Advances in Bioresearch
Adv. Biores., Vol 15 (3) May 2024: 220-226

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.3.220226

Advances in Bioresearch

ORIGINAL ARTICLE

In-vivo Antiulcer activity of Ethanolic extract of *Mussaenda erythrophylla* Schumach. & Thonn. leaves

P. Hari Krishna[1], E. Sujatha[1]*, and S. Karunakar[2]
[1] Department of Botany, University College of Science, Osmania University, Telangana, India, 500007
[2] Department of Botany, MALD Govt. Degree College, Gadwal, Jogulamba Gadwal district, Telangana, India,500007
***Corresponding Author's** Email: sujatha@osmania.ac.in

ABSTRACT
Mussaenda erythrophylla Schumach. & Thonn. (Referred as ME from now onwards) is a plant from Rubiacea family widely employed for its medicinal benefits in folklore. In the current study antiulcer activity of the ethanolic extract of the ME leaves was investigated. Three different models of the antiulcer evaluation (Drug induced, Ethanol induced, Stress induced) were employed to assess the potential of ME leaves as an alternative herbal remedy for the synthetic drugs. Phytochemical analysis of the ME leaves revealed the presence of the various secondary metabolites such as flavonoids, alkaloids, glycosides, terpenoids, tannins etc. the highest Percentage Inhibition of ulceration of ethanolic extract of ME leaves was found to be 62.59, 53.38, and 70.45 at a dose of 200mg/kg body weight against Drug, Ethanol, and Stress induced models. This study revealed the potential application of the ME plant as an herbal remedy for the ulcer, especially against stress-induced ulcers.
Keywords: *Mussaenda erythrophylla Schumach. & Thonn., Extraction, In vivo, Antiulcer.*

Received 23.02.2024 Revised 24.03.2023 Accepted 30.04. 2023

How to cite this article:
P. Hari Krishna, E. Sujatha, and S. Karunakar. *In-vivo* Antiulcer activity of Ethanolic extract of *Mussaenda erythrophylla* Schumach. & Thonn. Leaves. Adv. Biores., Vol 15 (3) May 2024: 220-226.

INTRODUCTION

Mussaenda erythrophylla Schumach. & Thonn. a member of the Rubiaceae family is a perennial evergreen shrub originally found in tropical regions of India and western Africa [1]. The plant has a complex branched taproot system and thrives best in warmly temperate and subtropical climates [2]. In cooler environments, it tends to be semi-deciduous. The shrub can grow up to 10 meters in height in its natural habitat, often climbing nearby trees [3]. However, under cultivation, it usually remains much more compact. Its distinct star-shaped flowers are about 10 mm in diameter and feature unique, brightly colored red, white, pink, or pale pink sepals [4].

The roots have traditional uses, and they are believed to be effective for treating coughs and jaundice, laryngopharyngitis, acute gastroenteritis, and dysentery and are thought to stimulate appetite when chewed. The plant has been the subject of numerous studies and contains various phytochemicals such as triterpenoids and glycosides, including specific compounds like Mussaendosides and Aureusidin. Research has reported diverse pharmacological activities, including diuretic, anti-inflammatory, anti-fertility, antioxidant, hepatoprotective, antithrombin, and antidiabetic activities, and has been consumed as food [5-9].

Ulcers, specifically peptic ulcers, are open sores that develop on the inner lining of the stomach, small intestine, or esophagus [10]. They are a global health concern with significant morbidity and mortality if left untreated [11]. The primary etiologic factors include *Helicobacter pylori* infection, prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs), and excess secretion of stomach acids [12]. Current pharmacological treatments, such as antacids and proton pump inhibitors, often provide temporary relief but come with side effects related to the digestive system, liver, and kidney [13, 14].

Inflammation is a key component of ulcer pathology; the anti-inflammatory attributes of ME make it a compelling subject for antiulcer research. This research aims to investigate the potential antiulcer properties of ME, leveraging its known pharmacological activities and phytochemical constituents. This plant offers a unique combination of anti-inflammatory and antioxidant properties, which may synergistically contribute to effective ulcer treatment. Investigating antiulcer activities with standard models could pave the way for a new generation of antiulcer medications with fewer side effects and higher efficacy.

