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Original Article

Rutin Attenuates Serotonin-Induced Gastric Ulceration in Rats *via* Antioxidant Defense Enhancement and Inhibition of Inflammatory Responses

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ABSTRACT

Background: Gastric ulcer is a disorder that occurs when the stomach's lining becomes damaged. This deterioration transpires when the gastric lining lacks sufficient strength to safeguard itself from external factors. The gastric lining deteriorates. This facilitates the potential for adverse elements to inflict damage. Serotonin is a substance that can exacerbate damage to the stomach lining. It achieves this by inducing stress and inflammation in the gastric region. We aimed to investigate whether a substance known as Rutin may safeguard the stomach against ulcer formation. We conducted an experiment on rats to see whether Rutin can prevent the exacerbation of ulcers. We administered a substance to the rats that would induce ulcers. Subsequently, we administered Rutin to observe the outcome. We believe that Rutin may assist due to its characteristics that might alleviate inflammation and stress. We seek to determine whether Rutin can effectively inhibit the advancement of ulcers. We are endeavoring to avert the occurrence of stomach ulcers through the application of Rutin. This research could yield significant new insights on the therapeutic application of Rutin in the management and prevention of gastric ulcers linked to serotonin creatinine sulphate.

Methodology: In experimental rats, the introduction of serotonin creatinine sulphate led to the formation of stomach mucosal ulcers. The rats were administered Rutin prior to that. The intensity of the ulcers was evaluated using the ulcer index. This indicates the degree of mucosal damage. Furthermore, we evaluated stress by the detection of reduced glutathione, catalase, superoxide dismutase and malondialdehyde. We investigated the tissues of the stomach for alterations. We ran analysis to identify the importance of the findings. P-value < 0.05 was determined to be statistically significant. The inquiry was focused on the evolution of stomach ulcers and putative protective impact of Rutin was given.

Results: The injection of serotonin creatinine sulphate has an effect on the stomach. It made the activities of superoxide dismutase, catalase and glutathione go down a lot. This signifies that the stomach lining became destroyed because of excessive oxygen. The levels of malondialdehyde and ulcer index also went increased a lot. When we provided Rutin before that it helped a lot. The ulcer index fell lowered. There was less harm to the fats in the body. This suggests that Rutin can help prevent gastric troubles. Rutin

also helped with inflammation. Increased the activity of natural antioxidant enzymes. When we looked at the stomach tissue we saw that the groups that had Rutin had a typical stomach anatomy. There were cells penetrating the stomach lining and the mucosal integrity was retained. This shows that Rutin can help prevent tissue damage and maintain the stomach structure stable. The serotonin creatinine sulphate caused a lot of damage. Rutin was able to counteract that. Rutin is particularly helpful, at supporting the stomach and keeping it healthy.

Conclusion: Rutin's powerful gastro protective function has been proven by its clear protective impact against serotonin creatinine sulphate -induced stomach ulcers. Its cytoprotective and anti-inflammatory characteristics imply probable therapeutic use for the treatment of stomach ulcers. Given its capacity to decrease mucosal damage and retain stomach integrity, our results underline the need for greater research into Rutin as a potential treatment for ulcers.

Introduction

Gastric ulcer disease is a long term condition that affects the stomach. It happens when the lining of the stomach gets injured. This damage can be caused by stomach acid, microorganisms like Helicobacter pylori and some kinds of pharmaceuticals such -steroidal anti-inflammatory medicines. The stomach normally has measures to protect itself such generating mucus and bicarbonate. When these protective mechanisms are not enough the stomach lining can get injured. This can lead to ulcers and other complications like bleeding and perforation. Gastric ulcer disease is an issue, for public health. Many people get it. It can come back. It also costs a lot to treat. Can impact a person's quality of life. Even though we have developed ways to cure some of the causes including getting rid of H. Pylori bacteria and using proton pump inhibitors stomach ulcers remain still an issue. Some things that can make it worse are taking -steroidal anti-inflammatory medicines for a long period getting older not eating well smoking, drinking too much alcohol and being worried. The typical therapies can help stop acid from developing in the stomach. They do not fix all the problems. They do not help with stress, inflammation or the stomach lining not recovering properly.

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Also taking these medicines for a while can have harmful effects and the ulcers can come back. So, we need to identify safer techniques to treat gastric ulcer illness that can target all the causes at the same time. Gastric ulcer disease needs to be treated in a way that addresses all these aspects to really benefit people who have it [8–10].

The thing that causes gastric ulcer disease is really complicated. It involves a lot of things happening together like biochemical and cellular and molecular events. Gastric acid and pepsin are still the things that cause damage. They only cause problems when the defense mechanisms of the mucosa are not working well. NSAIDs stop the cyclooxygenase enzymes, COX-1 which means they reduce the production of prostaglandin. When there is prostaglandin it means there is less mucus and bicarbonate secretion, less blood flow to the mucosa and it is harder for the epithelium to fix itself. This makes the gastric mucosa really vulnerable to getting hurt by acid. Gastric ulcer disease is also caused by H. Pylori bacteria. These bacteria disrupt the epithelium. Cause inflammation, which leads to the tissue getting destroyed over time. Oxidative stress is one of the reasons why the gastric mucosa gets injured. When there are many reactive oxygen species, like superoxide anion and hydroxyl radicals and hydrogen peroxide it causes a lot of problems. It damages the lipids and proteins and mitochondria and DNA. It even causes the death of gastric epithelial cells. Under normal physiological conditions, endogenous antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase, and reduced glutathione (GSH)

efficiently neutralize ROS and preserve cellular homeostasis. When gastric ulcer disease is happening these antioxidant defenses do not work as well. This means that the oxidative damage gets worse and it takes longer for the tissue to repair itself. So oxidative stress is a target for therapy in gastric ulcer research for both experiments and clinical trials, on gastric ulcer disease [16–34].

Oxidative injury is really connected to signaling. When there is much ROS it activates something called nuclear factor-kappa B or NF-κB for short which is like a switch that turns on lots of inflammatory genes, including tumor necrosis factor-alpha or TNF-α, interleukin-1β or IL-1β, interleukin-6 or IL-6 and a few others. When we have many of these things it makes the blood vessels leakier and it brings in more inflammatory cells, which makes things worse. It also makes myeloperoxidase and that just keeps on hurting the mucosal lining. The neutrophils and macrophages that are activated are like the guys they release more ROS and enzymes that break things down and that just makes a big cycle where oxidative stress and inflammation just keep on making each other worse. At the time the blood flow to the stomach is not working right so it does not get enough oxygen and food and it takes too long to get rid of the bad stuff and that slows down the healing of the mucosal lining [35–39]. There is a way to study ulcers in a lab using something called serotonin or 5-hydroxytryptamine or 5-HT for short to make ulcers in the stomach. Normally serotonin helps the stomach work properly it helps with movement, secretion feeling things and blood flow. But when there is much serotonin it really messes up the stomach it makes the blood vessels get smaller which reduces blood flow and that makes ischemia and ROS and it stops the antioxidants from working and it reduces the production of prostaglandin and mucus and bicarbonate. All of these changes make the mucosal lining not work right. That makes ulcers form faster. Serotonin also makes TNF-α, IL-1β and IL-6 which makes the inflammation worse and that makes it take longer to heal the ulcers. When we give serotonin to animals, in a lab it always makes these bad things happen. It is a great way to study what is going on and how to make it better. [20–39].

Natural things that come from the earth are looking like they could be really good at helping people feel better because they can do lots of things to help the body and they are usually safe to use. One type of thing that people are really interested in is called Flavonoids. They are good at stopping cells from getting hurt. They can also stop inflammation and catch bad things that might harm the body. Rutin is a type of Flavonoids that is found in lots of things like fruits and vegetables and plants that are used for medicine. Rutin is really good, at doing all these things because it is a kind of Flavonoids that comes from nature. Experimental evidence indicates that Rutin scavenges ROS, inhibits lipid peroxidation, restores endogenous antioxidant enzyme activity, suppresses NF-κB activation, decreases pro-inflammatory cytokine production, improves endothelial function, preserves gastric microcirculation, and accelerates mucosal healing. The protection that Rutin offers is not about reducing acid it is about protecting the stomach and digestive system in general. Rutin may be very good, at protecting the stomach and digestive system from harm. Despite increasing evidence supporting the pharmacological potential of Rutin, limited studies have comprehensively evaluated its protective efficacy against serotonin creatinine sulphate-induced gastric ulceration with simultaneous assessment of oxidative stress, inflammatory biomarkers, and histopathological alterations. Therefore, the present study was undertaken to investigate the gastro protective effect of Rutin in a serotonin-induced gastric ulcer model in rats. Particular emphasis was placed on evaluating ulcer severity, antioxidant defense systems, inflammatory mediators, and microscopic gastric tissue architecture. Understanding these mechanisms may contribute to the development of safer, mechanism-based therapeutic strategies for gastric ulcer disease and further support the clinical potential of naturally occurring flavonoids in gastro protection.

Gastric Ulcer: Pharmacological Treatment, Side Effects & Management

Peptic ulcer disease includes gastric ulcers as a subtype. When the stomach lining is compromised, gastric ulcers develop. This is known as a lesion. It occurs when a number of harmful substances, including pepsin, stomach acid and some medications, such as non-steroidal anti-inflammatory drugs. Helicobacter pylori, a bacterium and excessive stress can also cause gastric ulcers. The stomach is capable of self-defence. It produces mucus to protect the lining of the stomach. Additionally, the stomach produces prostaglandins and bicarbonate. For the stomach lining to remain healthy, there must be enough blood flow. When the harmful substances outweigh the stomach's defences, gastric ulcers become an issue [39, 40]. The treatment of Ulcer with medicines is divided into five types:

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Figure 1: Classification of Anti-Ulcer Drugs.

Proton Pump Inhibitors (PPIs)

PPIs, including as omeprazole, esomeprazole and pantoprazole, are prodrugs that are activated in the parietal cell canaliculi's acidic environment. After diffusing into parietal cells and building up in acidic canaliculi, PPIs are transformed into active sulphonamide form and covalently attach to the cysteine residues of H-ATPase. The last stage of acid secretion is blocked when PPIs bind irreversibly and inhibit the proton pump [40].

Pharmacodynamics

- There is a significant decrease in both baseline and induced acid secretion. This indicates a considerable reduction in both the normal generation of acid and the excess acid produced when it is stimulated. Both the induced acid secretion and the basal acid secretion are somewhat impacted.
- This is beneficial for mucous membrane healing and helps prevent clots from dropping. By ensuring that the clot remains in place and the mucous membrane heals, it does this. It is crucial that the clot remain stable. This is helpful in that regard. Additionally, it aids in the membrane's gradual healing. This aids in the mending of the mucosa, which is a necessary element of recovery [40].

Pharmacokinetics

• When you take the medication orally, it is absorbed by your body. It has a covering that improves its performance.
• Thirty to eighty percent of the medication truly enters your body and begins to function.
• Ninety-five percent of the time, the medication adheres to your body's proteins.
• CYP2C19 and CYP3A4 assist your liver in breaking down the medication.
• Your body retains the medication for one to two hours. Due to its irreversible binding to bodily components, it continues to function for 24 to 48 hours.
• The medication exits your body through your faeces and urine.

Therapeutic Uses

Many digestive issues are treated with this medication. It is beneficial for GERD, duodenal ulcers and ulcers. Zollinger-Ellison syndrome is another condition that is treated with the medication. NSAID medication can occasionally cause ulcers. Additionally, this medication helps with it. Additionally, it is used to eliminate the H. Pylori bacterium, which can lead to digestive issues. For H. Pylori elimination therapy, the medication is excellent.

Adverse Effects

Headache, nausea, vomiting, constipation, low magnesium and vitamin B12 levels, increased risk of fractures, Clostridium difficile infection and excessive acid production in the stomach when the medicine is discontinued.

Limitations

Long-term usage causes vitamin B12 deficiency. Fractures and infections also occur. The risk of fractures is further increased by prolonged usage. It can also result in a vitamin B12 shortage and infections [40]

H_2 Receptor Antagonists

Mechanism of Action

Histamine attaches itself to H_2 receptors. Adenylate cyclase is then activated as a result. It raises a pathway called cAMP in the body. This leads to the stimulation of a proton pump.
To inhibit this H_2 receptor, histamine and H_2 blockers compete [41].

Pharmacodynamics

• The medication aids in lowering the stomach's acid production when food is not being digested.
• When we eat and the stomach produces a lot of acid to digest the meal, it does not function efficiently.
• The medication effectively suppresses stomach acid while we are sleeping.

Pharmacokinetics

• When you take this medication orally, the body absorbs it efficiently.
• It is not very adherent to the proteins in your blood.
• This medication is eliminated via your kidneys.
• The medication's half-life, or half-life of this specific medication, is the amount of time it remains in your system—two to four hours.

Therapeutic Uses

Additionally, it is used to treat GERD, or gastroesophageal reflux disease. Stress ulcers are one of its uses. The medication relieves ulcers, GERD and stress ulcers, among other issues.

Adverse Effects

Headache, dizziness and diarrhoea are some side effects, elderly people might feel confused, Gynecomastia is an issue with cimetidine, this medicine can also interact with drugs especially when it inhibits CYP, some people may develop tolerance, over time, bradycardia is a possible side effect.

Limitations

Long-term usage of H_2 receptor antagonists causes tolerance and their therapeutic usefulness is further restricted by drug-drug interactions, especially with cimetidine [41].

Antacids

Through a chemical mechanism, it aids in reducing stomach acid. Magnesium hydrochloride, an antacid, is combined with hydrochloric acid in this method. They mix to create water and magnesium chloride, a salt. The PH level rises as a result. The symptoms of acidity and stomach ulcers are alleviated by this rise in PH. It's the stomach acid [42].
It aids in the stomach ulcer's healing. The magnesium. Together, hydrochloric acid and other substances create a solution.

Pharmacodynamics

• We get relief from symptoms away.
• The body's production of acid is unaffected.

Pharmacokinetics

• The body does not absorb the medication very well.
• It acts immediately on the stomach.

Therapeutic Uses

This drug is mostly used to treat dyspepsia; however, it can also help with moderate gastrointestinal irritation. In people with functional dyspepsia and associated gastrointestinal disorders, its therapeutic effect relieves symptoms and enhances patient comfort.

Adverse Effects

This medication is used to treat diarrhoea. It also relieves mild gastrointestinal distress. It is used by those who suffer dyspepsia to feel better. For those who suffer from dyspepsia and stomach irritation, it works well.

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Limitations

This product's short half-life and potential to lead to an electrolyte imbalance are its drawbacks. This medication has several limits, such as a duration and an electrolyte imbalance [42]

Cytoprotective Agents

Its short duration and potential to lead to an imbalance in your body's electrolytes are its drawbacks. This device's limits include things like an electrolyte imbalance and a duration. As an example: Misoprostol, Sucralfate [43].

Pharmacodynamics

This substance protects our body's membranes and has little effect on acid production. It strengthens the mucosal defence. There is little effect on the acid.

