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Screening of *catharanthus roseus* stem extract for anti-ulcer potential in wistar rat

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***Abstract*---**This study aims to evaluate the anti-ulcer effect of *Catharanthus roseus* Stem extract on gastric ulcers caused by forced swimming. It was investigated that *Catharanthus roseus* having many kinds of phytochemical constituents so it is responsible for different pharmacological actions. The ethanolic extract of *Catharanthus roseus* stem at 250 and 500 mg/kg orally (PO) significantly reduces the incidence of ulcers. In this investigation albino wistar rats induced by

forced swimming, an increase in the rate of ulcers was observed compared to the control group. The ethanol extract of *Catharanthus roseus* stem showed a significant decrease in the previous index at a dose of 500 mg/kg; it was comparable to the standard preparation ranitidine (5 mg/kg). The protection index of *Catharanthus roseus* stem extract was 65.4%, while the protection index of the standard preparation ranitidine was 76.8%.

Keywords*---Catharanthus roseus,*** Stem extract, anti-ulcer effect, pharmacological actions, gastric ulcer model.

Introduction

Ulcers are open sores of mucous membrane, caused by the damage of tissue. **[3]** They can be mucous membranes like the surface of the stomach or inside the mouth. Ulcers are commonly caused on the skin and gastrointestinal tract. When Ulcer caused on digestive tract are called as peptic ulcers or stomach ulcers. **[24]** Ulcer is a common disorder caused various problems. ulcer is commonly disorder of intestinal tract, caused uncomfortable to patient and ulcer disturb the daily routine. **[4]** Stomach ulcers are called peptic ulcers, and ulcers in the first part of the intestine are called duodenal ulcers. **[25]**

Peptic ulcer is the major disease of gastrointestinal disease, 10 % world populations are affected this disease. found the 18-19 cases of peptic ulcer out of 20. approx. 13000 death occurs in yearly. **[26]** Peptic ulcers are commonly two types first is gastric ulcers, second is Duodenal ulcers. **[1]** Peptic ulcers are commonly caused by imbalance invasive factor such as HCL, pepsin and reactive oxygen species and some include defensive factor like mucous bicarbonate barrier, prostaglandins (PGs), mucosal blood flow, cell renewal, migration, enzymatic and non-enzymatic antioxidants and some other growth factors are imbalance, then cause ulcers. H. Pylori infections and NSAIDS are responsible for peptic ulcer. **[27]** Ulcers are severing medicated problems. Approx. 500,000 new cases are found in every year and alone in USA 5 million peoples are affected. **[2]** ulcers are mainly older person between 50-65 age. Patients with Zollinger-Ellison condition have ulcers in the throat, stomach, or duodenum at the edge of the jejunal gastrointestinal stoma, joined by Meckel diverticulum and heterotopic gastric mucosa. **[28]**

Stomach ulcers are brought about by stomach corrosive. Ulcer is a genuine illness; gastric and duodenal ulcers are breaks in the covering of the stomach and duodenum. The width of the ulcer goes from 2.5 millimeters to a few centimeters. **[6]** Basic causes are Helicobacter pylori and non-steroidal mitigating drugs (NSAIDs). **[29]** Other more uncommon causes incorporate smoking, stress from genuine sickness, Bechet illness, Zollinger-Ellison disorder, Crohn's infection, and cirrhosis. **[30]**

The older are more delicate to the ulcerative impacts of non-steroidal mitigating drugs. The presence of indications affirmed by endoscopy or barium meal.[26] Helicobacter pylori can be analyzed by a counter acting agent blood test, a urea

breath test, a bacterial stool test, or a gastric biopsy. **[5]** Different conditions that cause comparable manifestations incorporate stomach malignancy, coronary corridor infection, and aggravation of the coating of the stomach or gallbladder. **[31].** The stomach related plot comprises of two sections: the throat, stomach, duodenum, and digestion tracts. Most ulcers happen in the duodenum. It is additionally called duodenal ulcer. The stomach is called peptic ulcer. **[7]** Different conditions that cause comparative manifestations incorporate stomach malignant growth, coronary conduit infection, and aggravation of the coating of the stomach or gallbladder. **[32]**

Ulcers can show different indications, like spots, indigestion, queasiness, and retching. In the event that the ulcer is serious, they may show different manifestations like melena or melena (dying), blood in the regurgitation, weight reduction, and extreme agony in the upper midsection. Medicines incorporate stopping smoking, stopping non-steroidal mitigating drugs, stopping liquor, and medications that decrease stomach corrosive. **[27]** The medications used to lessen corrosiveness are typically proton siphon inhibitors (PPI) or H2 blockers for the initial a month of treatment. **[26]** Helicobacter pylori ulcers are treated with a blend of medications like amoxicillin, clarithromycin, and proton siphon inhibitors. **[8]** Anti-toxin opposition is expanding, so treatment may not generally be powerful. Disappointment circumstance. **[28]**

Peptic ulcer is also called as acid peptic disease (APD), is an ulcer of the mucous membrane of the duodenum or esophagus. **[9]** n this new world of this, gastrointestinal disorders are a universally common problem. **[10]** Now-a-days people are under stress due to daily life routine and due to this lifestyle and they often enjoy fast food. **[11]** Ulcers are affected 9.5% women and 10.5% men. **[34]** In this modern era, gastrointestinal disorders are a common problem. Peptic ulcer is one of the major diseases affecting human numbers. **[12]** This acid, pepsin, h. Develops due to an imbalance between invasive factors such as pylori and bile salts, **[13]** and aggressive factors such as mucus, bicarbonate, blood flow, epithelial cell restoration, and prostaglandin. **[35]** Peptic ulcers are a disease that causes inflammation and lesions of the mucosa and tissue that protect our gastrointestinal tract. **[14]** Mucus causes peptic ulcer 2, damaging the body's membranes, which usually protect the esophagus, stomach, and duodenum from gastric acid and pepsin. **[36]** The major cause of ulcers is a bacterial infection called Helicobacter pylori (H. pylori). **[15]**

1.2.1 Variables that increment your danger of ulcers.

- Use of painkillers known as anti-inflammatory drug medication (NSAIDs), corresponding to Bayer, Naprosyn (Aleve, Anaprox, Naprosyn, and others), isobutylphenyl propionic acid (Motrin, Advil, some forms of Midol, and others), and lots of a lot of obtainable by prescription. Even safety-coated aspirin and aspirin will usually cause operated ulcers. **[16]**
- At the point when corrosive is delivered from the supermarket, tumors in the corrosive creating cells of the stomach increment corrosive creation. **[17]**
- Drink more. **[18]**
- Smoking, tobacco consumption. **[19]**
- Have a serious illness. **[20]**

- Radiation treatment in the field. **[21]**

1.2.2 Signs of ulcers may or may not be present. If symptoms occur, they may include:

- Pain or burning in the mid or upper abdomen between meals or at night;
- Swelling and pain; **[22]**
- Stomach irritation;
- Nausea or vomiting.
- Black stool (due to bleeding)
- Hematemesis ("may look like coffee grounds")
- Weight loss **[23]**
- Severe pain in the middle of the upper abdomen.**[28]**

Peptic ulcer disease (PUD) as a breach in the integrity of the gastric or duodenal mucosal lining secondary to depressed protective gastric mechanisms or overriding obnoxious inciting agents such as acid or pepsin. Historically, one of the main reasons for being PUD is that it has been associated with stress or alcoholism for a long time. This was proved wrong some years ago and stress and alcohol ingestion have not been applied as etiology factors to make PUD. **[37]**

