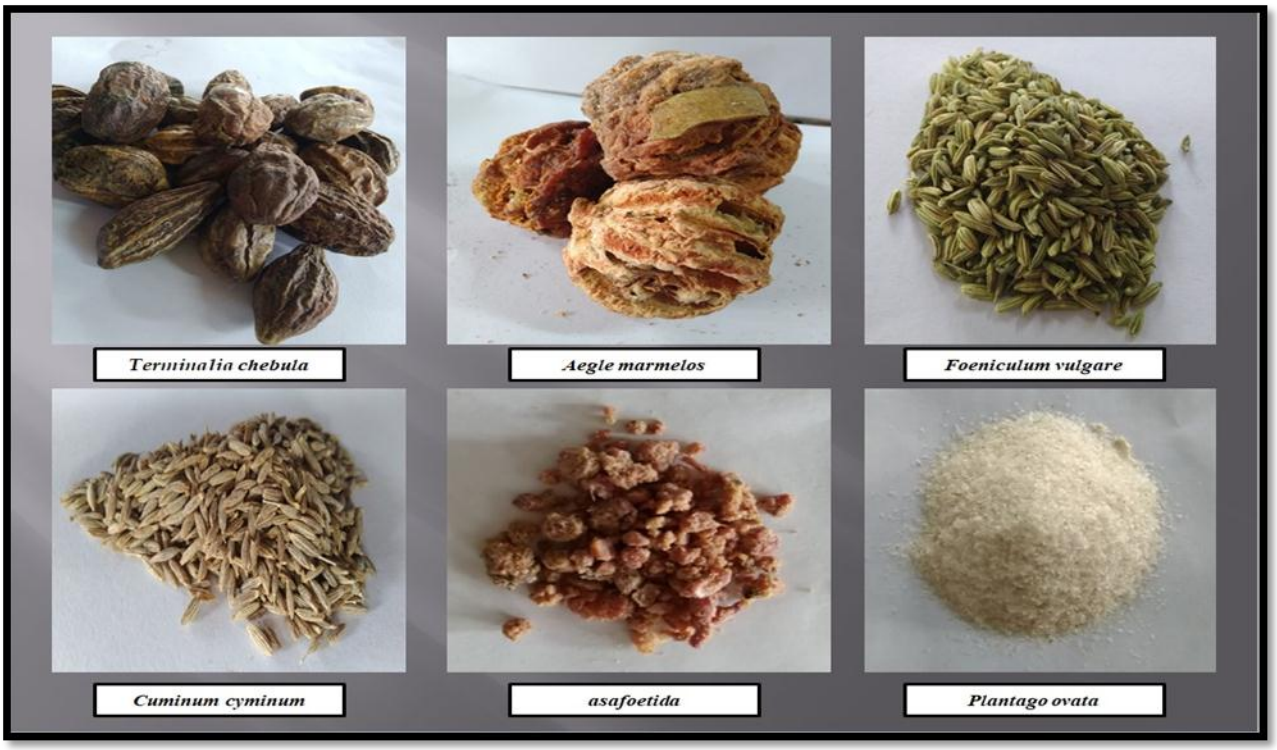


Figure No. 1: ALL SIX HERBAL DRUGS USED IN THE PREPARATION OF POLYHERBAL ETHANOLIC EXTRACT

MATERIALS AND METHODS:

MATERIAL:

Selection and collection of plant material: *Terminalia chebula*, *Aegle marmelos*, *Foeniculum vulgare*, *Cuminum cyminum*, *Ferula asafoetida*, and *Plantago ovata* were obtained from the Chandra Shekhar Azad University of Agriculture and Technology, Kanpur.

Selection of Experimental animals

The protocol of the experimental study design was approved by the animal ethical committee which was followed as per CPCSEA (Committee for Control & Supervision of Experiments on Animal) guidelines.

METHOD:

Preparation and Standardizations of Poly-herbal Extract

The raw material i.e. *Terminalia chebula*, *Aegle marmelos*, *Foeniculum vulgare*, *Cuminum cyminum*, *Plantago ovata* and *Ferula asafoetida* was powdered firstly in a mortar pestle and each of the herbs was then subjected to an electrical grinder followed bypassing through sieve no. 120 # for the formation of fine powder. The fine powder of each herb material was obtained and further mixed in a specific scale.

Properties of Polyherbal Powder

Organoleptic properties, Physical evaluation (Moisture Content, Total Ash value, Acid-insoluble ash, Water-insoluble ash), Rheological properties (bulk density, angle of Repose, Hausner's ratio, and Carr's index) were determined. [27]

Preparation of extract

The soxhlet extraction technique is effective for the extraction of phytocompound of plants or herbs and the system works in continuous mode. The powdered samples were loaded into the thimble which made of thick filter paper and were placed inside the soxhlet extractor or chamber. The isomantle brings heat to the solvent which is placed in a flask. The solvent begins to evaporate and is moved to the condenser. The condensate drips into the soxhlet chamber containing thimble. Once the solvent level reaches the siphon tube and empties with

the running of the solvent back to the flask. The cycle is allowed to repeat until the process of extraction has been done. [27, 28]

The soxhlet techniques used for the extraction of poly-herb. The polyherbal ethanolic extract was obtained after running the solvent at a temperature of 50°C with 4 cycles.

