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Evaluation of Protective Effect of Aegle Marmelos Fruit (BAEL) On Diclofenac Sodium Induce Gastrointestinal Toxicity

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ABSTRACT

Aegle marmelos, a native plant of India, is a significant medicinal plant with ethnomedicinal applications in traditional and folk systems. It is grown as a temple garden plant and its leaves are used to pray Lord Shiva. The plant is considered sacred by Hindus and its fruits are used in traditional medicine and as food. A study aimed to assess the protective effect of Aegle marmelos fruit on gastrointestinal toxicity in mice. The study investigated the phytochemical constituents and medicinal value of Aegle marmelos fruit extracts, conducting GC-MS chromatography and analyzing antioxidant, TPC, TFC, toxicity, and anti-gastrointestinal toxicity properties of these fruit extracts. Results showed that AEAM and MEAM were safe up to a dose of 2000 mg/kg body weight without causing drug-related toxicity, mortality, abnormal clinical signs, remarkable body weight, or gross pathological changes. AEAM and MEAM significantly influenced the volume of gastric juice, pH, and free acidity of gastric juice. The administration of Omeprazole and AEAM and MEAM significantly influenced oxidative stress parameters in mice. The study also revealed that diclofenac administration caused significant alterations in the EC group compared to normal rats. Pretreatment with AEAM and MEAM attenuated the damage due to diclofenac administration, with lesser degree of tissue necrosis and hemorrhage. In conclusion, AEAM and MEAM of Aegle marmelos fruits proved to be a Gastroprotective agent against Diclofenac-induced GIT toxicity.

Keywords: *Aegle marmelos; Diclofenac-induced GIT toxicity; Inflammatory cytokines, Oxidative stress parameters.*

1. INTRODUCTION

Aegle marmelos is a native plant of India. A. marmelos belongs to Rutaceae family and commonly known as also known as bael, Bengal quince, golden apple, stone apple, wood apple, bili. In India, A. marmelos is grown as a temple garden plant and the leaves are used to pray Lord Shiva. A. marmelos is an important medicinal plant with several ethnomedicinal applications in traditional and folk medicinal systems. Traditionally, A. marmelos is used in the treatment of diarrhea and dysentery. Leaves of this plant used to cause infertility/abortion in women. It is present throughout Southeast Asia as a naturalized species. The tree is considered to be sacred by Hindus. Its fruits are used in traditional medicine and as a food throughout its range (1-3). The study aims to assess the protective effect of Aegle marmelos fruit on gastrointestinal toxicity in mice. It will investigate the phytochemical constituents and medicinal value of Aegle marmelos fruit extracts, conduct GC-MS chromatography, and analyze antioxidant, TPC, TFC, toxicity, and anti-gastrointestinal toxicity properties of these fruit extracts.

2. MATERIALS AND METHODS

2.1 Acute Toxicity Study

Total 6 rats of 10-12 weeks age were selected and randomly divided into 2 groups. Group I was vehicle control group which received vehicle (gum acacia 1% w/v in distilled water) while group II was test group that received aqueous and methanolic extracts of Aegle marmelos fruit (AEAM and MEAM). Each group consisted of 3 animals (females). Females were nulliparous and non-pregnant [4].

2.2 Effect of aqueous and methanolic extracts of Aegle marmelos fruit (AEAM and MEAM) on Diclofenac Sodium induce gastrointestinal toxicity in mice

Animals were randomly allocated into 7 groups (70 rats each). Mice of the 1st group received only saline intraperitoneally and served as normal control group. Group 2 received diclofenac (25 mg/kg/day, I.P.) for 10 days and served as diclofenac-control group. Group 3 received diclofenac (25 mg/kg/day, I.P.) for 10 days and Omeprazole (40 mg/kg/day, P.O.). Groups 4 and 5 received diclofenac (25 mg/kg/day, I.P.) for 10 days and AEAM (150 and 300 mg/kg/day, P.O.) respectively for 30 days. Groups 6 and 7 received diclofenac (25 mg/kg/day, I.P.) for 10 days and MEAM (150 and 300 mg/kg/day, P.O.) respectively for 30 days. All animals were sacrificed 24 h after the last treatment after overnight fasting (5).