Extreme gastric corrosive discharge is a factor prompting gastric ulcer illness. Gastric ulcer is an overall term for ulcers that happen in the upper piece of the stomach or small digestive system. **[38]** A ulcer is a reasonable and complete space of the stomach related framework where the tissues are harmed and obliterated by gastric corrosive and stomach related compounds. Peptic ulcer is essential for a ulcer that happens in the stomach or upper piece of the stomach. Intestinal gastric ulcer is a sore on the mass of the gastrointestinal parcel. The corrosive stomach related proteins discharged by gastric cells can cause ulcers in the covering of the stomach or the upper piece of the small digestive tract (duodenum). This prompts disintegration of the mucous layers that help the stomach related framework. **[39]** Peptic ulcers are red sores. Stomach ulcer is known as the inward mass of the stomach (gastric ulcer) or small digestive system (duodenal ulcer). Duodenal ulcers will in general happen in individuals somewhere in the range of 25 and 75 years of age, and gastric ulcers will in general happen in individuals somewhere in the range of 55 and 65 years of age. age.Ulcers are open injuries. The expression "peptic" implies that corrosive is the reason for stomach ulcers. By and large, when a gastroenterologist discusses "ulcer", the specialist implies that he has a stomach ulcer. Stomach ulcers are available in the stomach. Duodenal ulcers are found in the small digestive system. **[40]**

1.2.3 Causes of Ulcers

Peptic ulcers start with normal lining of the stomach and small intestine. Peptic acid is caused by excessive production of acid in the stomach. firstly, the peptic ulcer found by Helicobacter pylori (H. pylori) in 20th century by Barry Marshall and Robin Warren. They got prize for this research. **[24]** Other factors caused the ulcer disbalance of digestive juice, stomach and duodenum. Mostly ulcers are caused different type of bacteria called as Helicobacter pylori. Sometime long time used of NSAIDS; these are caused the peptic ulcers. Excessive smoking, large

consumption of alcohol and new life styles are caused the ulcers. **[41]**

Digestive juices are produced by the human body. Which damages the internal and sensitive part of the lining of the stomach or duodenum, that causes pain. Ulcer is also caused by *H. Pylori* bacteria, it has been cleared that the *H. Pylori* infection is spread through oral contact, so people living in crowded areas have a higher chance of getting the infection. The method of pylori transmission has not yet been cleared, but it appears to be transmitted from person to person by oral. Major cause of peptic ulcer is pain relievers such as aspirin, ibuprofen, naproxen or other non-steroidal anti-inflammatory drugs. Pain-relieving medicine is highly acidic due to which the level of acid in the stomach is increased. This causes peptic ulcer production. **[42]** The genetic factor also has an important role in the pathogenesis of the disease. Ulcer disease is seen three to four times more than the general population today. About 20-50% suffer from duodenal ulcer disease, this is seen in the report of today's history. **[25]**

1.2.4 Symptoms:

Following symptoms of ulcers like

1. Abdominal pain with burning sensation
2. Swelling and pain
3. Stomach Irritation
4. Nausea or vomiting
5. Abdominal pain middle and upper part.

Other ulcer indications, like uneasiness, skin inflammation, and so on Stomach torment, for the most part in the upper midsection, is exceptionally identified with food consumption. For duodenal ulcers, agony will show up around 3 hours in the wake of eating.

- Abdominal swelling and bulging,
- Watery spit (which discharges salivation after a reflux scene to weaken the corrosive in the throat, yet is bound to be identified with gastroesophageal reflux illness),
- Severe sickness and retching,
- Loss of hunger and weight reduction.
- Vomiting blood (regurgitating blood); this might be because of direct draining from a gastric ulcer or harm to the throat brought about by serious/delayed spewing
- Moles (gum faces with a horrendous smell because of the presence of hemoglobin iron oxide);
- In uncommon cases, ulcers can puncture the stomach or duodenum, causing intense peritonitis and serious stinging
- Stomach aggravation brought about by potential ulcers; here and there stomach corrosive (particularly stomach corrosive) can amass and create a consuming uproar. **[43, 35]**

1.2.5 Treatment

In the treatment of ulcers, stop smoking, stop NSAIDs, stop alcohol and give the same stop medicines that are made in the stomach acid. This way, the ulcer can be cured. The drug used to reduce the acid is a proton pump inhibitor (PPI). H.

Treatment of Ulcers due to Pylori Ulcers can be cured using drugs like amoxicillin, clarithromycin and PPI. Anti-microbial opposition is on the ascent, and this kind of treatment may not generally be advantageous. **[28]** Hemorrhagic ulcers can be dealt with endoscopically; this is a fruitless open a medical procedure. The treatment of ulcers relies upon the lower part of the ulcer. In the event that you are tainted with Helicobacter pylori, your PCP may recommend anti-microbials to kill and kill the bacterial contamination. Hormonal microorganisms. **[27]**

1.2.6 Types of Peptic Ulcer

Peptic ulcers are caused by acid. On the basis of location, peptic ulcer is categorized as follows;

1. Gastric Ulcer- An ulcer in the stomach is known as a gastric ulcer. The presence of gastric ulcer is more normal in the old. Stomach ulcers, or ulcers that beginning from the stomach line, are called gastric ulcers. Ulcers or ulcers that happen in the small digestive tract are called duodenal ulcers.
2. Duodenal Ulcer- Duodenal ulcers will in general happen in youngsters and are equitably circulated among various financial gatherings. In these patients, the corrosive emission rate is higher than expected.
3. Acute Peptic Ulcer – These ulcers influence the profound submucosal tissues and can show up as single or numerous sores and can be found in numerous pieces of the stomach and the initial not many creeps of the duodenum.
4. Chronic Peptic Ulcer- These ulcers infiltrate the epithelium. What's more, the muscle layer of the stomach divider, which may incorporate the contiguous pancreas or liver.In most cases, they show up at the passage of the stomach and duodenal pylorus, individually.
5. Esophagus Ulcer - Esophageal ulcers or ulcers that happen in the throat are called esophageal ulcers. **[29]**