MATERIAL AND METHODS

Plant collection and Authentication

ME leaves were collected from the local regions of Hyderabad in September.

Extraction

The leaves were shade-dried and pulverized to a coarse powder before being extracted. Then, 1 kg of the coarse powder of the leaves was transferred to a Soxhlet apparatus and extracted with absolute ethanol. Later, the solvent ethanol was evaporated under the vacuum using a Heidolph rotary evaporator to get the crude extract of ME leaves, followed by lyophilization to get its solid form.

Phytochemical Analysis

Powdered extracts of ME leaves were subjected to chemical identification tests to analyze secondary metabolites such as alkaloids, flavonoids, glycosides, steroids, tannins etc. Standard protocols were employed to identify the secondary metabolites in ME leaves [15].

Acute Toxicity Studies

The toxicity and dose for the antiulcer activity were estimated through acute toxicity on Swiss Albino Mice. The mice were procured from Sainath agencies, Musheerabad, weighing about 25-35g. Polypropylene cages were used to store the animals, and they were acclimatized to standard laboratory conditions for a week with standard rodent pellets (Golden Mohur Lipton India Ltd.) and free access to water. The temperature (25±2^{0}C), relative humidity (60%±10%), and 12-hour dark/light cycle was maintained throughout the experiment. The Institutional Animal Ethics Committee (IAEC) approved the study protocol before the commencement of experimental studies (1292/ac09/ CPCSEA/2020/3). Acute toxicity experiments were conducted according to the OCED 423 guidelines [16, 17].

Antiulcer activity

The antiulcer activity of the ME leaves was evaluated employing three different models. The doses for antiulcer activity were calculated from the acute toxicity studies and remained constant in three antiulcer models. Female Wistar rats were selected for the antiulcer activity evaluation. The animals were randomly selected for all models and divided into 5 groups, each containing 6 rats [18].

- Group 1 was a normal control that received only normal saline.
- Group 2 was ulcer control that received an ulcer-causing agent.
- Group 3 and group 4 were extract groups that received extract at a dose of 100mg/kg and 200mg/kg body weight, respectively.
- Group 5 was a reference group that received standard drug treatment (Esomeprazole at 20mg/kg body weight).

In each of the three experimental setups, rats received oral doses of ME extract at concentrations of 100 and 200mg/kg body weight, administered two hours before initiating ulcer formation as per the established protocols for each model. After six hours, the rats were humanely euthanized through an overdose of inhaled diethyl ether. Their stomachs were subsequently dissected along the major curvature, cleaned with saline to eliminate residual gastric material or blood, and inspected under a dissecting microscope with a 20×6.3 magnification. The aggregate length of all observable lesions in each stomach was quantified and designated as the Ulcer Index (UI). The percentage of ulcer inhibition was then determined using a specific formula:

$$[(UI_{Control} - UI_{Treated})/UI_{Control}] \times 100.$$

Indomethacin-induced antiulcer activity model

Ulcers in the stomach were artificially initiated in animals through the intraperitoneal administration of Indomethacin at 30 mg/kg following a 24-hour fasting period [19].

Ethanol-induced antiulcer activity model

A 24-hour fasting followed the oral delivery of 96% ethanol to induce gastric ulcers in the experimental animals [20].

Stress-induced antiulcer activity model

An adapted version of the procedure developed by Takagi and Okabe (1968) was adopted. Rats weighing 160-200 grams were isolated in individual compartments of a stress cage measuring 4.5 cm x 4.5 cm x 18

cm. Subsequently, the rats were partially submerged in a water bath, maintained at 19-21°C, up to their xyphoid levels to induce stress ulcers. Test substances were orally administered to the rats two hours before their immobilization. The rats were euthanized via ether overdose six hours post-immersion [21].

Results and Discussion

Extraction yields and sample preparation

Ethanol extraction of the leaves of ME delivered a yield of 5.2% from 1000 grams of the dried leaves.

Phytochemical analysis

The current study employed chemical identification tests for phytochemical analysis. The results of the phytochemical analysis were enumerated in Table 1. The chemical tests revealed that ME leaves contain all major secondary metabolites, such as alkaloids, flavonoids, glycosides, terpenoids, tannins, and steroids.