The presence of compound: The phytochemical tests were followed by the ethanolic extract of the polyherbal powder by the standard method for the constituents' identification which include molish test, detection of reducing sugar, Benedict's test, legal test, Borntrager's test, Modified Borntrager's test, Baljit's test, Wager's test, Hager's test, Dragendroff's test, Mayer's test, Shinoda test, Lead acetate test, Alkaline reagent test, Zinc-HCl test, Xanthoproteic test, Biuret test, Millon test, Ninhydrin test, Ferric chloride test, Gelatin test, Foam test, Salkowski's test, and Copper acetate test. [27, 29, 30]

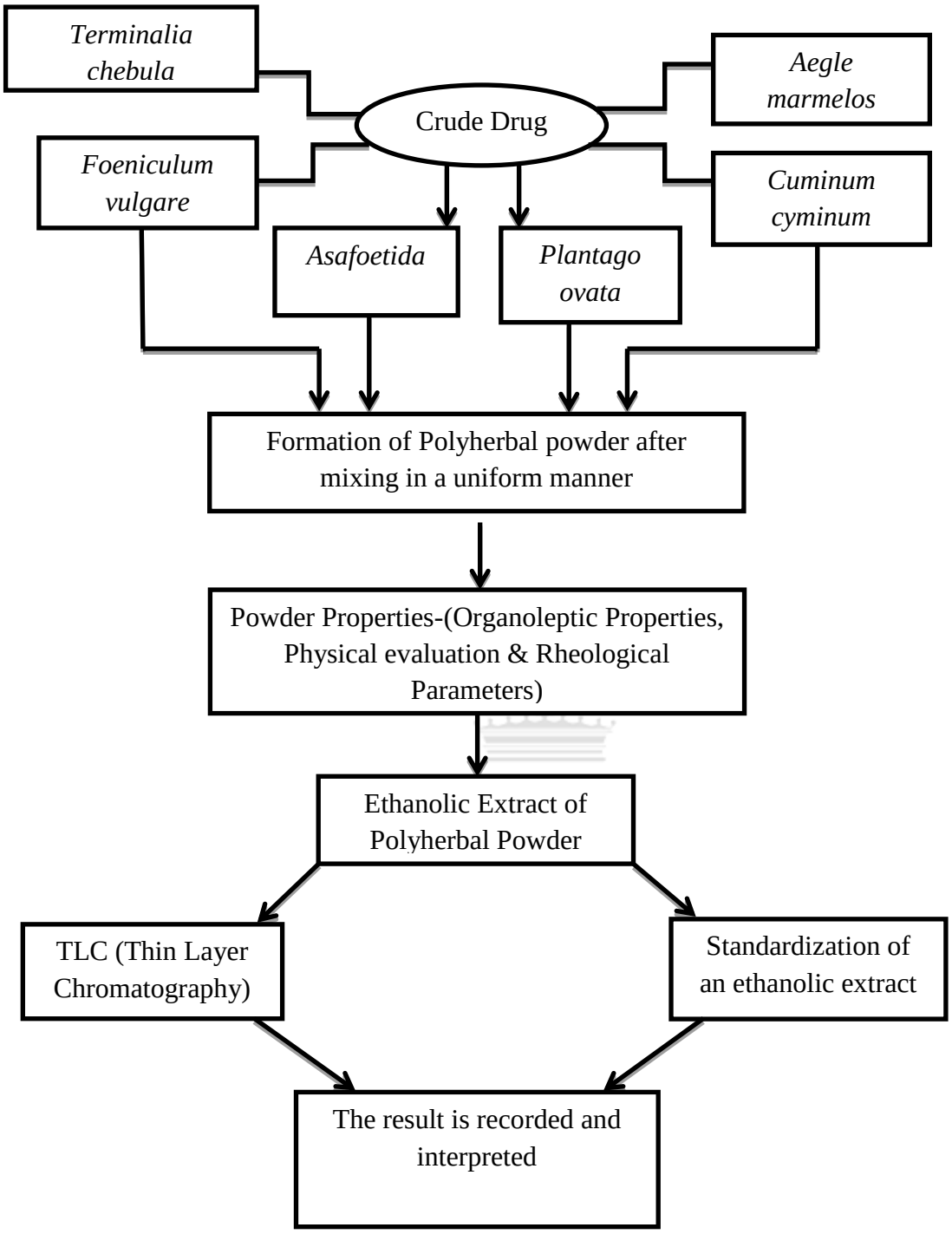
Thin-Layer Chromatography

The study was carried on the extract of the polyherbal drug on a prepared TLC plate (Silica Gel (G)). The different mobile phase of distinct polarities was also prepared for the study. By using a capillary tube the extract of the plant was applied in a TLC plate. In the iodine chamber, the observation was done. [27, 31] The movement of the sample as indicated by its retention factor (R_f value). The R_f value was calculated by:

R_f = distance traveled by solute/ distance traveled by the solvent

The Following Flow chart representation of work till chromatography and phytochemical parameters:

Flowchart of the Procedure till Present of Components



Acute Oral Toxicity studies (OECD test guideline No. 423)

To assess the therapeutic index or it mention as a Therapeutic ratio i.e. the ratio between the toxic dose / lethal dose and the pharmacological effect of dose (LD_{50}/ED_{50}). The compound is safe the therapeutic ratio is considerable. The experiments were performed as per OECD guidelines No. 423 for 14 days. The male Wistar rats were selected and weighed (120-150g).

Then the animals were divided into 5 groups (6 per group) and the doses of animals were selected on the bases of guideline i.e. 5 mg/kg, 50 mg/kg, 300 mg/kg, 2000 mg/kg and first group were a normal control group. Before the drug administration, the animals were kept for fasting (24 hrs.) with free access to water. [32] Acute oral toxicity groups of rats are given below:

Group I = Normal Control group

Group II = 5 mg/kg dose

Group III = 50 mg/kg dose

Group IV = 300 mg/kg dose

Group V = 2000 mg/kg dose

Sub-acute oral toxicity/Repeated dose (OECD test guideline No. 407)

The test substance was daily administered through the oral route for 28 days according to the OECD-407 guidelines. For the sub-acute toxicity study, healthy rats were taken (both male & female). A total of rats were distributed in separate 4 groups. Group 2nd received a dose of 125 mg/kg, 3rd were received 250 mg/kg, and 4thgroup received 500 mg/kg dose per day. Whereas group 1st one served as normal control. The treated animals were observed closely during the time of administration. The mortality or the euthanatized during the period of the test were necropsied. At the end of the experiment, the surviving animals were euthanatized and also necropsied. The organs of the euthanatized rat were separated, weighed, and observed for the sign of abnormality or toxicity. [33]

Sub-acute oral toxicity group are given below:

Group I = Normal Control group

Group II = 125 mg/kg dose

Group III = 250 mg/kg dose

Group IV = 500 mg/kg

On 28th day or termination day, the euthanized rats were carefully dissected to isolate the vital organs i.e. liver, heart, spleen, lungs, kidney, and weighed the organs (relative &

absolute organ weight) in grams. The relative organ weight (ROW) of each Wistar rats was calculated by a given formula;

$$\textbf{Relative Organ Weight (ROW)} = \frac{\textbf{Absolute organ weight (g)}}{\textbf{Body weight of rat on sacrifice day (g)}} \times \textbf{100}$$

The evaluation of biochemical parameters was also determined which include SOD, CAT, MAD, MPO, and GSH.