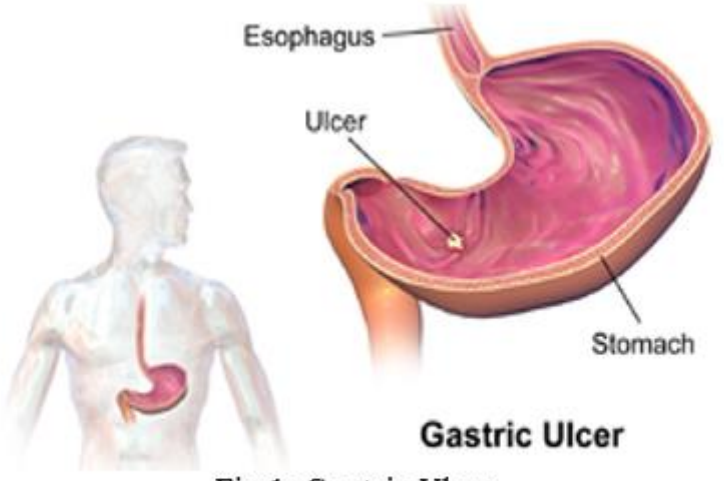


Fig 1: Gastric Ulcer

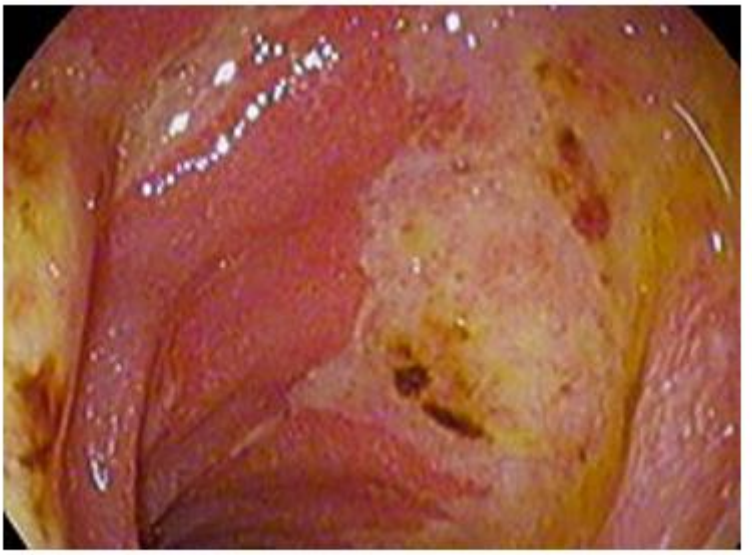


Fig 2: Chronic Duodenal Ulcer

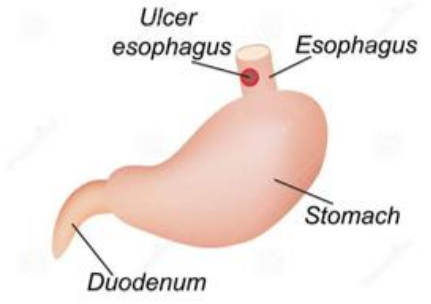


Fig 3: Esophagus ulcer

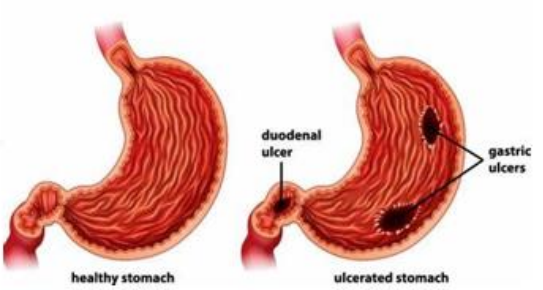


Fig 4: Healthy & Ulcerated Stomach

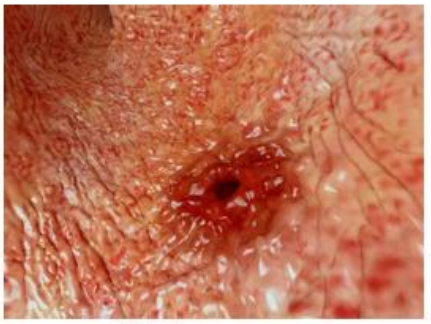


Fig 5

Fig 6

Fig 5 &6: Peptic Ulcer

Common symptoms of peptic ulcer such as fever, nausea, heartburn, nausea, vomiting, weight loss and chest pain.

1.2.7 Different Types of Ulcers:

Ulcer can appear anywhere in the outer layer of your skin in the abdomen or in the outer layer of your body. Sometimes some cases of ulcers heal on their own, but others require medical treatment to avoid the dreaded disease.

Many types of ulcers:

1. Arterial ulcers
2. Venous ulcers
3. Mouth ulcers
4. Genital ulcer

1.2.7.1 Arterial ulcers:

The lesion in an arterial (ischemic) ulcer is open. Arterial ulcers occur on the outer side of the toes and heel. Due to lack of blood flow in the tissue, arterial ulcers develop due to depletion of the arteries. Arterial ulcers take longer to heal and need better treatment to prevent infection.

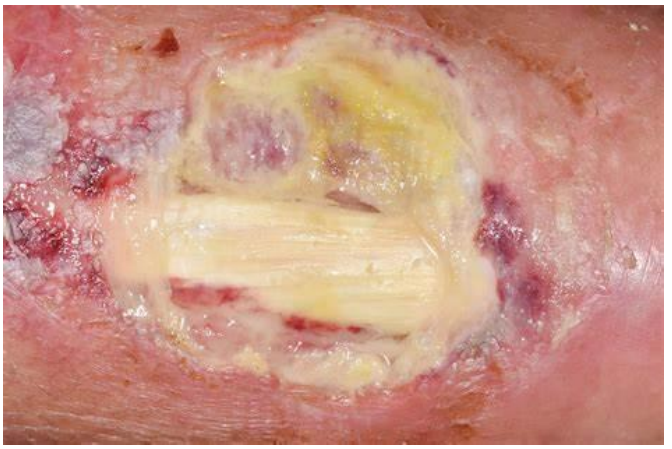


Fig 7: Arterial ulcers

Arterial ulcers are characterized by these common symptoms. which is like this -

1. Red, Yellow, or Black Wounds
2. Hairless skin
3. foot pain
4. Someone is bleeding
5. The affected area calms the touch with minimal blood circulation.

Treatment:
Blood circulation in the affected area has to be increased first of all to correct the arterial ulcer. Whereas antibiotics help reduce the symptoms of arterial ulcers.

1.2.7.2 Venous Ulcer
This ulcer occurs in an open wound in the foot, often on the foot, below the knee, and in the inside of the ankle. These ulcers are caused by a lack of blood flow. In some cases, venous ulcers have very little pain until they are infected. But other cases of this condition are very painful.
Some of its common symptoms are as follows –

- swelling
- swelling
- itchy skin

Treatment: improve the blood circulation to the affected area.

1.2.7.3 Mouth ulcers
Mouth ulcers are ulcers, small sores or wounds that occur in your mouth or in your gums. Mouth ulcers are also called canker sores.
These ulcers are caused by a number of reasons, including:

- Bite inside your cheek
- food allergies
- Brushing hard teeth
- Hormonal changes
- vitamin deficiency
- bacterial infection

- Diseases

Treatment: Mouth blisters are common and often disappear on their own within two weeks. They are very painful. If the mouth ulcer is corrected within 2 weeks, then contact the doctor immediately.

1.2.7.4 Genital ulcers: Genital ulcers are ulcers that cause lesions at the genital places, including the penis, vagina, anus, or the surrounding area. They are usually caused by sexually transmitted infections (STIs). It is very dangerous. Genital ulcers can be caused by trauma, inflammatory diseases, or skin allergies. Common symptoms of genital ulcer are-

- Rash or bumps in the affected area
- Pain or itching
- Swollen glands in the gorge area
- Fever

Treatment: To cure genital ulcer, the doctor performs an anti-viral order antibiotic which reduces the infection. **[44, 45, 46]**

Materials & Methods

5.1 Identification, collection and authentication of plant and its stem
5.1.1 *Catharanthus roseus* (L.)

Catharanthus roseus (L.) is a significant therapeutic plant in the Apocynaceae family, used to treat numerous lethal infections. It's anything but a great deal of advantageous alkaloids and can be utilized to treat diabetes, pulse, asthma, stoppage, disease and feminine problems. There are two normal assortments of *C. roseus*, whose names are named by the shade of the blossoms: pink roses and white Alba. Catharanthus roseus, gladly known as the Madagascar periwinkle, is a periwinkle that is local to Madagascar. Equivalent words of plant names are Vinca rosea, Ammocallis rosea, and Lochnera rosea. Other English names some of the time utilized for plants are Cape Periwinkle, Rose Periwinkle,Pink evergreen trees and old virgins. **[58]**

5.1.2 Possibly dynamic synthetic compounds: Researchers contemplating its restorative properties have found that it's anything but a gathering of alkaloids, which, albeit incredibly poisonous, might be utilized to treat malignancy. Plants can integrate an assortment of mixtures, which are utilized to perform significant organic capacities and oppose assaults from hunters like bugs, parasites and herbivorous warm-blooded animals, carbs, flavonoids, saponins and rose alkaloids. Alkaloids are conceivably the most dynamic compound parts in *Catharanthus roseus*. The plant contains 400 sorts of alkaloids, which can be utilized as meds, agrochemicals, flavors and aromas, fixings, food added substances and pesticides. Alkaloids, for example, plastid actineum, vinblastine, vincristine, vindesine, and vinblastine Taibanin are basically present in the respiratory lot. There are ajmalicin, vinchein, vinamine, raubazine, reserpine, isolate, and so on in the root and base stem. Rosin is an anthocyanin shade found in rose blossoms. **[59]**

5.1.3 Acclaimed clinical use:

- In India, leaf juice is utilized for honey bee/wasp stings.
- In the Philippines, leaf decoction is utilized to treat diabetes, delicate leaf decoction is utilized to treat stomach issues, and root decoction is utilized to treat intestinal parasites.
- In Madagascar, the external leaves are utilized as an emetic, and the roots are utilized as a diuretic, creepy crawly repellent, cleanser, hemosAtatic specialist, and toothache. Leaf juice is utilized for acid reflux and indigestion.
- The plant is utilized to treat diabetes in the West Indies and Nigeria.
- In Cuba and Jamaica, blossom secludes are utilized to clean kids' eyes.
- In the Bahamas, Huatang is utilized to treat asthma, tuberculosis and tuberculosis.
- In Malaysia, this plant is utilized to treat diabetes, hypertension, absence of rest and perilous development.
- In Hawaii, invaded plant secludes are utilized to stop work.
- In Africa, the leaves are utilized to treat menorrhagia and increment hardness.

