ISSN: 2977-6147

Research Article

Open Access Journal of Clinical Images

Haemotoxicological Studies of Ethanolic Extract of Pericarp of Dried Mature Fruits of Emblica Officinalis Gaertn. In Swiss Albino Rats

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Received: October 28, 2025; **Accepted:** November 10, 2025; **Published:** November 16, 2025

ABSTRACT

Embilica officinalis has been traditionally used for many purposes through decades. This study aims to evaluate the safety of the ethanolic fruit extract of the dried mature Pericarp of Embilica officinalis. The extract was administered for 28 days at 50 mg/kg and 400 mg/kg in Swiss albino rats. After 28 days of the administration, the rats were sacrificed to evaluate the haematological profile. The result shows that, in case of WBC parameter, the extract has a highly significant effect on total WBC (↑ 24.01% increase in 50mg/kg and ↓66.4% decrease in 400mg/kg), Neutrophil (↓ 24.16% decrease in 50 mg/kg and 34.61% in decrease in 400 mg/kg) and Lymphocyte (15.16% increase in 500 mg/kg); in case of RBC, there was significant effect on Hematocrit (8.7% decrease in 50 mg/kg); in case of platelet, there is significant effect on the total platelet (10.53% decrease in 400 mg/kg) and Plateletcrit (11.37 % decrease in 400 mg/kg). From the data, it can be safely concluded that this medication has a direct effect on haematopoiesis or is an immune booster, but in case of prolonged use at a higher dose, one should look for thrombocytopenia or any problem associated with it.

Keywords: Haematology, WBC, RBC, Platelet, Safety, Toxicity

Introduction

Embilica officinalis belongs to the family Euphorbiaceae and is also known as amla, aonla, and Indian gooseberry. It is widespread in tropical and subtropical countries like Bangladesh, Myanmar, India, Pakistan, Sri Lanka, and Uzbekistan [1]. The 2010-2030 UN decades on ecosystem restoration strongly emphasize the need to support sustainable development, boost biodiversity, fight climate change, and restore ecosystems. One of the best methods to accomplish these objectives is to cultivate and preserve the native, phytochemically rich plant amla. A mainstay of ancient medical systems, especially Ayurveda, for millennia, amla is well-known for its abundant nutritional profile and therapeutic qualities [2]. In Ayurveda, Embilica officinalis is regarded as an immune-modulator and revitalizing plant. Amla has long been a mainstay in traditional medical systems, especially Ayurveda, due to its rich nutritional profile and therapeutic qualities [3]. In Ayurveda, amla is primarily used in combination with two other medicinal species, Bibhitaki (Terminalia bellirica) and Haritaki (Terminalia chebula), known as "Triphala." Amla has deeply rooted ethnomedicine properties, and researchers have extensively studied the medicinal properties of its various parts, particularly the Pericarp (the outer layer of the fruit). Preclinical studies have supported the traditional use of Amla for treating carcinogens, tumours, and genotoxicity due to its anti-inflammatory and antioxidant properties [4]. In addition to treating scurvy, diarrhoea, and dysentery, it is also used with iron to treat jaundice, anaemia, and dyspepsia. A significant amount of tannin and flavonoid, vitamin C, embicol, gallic acid, phyllembin, phyllembic acid, micic acid, and fat are among its other nutrients [5].

To identify the underlying toxicity mechanism and offer recommendations for suitable dosage and safety profiling, hemotoxicology investigates the detrimental effects of different chemicals on blood components and makers. It aids in figuring out whether the plant's bioactive substances negatively impact

Citation: Kamrun Nahar, Md Shanawes Hossen Rasel, Mohammad Abbas Gani, Fatema Tuj Johora Faria, Anika Tabassum Sumaya, et al. Haemotoxicological Studies of Ethanolic Extract of Pericarp of Dried Mature Fruits of Emblica Officinalis Gaertn. In Swiss Albino Rats. Open Access J Clin Images. 2025. 2(4): 1-6.

DOI: doi.org/10.61440/OAJCI.2025.v2.24

blood cells and overall haematological health. This is crucial to verify the safety of traditional medicines and ensure that using them therapeutically doesn't have any unintended toxic effects [6,7].

