



Evaluation and Comparison of the effects of Low and High doses of *Emblica officinalis* (Amla) on White Cell Parameters in Animal Model

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To study and compare the effects of low and high doses of Emblic myrobalan (Amla) extract on the white blood cells in laboratory animals. Experimental study on animal model carried out in the department of Pharmacology and animal house of Baqai Medical University, Karachi from July 2017 to August 2018. Twenty-seven male rabbits of 1-2 kg body weight were taken and equally divided into 3 groups (group A, B and C). Acute inflammation was induced in group B and C animals by injecting commercially available bacterial lipopolysaccharide solution. Group A served as control group, group B (Low dose group) was receiving oral *Emblica officinalis* drug in dose of 2g/Kg body weight while group C (high dose group) was administered a drug dose of at 4g/Kg body weight. Powdered *Emblica officinalis* was mixed with water and administered orally with syringe for 60 days. Blood samples were drawn via cardiac puncture on day 61 for determination of white cell parameters. The results were analyzed using Statistical Package for Social Sciences (SPSS) v21. Administration of low dose amla extract resulted in no change in WBCs count (p- value =0.363) and increase in lymphocyte (p- value = 0.05). Administration of high dose amla produced significant decrease in WBCs count (p- value = 0.03) and lymphocytes parameters (p- value = 0.05). Amla may be used to decrease white cells count lymphocytes in acute and chronic infections.

Keywords: *Emblica Officinalis*; White Cells; Lymphocytes; Herbal Medicines

INTRODUCTION

Acute and chronic infections manifest to increase in white blood cells especially lymphocytes, which causes inflammation and destruction of vessels. It is characterized by lymphocytosis, neutrophilia and monocytosis on peripheral blood smear. The etiology of leukocytosis includes both benign to malignant pathologies i.e., common cold fever, bacterial and viral infections to leukocytosis in leukemia patients (1). The most common benign cause of leukocytosis is acute infection. The acute inflammatory response causes small blood vessel dilation, increased vascular permeability, and leukocyte emigration from circulation to the site of injury. Despite the fact that circulating neutrophils are the first responders to acute infection, Furze and Rankin discovered that the bone marrow release of mature granulocytes from storage reservoirs increases within the first 2 hours after acute insult (2). In moderate to severe infections, ongoing inflammatory mediator stimuli (predominantly interleukin-1 and tumor necrosis factor) promote both differentiation and proliferation of granulocytic progenitors in the bone marrow, resulting in a subsequent increase in neutrophil production over a few days. The marrow's release of immature band forms and precursor metamyelocytes causes a left shift, which may help distinguish infection from other nonpathogenic causes of leukocytosis (3). Chronic inflammation can also lead to excessive or unwarranted immune system activation due to noninfectious causes such as autoimmune disorders, inflammatory bowel disease, and chronic allergic conditions. Chronic inflammation and subsequent leukocytosis are caused by an inappropriate activation of the immune response against normal body tissue in many autoimmune diseases such as rheumatoid arthritis, lupus, and multiple sclerosis (4). Infectious or autoimmune vasculitis can cause severe leukocytosis. Although the etiology of inflammatory bowel disease is yet unknown, uncontrolled immune responses against colonial microorganisms are thought to contribute to persistent inflammation. Immune reactions to nonpathogenic environmental stimuli can cause allergic disorders (ie, asthma and chronic urticaria) (5).