2.3 Evaluating Parameters

The examination of Volume and pH of gastric juice, Total acidity and free acidity of gastric juice, Mucin content of gastric juice and Pepsin contents of gastric juice. Oxidative stress parameters in homogenate are estimated using a radio immunoassay technique. The study aims to provide valuable insights into the health and lipid profile of rats and their potential treatment options. The study involved preparing a suspension of stomach tissue. The tissue was washed, homogenized, and centrifuged to prepare a 10% w/v suspension. The supernatant was used to assess lipid peroxidation (LPO), catalase (CAT), superoxide dismutase (SOD) activity, and reduced glutathione (GSH) content. LPO was assessed using a method that involved adding sodium dodecyl sulfate, acetic acid, and thiobarbituric acid to the tissue homogenate. SOD activity was measured using a spectrophotometer, and catalase activity was measured using a spectrophotometer. Reduced glutathione content was measured using precipitating buffer and a test tube containing phosphate buffer and DTNB reagent. The concentrations of TNF-a, IL-6, and IL-10 in brain supernatants were estimated using commercial ELISA kits. Stomach were preserved in 10% buffered formalin and processed using a tissue processor. The tissues were then embedded in paraffin and sections were cut into sections. A scoring system was used to evaluate the loss of stomach and the appearance of hypertrophy stomach [6-9].

3. RESULTS AND DISCUSSION

3.1 Acute Toxicity Study

It was noted that the LD50 of the test substance (aqueous and methanolic extracts of Aegle marmelos fruit (AEAM and MEAM) was greater than 2000 mg/kg of body weight. Based on the observation that was made during the toxicity studies, it is possible to draw the conclusion that AEAM and MEAM were safe up to a dose of 2000 mg/kg body weight. This conclusion is based on the fact that an oral dose of 2000 mg/kg body weight did not cause drug-related toxicity and mortality, abnormal clinical signs, remarkable body weight, or gross pathological changes in the animals. The test chemical is categorised as "unclassified" or "category - 5" in accordance with the Globally Harmonised approach. This is due to the fact that its LD50 was shown to be greater than 2000 mg/kg body weight.

3.2 Effect of aqueous and methanolic extracts of Aegle marmelos fruit (AEAM and MEAM) on Diclofenac Sodium induce gastrointestinal toxicity in mice

The study found that AEAM and MEAM significantly influenced the volume of gastric juice, with a significant decrease observed after pretreatment with AEAM and MEAM (300 mg/kg). However, lower doses (150 mg/kg) did not show a significant decrease in volume compared to the control group (NC). Omeprazole also showed a significant decrease in volume compared to the NC group. Pretreatment with Omeprazole and AEAM and MEAM increased the pH of gastric juice, while lower doses (150 mg/kg) did not show any significant effect. Omeprazole and AEAM and MEAM significantly decreased the total acidity of gastric juice, while lower doses (150 mg/kg) did not show a significant decrease. Furthermore, Omeprazole and AEAM and MEAM significantly decreased the free acidity of gastric juice, with a significant decrease observed after pretreatment. However, lower doses (150 mg/kg) did not show a significant decrease in free acidity compared to NC (Table 1).