Ayurvedic medications and herbs are comparatively reliable and cost-effective. The Health and Population Service Program (HPSP) began implementing a strategy in 1998 to integrate alternative medicine into 30 district hospitals to introduce and establish the Ayurvedic Medical System. Hundreds of manufacturers have participated in producing and marketing ayurvedic medications, using herbs as key components. Based on the aforementioned facts and factors, this research has examined the chronic toxicological profile of Emblica officinalis Gaertn, a commercially available Ayurvedic therapeutic component [8,9].

The growing use of herbal remedies and supplements in modern healthcare further demonstrates the importance of this study. The Pericarp of dried ripe fruits of Emblica officinalis has been extensively studied for its medicinal benefits; nevertheless, the extract's hemotoxicological effects have not been sufficiently examined. Its safety profile needs to be thoroughly investigated, especially in light of blood-related toxicity. This study analyses several haematological measures to determine the relationship between Amla and its effects on blood parameters. The goal is to understand better the plant's hemotoxicological effects and potential therapeutic advantages while ensuring the safe usage of Emblica officinalis pericarp in clinical settings.

Materials and Methods

Collection and Extraction of Emblica Officinalis Pericarp of Dried Mature Fruits

For this study, Emblica officinalis pericarp of dried mature fruits were collected from Chittagong at Sri Kundeswari Aushadhalaya Ltd, For the toxicological experiment, 1 kg of Emblica officinalis pericarp of dried mature fruits was extracted with ethyl alcohol to yield 30 g of extract, and suspension of this resinous extract was administered at a suitable volume which allows optimal dosage accuracy without affecting the total increase in the body fluid.

Route of Administration

The drug was administered orally at 50 and 400 mg/kg body weight, and ketamine was administered intra-peritoneally (500 mg/kg i.p).

Experimental Animal

Healthy Albino male rats of eight-week-old (50-70 g) were maintained at the Animal House of the Department of Pharmacy, Jahangirnagar University. Prior to the experiment, rats were randomly divided into 2 groups of 5 animals each. Thus, eight rats were taken for the control and five rats for each group of the low and high dose groups of the experimental animals.

Control Groups

A group of eight rats was concurrently employed in the experiment, and this group served as the control. They were administered with distilled water as a placebo in the same volume as the drug-treated group for the same number of days.

Animal Care

All rats were kept in plastic cages with dimensions of 30 x 20 x 13 cm, and soft wood shavings were employed as bedding in the cages. Feeding of animals was done ad libitum, along with drinking water, and maintained at a natural day-night cycle. The animals were housed in a well-ventilated, hygienic experimental animal house. Constant environmental parameters with adequate nutritional conditions were maintained. The rats were fed with "mouse chow" (prepared according to the formula developed at BCSIR, Dhaka). All experiments on rats were carried out in absolute compliance with the ethical guidance for the care and use of laboratory animals. The experimental animals were marked carefully on the tail, which helped to identify a particular animal. Using identification marks, responses were noted separately for a specific rat before and after the administration.

Toxicological Experiment

An intra-gastric syringe has been used for the administration of the medicinal preparation. Administration of the drug has been carried out between the hours of 10 AM and noon.

Animal Treatment

At the end of the 28-day treatment period, the animals were fasted for 18 hours and twenty-four hours after the last administration. Ketamine (500 mg/kg i.p.) was administered for the purpose of anaesthesia.

Blood Samples Collection

Whole blood samples were collected from the post vena-cava and transferred to EDTA-added tubes immediately. All analyses were completed within 12 h of sample collection.

Results

After 28 days of chronic administration, haematological studies involved determination of toxicological aspects, those are- total WBC and differential count, various erythrocytic parameters, various platelet parameters, erythrocyte sedimentation rate. The results of the haemo-toxicological studies are given below.