Ulcerative Colitis Healing Activity (Pharmacological Activity of Ulcerative Colitis): The induction of Ulcerative colitis was done by the following two models:

TNBS (2, 4, 6-trinitrobenzene sulfonic acid) induced Colitis: Before the induction of colitis in rat, a fasting should be for 24 hrs. Ether or Halothane can be used for mild anesthesia to avoid pain. A medical-grade polyurethane or polypropylene catheter (2 mm diameter) is suitable for the administration of TNBS through anal verge. For the induction of colitis, Hapten reagent TNBS (2mg) was mixed in ethanol (0.1 ml of 50%). Then the mixture was slowly administered into the colon with a catheter which was attached with 1ml of a syringe. After that, the rat was maintained the vertical position also refers to the supine Trendelenburg position to avoid leakage and also ensure the distribution of chemicals in the colon. [34, 35]

The rats were divided into six groups (four animals in each) as follow:

Group I = Normal Control

Group II = Disease group / TNBS induced group

Group III = Standard Group (Prednisolone Drug)

Group IV = 300 mg/kg of body weight/treated group 1

Group V = 500 mg/kg of body weight/treated group 2

Acetic acid Induced Colitis

Before the induction of experiment colitis, the animal was kept for fasted for 24 hrs. The rat's bowel was cleaned by cotton. Under the anesthetic (Ether or halothane) condition, a polyurethane or polypropylene catheter about 2mm in diameter was inserted carefully into the

rectum and was placed about 8 cm proximal to the anus verge. In the next step, Acetic acid (2ml of 3%) was given or administered through the rectum via the catheter which was fitted with a syringe for 1-2 mins. The rat has maintained the vertical position (Supine Trendelenburg position) to avoid the backflow of the fluid and also for proper distribution of fluid. [36]

The Wistar rats were divided into six groups four in each group were as follow:

Group I = Normal Control

Group II = Disease group / Acetic acid-induced

Group III = Standard Group (Prednisolone drug)

Group IV = 300 mg/kg of body weight (Treated group 1)

Group V = 500 mg/kg of body weight (Treated group 2)

Assessment of Inflammation

The tissue of the colon was excised which is about 8 cm in length and approx. 2 cm to the anus, then the colon tissue washed thoroughly in phosphate-buffered saline having pH 7.4.

Colon weight: On the day of termination, the rat was euthanized and dissected. The colon was excised and opened longitudinally. The feces were removed by cleaning gently under tap water. Then the colon was placed on a clean and unabsorbing surface. Thereafter colon weight was assessed and recorded. [37]

DAI: Weight of body, consistency of stool, and blood in feces was recorded daily for the ascertainment of DAI. Disease Activity Index was calculated by scoring the changes in stool consistency, bloody fecal, and body weight. [38] The Scoring system for stool consistency & body weight were given in Table 1:

Table No. 1: DAI SCORE-A 12 point score

% fall in b. wt.	Score	Stool Consistency
0%	0	Normal
1-5%	1	-
6-10%	2	Pasty & Semi-solid
11-20%	3	-
>=21%	4	Liquid

The scoring limitation ranged between 0-12 (total score). The body weight changes were calculated by the given formula:

$$\textbf{Bodyweight} = \frac{(final\ weight - initial\ weight)}{initial} \times 100$$

Ulcer Index: The percentage of impediment against ulceration was evaluated by using the given expression: [39]

$$\textbf{UI} = (\text{Ulcerated Area} \div \text{Total Colon Area}) \times 100$$

$$\textbf{UI \%} = [\textbf{UI}_{(control)} - \textbf{UI}_{(test)} / \textbf{UI}_{(control)}] \times 100$$

Biochemical parameters of Pharmacological activity

The colon tissue or ileum tissue were washed and homogenized on Tris-HCl buffer having pH 7.4 (0.01M). Then the homogenate was used to evaluate the following:

SOD activity: Superoxide dismutase is a cytoplasmic enzyme and an important antioxidant that helps to protect the cell from destruction by destroying superoxide anions (O_2). The decreased in the activity of SOD leads to an increasing level of superoxide. In the reaction mixtures, adrenochrome production contains 3×10^{-4} M. The 150µl of chloroform ice-cold and 750µl ethanol was mixed with 500 µl homogenate and centrifuged (2000 rmp) at 25°C for 20 mins. To 500µl supernatant, 1ml of carbonate bicarbonate buffer at pH 10.2 (0.1M) and 500µl EDTA (Ethylene diamine tetraacetic acid) 0.6mM was added. After the addition of 50µl of adrenaline (1.3mM), the reaction was begun. The absorbance of SOD was measured in the opposite of a blank at 480nm for four minutes by spectrophotometer. The SOD enzyme

was determined as the total of enzymes that impeded the adrenaline autoxidation at 25°C by 50%. [37, 40] The formula of inhibition of adrenaline autoxidation:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance (test)} - \text{Absorbance (blank)})}{\text{Absorbance (test)}} \times 100$$

$$\text{Unit of SOD activity/mg protein} = \left\{\frac{(Percentage\ inhibition)}{50 \times weight\ of\ protein}\right\} \times 100$$

GSH activity: Glutathione (g-glutamylcysteinyl-glycine) is a tripeptide and most abundant antioxidant in mammalian cells. GSH plays an important role in the inhibition of lipid peroxidase and detoxification of H_2O_2 (Hydrogen peroxidase) by the enzyme glutathione peroxidase (GP). The concentration range of GSH is 0.5 to 10 mM in a healthy cell or tissue. GSH exists in two forms i.e. reduced GSH and oxidized GSH (GSSG; glutathione disulfide). The majority of GSH that exists in a healthy cell is in reduced GSH form. The reduced GSH level decreased and increased in the GSSG level during oxidative stress that leads to the cause of some diseases.