Table 1: Effect on various parameters on Diclofenac Sodium induce gastrointestinal toxicity in mice

Group	Group Name	Volume of gastric juice (mL/100 g)	pH of gastric juice	Total Acidity (meq/L/100 g)	Free Acidity (meq/L/100 g)
G-I	Normal Control	3.56 ± 0.124	1.9 ± 0.154	50.99 ± 0.122	23.89 ± 0.124
G-II	Diclofenac Control	4.15 ± 1.254	0.5 ± 0.254	55.14 ± 0.542	26.91 ± 0.254
G-III	Omeprazole	1.99 ± 0.456	2.5 ± 0.356	47.44 ± 0.446	22.99 ± 0.356

	(40 mg/kg, BW)				
G-IV	AEAM-I (150 mg/kg, BW)	3.99 ± 0.488	1.9 ± 0.088	51.25 ± 0.901	23.44 ± 0.088
G-V	AEAM-II (300 mg/kg, BW)	2.84 ± 0.351	2.1 ± 0.301	48.45 ± 0.771	22.09 ± 0.301
G-VI	MEAM-I (150 mg/kg, BW)	3.45 ± 0.111	1.8 ± 0.191	50.41 ± 0.411	29.88 ± 0.454
G-VII	MEAM-II (300 mg/kg, BW)	2.04 ± 0.125	2.4 ± 0.335	46.99 ± 0.512	21.08 ± 0.125

Values are mean ± SEM

3.3 Effect on Ulcer Score, Ulcer Index and pepsin content of gastric juice

The study found that Omeprazole (40 mg/kg) and AEAM and MEAM (300 mg/kg) significantly influenced the ulcer score and ulcer index. Pretreatment with lower doses of AEAM and MEAM did not show a significant decrease in ulcer score compared to NC. However, the ulcer index decreased significantly with pretreatment with lower doses (150 mg/kg). Additionally, Omeprazole and AEAM and MEAM significantly decreased pepsin content in the gastric juice, while lower doses did not show a significant decrease in pepsin content compared to NC. These findings suggest that these medications may have potential therapeutic benefits for ulcer treatment (Figure 1-3).

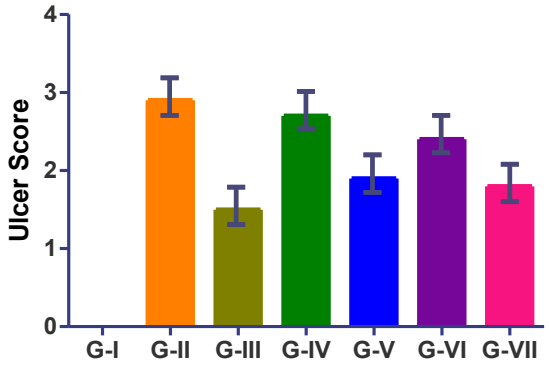

Figure 1: Effect on Ulcer Score (G-I, G-II, G-III, G-IV, G-V, G-VI and G-VII control groups, including normal control, Diclofenac control, omeprazole, AEAM-I, AEAM-II, MEAM-I, and MEAM-II, respectively).

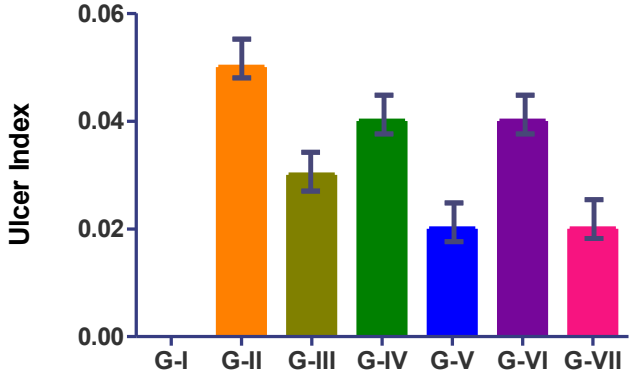

Figure 2: Effect on Ulcer Index (G-I, G-II, G-III, G-IV, G-V, G-VI and G-VII control groups, including normal control, Diclofenac control, omeprazole, AEAM-I, AEAM-II, MEAM-I, and MEAM-II, respectively).