Table 1: Effect of Emblica Officinalis Gaertn. (Fam. Euphorbiaceae) Pericarp of Dried Mature Fruits on WBC in Male Rats

Group	WBC	Neutrophil	Eosinophil	Lymphocyte	Monocyte
Control	5.93±0.33	29.67±0.84	1.29±0.18	66.17±0.87	2.57±0.48
EMB-OFF 50 mg/kg	7.35±0.72	22.50±1.26***	1.20±0.20^{ns}	71.60±1.21^{ns}	3.60±0.60^{ns}
	↑ 24.01%	↓ 24.16%	↓ 6.67%	↑ 8.21%	↑ 40.00%
EMB-OFF 400 mg/kg	9.87±0.56**	19.40±1.44***	1.20±0.20^{ns}	76.20±1.71***	3.20±0.73^{ns}
	↓66.4%	↓ 34.61%	↓ 6.67%	↑ 15.16%	↑ 24.45%

Values are presented as mean±S.E.M (n=8). One-way ANOVA followed by Dunnet's multiple comparisons was performed to analyze this dataset when compared against control. For Dunnet test, *P<0.05, **P<0.01, ***P<0.001, ^{ns}Not significant

At a low dose, there is an [24.01 %] increase in the white blood cell count of the male rat (table 1); the increase, though not significant, was prominent (p=0.132), whereas, at the higher dose, there is a statistically very highly significant (p=0.001) increase in the white blood cell count of the male rat [66.40 % increase] which is shown in figure 1. In the case of the neutrophil count, both at the low dose and the high dose, there was a statistically very highly significant decrease in the count (figure 2), which is 24.16 % and 34.61 % respectively. For the lymphocyte count, both at the low dose and the high dose, there is an [8.21 % and 15.16 %, respectively] increase in the percentage of lymphocyte count of the male rat (Figure 3); the increase was statistically significant (p=0.017 and p=0.001 respectively). In the case of eosinophil and monocyte count, there were no significant changes

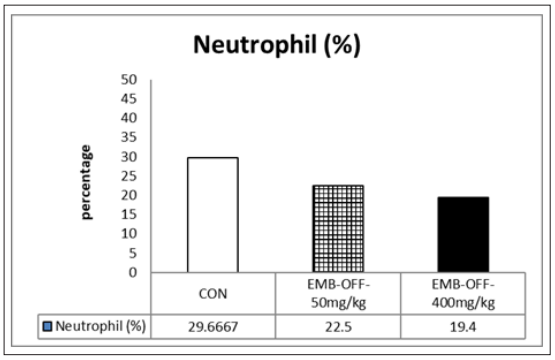

Figure 2: Effect of Emblica Officinalis Gaertn. (Fam. Euphorbiaceae) Pericarp of Dried Mature Fruits on Neutrophil (percentage) in Male Rats

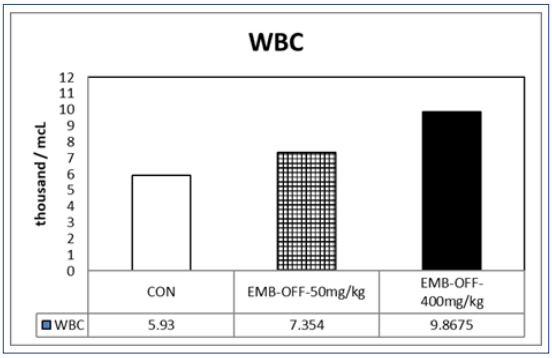

Figure1: Effect of Emblica Officinalis Gaertn. (fam. euphorbiaceae) Pericarp of Dried Mature Fruits on white Blood Cell (wbc) Count in Male Rats

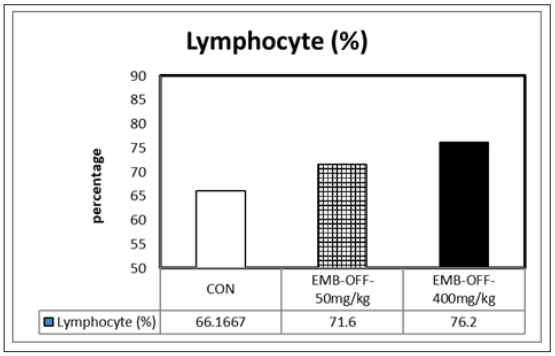

Figure 3: Effect of Emblica Officinalis Gaertn. (Fam. Euphorbiaceae) Pericarp of Dried Mature Fruits on Lymphocyte (percentage) in Male Rats

Table 2: Effect of Emblica officinalis Gaertn. (Fam. Euphorbiaceae) Pericarp of Dried Mature Fruits on RBC in Male Rats