To 0.5ml of supernatant (tissue homogenate) was added in tris-buffer(0.2M, pH 8.2) containing 0.1ml of 0.01m DNBS [5,5'-dithiobis – (2-nitro – benzoic acid)] or Ellman's reagent. Then the sample was centrifuged at room temperature (15 mins.). The GSH was determined by using a spectrophotometer at 412nm. [41] The following equation to calculate the reduced GSH concentration:

Reduced GSH concentration = Total GSH – Oxidative GSH (GSSG)

To calculate the total GSH concentration of the sample by the endpoint method:

$$\text{Total GSH} = \frac{(Absorbance\ at\ 414\ nm) - y - interpret}{Slop} \times 2^{*} \times \text{sample dilution}$$

*****Whole equation multiply by 2 as 1 GSSG = 2GSH

MPO activity: Myeloperoxidase is a pro-inflammatory enzyme or lysosomal protein mainly stored in neutrophils. MPO catalyzes the hypochlorous acid which is formed from H_2O_2

(Hydrogen peroxidase) and also generates the molecules which are highly reactive i.e. tyrosyl radicals & cross-linked protein. These highly cytotoxic products are released from the tissue or cell for the destruction of foreign microorganisms. These agents are also involved in inflammation and can damage the normal cells.

To evaluate the MPO activity the homogenate was integrated with a solution that was filled with 50mM potassium phosphate buffer (pH 6.0) adding 0.5% Hexdecyltrimethyl ammonium bromide (HTAB) and 10mM EDTA. Before sonication, the homogenate was freeze-thawed (3 times) then centrifuged at 15000 rpm for 15 mins. At 450nm MPO activity was steady by spectrophotometer. For this ground, 0.1 ml of supernatant was involved with 2.9 ml of phosphate buffer (50mM), 0.167 mg/ml O-dianisidine HCl and 0.0005% of H_2O_2. The MPO activity of the enzyme was determined by degrading one mM of peroxide per min. at 25°C. [42] Calculation of MPO by the given formula:

$$\textbf{MPO activity} = \frac{\textbf{X}}{\textbf{weight of the sample tissue}}$$

Where X;

X = (10 × change in absorbance per min.) / volume of supernatant in the final reaction (ml)

MDA activity: Lipid peroxidase has also represented the destruction of tissue because of inflammation. Unrestricted lipid peroxidase leads to an increase in the level of MDA. MDA is an expression or end product of lipid peroxidation level in the solution of plasma. One ml of supernatant was assorted with 1.5 ml of 20% acetic acid, 0.2 ml of 8.1% SDS (Sodium Dodecyl Sulphate) containing 1.5 of 0.8% of TBA (aqueous solution). The sample of the mixture was heated at 95°C for 30 mins. Then centrifuged at 2000 rpm (10 mins.) after cooled in an ice bath. The measurement of absorbance of the MDA-TBA complex was developed in opposition to the blank reagent (532nm). MDA was calculated by using the molar coefficient i.e. $1.56 \times 10^{-5}M^{-1}$. [37]

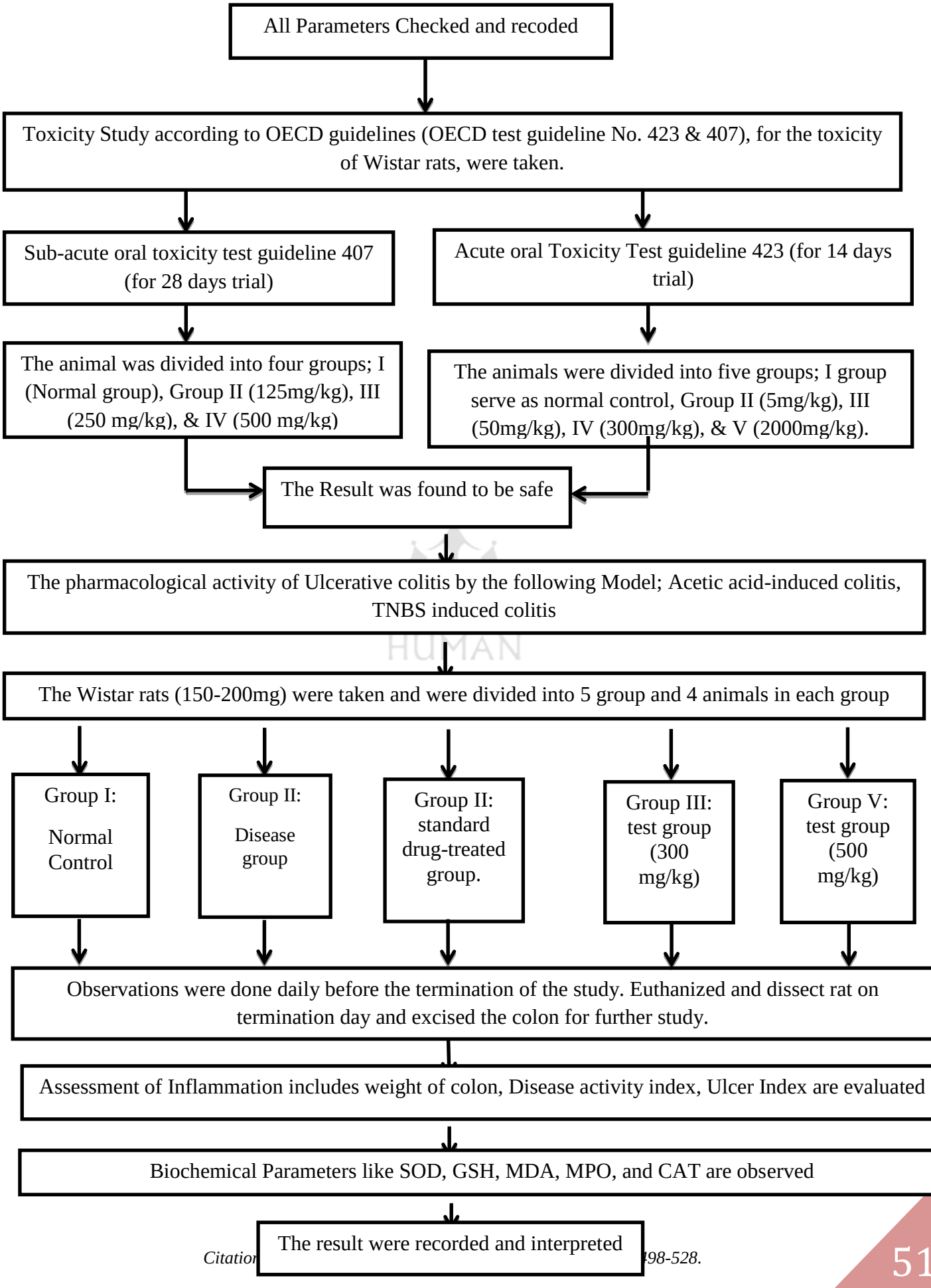
CAT activity: In mitochondria, CAT is a cytoplasmic enzyme that helps in the decomposition of H_2O_2 (Hydrogen peroxidase) in water and molecular oxygen.

