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Original Article

Development and Characterization of Catechin-Loaded Self-Nanoemulsifying Drug Delivery System: Pharmacokinetics, Toxicity Assessment, and *in vivo* Anti-Ulcer Activity Evaluation

Rashmi Pathak*, Phool Chandra

Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, INDIA.

ABSTRACT

Purpose: This research aimed to prepare catechin-loaded SNEDDS that would improve the oral therapeutic efficacy of catechin. **Materials and Methods:** Catechin-SNEDDS was prepared and characterized using emulsification time, percent transmittance, thermodynamic stability, droplet size, polydispersity index, and morphological characterization. Tween 80 (surfactant), propylene glycol (co-surfactant), and olive oil (oil phase) with the highest solubility of catechin were used to prepare catechin-SNEDDS. Out of two catechin-SNEDDS (ME2F1 and ME2F2), ME2F2 passed the thermostability test and was further tested. **Results:** The obtained ME2F2 had a droplet size <100 nm, percent transmittance 99.60±0.01%, emulsification time 10s, PDI 0.232, and was morphologically spherical. Further evaluations showed that ME2F2 has no toxicity, and pharmacokinetic study showed the AUC0-t is 1.65 µg/mL*h for catechin and 2.14 µg/mL*h for ME2F2. As a result, higher C_{max} and AUC for ME2F2 in comparison to catechin are evidence of improved systemic drug absorption, which increases oral bioavailability. Further *in vivo* anti-ulcer activity showed that ME2F2 reduces the formation of ulcers, gastric juice volume, ulcer index, and increases pH in comparison to the ulcer control group. Histopathological estimation of stomach ulcers showed a reduction in ulcer and inflammation in the gastric layer in the ME2F2 treated group. *In vivo* antioxidant activity on stomach tissue for ME2F2 showed an increase in CAT, SOD, and GSH levels in response to oxidative stress and a decrease in LPO levels, indicating the formulation's antioxidant activity. **Conclusion:** Catechin's gastroprotective properties and anti-oxidative efficacy are improved by SNEDDS. According to research, it has a good probability of becoming a bioactive substance used as an anti-ulcer agent.

Keywords: Catechin, SNEDDs, Bioavailability, Anti-ulcer, Antioxidant.

Correspondence:
Ms. Rashmi Pathak
Research Scholar, Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad-244001, Uttar Pradesh, INDIA.
Email: rashmipathak963@gmail.com

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INTRODUCTION

A flavan-3-ol, catechin (Figure 1) may be found in a variety of foods, green tea, fruits, red wine, cocoa, beer and chocolate, among other foods.[1] It comprises one of the basic structural components of proanthocyanidins, a class of polyphenols called tannins. Phenolic compounds with a relatively large molecular weight that have a significant affinity for proteins and carbohydrates are known as tannins.[2] Tannins are beneficial bioactive substances for foods, cosmetics, and medications because of their antibacterial, radical-scavenging, antiviral, antioxidant, enzyme-inhibiting, and antimutagenic qualities.[3]

However, catechin's poor water solubility restricts its effectiveness through oral absorption by rendering it insoluble in the gastrointestinal tract.[4] Catechin's 5% absolute oral bioavailability significantly diminishes its potency as a medication.[5] Consequently, catechin's oral bioavailability and solubility need to be improved.

Because of their nano size, nano-formulations such as lipid nanocarriers, carbon nanotubes, polymeric micelles, dendrimers, nanocrystals, nanoemulsions, and polymeric nanoparticles are recognized for their capacity to get around the low oral bioavailability of insoluble medications.[6-9] For example, imprinted biopolymeric micelles were created to increase curcumin's poor solubility from 11 to 0.5 mg/mL, which doubles the AUC in rats when compared to the drug in its free form.[10] Similar to this, furosemide-caffeine nano-cocrystals were created to increase the solubility of furosemide from 17 to 30 µg/mL. Additionally, the dissolution rate of the nano-cocrystals was almost six times more

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than that of the drug.[11] All things considered; these changes have demonstrated that nano-formulations offer workable methods for improving the bioaccessibility of insoluble drugs.

One of the previously researched nano-formulations, SNEDDS, has attracted a lot of attention because of its potential to maximize the bioaccessibility of insoluble medications, particularly flavonoid compounds.[9,12] Li *et al.*, proved that self-nanoemulsifying drug delivery systems enhanced quercetin's solubility from 5 to 5 mg/mL, resulting in a 1.5-fold higher AUC after oral treatment in beagle dogs who were fasting.[13] According to earlier research, SNEDDS increases the oral bioavailability of flavonoid medications including rutin and naringenin.[14,15] To date, the pharmaceutical industry and academics have paid more attention to SNEDDS in boosting the oral bioavailability of insoluble medications. Three SNEDDS medications are now available for purchase: Norvir® (ritonavir), Sandimmun Neoral® (cyclosporin A), and Fortovase® (saquinavir).[16]

One serious medical problem is peptic ulcer disease. Five million individuals are affected by the approximately 500,000 new cases that are reported in the US each year. Keep crucial to keep in mind that the risk of developing peptic ulcer disease was highest for those born in the middle of the 20th century. The frequency of ulcer disease rises between the ages of 55 and 65 since it now mostly affects the elderly.[17,18] The development of sores or ulcers in the stomach or duodenal lining is often linked to the gastrointestinal condition known as Peptic Ulcer Disease (PUD). PUD is brought on by an imbalance between the gastro-duodenal mucosa's capacity for self-healing and self-defence and hostile substances like stomach acid and pepsin. The main causes of peptic ulcer include excessive alcohol use, the use of NSAIDs and *Helicobacter pylori* infection.[19]

To improve catechin's solubility and bioavailability for oral administration, the goal of this study is to develop an appropriate SNEDDS. In short, through testing catechin's solubility in different excipients and developing a ternary phase diagram, stable SNEDDS formulations of catechin were formulated. The resulting formulations were then evaluated for surface morphology, particle size, and emulsification efficiency. Afterwards, *in vivo* pharmacokinetic properties, Acute oral toxicity, *in vivo* antiulcer activity, and *in vivo* antioxidant activity of optimized formulation were determined.

MATERIALS AND METHODS

Materials

Catechin was isolated from *Myrica esculenta* and characterized[20] and used as active ingredient in the formulation. Span 80 (Loba Chemie Pvt. Ltd., Mumbai, India), Tween 80 (BRM chemical, Delhi, India), Tween 20 (BRM chemical, Delhi, India) was used as surfactant in formulation. Glycerine (Paskem Fine Chem Pvt. Ltd., Ghaziabad, India), propylene glycol (ASES Chemical Works, Jodhpur-Rajasthan, India), PEG 400 (Pure Chem, New Delhi, India) was used as co-surfactant in the formulation. Olive oil (BRM chemical, Delhi, India), corn oil (The Wholesaler Co, Noida, India), sunflower (Pure Chem, New Delhi, India) was used as oil in the formulation. Ethanol was purchased from Bio Liqua Research Private Limited, Karnataka, India and used to induce ulcer in rat's stomach. Indomethacin (Sun Pharma, Goregaon, Mumbai, India) was used to induce ulcer in rat's stomach. Omeprazole (Cipla Ltd., Mumbai) was used as standard drug to treat ulcer in rats.

Formulation of Catechin-Loaded Self-Nanoemulsifying Drug Delivery System (SNEDDS)

Determination of Solubility of Catechin

Suitable surfactants, cosurfactants, and oils must be used while developing the SNEDDS to improve the loading efficiency and the solubility of the active ingredients. The components of self-nanoemulsifying drug delivery systems must improve drug solubility and have good compatibility with one another to create a stable formulation.[21] Thus, catechin's solubility was initially assessed in oils (olive, sunflower, and corn oil), co-surfactants (glycerine, propylene glycol, and PEG 400), and surfactants (Span 80, Tween 80, and Tween 20). In short, two millilitres of each excipient were mixed with an excess of catechin. The resulting mixtures were combined with a vortex mixer and then stored for 48 hr at 37±2°C and 100 rpm in a shaking water bath. After reaching a state of equilibrium, the sample was centrifuged for 20 min at a speed of 8000 revolutions per minute. The supernatant was collected, and any extra undissolved catechin was disposed of. High-performance liquid chromatography was used to analyse the catechin concentration. Three duplicates of each experiment were conducted. The ternary phase diagram was created using the excipient that catechin was most soluble in.[22,23]

Pseudo-ternary Phase Diagram

Without catechin, ternary phase diagrams were created to identify the self-emulsifying zones. Based on catechin's solubility in several excipients, propylene glycol (co-surfactant), Tween 80 (surfactant), and olive oil (oil phase) were chosen to prepare the formulation. Oil, surfactant, and cosurfactant systems' ternary phase diagrams revealed the presence of self-emulsifying fields that, when diluted and gently swirled, create a transparent emulsion.[24] A magnetic bar was used to gently agitate 300 mL of distilled water at 37°C in a glass beaker while 0.2 mL of each formulation with different component ratios was introduced. The development of emulsion droplets and the propensity to emulsify spontaneously were noted. An emulsion's formation ability was rated as "good" if the oil droplets readily dispersed in distilled water and created a fine, milky emulsion; it was rated as "bad" if the droplets immediately solidified and little to no emulsion development occurred, particularly after stirring was stopped.