Catharanthus roseus is used as a preliminary have for plant matter in plant pathology. Signs, for instance, fronds and leaf size are in a general sense diminished. **[60]**

5.2 Plant Material

Catharanthus roseus was assembled in late July and early August of 2019 from semi-dry, unshaded land close the Translam institute, Meerut (UP), India. The plant was taken (Fig-8, 9 & 10) to the examination office and was affirmed by Dr. Nasiruddin Ahmad Farooqui.

- Stems were washed autonomously first under running faucet water, followed by sanitized refined water.
- The current investigation zeroed in on plant which was *Catharanthus roseus*.
- Synthetics methanol, ethanol, H_2SO_4, $(CH_3)2CO$, Wagner's reagent (Iodine in potassium iodide), NaOH, HCl, $FeCl_3$, chloroform, cold acidic corrosive, tannic corrosive, quercetin and ninhydrin.

5.2.1 Large scale morphology of leaf: The boundaries considered were structure, shape and surface characters of medication. **[61]**

Fig 8: Stem Part of *Catharanthus roseus*

Fig 9: Leaves Part of *Catharanthus roseus*

Fig 10: Plant of *Catharanthus roseus*

Table-1: Macro morphology of the leaf of Catharanthus *roseus (L.) G.*

S. No.	*Don* Properties	Inference
1	Colour	Green
2	Taste	Bitter
3	Odour	Characteristic
4	Shape	Petiolate, Ovate or Oblong
5	Appearance	Glossy
6	Margin	Centric
7	Apex	Acute

5.3 Fluorescence investigation

Many homes grown medications when presented to enlightenment discharge light of various shading. The fluorescence investigation assists with recognizing the medication with explicit fluorescence. It likewise assists with distinguishing fluorescent debasements. This strategy can be utilized as a demonstrative apparatus for testing debasement. **[62]**

- This technique has been finished by treating the leaf powder alongside 1N HCl, 1 N NaOH, half HCl, half H2SO4, half HNO3 and Methanol was seen under the short UV light (254nm) and long UV light(365nm).

Table 2: The fluorescence analysis of the leaf powder of Catharanthus *roseus (L.) G*

S. No.	*Don* Reagents Used	Day light	Lower UV (320-400nm)	Short UV (280-320nm)
1	Powder Drug	Light Green	Dark Green	Black
2	Powder+ ethanol	Yellowish Orange	Yellowish Green	Pale Green
3	Powder+50% HNO3	Reddish Orange	Dark Green	Pale Green
4	Powder+1N HCl	Pale Yellow	Pale Green	Dark Green

5.4 Cross over segment of stem

Clean the handle and fix it with formalin, destructive acid and ethanol. After fixation for 24 hours, dry the model after the evaluation of the action of tert-butanol. The parts are colored with toluidine blue, safranin, true green and iodine. The transient process of sliding action is carried out by glycerin.

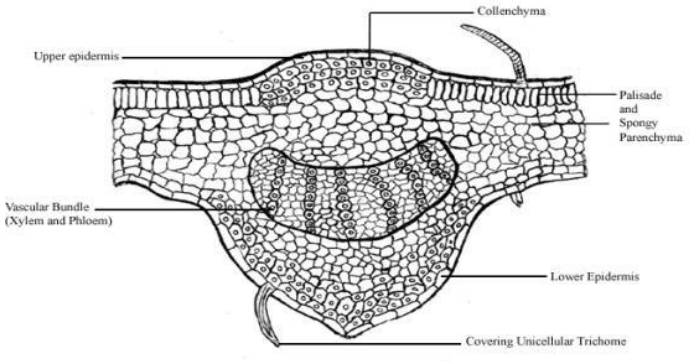


Fig 11: Diagram of TS of Catharanthus *roseus (L.) G. Don*

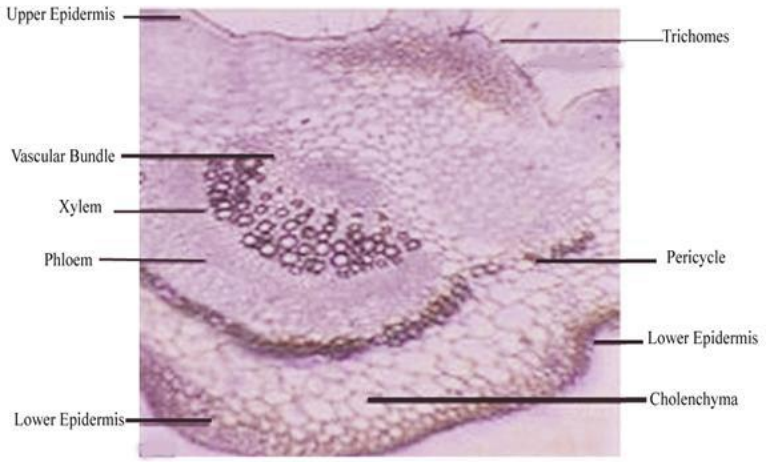


Fig 12: Photomicrograph of TS of Catharanthus *roseus (L.) G. Don*

5.5 Powder microscopy

The plant stems dried in the shade are ground into a fine powder with an electric processor, and introduced under a powder magnifying lens utilizing the previously mentioned different shading designers.

Fig 13: Trichome

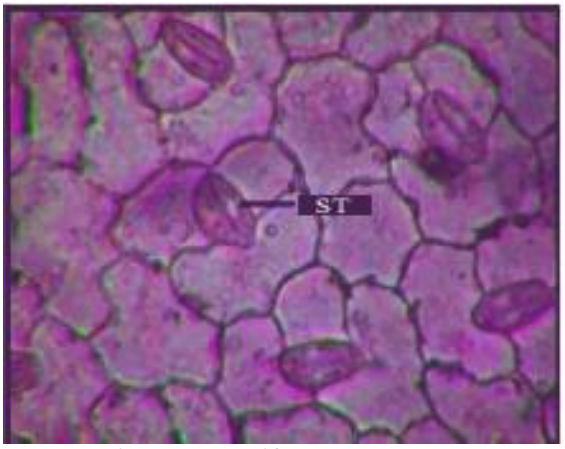


Fig 14: Cruciferous Stomata

5.6 Affirmation of physico substance limit

Catharanthus stem seek after various normalization restricts because of their alert and strength. As indicated by the 1998 WHO rules, different cutoff points are set, for example, debris worth and dampness misfortune during drying.