Catalase activity of one unit was described as the total enzyme that required causing putrefaction of 1µmol H_2O_2/min/mg protein (pH 7.0) at temperature 25°C. The mixture of

assay carry 1 ml of potassium phosphate buffer (0.01M) having pH 7.0, 500µl hydrogen peroxide (1.18M) and water (400µl) was put in the supernatant and initiate the incubation reaction for 5 mins. at 28°C. One ml reaction was terminated after the addition of a 2 ml acetic acid-dichromate mixture. The chromic acetate absorbance was determined by spectrophotometer at 620nm. The catalase enzyme activity was shown in the title of its molar extinction coefficient of 39.4 $M^{-1}cm^{-1}$[37]. Determination of CAT by the given formula:

$$\textbf{mUnit CAT mg/protein} = \left[\frac{Absorbance\ (620\ nm)}{(3.94)\times \text{weight of protein}}\right] \times 1000$$

The whole procedure of the experiment was summarized in the following flow chart:



RESULTS AND DISCUSSION:

RESULT

Properties of Polyherbal Powder: The result or value of organoleptic properties, physical evaluation, and rheological properties are given in table 2 and table 3 respectively.

Table No. 2: Organoleptic Properties of Polyherbal Powder

S. No.	Properties	Results
1.	Appearance	Powder
2.	Texture & Particle Size	Moderate Fine & 45#
3.	Color	Brown or Dull brown
4.	Odor	Unpleasant
5.	Taste	Bitter/sour

Table No. 3: Physiochemical and Rheological Properties of Polyherbal Powder

S. No.	Parameters	Results
1.	Moisture Content	11.2%
2.	Total Ash	0.094 g
3.	Acid insoluble Ash%	3.15%
4.	Water-insoluble Ash%	1.6%
5.	Angle of Repose	39.88°
6.	Bulk Density	0.512 g/ml
7.	Tapped density	0.645 g/ml
8.	Carr's Index	20.62
9.	Hausner's Ratio	1.25

The presence of Components in polyherbal ethanolic extract: Phytochemical screening of ethanolic extract disclosed the presence of many phytochemical components which are shown in table 4.

Table No. 4: Phytochemical Screening of Polyherbal Ethanolic Extract

S. No	Phytochemical component	Observation/Results
1.	Detection of Carbohydrates	
	a. Molish's test	+
2.	Detection of Reducing Sugar	
	a. Fehling Solution	+
	b. Benedict's test	+
3.	Detection of Cardiac Glycoside	
	a. Legal test	+
4.	Detection of Anthraquinone	
	a. Bontrager's test	+
	b. Modified Bontrager's test	+
	c. Baljit's test	+
5.	Detection of Alkaloids	
	a. Wager's test	+
	b. Hager's test	+
	c. Dragendroff's test	+
	d. Mayer's test	+
6.	Detection of Flavonoids	
	a. Shinoda test	+
	b. Lead acetate test	+
	c. Alkaline reagent test	-
	d. Zinc-hydrochloric acid test	+
	Detection of Amino acid/Protein	
7.	a. Xanthoproteic test	+
	b. Biuret test	+
	c. Millon test	+
	d. Ninhydrin test	-
8.	Detection of tannins & Phenol	
	a. Ferric Chloride test	+
	b. Gelatin test	+
	Detection of Saponins	
9.	a. Foam test	+
10.	Detection of terpenoids& steroids	
	a. Salkowski's test	+
	b. Copper acetate test	+

(-) absent & (+) presence

TLC (Thin Layer Chromatography)

The ethanolic extract of combined six drugs was subjected to TLC (Thin Layer chromatography) using Methanol: Chloroform as a solvent system and observation was done under iodine chamber. The R_f (refractive index) value was calculated:

R_f = Distance traveled by the substance/distance traveled by the solvent

R_f = 5.0 / 6.0

$R_f = 0.83$

Acute Oral Toxicity

The doses taken for acute toxicity are between 5mg/kg-2000mg/kg. The deaths were not observed during the experiment of 14 days. The animal shows no toxicity in any of the given groups. According to OECD guidelines some parameters observed during the study like fecal, salivation, urination, food and water taken, etc. are given in table 5. The study shows that the dose administration up to 2000 mg/kg did not show any toxic effect on the body.

Table No. 5: Results obtained post-acute toxicity studies of the oral polyherbal ethanolic extract on Wistar rats

S. No.	Response	Control Group	5 mg/kg	50 mg/kg	300 mg/kg	2000 mg/kg
1.	Mortality	×	–	–	–	–
2.	Pupils	Regular	Regular	Regular	Regular	Regular
3.	Touch response	Regular	Regular	Regular	Regular	Regular
4.	Water & Food intake	Regular	Regular	Regular	Regular	Regular
5.	Convulsion	–	–	–	–	–
6.	Alertness	Regular	Regular	Regular	Regular	Regular
7.	Skin Color	Regular	Regular	Regular	Regular	Regular
8.	Urination	Regular	Regular	Regular	Regular	Regular
9.	Griping	Regular	Regular	Regular	Regular	Regular
10.	Saliva	Regular	Regular	Regular	Regular	Regular
11.	Fur shielding/density	Regular	Regular	Regular	Regular	Regular
12.	Feces blood	–	–	–	–	–
13.	Tremors	–	–	–	–	–
14.	Grooming & writhing	–	–	–	–	–
15.	Corneal Reflex	–	–	–	–	–

('×' not applicable)

Sub-Acute oral toxicity study

It also refers to a repeat dose because the only period of the dose was extended to 28th days. In sub-acute toxicity, the doses of 150 mg/kg, 250 mg/kg and 500 mg/kg were shown no sign of toxicity or morbidity and mortality. There is also no death was recorded and no change in behavior was observed during the experiment as compared to the control group. This study also showed a gradual increment of body weight as compared with the control one ($p<0.05$).