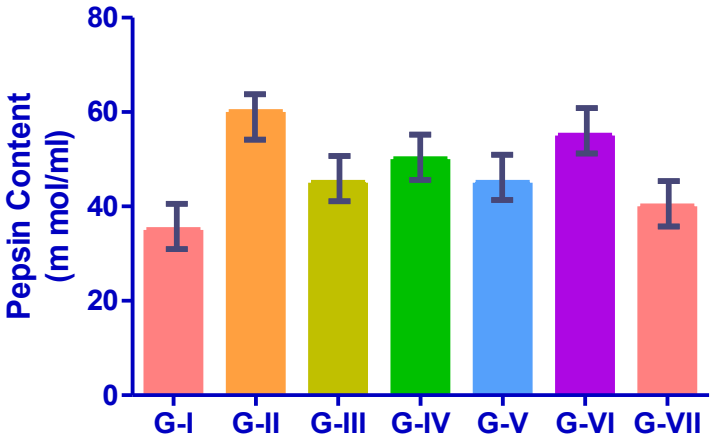

Figure 3: Effect on pepsin content of gastric juice (G-I, G-II, G-III, G-IV, G-V, G-VI and G-VII control groups, including normal control, Diclofenac control, omeprazole, AEAM-I, AEAM-II, MEAM-I, and MEAM-II, respectively).

3.4 Oxidative Stress Parameters

One-way ANOVA showed that administration of Omeprazole (40 mg/kg) and AEAM and MEAM (300 mg/kg) exhibited significantly ($P<0.01$-$P<0.01$) influenced the Oxidative stress parameters level in mice. The post hoc test exhibited that there is significant ($P<0.001$) altered the Oxidative stress parameters level in Experimental Control mice compared to Normal Control mice. Concurrent treatment with Omeprazole (40 mg/kg) and AEAM and MEAM (300 mg/kg) exhibited significant ($P<0.01$-$P<0.01$) decline in Oxidative stress parameters level as compared to EC group mice. The treatment also significantly ($P<0.001$) affects the Oxidative stress parameters level as compared to EC mice (Figure 4-7).

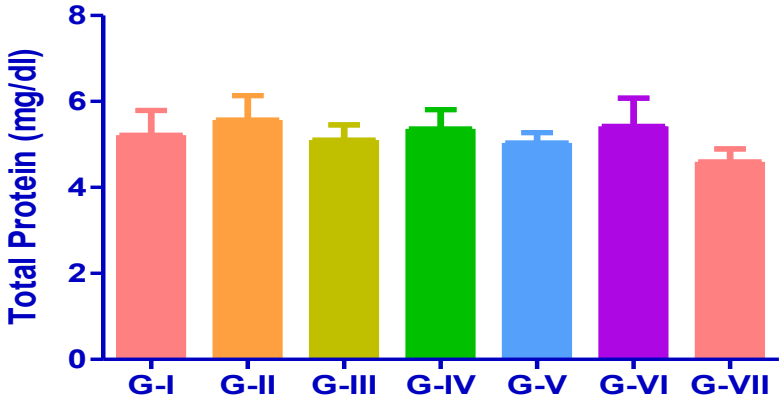

Figure 4: Estimation of Total Protein in Stomach Homogenate

(G-I, G-II, G-III, G-IV, G-V, G-VI and G-VII control groups, including normal control, Diclofenac control, omeprazole, AEAM-I, AEAM-II, MEAM-I, and MEAM-II, respectively)

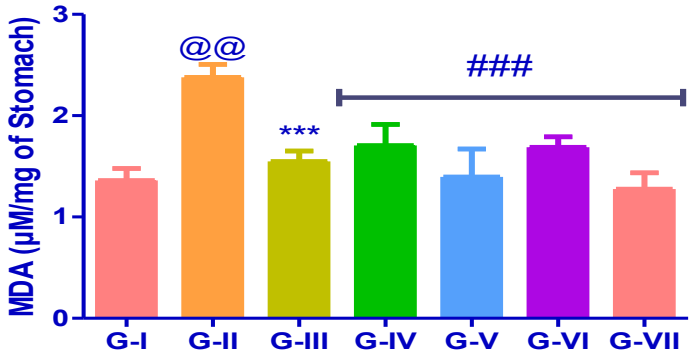

Figure 5: Estimation of LPO in Stomach Homogenate

(G-I, G-II, G-III, G-IV, G-V, G-VI and G-VII control groups, including normal control, Diclofenac control, omeprazole, AEAM-I, AEAM-II, MEAM-I, and MEAM-II, respectively)