Finding the "good" self-emulsifying zone allowed for the creation of phase diagrams. Every experiment was conducted three times, and following an infinite dilution with purified water, the formula's self-emulsifying capabilities were evaluated visually.[25,26]

Preparation of Catechin-Loaded SNEDDS

From each constructed phase diagram, a variety of formulations were selected from the zone of nanoemulsion. According to the phase diagrams that were produced, the ratio of surfactant, co-surfactant and oil gave the maximum area of nanoemulsions, indicating appropriate ratios for SNEDDS synthesis.[22,27] Tween 80 as surfactant, propylene glycol as co-surfactant and olive oil as oil phase were combined by the formula ratio to create the drug-free SNEDDS (blank) (Table 1). To create drug-loaded SNEDDS, 10 mg/mL of catechin was added to each blank SNEDDS individually, and they were constantly stirred for 30 min at 40°C. A transparent mixture was obtained by separating the surplus undissolved catechin. For additional research, the catechin-loaded SNEDDS were stored at room temperature in hermetically sealed glass containers.[28,29]

Characterization of Catechin-SNEDDS

Determination of Emulsification Efficacy

The emulsification efficacy of the SNEDDS was assessed by dissolving apparatus II. Finally, 0.5 mL of SNEDDS was combined dropwise with 500 mL of distilled water at 37°C. A straightforward stainless-steel dissolving paddle spinning at 50 rpm supplied mild agitation. Visual examination was used to determine the emulsification time.[22,30]

Percent Transmittance

The clarity-turbidity of SNEDDS is determined by the transmittance, which reveals optical isotropy and thermodynamic stability. A percent Transmittance (%T) nearer 100% indicates an isotropic nature and the development of globules in the nanoscale range. A UV-vis spectrophotometer was used for spectrophotometry to ascertain the optical clearness of the SNEDDS. At 500 nm, the percentage transmittance of SNEDDS, diluted with deionized water at a 1:100 v/v ratio was determined using it as a blank. Four analyses were performed on each sample.[22,23]

Thermodynamic Stability

To produce stable nanoemulsions free of creaming, breaking, or precipitation, SNEDDS must be dissolved. As a result, several concerns must be considered while evaluating thermodynamic stability.[22] Different test like Centrifugation,[22] Heating cooling cycle,[22] Freeze-thaw cycle,[22] were performed to evaluate the stability of the formulation.

Measurement of the Droplet Size, Polydispersity Index and Zeta Potential

A Malvern zeta sizer nano ZS-90 was utilized to determine the mean droplet size (z-ave), Polydispersity Index (PDI), and Zeta Potential (ZP) by shining a laser light with a potential of 50 mV through samples that were placed in polystyrene cuvettes at a 90-degree angle. A 0.2 µm syringe filter was used to filter the optimal formulation mixture (0.1 mL), which had been diluted with 100 mL of distilled water. A diluted sample of one millilitre

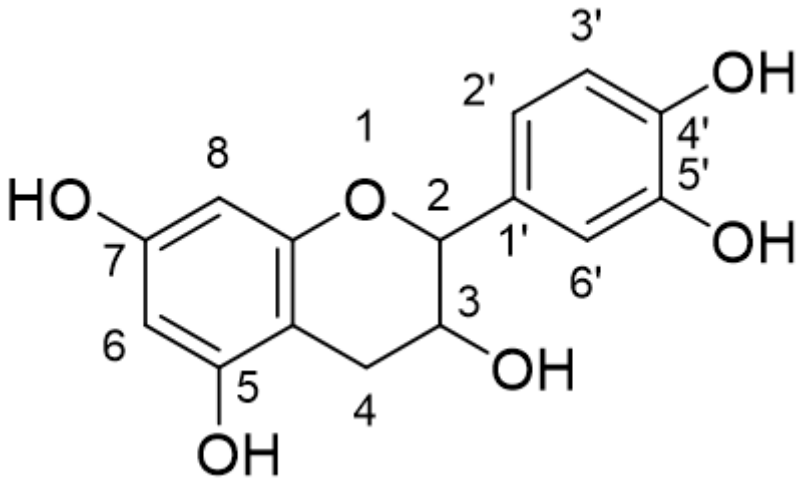

Figure 1: Structure of Catechin.

Table 1: Compositions of formulations of catechin-loaded SNEDDS.

Sl. No.	Formulation	Percentage of Oil (v/v)	Percentage of Surfactant v/v)	Percentage of Co-Surfactant (v/v)	Drug (mg)
1	ME2F1	20	50	30	500
2	ME2F2	10	60	30	500

was collected and examined in the sample cell. A temperature of 25° C was used for the procedure.[31,32]

Morphological Characterization- HR-TEM

The JEOL JEM 2100 Plus HR-TEM was used to assess the surface structure of the optimized SNEDDS formulation. The pre-concentrate SNEDDS (1 mL) was mixed in water till the nanoemulsion with the correct droplet size was formed. 1 drop of the prepared nanoemulsion was left on the metal plate, and the extra formulation was wiped away using filter paper. When the squares were dried the High-Resolution Transmission Electron Microscope image was captured.[22,28,30]

Experimental Animals

Healthy male and female Wistar rats weighing 150-250 g was obtained. The rats were kept in polypropylene cages with a 12:12 hr dark/light cycle, 28±2°C and 60-70% relative humidity. The rats were given unlimited access to a standard pellet diet throughout the study. The rats had free access to mineral water. With reference number DVCP/IAEC/2023/05, the Institutional Animal Ethical Committee (IAEC) approved the acute toxicity and pharmacology procedures and followed ethical guidelines.

Acute Oral Toxicity Test

The negative effects that follow a single dose of a medicine or several doses given over a day are referred to as acute oral toxicity. In compliance with OECD guideline 423, the highest feasible dose of 2000 mg/Kg was used. Three rats were employed in the experiment, and each one received a dosage 48 hr apart. Changes are noted once a day in the cage in the following areas: fur, eyes, autonomic salivation, skin, mucous membrane (nasal), perspiration, piloerection, lacrimation, feces, and urine incontinence. Mortality was found after two weeks.[33] Hematological and histopathological parameters were also evaluated.[34] Table 2 shows the animal grouping for acute toxicity studies.

Pharmacokinetic Study

To evaluate catechin and catechin-SNEDDS bioavailability in the plasma, rats fasted for 16 hr before administration. Catechin and catechin-SNEDDS (ME2F2) were given orally at doses of 30 mg/Kg body weight. The blood samples were withdrawn through the retro-orbital route from rats at 0, 30, 60, 90, 120, 150, 180, and 210 min after oral administration. After 5 min, blood samples were centrifuged for 15 min at 3500 rpm to collect plasma. HPLC

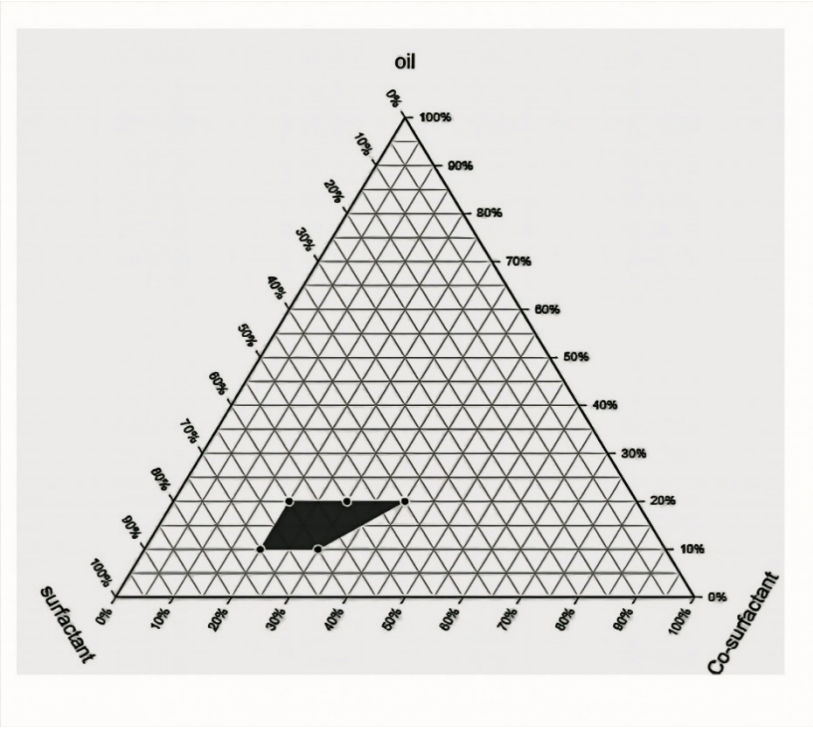

Figure 2: Pseudo-ternary phase diagram for selection of SNEDDS excipients.