Pharmacokinetics

• When it comes to Sucralfate, the medication acts locally.
• The body absorbs misoprostol when you take it and enters the system.

Therapeutic Uses

• The medicine works locally which is the case with Sucralfate.
• When you take misoprostol, it gets absorbed by the body and goes into the system.

Adverse Effects

Diarrhoea, Abdominal pain, Nausea, Uterine contraction from misoprostol, Constipation, from Drug interactions that affect binding, Flatulence and Dizziness.

Limitations

It causes diarrhoea and cramps [43]

Antibiotics (*H. pylori*): Examples- Clarithromycin, Amoxicillin, Metronidazole.

It helps with:

• Killing bacteria
• Reducing swelling
It is used to treat bacterial infections and helps with reducing inflammation. This treatment also helps prevent recurrence of infections [44].

Pharmacodynamics

• It aids in reducing the possibility of an event.

Pharmacokinetics

• It changes from one drug to another
• The body absorbs it well when taken orally.
Therapeutic Uses

It is used to treat the H. pylori infection.

Adverse Effects

Nausea, Diarrhea, Antibiotic resistance, problems with taste, Superinfection, damage to the liver and interactions with other medicines.

Anticholinergics Drugs

Anticholinergic drugs work by blocking a chemical called Acetylcholine at receptors specifically M_3 receptors, found on stomach cells and nerve cells in the gut. In body function the Vagus nerve releases Acetylcholine, which then binds to these M_3 receptors. This binding activates an enzyme called phospholipase C or PLC. The activation of PLC increases the levels of a chemical called Ca^{2+} inside the cells. As a result, it triggers the H^+/K^+-ATPase, also known as the proton pump, which in turn causes the stomach to release acid. Acetylcholine plays a role, in this process and Anticholinergic drugs block Acetylcholine from working properly. The M_3 receptors are crucial for Acetylcholine to have its effect and blocking them affects how the stomach secretes acid [45].

With Anticholinergic Drug

Drug blocks M_3 receptors. Stops acetylcholine from binding. This decreases, in Ca^{2+} signalling results in less activation of the proton pump. As a result, gastric acid secretion decreases. The drug works by targeting M_3 receptors. It prevents acetylcholine from interacting with these receptors. This is how it reduces gastric acid secretion [44].

Pharmacokinetics

• Absorption: This thing gets absorbed when you take it by mouth
• Distribution: The drug goes to parts of the body
• Metabolism: The liver breaks it down
• Excretion: The kidneys get rid of it
• Duration: The drug lasts for an amount of time
Therapeutic Uses

This medicine is used to help people with peptic ulcer disease. Doctors also use it with medicines to treat the disease.

Limitations and Adverse Effects

This medication has several potential side effects. Maybe you'll get a mouth. You may get hazy vision. Constipation may occur. It might be challenging to use the restroom. Your heart may beat quickly, a condition known as tachycardia. You may experience confusion or sedation, particularly if you are older. Sweating will be less than normal. Glaucoma patients may have a rise in intraocular pressure. Xerostomia is another term for dry mouth [45].

Natural Agent: Rutin

Mechanism of Action:

1. The Antioxidant Mechanism of the Rutin is really important.
• The antioxidant helps get rid of things like ROS, which includes O_2^- and OH•.
• The antioxidant also helps increase some good things in our body, such as SOD, CAT and GSH.
•The antioxidant reduces lipid peroxidation, which means it reduces MDA.
2. The Anti-inflammatory Mechanism of the Rutin is also very important.
• The anti-inflammatory stops the NF-κB pathway from working.
• The anti-inflammatory reduces some things in our body like TNF-α, IL-1β and IL-6.
•The -inflammatory also reduces the number of neutrophils that enter our body tissues.
3. The Gastro protective Mechanism of the Rutin is really good for our stomach.
• The gastro protective helps our stomach make mucus, which is a good thing.
• The gastro protective also helps our stomach make prostaglandin, which helps our stomach feel better.
• The gastro protective increases the blood flow, to the mucosal area of our stomach which helps keep it healthy [44].

Pharmacodynamics

There are several implications from this. It can simultaneously lessen inflammation and aid in cell protection. The antioxidant component is especially excellent as it protects the body from damage. Therefore, this antioxidant's multi-target effect combined with anti-inflammatory or cytoprotective drugs is very beneficial to human health. Its cytoprotective and anti-inflammatory properties provide it advantages.

Pharmacokinetics

The absorption of this thing is moderate. It gets broken down in the intestines and the liver. When it is in the form of a glycoside it is not very good, at getting into the body. The body gets rid of it through the urine.

Uses

Oxidative stress problems are something I occasionally have to cope with. All of these factors are connected to oxidative stress disorders, inflammatory illnesses and ulcers. Oxidative stress problems, inflammatory illnesses and stomach ulcers are all very detrimental to my health.

Adverse Effects

Mild nausea, headache, reactions, stomach discomfort, diarrhoea, feeling dizzy, drug interaction and low blood pressure are some side effects. These include nausea and headache. Other side effects are reactions GI discomfort and diarrhoea. Some people may also experience dizziness. Additionally, drug interaction and hypotension can occur [45].

Current Treatment Strategies and Limitations

The main goal of gastric ulcer disease treatment is to decrease stomach acid, promote healing of the stomach lining and eliminate the cause, which may be Helicobacter pylori infection. Stomach ulcers are far more effectively treated thanks to the development of pharmaceutical drugs. Pantoprazole and omeprazole are examples of proton pump inhibitors. These proton pump inhibitors are still the treatment for stomach problems because they really stop the stomach from making acid. They do this by suppressing the gastric H+/K+ ATPase, which helps the stomach lining heal. Proton pump inhibitors are very good, at what they do. However people who take proton pump inhibitors for a time can have some problems. They might get effects and their ulcers might come back. So stomach ulcer disease is still a problem to solve. We need to find ways to make treatment better and safer. For example people who take proton pump inhibitors for a time might not have enough vitamin B12. They might also get sick easily. Some people might even have kidney problems. When people use proton pump inhibitors for a time a lot of things can go wrong. There are kinds of drugs that help with ulcers too. These are called H_2 receptor antagonists. Famotidine and ranitidine are examples of H_2 receptor antagonists. These drugs help the stomach by blocking the thing that makes it produce acid. This means that the stomach lining does not get as damaged. Despite their effectiveness, they are not as strong as proton pump inhibitors and long-term use may cause tolerance, which eventually reduces their therapeutic value. As a result, H_2 receptor antagonists will eventually lose their effectiveness [46]. Antibiotics are required for H. Pylori infection-related ulcers. A

combination of antibiotics, such as amoxicillin and clarithromycin, plus a PPI is the standard course of therapy. For H. Pylori ulcers, this is referred to as treatment. Antibiotic-resistant H. Pylori ulcers are growing more and more common, which is a serious issue. This indicates that there is no effective therapy for H. Pylori ulcers. [47-48]. To make individuals feel better, antacids and some other medications that protect the stomach are utilized.

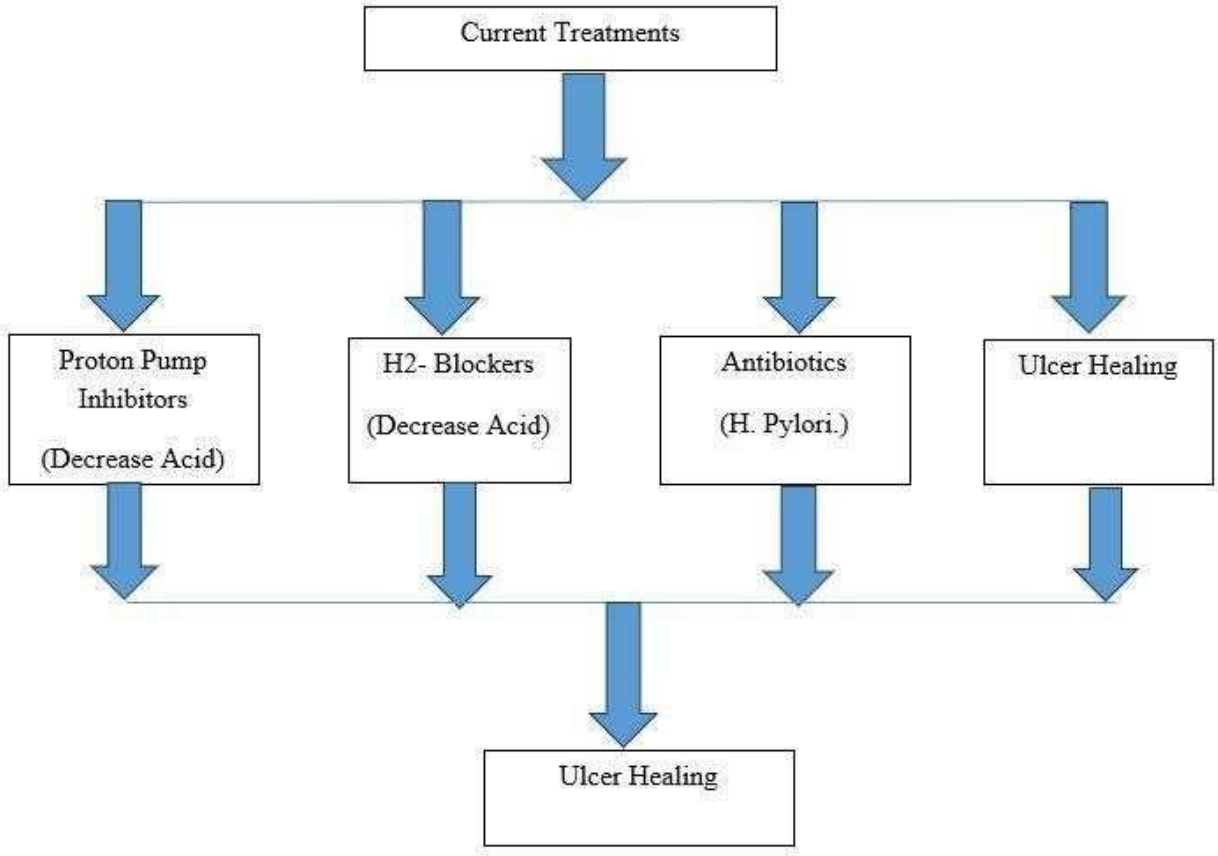

Figure 2: Current Treatment Strategies [47-48].

Antacids and acid-suppressive medications are examples of conventional treatments that do not directly target the underlying pathogenic processes of gastric ulcer disease. As a result, these medications are frequently used in conjunction with other treatments to relieve symptoms. There are, however, still many limitations as long-term use of acid-suppressive drugs can have adverse side effects and disrupt normal stomach function, which will make it harder to treat long term. Many patients have persistent risk factors (NSAID use or H. Pylori infection) and continue to develop ulcers. Additionally, patients may not take their medications as directed since certain medications don't work well together and therapy can be costly, making it more difficult to manage [49]. Conventional ulcer treatments focus mainly on decreasing stomach acid production, rather than targeting the underlying pathological causes of inflammation and oxidative stress. These mechanisms are important in the pathogenesis of ulcers as they injure the mucosa and impair its healing. To address the multiple factors involved in ulcer genesis, a growing interest has been seen in the use of therapies that act on multiple pathways, such as antioxidant and anti-inflammatory mechanisms [49]. The use of natural substances and phytochemicals has increased greatly in recent years. Some believe they can benefit us due to their potential to provide protection of our cells and to help reduce inflammation. Rutin and other flavonoids are substances that have been well investigated. They appear to function well in experiments. Our bodies can help us deal with stress, inflammation and fortify our stomach and intestinal lining with natural substances such as Rutin. Phytochemicals and natural substances are currently being researched; they appear to have a lot of potential. Flavonoids are a group of compounds

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which include Rutin and which offer a number of health benefits to the human body. Current ulcer therapies are generally effective, although they have certain drawbacks. We need therapies that're safer and that really get to the bottom of the problem. When it comes to treating ulcers we have to do it in a way that looks at everything. To treat ulcers we need to have a plan that includes many things. Gastric ulcers require a strategy to really address the issue of ulcers. We should look at all the things that can help us treat ulcers. Treating ulcers is not easy but we can do it if we have a good plan to treat gastric ulcers [50-51].

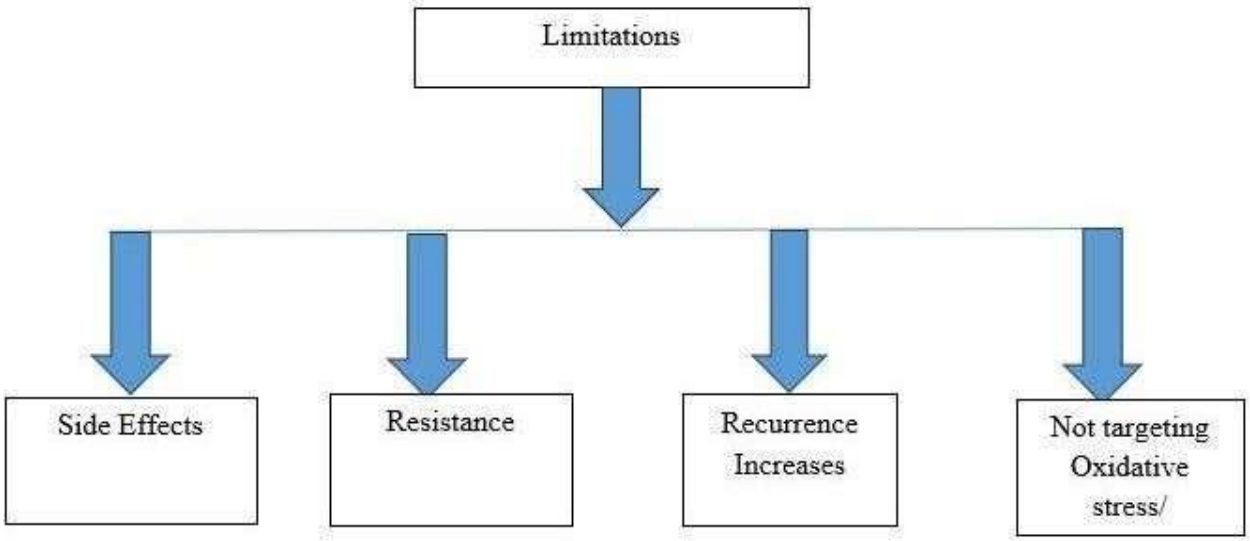

Figure 3: Limitations of Current Treatment [51].