Herbal medicine is a vital part of supplementary medicine. Many studies have shown the role of herbs in reducing inflammation. Natural remedies are rapidly advancing fields of medicine, and we must, of course, enhance our knowledge of them. While complementary, alternative, and traditional medicine are critical sources of herbal prescription guidance, mainstream medicine must certainly validate these principles scientifically before implementing them in practice. Herbal supplements possess multiple advantages over the conventional anti-inflammatory drugs, in terms of tolerability and safety profile (6). *Emblic officinalis* belong to the family Euphorbiaceae (**Table 1**). It is also known as Amla in Urdu and Emblic myrobalan in English language respectively. Its fruits are of small size having greenish yellow color, containing tannin, water, amino acid, gallic acid, flavonoids, poetin, chromium, zinc and copper. All these ingredients are active pharmacologically (7). It is a rich source of vitamin C and plays protective role in liver, cardiovascular and hematological disorders. It also strengthens the organ systems and the immune system in the body. It helps in the synthesis of collagen which is responsible for bone strength and wound healing (8). It also helps in regulation of blood sugar and lipid profile in patients with type 2 diabetes mellitus (9). The main objective of the study was to evaluate and compare the effects of *Emblca officinalis* solution on white blood cell parameters of rabbits with acute inflammation.

Table 1: The taxonomical Position of *Emblica officinalis*

Taxonomy of <i>Emblica officinalis</i>	
Kingdom:	Plantae
Order:	<i>Euphorbiales</i>
Family:	<i>Euphorbiaceae</i>
Genus:	<i>Emblica</i>
Species:	<i>Emblica officinalis</i>

MATERIAL & METHODS

A laboratory based experimental study was designed to achieve our goal. The study was conducted in the Department of Pharmacology, Baqai Medical University, Karachi in collaboration with animal house and pathology department of Baqai Medical University, Karachi from July 2017 to August 2018. All the procedures were approved by the institutional review board of the university. 27 male rabbits having weight of 1-2 kg were selected via convenient non-probability sampling. Female rabbits and those with visible disease features were excluded from the study. Rabbits were divided into 3 groups, each containing 9 rabbits. The group A was kept as control while the other two groups were kept as test drug groups i.e., B and C. Acute inflammation was induced 24hrs before starting experiment in group B and group C animals. This was achieved by injecting 2mL of lipopolysaccharide solution derived from *E-coli* (LPS: 0111: B4, Sigma-Aldrich® Chemicals, China) in dose of 0.5mg/Kg. After that, group B animals were fed with 2g/kg /day of *Emblc officinalis* solution for 60 days while Group C rabbits were fed with 4 g/day for 60 days. Powdered form of the drug was mixed with water and administered orally with syringe without needle. At the end of study, blood samples were drawn through cardiac puncture and tested for white blood cells i.e., lymphocytes, neutrophils, eosinophils and monocytes. All the parameters were analyzed using statistical package for social sciences (SPSS) version 21. Paired *t*-test was applied to analyze the differences among the groups.

RESULTS

In our current study, the effects of low dose and high dose of *Emblc officinalis* solution on white blood cells count was determined in laboratory animals. When the solution was given orally in low dose i.e., 2 mg/Kg per day (group B) to rabbits, there was a slight but non-significant increase in WBCs count, while a significant increase in lymphocytes and significant decrease in eosinophils count was observed as compared to control group A. However, a non-significant decrease in neutrophils count while no change in monocytes count was observed. The results are summarized in **Table 2**. On the other hand, a significant decrease in white blood cells count was noted with administration of *Emblc officinalis* solution in high doses i.e., 4mg/Kg per day (group C). **Table 3** shows comparison of white cell parameters of control group A with group C animals. Compared to control group, a significant decrease in lymphocytes count was found in group C animals.

Table 2: Comparisons of hematological parameters with low dose of *Emblic officinalis*

Hematological Parameters	Controls (n=9)	Low dose of <i>Emblic officinalis</i> (n=9)	p-value
	Mean $\pm$ SEM	Mean $\pm$ SEM	
WBC ($10^3/\text{ul}$)	6.3 $\pm$ 0.16	6.5 $\pm$ 0.11	0.036
Lymphocyte (%)	38.9 $\pm$ 4.00	46.8 $\pm$ 0.78	0.05
Neutrophil (%)	56.4 $\pm$ 4.13	58.4 $\pm$ 0.77	0.173
Eosinophil (%)	3.0 $\pm$ 0.24	3.2 $\pm$ 0.22	0.50
Monocytes (%)	2.0 $\pm$ 0.00	2.2 $\pm$ 0.1	1.000