Table 1. Phytochemical analysis

S. No	Secondary metabolite	Ethanol extract
1	Alkaloids	+++
2	Flavonoids	+++
3	Glycosides	+
4	Terpenoids	++
5	Steroids	++
6	Saponins	++
'+' Present in Trace Amount '++' and '+++' Present in higher amounts		

Acute toxicity study

The outcomes of the acute toxicity studies for the ethanolic extract of ME are presented in Table 2. Following the administration of acute dosages, no discernable clinical toxicity or fatal symptoms were observed, even at elevated concentrations of 1000 mg/kg body weight. The treatment groups' consumption patterns were remarkably similar to those seen in the control group. These findings suggest that ME has a wide margin of safety.

Table 2. Effect of ME ethanolic extract on body weight in Swiss albino mice

Dose in mg/kg	Body weight in grams			Survived out of six animals
	Day 0	Day 7	Day 14	
100	30	32	30	6
250	29	30	3	6
500	32	29	33	6
1000	30	32	30	6
1500	27	30	33	4
2000	32	32	33	3

Antiulcer activity

Based on the results of acute toxicity studies, the dosage levels for evaluating antiulcer activity were chosen. These studies showed that 1000mg/kg of body weight of the ethanolic extract was safe. As a result, according to OECD guidelines, we chose to evaluate antiulcer activity in all experimental models at concentrations of 100 mg/kg and 200 mg/kg of body weight. In all test models, esomeprazole was given at a standard dosage of 20 mg/kg of body weight for comparison. The effects of this standard drug were then compared to the ulcer index and the rate of ulcer inhibition attributable to the tested extract.

Drug-Induced antiulcer activity

Table 3 and Figure 1 represent the data regarding the antiulcer potential of ME ethanol extract (MEE) compared to a control group with induced ulcers and a reference standard medication (Esomeprazole).

The Ulcer Index (UI) serves as a quantifiable metric for evaluating the severity of ulceration within the test subjects. Without any treatment, the Ulcer Control group (Group 2) displayed a UI of 13.21, signifying a high level of ulceration. Treatments with ME ethanol extract (MEE) showed a dose-dependent reduction in UI. When treated with a 100mg/kg dose of MEE, the UI reduced to 8.89 (Group 3) and further decreased to 5.64 when the dose was increased to 200mg/kg (Group 4). The reference standard, Group 5, had the lowest UI of 2.44, serving as the optimal benchmark for ulcer alleviation.

The percentage of Ulcer Inhibition provides another lens to assess the effectiveness of the treatments. The 100mg/kg MEE treatment in Group 3 achieved a moderate ulcer inhibition of 32.70%, indicating some efficacy but leaving room for improvement. On the other hand, the 200mg/kg MEE treatment in Group 4 was far more effective, inhibiting ulcers by 57.31%, thereby displaying significant therapeutic potential.

The reference standard, represented by Group 5, achieved an impressive 81.52% ulcer inhibition, setting a high bar for the effectiveness of any prospective antiulcer agents.

Table 3: Effect of ME on drug-induced Ulcer in rats

Group	AVERAGE (UI)	Standard Error Mean (SEM)	%Ulcer Inhibition
Group 1 (Normal Control)	0	0	--
Group 2 (Ulcer Control) (Indomethacin 30mg/kgb.w.)	13.21	0.92	--
Group 3 (Indomethacin 30mg/kgb.w.) + MEE 100mg/kgb.w.	8.89	0.37	32.70
Group 4 (Indomethacin 30mg/kgb.w.) + MEE 200mg/kgb.w.	5.64	0.40	57.31
Group 5 (Ref.Std) (Indomethacin 30mg/kgb.w.) + Esomeprazole 20mg/kgb.w.	2.44	0.10	81.52