Rutin: A Potential Therapeutic Flavonoid

Source and Chemical Nature of Rutin

Rutin is a naturally occurring glycoside of the flavonoids which can be obtained from various food and medicinal plants including buckwheat, citrus fruits, apple and tea. It is common in phototherapeutic agents and food and it is important as a bioactive compound with potential gastro protective properties. Rutin can also be known as quercetin-3-O-rutinoside. This word is used by scientists to describe the make-up of Rutin. It's rich in a type of sugar and quercetin. The glyosidic part of Rutin (rhamnose and glucose) [51] enhances the water solubility and extends its biological activity, as compared to quercetin alone. Due to its structure, Rutin has superior pharmacological properties in several aspects, thus making it a promising medicinal compound. Rutin is classified as a polyphenolic compound, which has a backbone of flavonoids. The usual C6–C3–C6 structure for this backbone of two benzene rings linked by a three-carbon bridge. The presence of many hydroxyl groups, which boost antioxidant capacity and contribute to Rutin's varied pharmacological effects, is one of its distinguishing characteristics. These organizations help to make Rutin effective against radicals. As a result, people believe that Rutin might be a medicine for various diseases [52].

Pharmacological Properties of Rutin

Among the many advantageous pharmacological effects of Rutin is the scavenging of dangerous reactive species that endanger the integrity of cells. It has neuroprotective and cardio protective benefits in addition to anti-oedematous qualities. Rutin plays a major role in preserving homeostasis and boosting tissue resilience by regulating physiological reactions in stressful or injured situations. This reduces inflammation and stress, for example. Rutin has beneficial impacts on our bodies, such as reducing inflammation and acting as an antioxidant. It may even aid in preventing damage to our brains and hearts [53, 54].

Antioxidant Activity

By neutralizing free radicals, such as hydroxyl radicals and superoxide anions, Rutin demonstrates strong cytoprotective effect and prevents oxidative damage to cellular structures. Rutin strengthens the body's ability to combat oxidative stress and preserve cellular integrity by boosting endogenous antioxidant defenses in addition to its radical-scavenging capabilities. These

substances are called as glutathione, catalase and superoxide dismutase. Rutin improves these mechanisms so that the substances that might harm our cells [55].

Rutin has strong cytoprotective properties by protecting cells' ultrastructural elements from oxidative damage. Rutin does a job of stopping bad things from hurting our cells. It helps keep the cells in our gut healthy. When these cells are healthy our stomach works a lot better. Rutin is really good at protecting these cells from things that can harm them. This is important because it helps keep our gut tissue strong and working well. Rutin is good for the cells that line our gut. It helps keep them safe, from damage. This is very important when we suffer from gastrointestinal problems such as ulcers. These issues may arise when there is a lot of stress on the body from substances that can damage the cells. By preventing oxidative and inflammatory damage to stomach mucosal cells, Rutin protects them. The cytoprotective effect highlights the potential therapeutic value of Rutin in gastric ulcer disease, as it may help to maintain the integrity of gastric cells and promote mucosal health [55].

Anti-inflammatory Activity

Rutin is very effective in reducing inflammation. By inhibiting the synthesis of important pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6), Rutin has strong anti-inflammatory effects. Rutin has been found to successfully reduce the inflammatory response by reducing these mediators, thus reducing tissue damage and helping heal the mucosal lining. The cytokine-modulatory effect of Rutin is a therapeutic relevance in the gastric ulcer disease. Inflammatory response is the response of the body when it is hurt or sick and Rutin helps to make it less severe [56]. Rutin has also shown strong anti-inflammatory activity, acting on several pathways that are involved in inflammatory processes, such as the NF-κB signaling pathway. It does this by downregulating the expression of pro-inflammatory enzymes such cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS).

Gastro protective Effects

Rutin works as a powerful gastro protective agent by strengthening the defense systems of the stomach mucosa. It strengthens the stomach lining and maintains the integrity of the mucosa by stimulating the formation of mucus. In order to preserve stomach health and prevent ulcer development, this preventive function is essential. Additionally, Rutin aids in the flow of blood to the stomach lining, which is essential for the stomach to get the nutrients required for healing. Rutin has been demonstrated to lessen stomach stress and inflammation, which helps shield the stomach lining from damage [57, 58]. Rutin prevented the stomach blood vessels from shrinking and prevented oxidative damage in several instances when serotonin was the cause of ulcers. Inflammation of the stomach was prevented by it. Rutin therefore aids in the healing of ulcers and prevents gastrointestinal damage. The lining of the stomach is well protected by Rutin. This explains why it can be used to heal stomach ulcers [59].

Figure 4: Dried Leaves of *Moringa oleifera Lam* leaves.

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Material and Methods

Plant Work

Collection and Identification of plant

Moringa oleifera Lam. leaves were picked from the Herbal Garden of Metro College of Health Sciences and Research in Greater Noida. The reference number, for the authenticated plant material specimen is-BSI/BGIR/1/TECH./2026/334.

Extraction and Isolation of Rutin from *Moringa oleifera* Leaves

Collection and Preparation of Plant Material:

After gathering fresh *Moringa oleifera* leaves, they were meticulously cleansed with distilled water to get rid of any dust or other unwanted objects. The leaves were dried at ambient temperature in the shade and not in the sun where delicate phytoconstituents could be degraded. This stage is particularly important as flavonoids such as rutin can be destroyed by high temperatures and light. The leaves were completely dried and then ground in a mechanical grinder to a coarse powder. The powder was sieved to ensure uniformity of particle size. Overall, a homogenous powder results in a greater extraction yield of phytochemicals due to better penetration of the solvents during extraction [91].

Defatting of Plant Material

Defatting procedure was performed before the target component was extracted. Petroleum ether (boiling range: 60–80°C) was used to extract around 100 g of the powdered leaves from a Soxhlet device. The extraction was performed for approximately six to eight hours or until the solvent from the siphon tube was almost colorless. This indicated that most of the lipophilic substances (lipids, waxes, chlorophyll) were removed. After the completion of the process the defatted plant material (marc) was removed and air-dried at room temperature to allow any traces of solvent to evaporate. Flavonoids are polar molecules and so it is important to remove non-polar contaminants prior to extraction to improve the efficiency of the extraction and purification process [92].

Extraction of Flavonoid-Rich Fraction

After that, a polar solvent solution was used to extract the defatted plant material. Because of its well-known capacity to efficiently dissolve flavonoids, methanol was chosen as the main solvent. In some cases, a hydroalcoholic combination (70-80% ethanol) may also be used with equally good results. A Soxhlet equipment was used for the extraction, which took six to ten hours. The solvent is continually circulated through the plant material, which gradually dissolves the chemicals in the leaves, known as flavonoids. As an alternative, maceration can be done in labs without Soxhlet equipment. This method is based on the extraction of the plant powder with methanol for 2-3 days with intermittent stirring. This gives an even yield but is slower.

Figure 5: Extraction of Rutin from *Moringa oleifera* Leaves.

After extraction, the mixture was filtered and concentrated in low-pressure rotary evaporator. This process removes the solvent but does not heat the extract. Because of their polarity and affinity with phenolic chemicals, methanol and aqueous alcohol solutions have been shown to be very useful for extracting flavonoids from *Moringa oleifera* leaves [93].

Fractionation of the Crude Extract

At this point, a variety of phytochemicals can be found in the crude extract. In order to separate the compounds according to their polarity, a fractionation stage was carried out. Before being put into a separating funnel, the concentrated extract was dissolved in distilled water. It was then successively fractionated by successively more polar solvents:

- Petroleum ether → to eliminate any residual non-polar contaminants
- Chloroform → to get rid of chemicals that are somewhat non-polar
- To extract flavonoids, such as rutin, use ethyl acetate.

The proportion of ethyl acetate was meticulously gathered and concentrated. Flavonoids are generally abundant in this fraction because they are soluble in moderately polar solvents.

Similar methods have been applied in investigations on flavonoid separation where it has been discovered that ethyl acetate fractions contain important flavonoid ingredients.

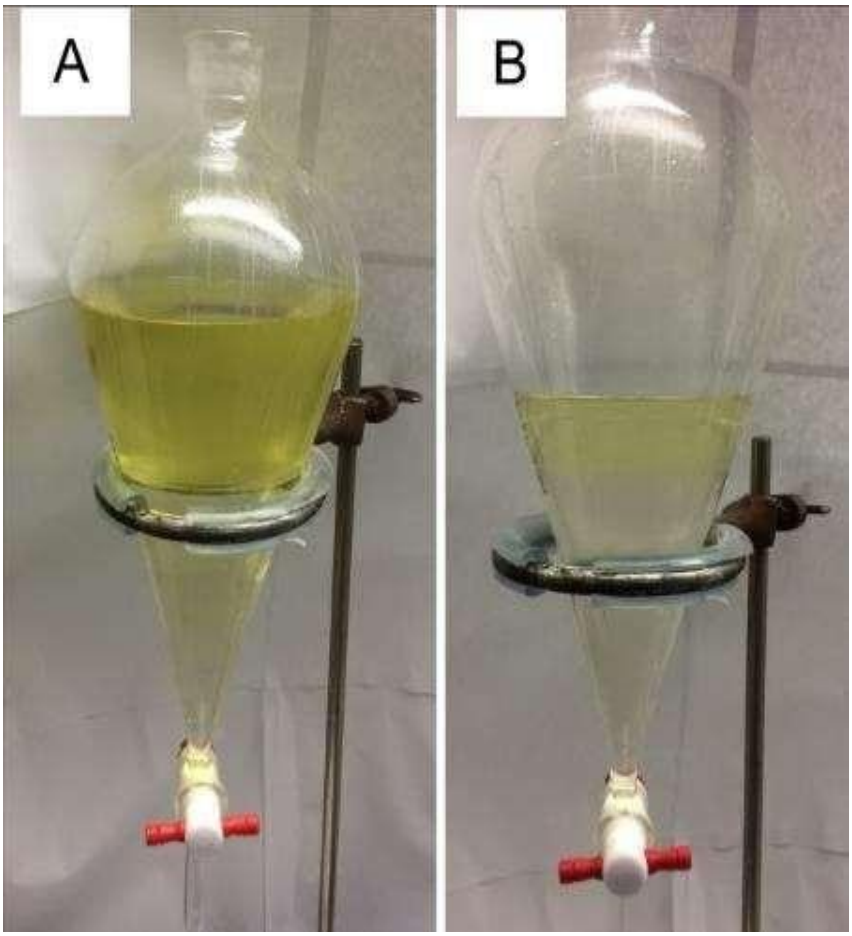

Figure 6: Separating Funnel; A= Mixture before phase separation in separating funnel; B=Formation of distinct aqueous and organic layers after extraction.

Isolation of Rutin by Column Chromatography

Rutin was separated from the flavonoid-enriched fraction by open column chromatography on silica gel (60-120 mesh) as stationary phase. A silica gel slurry made in chloroform was evenly packed into the chromatographic column to provide consistent packing and avoid channel development. Without disturbing the stationary phase, the flavonoid-rich fraction was pre-adsorbed onto a little amount of silica gel, properly dried and then gently put onto the top of the packed column. Elution was performed using a gradient solvent solution of methanol and chloroform which increased in polarity. Chloroform: methanol solutions in the ratios of 95:5, 90:10, 85:15, 80:20 and 70:30 (v/v) were added

after 100% chloroform was used to start the elution sequence. Fractions were methodically collected at a regulated flow rate of around 1-2 mL/min. Thin-layer chromatography (TLC) utilising pre-coated silica gel 60 F254 plates was used to analyse the eluted fractions. The composition of the mobile phase was 100:11:11:26 (v/v/v/v) ethyl acetate, formic acid, acetic acid and water. The plates were air dried after the chromatograms were developed and then subjected to UV light (254 and 366 nm). A distinctive yellow fluorescent band that matched the retention factor (Rf) value of Rutin was seen. Fractions having similar TLC profile were pooled and concentrated under low pressure using rotating vacuum evaporator to get a semi-purified rutin fraction suitable for further characterisation and pharmacological evaluation [94].

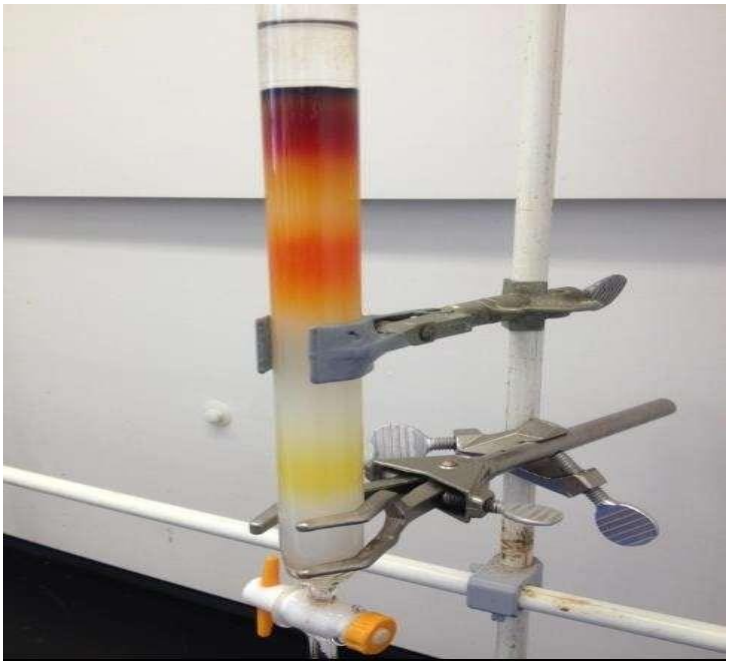

Figure 7: Isolation of Rutin by Column Chromatography.

Purification of Rutin

The semi-purified extract was further purified by recrystallization. The recrystallizing solvents used were either methanol or aqueous ethanol. The chemical was dissolved by lightly heating the solution and allowed to cool slowly. It was found that the yellow crystalline solids which formed with time were Rutin. The crystals were filtered, dried and stored in a desiccator to prevent moisture absorption.

The amount of product obtained after the reaction or extraction including purification process is called as the yield value. It is often expressed as a percentage yield, that is, the percentage of the actual amount of product obtained compared to the original amount of the starting material, to indicate how efficient the process [95].

Rutin % Yield: Weight of pure rutin obtained/ Weight of dried plant material *100 = 600/5000*100 = 12 %

Identification and Confirmation Tests for Rutin

High-Performance Thin Layer Chromatography (HPTLC) Analysis of Rutin

To investigate the presence of rutin in the plant extract prepared, High-Performance Thin Layer Chromatography (HPTLC) was performed. The sensitivity, accuracy and reproducibility of HPTLC make it an efficient and reliable analytical tool for separation and characterization of phytochemical constituents in herbal materials. The chromatographic profile of the extract was compared with the standard rutin in the present study to confirm the presence of the marker compound (flavonoid compound). The experimental analysis was done in School of Pharmaceutical Education and Research (SPER), Jamia Hamdard.