Table 3: Physico-chemical constants

S. No.	Parameters	Value obtained
1	Total Ash	0.3% w/w
2	Acid insoluble ash	0.58% w/w
3	Water soluble ash	1.48% w/w
4	Sulphated ash	4.04% w/w
5	Solubility Water soluble extractive Alcohol soluble extractive	 6.26% w/w 4.5% w/w
6	Moisture content	9.09% w/w
7	Loss on drying	4.01% w/w
8	Foaming index	0.7cm height
9	Swelling index	0.6 g

5.7 Preparation of plant extract and phytochemical screening

The leaves of the picked plants were assembled and thereafter washed under running fixture water followed by flush using refined water for the ejection of build-up and soil particles. The leaf tests were then dried under hide at room temperature and crushed to fine powder and kept in fixed shut polythene packs for future occupations. **[63]**

5.7.1 The concentrate is set up in two distinct manners:

1. Separate new plant material without drying;
2. Remove in the wake of drying each piece of the plant.

5.7.2 Concentrate with high temp water:
Each piece of the plant increments by 10 grams. Mix persistently in 100 ml of cleansed water for 30 minutes. Permit the interaction to cool to room temperature, then, at that point channel with a muslin material. Axis the filtrate at 5000 rpm for 15 minutes. Under incredibly sterile conditions, use Whattman No. 1 channel to channel the supernatant again. Collect the filtrate in another, spotless glass chamber and store at 4°C until use**.**

5.7.3 Cold water extraction:
Soak 10 g of each plant section a mortar and pestle with 100 ml of cleaned water at room temperature, and afterward separate them with a muslin material. They were gathered in another, spotless glass tube and utilized inside 24 hours to assess antimicrobial turn of events.

5.7.4 Organic Solvent Extraction
Blend 10 grams of each plant part with 100 milliliters of solvent standard arrangements (ethanol and methanol). The blend is along these lines sifted through a muslin design, and afterward separated through a Whattman No. channel once more, broken up and totally scattered at room temperature to acquire an unadulterated concentrate. By completely blending the fitting proportion of dry concentrate with solvent supplies to give a last combination of 100 mg/ml, an inconvenient concentrate renewal plan has been set up. Store in a perfect glass tube at 4°C until use.

5.7.5 Dry Powder Extractions
Re-vanishing and residue expulsion are accomplished by first air-drying the plant material, and afterward showering it's anything but a perfect mortar and pestle under severe aseptic conditions. As of now shown, for conventional fluid and dissolvable extraction, powders are additionally dealt with mistakenly.

5.8 Photochemical screening

1) **Alkaloid test (Wagner's reagent):** The concentrate (23 ml) is treated with around 1 ml of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml of water), and the light red impact is debilitated. Initiate (or shading) sand designs (Kokate et al., 2001). **[64]**
2) **Test for amino acids and proteins (1% ninhydrin solution):** 2-5 drops of ninhydrin game plan were added into 2 mL of moves set in a hot water shower at 100ºC for 1-2 minutes and were seen for the improvement of purple colouration (Singh et al., 2013). **[65]**
3) **Carbohydrate's test (Fehling's Test):** Fehling A and B reagents were mixed in with 2 mL of amass and rose in water shower for 10 minutes. Speeds up of cuprous oxide were molded, if diminishing sugars were accessible, which were block red in concealing.
4) **Test for cardiac glycosides (Keller Kelliani's test):** Concentrates (2 mL) were treated with cold acidic destructive with few drops of 5% ferric chloride game plan, warily under laid with 1 mL concentrated H2 SO4 . Reddish gritty shaded ring at the interface showed the presence of cardiovascular glycosides (Kumar et al., 2013). **[66]**

5) **Flavonoid's test (Alkaline reagent test):** The concentrate (2ml) was treated with a relatively small amount of 20% sodium hydroxide droplets. Mode of action: The extremely yellow color disappears with the swelling of the destructive and debilitating hydrochloric acid, indicating the presence of flavonoids. **[67]**
6) **Test for phenols (Ferric chloride test):** Concentrates (2 mL) were treated with 0.5 mL of liquid 5% ferric chloride and saw for course of action of dull blue or dim colouration, which confirms the presence of phenols (Hema et al., 2012). **[68]**
7) **Test for phlobatannins (Precipitate test):** Concentrate (2 mL) was flooded with 1mL of 1% HCl, declaration of a red speed up showed the presence of phlobatannins (Ayoola et al., 2008). **[69]**
8) **Test for saponins (Foam test Refined water (6 mL):** Test for saponins was incorporated 2 mL of concentrate. The mix was shaken totally in graduated chambers for 15 minutes and saw for the advancement of consistent foam to confirm the presence of saponins (Dubey and Sushma, 2014). **[70]**
9) **Test for tannins (Braymer's test):** Ferric chloride course of action (10%) was added to 2 mL of concentrate and saw for advancement of blue or greenish concealed plan.
10) **Test for terpenoids (Salkowki's test):** Chloroform (2 mL) was added to 2 mL of concentrate and two or three drops of concentrated H2 SO_4. The mix was shaken well. A rosy natural shaded speed up conveyed rapidly exhibits the presence of terpenoids (Mir et al., 2013). **[71]**
11) **Test for quinones:** Concentrates (2 mL) were added two or three drops of concentrated HCl; course of action of yellow speed up (or colouration) exhibits the presence of quinones (Ugochukwu et al., 2013). **[72]**
12) **Estimation of total phenolic content:** The hard and fast phenolic content (TPC) was surveyed by spectrophotometer using Folin-Ciocalteu strategy (Singleton and Rossi, 1965). FolinCiocalteu's reagent (5mL) (1:10 debilitated) was added with 200 µL of debilitated model. By then 4 mL of 7% sodium carbonate course of action was added following 4 minutes. The mix was mixed through and through by vortex for 2 minutes and a while later kept at 40°C for 30 minutes, after which the absorbance was assessed at 765 nm. The TPC was surveyed by using tannic destructive (0.02–0.1 mg/mL) as a standard arrangement twist. The results were conveyed as milligram of tannic destructive same (TAE) per gram of dried plant test. **[73]**
13) **Estimation of total flavonoid content:** Flavonoid's content was expected by the aluminum chloride strategy (Park et al., 2008). Concentrate (0.3mL), 0.15 mL of NaNO2 (0.5 M), 3.4 mL of 30% methanol and 0.15 mL of 0.3 M AlCl3 .6H2O were taken in a test tube. By then 1 mL of 1M NaOH was added following 5 minutes. The mix was totally mixed and absorbance was recorded against the unmistakable reagent at 506 nm. Quercetin was used as a standard plan (0 to 100 mg/L) to get standard curve. The full-scale flavonoids were imparted as milligrams of quercetin reciprocals per gram of dried plant test. **[74]**

Table 4: Phytochemical screening

S. No.	Test	Petroleum ether	Acetone	Chloroform	Ethanol	Aqueous
1	Alkaloid	Positive	Positive	Positive	Positive	Positive
2	Glycoside	-	-	-	Positive	Positive
3	Terpenoids	-	Positive	Positive	Positive	Positive
4	Flavonoids	-	-	-	Positive	Positive
5	Phenols	-	-	-	Positive	-
6	Tannins	-	-	-	Positive	Positive
7	Carbohydrates	-	-	-	Positive	Positive
8	Saponins	-	-	-	Positive	-
9	Phytosterols	-	-	-	Positive	Positive
10	Protein and amino acids	-	-	-	Positive	-
11	Fixed oil and fats	-	-	-	-	-
12	Resin	-	-	-	-	-

5.9 Acute toxicity studies as per OECD guidelines

The risk score is utilized to decide the security of the remedy to the human body. In the drug business, thought of outrageous harm is normally dispensed with before all plans are accessible. Degradability is a state where a substance is hurtful and produces results because of the connection among poisons and cells. High groupings of certain plant concentrates may have harmful impacts. Be that as it may, harm testing isn't sufficient, and different imperatives (like biochemical and histological tests) are performed together to choose reasons. Harm and passing of explicit organs. Harm tests and photomicrographs of treated and stained tissue regions demonstrate dangerous impression of medications or plants. (Dapar, L.P.M., 2007). **[75]**

5.9.1 Preparation of ethanol extract: Take out a sample of C. roseus (41.5 g) with 600 ml of 95% ethanol and place it until further notice, and then filter it with Whatman drainage paper. In addition, use a hot plate to remove the filtrate and disperse the cones to dryness. Like a cup.