Table 2: Experimental Design for Acute oral toxicity.

Animals Required for Acute Oral Toxicity			
Model	Formulations	Group	Animals
Acute Oral Toxicity	ME2F2	3	3

was used to extract and analyse the 250 µL supernatant. Next, measurements were made of the pharmacokinetic parameters.[35]

In vivo Antiulcer Activity

Table 3 shows the experimental design and groups of rats divided to evaluate the anti-ulcer activity using ethanol-induced gastric ulcers, stress-induced gastric ulcers, NSAIDs induced gastric ulcers.

Ethanol-induced Gastric Ulcers

The gastroprotective effectiveness was examined using the ethanol-induced gastric ulcer model.[36] Rats (*n*=6) were divided into six groups and fasted for a full day before the test. The formulation was administered to the test groups (ME2F2 30 mg/Kg), omeprazole (30 mg/Kg) was administered to the standard group, and the vehicle (NaCl 9%, 2.5 mL/Kg) was administered intraperitoneally to the control group. After 30 min, ethanol (5 mL/Kg) was given orally to each group to cause stomach ulcers.[36-38] Before the animals were euthanized an hour later, their stomachs were taken out, opened along the large curve, cleaned, and stretched on cork plates. Lesion presence was assessed on the surface, and the size of the lesions was quantified. As a lesion index, the total length of the stomach lesions was measured in millimetres.[39-41]

Stress-induced Gastric Ulcers

Before the test, the rats were fasted for 24 hr and divided into six groups of six. The test groups received the formulation (ME2F2 30 mg/Kg), the control group received an intraperitoneal dose of vehicle (NaCl 9%, 2.5 mL/Kg), and the standard group received omeprazole (30 mg/Kg). The rats starved for the entire night. For 1 hr, the animal was vertically submerged in water that was kept at 10°C±1°C until it reached the sternum xiphoid. Rats in the unstressed state went through the identical process without being exposed to cold water.[42] Rats that had been acutely submerged in cold water were promptly euthanized, and their intestinal tissues and serum were taken for further analysis.[43]

NSAIDs-induced Gastric Ulcers

24 hr before the ulcer was induced; they were given free access to water but no food. A single oral dosage of indomethacin (30 mg/Kg B.W.) was given to the rats. 4 hr after the indomethacin was administered, different levels of ulceration appeared. Four equal groups of six rats each were created from the rats. All that was given to the normal control animals was distilled water. Rats in group II (ulcer control) received just indomethacin, whereas the treatment groups received formulation (ME2F2 30 mg/Kg) and the standard group received omeprazole (30 mg/Kg). On the fifteenth day, 4 hr after the ulcer was produced, the animals were euthanized. The stomach was removed when the abdomen was opened. The stomach was then opened along its larger curvature, and the contents were poured into a centrifuge tube. The cleaned stomachs were initially stored in phosphate saline buffer (0.1 M, pH 7.4, 1:4 w/v) before to being viewed under a microscope and homogenized.[44,45]

Parameters Studied

Determination of Ulcer index

After cutting the stomach open along its larger curvature, the mucosa was cleaned with slow-flowing tap water to get rid of any blood clots or stomach contents. The mucosa was then inspected under a 10X magnifying glass to see whether an ulcer had formed. The numbers of ulcers were counted and ulcer index was determined.[46,47]

Table 3: Experimental design for *in vivo* anti-ulcer activity.

Sl. No	Groups	Drugs	Doses	Animals
1	Normal control	Normal Saline Solution		6
Ethanol Induced Gastric Ulcers				
2	Control	Ethanol	5 mL/Kg	6
3	Standard	Omeprazole	30 mg/Kg	6
4	Treated	ME2F2	30 mg/Kg	6
Stress Induced Gastric Ulcers				
8	Control	Water-immersion stress		6
9	Standard	Omeprazole	30 mg/Kg	6
10	Treated	ME2F2	30 mg/Kg	6
NSAIDs Induced Gastric Ulcers				
14	Control	Indomethacin	30 mg/Kg	6
15	Standard	Omeprazole	30 mg/Kg	6
16	Treated	ME2F2	30 mg/Kg	6
Total				60

Volume of gastric juice

The volume was recorded after the contents were emptied into tubes and centrifuged for ten min at 1000 rpm.[48]

Determination of pH

Following centrifugation, gastric juice (1 mL) was diluted with distilled water (1 mL), and a pH meter was used to determine the solution's pH.[48]

In vivo Antioxidant Activity

Getting the homogenate ready. 10% (w/v) gastric tissue homogenate was made in ice-cold phosphate buffer (50 mM, pH 7.4) with a cocktail of mammalian protease inhibitors, and it was centrifuged for 10 min at 4000 rotation per minute (4uC).[49] The gastric tissue homogenate was utilized to estimate Superoxide Dismutase (SOD) activity,[50] Catalase (CAT) activity,[51] lipid peroxidation (MDA),[52] total Glutathione (GSH).[53,54]

Histopathological Examination

Small pieces of tissue were collected in a 10% formalin solution after the rats' stomachs were removed and cleaned in regular saline while they were under mild ketamine anaesthesia. Stomach histopathological analyses were performed, and the alterations were noted.[34]

Statistical Analysis

The findings were shown as Mean±SEM. All of the gathered data was statistically assessed using ANOVA (Dunnett's test). At $p<0.05$, the differences were deemed significant. The Graph Pad Prism 5 program was utilized to do the statistical study.

RESULTS

Formulation of Catechin-Loaded Self Nanoemulsifying Drug Delivery System

Determination of Solubility of Catechin

Table 4 displays catechin's solubility in different vehicles. One of the key elements that keeps the drug in its solubilized state in nanoemulsions is the drug's high solubility in the oil phase. With a solubility of 72.67±1.76 mg/mL, catechin was the most soluble in olive oil among the other oils. Olive oil was thus selected as one of the oil components in SNEDDS. Catechin's solubility in corn and sunflower oils was similar but significantly less than that of olive oil. The solubility of catechin was assessed using a variety of cosurfactants and surfactants, such as Tween 80, Tween 20, glycerine, Span 80, PEG 400, and propylene glycol. The findings demonstrated that the HLB of the surfactant influenced catechin's solubilizing ability. Catechin solubilization was facilitated by surfactants with high HLB values. Catechin has a solubility of 62.67±1.76 mg/mL in the surfactant Tween 80. Similarly, catechin has a solubility of 114.00±2.08 mg/mL in the co-surfactant propylene glycol. According to the study, catechin was most soluble in Tween 80, propylene glycol and olive oil; these were selected to create a self-nanoemulsifying drug delivery system as the surfactant, cosurfactant, and oil respectively.

Pseudo-ternary Phase Diagram

Formulations of liquid SNEDDS were created, and their capacity to self-emulsify was assessed visually. To determine the self-emulsifying areas and optimize the ratio of oil,

Table 4: Solubility of catechin in excipients.

Excipients	Solubility of Catechin at 37°C (mg/mL)
Oils	
olive oil	72.67±1.76
corn oil	0.97±0.15
sunflower oil	10.33±0.88
Surfactants	
Tween 80	62.67±1.76
Tween 20	27.00±1.73
Span 80	6.67±0.88
Co-surfactants	
Glycerine	19.67±1.45
PEG 400	62.33±0.88
Propylene glycol	114.00±2.08

Data are shown as mean±SD (n=3).

Table 5: Ratio of oil, surfactant, co-surfactant, and used to plot ternary phase diagram.

Oil % (v/v)	Surfactant % (v/v)	Co-Surfactant % (v/v)	Result
10	70	20	Good
10	60	30	Good
10	45	45	Bad
20	40	40	Good
20	50	30	Good
20	60	20	Good
30	40	30	Bad
20	40	40	Bad

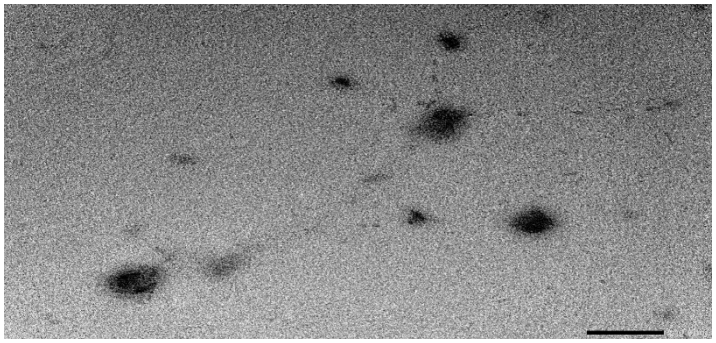

Figure 3: TEM image of catechin-loaded SNEDDS (ME2F2).

Table 6: Measurement of emulsification time of catechin-loaded SNEDDS.