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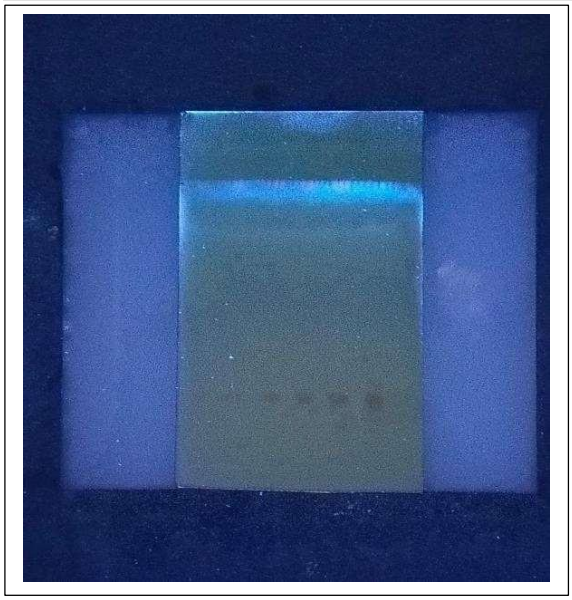


Figure 8: HPTLC plate containing sample

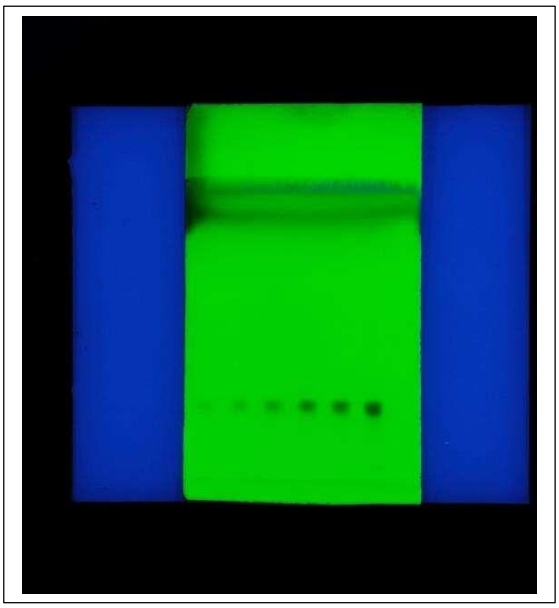


Figure 9: HPTLC plate at wavelengths of 368 nm

The stationary phase used was precoated silica gel 60 F254 aluminum plates. The optimized mobile phase was made up of ethyl acetate: formic acid: acetic acid: water (100:11:11:26, v/v/v/v) which gave satisfactory separation of phytoconstituents. The chamber was pre-saturated with the mobile phase for about 20 min before the chromatographic development to ensure uniform movement of the solvents and reproducible chromatographic resolution. Both standard rutin solution and the plant extract were precisely applied as bands on the chromatographic plates under controlled conditions. The plates were air dried after the completion of the chromatographic development and then were viewed under UV light at 368 nm. The chromatogram of rutin standard gave a well-separated and compact fluorescent spot at Rf 0.68. Similarly, the plant extract showed a band with fluorescent value of 0.67 Rf which was close to the standard band of rutin, thus showing the presence of rutin in the extract.

Table 1: Experimental Conditions for HPTLC Analysis.

Parameters	Experimental Conditions
Analytical technique	High-Performance Thin Layer Chromatography
Stationary phase	Silica gel 60 F254 precoated aluminium plates
Mobile phase	Ethyl acetate: formic acid: acetic acid: water
Mobile phase ratio	100:11:11:26 (v/v/v/v)
Standard compound	Rutin
Sample analysed	Plant extract
Chamber saturation time	20 min
Detection wavelengths	368 nm
Visualization method	UV illumination
Sample application	Band application

The chromatogram of standard Rutin showed a sharp and well-resolved fluorescent band at an Rf value of 0.68 under UV illumination, confirming the chromatographic identity of Rutin.

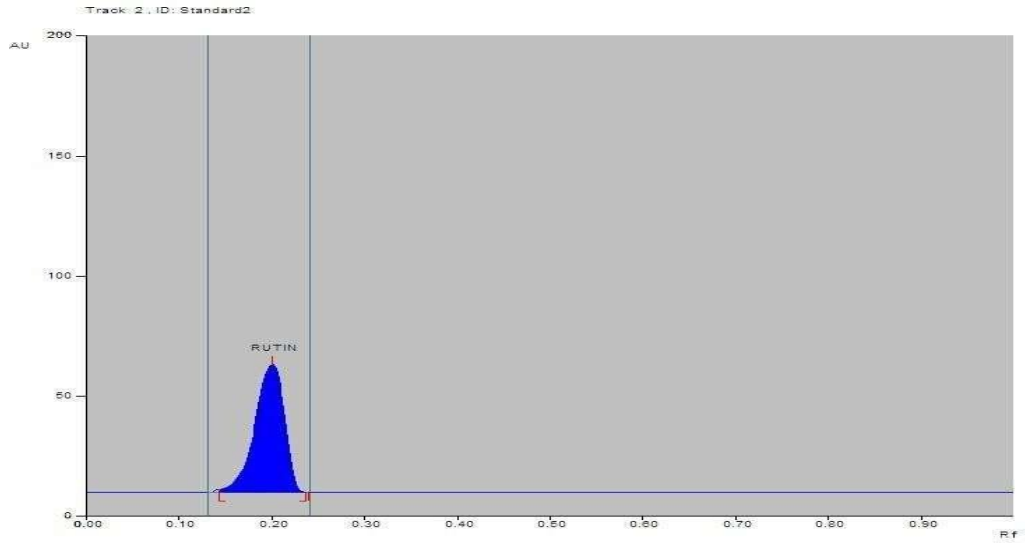


Figure 10: HPTLC Chromatogram of Standard Rutin (T2 STD).

The chromatographic profile of the plant extract revealed a fluorescent band at an Rf value of 0.67 corresponding closely to the standard Rutin, thereby confirming the occurrence of Rutin in the extract.

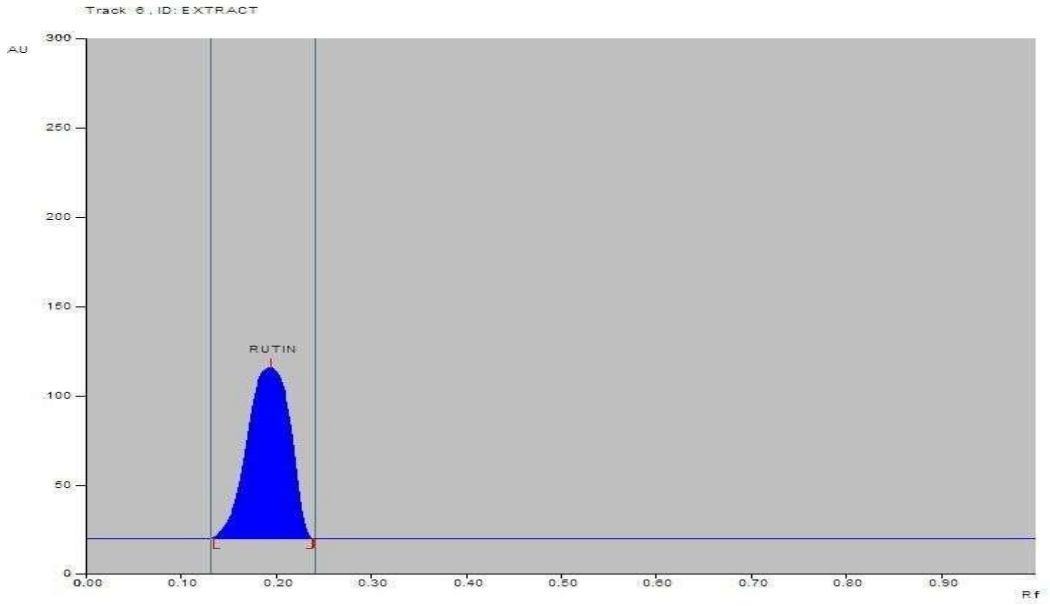


Figure 11: HPTLC Chromatogram of Standard Rutin (T6 EXTRACT)

Table 2: Comparative HPTLC Report of Standard Rutin and Plant Extract.

Sample	Rf Value	Observation	Interpretation
Standard Rutin (T2 STD)	0.68	Sharp fluorescent band observed under UV light	Characteristic chromatographic profile of Rutin confirmed
Plant extract (T6 EXTRACT)	0.67	Corresponding fluorescent band observed	Presence of Rutin in the extract confirmed

The present investigation was aimed to determine the phytochemical profile of the plant extract by HPTLC fingerprint analysis. The chosen chromatographic conditions yielded good separation and resolution of

phytochemical constituents present in the sample matrix. The RF value for the fluorescent band of standard Rutin was 0.68, which showed suitable chromatographic behavior of standard and the suitability of the mobile phase system used. When compared, the plant extract showed a band of fluorescence with an Rf value of 0.67 which was very close to that of standard Rutin. Based on the similarity of the chromatographic migration, it was assumed that Rutin was one of the important flavonoids present in the extract under study. HPTLC fingerprinting is widely used for the authentication and standardization of herbal drugs as it gives reliable information about the chemical composition of the plant materials. The identification of Rutin is of particular pharmacological importance as Rutin has been reported to possess antioxidant, anti-inflammatory and gastro protective activities. Hence, it can be concluded that Rutin present in the extract might play a major role in its therapeutic efficacy to protect gastric mucosal tissues against ulcerative damage. The developed chromatographic method was also found to be satisfactory for phytochemical analysis with respect to its reproducibility and specificity. Therefore, the obtained chromatographic fingerprint can be useful to future standardization and quality control studies of the investigated plant extract. The comparative chromatographic evaluation of the plant extract with standard Rutin confirmed the presence of Rutin in the plant extract in the HPTLC study. The extract showed a characteristic fluorescent band whose RF value was almost same as the standard compound, thus confirming the phytochemical authenticity of the extract. The results also confirmed the presence of bioactive flavonoids constituents which may be responsible for the pharmacological activity of the extract investigated.

TLC: Yellow fluorescent spot under UV light at 366 nm and calculated Retention factor (Rf) was found to be 0.67.

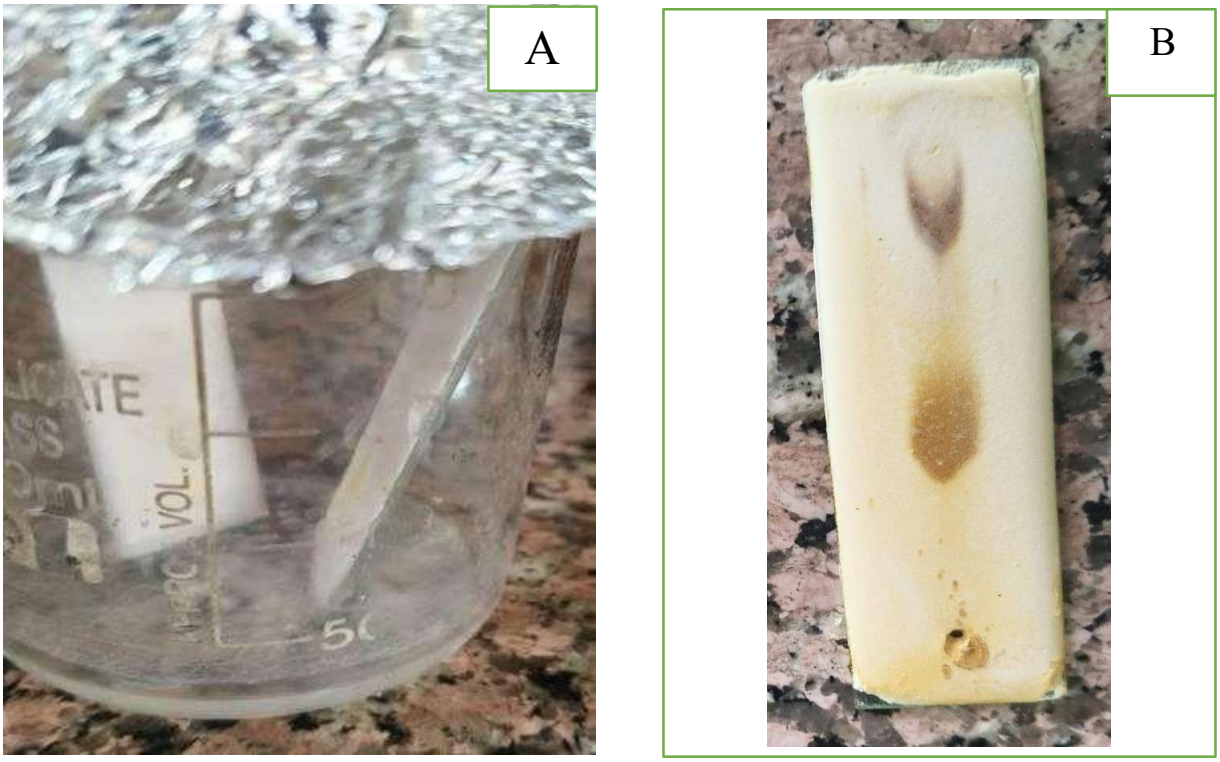


Figure 12: Identification and Confirmation of Rutin by TLC.

A indicates TLC plates dipped in the Ethyl acetate: Formic acid: Acetic acid: Water (10:1:1:1)
B indicates yellow fluorescent spot under UV light at 366 nm and calculated Rf value 0.6.

Biological Evaluation

Animals Section

Experimental Animals

For this investigation, we utilized adult albino Wistar rats in good health. These animals were purchased from

a licensed vendor (Biotenic Pharma Private Limited) who offers research animals for sale. They were maintained under standard conditions in the laboratory.

Details of Animals

Table 3: Details of Animals.

Animal	Albino Wistar Rats
Sex	Male
Age	Adult rat (8-10 weeks)
Body weight	180-250 g
Number of Animals	35 Rats

Grouping of Animals

Five groupings were formed from the animals. There were seven rats in each group. Each group had seven rats, for a total of seven rats per group.

Table 4: Grouping of Animals.

Group I	Control Group (Normal Saline)
Group II	Negative (Serotonin) Group
Group III	Standard (Esomeprazole + Serotonin) Group
Group IV	Test (Rutin 10 mg/kg) Group
Group V	Test (Rutin 20 mg/kg) Group

Animal Housing and Husbandry

Table 5: Animal Housing and Husbandry.

Room Temperature	24±2 degree Celsius
Relative Humidity	45-64 %
Light/ dark Cycle	12 Hourly
Feed	The animals will be provided with a commercially available standard laboratory animal feed ad libitum, allowing unrestricted access to food throughout the experimental period.
Water	Filtered drinking water will be provided and libitum.

Experimental Studies

To figure out how stomach ulcers happen and to find treatments we need to do a lot of research on medicines that can help with ulcers. Scientists have come up with ways to study ulcers in a way that is similar to what happens in people. One way they do this is by using rats and giving them something called serotonin creatinine sulphate to cause ulcers. This method is useful because it shows what happens when there is inflammation, stress and damage, to the lining of the stomach. These methods are used to see if the medicines that are supposed to help with ulcers really work on stomach ulcers and antiulcer medicines [154,155].