Group	RBC	HGB	HCT	MCV	MCH	MCHC	RDW-SD	RDW-CV
Control	7.50±0.12	13.00±0.16	47.30±0.75	62.60±0.32	17.25±0.13	28.07±0.45	35.33±0.99	19.41±0.51
EMB-OFF 50 mg/kg	7.06±0.11^{ns}	12.24±0.39^{ns}	43.18±1.25*	61.12±0.78^{ns}	17.34±0.26^{ns}	28.32±0.18^{ns}	36.66±0.95^{ns}	19.62±0.45^{ns}
	↓ 5.9%	↓ 5.8	↓ 8.7%	↓2.4	↑ 0.5%	↑0.9 %	↑3.6%	↑1.1%
EMB-OFF 400 mg/kg	7.36±0.13^{ns}	12.86±0.26^{ns}	45.58±0.93^{ns}	61.98±1.70^{ns}	17.48±0.44^{ns}	28.22±0.45^{ns}	33.63±0.95^{ns}	19.12±0.50^{ns}
	↓ 1.9%	↓ 1.1	↓ 3.6%	↓0.9%	↑ 1.3%	↑0.5%	↑4.8%	↑1.5%

Values are presented as mean±S.E.M (n=8). One-way ANOVA followed by Dunnet's multiple comparisons was performed to analyze this dataset when compared against control. For Dunnet test, *P<0.05, **P<0.01, ***P<0.001, ^{ns}Not significant

Table 3: Effect of Emblica officinalis Gaertn. (Fam. Euphorbiaceae) Pericarp of Dried Mature Fruits on ESR in Male Rats

Group	ESR
Control	2.50±0.56
EMB-OFF 50 mg/kg	1.60±0.40^{ns}
	decr 36%
EMB-OFF 400 mg/kg	3.80±0.58^{ns}
	incr52%

Values are presented as mean±S.E.M (n=8). One-way ANOVA followed by Dunnet's multiple comparisons was performed to analyze this dataset when compared against control. For Dunnet test, *P<0.05, **P<0.01, ***P<0.001, ^{ns}Not significant

In table 2, it can be seen that both at the low dose and the high dose, there is a [5.92 % and 1.90 % respectively] decrease in the total numbers in the red blood cells of the male rat, the decrease though not statistically significant (p=0.054 and p=0.671 respectively). In the case of haemoglobin content, at a low dose, there was a prominent decrease [5.85 %] in the haemoglobin content of the blood of the male rat, and at the higher dose, the decrease [1.08 %] was negligible. For the haematocrit level, at the low dose, there is a statistically significant (p=0.015) decrease (8.71 %) in the haematocrit level of the blood of the male rat, whereas, at the higher dose, there is a prominent [3.64 %] decrease in the haematocrit level of the blood (Figure 4). The effect on mean corpuscular volume (MCV), a red cell index, and mean corpuscular haemoglobin (MCH) shows a negligible decreasing effect at both dosages; mean corpuscular haemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) has a negligible increasing effect at both dosages; whereas red cell volume distribution width (RDW-

SD) and RDW-CV has increasing effect at a lower dose and has decreasing effect at higher dose.

At a low dose, there is a [36.00 %] prominent decrease in Erythrocyte sedimentation rate in blood from the male rat, whereas on the contrary, at the higher dose, there is an [52.00 %] prominent increase in ESR. (Table 3)

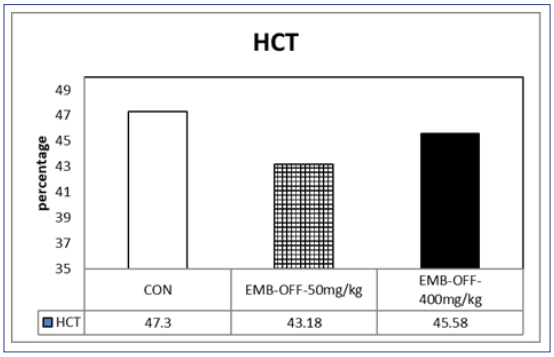

Figure 4: Effect of Emblica Officinalis Gaertn. (Fam. Euphorbiaceae) Pericarp of Dried Mature Fruits on Haematocrit (HCT) Level in Male Rats