Sl. No.	Formulation	Percentage of Oil (v/v)	Percentage of Surfactant (v/v)	Percentage of Co-Surfactant (v/v)	Drug (mg)	Self-emulsification Time (s)
1	ME2F1	20	50	30	500	20±2.9
2	ME2F2	10	60	30	500	10±0.6

cosurfactant, and surfactant in the formulations of liquid self-nanoemulsifying drug delivery systems, a ternary phase diagram was created without catechin. Figure 2 displayed the ternary phase diagrams of the systems that contained propylene glycol (co-surfactant), Tween 80 (surfactant), and olive oil (oil phase). The more surfactant added to the liquid SNEDDS formulation, the greater the likelihood that an emulsion would develop spontaneously inside the self-emulsifying zone (Table 5). The surfactant phase needs to include at least 40% of the formula for self-emulsification; a ratio of 40-70% was optimal. The co-surfactant ratio in the formulation was not an important component for self-emulsification, even though a ratio of about 20-40% was preferred. Overall emulsification efficiency was "good" when the surfactant:co-surfactant ratio exceeded 80% of the self-nanoemulsifying drug delivery system formulation. The drug in SNEDDS may affect the self-emulsifying qualities, according to reports. Catechin's inclusion, however, did not appear to change the self-emulsifying effectiveness in our study. Since it seemed somewhat milky and did not separate, the oil:surfactant:co-surfactant ratios of 10:60:30 and 20:50:30 were used to create the SNEDDS formulation.

Preparation of Catechin-Loaded SNEDDS

From ternary phase diagram, a variety of formulations were selected from the zone of nanoemulsions. Tween 80 as surfactant, propylene glycol as co-surfactant and olive oil as oil phase were combined to create the drug-loaded SNEDDS using the formula ratio Table 1. For additional research, the catechin-loaded SNEDDS were stored at room temperature in hermetically sealed glass containers.

Table 7: % Transmittance of the catechin compound-loaded SNEDDS.

Sl. No.	Formulations	% transmittance
1	ME2F1	99.20±0.01
2	ME2F2	99.60±0.01

Characterization of Catechin-SNEDDs

Self-emulsification Time

According to Table 6, the formulations had an excellent capacity to generate nanoemulsions with a self-emulsification time of less than 25 sec. The quickest self-emulsification time 10 s, was demonstrated by formulation ME2F2. It was discovered that the ratio of surfactant, cosurfactant and oil composition affected the self-emulsification time.

Percent Transmittance

Table 7 displays the catechin-loaded SNEDDS's %T. The percentage T for each formulation was between 99.15 and 99.60%, suggesting that isotropic nanoemulsions were formed.

Thermodynamic Stability

Table 8 displays the catechin-loaded SNEDDS's thermodynamic stability data. Following centrifugation, heating and cooling, and freeze-thaw cycles, the ME2F2 was determined to be thermodynamically stable. The test of the freeze-thaw cycle was unsuccessful for formulation ME2F1. The formulation ME2F1's turbidity increased after passing the freeze-thaw tests, which may have been caused by insufficient surfactant and cosurfactant levels to preserve catechin's full solubility in the SNEDDS formulation.

Determination of the Polydispersity Index, Droplet Size, and Zeta Potential

Table 9 displays the catechin-loaded SNEDDS's droplet size and polydispersity index. All diluted SNEDDS formulations ME2F2

had mean droplet sizes that were within the nanometre (<100 nm) range. The homogeneity of the particle size was shown by the formulation's PDI values, which were less than or around 0.3. Clear solutions are provided for the formulation.

Table 9 displays the catechin-loaded SNEDDS's zeta potential values. The resultant nanoemulsion droplets exhibited a very low charge, as indicated by the formulation ME2F2's zeta potential value of -14.7 mV. The OH group in catechin may be the cause of this outcome as it may provide the surface of the generated nanoemulsions with negative charges.

HR-TEM Analysis

Figure 3 displays the High-Resolution Transmission Electron Microscopy (HR-TEM) picture of a diluted SNEDDS formulation ME2F2. The nanoemulsion generated from the diluted formulation had a consistent diameter and a spherical shape. Following the findings from the zeta potential, the size of the droplet was found to be in the nanoscale range.

Acute Oral Toxicity Study

For the acute oral toxicity trial in this investigation, 2000 mg/Kg of the ME2F2 formulation was utilized. No toxicity of any kind was detected in the animals that were given the formulation. Thus, as seen in Table 10, formulation was employed for more research.

Determination of Acute Oral Toxicity

Hematological Study for Acute Oral Toxicity Studies

Table 11 shows the evaluation of the formulation's hematological study for the evaluation of acute oral toxicity.

Histopathological Examination

Under a microscope, the histopathology of several rat organs, including the kidney, liver, stomach, and brain, was examined for the alterations depicted in Figure 4.

(A1) Normal stomach layer histology, with mucosa indicated by arrows; (A2) liver with hepatocytes which appear normal (arrow); (A3) the renal parenchyma is well intact in the renal tissue. It seemed as though the renal tubules around the renal corpuscles were normal. There are no obvious signs of inflammation in the renal parenchyma; (A4) Normal white matter shows oligodendrocytes (arrow), which have round dark nuclei often with a slight perinuclear halo, and astrocytes.

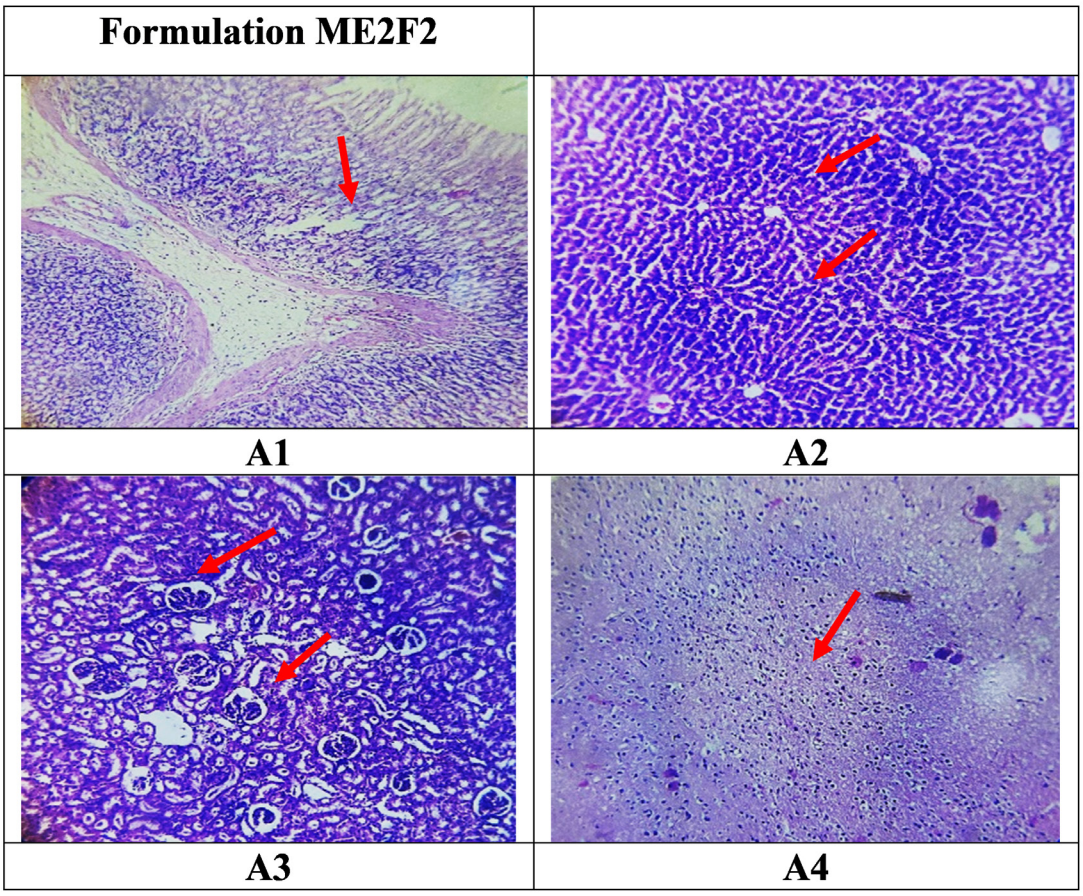

Figure 4: Histological study of rat's organs for acute oral toxicity study.