5.9.2 Test animals: Wistar albino rodents were used as experimental animals. These rodents were purchased from a Meerut distributor and stayed in the institute; the rodents obtained were a mix of male and female, a total of 50 Wistar albino rodents, these rodents were processed and domesticated in the laboratory They were reared under conditions, and they were divided into 4 circles, each with 4 rodents. Already, during and after treatment.

5.9.3 Acute toxicity study:

- The serious damaging force of *C. roseus* was concentrated in rodents (180-200 grams).
- Four authorities and 4 rodents, each arbitrarily chosen dependent on their common body weight, gotten ethanol end in Part 0 (Group A), and if the driving master is water, it is assigned as a control regardless; 1900 (Group

B), 3000 (Group C), and 5000 (Group D), all plan to utilize mg kg G1 body weight as a unit, and use needles to give rodents (oral) fixation after a brief time of fasting.

- Continue to notice the general advantage to the creature for the following 24 hours, and a peculiar idea shows up in the following 4 hours, and afterward for a sum of 14 days.
- The one-of-a-kind handle has caused huge shortage and thirst in rodents.
- Made an intriguing point about running and running, however there was no conspicuous change.
- The enduring creature was tried thoughtfully and deserted following 14 days, and a blood test was requested for hematology (blood) and histology (kidney) assessments.

5.9.4 Biochemical examination:

- For biochemical assessment, serum is acquired by centrifuging and holding the blood test multiple times until estimation can be performed.
- The all-out protein fixation is resolved utilizing Lu23, aspartate aminotransferase (AST) and alanine strategies. The degree of transaminase (ALT) is dictated by colorimetry, as displayed in the figure-17.

5.9.5 Histological examination:

- Take out the kidney for histological assessment, fix it in 10% formaldehyde, then, at that point perform 3 changes of liquor and xylene, measure and insert it in paraffin.
- The rear of the tissue block is 5 μm thick and stained with hematoxylin and eosin.
- Microscopic pictures of tissue sections were taken with AmScope reformist magnifier and Olympus magnifier.

5.10 Forced Swim induced ulcer model

- The creatures (Wistar Albino rodents) were set in a grid pen to try not to swallow dung, and were partitioned into four batches, that is, batch-I got an ordinary eating routine, and batch-II got 5 mg/kg ranitidine orally as the standard medication.
- The oral portion of *Catharanthus roseus* stem extract is 250 mg/kg or 500 mg/kg for batch-III and batch-IV.
- As indicated by the creature's body weight (Wistar Albino rodents), all concentrates and controls were broken up in water and directed.
- Under typical food admission, this cycle can require as long as 14 days before the keep going portion is required on the fifteenth day.
- The Wistar Albino rodents were not taken care of for 18 hours, and the rodents had to swim for 2 hours.
- The Wistar Albino rodent is anesthetized, the midsection is opened, the stomach is taken out and etched.
- Huge. (Fundus) Measure the ulcer record. **[76]**

Fig 15: Control

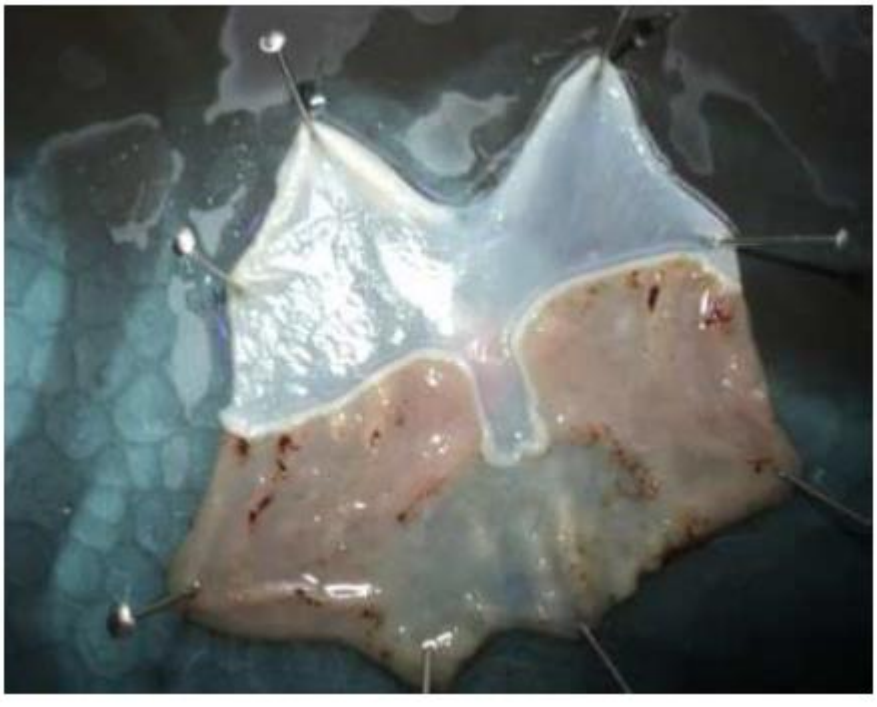


Fig 16: *Catharanthus roseus* Stem extract (250mg/kg P.O)

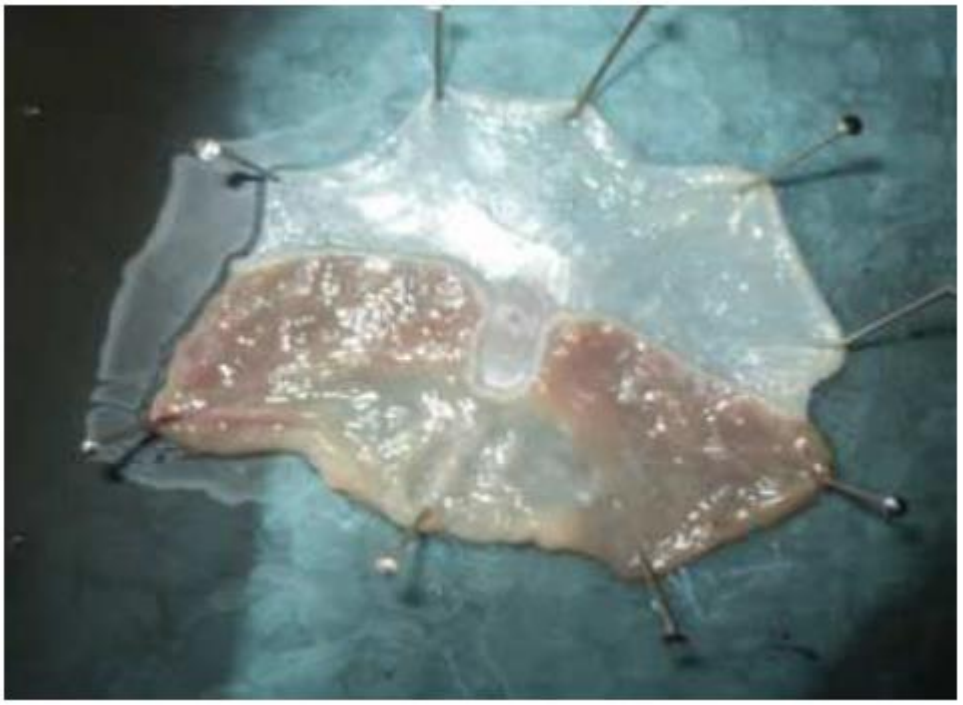


Fig 17: *Catharanthus roseus* stem extract (500mg/kg P.O)