The data of table 4 shows that, no change was noticed in the platelet count of the male rat but at the higher dose, there was a statistically significant (p=0.028) decrease in the platelet count (Figure 5) of the male rat [10.53 % decrease]. At a low dose, there was no change noticed in the mean platelet volume (MPV) of the male rat but at the higher dose, there is a negligible [2.03 %] increase in the mean platelet volume of the male rat. At a low dose, there is a [5.07 %] insignificant decrease in the Plateletcrit value (PCT) of the blood of the male rat whereas at the higher dose, there is a statistically significant (p=0.028) decrease in the Plateletcrit value of the blood of the male rat. [11.37 % decrease] (Figure 6).

At a low dose, there is a negligible [1.63 %] decrease in the platelet volume distribution width (PDW), whereas on the contrary at the higher dose, there is an [8.29 %] prominent increase in the platelet volume distribution width of the male rat. Both at the low dose and the high dose, there is a [13.59 % and 16.92 % respectively] increase in the Platelet-Large cell ratio (P-LCR) of the male rat, the increase though not significant yet it was prominent (p=0.362 and p=0.265 respectively).

Table 4: Effect of Emblica Officinalis Gaertn. (Fam. Euphorbiaceae) Pericarp of Dried Mature Fruits on Platelet in Male Rats

Group	Platelet	MPV	PCT	PDW	P-LCR	MCHC	RDW-SD	RDW-CV
Control	648.80±18.70	8.43±0.24	0.56±0.02	9.27±0.27	12.17±1.09	28.07±0.45	35.33±0.99	19.41±0.51
EMB-OFF 50 mg/kg	648.67±11.29^{ns}	8.44±0.12^{ns}	0.53±0.01^{ns}	9.12±0.26^{ns}	13.82±0.93^{ns}	28.32±0.18^{ns}	36.66±0.95^{ns}	19.62±0.45^{ns}
	↓ 0.02%	↑ 0.14%	↓ 5.07%	↓ 1.63%	↑ 13.59%	↑0.9 %	↑3.6%	↑1.1%
EMB-OFF 400 mg/kg	580.50±15.09*	8.60±0.15^{ns}	0.495±0.01*	10.04±0.32^{ns}	14.23±0.16^{ns}	28.22±0.45^{ns}	33.63±0.95^{ns}	19.12±0.50^{ns}
	↓ 10.53%	↑ 2.03%	↓ 11.37%	↑ 8.29%	↑ 16.92%	↑0.5%	↑4.8%	↑1.5%

Values are presented as mean±S.E.M (n=8). One-way ANOVA followed by Dunnet's multiple comparisons was performed to analyze this dataset when compared against control. For Dunnet test, *P<0.05, **P<0.01, ***P<0.001, ^{ns}Not significant

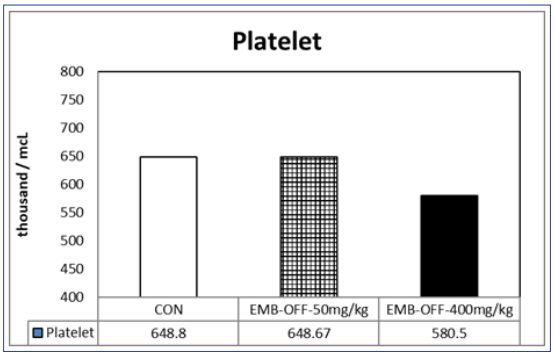

Figure 5: Effect of Emblica Officinalis Gaertn. (Fam. Euphorbiaceae) Pericarp of Dried Mature Fruits on Platelet Count in Male Rats

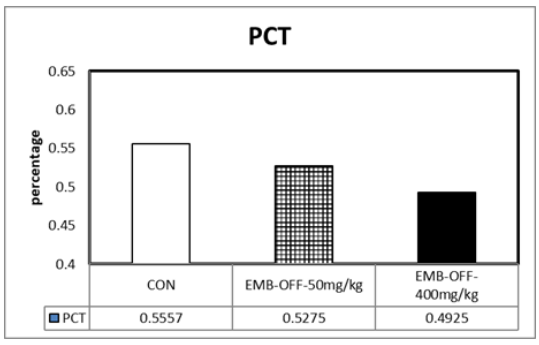

Figure 6: Effect of Emblica Officinalis Gaertn. (Fam. Euphorbiaceae) Pericarp of Dried Mature Fruits on Plateletcrit (PCT) in Male Rats