The changes in body weight are shown in table 6.

Table No. 6: Difference in Wistar rats' body weight that found on termination day of sub-acute toxicity test

Period	Control Group	125 mg/kg	250 mg/kg	500 mg/kg
Initial Weight	145.70 ± 22.80 g	144.71 ± 23.70 g	147.40 ± 10.92 g	137.82 ± 8.24 g
Final Weight	204.20 ± 32.09 g	203.21 ± 31.10 g	204.90 ± 15.19 g	192.40 ± 15.60 g

(n=6) ($p<0.05$)

After calculation of weight the dissection of wistar rat were done with the dissection kit and after that seperation, observation and weighing of other organs (like lungs, liver, spleen, kidney and heart) were done carefully. (Image of dissected rat and separated organs are given in fig. 2 and fig. 3 respectively).

FIGURE NO. 2: WISTAR RAT BEFORE AND AFTER DISSECTION

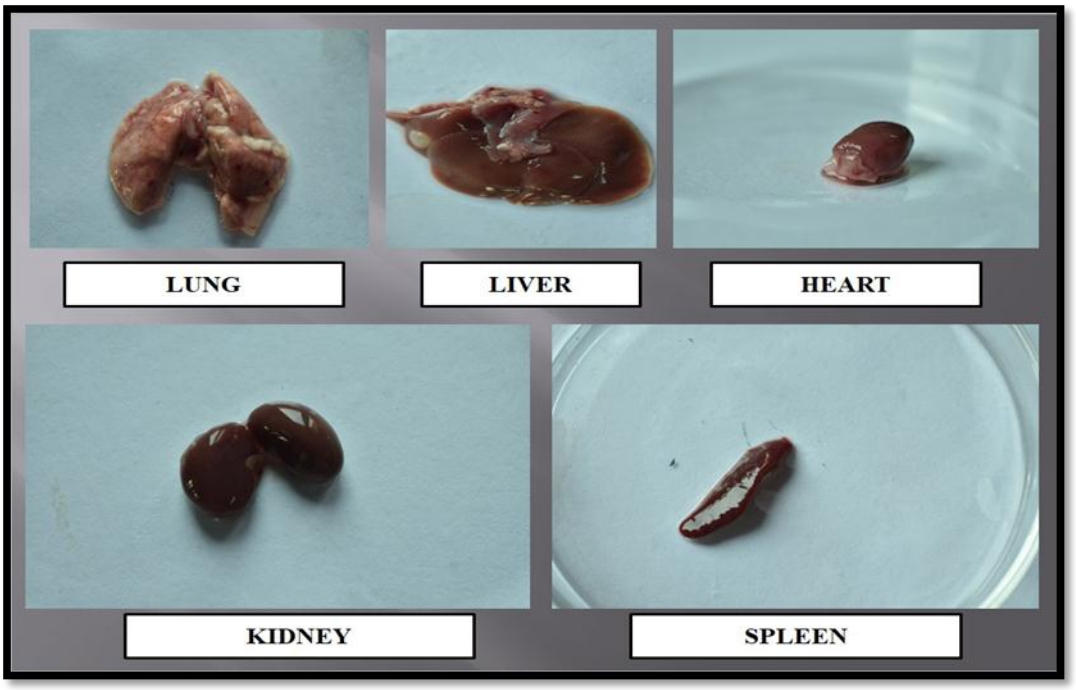


FIGURE NO. 3: DISSECTED ORGANS OF WISTAR RAT

In ROW, There is no remarkable changes were seen in liver, lungs, heart, kidney and spleen. (Table 7).

Table No. 7: Effect of polyherbal ethanolic extract on the ROW of rats- (g/100g body weight)

Doses	Lungs	Liver	Heart	Kidney	Spleen
Control	0.73 ± 0.03	3.87 ± 0.23	0.4 ± 0.07	0.64 ± 0.09	0.43 ± 0.11
125 mg/kg	0.90 ± 0.08	3.88 ± 0.11	1.42 ± 0.04	1.74 ± 0.04	1.47 ± 0.04
250 mg/kg	0.87 ± 0.10	3.90 ± 0.17	0.44 ± 0.04	0.75 ± 0.02	0.53 ± 0.02
500 mg/kg	0.84 ± 0.7	3.89 ± 0.14	0.45 ± 0.06	0.88 ± 0.04	0.75 ± 0.08

(n=6) ($p<0.05$)

The biochemical parameters were analyzed at the end of the sub-acute oral toxicity study. The enzymatic activity (SOD, CAT & GSH) and MPO and MDA level are shown near to the control group. (Table 8)

Table No. 8: Effect of polyherbal ethanolic extract at the end of sub-acute oral toxicity study in Wistar rats by biochemical parameters

Doses	SOD (mU/mg Protein)	CAT (mU/mg Protein)	MPO (mU/mg Protein)	MDA (nmol/g protein)	GSH (nmol/g protein)
Control	185.3 ± 22.0	2.34 ± 0.23	7.81 ± 0.35	3.64 ± 2.02	8.89 ± 0.60
125 mg/kg	186.7 ± 24.0	3.36 ± 0.22	8.83 ± 0.32	4.63 ± 1.01	9.92 ± 0.63
250 mg/kg	186.8 ± 25.0	2.37 ± 0.24	7.82 ± 0.34	3.62 ± 2.03	8.90 ± 0.61
500 mg/kg	187.6 ± 23.0	2.40 ± 0.21	7.80 ± 0.30	3.60 ± 2.04	8.87 ± 0.59

(n=6) ($p<0.001$)

ULCERATIVE COLITIS HEALING ACTIVITY (PHARMACOLOGICAL ACTIVITY OF ULCERATIVE COLITIS)

Some parameters are related to the inflammation of colitis directly whereas some are indirectly related to the inflammatory action of ulcerative colitis like SOD, MDA, MPO, GSH, and CAT.