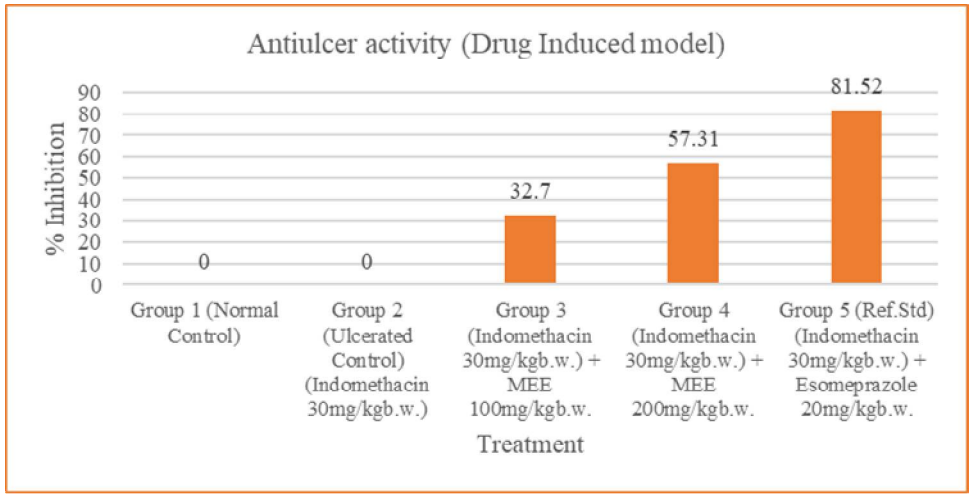

Figure 1: Graphical representation of results of the Antiulcer activity of ME leaves ethanol extract in the Stress-induced Ulcer model.

Ethanol Induced Antiulcer activity

The results of antiulcer activity of ethanolic extract of ME leaves against the drug-induced ulceration were enumerated in Table 4 and Figure 2. The group with ulcer induced by 96% ethanol (Group 2) had a significant UI of 12.18, serving as the control to measure the efficacy of treatments. The subsequent groups, treated with ME ethanol extract (MEE), showed a considerable reduction in UI in a dose-dependent manner. Specifically, Group 3, treated with a 100mg/kg dosage of MEE, exhibited a reduced UI of 8.78. More impressively, the UI further dropped to 6.23 in Group 4 when the MEE dosage was increased to 200mg/kg. The reference standard, Group 5, which was treated with Esomeprazole, had the least UI, coming in at 2.47, representing the most effective treatment in the study.

The percentage of Ulcer Inhibition provides an additional metric to assess the efficacy of the different treatments. The 100mg/kg dosage of MEE (Group 3) achieved 27.93% ulcer inhibition, indicating moderate therapeutic effectiveness. However, the 200mg/kg dosage of MEE (Group 4) performed even better, achieving nearly 48.81% ulcer inhibition, which showcases its substantial potential as an antiulcer agent. The reference standard, represented by Group 5 (Esomeprazole), achieved the highest efficacy with 79.70% ulcer inhibition.

Table 4: Effect of ME on Ethanol-induced Ulcer in rats

Group	AVERAGE (UI)	%Ulcer Inhibition or (%Biological action)
Group 1 (Normal Control)	0	--
Group 2 (Ulcerated Control) (96% Ethanol)	12.18±0.56	--
Group 3 (96% Ethanol) + MEE 100mg/kgb.w.	8.78±0.55	27.93
Group 4 (96% Ethanol) + MEE 200mg/kgb.w.	6.23±0.22	48.81
Group 5 (96% Ethanol) + Esomeprazole 20mg/kgb.w.	2.47±0.17	79.70

