

## RESEARCH ARTICLE

# Ameliorative role of leaf extract of *Pithecollobium dulce* on chloramphenicol induced hematological changes in *Mus musculus*

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**ABSTRACT**

Leaf extract of *Pithecollobium dulce* was evaluated for its protective effect against chloramphenicol induces hematological changes like aplastic- anaemia, leucocytosis, thrombocytosis, etc. The dose of chloramphenicol i. e. 500 mg/1 kg. body weight of mice for was administered orally for 14 days. On day 15 hematological parameters like Haemoglobin level (Hb), red blood cell (RBC) count, white blood cells (WBC) count, blood platelets (thrombocytes) count, differential WBC count were declined in toxin group except the lymphocytes level was increased. But the methanolic leaf extract of *Pithecollo biumdulce* showed significantly increase in RBC count, total WBC count, platelets count and some leucocytes. While decrease in Haemoglobin (Hb) level, neutrophils etc. The result indicates that the leaf extract of *Pithecollo biumdulce* significantly recovered the altered values of blood cells due to chloramphenicol induced toxicity in bone marrow as well as haematological parameters.

**Keywords:** Chloramphenicol, *Pithecollobium dulce*, aplastic anaemia, haematological changes.

**INTRODUCTION**

Chloramphenicol is an antibiotic useful for treatment of a number of bacterial infections. This includes meningitis, plague, cholera, and typhoid fever. It is recommended when safer antibiotics cannot be used. However, it leads to toxic effect to blood parameters like aplastic anaemia (Rich *et al.*, 1950), leukemia (Shu *et al.*, 1987), bone marrow suppression due to direct toxic effect of the drug on human mitochondria (Yunis, 1989). The anaemia is fully reversible once the drug is stopped and does not predict future development of aplastic anaemia. Studies in mice have suggested existing marrow damage may compound any marrow damage resulting from the toxic effects of chloramphenicol (Morley, 1976).

*Pithecollobium dulce* has been commonly used for fencing and tanning, as fodder for feed and pods for food. Infusions of different parts of *P. dulce* have been traditionally used to treat diseases, for example, skin of the stem is used for dysentery, leaves for intestinal disorders and seeds for ulcers (Zapesochnya *et al.*, 1980; Rzedowski and Rzedowski, 1985).

## MATERIALS AND METHODS

**Plant material and authentication:** The fresh leaves were collected from the local area of Kalyan, Maharashtra, India and washed the leaves in order to wash the dust particles. The plant was identified at BLATTER HERBARIUM, ST. Xavier's college, Mumbai-400001.

**Preparation of extraction:** The leaves were dried in sun for 7 days continuously and made powder form. The powdered mass of leaves was defatted with petroleum ether (60° to 80° c). It is followed by extraction with methanol (95 % v/v) for about 18 hour by using soxhlet apparatus. The extract was filtered and the filtrate was concentrated under reduced pressure using rotary evaporator to obtain the extract as solid as residues. The extraction value (% w/w) of methanol was 18 (Sugumaran *et al.*, 2008).

**Experimental animals- Mice:** Swiss albino mice of both sexes weighing from 30-35 gm were used for the study, obtained from Haffkins Institute, Parel (E), Mumbai-400012. The house was maintained at 28±20 c and exposed to 10-12 hours of day light and a relative humidity of 30-70 %. The animals were provided with drinking water ad libitum and fed on commercially available feed supplied by Amrut feed.

**Chloramphenicol palmitate:** It was procured from Mehta pharmaceutical limited, 315, Jankicentre, plot no. 29, Shah Industrial Estate, Off. Veera Desai road, Andheri (W), Mumbai, India. It was kept below room temperature.

### Experimental design

**Group I** (6 mice) were used as control

**Group II** (6 mice) received 500 mg/kg chloramphenicol.

**Group III** (6 mice) received 200 mg/kg leaf extract of *Pithecollobium dulce*.

**Group IV** (6 mice) received 500 mg/kg chloramphenicol as well as 200 mg/kg leaf extract of *Pithecollobium dulce*.

### Blood sample collection and analysis

Blood sample was collected by puncture of retro-orbital vein and put the blood in EDTA vial for all haematological analysis like (RBC) count was done using the methods by Dacie and Lewis (2001); Antai *et al.* (2009) Blood was diluted to 1:200 with Hayem's fluid which preserved the corpuscles and then counted with Neubauer counting chamber under a light microscope. Sahli's hemoglobinometer was employed for estimation of hemoglobin (Hb) content of the blood. WBC count, differential WBC count and thrombocyte count were done as per the standard method (Schalm *et al.*, 1975).