Results of TNBS (2, 4, 6-trinitrobenzene sulfonic acid) induced Colitis model:

In experimentation, After the TNBS administration, the estimation of DAI can be achieved by stool consistency, bleeding, and changes in body weight after the induction of colitis in rats.

The treated groups of all doses (300 mg/kg, & 500 mg/kg) are showed notable improvement in DAI in comparison to the disease group. In UI, treatment group 1 (5.3 ± 0.35) and treatment group 2 (4.3 ± 0.36) shows equal action as compared to the standard group (3.4 ± 0.27) whereas the colon weight & length of treatment group 1 (148.3 ± 13.3) are significantly equal to standard drug group (146.4 ± 18.3) also the treatment group 2 (145.5 ± 18.1) are precise than standard group and the effect was improved when compared to diseased group (201.4 ± 9.8). The benefits of polyherbal ethanolic extract were confirmed by inflammation assessment (table 9).

Table No. 9: Assessment of Inflammation by help of various parameters

S. No.	Groups	Disease activity Index	Ulcer Index	Colon weight & length (g/cm)
1.	Normal Control	0.0± 0.0	0.0 ± 0.0	70.2 ± 2.1
2.	Disease group	8.0 ± 0.42[a]	11.3 ± 0.64	201.4 ± 9.8
3.	Standard drug group	0.1 ± 0.1[c]	3.4 ± 0.27[b]	146.4 ± 18.3[*]
4.	Treatment group 1 (300 mg/kg)	0.6 ± 0.25[c]	5.3 ± 0.35[a]	148.3 ± 13.3[*]
5.	Treatment group 2 (500 mg/kg)	0.2 ± 0.5[c]	4.3 ± 0.36[a]	145.5 ± 18.1[*]

(n=6) ([*]p<0.05) ([a]p<0.01) ([b]p<0.001) ([c]p<0.0001)

In biochemical parameters; SOD of treatment group 2 (230.1 ± 23.7) is significantly near to the activity of the standard drug group (233.4 ± 18.1) whereas improved in contrast to the diseased group (45.8 ± 8.09). GSH activity of treatment group 1 (7.18 ± 0.63) significantly equal to the standard drug group (7.03 ± 0.42) whereas treatment group 2 (8.17 ± 0.64) is more precise. CAT enzymatic activity in treatment group 3 (4.12 ± 0.17) is significantly equal to standard group (4.18 ± 0.21) activity and shows improvement in correspondence to the diseased group (1.27 ± 0.05). MDA activity in treatment group 2 (3.64 ± 0.17) is more precise and significantly improved in comparison to a standard group (5.74 ± 1.04) and diseased group (7.86 ± 0.54) respectively. MPO activity in both treatment group i.e. group 1 (16.8 ± 1.84) & group 2 (15.0 ± 1.85) significantly more accurate than the standard group (18.0 ± 3.18) and also showed an improvement when compared to the diseased group (4.12 ± 0.17) as in table 10.

Table No. 10: Effect of polyherbal ethanolic extract and standard drug on TNBS-induced colitis in Wistar rats by biochemical parameters at the end of the study

S. No.	Groups	SOD (mU/mg Protein)	MDA (nmol/g protein)	MPO (mU/mg protein)	GSH (nmol/mg Protein)	CAT (mU/mg protein)
1.	Normal Control	184.3 ± 21.0	3.66 ± 1.02	7.82 ± 0.36	8.70 ± 0.70	2.36 ± 0.22
2.	Disease group	45.8 ± 8.09*	7.86 ± 0.54a	61.8 ± 1.40*	4.40 ± 0.36*	1.27 ± 0.05*
3.	Standard drug group	233.4 ± 18.1*	5.74 ± 1.04a	18.0 ± 3.18*	7.03 ± 0.42*	4.18 ± 0.21*
4.	Treatment group 1 (300 mg/kg)	228.2 ± 24.6*	5.87 ± 0.25a	16.8 ± 1.84*	7.18 ± 0.63*	3.24 ± 0.16*
5.	Treatment group 2 (500 mg/kg)	230.1 ± 23.7*	3.64 ± 0.17a	15.0 ± 1.85*	8.17 ± 0.64*	4.12 ± 0.17*

(n=6) (*p<0.001) (ap<0.05)

Results of Acetic acid Induced Colitis model:

The acetic acid-induced colitis study shows that the doses of 300 mg/kg and 500 mg/kg of the polyherbal ethanolic extract are effective and evaluated in colitis test. In DAI, all the treatment groups [group 1 (2.0 ± 0.45), & group 2 (0.1 ± 0.4)] showed a recovery action when compared with the disease control group (6.75 ± 0.47). In UI, the treatment group 1 (4.40 ± 0.32) shows the same effect as the standard group (4.73 ± 0.24) and significantly improved in comparison to the diseased group (10.00 ± 0.61) also the treatment group 2 (3.48 ± 0.30) are more accurate than the standard group. Colon weight & length of treatment group 2 (0.41 ± 0.05) are significantly improved when compared to the diseased group (0.96 ± 0.06) and more précised than the standard drug group (0.43 ± 0.01). The Acetic acid parameter results are shown in Table 11.