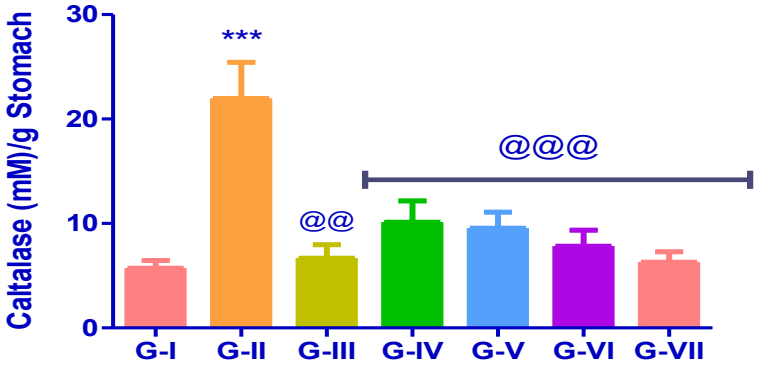

Figure 6: Estimation of Catalase in Stomach Homogenate

(G-I, G-II, G-III, G-IV, G-V, G-VI and G-VII control groups, including normal control, Diclofenac control, omeprazole, AEAM-I, AEAM-II, MEAM-I, and MEAM-II, respectively)

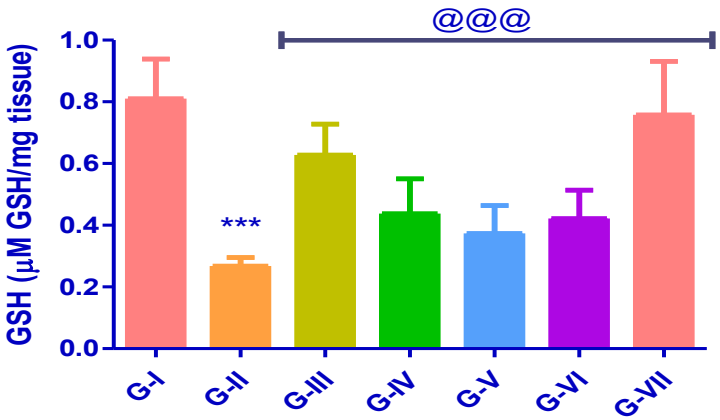

Figure 7: Estimation of Reduced Glutathione (GSH) in Stomach Homogenate (G-I, G-II, G-III, G-IV, G-V, G-VI and G-VII control groups, including normal control, Diclofenac control, omeprazole, AEAM-I, AEAM-II, MEAM-I, and MEAM-II, respectively)

Effect on Histology of Stomach

Kruskal-Wallis analysis of total histomorphological score and the photomicrographs illustrated that diclofenac administration (Figure 8B) caused significant ($P<0.001$) alterations as indicated by severe thickened mucosa, infiltration of inflammatory cells, sloughing of mucosa and tissue necrosis and mild hemorrhage in EC group as compared to those of normal mice (NC)

(Figure 8A) which showed normal architecture. Pretreatment with AEAM and MEAM attenuated ($P<0.05$) the damage due to diclofenac administration as indicated by lesser degree of tissue necrosis and hemorrhage and prevention of the damage (Figure 8D and 8E) compared to EC group (Figure 8B) while AEAM and MEAM at low doses showed non-significant ($P>0.05$) prevention of damage. Omeprazole also exhibited ($P<0.01$) marked reduction in the damage caused by diclofenac administration (Figure 8C). The histological observations quantified as histomorphological score are indicated in Table 2.