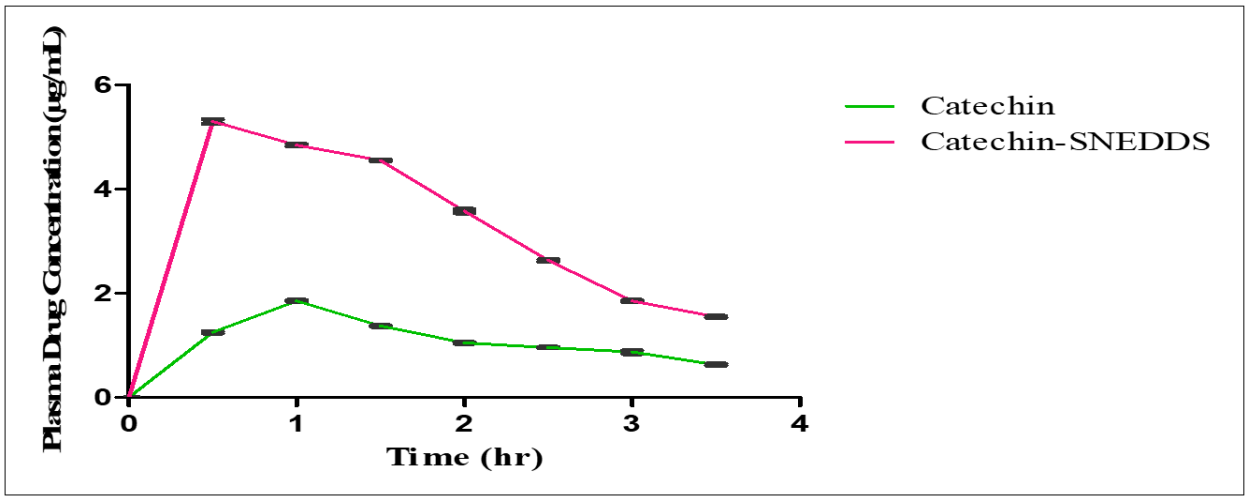

Figure 5: Graph showing bioavailability of Catechin and Catechin-SNEDDS in plasma.

Table 8: Thermodynamic stability study of formulations.

Sl. No.	Formulations	Centrifugation	Heating and cooling cycle	Freeze-thaw cycle	Inference
1	ME2F1	Separation	Separation	Turbidity	Fail
2	ME2F2	Stable	Stable	Stable	Pass

Table 9: Droplet size, PDI, and ZP of the Catechin-loaded SNEDDS.

Sl. No.	Formulation	Droplet Size Z-Average (d.nm)	Polydispersity index	Zeta potential (mV)
1	ME2F2	45.21	0.232	-14.7±6.37

Pharmacokinetic Study for Catechin-SNEDDS (ME2F2)

The important step of this study was to find the estimation of the bioavailability of developed formulations of SNEDDS in the plasma. The outcomes are shown in Table 12 and Figure 5. The results showed that the T_{max} of catechin was found to be 1 hr in plasma, whereas it was 30 min in the case of catechin-SNEDDS in plasma. It indicates the delay in the absorption of catechin from the stomach to the systemic circulation due to its poor aqueous solubility. The AUC_{0-t} 1.65 µg/mL*h for catechin and 2.14 µg/mL*h for catechin-SNEDDS. As a result, higher C_{max} and AUC for catechin-SNEDDS comparison to catechin are evidence of improved systemic drug absorption, which increases oral bioavailability.

In vivo Antiulcer Activity

Estimation

of pH and Volume of Gastric Juice, Ulcer Index and % of Inhibition for Formulation

Table 13 shows the pH and Volume of Gastric Juice, Ulcer Index and % of Inhibition determined for the ME2F2 formulation. ME2F2 showed a decrease in the volume of gastric juice, ulcer index and an increase in pH of gastric juice in comparison to the ulcer control group in all three models.

Histopathological Studies

Ethanol-induced Antiulcer Activity

Figure 6 shows the histopathological of the stomach in ethanol induced ulcer model, in which the formulation ME2F2 treated group showed the recovery of ulcer and inflammation when compared to the ulcer control group.

Rat stomach parts histopathological study: (A) Normal control displaying the normal histological structure of the stomach layers; (B) Ulcer control group, displaying ulceration (black arrow), necrosis of the stomach mucosa, submucosal oedema (yellow arrow), and congested blood vessel (green arrow); (C) Standard group treated with omeprazole showed slight inflammation in mucosa (Red arrows) and showed normal gastric layers; (D) treated group 2 ME2F2, Showed moderate hyperplasia (Black arrows) and oedema (Red arrows).

Stress-induced Gastric Ulcers

Figure 7 shows the histopathological of the stomach in the stress-induced ulcer model, in which the formulation ME2F2

treated group showed the recovery of ulcer and inflammation when compared to the ulcer control group.

Histopathology of stomach sections of rats: (A) The normal control displays the usual histology of the stomach layers.; (B) Ulcer control group, showed necrosis of mucosal glands, submucosal oedema (yellow arrow), ulcers (Black arrow), and hyperplasia (green arrow); (C) Standard group treated with omeprazole showed mild inflammation in mucous (Red arrows) and showed normal tissue with mild ulcer lesion (black arrow); (D) treated group 2 ME2F2, showed mild erosion of surface epithelium (Red arrows).

NSAIDs-induced ulcers

Figure 8 shows the histopathological of the stomach in the NSAIDs-induced ulcer model, in which the formulation ME2F2 treated group showed the recovery of ulcer and inflammation when compared to the ulcer control group.

Histopathology of stomach sections of rats

(A) Normal control showed normal gastric layers; (B) Ulcer control group, showed ulcers (Black arrow) with inflammatory mucosa (Red arrow) and congested blood vessel (green arrow); (C) Standard group treated with omeprazole showed tissue with no ulcer lesion and slight inflammatory cells (Green arrow) (D) treated group 2 ME2F2, showed mild erosion of surface epithelium (Red arrows) and muscularis oedema (Black arrow).

In vivo Antioxidant Activity

Formulation (ME2F2)-Ethanol Induced Antiulcer Activity

Measurement of Superoxide Dismutase (SOD) Activity

Rats in the ulcer control group had significantly lower levels of superoxide dismutase in their stomachs (3.17±0.40 mmol/min/mg tissue) than rats in the normal group (7.83±0.60 mmol/min/mg tissue). In contrast, SOD levels in the treated group rats (ME2F2) increased to 6.33±0.21 mmol/min/mg tissue, indicating its antioxidant activity as shown in Figure 9.

Table 10: Acute oral toxicity study of formulation in Rats.

Parameters	Effect of Formulation ME2F2
Eyes	No variation
Fur	No variation
Skin	No variation
Mucous membrane	No variation
Salivation	No variation
Lacrimation	No variation
Perspiration	No variation
Piloerection	No variation
Urinary	No variation
Incontinence	No variation
Defecation	No variation
Mortality	No mortality

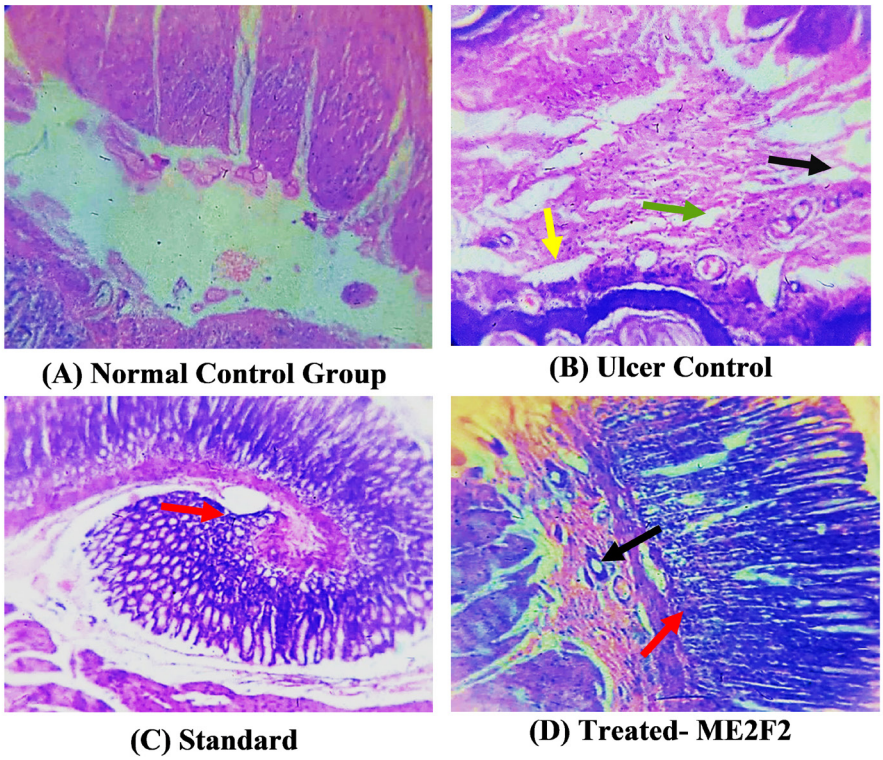

Figure 6: Histopathological study of the stomach for ethanol induced gastric ulcers.

Table 11: Hematological assessment for acute oral toxicity study.