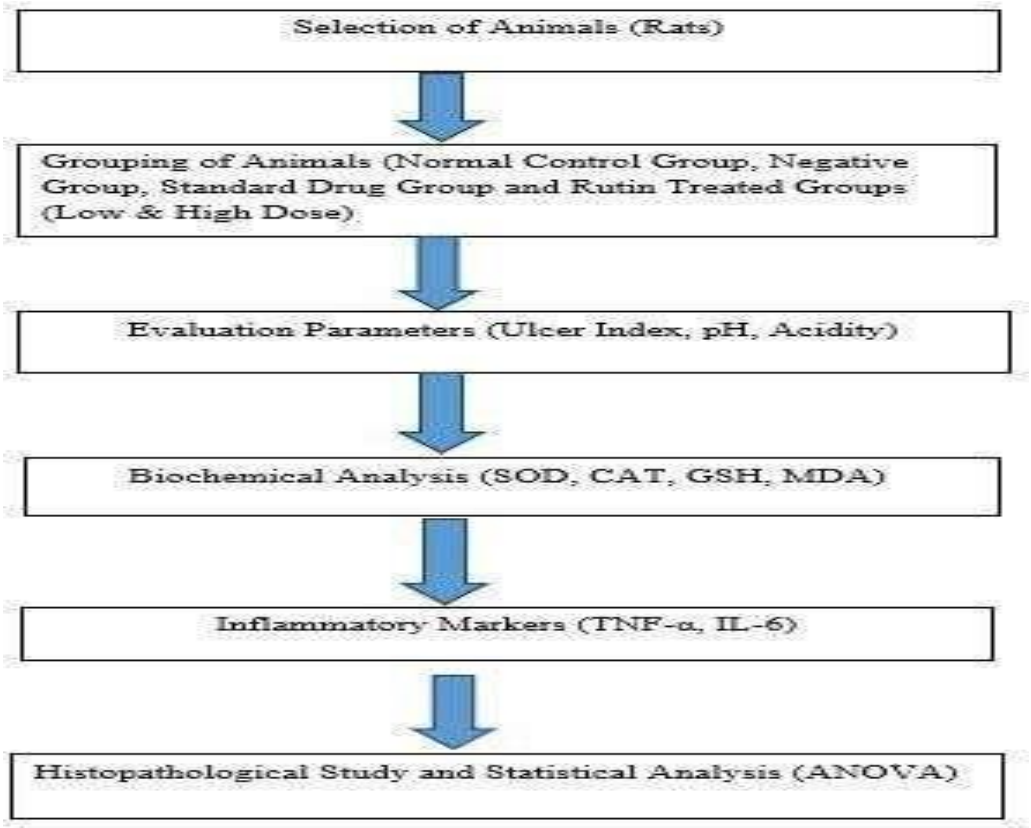


Figure 13: Experimental Study Design.

Rats with stomach ulcers caused by serotonin creatinine sulphate are a model because they are similar to human ulcers. Scientists can use this model to learn more about ulcers and develop treatments for ulcers. The medications that help with ulcers are very important because they can help people who suffer from ulcers. Serotonin, also known as 5-hydroxytryptamine has been linked to stomach problems because it stimulates pathways in the body. When these pathways are stimulated they increase the production of things that can harm the body called reactive oxygen species and they reduce blood flow to the stomach lining. This causes damage to the stomach tissues. In experiments animals are usually divided into groups, including those with ulcers, healthy animals and animals that receive treatment. Scientists use serotonin creatinine sulphate to cause ulcers in the animals. Then they give them potential new treatments, such as the flavonoid Rutin, to see if they can help protect the stomach [156-157]. To see if new treatments work scientists use tests to look at the stomach tissues and check for damage. They also use tests to measure the level of damage in the stomach including how acidic it's how big the ulcers are. At the time they check for signs of oxidative stress, such as the level of malondialdehyde and the activity of important enzymes like glutathione, catalase and superoxide dismutase. All these things help scientists understand if a new treatment is working and if it can affect the pathways that cause stomach ulcers those that involve serotonin and the ulcers that it causes. Stomach ulcers are a problem and serotonin creatinine sulphate-induced stomach ulcers in rats are a good way to study them and find new treatments, for ulcers. Rutin is a kind of food thing that is good for us. It helps keep our stomach and body healthy. Rutin does this by working in different ways. Lots of studies have shown that Rutin can help stop things from happening in our body. It does this by making our body's own defenses stronger and getting rid of stuff. Rutin is also very good at reducing swelling and pain. It can stop some of the things that make us sick like tumor necrosis factor alpha and interleukin 6. Rutin also stops a pathway in our body that can make us sick. This helps reduce the things that happen when we get hurt. All of these things that Rutin does show that it could be a helpful medicine. Rutin helps our stomach heal when it is hurt. It also helps keep the inside of our stomach healthy. Rutin does this by reducing the stuff that can hurt our stomach and by helping our stomach heal faster. When we look at the stomach tissue under a microscope we can see that Rutin helps make the stomach healthier. The ulcers are not as bad. The stomach looks better. To make sure these results are real

we use math techniques like analysis of variance. All of the data shows that Rutin is very good at keeping our stomach healthy. It can stop stomach damage caused by serotonin creatinine sulphate. Rutin does this by reducing the things that make us sick and by making our body's defenses stronger. Rutin is a promising medicine that can help keep our stomach healthy. The data on Rutin shows that it is very good at protecting our stomach. This is why Rutin is so important, for our health [158-161].

Experimental Procedure [161]

Table 6: Experimental Design and Animal Grouping.

Animal	Albino Wistar Rats
Sex	Male
Age	Adult rat (8-10 weeks)
Body weight	180-250 g
Number of Animals	35 Rats
Number of animals	07 per groups
Number of groups	05

For Serotonin- Induced gastric Ulcers [162-165]

Table 7: Experimental Procedure.

Groups	Number of animals	Treatment	Route of Administration	Duration	References
Control Group	7	Normal Saline	Oral	14 days	[162]
Negative Group	7	Serotonin creatinine sulphate (20mg/Kg)	Subcutaneous	Animals received a 0.9% NaCl solution (5 ml/kg) orally for 14 days. On the final day of the Group study, the animals were given Serotonin creatinine sulphate (20 mg/kg) s.c. following a 24-h fast	[163]
Standard Group	7	Esomeprazole (20 mg/kg) + serotonin creatinine sulphate (20 mg/kg)	Subcutaneous (s.c)	Animals were given Esomeprazole (20 mg/kg) orally once day for 14 days. Following a 24-h fast, the animals were administered serotonin creatinine sulphate (20 mg/kg) s.c on the final day	[164]
Groups IV and V (Test Groups)	7	Rutin (10mg/kg) and (20 mg/kg)	Oral	Animals were given Rutin (10mg/kg) and (20 mg/kg) orally daily for 14 days. On the final day, serotonin creatinine sulphate (20mg/kg) was administered subcutaneous to starving animals for 24 hours.	[165]

Procedure

Each of the animals was divided into five groups of seven rats. Rutin and Serotonin creatinine sulphate were dissolved in a 0.9% sodium chloride (NaCl) solution. Group I (Control): The animals received 5 ml/kg of 0.9% NaCl orally for a period of 14 days. For 14 days, animals in Group II (Negative Group) were given an oral 0.9% NaCl solution (5 ml/kg). The mice were administered Serotonin creatinine sulphate (20 mg/kg) subcutaneously on the last day of the research after a 24-hour fast. Esomeprazole (20 mg/kg) was administered orally to animals in Group III (Standard Group) once daily for a period of 14 days. On the last day, the animals were given 20 mg/kg of Serotonin creatinine sulphate subcutaneously after a 24-hour fast. Rutin (10 mg/kg) and (20 mg/kg) were administered orally to the animals in Groups IV and V (Test Groups) for a duration of 14 days. A subcutaneous injection of Serotonin creatinine sulphate (20 mg/kg) was given on the last day to animals that are starved for a full day. The PGE 2 level, stomach acidity and ulcer index were assessed. Histological analysis, oxidative stress levels (MDA, GSH, CAT and SOD) and inflammatory markers (IL-1β, IL-6 and TNF-α) were also evaluated [162-165].

Result

Macroscopic Features of Rutin

Rutin is a naturally occurring flavonoid glycoside that is typically obtained as a crystalline powder that ranges in colour from yellow to greenish-yellow. As is characteristic of polyphenolic substances, it has a mildly bitter taste and is usually odourless. The substance typically takes the form of small, needle-shaped crystals or an amorphous powder that has a smooth, dry and fluid feel. These physical traits are crucial for Rutin's initial identification and standardisation in Pharmacognostical studies. Rutin is more soluble in organic solvents like ethanol and methanol than it is, in water. The way Rutin behaves and how stable it is depends on the moiety and all the hydroxyl groups it has. Generally Rutin is stable when you store it like you normally would. If Rutin is exposed to heat, moisture and direct light for a long time it can break down and become less stable. Rutin can degrade if it is not stored properly so it is important to keep Rutin from things that can cause it to degrade. The macroscopic and physicochemical characteristics of Rutin-containing herbal and pharmaceutical preparations are frequently used in quality control investigations. Research has shown that Rutin's distinctive yellow color and crystalline structure are related to its conjugated aromatic system and flavone structure. These characteristics are frequently used in natural product research processes such as phytochemical screening and analytical standardization.

Table 8: Macroscopic Characteristics of Rutin.

Sr. No.	Parameters	Macroscopic Characteristics of Rutin
1.	Appearance	Fine crystalline powder
2.	Colour	Yellow to greenish-yellow
3.	Odour	Odourless or faint characteristic odour
4.	Texture	Smooth and free-flowing powder
5.	Nature	Non-hygroscopic crystalline compound
6.	Shape of Crystals	Needle-Shaped crystals
7.	Solubility Character	Slightly soluble in water; soluble in ethanol and methanol
8.	Surface Characteristics	Dry and uniform appearance
9.	Stability	Stable under normal storage conditions but sensitive to prolonged light and moisture exposure

Extraction yield of plant

The amount of product obtained after the reaction or extraction including purification process is called as the yield value. It is often expressed as a percentage yield, that is, the percentage of the actual amount of product obtained compared to the original amount of the starting material, to indicate how efficient the process.

Rutin % Yield: Weight of pure rutin obtained/ Weight of dried plant material *100 = 600/5000*100 = 12 %.

Table 9: Extraction yield of plant.

Extract	Extraction yield (%)	Appearance
Ethanolic Extract of *moringa oleifera*	12%	Yellow to greenish-yellow

Isolation of Rutin by Column Chromatography

Open-column chromatography helped separate Rutin from the flavonoid-enriched fraction. It used silica gel, which had a mesh size of 60 to 120 as the phase. The process involved using chloroform and methanol solvents. These solvents had increasing levels of polarity. This helped get fractions, with different phytoconstituents. The fractions collected during this process were then tested using thin-layer chromatography. This was done to monitor and identify which fractions contained rutin. Rutin was successfully separated using this method. The fractions were checked to see if they had Rutin. The right parts were collected and then they were analysed using a mix of ethyl acetate, formic acid, acetic acid and water. This mix was made up of these things in a ratio: 100 parts of ethyl acetate 11 parts of formic acid 11 parts of acetic acid and 26 parts of water. When this mix was used to analyse the flavonoid compounds, it worked well. When the compounds were looked at under a light one that shines at 254 nm and another one that shines at 366 nm a yellow band that glowed was seen and it was rutin. The compound that was found had the characteristics as standard rutin, which meant that rutin was really, in the collected parts. Fractions that showed TLC profiles and the same Rf values were combined and concentrated using a rotary vacuum evaporator. The concentrated fraction looked like a yellowish -purified flavonoid residue. This indicated that rutin was successfully enriched from the plant extract. The chromatographic separation showed that rutin was isolated efficiently. This makes it suitable for investigations on its effects, on health and detailed analysis. The whole chromatographic process yielded 12% rutin. This suggests that a satisfactory amount of the flavonoid was recovered from the selected plant material.

Identification and Confirmation Tests for Rutin by HPTLC

To investigate the presence of rutin in the plant extract prepared, High-Performance Thin Layer Chromatography (HPTLC) was performed. The stationary phase used was precoated silica gel 60 F254 aluminum plates. The optimized mobile phase was made up of ethyl acetate: formic acid: acetic acid: water (100:11:11:26, v/v/v/v) which gave satisfactory separation of phytoconstituents. The chamber was pre-saturated with the mobile phase for about 20 min before the chromatographic development to ensure uniform movement of the solvents and reproducible chromatographic resolution.

Table 10: Comparative HPTLC Report of Standard Rutin and Plant Extract.

Sample	Rf Value	Observation	Interpretation
Standard rutin (T2 STD)	0.68	Sharp fluorescent band observed under UV light	Characteristic chromatographic profile of rutin confirmed
Plant extract (T6 EXTRACT)	0.67	Corresponding fluorescent band observed	Presence of rutin in the extract confirmed

The chromatogram of standard rutin showed a sharp and well-resolved fluorescent band at an Rf value of 0.68 under UV illumination, confirming the chromatographic identity of rutin.

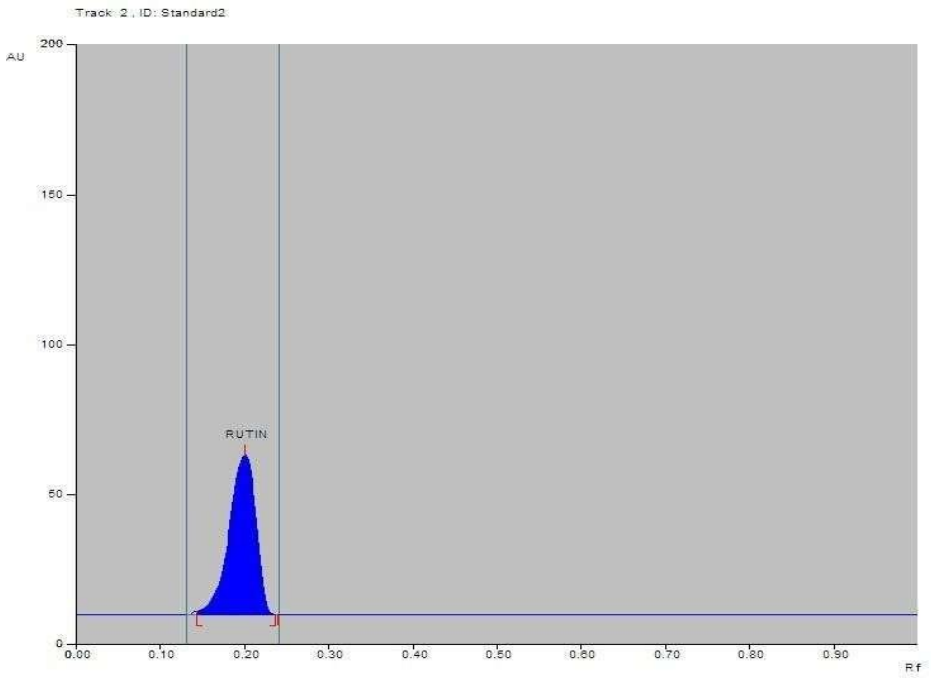


Figure 14: HPTLC Chromatogram of Standard Rutin (T2 STD).

