

## Impact of Aqueous Fruit Extract of Haritaki (*Terminalia chebula*) on Blood Parameters of Mammalian Model (Albino Rats)

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**Abstract:** The effect of aqueous *Terminalia chebula* fruit extract on hematological parameters in rat during a seven day oral administration of the low dose of 250mg/kg and high dose of 500 mg/kg of body weight was investigated. The parameters evaluated include red and white blood indices. The result showed significant increase in TLC, neutrophil, eosinophil, total RBC, (Haemoglobin) Hb, (Mean corpuscular haemoglobin concentration) MCHC at the dose of 250mg/kg and 500mg/kg body weight when compared with control. Whereas lymphocyte, monocyte, basophil significantly varied and (Packed cell volume) PCV, (Mean corpuscular volume) MCV, (mean corpuscular haemoglobin) MCH and platelets showed non-significant changes in low dose as compared to control. The results of this study suggest that the fruit extract may have beneficial effect on leucocytosis and blood dependent disorders.

**Key words:** *Terminalia chebula* • Haematology • Leucocytosis • PCV • MCHC • MCV

### INTRODUCTION

Herbal medicines are in great demand in the developed world for primary health care due to their efficacy, safety and lesser side effects [1]. Herbal medicines are as effective as their counterpart conventional medicines, this is one of the main reasons, why medicines of plant origin are gaining grounds rapidly [2, 3]. In 2001, researchers identified 122 compounds used in modern medicine which were derived from 'ethnomedicinal' plant sources; 80% of these have had an ethnomedical use identical or related to the current use of the active elements of the plants [4]. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80% [5]. This is because the herbal medicines are cheap and have natural origin with higher safety margins and lesser or no side effects [6].

Fruits are amongst the first food items known to human beings. Fruits, whether fresh or dried, have always formed a part of the staple diet of human beings. The reason for this is that they are rich in nutrients and provide some of the essential minerals, vitamins, etc. to

our body. Apart from that, they also help in curing a number of diseases [7]. *Terminalia chebula* Retz (commonly known in India as Triphala) is a plant species belonging to the family Combretaceae. It is a flowering evergreen tree known as Black Myrobalan in English and Haritaki in Sanskrit and west China stretching all along South Kerala and even Srilanka where it is called as Ara [8-11]. The fruit extract has many pharmacological uses for treatment of a number of diseases [12]. *T. chebula* uses has been reported in treatment of diabetes [13-15], digestion, cough, pitta, jaundice, oedema, asthma and constipation [16], antioxidant [17], cardio protective [18] and immunomodulatory [19]. Despite its extensive medicinal use no information is available related to its effects on hematological profile. Hence the present work investigated the effect of *T. chebula* fruit extract on hematology profile of albino rats.

### MATERIALS AND METHODS

**Collection of Plant Material:** The fresh fruits of *Terminalia chebula* were collected from Ranchi, dried in shade six to seven days and then crushed into coarse powdery substance by using electric grinder. The coarse

powdery substance was dried again and was then sieved to get fine powder using the fine plastic sieve and stored in an air tight bottle in the laboratory until required [20-22].

**Extract Preparation:** 50 g of the sieved powder was subjected to extraction in a Soxhlet apparatus at room temperature using ~350 mL distilled water. The extract obtained was filtered, concentrated in rotary flash evaporator and maintained at 45°C the percentage yield of each extract was calculated [23, 24].

**Animals:** Male Albino rats (175-200 g) were used in the study. They were maintained under standard laboratory conditions at ambient temperature of 25±2°C and 50±15% relative humidity with a 12-h light/12-h dark cycle. Animals were fed with a commercial pellet diet and water *ad libitum*. The experiments were performed after prior approval by the institutional animal ethics committee of Ranchi University, Ranchi.

**Acute Toxicity Studies:** Acute toxicity studies were determined by using fixed dose method according to OECD guidelines. Healthy adult mice, weighing 175-200g were used. 20 albino rats of either sex were used to determine the LD<sub>50</sub> of the aqueous extract of fruit of *Terminalia chebula*. The animals were randomly [25] divided into two groups of 10 rats each and administered and observed for 90 days as follows:

**Group 1:** Received 1 ml of distilled water orally.

**Group 2:** Received 250 mg/kg body weight of extract orally.

**Group 3:** Received 500 mg/kg of body weight of extract orally

Mortality was not observed up to 500 mg/kg of body weight in case of aqueous fruit extract of *Terminalia chebula*.

**Sample Collection:** At the end of each experimental period, the rats were reweighed, starved for 24 hours and sacrificed under chloroform anesthesia. 5mL of blood was collected from each animal by cardiac puncture using sterile needle and syringe. Part of the blood sample was put into test tubes and allowed to clot for 30 minutes before centrifuging at 800g (Wisperfuge, 1384, Samson, Holland) for 5 minutes. The supernatant

was used for the lipid analysis. The remaining blood sample was put in an EDTA bottles for hematological determinations.

### Analytical Procedure

**Estimation of Hematological Profile:** The hemoglobin (Hb) level was measured by the cyanmethaemoglobin method. The Red blood cell (RBC) and Reticulocyte counts were determined by visual method [26]. Packed cell volume (PCV) was measured using microhematocrit method and total white blood cell (WBC) count was estimated by visual method [27]. The RBC indices were calculated from the RBC count, Hb level and PCV estimations [26, 27].