Test Name	Formulation ME2F2	Units	Bio. Reference Interval
Complete Blood Count			
Haemoglobin	14.22±0.39	g/dL	10.6-15.6
Packed Cell Volume	39.50±1.38	%	32.7-44.8
Total Leucocyte Count (TLC)	4.98±0.52	thou/mm^3	3.3-8.7
RBC Count	5.82±0.80	mill/mm^3	5.5-9.3
MCV	50.67±2.56	fL	43.5-62.7
MCH	14.98±0.52	pg	15.8-19.9
MCHC	37.67±1.48	g/dL	31.4-36.0
RDW	14.60±0.82	%	11.9-16.1
Platelet Count (Electrical Impedance)	728.33±70.65	thou/mm^3	493-1124
Mean Platelet Volume (Calculated)	17.88±2.02	fL	
Differential Leucocyte Count (DLC)			
Neutrophils	16.72±2.60	%	3.3-26.6
Lymphocytes	67.22±1.77	%	
Monocytes	1.92±0.48	%	0-4.1
Eosinophils	2.42±0.70	%	0-5.0
Basophils	0.52±0.23	%	0-1.0
Absolute Leucocyte Count			
Neutrophils	0.85±0.31	thou/mm^3	
Lymphocytes	2.42±0.37	thou/mm^3	3.9-5.1
Monocytes	0.42±0.15	thou/mm^3	0-0.3
Eosinophils	2.37±0.83	thou/mm^3	0-6

Bars are expressed as Mean±SEM (n=6). Statistical analysis was done using one-way Analysis of Variance (Dunnett's test). Significance is indicated as a p<0.05 versus the normal control, while *p<0.05, **p<0.01, and ***p<0.001 represent a comparison with the ulcer control group.

Determination of Catalase Activity

Rats in the ulcer control group had significantly lower levels of catalase in their stomachs (288.50±2.25 mmol/min/mg tissue) than rats in the normal group (434.67±1.45 mmol/min/mg tissue). On the other hand, CAT levels in the treated group rats (ME2F2) increased to 404.33±2.16 mmol/min/mg tissue, indicating its antioxidant activity (Figure 10).

Bars are expressed as Mean±SEM (n=6). Statistical analysis was done using one-way Analysis of Variance (Dunnett's test). Significance is indicated as a p<0.05 versus the normal control, while *p<0.05, **p<0.01, and ***p<0.001 represent a comparison with the ulcer control group.

Measurement of Lipid Peroxidation (MDA)

A significant increase in LPO was recorded in the stomach of ulcer control group rats (82.17±1.97 nmol/g tissue) when compared to the normal group (57.50±1.38 nmol/g tissue). Whereas the

Table 12: Pharmacokinetic parameters of Catechin and Catechin-SNEDDS.

Parameters	Catechin	Catechin-SNEDDS (ME2F2)
C_{max} (µg/mL)	1.85±0.015	5.30±0.043
T_{max} (hr)	1	0.5
AUC0-t (µg/mL*h)	1.65	2.14

treated group rats (ME2F2) have shown a decrease in LPO levels 67.00±1.51 nmol/g tissue which indicates its antioxidant activity as shown in Figure 11.

Bars are expressed as Mean±SEM (n=6). Statistical analysis was done using one-way Analysis of Variance (Dunnett's test). Significance is indicated as a p<0.05 versus the normal control, while *p<0.05, **p<0.01, and ***p<0.001 represent a comparison with the ulcer control group.

Determination of Total Glutathione (GSH)

Rats in the ulcer control group had significantly lower levels of GSH in their stomachs (37.67±2.85 mmol/g tissue) than rats in the normal group (58.17±1.74 mmol/g tissue). On the other hand, GSH levels in the treated group rats (ME2F2) increased to

Table 13: pH and Volume of Gastric Juice, Ulcer Index and % of Inhibition for Formulation ME2F2.

Ulcer model	Group	Treatment	Dose	Gastric juice volume (mL)	Gastric juice pH	Ulcer index	% inhibition
Ethanol induced ulcer	Normal control	Normal Saline Solution	2.5 mL/Kg	2.88±0.23	3.27±0.17	0±0	-
	Ulcer Control	Ethanol	5 mL/Kg	4.57±0.16[a]	2.37±0.08[a]	23.92±0.72[a]	-
	Standard	Omeprazole	30 mg/Kg	3.35±0.10***	4.35±0.13***	8.25±1.98***	65.51
	Treated 2	ME2F2	30 mg/Kg	3.30±0.11***	3.35±0.11***	12.41±2.77***	48.11
Stress Induced Gastric Ulcers	Normal control	Normal Saline Solution	2.5 mL/Kg	3.02±0.15	3.00±0.04	0±0	-
	Ulcer Control	Water avoidance stress	-	4.50±0.12[a]	2.45±0.14[a]	23.17±0.93[a]	-
	Standard	Omeprazole	30 mg/Kg	3.32±0.11***	4.37±0.14***	5.08±1.11***	78.07
	Treated 2	ME2F2	30 mg/Kg	3.38±0.17***	3.58±0.16***	7.83±1.33***	66.19
NSAIDs Induced Gastric Ulcers	Normal control	Normal Saline Solution	2.5 mL/Kg	2.80±0.17	2.93±0.10	0±0	-
	Ulcer Control	Indomethacin	30 mg/Kg	4.75±0.20[a]	2.32±0.08[a]	16.58±1.37[a]	-
	Standard	Omeprazole	30 mg/Kg	3.00±0.24***	4.30±0.10***	4.58±0.79***	72.38
	Treated 2	ME2F2	30 mg/Kg	2.98±0.08***	3.83±0.12***	8.00±1.62***	51.76

Values are presented as Mean±SEM (n=6). Statistical analysis was done using one-way Analysis of Variance (Dunnett's test). Significance levels were shown as *p<0.05, **p<0.01, and ***p<0.001 versus the ulcer control group, while [a]p<0.05 represents significance compared to the normal control group.

46.00±1.75 mmol/g tissue, indicating antioxidant activity (Figure 12).

Bars are expressed as Mean±SEM (n=6). Statistical analysis was done using one-way Analysis of Variance (Dunnett's test). Significance is indicated as a p<0.05 versus the normal control, while *p<0.05, **p<0.01, and ***p<0.001 represent a comparison with the ulcer control group.

DISCUSSION

The research successfully formulated a Self-Nanoemulsifying Drug Delivery System (SNEDDS) for catechin, a bioactive compound with potential therapeutic effects. Catechin is poorly aqueous soluble which limits its bioavailability and SNEDDS formulations are increasingly recognized as an effective strategy to overcome this limitation by enhancing stability and solubility.[5]

To prepare the catechin-SNEDDS formulation, firstly the solubility of catechin in excipients was evaluated.[21-23] Catechin's solubility in corn (0.97±0.15 mg/mL) and sunflower oil (10.33±0.88 mg/mL) was similar, but much less than that of olive oil (72.67±1.76 mg/mL). The solubility of catechin was assessed using a variety of surfactants and cosurfactants, such as Tween 80, Span 80, Tween 20, glycerine, PEG 400, and propylene glycol. Catechin was more soluble in surfactant Tween 80 with a solubility of 43.33±2.40 mg/mL and in co-surfactant propylene glycol with a solubility of 96.00±0.58 mg/mL. Consequently, Tween 80, propylene glycol, and olive oil were chosen to create a self-nanoemulsifying drug delivery system as the surfactant, cosurfactant, and oil, respectively, since they demonstrated the highest solubilization for catechin. To identify the regions that self-emulsify and optimize the ratio of oil, surfactant, and cosurfactant in liquid SNEDDS formulations, a pseudo-ternary phase diagram was produced without catechin after excipient selection.[24,25] Following that, several formulations were carefully chosen from the constructed zone of nanoemulsions in each phase diagram. The drug-loaded SNEDDS was made by combining Tween 80 (surfactant), propylene glycol (co-surfactant), and olive oil (oil phase) in the recommended amounts.[22,28,29] The best formulation of catechin-loaded SNEDDS was ME2F2 showing good physicochemical properties and thermodynamic stability. The Catechin-SNEDDS ME2F2 was found to have a droplet size of less than 100 nm, percent transmittance of 99.55% and emulsification time 10s when evaluated. The nanoemulsions

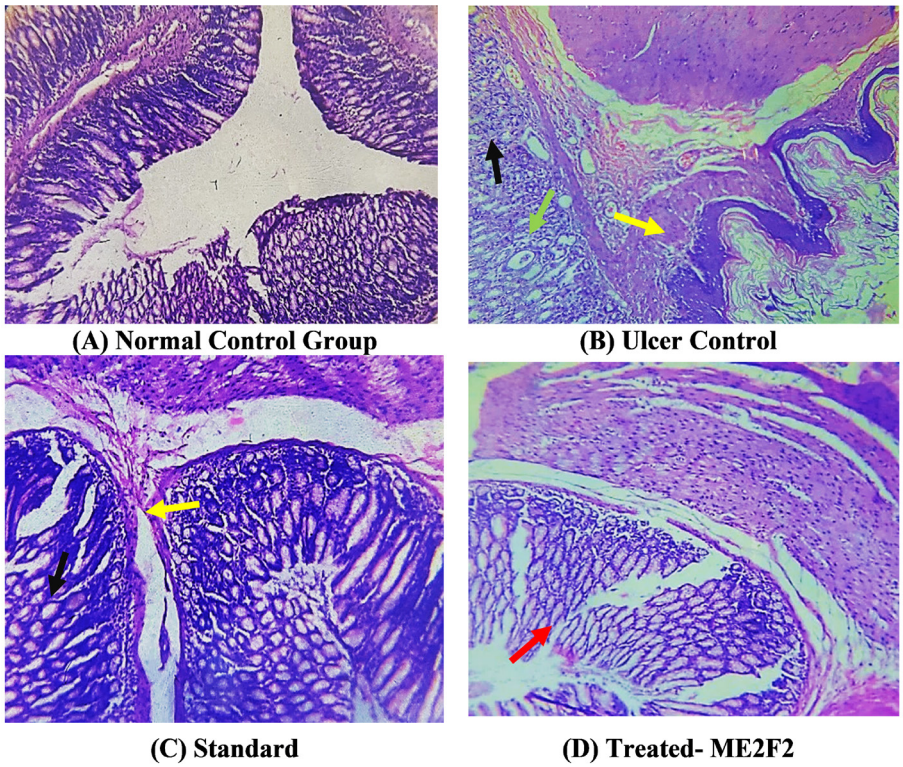

Figure 7: Histopathological study of stomach for stress induced gastric ulcers.