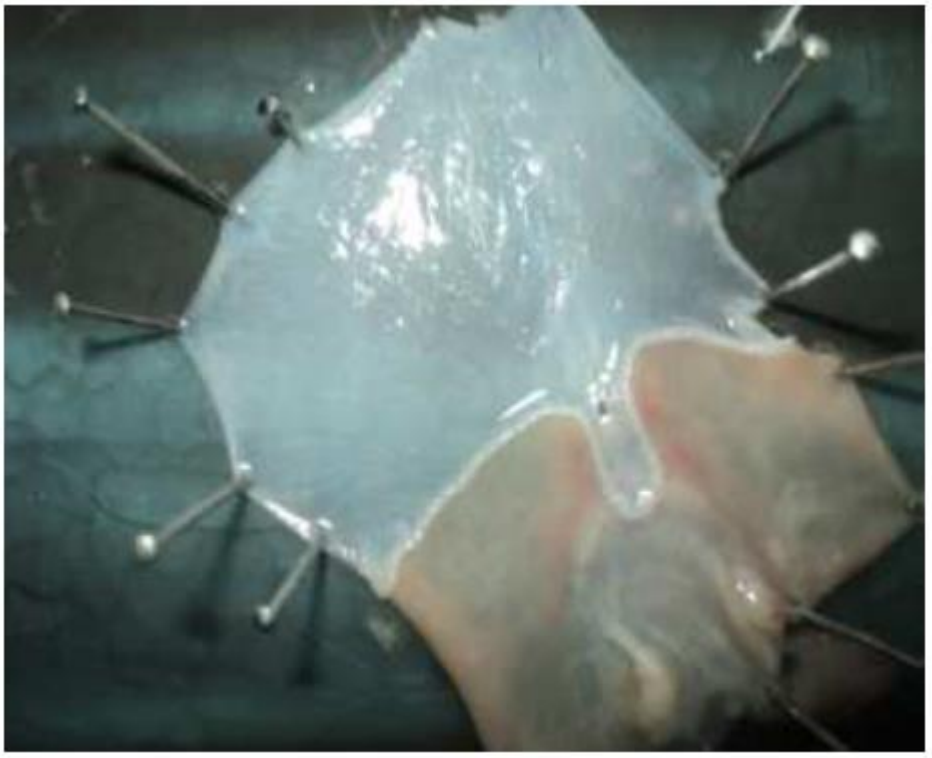


Fig 18: Standard (Ranitidine 5mg/kg P.O)

5.11 Evaluation of anti-ulcer activity:

- In this study, the anti-ulcer activity of the ethanol extract of *Catharanthus roseus* was compared with the gastric ulcer model induced by forced swimming.
- The results of the study are shown in Table 5.
- Significant gastroprotective activity was observed in kilograms.
- Compared with the standard preparation ranitidine 5 mg/kg, the ethanol extract of *Catharanthus roseus* at both doses showed a significant decrease in ulcer index.
- The protective effect of *Catharanthus roseus* ulcer on rats with forced swimming ulcer.
- The ethanol extract of *Catharanthus roseus* has an antiulcer effect on forced swimming ulcers in rats.
- Table 5 shows the results obtained with the ethanol extract of *Catharanthus roseus* stem of albino rats at 250 and 500 mg/kg body weight, showing significant gastroprotective effects.
- It was found that part of the ranitidine treatment group had normal mucosa (Figure 18) and ulcers caused by forced swimming (Figures 16 and 17), accompanied by mucosal ulcers and bleeding. **[47]**

Table 5. Effect of ethanolic extract of *Catharanthus roseus* stem in Constrained swimming ulcer model

Batch No.	Body weight	Treatment	Normal colored stomuch	Red coloration	Spot Ulcer	Hemorrhagic streaks	U≥3≤5	U>5	Total Score	Mean Ulcer ± SEM (Standard error of mean)	Total protection (%)
Batch-1	163	Control	-	0.6	1	2	2.5	-	6.1		
	172		-	0.6	1	-	2.5	-	4.1		
Control	155		-	0.6	1	-	-	-	1.6	3.5 ±	-
	170		-	0.6	1	2	2.5	-	6.1	1.927	
	184		-	0.6	1	-	-	-	1.6		
	133		-	0.6	1	2	-	-	3.6		
Batch-2	165	Ranitidine	-	0.6	1	2	-	-	3.6		
	180	(5mg/kg)	-	0.6	1	2	-	-	3.6		
Standard	146		-	0.6	1	-	-	-	1.6	5.003±	76.8
	138		-	0.6	1	2	-	-	3.6	2.453	
	146		-	0.6	1	-	-	-	1.6		
	148		-	0.6	1	-	-	-	1.6		

Batch-3	150	Stem	-	0.6	1	-	-	-	1.6		
	173	extract	-	0.6	1	-	-	-	1.6		
Low dose	146	(250mg/kg)	-	0.6	1	-	-	-	1.6	4.5±	32.6
	176		-	0.6	1	2	-	-	3.6	1.54	
	165		-	0.6	1	2	-	-	3.6		
	154		-	0.6	1	-	-	-	1.6		
Batch-4	166	Stem	-	0.6	1	2	-	-	3.6		
	185	extract	-	0.6	1	-	2.5	-	4.1		
High	140	(500mg/kg)	-	0.6	1	2	-	-	3.6	3.9±	65.4
dose	135		-	0.6	1	2	2.5	-	6.1	1.44	
	146		-	0.6	1	-	-	-	1.6		
	148		-	0.6	1	-	-	-	16		

Calculation of Ulcer index: It changed into calculated through following formula

$$\textbf{Ulcer index } U_I = U_N + U_S + U_P \times 10^{-1}\ U_I$$

Where, U_N = Average range of ulcer consistent with animal
U_S = Average range of severity score
U_P = Percentage of animal with ulcer

Determination of percent protection: It is calculated through formula

% Protection = Control imply ulcer index – check imply ulcer index/ Control imply ulcer index

Result and discussion

After toxicity studies

Change in body weight of Wistar Albino rodents after treating with C. roseus stem extract

Wistar Albino rodents were weighed when association of ethanol leaf plant eliminate. Table 6 Wistar Albino rodents versus body weight *C. roseus* eliminate. Weight obtains, including start, end, and weight change in the control and treatment get-togethers, was more imperative (high) in treatment of Batch C and D and the benchmark (control) batch, anyway low in treatment of batch B.

Table 6: Change in body weight of Wistar Albino rodents after treating with C. roseus stem extract dosages of 1900, 3000, and 5000 mg kg G^1

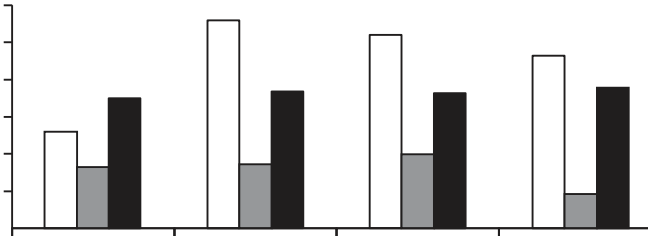
Batch	Treatment (mg kg G^1)	Initial weight (g)	Final Weight	Weight change
A	0	170	235	65
B	1900	170	210	40
C	3000	170	270	100
D	5000	170	270	100

Physiological and behavioural changes

No gigantic changes before atonement. Found in control Wistar rodents, Wistar rodents are dynamic in this social event for the term of the examination. There was no anorexia and they responded well to upgrades, anyway during the underlying 4 hours after utilization of the ethanol concentrate of *C. roseus*., the Wistar rodents in the treatment pack were shivering, aggravated and fretful. Rest, apathetic appearance, infection, general deficiency in the body, and some of them endeavor to escape from us. No mortality was found in the treatment and control social occasions, and the proportion of signs/impacts saw depended upon the centralization of the coordinated concentrate, which is the LD50 or *C. roseus* used in this assessment., which implies much higher than the centers used in this examination. Wistar rodents treated with blossom petal remove had an immense change in body weight diverged from the run of the mill gathering.