**Statistical evaluation:** All the values were expressed as mean ± SD for 6 mice. Statistical significance of differences between the control and experimental groups was assessed by Analysis of variance (ANOVA) two ways without replication. The value of probability less than 5 % (P < 0.05) was considered statistically significant.

## RESULTS

Table 1 shows the effect of leaf extract of *Pithecollobium dulce* on hematological parameters of mice like Hb, RBC, WBC and thrombocytes count. While table 2 shows differential WBC count. Hb, RBC, WBC, thrombocytes, eosinophils, lymphocytes and monocytes were significantly increased (P < 0.05), while neutrophils value was decreased in prophylactic group of mice fed with chloramphenicol as well as leaf extract of *Pithecollobium dulce*. There was significant difference in Total WBC count as well as neutrophils and monocytes.

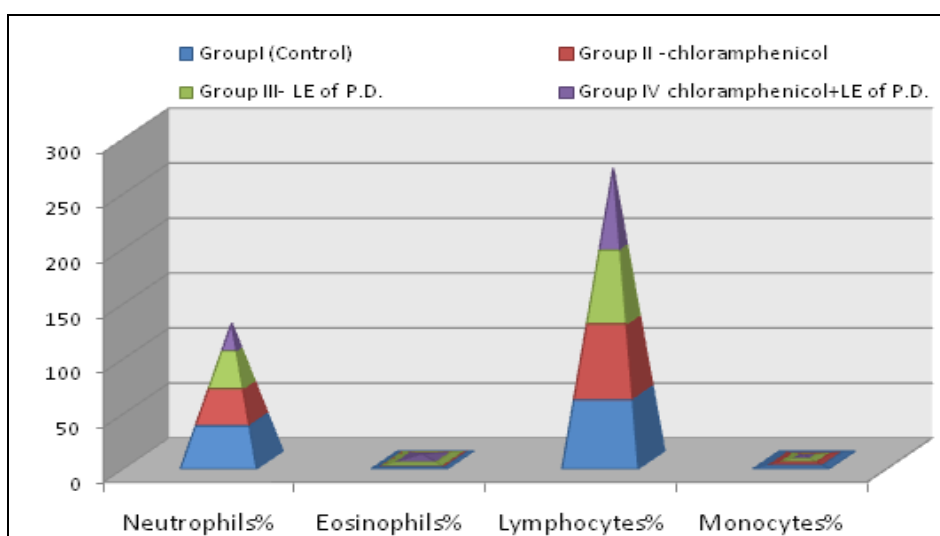
**Table 1:** Haematological observations after treatment chloramphenicol and recovery with the help of leaf extract of *Pithecollobium dulce* in mice.

	Hb gm %	RBC × 10 <sup>6</sup>	TLC × 10 <sup>3</sup>	Platelets × 10 <sup>5</sup>
Group I (Control)	14.58±0.55	6.5 ± 0.18	8.55±	7.68 ± 0.79
Group II -chloramphenicol	14.22±0.9	6.4 ± 0.48	7.78±	7.26 ± 1.37
Group III- LE of P.D.	14.25±1.87	7.83 ± 0.28	10.45±	6.85 ± 0.78
Group IV chloramphenicol+LE of P.D.	16.08±0.56	7.2 ± 0.57	9.48±	7.6 ± 0.71