The values are expressed as Mean±SEM for

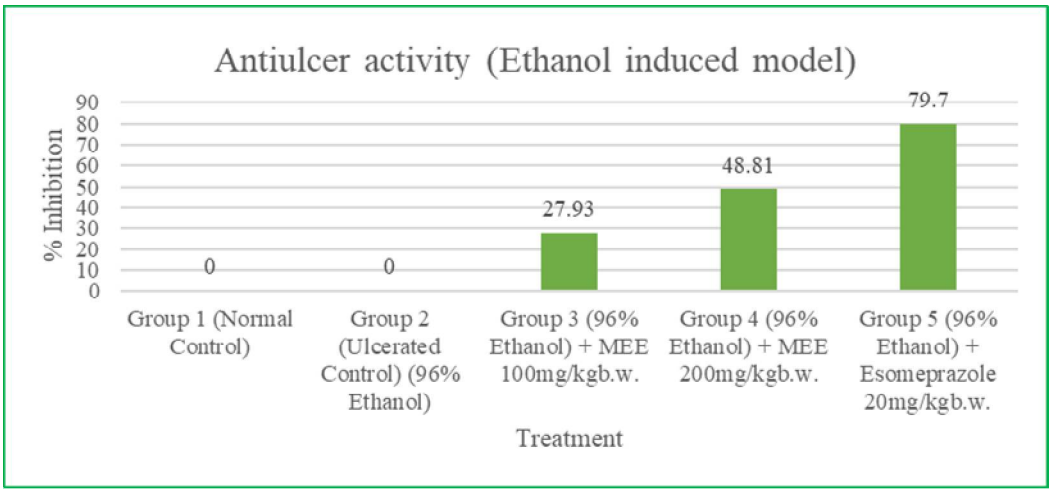

Figure 2: Graphical representation of results of the Antiulcer activity of ME leaves ethanol extract in the ethanol-induced Ulcer model.

Stress-Induced Antiulcer activity

The results of the antiulcer activity of ethanolic extract of ME leaves against the Stress-induced ulceration were enumerated in Table 5 and Figure 3. In the Stress-Induced Ulcer model, the Ulcer Index (UI) quantitatively measures ulcer severity. The Ulcerated Control group (Group 2), exposed to stress, had a notable UI of 11.89. This serves as the baseline against which the efficacy of various treatments is compared. The group treated with 100mg/kg of ME ethanol extract (MEE) (Group 3) saw a UI reduction to 7.76, suggesting a moderate antiulcer effect. This effect was considerably amplified in Group 4, where a 200mg/kg dosage of MEE resulted in a much lower UI of 3.38. The Esomeprazole-treated group (Group 5) represented the gold standard and had the lowest UI of 2.37, indicating the highest level of ulcer alleviation.

The treatment with 100mg/kg MEE (Group 3) achieved an ulcer inhibition of 34.76%, showing some therapeutic promise. However, the more prominent result came from the 200mg/kg MEE treatment (Group 4), which resulted in 71.52% ulcer inhibition, nearly matching the efficacy of the standard Esomeprazole treatment (Group 5), which had 80.07% ulcer inhibition.

Table 5: Effect of ME on Stress-induced Ulcer in rats

Group	AVERAGE (UI)	Standard error mean (SEM)	%Ulcer Inhibition or (%Biological action)
Group 1 (Normal Control)	0	0	--
Group 2 (Ulcerated Control) (Stress)	11.89	0.60	--
Group 3 (Stress) + MEE 100mg/kgb.w.	7.76	0.38	34.76
Group 4 (Stress) + MEE 200mg/kgb.w.	3.38	0.23	71.52
Group 5 (Stress) + Esomeprazole 20mg/kgb.w.	2.37	0.16	80.07

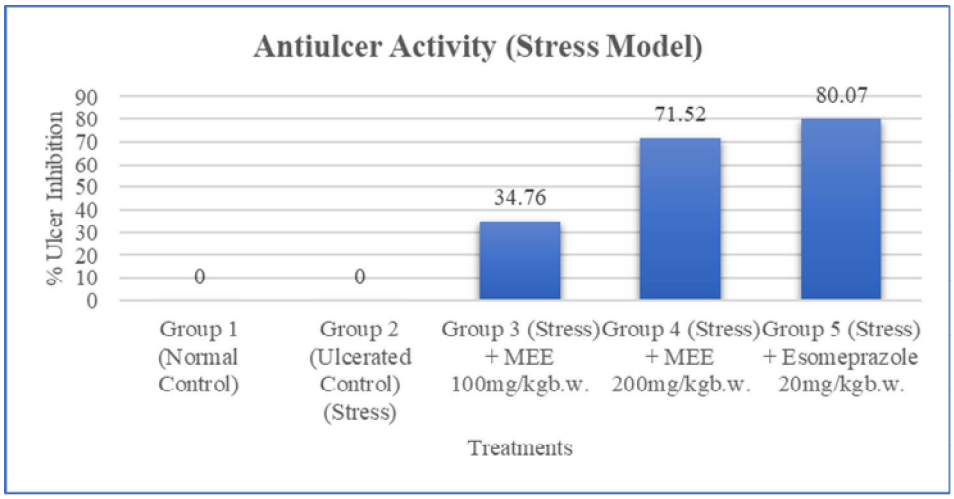

Figure 3: Graphical representation of results of the Antiulcer activity of ME leaves ethanol extract in the Stress-induced Ulcer model.