**Statistical Analysis:** All results were expressed as mean ± standard error of mean (S.E.M). Data was analyzed using one-way ANOVA followed by Dennett's- test, p<0.05 was considered as statistically significant.

## RESULTS AND DISCUSSION

The effects of oral administration of aqueous extract of *T. chebula* fruit on Hematological Indices are presented in Table 1.

The total leucocyte count (TLC), eosinophil, basophil, Hb, (packed cell volume) PCV and Platelet count showed significant increase in case of low dose (group 2) as compared to the control (group 1). The neutrophils, lymphocytes, monocytes and MCV showed significant decrease in case of both low dose and high dose (group 2 and group 3 respectively) as compared to the control (group 1). No significant change was observed in case of total RBC and MCHC.

Table 1: Effect of *Terminalia chebula* extract on hematological profile of Rats (value are expressed as mean±SD, n=6, statistical significance was considered to be <sup>a</sup> p<0.10, <sup>b</sup> p<0.25, <sup>c</sup> p<0.025, <sup>d</sup> p<0.05, <sup>e</sup> p<0.0001, <sup>f</sup> p<0.0025, <sup>g</sup> p<0.01, <sup>h</sup> p<0.005 relative to control)

PARAMETERS	GROUP 1	GROUP 2	GROUP 3
TLC(μL)	6.6±0.236	7.441±0.11 <sup>a,e</sup>	6.953±0.01 <sup>a,b</sup>
NEUTROPHILL (%)	56.71±0.116	41.61±1.21 <sup>b,e</sup>	42.36±0.98 <sup>b,e</sup>
LYMPHOCYTE (μL)	32.61±0.194	24.33±1.49 <sup>b,a</sup>	21.5±1.70 <sup>b,c</sup>
MONOCYTE (/μL)	32.61±0.19	6.566±0.26 <sup>c,f</sup>	7.55±0.170 <sup>c,g</sup>
EOSINOPHILL (/μL)	0.65±0.187	3.293±0.04 <sup>d,e</sup>	1.555±0.01 <sup>d,d</sup>
BASOPHILL (/μL)	0.275±0.010	1.423±0.01 <sup>d,h</sup>	1.251±0.02 <sup>d,c</sup>
TOTAL RBC (/μL)	4.258±0.031	4.383±0.03 <sup>b,e</sup>	4.418±0.05 <sup>b,b</sup>
HB (g/dL)	11.61±0.172	14.03±0.02 <sup>a,e</sup>	14.31±0.043 <sup>a,c</sup>
PCV (%)	37±2.607	43.47±1.38 <sup>b,b</sup>	43.15±0.60 <sup>b,b</sup>
MCV (μL)	98.33±2.065	93.08±1.00 <sup>b,b</sup>	93.43±0.93 <sup>b,b</sup>
MCH (pg)	30.73±0.695	31.5±0.45 <sup>c,b</sup>	27.06±0.59 <sup>c,a</sup>
MCHC (g/DL)	31.06±0.014	32.65±0.17 <sup>b,e</sup>	31.38±0.50 <sup>b,b</sup>
PLATELET COUNT (/μL)	335.8±2.639	339±1.29 <sup>c,b</sup>	32.31±0.21 <sup>e,h</sup>

The *T. chebula* decreased WBC count which helps in blood flow regulation [28]. High levels of WBC count is leucocytosis. The reduction in WBC by the extract shows that it can be used as a tool against leucocytosis. Therefore the high dose (500mg/kg body weight) of the *T. chebula* extracts useful in reducing the total leucocyte which in turn might be useful in leucocytosis, lymphocytosis, monocystosis, eosinophilia and basophilia [29]. It is frequently a sign of an inflammatory responses most commonly the result of infection, it may also occur after strenuous exercise, convulsions such as epilepsy, emotional stress, pregnancy and labour, anesthesia and epinephrine administration. It is characterized by acute bacterial infections, tissue necrosis, allergic disorders like asthma, hay fever, drug allergies, allergic skin diseases [29]. Similar alterations found in *P.guajava* when low dose and high dose was compared to control [30]. The *T. chebula* extract show the platelet significantly increased ( $p < 0.05$ ) in rats treated with both the doses [30]. The results obtained shows significant values of WBC, therefore it is clear that an increase in the number of WBC is a normal reaction of rats to foreign substances, which alter their normal physiological processes. Platelets play a major role in the development as well as in the stability of atherosclerotic plaques and as a consequence, anti-platelet agents have been used clinically in patients at risk for myocardial ischemia, unstable angina and acute myocardial infarction [31, 32] but in high dose (500 mg/kg body weight) of the *P. guajava* extracts were useful in reducing the platelets which in turn might be useful in reducing the cardiovascular diseases as some studies suggested various mechanisms by which flavonoid exert its anti platelet property by lowering intracellular  $Ca^{2+}$  levels; alteration in the metabolism of cAMP and thromboxane  $A_2$ [27]. The haemoglobin content, RBC and PCV has also significantly increased stimulate erythropoietin release in the kidney which is the humoral regulators of RBC production [33, 34]. The result of this study suggested that *T. chebula* extract studied showed positive hematological activities in rats and can be recommended in the management of anemia and other blood dependent disorders.

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