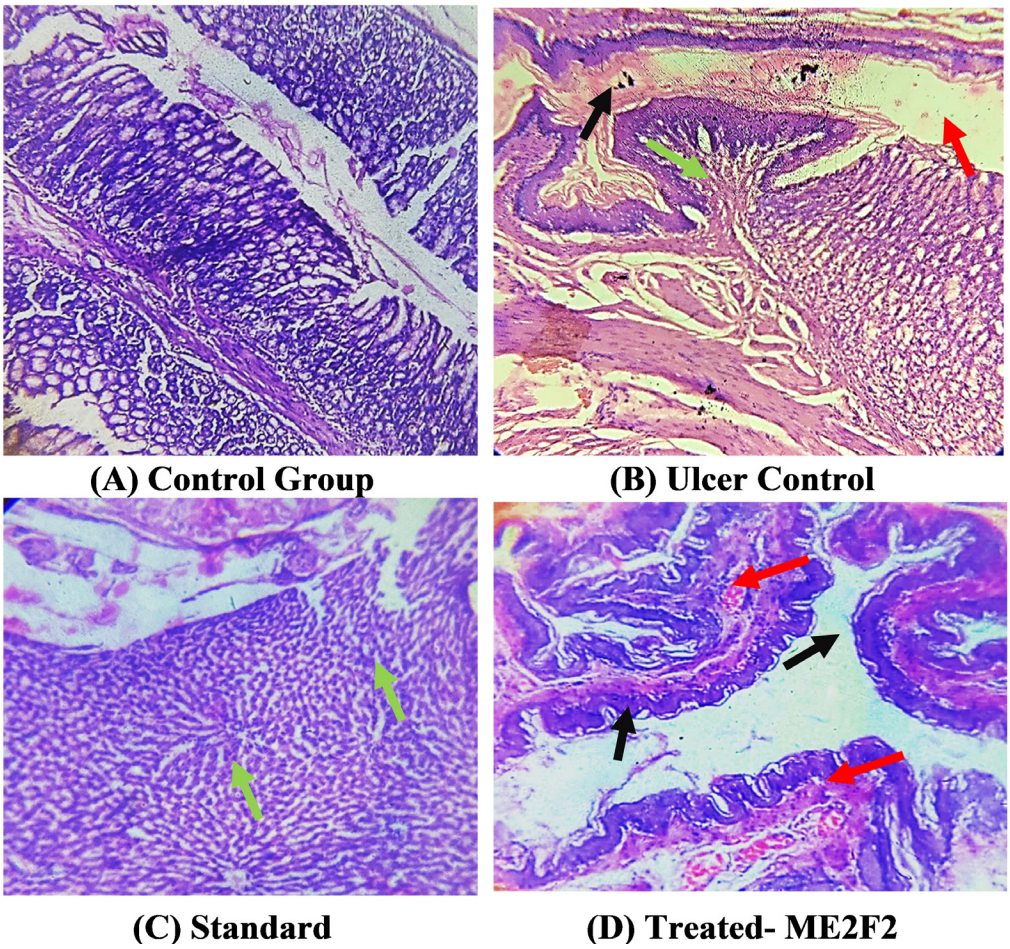

Figure 8: Histopathological study of stomach for NSAIDs induced gastric ulcers.

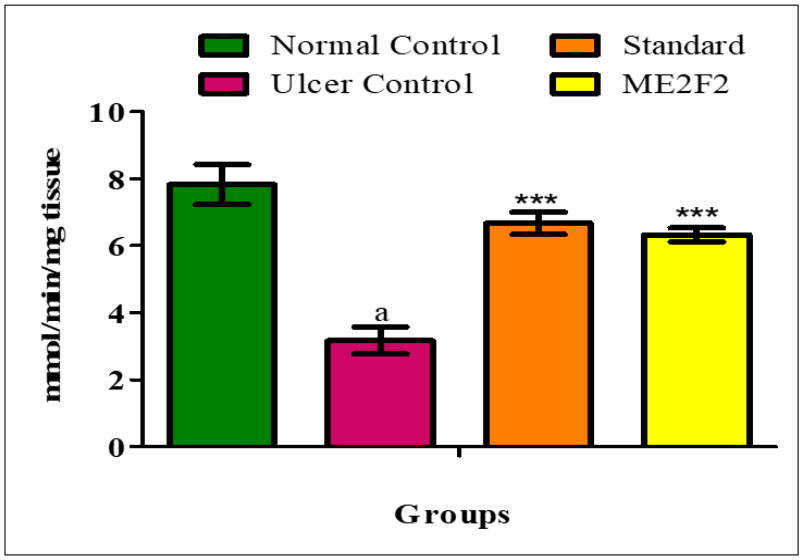

Figure 9: Effect of ME2F2 on the status of the level of SOD in ethanol induced ulcer.

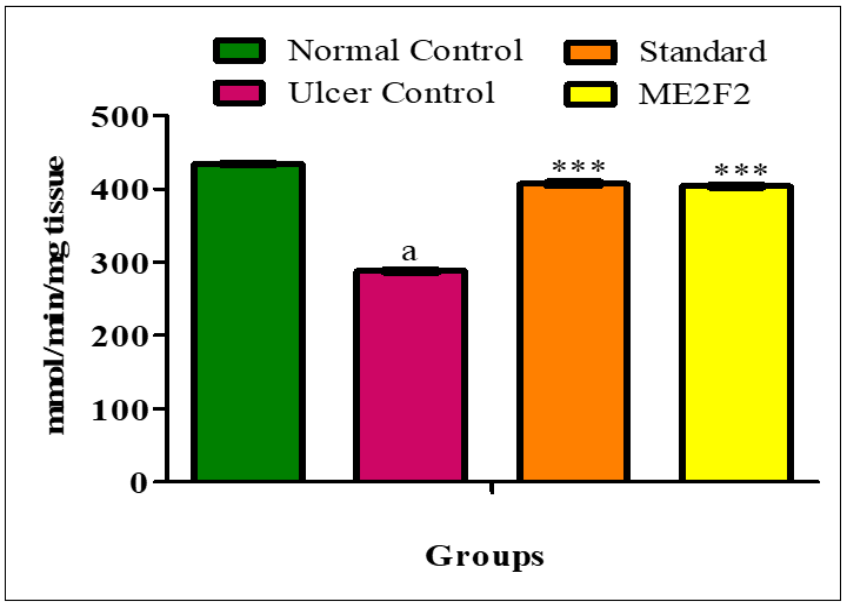

Figure 10: Effect of ME2F2 on the status of the level of CAT in ethanol induced ulcer,

generated from the diluted SNEDDS had a consistent diameter and a spherical shape when morphological study (TEM) was done. The SNEDDS formulation process is adaptable to large scale manufacturing using standard pharmaceutical techniques such as higher shear mixing and self-emulsification, ensuring its feasibility for industrial production.[55] Additionally, the excipients used (olive oil, Tween 80 and propylene glycol) are widely available and commonly used in oral formulations, making large-scale production feasible.[56] Since SNEDDS formulations contain GRAS (Generally Recognized as Safe) excipients, the regulatory pathway is expected to be less complex. However, regulatory approval will require further stability studies, bioequivalence testing, and clinical validation to ensure safety, efficacy, and quality consistency. The formulation aligns with ICH Q8 (Pharmaceutical Development) and Q9 (Quality Risk Management) guidelines, ensuring compliance with international regulatory frameworks. SNEDDS offers enhanced oral bioavailability, reduced dosing frequency, and improved gastrointestinal tolerance, which can significantly enhance patient adherence to therapy.[57] Additionally, the self-emulsifying nature of SNEDDS allows for easy oral administration without the need for specialized handling, making it suitable for routine clinical use.[58]

A further acute oral toxicity study for ME2F2 was carried out, and the formulation did not reveal any toxicity in the animals that were treated.[33] Therefore, the formulation was used for further study. Then pharmacokinetic study for testing its bioavailability was performed[35] and the results showed that the T_{max} of catechin was found to be 1 hr in plasma, whereas it was 30 min in the case

of ME2F2 in plasma. It indicates the delay in the absorption of catechin from the stomach to the systemic circulation due to its poor aqueous solubility. The AUC_{0-t} is 1.65 µg/mL*h for catechin and 2.14 µg/mL*h for catechin-SNEDDS. As a result, higher C_{max} and AUC for ME2F2 in comparison to catechin are evidence of improved systemic drug absorption, which increases oral bioavailability.