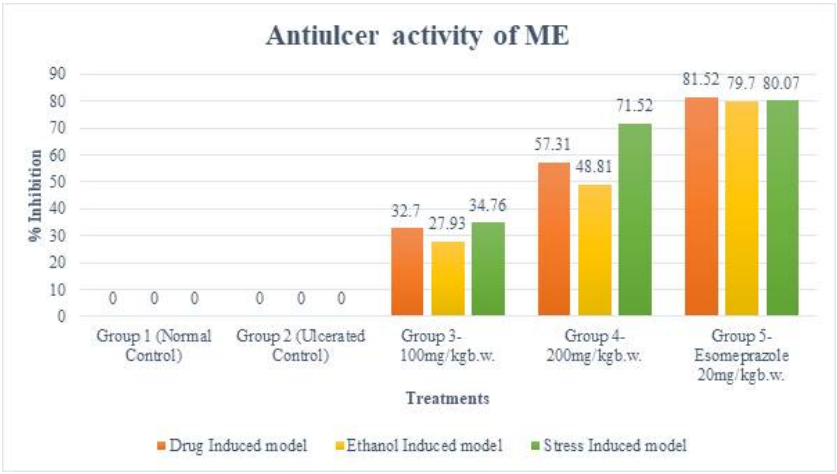

Figure 4: Relative Graphical representation of results of Antiulcer activity of ME leaves ethanol extract in all tested models.

In a comprehensive analysis of the anti-ulcerative effects of ME ethanol extract (MEE) across three different ulcer models consistently demonstrated its ability to reduce ulcer severity, as measured by the Ulcer Index (UI), and increase ulcer inhibition percentage. This efficacy was most notably dose-dependent, meaning higher doses significantly reduced UI and ulceration (Figure 4).

The stress-induced ulcer model revealed the most promising results, where MEE achieved an ulcer inhibition rate close to standard antiulcer medication, Esomeprazole. This suggests that MEE may have specific therapeutic components that are highly effective against stress-related gastric ulceration.

In the drug-induced and ethanol-induced models, while the efficacy of MEE was less than that of Esomeprazole, the results were still significant. Achieving up to 57.31% and 48.81% ulcer inhibition suggests that MEE could serve as an alternative or complementary treatment in cases where conventional medications may not be suitable or where a natural remedy is preferred.

The consistency of MEE's effectiveness across multiple ulcer-inducing models suggests that the plant extract could have broad-spectrum antiulcer properties. The dose-dependent nature of its efficacy indicates the potential for optimization in therapeutic applications. While it may not fully replace standard treatments, MEE is a promising candidate for developing new antiulcer medications or as a complementary therapy.

CONCLUSION

Across drug-induced, ethanol-induced, and stress-induced ulcer models, ME ethanol extract (MEE) consistently reduced ulcer severity dose-dependently. Its nearly 72% ulcer inhibition in the stress-induced model was most promising. With up to 57% and 49% inhibition in drug and ethanol models respectively, MEE emerges as a viable alternative or supplement to conventional treatments. Its consistent efficacy across models suggests broad-spectrum antiulcer potential. MEE's significant, dose-

dependent efficacy makes it a candidate for further research in developing alternative or complementary antiulcer therapies.