The chromatographic profile of the plant extract revealed a fluorescent band at an Rf value of 0.67 corresponding closely to the standard rutin, thereby confirming the occurrence of rutin in the extract.

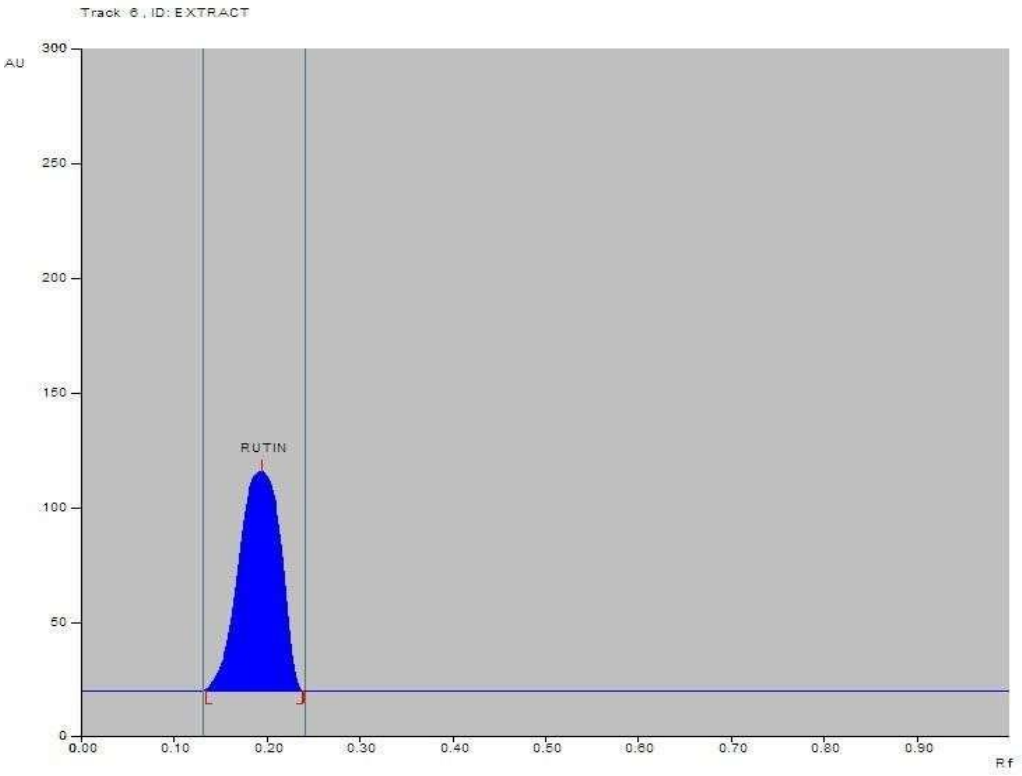


Figure 15: HPTLC Chromatogram of extract Rutin (T6 EXTRACT).

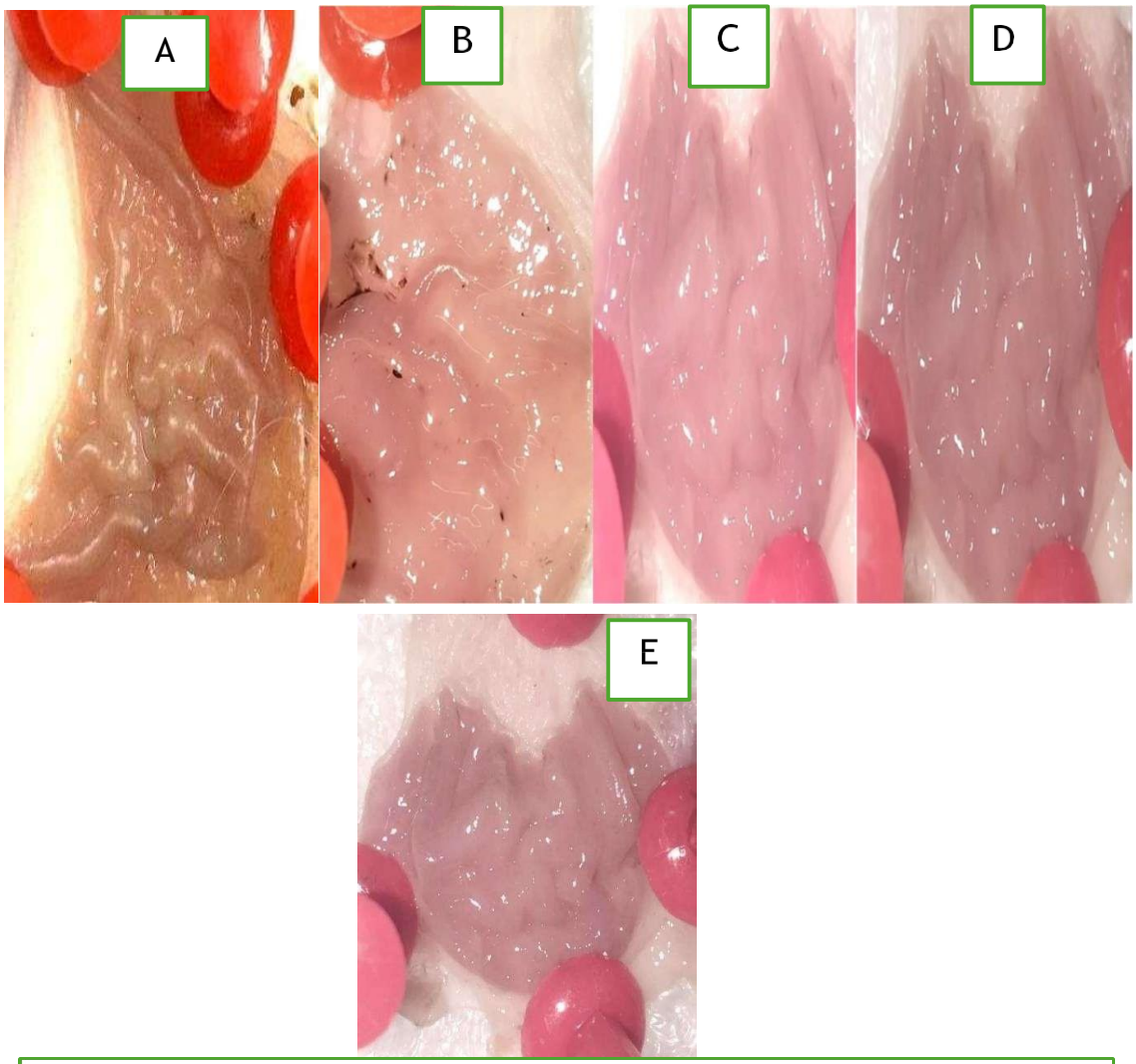
The Rf value for the fluorescent band of standard rutin was 0.68, which showed suitable chromatographic behavior of standard and the suitability of the mobile phase system used. When compared, the plant extract showed a band of fluorescence with an Rf value of 0.67 which was very close to that of standard rutin. Based on the similarity of the chromatographic migration, it was assumed that rutin was one of the important flavonoids present in the extract under study.

Gross Morphological Evaluation of Gastric Mucosa

The excised stomach from different experimental groups were examined for visible ulcerative lesions and hemorrhagic changes:

Table 11: Gross Morphological Evaluation of Gastric Mucosa

Experimental Group	Treatment	Gross Morphological Observations	Interpretation
Control Group (shown in fig. A)	Normal saline	The gastric mucosa showed no erosions, haemorrhagic streaks, or ulcerative lesions; it was smooth, glossy and intact. The thickness and colouration of the stomach wall were found to be normal.	It shows that there is no mucosal damage and that the stomach architecture is normal.
Negative Group (shown in fig. B)	Serotonin creatinine Sulphate (20 mg/kg)	Significant haemorrhagic streaks, many ulcerative lesions, mucosal congestion, patchy necrotic regions and loss of surface integrity were all signs of severe stomach mucosal damage.	It confirms that serotonin has successfully caused stomach ulcers.
Standard Group (shown in fig. C)	Esomeprazole + Serotonin creatinine Sulphate (20mg/Kg)	There were relatively few superficial erosions and minor erythematous spots on the gastric mucosa. With less congestion and maintained mucosal smoothness, the stomach surface stayed mostly unaltered.	It shows that esomeprazole has a substantial gastroprotective effect.
Test Group IV (shown in fig. D)	Rutin (10 mg/kg) + Serotonin creatinine Sulphate (20mg/Kg)	When compared to the negative group, there were fewer haemorrhagic streaks and less mucosal congestion. Mucosal integrity was partially preserved whereas small, localised erosions continued to occur.	It shows that rutin at 10 mg/kg has a modest gastroprotective effect.
Test Group V (shown in fig. E)	Rutin (20 mg/kg) + serotonin creatinine sulphate	There were few superficial lesions and the gastric mucosa looked almost normal. The mucosal surface was smooth; glossy and undamaged and haemorrhagic streaks were much diminished.	Rutin has a potent dose-dependent gastroprotective effect that is equivalent to esomeprazole.



A-Control Group B- Negative Group C- Standard Group D- Test Group IV
E- Test Group V

Quantitative estimation of gastric ulcer index, gastric mucosal myeloperoxidase activity, total nitrite/ nitrate contents and gastric juice acidity.

Serotonin creatinine sulphate-induced haemorrhagic and ulcerative lesions were measured using the stomach ulcer index, which was determined by adding the lengths of each lesion in millimetres (fig 16A). In the stomach mucosa of the group receiving serotonin, the ulcer index (mm) was 63 ± 0.54. Rutin pretreatment significantly decreased the ulcer index to 3.0 ± 0.5, resulting in about 83% fewer gastric lesions in rat stomachs caused by serotonin creatinine sulphate (fig. 16A). Rats given rutin orally by itself did not exhibit haemorrhagic or ulcerative lesions, much as the vehicle control group (fig. 16A).

When compared to the control group, rats given oral serotonin creatinine sulphate exhibited a four-fold increase in myeloperoxidase activity (measured as a percentage of control), yielding a relative value of 403 ± 0.32, suggesting an increase in neutrophil infiltration into mucosal tissues. When rutin was administered one hour prior to serotonin creatinine sulphate, the increased myeloperoxidase activity decreased to 170 ± 0.16 (fig. 16B). Furthermore, following the administration of serotonin creatinine sulphate, total nitrite/nitrate levels ($nmol^2g$ tissue) decreased by almost 40%, resulting in a value of 210 ± 0.22 compared with the control (350 ± 0.12). Rutin pretreatment considerably increased the gastric mucosa's lowered total nitrite/nitrate levels to 310 ± 0.13 (fig. 16C). The total nitrite nitrate levels were raised by rutin alone from 350 ± 0.12 to 340 ± 0.10, although this increase was not significant when

compared to the control (fig. 16C). Thus, nitric oxide synthesis might be indicated by the word's nitrite × nitrate. Following serotonin creatinine sulphate administration, gastric mucosal acidity (mEq¹l) increased to 85 ± 0.7 from the control value of 45 ± 0.4. However, there was no discernible decrease in stomach acidity when rats were pre-treated with rutin (fig. 16D). Myeloperoxidase activity and stomach acidity were not significantly affected by oral rutin treatment alone (fig. 16B and 16D).

Evaluation of oxidative stress biomarkers (glutathione, Thio barbituric acid reactive substance, superoxide dismutase) in gastric mucosal tissues:

In this investigation, glutathione levels (nmol/g tissue) dropped from 0.47 ± 0.03 to 0.25 ± 0.02 due to serotonin creatinine sulphate. Rutin stopped the decrease in glutathione content caused by serotonin creatinine sulphate and brought it back to normal at 0.46 ± 0.02 (fig. 17A). Additionally, serotonin creatinine sulphate increased the amounts of lipid peroxide (Thio barbituric acid reactive material) in stomach tissues from 3.2 ± 0.2 to 6 ± 0.3 (nmol ¹g tissue). The increased levels of Thio barbituric acid reactive material were normalised by rutin to 4.2 ± 0.3 (fig. 17B). Rats treated with serotonin creatinine sulphate showed a reduction in superoxide dismutase activity (U mg protein) from 55 ± 0.2 to 30 ± 0.2 in their stomach tissues. However, following rutin administration, the decreased superoxide dismutase activity was raised to 35 ± 0.2 (fig. 17C). Glutathione, Thio barbituric acid reactive material levels and superoxide dismutase activity were not significantly affected by rutin administration (fig. 17A, 17B and 17C).

Effect of Rutin on Inflammatory Markers

Serotonin creatinine sulphate (SERO)-induced gastric ulcers are associated with a significant increase in pro-inflammatory cytokines, indicating enhanced inflammatory response in gastric mucosal tissues. In the ulcer control group, TNF-α levels were significantly elevated ($p < 0.001$) by approximately 2.14-fold compared to normal control rats, demonstrating severe inflammatory damage. Treatment with Rutin (10 mg/kg and 20 mg/kg) resulted in a significant reduction in TNF-α levels by 1.44-fold and 1.94-fold, respectively, when compared to the SERO-treated group. Similarly, the standard drug (ESO) and RUTIN at 20 mg/kg showed almost comparable effects, reducing TNF-α levels by approximately 2.00-fold and 1.94-fold, respectively. IL-1β levels were also significantly increased ($p < 0.001$) in the SERO group by 2.62-fold compared to control rats. However, treatment with ESO and RUTIN (10 mg/kg and 20 mg/kg) significantly suppressed IL-1β levels by 2.73-fold, 1.54-fold and 2.60-fold, respectively, in comparison to the SERO group. Furthermore, IL-6 levels were markedly elevated ($p < 0.001$) in the SERO group by 3.22-fold relative to the control group. Administration of ESO and RUTIN significantly reduced IL-6 levels to 2.73-fold, 1.54-fold and 2.60-fold, respectively, compared to the SERO group. Overall, the results indicate that RUTIN exhibits strong anti-inflammatory activity by significantly reducing pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) in serotonin-induced gastric ulcer models.