Discussion

The research indicates that the overall WBC count increases statistically significantly at higher doses. It can imply that the medication directly affects hematopoiesis or boosts the immune system. Nonetheless, a significant decrease in neutrophil counts at both low and high doses indicates that the extract is inhibiting the immune system in some ways, which could make people more vulnerable to illness and possibly help prevent it. Furthermore, a statistically significant rise in lymphocyte count at higher doses could be a compensation reaction to the decrease in neutrophils or a sign that the immune response has changed from an innate (decreased neutrophil count) to an adaptive (increased lymphocyte count) or could indicate a specific effect of the drug that enhances lymphocyte activity or proliferation.

Significant reductions in total platelet count and Plateletcrit value suggest that the extract may have a dose-dependent effect that, at larger doses, may cause thrombocytopenia and raise concerns about bleeding risk. However, the extract has no significant impact on the platelet's variability in size (PDW) or average

size (MPV), which may indicate that it does not affect platelet size heterogeneity. Additionally, there is a noticeable increase in bigger platelets (P-LCR), which are frequently more reactive and might represent a compensatory reaction to the decline in the overall platelet count.

In the case of RBC data, the drug appears to have complex dose-dependent effects on haematological parameters. Low doses may reduce hemoglobin levels and cause microcytosis (decreased MCV), potentially due to impaired RBC development or maturation. In contrast, at high doses, it prominently affects Hematocrit and RDW-SD. Still, it does not significantly impact hemoglobin or RBC count while producing narrower (RDW-SD) but uniform (RDW-CV) sized RBC. The prominent decrease in hemoglobin at a lower dose is suggestive of decreased production or increased destruction of hemoglobin, which is aligned with a significant reduction in hematocrit level. In comparison, at a higher dose, the extract may have a compensatory mechanism. It suggests that the drug may have distinct biological effects at different concentrations, affecting RBC morphology and hemoglobin synthesis differently depending on the dose.

At lower dose the extract may have anti-inflammatory response as indicated by fall in ESR but at higher dose the extract may alter the theological properties of the blood leading to increased sedimentation hence increased ESR [10-13].

Conclusion

Emblica officinalis gaertn. Offers a safer, cost effective and excellent alternative for the resource limited countries. However, further investigation would be necessary to ensure patient safety, optimize treatment outcomes, and understand the underlying mechanisms of these changes. It is also essential to understand the clinical significance of these findings and whether they correlate with any therapeutic or adverse effects of the drug. Furthermore, it would be crucial to look for any possible dangers linked to the decline in neutrophils, such as a higher possibility of infection, any indications of thrombocytopenia or problems associated with it, particularly in groups that may already be at risk for bleeding disorders or other haematological problems. Strategies to reduce dangers while maximizing the anti-inflammatory effects must be implemented. This may require exploring adjunct therapies and different dosage schedules. Conducting studies exploring the relationship between dosages, platelet dynamics, and inflammation markers would be essential.

Declarations

Ethics Declarations

The experiment was conducted with the prior approval of Institutional Ethical Approval Committee (IEAC), Primeasia University (Reference no: PAU/IEAC/25/132).

Acknowledgements

This work was conducted with the affiliation of Primeasia University, Banani, Dhaka and Jahangirnagar University, Savar, Dhaka.

Credit Author Contributions Statement

Kamrun Nahar: Conceptualization, Data curation, Formal analysis, Investigation Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing.

Md. Shanawes Hossen Rasel: Funding acquisition, Investigation Methodology, Project administration, Resources, Writing – original draft.

Mohammad Abbas Gani: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Validation, Visualization.

Fatema Tuz Johora Faria: Funding acquisition, Investigation Methodology, Project administration, Resources.

Anika Tabassum Sumaya: Funding acquisition, Investigation Methodology, Project administration, Resources.

Funding statement

This research did not receive any specific grant/ funding.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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