Table 2: Summary of Histological Changes

Parameters	Groups						
	G-I	G-II	G-III	G-IV	G-V	G-VI	G-VII
Thick Mucosa	4.0 ± 1.024	0 ± 0.569	3.55 ± 0.598	2.88 ± 1.111	3.45 ± 0.598	2.77 ± 1.007	3.67 ± 0.984
Inflammation	3.55 ± 0.541	0.17 ± 0.447	2.99 ± 0.333	2.44 ± 0.024	3.01 ± 0.111	2.88 ± 1.024	3.41 ± 0.651
Hemorrhage	3.78 ± 1.024	0.5 ± 0.024	3.88 ± 0.541	2.11 ± 0.398	3.01 ± 1.024	2.99 ± 1.024	3.44 ± 0.484
Degenerative Changes	4.1 ± 0.651	1.21 ± 0.398	3.98 ± 0.417	2.5 ± 0.441	3.8 ± 0.111	2.9 ± 0.099	3.7 ± 1.024
Necrosis	4.0 ± 0.444	0 ± 0.441	3.77 ± 0.111	2.44 ± 1.024	3.44± 0.651	3.01 ± 0.398	3.88 ± 0.541
Total Histological Score	17.11 ± 0.988	1.99 ± 1.024	15.10 ± 0.417	10.59 ± 0.786	14.59 ± 0.444	11.59 ± 0.441	14.88 ± 0.222