REFERENCES

1. Singh V, Pande, PC, Jain DK. A text book of botany-Angiosperms, Ist edition; 2000: pp. 118-122.
2. Nadkarni K.M., Nadkarni A.K., Chopra R.N. Indian Materia Medica, Vol. I Popular Prakashan: Bombay,1976: pp. 548-550.
3. Xu Junping, Xu Rensheng, Luo Zi, Dong Jieying. New Triterpenodal Saponins from Folk Contraceptive Medicine, *Mussaenda Pubesecus*. Acta Chimica Sinica, June 15, 1991.
4. Raju, N. Jaya, B. Ganga Rao. (2011). Anthelmintic activities of *Antigonon leptopus* Hook and *Mussaenda erythrophylla* Lam. International Journal of Pharmacy and Pharmaceutical Sciences 3, no. 1: 68-69.
5. Hariharan, M., A. Rajan. (2009). Biomarker For Genotoxicity-Evaluation Of The Antineoplastic Activity of *Mussaenda Erythrophylla* In Cultured Hela Cells. New Biotechnology 25: S284.
6. D. Vinothkumar, S. Murugavelh, A. Kethsy. (2011). Prabhavathy Phytosociological and ethnobotanical studies of sacred groves in Pudukottai district, Tamil Nadu, India Asian Journal of Experimental Biology Science, 2; pp. 306-315
7. D. Amutha, S. Shanthi, V. Mariappan. (2012). Anti-inflammatory effect of *Mussaenda erythrophylla* (L) in carrageenan-induced lung inflammation International Journal of Pharmacy and Pharmaceutical Sciences, 4, pp. 344-347.
8. S. Deborah, S.P. Anand, G. Velmurugan. (2017). Evaluation of in vitro anticancer activity of *Mussaenda erythrophylla* (L.) fruits ethanolic extract against human breast cancer Journal of Herbal Medicine, 5, 110-113
9. Suresh Arumugam et al. (2021). Pharmacological Evaluation of *Mussaenda erythrophylla* Leaves Extract for Analgesic, Anti-Inflammatory, and Antipyretic Activities. Ijppr.Human, Vol. 22 (2): 114-127
10. Narayanan M, Reddy KM, Marsicano E. (2018). Peptic Ulcer Disease and Helicobacter pylori infection. Mo Med.;115(3):219-224
11. Yibirin M, De Oliveira D, Valera R, Plitt AE, Lutgen S. Adverse Effects Associated with Proton Pump Inhibitor Use. Cureus. 2021 Jan 18;13(1):e12759.
12. Drini M. Peptic ulcer disease and non-steroidal anti-inflammatory drugs. Aust Prescr. 2017 Jun;40(3):91-93.
13. Dixon MF. Pathology of Gastritis and Peptic Ulceration. In: Mobley HLT, Mendz GL, Hazell SL, editors. *Helicobacter pylori*: Physiology and Genetics. Washington (DC): ASM Press; 2001. Chapter 38.
14. Barkin, J. (1998). The relation between *Helicobacter pylori* and non-steroidal anti-inflammatory drugs. The American Journal of Medicine, 105(5), 22S-27S.
15. J.B. Harborne.(1984). Phytochemical methods Chapman and Hall Ltd, LondonNew York. pp. 49-188
16. Variya, B.C., Bakrania, A.K., Madan, P. and Patel, S.S., (2019). Acute and 28-days repeated dose sub-acute toxicity study of gallic acid in albino mice. Regulatory Toxicology and Pharmacology, 101, pp.71-78.
17. OECD (2002), Test No. 423: Acute Oral toxicity - Acute Toxic Class Method, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.
18. Zewdu, W.S. and Aragaw, T.J., (2020). Evaluation of the antiulcer activity of hydromethanolic crude extract and solvent fractions of the root of *Rumex nepalensis* in rats. Journal of Experimental Pharmacology, 12, p.325.
19. Borra, S.K., Lagisetty, R.K. and Mallela, G.R., (2011). Antiulcer effect of Aloe vera in non-steroidal anti-inflammatory drug induced peptic ulcers in rats. African Journal of Pharmacy and Pharmacology, 5(16), pp.1867-1871.
20. Sistani Karampour, N., Arzi, A., Rezaie, A., Pashmforoosh, M., & Kordi, F. (2019). Gastroprotective Effect of Zingerone on Ethanol-Induced Gastric Ulcers in Rats. Medicina (Kaunas, Lithuania), 55(3), 64.
21. Di Cerbo, A., Carnevale, G., Avallone, R., Zavatti, M. and Corsi, L., (2020). Protective Effects of *Borago officinalis* (Borago) on Cold Restraint Stress-Induced Gastric Ulcers in Rats: A Pilot Study. Frontiers in Veterinary Science, 7. https://doi.org/10.3389/fvets.2020.00427