Discussion

Non-steroidal anti-inflammatory medications are effective in treating pain, fever and inflammation, they are often recommended in clinical settings. NSAID usage, however, is linked to serious gastrointestinal side effects include bleeding, perforation, ulceration and erosion of the stomach mucosa. In comparison to the control group, the administration of serotonin creatinine sulphate resulted in significant mucosal injury both morphologically and histologically. Depletion of gastric mucosal blood flow and suppression of prostaglandin production have been identified as the pathophysiology of serotonin creatinine sulphate-induced gastropathy. Leucocytes and neutrophils invade stomach tissue when mucosal blood flow is reduced. Activated leucocytes often release the myeloperoxidase enzyme. Inflammatory disorders are frequently linked to its overexpression. As a result, it is regarded as a trustworthy indicator of leucocyte and neutrophil infiltration. Myeloperoxidase activity has been shown to increase following serotonin creatinine sulphate injection in a number of investigations, suggesting that neutrophil infiltration plays a role in serotonin creatinine sulphate-induced gastropathy. According to a recent research, neutrophil infiltration and disruption of nitric oxide generation are necessary for serotonin creatinine sulphate-induced gastropathy. This might account for the rise in myeloperoxidase activity and neutrophil infiltration in the stomach tissue of rats given serotonin creatinine sulphate. Ironically, nitric oxide adds to mucosal injury even though it mediates gastrointestinal mucosal defence. The enzyme NOS, which is found in gastric mucosa in two constitutive (cNOS) isoforms—endothelial (eNOS) and neuronal (nNOS)—mediates the generation of nitric oxide. Neutrophils and macrophages both contain the inducible (iNOS) enzyme. Nitric oxide generated by iNOS is cytotoxic in the digestive system, whereas nitric oxide generated by cNOS is cytoprotective. By enhancing the mucosal blood flow in the GIT system, nitric oxide at low concentrations (from cNOS)

contributes to the preservation of epithelial tissue integrity. Nitric oxide has a protective impact because it inhibits leucocyte activation, adhesion and migration in the inflammatory region. It has been found that serotonin creatinine sulphate increases iNOS-derived nitric oxide and decreases tissue cNOS-derived nitric oxide. iNOS-generated nitric oxide is implicated in stomach injury caused by serotonin creatinine sulphate using either pharmacological iNOS inhibitors or genetically inducible nitric oxide synthase (iNOS) defective animals. Peroxy-nitrite, a strong cytotoxic oxidant that damages the stomach, is created when nitric oxide, which is generated when iNOS is activated, combines directly with superoxide. Concurrently, serotonin up-regulates endothelin-1, which reduces gastric mucosal cNOS synthesis. This suppresses the amount of cytoprotective nitric oxide in the stomach mucosa, which increases neutrophil infiltration. This could account for the drop in nitrite-nitrate levels (a measure of nitric oxide production) in the gastric mucosa of rats treated with serotonin creatinine sulphate when compared to the control group. This could be because gastric tissue produces less cNOS, while macrophages and neutrophils produce more iNOS (nitric oxide used to form peroxy-nitrite). Our findings are in line with several studies that show a substantial drop in stomach mucosal nitrite/nitrate levels is linked to serotonin creatinine sulphate-induced gastropathy. The inhibitory effects of serotonin creatinine sulphate on prostaglandin synthesis may be the cause of this increase in stomach acid output. Numerous researchers have proposed a connection between the production of free radicals and leucocyte infiltration triggered by serotonin creatinine sulphate. The data showed a decrease in glutathione levels in the stomach tissues of rats treated with serotonin, further corroborated our findings. Gastric damage is made worse by the free radicals created by neutrophil infiltration, which cause lipid peroxidation and alter the lipid content of membranes. Increased lipid peroxidation products in the stomach tissues of rats given serotonin provide credence to this theory. In line with this discovery, we discovered that the serotonin creatinine sulphate-treated group had higher lipid peroxide levels, which were linked to stomach mucosal necrosis and muscularis hypertrophy. Superoxide dismutase is an enzyme that catalyses the dismutation of superoxide to H_2O_2, hence scavenging superoxide radicals. It has previously been documented that serotonin therapy reduces superoxide dismutase activity implying that one of the reasons for serotonin-induced gastropathy is oxidative damage. Among the flavonoids with antioxidant and free radical scavenging properties is rutin. Furthermore, it was observed that rutin prevents neutrophil infiltration, as evidenced by a decrease in human myeloperoxidase activity in both activated human neutrophils and isolated enzyme. Rutin has the capacity to prevent neutrophil infiltration in stomach tissue, as demonstrated by the considerable reduction of increased myeloperoxidase activity in serotonin-treated rats. One of the reasons of neutrophil infiltration is the reduction of mucosal blood flow following serotonin treatment. According to reports, rutin increases cNOS activity, which raises nitric oxide levels in stomach mucosal tissues.

Table 12: Effect of Serotonin, Esomeprazole, and Rutin on Gastric Parameters in Rats

Groups	Gastric Ulcer Index (mm)	Gastric Myeloperoxidase Activity (% of Control)	Total Nitrite/Nitrate Content (nmol/g tissue)	Gastric Juice Acidity (mEq/l)
Normal Control	0.5 ± 0.2	100 ± 0.5	350 ± 0.12	45 ± 0.4
Sero	63 ± 0.54***	403 ± 0.32***	200 ± 0.8***	85 ± 0.7***
Sero + Eso (20 mg/kg)	10 ± 0.9**	170 ± 0.16**	250 ± 0.10**	75 ± 0.3**
Sero + Rutin (10 mg/kg)	3.0 ± 0.5**	210 ± 0.22**	310 ± 0.13**	70 ± 0.2**
Sero + Rutin (20 mg/kg)	1.5 ± 0.3**	200 ±0. 7**	340 ± 0.10***	60 ± 0.3**

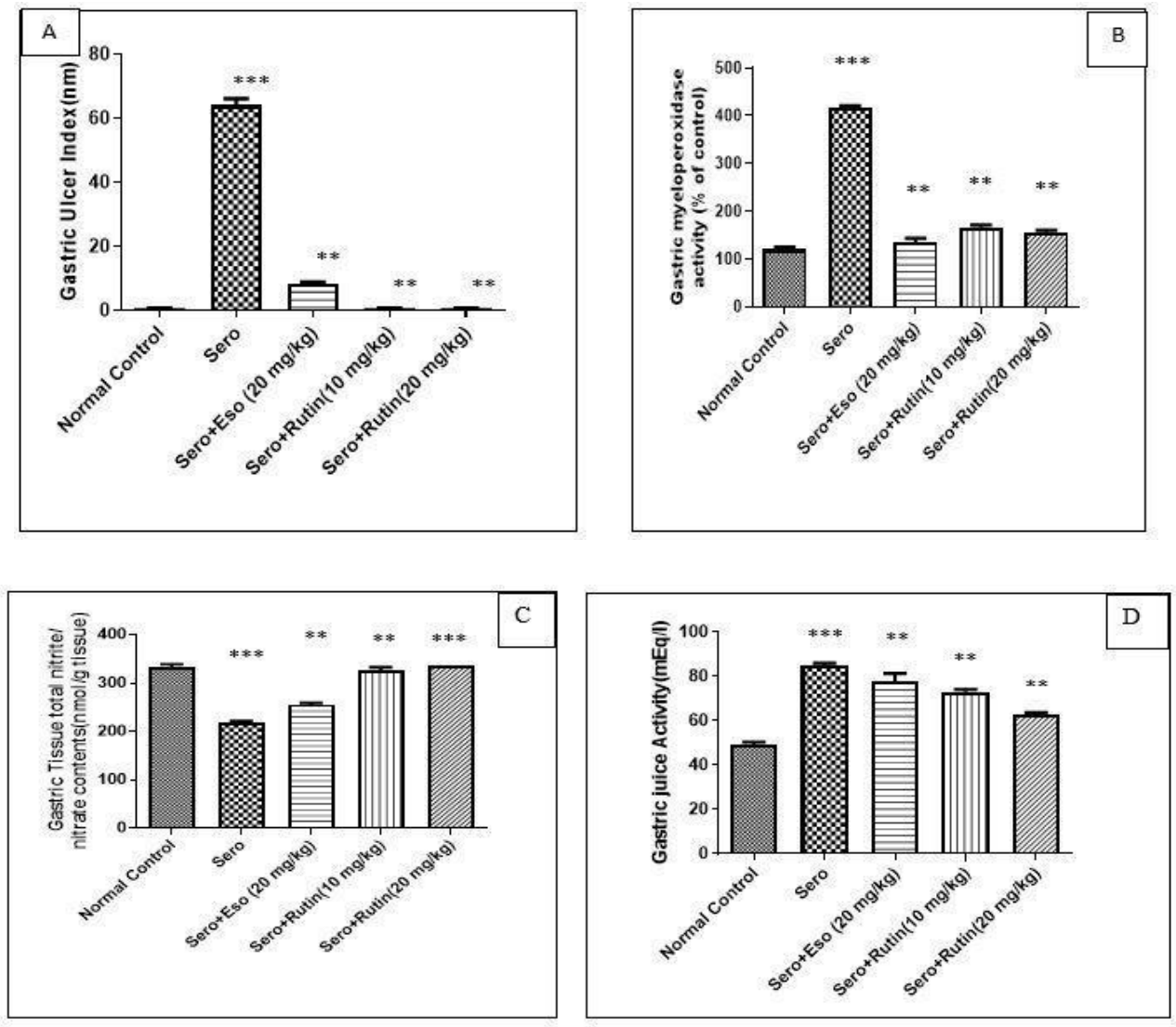


Figure 16: Serotonin creatinine sulphate (Sero), Sero+ Eso (serotonin creatinine sulphate + Esomeprazole), Sero + Rutin (serotonin creatinine sulphate + Rutin) 10 mg/ kg and their combination (RU+SERO) 20 mg/kg on gastric ulcer index (A), gastric mucosal myeloperoxidase activity (B), total nitrite/nitrate contents (C) and gastric juice acidity (D) compared with control (CO) group.

The data were expressed as mean ± SD. The statistical significance of differences between the Rutin treated groups, control groups and SERO group, was determined by tested by using one way ANOVA followed by Dunnett's test.
*Significant means ($p < 0.05$) ** Significant means ($p < 0.01$) *** Significant means ($p < 0.001$)

Table 13: Effect of Serotonin, Esomeprazole, and Rutin on Gastric Antioxidant Parameters in Rats.

Groups	GSH (µg/mg protein)	TBARS (nmol/g tissue)	SOD (U/mg protein)
Normal Control	0.47 ± 0.03	3.2 ± 0.2	55 ± 0.2
Sero	0.25 ± 0.02*	6.0 ± 0.3*	30 ± 0.2*
Sero + Eso (20 mg/kg)	0.45 ± 0.02**	3.0 ± 0.2**	40 ± 0.2**
Sero + Rutin (10 mg/kg)	0.46 ± 0.02**	4.2 ± 0.3***	35 ± 0.2**

Sero + Rutin (20 mg/kg)	0.55 ± 0.03**	2.8 ± 0.2**	45 ± 0.2**

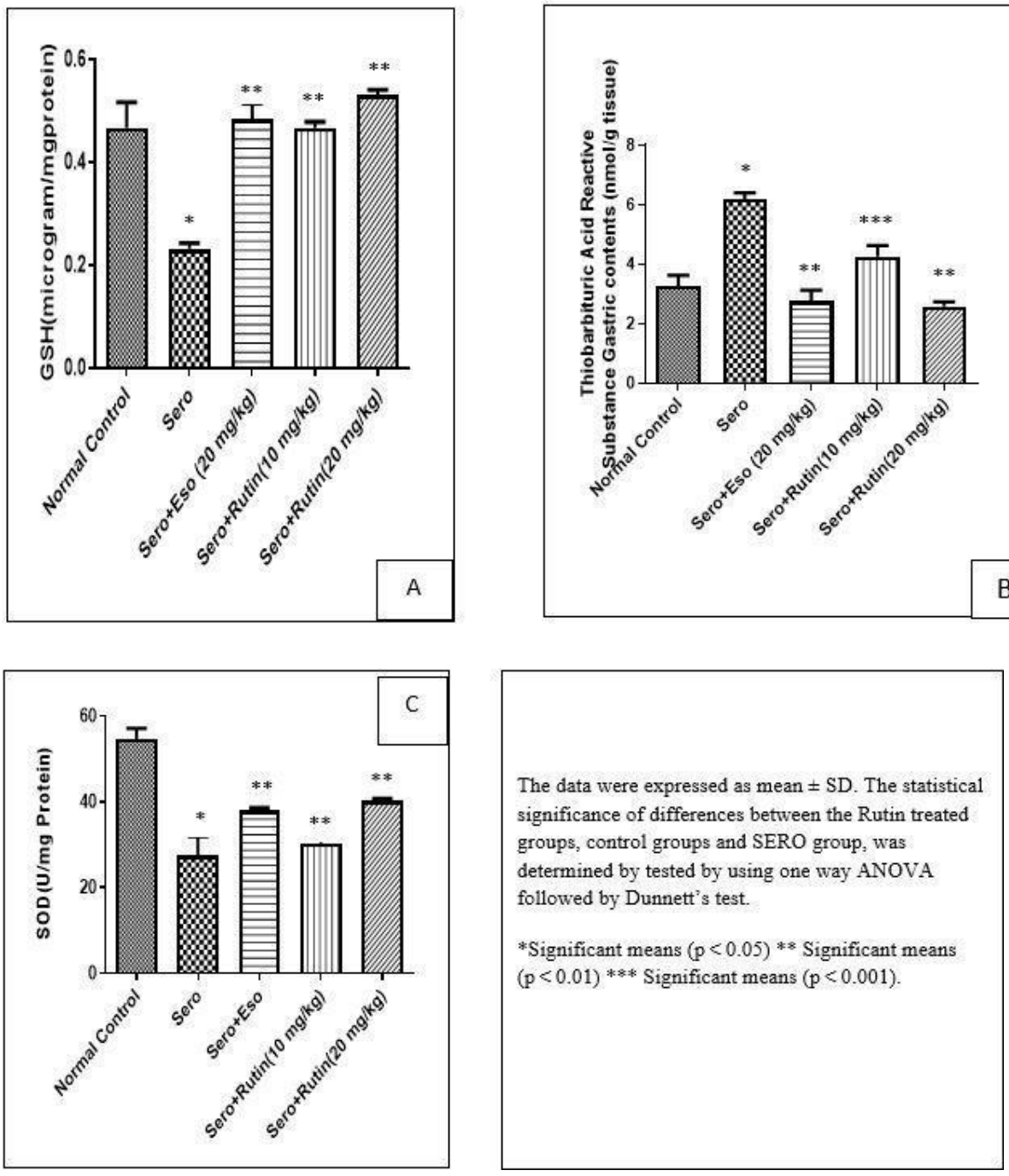


Figure 17: Effects of serotonin creatinine sulphate (Sero), serotonin creatinine sulphate + Esomeprazole (Sero+ Eso), Sero+ Rutin (10 mg/kg), Sero+ Rutin (20 mg/kg) and their combination (RU+SERO) on reduced glutathione contents (A), Thio barbituric acid reactive substance contents (B) and superoxide dismutase activity (C) in gastric tissues compared with control (CO) group.