Table 3: Comparisons of hematological parameters with high dose of *Emblic officinalis*

Hematological parameters	Controls (n=9)	High dose of <i>Emblic officinalis</i> (n=9)	p-value
	Mean $\pm$ SEM	Mean $\pm$ SEM	
WBC ($10^3/\text{ul}$)	6.3 $\pm$ 0.16	6.0 $\pm$ 0.10	0.03
Lymphocyte (%)	38.9 $\pm$ 4.00	34.1 $\pm$ 1.02	0.05
Neutrophil (%)	56.4 $\pm$ 4.13	48.3 $\pm$ 1.04	0.079
Eosinophil (%)	3.0 $\pm$ 0.24	2.4 $\pm$ 0.18	0.077
Monocytes (%)	20.0 $\pm$ 0.00	20.0 $\pm$ 0.00	1.000

DISCUSSION

Herbal supplements play significant role in improving the hematological parameters. *Emblica officinalis* (Amla) had been extensively used in ancient Indian medicine. Liquid chromatography-mass spectrometry (LC-MS) analysis of the extract revealed the presence of gallic acid, quinic acid, and quercetin as the major constituents in addition to the other flavonoids (10). The *Emblica officinalis* shows antioxidant, anticarcinogenic, antitumor, antigenotoxic, anti-inflammatory activities. As per studies, *Emblica officinalis* gives 3,000 mg of vitamin C per fruit, making it one of the highest natural sources of the vitamin C.⁶ It helps to decrease the risk of cardiovascular abnormalities such as atherosclerosis as its juice contains high fraction of polyphenol acting as antioxidant. Similarly, the powdered drug is also used traditionally for relief of neuropathic pain in diabetic patients. It is also a proven fact that the amla (*E. officinalis*) extract in organic solvents (such as CHCl_3 and CH_3OH) is effective against some Gram-positive and Gram-negative bacteria and helps in decreasing white cell counts in acute as well as chronic infections. As per different studies, the extract of *Emblica officinalis* showed anticancer activity due to its polyphenol contents. Similarly, the drug also showed radiation retardant activity in patients undergoing radiotherapy against cancer (11, 12).

This study was conducted on rabbits using *Emblica officinalis* (Amla). The study is unique as no previous study has been conducted in past to observe the effects of low and high doses of *Emblica officinalis* solution on hematological parameters in rabbits. As per our study, there was a non-significant increase in WBCs count including neutrophils, eosinophils and monocytes count in rabbits with acute inflammation. However, there was a significant increase in lymphocytes count in group B rabbits. This may be due to the fact that low doses (2mg/Kg) of *Emblica officinalis* solution had no effects on WBCs count in acute inflammatory processes. Furthermore, significant changes were found in the hematological parameters of test groups administered with high doses of the drug compared to the control group A. Administration of high dose *Emblica officinalis* solution to rabbits led to significant decrease in WBCs and lymphocytes count (p -value = 0.05) in rabbits with acute inflammation as compared to control group. The probable reason for this improvement may be due to stabilizing action of *Emblica officinalis* on WBCs cell membrane (13). Another explanation for this is that the methanolic extract of *Emblica officinalis* fruit also possesses antimicrobial activity against *E-coli*, suggesting its indirect role in decreasing WBCs count in our study as showed by Anbukkarasi *et al* (14). Similarly, as per Singh *et al.*, the fruit extract of *Emblica officinalis* reduces the migration of WBCs and other immune cells to free radicle's affected organ, resulting in decrease in pro-inflammatory response (15). According to our results, the effects of high dose of *Emblica officinalis* solution on neutrophils and eosinophils count was found to be non-significant, while the number of monocytes remain unchanged.

CONCLUSION

Emblica officinalis is helpful in improving white cell parameters. However, it is very early to recommend the use of the drug in patients with acute and chronic infections. However further studies and trials are required to determine uses in safe doses.

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