Table No. 11: Assessment of Inflammation on AA-induction of colitis

S. No.	Groups	Disease activity Index	Ulcer Index	Colon weight & length (g/cm)
1.	Normal Control	0.0 ± 0.0	0.0 ± 0.0	0.35 ± 0.4
2.	Disease group	6.75 ± 0.47[a]	10.00 ± 0.61	0.96 ± 0.06
3.	Standard drug group	0.2 ± 0.2[c]	4.73 ± 0.24[b]	0.43 ± 0.01[*]
4.	Treatment group 1 (300mg/kg)	2.0 ± 0.45[a]	4.40 ± 0.32[*]	0.47 ± 0.05[*]
5.	Treatment group 2 (500mg/kg)	0.1 ± 0.4[a]	3.48 ± 0.30[*]	0.41 ± 0.05[*]

(n=6) ([*]p<0.05) ([a]p<0.01)([b]p<0.001) ([c]p<0.0001)

In Biochemical parameters, the SOD activity of the treatment group 2 (5.25 ± 0.07) is significantly equal to the standard drug group (5.20 ± 0.04) and showed a modified action in comparison to the diseased group (2.52 ± 0.03). GSH enzyme activity in treatment group 2 (8.70 ± 0.60) is significantly improved when compared to the diseased group (3.40 ± 0.20) and near to the activity of the standard drug group (7.90 ± 0.38). CAT activity of the given treatment groups i.e. group 1 (16.3 ± 0.27) & group 2 (18.7 ± 0.29) are significantly equal to the standard drug group (15.8 ± 0.17) and also upgraded in comparison to the diseased group (09.2 ± 013). In the activity of MDA, all the treatment groups [group 1 (63.61 ± 2.32) & group 2 (61.60 ± 2.30)] showed significant action in comparison of both standard drug group (71 ± 5.10) and diseased group (117.20 ± 4.50). MPO activity in treatment group 2 (08.8 ± 3.47) significantly precise to the standard drug group (8.06 ± 1.62), both treatment group 1 (10.7 ± 2.48) and group 2 (08.8 ± 3.47) significantly modified in correspondence to the diseased group (75.0 ± 4.54) as in table 12.

Table No. 12: Assessment of Biochemical parameters in the end of the study to analyze the effect of polyherbal ethanolic extract and standard drug

S. No.	Groups	SOD (mU/mg protein)	MDA (nmol/g protein)	MPO (mU/mg protein)	GSH (nmol/mg Protein)	CAT (mU/mg protein)
1.	Normal Control	07.24 ± 0.07	58.02 ± 3.80	07.24 ± 1.17	8.70 ± 0.70	22.7 ± 0.24
2.	Disease group	2.52 ± 0.03[a]	117.20 ± 4.50	75.0 ± 4.54[*]	3.40 ± 0.20[a]	09.2 ± 013[a]
3.	Standard drug group	5.20 ± 0.04[a]	71 ± 5.10	8.06 ± 1.62[a]	7.90 ± 0.38[a]	15.8 ± 0.17[a]
4.	Treatment group 1 (300mg/kg)	4.42 ± 0.03[a]	63.61 ± 2.32	10.7 ± 2.48[a]	4.18 ± 0.62[a]	16.3 ± 0.27[a]
5.	Treatment group 2 (500 mg/kg)	5.25 ± 0.07[a]	61.60 ± 2.30	08.8 ± 3.47[a]	8.70 ± 0.60[a]	18.7 ± 0.29[a]

(n=6) ([*]p<0.001)([a]p<0.05)

DISCUSSION:

After the estimation of all the parameters i.e. physicochemical, rheological, and phytochemical, the study includes the toxic effect of the ethanolic extract of polyherbal according to OECD guidelines. This study also executes to determine the efficacy of ethanolic extract of polyherbal in the induction of ulcerative colitis in Wistar rats by the two models viz. TNBS and AA-induced colitis. Additionally, we also determined the disease-modifying activity of ethanolic extract of polyherbal by the assessment of inflammation and assessment of biochemical activity which includes different parameters respectively.

Administration of polyherbal ethanolic extract at a dose of 2000 mg/kg did not produce any abnormal behavior in the Wistar rats during the experiment period with any mortality was observed for 14 days. The chronic administration of the polyherbal ethanolic extract at a dose of 500 mg/kg for 28 days also did not produce any significant physiological changes when was compared with the control group. TNBS and AA are chemically induced colitis in Wistar

rats by intrarectal administration. In our study, the ethanolic extract of polyherbal demonstrate a significant effect in induced colitis models and was also found to be superior to prednisolone during the observational period. Our study showed a consistent increase in body weight and a decrease in UI and colon weight and length in all treatment groups. The model also certified by the biochemical parameters which found to be more precise when was compared with the standard drug.

CONCLUSION:

Ulcerative colitis is common dementia and also limited numbers of therapy or therapeutic options are currently available to alter the demonstration of the disease. Ethanolic extract of polyherbal showed potent inhibition against the various pathological changes caused by the administration of AA and TNBS chemical through intrarectal. Many different components which are present in the ethanolic extract of polyherbal-like flavonoids may possess anti-inflammatory as well as antioxidant potential against the TNBS and AA-induced colitis.

FUTURE ASPECT: Further studies must be carried out or concentrate on the pharmacokinetics of the biochemical active components of herbal plants and their interaction along with their effect in Ulcerative colitis and other diseases. Synthetic medicines should also be examined in detail.

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CONFLICT OF INTEREST

The authors verified that there is no conflict of interest.

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CONSENT FOR PUBLICATION: Yes

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CONTRIBUTION OF AUTHORS

Dr. Pranay Wal and Dr. Ankita Wal conceived of the presented idea and supervised the work. Miss. Sonali Dubey performed the experiments. Dr. Pranay Wal, Dr. Ankita Wal and Miss. Sonali Dubey contributed to the interpretation of the results. Miss. Sonali Dubey wrote the Manuscript after input from all authors.

Abbreviations:

IBD: Inflammatory Bowel Diseases

UC: Ulcerative colitis

5-ASA: 5-aminosalicylates

TLC: Thin Layer chromatography

R_f: Refractive index

LD_{50}: Lethal Dose

ED_{50}: Effective Dose

TNBS: 2, 4, 6, tri-nitrobenzene sulfonic acid

AA: Acetic Acid

DAI: Disease Activity Index

UI: Ulcer Index

ROW: Relative Organ Weight

SOD: Superoxide dismutase

EDTA: Ethylene diamine tetraacetic Acid

GSH: Glutathione

DNBS: 5, 5'-dithiobis – (2-nitro–benzoic acid)

MPO: Myeloperoxidase

TBA: Thiobarbituric acid

MDA: Malondialdehyde

CAT: Catalase

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