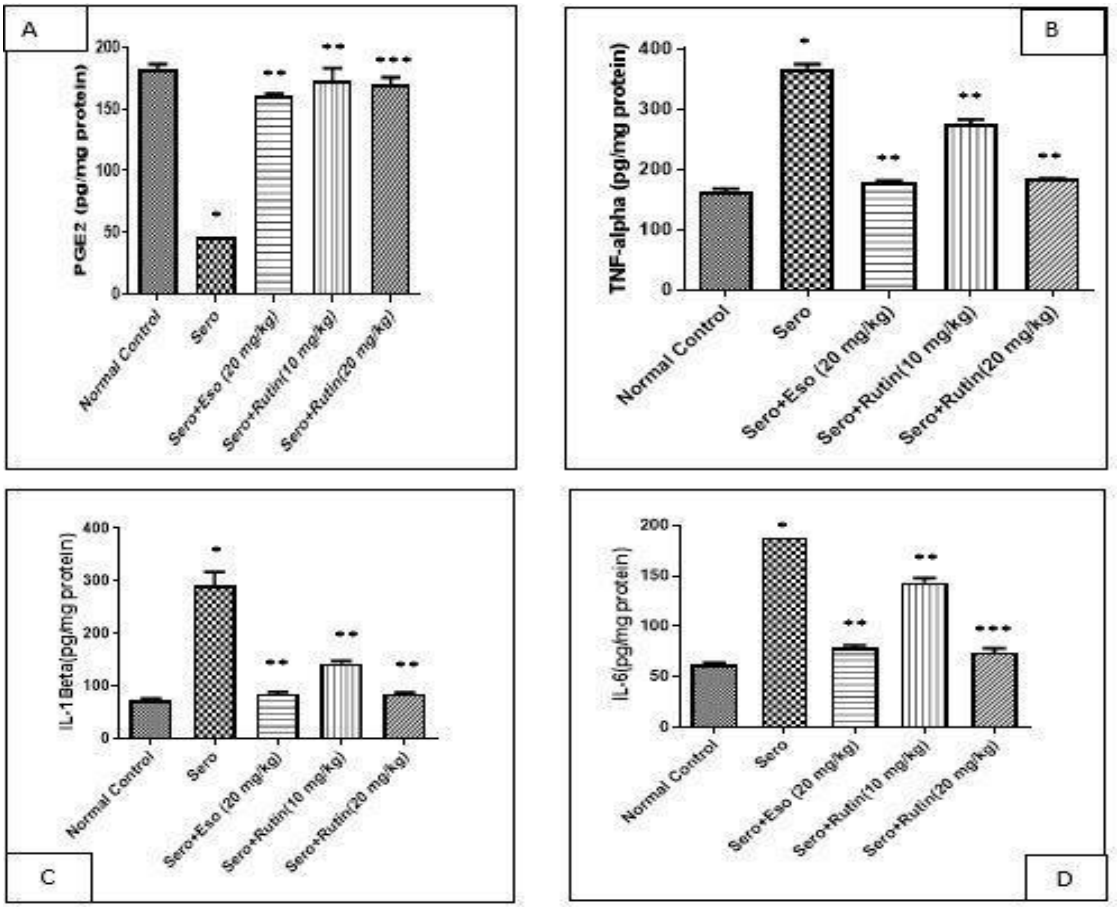
Nitric oxide produced by cNOS enhances tissue perfusion and mucosal blood flow, which reduces neutrophil infiltration. Because rutin promotes cNOS in gastric tissue, the rise of gastric mucosal nitrite-nitrate levels in serotonin-treated rats in this research may be explained. Rutin is a really gastroprotective drug

because it had no effect on either the high stomach acidity or the basal normal acid level. Due to their strong anti-secretory properties, proton pump inhibitors like lansoprazole and omeprazole are also useful in treating NSAID-induced gastrointestinal damage. Independent of their anti-secretory effects, proton pump inhibitors also have a gastroprotective effect that is likely mediated by antioxidant activities. Proton pump inhibitors, however, have been associated with a number of studies with increased risks of stomach acid inhibitory-related illnesses, including hip fractures, community-acquired pneumonia, enteric infections (Clostridium difficile) and nutritional deficiencies. Unlike proton pump inhibitors, which cause a decrease in stomach acidity, rutin may reduce serotonin-induced ulcers without having the negative effects of these drugs. The oxidative stress indicators that were negatively impacted by serotonin administration were considerably improved by rutin's antioxidant activity. By scavenging hydroxyl radicals, glutathione plays a crucial function in preventing damage from oxygen-free radicals. Rutin replenished the lower glutathione levels in the stomach mucosa that serotonin had lowered. Additionally, serotonin decreased the activity of superoxide dismutase in stomach tissues, whereas rutin pretreatment brought the activity back to normal. Rutin and other polyphenolic compounds have been demonstrated to have direct antioxidant actions by scavenging free radicals and quenching singlet oxygen. Consequently, rutin can either directly or indirectly counteract serotonin-induced gastropathy. Directly, it can do this by collecting superoxide anions, which will then stop it from interacting with produced nitric oxide to create peroxy-nitrite, maintaining the beneficial effects of nitric oxide. indirectly by raising nitric oxide generated by cNOS, which enhances tissue perfusion and reduces neutrophil infiltration and the production of free radicals. Additionally, rutin is a potent peroxy-nitrite radical capturer. At a single daily dose of 2 g, it also protects the distal venous system from deteriorating and lessens venous ulcers in patients with diabetic micro-angiopathy with high compliance and no side effects or intolerance. Rutin should be translated from animal doses to human doses for therapeutic application in humans. The body surface area normalisation approach is recommended for more accurate medication dosage translation from animal research to human studies. This approach shows that 20 mg ×kg of rutin used in rats is similar to 32.4 mg ×kg in adults (about 1.9 g per 60 kg). The dose of rutin employed in this investigation (20 mg/kg) might be used safely and successfully in people (32.4 mg/kg) for the prevention and treatment of stomach damage caused by serotonin. This issue needs to be evaluated by more human clinical research.

The results from the tests showed that the bad things that cause inflammation were really high and the prostaglandin E2 levels were low. This was a change from the normal group where the levels were around 180 pg/mg protein to the Sero group where the levels were around 48 pg/mg protein. This means that giving serotonin really messed up the balance in the stomach lining. When we gave serotonin the IL-6 levels went from around 48 pg/mg protein to than 180 pg/mg protein, which is a big jump. The TNF- α levels also went up a lot from around 150 pg/mg protein in the group to around 380 pg/mg protein, in the Sero group. PGE2 concentrations were brought back to almost normal levels (~160-170 pg/mg protein) following treatment with Sero+ esomeprazole (20 mg/kg) and rutin (10 and 20 mg/kg), suggesting that prostaglandin-mediated cryoprotection was preserved. In comparison to the serotonin group, both drugs dramatically decreased TNF α and IL 6 levels at the same time with rutin at 20 mg/kg exhibiting the most impact (TNF α decreased to ~200 pg/mg protein and IL 6 decreased to 100 pg/mg protein). All of these results show that rutin, like the common anti-ulcer medication esomeprazole, has dose-dependent gastroprotective benefits by strengthening mucosal defence and reducing inflammatory cytokine production. PGE2 concentrations were returned to normal by esomeprazole and rutin (at 10 and 20 mg/kg), suggesting their protective function in preserving prostaglandin-mediated mucosal defence.

Table 14: Effect of Serotonin, Esomeprazole, and Rutin on Gastric Inflammatory and Cytoprotective Markers in Rats.

Groups	PGE_2 (pg/mg protein)	TNF-α (pg/mg protein)	IL-1β (pg/mg protein)	IL-6 (pg/mg protein)
Normal Control	180 ± 0.5	150 ± 0.8	120 ± 0.6	75 ± 0.4
Sero	48 ± 0.3*	380 ± 0.10*	280 ± 0.9*	200 ± 0.8*
Sero + Eso (20 mg/kg)	160 ± 0.6**	200 ± 0.7**	130 ± 0.6**	110 ± 0.5**
Sero + Rutin (10 mg/kg)	175 ± 0.5**	250 ± 0.8**	160 ± 0.7**	130 ± 0.6**
Sero + Rutin (20 mg/kg)	170 ± 0.6***	200 ± 0.7**	120 ± 0.6**	100 ± 0.5***



The data were expressed as mean ± SD. The statistical significance of differences between the Rutin treated groups, control groups and SERO group, was determined by tested by using one way ANOVA followed by Dunnett's test. *Significant means ($p < 0.05$) ** Significant means ($p < 0.01$) *** Significant means ($p < 0.001$)
*** Significant means ($p<0.001$)

Figure 18: Effects of Serotonin (Sero), Serotonin+ Esomeprazole (Sero+ Eso), Sero+ Rutin (10 mg/kg), Sero+ Rutin (20 mg/kg) and their combination (RUTIN+SERO) on PGE2 (pg/mg protein) (A), TNF-alpha (pg/mg protein) (B) and IL-1Beta (pg/mg protein) (C) and IL-6(pg/mg protein) (D) in gastric tissues compared with control (CO) group.

Histopathological photomicrograph of stomach tissue of Albino Rats

Administration of serotonin to rats produced marked ulcerative lesions in the glandular region of the gastric mucosa. The mucosal damage was characterized by extensive loss of glandular cells and the presence of multiple hemorrhagic patches of varying sizes distributed throughout the glandular stomach. Histopathological evaluation revealed pronounced hemorrhagic gastric pits, inflammatory cell infiltration, disruption of the surface epithelium, vacuolization of epithelial cells, and elongation of micro vessels. However, pretreatment with rutin significantly attenuated serotonin-induced gastric injury, reducing both the extent and severity of mucosal lesions and preserving the structural integrity of the gastric mucosal architecture. These findings indicate improved mucosal protection and recovery against serotonin-induced gastric alterations. The protective effect was more evident at the higher dose of rutin (20 mg/ kg), showing better preservation of gastric tissue morphology.

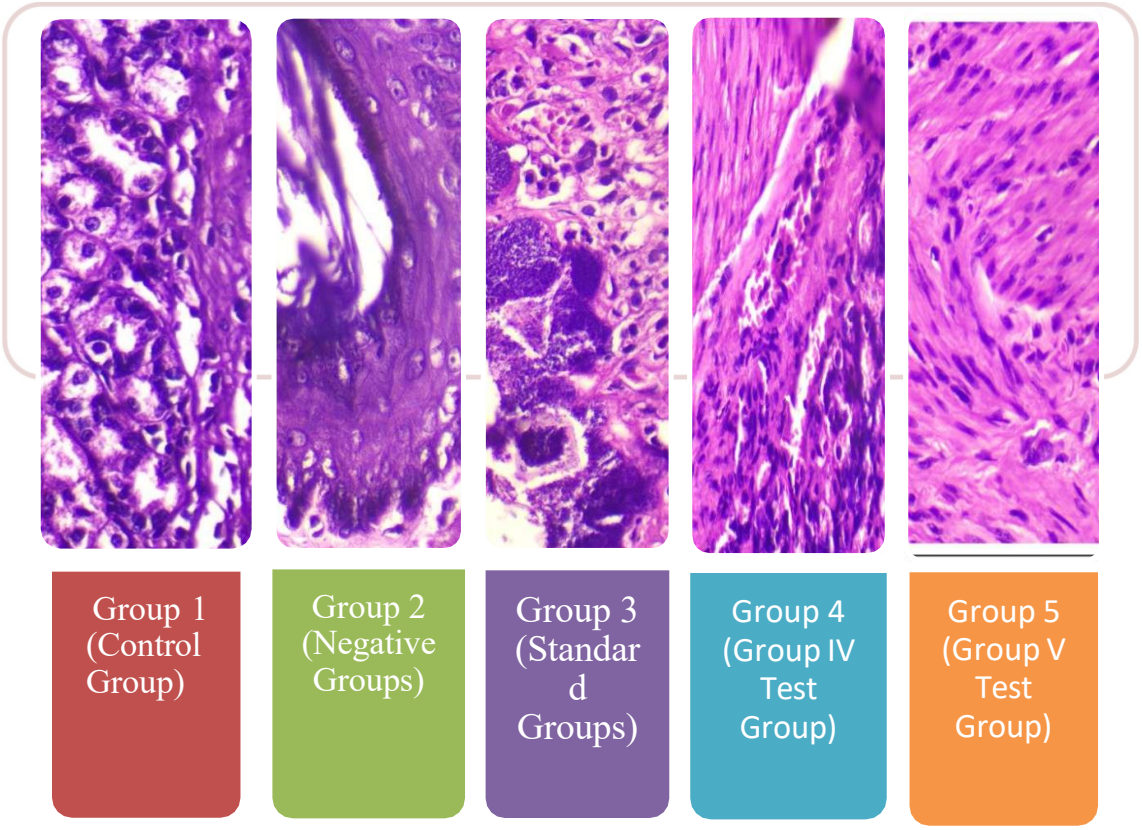


Figure 19: Histopathological photomicrograph of stomach tissue.

Conclusion

The present study was able to prove the gastroprotective potential of rutin in serotonin creatinine sulphate induced gastric ulceration in experimental rats. Serotonin administration caused severe gastric mucosal injury, as evidenced by the elevated ulcer index, oxidative stress, inflammatory response and histopathological changes in the gastric tissues. The results clearly showed that the serotonin-mediated ulcerogenesis was linked to an increased production of ROS, increase of lipid peroxidation, reduction of endogenous antioxidant enzymes and activation of inflammatory mediators, which all led to gastric mucosal damage and disruption of tissue integrity. Gastric lesions were significantly decreased and gastric mucosa architecture was maintained by pretreatment with rutin. Rutin produced a significant restoration of the levels of the endogenous antioxidant enzymes such as superoxide dismutase, catalase and reduced glutathione and a significant decrease in malondialdehyde, suggesting attenuation of oxidative stress and lipid peroxidation. Rutin also exhibited significant anti-inflammatory effect by inhibiting inflammatory mediators and inhibiting inflammatory cell infiltration in gastric tissue. Histopathological observations also confirmed the protective effect of rutin as there was less epithelial degeneration, less haemorrhage, less oedema and preservation of gastric glandular structure in rutin treated groups. Thus, the gastroprotective activity of rutin could be explained by its antioxidant, anti-inflammatory and cytoprotective activity. Rutin was able to effectively reduce the gastric damage induced by serotonin by enhancing the mucosal defence mechanisms, maintaining the blood flow in the stomach, scavenging free radicals and inhibiting inflammatory pathways. The results strongly suggest the therapeutic potential of rutin, a natural flavonoid for gastric ulcer disease prevention and management. In conclusion, the study emphasizes the need to include the oxidative stress and inflammation as therapeutic targets in the management of ulcer and proposes rutin as a safer and effective alternative or adjunct to the current anti-ulcer drugs. Long-term efficacy, safety and molecular mechanism of action have yet to be investigated in the management of gastric ulcers and further experimental and clinical studies are warranted.

Ethical Approval

We ensured that all animal studies were conducted in accordance with the regulations established by the Government of India's Committee for Control and Supervision of Experiments on Animals, or CCSEA. Our study proposal was reviewed by the members of our institution's Institutional Animal Ethics Committee, or IAEC. Before we began the tests, he gave his approval.

IAEC Approval No.: IAEC/04/08/2026

In order to ensure that all animal studies are conducted in a way that is fair, compassionate and logical from a scientific standpoint, the IAEC complies with the guidelines established by the CCSEA. In order to prevent the animals in the lab from experiencing excessive pain, distress, or discomfort throughout the trial, our study took care of them.

Funding

None.

Conflict of Interest

None.

Author Contributions

All the authors contributed to the study.

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