The anti-ulcer activity was carried out with the help of three models- Ethanol induced ulcers,[36] Stress Induced Gastric Ulcers,[40] and NSAIDs Induced Gastric Ulcers.[45] Because it considerably reduces the formation of ulcers, the findings of this experiment suggest that ME2F2 have a gastroprotective function. Comparing ME2F2 to the ulcer control group, ME2F2 demonstrated considerable protection against stomach ulcers caused by ethanol, stress, and NSAIDs, with percentage inhibitions of 48.11%, 66.19%, and 51.76%, respectively. The ME2F2 treated group's pH rose while its gastric juice volume and ulcer index decreased in comparison to the ulcer control group. The results show that the ME2F2 successfully reduces the risk of stomach ulcers. The histopathological estimation of stomach ulcers showed a reduction in ulcer and inflammation in a gastric layer in ME2F2 treated group.[49]

The results indicate the formulation's antioxidant activity. *in vivo* antioxidant activity was evaluated in the stomach tissue of rats by estimating SOD, CAT, LPO, and GSH levels.[50-52] ME2F2 showed an increase in SOD, CAT, and GSH levels in response to oxidative stress- 6.33±0.21 mmol/min/mg tissue, and 404.33±2.16 mmol/min/mg tissue, 46.00±1.75 mmol/g tissue respectively when

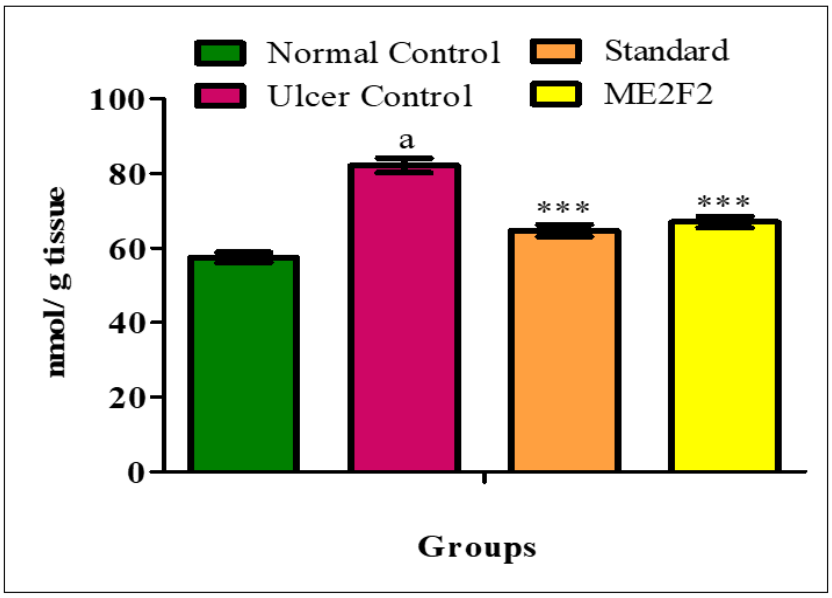

Figure 11: Effect of ME2F2 on the status of the level of LPO in ethanol induced ulcer.

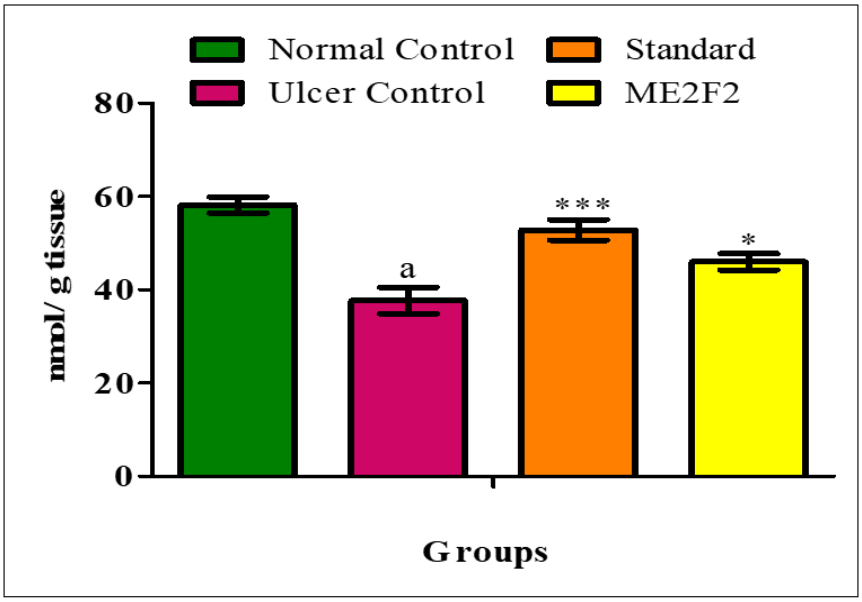

Figure 12: Effect of ME2F2 on the status of level of GSH in ethanol induced ulcer.

compared to the ulcer control group rats: 3.17±0.40 mmol/min/mg tissue, 288.50±2.25 mmol/min/mg tissue, and 37.67±2.85 mmol/g tissue respectively. Superoxide is converted by SOD to H_2O_2, which is then converted to water by glutathione peroxidase in the mitochondria or catalase in the lysosomes.[59] Because of its antioxidant qualities, GSH and its related enzymes are recognized as significant tissue-protecting agents.[60,61] ME2F2 indicated a decrease in LPO levels 67.00±1.51 nmol/g tissue when compared to the ulcer control group rats (82.17±1.97 nmol/g tissue). Lipid peroxidation produces MDA, which is used to measure the amount of lipid peroxidation.[62] Reduction of the fluidity of the membrane, compromised ion transport, compromised integrity of the membrane, and eventually, a loss of function of cells are all consequences of LPO.[63]

CONCLUSION

The formulation and characterization of Catechin-SNEDDS have shown its capability to enhance the stability, solidity and bioavailability of catechin. *In vivo* pharmacokinetic studies confirm that Catechin-SNEDDS could effectively deliver therapeutic concentration of catechin offering significant anti-ulcer and antioxidant activity. This formulation strategy could be extended to other poorly soluble compounds making SNEDDS a versatile and powerful drug delivery system for bioactive compounds with limited aqueous solubility.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

SNEDDS: Self-Nanoemulsifying Drug Delivery System; **AUC:** Area Under Curve; **PUD:** Peptic Ulcer Disease; **NSAIDs:** Nonsteroidal Anti-inflammatory Drugs; **PEG:** Polyethylene Glycol; **UV-vis:** Ultraviolet-visible; **HR-TEM:** High-Resolution Transmission Electron Microscope; **IAEC:** Institutional Animal Ethical Committee; **OECD:** Organization for Economic Co-operation and Development; **HPLC:** High-performance Liquid Chromatography; **B.W.:** Body Weight; **SOD:** Superoxide Dismutase; **CAT:** Catalase; **MDA:** Malondialdehyde; **GSH:** Glutathione; **LPO:** Lipid Peroxidation; **HLB:** Hydrophilic-lipophilic Balance; **RBC:** Red Blood Cell; **MCV:** Mean Corpuscular Volume; **MCH**: Mean Corpuscular Haemoglobin; **MCHC:** Mean Corpuscular Haemoglobin Concentration; **RDW:** Red Cell Distribution Width; **ANOVA:** Analysis of Variance.

ETHICAL STATEMENTS

The ethical approval was given by Institutional Animal Ethics Committee with reference no. DVCP/IAEC/2023/05.

AUTHOR CONTRIBUTIONS

RP: Data curation, Writing-original draft, Methodology, Software. PC: Conceptualization, Methodology, analysis of the data and tools and editing, Supervision. Both authors have read and approved the submitted manuscript.

SUMMARY

The study successfully developed a Self-Nanoemulsifying Drug Delivery System (SNEDDS) for catechin, a bioactive compound with poor aqueous solubility, to enhance its stability, solubility, and bioavailability. Acute toxicity studies confirmed the safety of Catechin-SNEDDS, while pharmacokinetic analysis demonstrated improved bioavailability, with a higher C_{max} and AUC compared to pure catechin. In anti-ulcer activity tests using ethanol, stress, and NSAID-induced gastric ulcer models, Catechin-SNEDDS significantly reduced ulcer formation and improved gastric parameters. Histopathological studies confirmed its gastroprotective effects. Furthermore, *in vivo* antioxidant studies in rat stomach tissue showed Catechin-SNEDDS enhanced levels of SOD, CAT, and GSH while reducing LPO levels, indicating its strong antioxidant potential. These findings suggest that Catechin-SNEDDS effectively improves catechin's therapeutic efficacy and offers promising gastroprotective and antioxidant benefits.

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