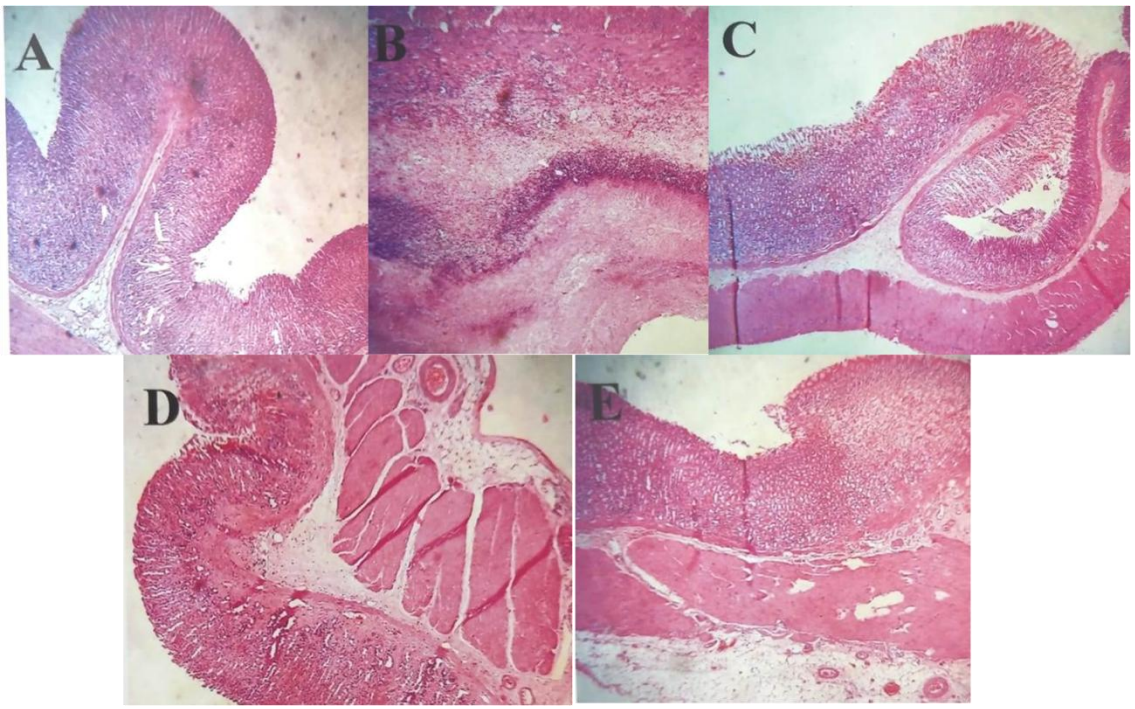

Figure 8: Effect of AEAM and MEAM (300 mg/kg, BW) on Histology of Stomach

4. CONCLUSION

The study investigated the effects of Aegle marmelos (L.) fruits extracts (AEAM and MEAM) on mice' body weight. Results showed that AEAM and MEAM were safe up to a dose of 2000 mg/kg body weight, without causing drug-related toxicity, mortality, abnormal clinical signs, remarkable body weight, or gross pathological changes. AEAM and MEAM significantly influenced the volume of gastric juice, pH, and free acidity of gastric juice. The administration of Omeprazole and AEAM and MEAM significantly influenced oxidative stress parameters in mice. Post hoc tests showed a significant increase in TNF-α and IL-6 levels in Experimental Control mice compared to Normal Control mice. Concurrent treatment with Omeprazole and AEAM and MEAM (300 mg/kg) showed a significant decline in TNF-α levels compared to EC group mice. The study also revealed that diclofenac administration caused significant alterations in the EC group compared to normal mice. Pretreatment with AEAM and MEAM attenuated the damage due to diclofenac administration, with lesser degree of tissue necrosis and hemorrhage. Omeprazole also exhibited a marked reduction in the damage caused by diclofenac administration. In conclusion, AEAM and MEAM of fruits of Aegle marmelos (L.) proved to be a Gastroprotective agent against Diclofenac-induced GIT toxicity.

5. CONFLICT OF INTEREST

None

REFERENCES

[1] Sabu MC, Kuttan R. Antidiabetic activity of Aegle marmelos and its relationship with its antioxidant properties. Indian Journal of physiology and pharmacology. 2004 Jan 1;48(1):81-8.

[2] Kushawaha H, Singhai AK. Aegle marmelos: A Medicinal Plant from a Traditional Indian Perspective. AJBR. 2024 Nov. 16;27(4S):15308-16.

[3] Kushawaha H, Singhai AK. Pharmacognostical Evaluation of Methanolic and Aqueous Extract of Aegle marmelos L. J Neonatal Surg [Internet]. 2025 Jun.18;14(32S):754-9.

[4] Halim SZ, Abdullah NR, Afzan A, Rashid BA, Jantan I, Ismail Z. Acute toxicity study of Carica papaya leaf extract in Sprague Dawley rats. Journal of Medicinal Plants Research. 2011 May 18;5(10):1867-72.

[5] Omer D, Tijen KT, Fatma S, Kaplan Yusuf C, Baris K, Selen AB, Gokhan K. Effect of diclofenac on colonic motility in experimental irritable bowel syndrome. Indian Journal of Experimental Biology. 2025 Apr 1;63(4).

[6] Elbouri N, Alsherif N, Elbadri S, Mohamed S. Exploring the Hematological Risks of Diclofenac Sodium: An Experi-mental Study in Wistar Rats. Libyan Medical Journal. 2025 May 4:178-84.

[7] Arendt N, Lennernäs H, Heindryckx F, Sjöblom M. Irinotecan induces intestinal atrophy and compromises duodenal motility and mucosal permeability: in vivo and in vitro evaluations. bioRxiv. 2025 Mar 17:2025-03.

[8] Nausheen N, Ahmed MM, Rahaman MM. Evaluation Of Anti-Arthritic Activity Of Proton Pump Inhibitors In Freund's Adjuvant Induced Arthritic Rats. Journal of Contemporary Clinical Practice. 2025 May 15;11:286-91.

[9] OMORODION N, BLANKSON E. HISTOPATHOLOGICAL ASSESSMENT OF PAX HERBAL'S EFFECTIVENESS IN TREATING DICLOFENAC-INDUCED GASTRIC ULCERS IN ALBINO RATS. African Journal of Health, Safety and Environment. 2025 Jun 4;6(1):89-99.