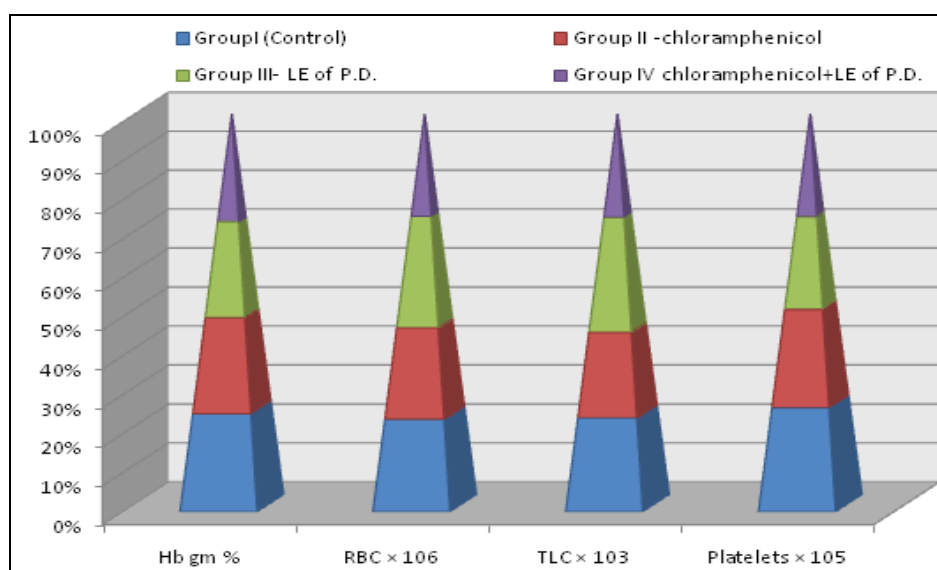
**Table 2:** Haematological observations after treatment chloramphenicol and recovery with the help of leaf extract of *Pithecollobium dulce* in mice.

	Neutrophils%	Eosinophils%	Lymphocytes %	Monocytes %
Group I (Control)	36.83 ± 5.47	0.66 ± 0.48	61 ± 6.1	1.16±0.37
Group II -chloramphenicol	31.83 ± 5.43	0.5 ± 0.5	67 ± 5.5	1±0
Group III- LE of P.D.	32.33 ± 7.15	1.33 ± 1.79	65.17 ± 8.03	1.17±0.69
Group IV chloramphenicol+ LE of P.D.	23.67 ± 3.4	3 ± 2	72.33 ± 4.1	1±0

P values < 0.05 by 'F' test. The values are expressed as Mean ± SE from 6 rats in eachgroup.  
LE of P.D. means leaf extract of *Pithecollobium dulce*.



**Fig. 1:** Effect of methanol extract of *Pithecollobium dulce* leaves on haematological parameters of mice with chloramphenicol induced haemato toxicity.



**Fig. 2-** Effect of methanol extract of *Pithecollobium dulce* leaves on haematological parameters of mice with chloramphenicol induced haemato toxicity.

Medicinal drugs or chemicals induced anaemia (Lewis *et al.*, 2002) other haematological disorders, tissue injuries etc. have been known. In the present study it was found that the leaf extract of *Pithecollo biumdulce* recovered the hematological variations induced by chloramphenicol. It was observed that the treatment of leaf extract of *Pithecollo biumdulce* significantly reduced the tissue damaged in bone marrow that leads to decrease the number of erythrocytes, variations in the leucocytes and thrombocytes.

The results showed that RBC, Hb level were significantly higher in the leaf extract of *Pithecollo biumdulce* feed groups and compared these values with control, toxin and prophylactic group of mice fed with chloramphenicol as well as leaf extract of *Pithecollo biumdulce*. This suggests that the flower extract contain agents that induced the production of red blood cells or enhances erythropoiesis by bone the marrow.

Total leucocyte count increased in only in prophylactic group of mice fed with chloramphenicol as well as leaf extract of *Pithecollo biumdulce*. Increase in total WBC count may be beneficial as they are vital in the body's defence mechanism (Gilani *et al.*, 2000).

The platelets level was also increased except the group III. When study separately the differential WBCs count, neutrophil value was not much recovered as compare to group I (control). The eosinophil value was elevated much more as compare to other groups. The level of lymphocytes was increased in all the groups II, III and IV as compare to group I. The value of monocytes was near about similar means it was not affected by chloramphenicol as well as leaf extract of *Pithecollo biumdulce*. It means the chloramphenicol + leaf extract of *Pithecollo biumdulce* group exhibited mark changes in the haematological parameters indicating that when chloramphenicol with extract diet fed mice has been ameliorated.

Scientists continue to investigate that when drug entered in body the free radicals are formed which are damaging the bone marrow. But antioxidants present in the leaf extract of *Pithecollo biumdulce* that act as defense system against free radicals.

In conclusion, *Pithecollo biumdulce* methanolic extract of leaves given to mice orally at the dose of 200 mg/kg body weight improved full blood count in mice. (Simplicio *et al.*, 1996) The extract should be taken with caution despite of its antioxidant and hematopoietic effect.

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