Biochemical assessment

Figure 19 shows the eventual outcomes of changes in biochemical limits of the pale cleaned individual Wistar albino rodents used in the examination. The limits investigated were aspartate aminotransferase (AST), alanine aminotransferase (ALT) and hard and fast protein. AST levels were through and through higher in the treatment batch than in the benchmark batch. It was generally raised in batch B (112 µLG1), batch C (104 µLG1) and batch D (93 µLG1). Changes in ALT and outright protein levels were basically lower in the treatment pack than in the benchmark batch (19 µLG1) and not in the treatment batch beside treatment batch D, which had a relative full-scale protein was Low.



	Control	B (1.9)	C (3 mL)	D (5 m)
AST (U LG^{1})	52	112	104	93
ALT (U LG^{1})	33	35	40	19
Total (G LG^{1}) protein	70	74	73	76

Fig. 19: Changes in biochemical parameters in the Wistar rats studied

Histopathological discoveries

Batch A (control) shows segments of typical liver tissue, including hepatocytes, sinuses, and focal veins. Batch B (treated with 1900 mg/kg ethanol concentrate of *C. roseus*) showed tissue discontinuity with slight lysis, including lumen. Tissue areas showing batch C ethanol leaf separate (treated at 3000 mg/kg), focal vessel blockage, vacuolation, break, adjustment, and corruption of hepatocytes and connective tissue. Notwithstanding, as demonstrated in Fig. 20, complete liver tissue harm with corruption was seen in batch D (5000 mg/kg).

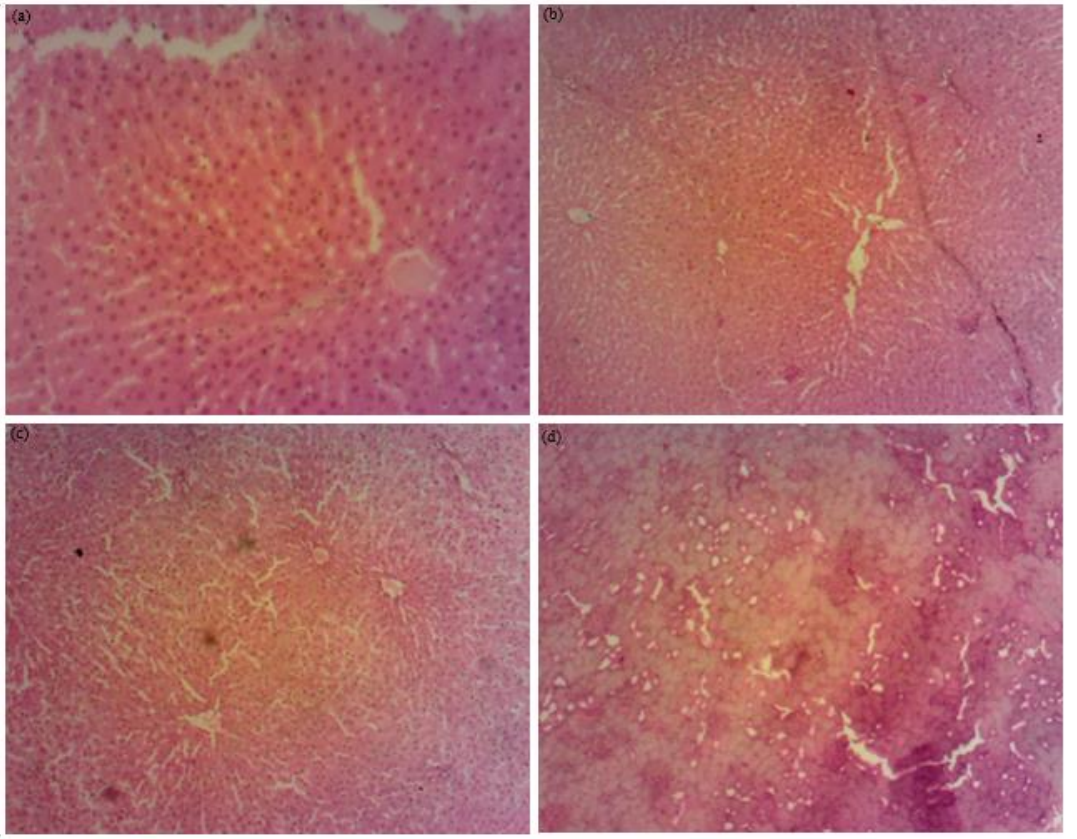


Fig. 20: Effect of an ethanolic extract of *C. roseus* on the liver of Wistar rats

Group A (control): normal liver tissue with hepatocytes, sinusoids and central veins (cv),
Group B: mild degeneration with vacuoles, tissue division,
Group C: central venous overload, vacuolation, changes in adipose tissue, necrosis of hepatocellular and connective tissue,
Group D: complete tissue damage with necrosis

During the investigation time (toxicity studies) frame, plant removes were seen to can get in shape in treated creatures. Huge weight reduction was seen in Wistar rodents treated with portions (1900 and 3000 mg/kg body weight) contrasted with rodents treated with 5000 mg/kg/g. This was not related with the lessening in absolute protein levels saw in rodents treated with 5000 mg/g. Concentrates can meddle with protein combination, and histological assessment of the livers of these creatures uncovers unblemished histological highlights. Histological segments of the treated rodent liver gave no indications of cell harm as no disintegration and denitrification were found in the sinuses of the treated creature organs. No hepatocyte corruption or edema was seen besides in batch C and D. The entryway vein is known to be non-incendiary, and chemicals enter the circulatory system when certain cell types are harmed. An increment in these chemicals is utilized as a marker of harm to these organs.

Alanine aminotransferase (ALT) is one of the chemicals that increments significantly in extreme liver harm. The protein aspartic corrosive aminotransferase (AST) assumes a comparable part however is found in different tissues and organs like the liver, heart, cerebrum, lung, muscle, and liver. Significant degrees of AST in this investigation propose that the compound is from tissues and organs other than the liver. Along these lines, estimating ALT action in some creature species is a generally delicate pointer of liver harm and may help decide the requirement for extra analytic tests. The discharge of ALT by the cytoplasm might be auxiliary to cell putrefaction or may result from cell harm because of layer harm and rankling.

The consequences of this examination recommend that *C. roseus* stem extract is generally protected at these portions (1900-5000 mg/kg), however may have poisonous impacts at higher measurement. Subsequently, it is important to avoid potential risk while mishandling these items. This may incorporate checking serum levels of proteins and milk items like *C. Roseus* alkaloids. Further examinations should zero in on the impacts of high-portion *C. roseus* to decide the LD50 of plants.

Conclusion

The ethanolic extract from *Catharanthus roseus* stem showed huge, separated and portion subordinate antiulcer action in skimming ulcers initiated by rodents. In the constrained gastric ulcer model, the methanol concentrate of Catharanthus roseus leaves was utilized at a portion of 250 mg/day. Contrasted and the benchmark group with ranitidine 5 mg/kg as the norm, the oral organization of kg and 500 mg/kg has altogether unique and portion subordinate antiulcer and antisecretory exercises. Hence, it tends to be closed from this investigation that the methanol extricate from Catharanthus roseus leaves has against refinement and antisecretory impacts on ulcers brought about by constrained swimming in rodents.

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